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LDL-oxidation, serum uric acid, kidney function and pulse-wave velocity: Data from the Brisighella Heart Study cohort

Arrigo F.G. Cicero [a,*,1], Masanari Kuwabara [b,d,1], Richard Johnson [c,1], Marilisa Bove [a,1], Federica Fogacci [a,1], Martina Rosticci [a,1], Marina Giovannini [a,1], Sergio D'Addato [a,1], Claudio Borghi [a,1], Brisighella Heart Study group

[a] Medical and Surgical Sciences Dept., University of Bologna, Italy
[b] Department of Cardiology, Toranomon Hospital, Tokyo, Japan
[c] Division of Renal Diseases and Hypertension, School of Medicine, University of Colorado Denver, Aurora, CO, United States
[d] Department of Cardiology, Cardiovascular Center, St. Luke's International Hospital, Tokyo, Japan.

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ABSTRACT

Background: Serum uric acid (SUA) and oxidized LDL (oxLDL) may be associated with arterial aging. The aim of our study was to evaluate the relationship between SUA, oxLDL and arterial stiffness in subjects with normal renal function and in patients with mild or moderate renal impairment.
Methods: From the database of the 2012 Brisighella Heart Study, we compared age-matched adult, non-smoker subjects without cardiovascular disease and with normal renal function ($n = 205$), subjects with stage II chronic kidney disease (CKD) ($n = 118$) and subjects with stage III CKD ($n = 94$). All subjects underwent a determination of the LDL oxidative susceptibility, oxLDL levels, SUA and Pulse Wave Velocity (PWV).
Results: By univariate analysis, PWV correlated with a large number of clinical, haemodynamic and metabolic parameters, including estimated glomerular filtration rate (eGFR) in subjects with normal renal function and in those with stage II or III CKD. Stepwise multiple regression analyses showed that in the presence of normal renal function or stage II CKD, the main predictors of PWV were age, systolic blood pressure (SBP), ox-LDL, apolipoprotein B and SUA ($p < 0.05$), while in the presence of stage III CKD only age, SBP and apolipoprotein B remained significant ($p < 0.05$).
Conclusion: Both ox-LDL and SUA independently predicts PWV only in subjects with normal or mildly reduced renal function, but not in the subjects with more compromised eGFR. This study confirms the complex relationship of SUA with cardiovascular and metabolic disease in the patient with established renal disease.

1. Introduction

The morbidity and mortality rates related to cardiovascular diseases are still very high in both developing and rich countries as a response to many different pathophysiological mechanisms [1,2]. Oxidative burst is an innate immune response to infections aimed at neutralizing the endotoxin dangerous effects. The consequences of oxidative activation are not limited to physiology and also involve the development of several pathological conditions. In particular, the onset and progression of the atherosclerotic lesions is related to different oxidative reactions, such as the oxygen and peroxyl radical synthesis and the oxidation of low-density lipoprotein (LDL) cholesterol, closely associated with the circulating free radical damage [3]. Lipid oxidation is essential for the formation and progression of the atherosclerotic plaque. The production of reactive oxygen compounds raises the number of the oxidized LDL (ox-LDL) particles that accumulate in macrophages leading to the formation of the foam cells and the stem cell proliferation, promoting the atherosclerosis progression [4]. Ox-LDL's also induce the maturation of dendritic cells and the activation of the alternative (M2) macrophage and T helper 2 response, that represent an important link between the innate and the adaptive immunity and favour the atheroma lesions' evolution [5].

Uric acid is a complex molecule that can act as either an antioxidant or prooxidant. It can act as an antioxidant by directly reacting with superoxide anion to generate allantoin [6] and peroxynitrite to generate triuret [7,8], its reaction with the latter also generates radicals in the process [7,9]. Moreover, it can generate hydroperoxides on reaction with myeloperoxidase and hydrogen peroxide [10,11], and can also directly inactivate nitric oxide to form 6-aminouracil [12,13]. Intracellular uric acid can also stimulate NADPH oxidase, leading to both intracellular and mitochondrial oxidative stress [14,15]. Indeed, uric acid can accelerate the oxidation of LDL under certain conditions, thereby countering the effects of ascorbic acid [16].

* Corresponding author at: Atherosclerosis Research Unit - Medical and Surgical Sciences Dept., Sant'Orsola-Malpighi Hospital - University of Bologna, Via Albertoni, 15, 40138 Bologna, Italy.
E-mail address: arrigo.cicero@unibo.it (A.F.G. Cicero).
[1] This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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It is perhaps not surprising, therefore, that an elevated SUA is associated with early markers of vascular stiffness [17,18] and is an independent predictor of increased cardiovascular risk [19] also in children [20] and healthy subjects [21]. The plasma levels of ox-LDL level [22] and serum uric acid (SUA) [23] are concomitantly elevated in patients with chronic kidney disease (CKD) and their interaction could be, at least in part, responsible for the increased risk of cardiovascular disease in these patients.

The aim of our study was to therefore evaluate the relationship between SUA, LDL oxidation and arterial stiffness in subjects with a normal or moderately reduced renal function.

2. Materials and methods

The Brisighella Heart Study (BHS) is a longitudinal population model active since 1972 on a randomized sample, which is representative of the population of Brisighella, a rural North-Italian village. At the baseline, it involved 2939 Caucasian subjects (1491 men and 1448 women), aged 14–84 and without history of cardiovascular disease at enrolment. The complete study protocol has been previously described elsewhere [24]. Briefly, participants were clinically evaluated at baseline and every 4 years thereafter, by collecting a large setting of clinical data and biochemical parameters. Mortality and morbidity data, as well as the incidence of the main cardiovascular risk factors, were recorded throughout the entire study [24].

For the purpose of the present study, we selected from the general database of the Brisighella Heart Study three groups of age- and sex-matched subjects with different levels of renal function and examined during the 2012 population survey. From the studied population we excluded patients with active and previous smoking habit, previous cardiovascular disease, confirmed type 2 diabetes, history of rheumatological diseases (including gout), active kidney disease other than CKD or severe CKD (G4–G5: eGFR < 30 ml/min/1.73 m^2), and current use of any antioxidant dietary supplement or vasodilating drugs, as well as subjects with antihypertensive therapy not stabilized from at least 3 months. Pregnant or nursing mothers and women taking oestrogens or oral contraceptives were also excluded from the analysis (Fig. 1). The selected subjects' characteristics were then compared to the one of the whole cohort in order to verify that they maintained their representativity of the original cohort.

The study has been carried out in agreement with the declaration of Helsinki and the protocol has been approved by the institutional ethical board of the University Hospital of Bologna (Code: BrixFollow-up_1972–2024). All involved subjects signed an informed consent form.

For every subject we recorded a detailed personal and family history (with specific attention to lifestyle and dietary habits, smoking status and pharmacological treatments), a physical examination (including anthropometric data), resting blood pressure and heart rate, a fasting blood sample and a 12 lead electrocardiogram (Minnesota-coded) [25]. Waist circumference (WC) was measured as the narrowest body diameter between the arcus costarum and the crista iliaca. Height was evaluated with the person standing erect, bare foot together and eyes directed straight ahead. Weight was measured twice, and the average of these two measures is used. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Systolic (SBP) and diastolic (DBP) blood pressure were measured three times at 1-minute interval with a standard sphygmomanometer and with the subject in the seated position and after 5 minutes of quiet rest. The average value of the three measurements was taken as individual blood pressure value.

The biochemical analyses were carried out on venous blood from the basilic vein. Subjects were fasted for at least 12 h at the time of sampling. All available routine laboratory parameters were sampled with standardized methods by trained personnel [26], evaluating fasting plasma glucose (FPG), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), LDL-C, apolipoprotein AI (apoAI), apolipoprotein B-100 (apoB), lipoprotein(a), aspartate aminotransferase (ALT), alanine aminotransferase (AST), gamma-glutamyl-transferase (GGT), total bilirubin (TB), creatinine, estimated glomerular filtration rate (eGFR) [27], SUA and creatinine phosphokinase (CPK). The renal function degree was classified on the basis of the KDIGO guidelines [28], dividing subjects with normal renal function (G1: eGFR ≥ 90 ml/min/1.73 m^2), with mildly impaired renal function (G2: eGFR = 60–90 ml/min/1.73 m^2) and those with more severely impaired renal function (G3: eGFR = 30–60 ml/min/1.73 m^2).

Oxidative susceptibility of LDL was obtained following the method of Esterbauer et al. [29] by monitoring the LDL oxidation kinetic by the change of the 234 nm diene absorption. The time-course showed three consecutive phases: 1) a lag-phase, in which no oxidation occurred, which was measured in minutes and was proportional to the LDL resistance to oxidation, 2) a propagation phase, with a rapid increase of conjugated dienes production and absorption, 3) a diene decomposition phase, characterized by a slow absorbance increase when the conjugate diene on LDL (diene mol/LDL mg) was calculated. The lag time was used in order to evaluate the oxidative susceptibility of LDL after ultra-centrifuge isolation. The oxidized LDL dosage was obtained using a standardized ELISA kit (Mercodia, Uppsala, Sweden) including monoclonal antibodies against specific antigenic determinants on the

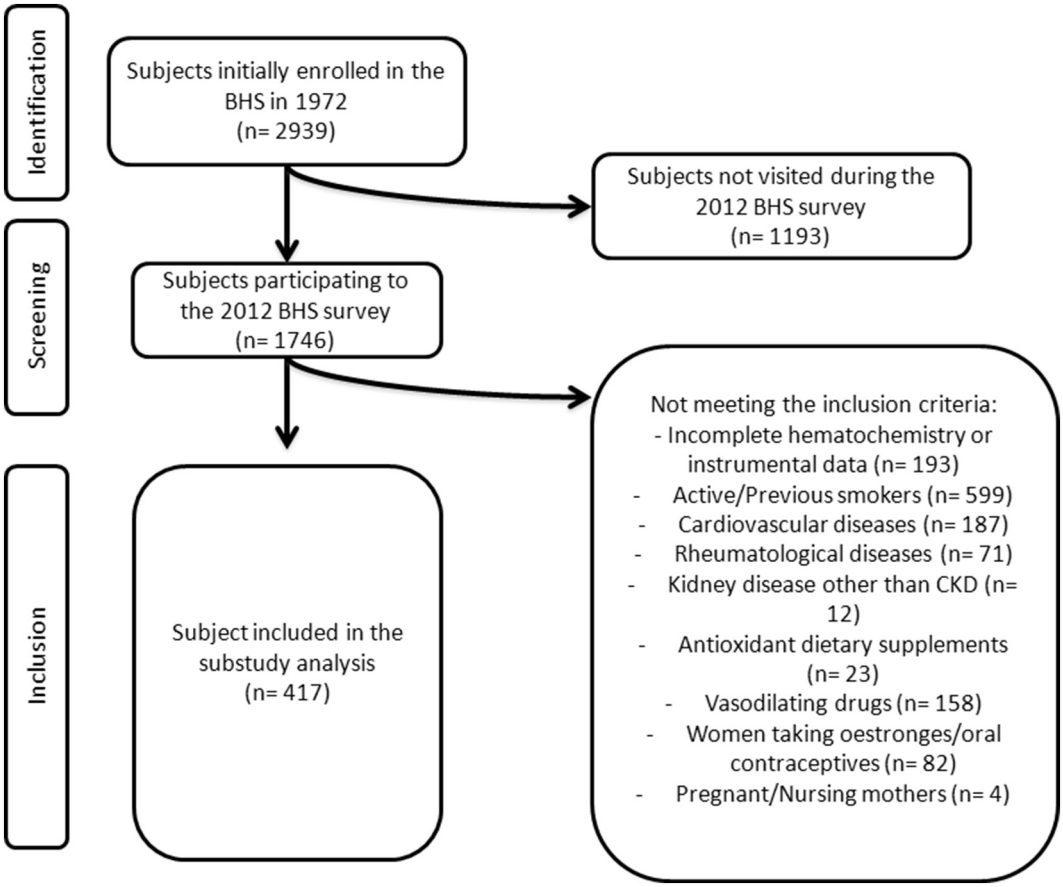

Fig. 1. Flow-chart resuming the selection criteria for the sub-study analysis.

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