Subclinical atheromatosis localization and burden in a low-to-moderate cardiovascular risk population: The ILERVAS study

ILERVAS PROJECT INVESTIGATORS

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1. SUPPLEMENTARY MATERIAL AND METHODS

1.1. Statistical analysis

Post-stratification weights were defined by the inverse of the selection probability for each stratum and year of study (see supplementary materials for further details). These weights were applied in all statistical analyzes to take into account the complex design implemented in the sampling phase, and to provide representative results of the actual population distribution in the province of Lleida.

The descriptive analysis included absolute and relative frequencies of qualitative variables, and means and standard deviations for continuous variables following a normal distribution (or median and interquartile range otherwise). Its distribution by gender was compared with the chi-square test for qualitative variables, and student t-test for quantitative variables that were normally distributed, or failing that, the non-parametric U Mann-Whitney was used. The “prevalence” of each medical conditions screened was estimated, together with its confidence interval for the whole population of Lleida. The statistical significance was set at a p-value <.05. The R software and its library ‘survey’ were used for data analysis.

1.2. Sampling strategy details

Strata were defined by medical center as defined in the database (medical office, primary care center, or basic health area). The sampling process was performed semiannually, excluding people who had participated or had been excluded in previous samples. To give the opportunity to participate in the study to all the inhabitants of the province of Lleida, even those living in small rural areas, the sample size to be selected in each stratum was not proportional to the population served, but a complex sampling process was used instead with the restriction of selecting less than 20 patients per workday, the capacity per workday. A drop-out rate of 20% was anticipated. Therefore, the number of patients selected per day was increased in the same proportion.

The sample size to be selected in each stratum was not strictly proportional to the population served, but selected in order to have representative samples for the 13 counties or local districts, and taking into account the capacity of visiting participants per day. Each stratum in which to

perform the random selection was identified with a medical center, except in the case that it had less than r+1 candidates, with r equals to 144, or the minimum number of candidates required (considering intervention and control groups, along the duration of the study) to perform the selection to participate 1 day per year along the study. Thus, the selection strategy was defined depending on two criteria:

If the medical centers are settled in region capitals:

- City of Lleida: 12 days in total (undersampled);
- Other region capitals: 7 days in each one, with the exception of Solsona and Vall d'Aran with 6 days in each one, and Cerdanya with 4 days since it is divided in half between Catalan provinces of Lleida and Girona.

Otherwise:

- Medical centers with more than 6r candidates: 6 days each one;
- Medical centers with between 5r+1 and 6r candidates: 5 days each one;
- Medical centers with between 4r+1 and 5r candidates: 4 days each one;
- Medical centers with between 3r+1 and 4r candidates: 3 days each one;
- Medical centers with between 2r+1 and 3r candidates: 2 days each one;
- Medical centers with between r+1 and 2r candidates: 1 day each one;

1.3. Post-stratification weights details

Post-stratification weights for the province of Lleida or the whole population (w):

They were computed as $w = (N_h / n_h) * (n / N)$, where N and N_h are the population size of the whole province and of each stratum h, while n and n_h are the sample size of the whole province and of each stratum h, respectively.

Post-stratification weights for each comarca R or each administrative region (w_R):

They were computed as $w_R = (N_h / n_h) * (n_R/N_R)$, where N_R and N_h are the population size of the administrative region R and of each stratum h, while n_R and n_h are the sample size of the administrative region R and of each stratum h, respectively.

1.4. Electronic Medical Record follow-up group

The Ethics Committee of the Hospital Arnau de Vilanova approved the protocol with the instruction to delay the selection of the Electronic Medical Record follow-up group until the end of recruiting the intervention group, to guarantee the opportunity to participate in the study to all inhabitants of the province of Lleida. Therefore, in the Electronic Medical Record follow-up group, subjects will be enrolled with the same inclusion and exclusion criteria. Sociodemographic (age, sex, race, marital status, and education), clinical, and anthropometric data will be electronically collected based on their electronic medical records. Allocated participants in this group will be followed through their electronic medical records for the same follow-up period as the intervention group.

1.5. Source of Information and Data Collection

Sociodemographic variables (age, sex, and race), clinical history of cardiovascular risk factors and medical treatments were collected from the electronic medical record database of Primary Care. The other variables such as anthropometric data, smoking habit, lifestyle parameters (physical activity and adherence to Mediterranean diet), respiratory parameters (spirometry and somnolence), biochemical parameters, and vascular parameters were only collected in the Mobile Unit follow-up group. This Mobile Unit is formed by a highly medicalized bus, a caravan, and a qualified research nurse team. The protocols of anthropometric, lifestyle, respiratory, and biochemical evaluation are described in the supplementary material.

1.6. Anthropometric data and lifestyle parameters

Weight and height were measured without shoes and in light clothing, and BMI was obtained. Underweight was defined as a BMI <18.5 kg/m^2, normal weight as 18.5-24.9, overweight 25-29.9, and obesity ≥30. Neck and waist perimeter were measured with a non-stretchable tape with a precision of 0.1 cm. Blood pressure was determined in triplicate, after 5 minutes' rest using an automated device (Omron M6 Comfort, Omron Healthcare, Japan) at 2-minutes intervals, and the mean of the three recordings was calculated. According to guidelines, blood pressure was classified as optimal, normal, high normal, or hypertension[1]. Optimal blood pressure was defined as SBP <120 mmHg and DBP <80; normal as SBP 120-129 and/or DBP 80-84; high normal as

SBP 130-139 and/or DBP 85-89; hypertension as SBP ≥140 and/or DBP ≥90. Abdominal adiposity was defined as a waist perimeter ≥88 cm in women and ≥102 cm in men.

Physical activity was evaluated according to the short version of the International Physical Activity Questionnaire (IPAQ). Briefly, this questionnaire evaluates physical activity and inactivity in adults. Detailed types of physical activity (walking, moderate, and vigorous intensities), and the metabolic equivalent of task (METs)-minute per week were assessed. Following IPAQ guidelines, participants were classified as low, moderate, or vigorous physical activity[2].

The validated 14-item Mediterranean Diet Adherence Screener (MEDAS) was used to assess the adherence to MedDiet. This questionnaire was designed in the *Prevención con Dieta Mediterránea* (PREDIMED) trial. Briefly, the frequencies of consumption of olive oil, wine, fruits, vegetables, fish, legumes, and nuts were evaluated. The intake of meat or meat products, butter, and bakery products were also considered in the composite score[3]. Participants were classified according to their score: adherence (score ≥10 points), low adherence (7-9 points), and very low adherence (<7 points).

1.7. Atheromatous plaque assessment by vascular ultrasound

Arterial ultrasound was performed in 12 territories: both carotid (common, bifurcation, internal, and external) and femoral (common and superficial) arteries[4]. The VIVID *i* BT09 model ultrasound system (GE Healthcare), equipped with a 12L-RS linear probe (6–13 MHz) and a pulsed Doppler ultrasound were used to assess hemodynamic abnormalities. Subclinical atheromatosis was defined as the presence of any plaque in the twelve explored areas. According to Mannheim consensus, an atheroma plaque was defined as a focal encroachment into the lumen of the artery ≥1.5 mm[5]. All plaques were measured and plaque area (cm^2) was assessed. Standardized scanning and reading protocols were used to decrease inter-operator variability and type 2 errors (see supplementary materials).

1.8. Internal validation of atheromatous plaque assessment

To measure intra- and inter-reader absolute agreement, Fleiss' Kappa for plaque presence, and interclass correlation coefficient for plaque area quantification were obtained. Overall interrater reliability of all examiners for plaque presence was .915 (95% CI: .899-.941; 959 observations).

Overall interrater reliability of all examiners for plaque area quantification was .942 (95% CI: .921-.959; 86 observations). Readers were unaware of patients' clinical history.

1.9. Respiratory parameters

Forced spirometry was performed using a portable ultrasonic spirometer (Datospir®, silbelmed Barcelona; Spain) in 6209 participants. Pulmonary function tests were performed in agreement with the European Respiratory Society Guidelines and the American Thoracic Society. Participants performed at least three reproducible measurements, and the output that produced the highest forced vital capacity (FVC) and forced expiratory volume in the first second (FEV1) were selected for analysis. A bronchodilator test was not included in the evaluation process. According to the European Respiratory Society criteria, spirometric parameters were measured as a percentage of the predicted values and included FVC, FEV1, and the ratio FEV1/FVC[6]. An anomalous FEV1 was defined as a value lower than 80% of the predicted. Moreover, a restrictive spirometric pattern was defined as FVC <80% of the predicted value with a FEV1/FVC ratio ≥70%, with a flow-volume curve showing a convex pattern. Finally, an obstructive spirometric pattern was defined as a FEV1/FVC <70% according to the global Initiative for the Chronic Obstructive Lung disease (GOLD)[7].

The Berlin questionnaire and the Epworth sleepiness scale were used as a screening for obstructive sleep apnoea (OSA), as it has been associated with increased cardiovascular risk. Briefly, the Berlin questionnaire incorporates questions about snoring (category 1), daytime somnolence (category 2), hypertension, and BMI (category 3). According to authors, patients with a positive score on two or more categories were classified as high risk of OSA; while those with a negative score on two or more categories were classified as low risk of OSA. Otherwise, patients' classification was missed[8]. The Epworth sleepiness questionnaire has eight items to measure daytime sleepiness. The score ranges from 0 to 24. A score ≥11 indicates excessive daytime sleepiness[9].

1.10. Biochemical parameters

Creatinine, uric acid, and total cholesterol levels were assessed in all participants, while the entire lipid profile (HDL cholesterol, LDL cholesterol, and triglycerides) was evaluated only in subjects

in whom total cholesterol was ≥ 200 mg/dL after 6 hours fasting or when total cholesterol was ≥ 250 mg/dL regardless of fasting hours. A dried blood spot sample collected by a fingertip puncture was obtained according to standard protocols. Determinations were performed with the REFLOTRON Plus system (Roche Diagnostics, Germany). This system is a highly validated clinical chemistry system which results highly correlate to well standardized laboratory methods[10-12].

The HbA1c test was performed using a point-of-care instrument (Cobas B101®, Roche Diagnostics, Germany). This method meets the generally accepted performance criteria for HbA1c. According to the European guidelines, if HbA1c was 5.7- <6.5%, participants were classified as prediabetics; whereas if ≥6.5% participants were classified as diabetics[13].

Glomerular filtration rate (GFR) was estimated according to international guidelines using the CKD-EPI equation[14]. Albumin/creatinine ratio (ACR) was determined in all participants from a spontaneous urine sample obtained in the mobile Unit. The CLINITEK microalbumin 2 Reagent strips (Siemens Healthinneers) were analysed with the CLINITEK status (Siemens Healthinneers). According to guidelines, patients were classified according to their ACR as A1 (<30 mg/g), A2 (30-299 mg/g), and A3 (≥300 mg/g)[15].

1.11. Transcranial ultrasound

The arteries of the circle of Willis and their branches were analysed in 6301 participants. The Doppler spectrum of each intracranial artery was determined using the colour-coded signal. Flow direction, peak systolic velocity, mean flow velocity, and diastolic flow velocity were determined. The intracranial carotid artery, the medial cerebral artery in the M1 and M2 segments, the anterior cerebral artery (segment A1), and the posterior cerebral artery in segments P1 and P2 were studied through the transtemporal acoustic window. According to Baumgartner's criteria, the corresponding peak systolic velocity cut-offs for ≥50%/<50% stenosis were ≥155/≥120 cm/s (anterior cerebral artery), ≥220/≥155 cm/s (middle cerebral artery), and ≥145/≥100 cm/s (posterior cerebral artery)[16].

1.12. Abdominal aortic aneurysm screening

In male participants with ≥60 years, the diameter of abdominal aorta was determined. Abdominal aorta was explored from the xiphoid process of the sternum until the aorta bifurcation in both common iliac arteries in supine decubitus. Two images were captured at the point with a greater diameter. The anterior-posterior and transverse diameters were measured. An abdominal aortic aneurysm was considered when the diameter was ≥3 cm.

1.13. Ankle-brachial index (ABI)

A continuous Doppler (Hadecco ES-100VX Minidop), sphygmomanometer, and blood pressure cuffs (Riester minimus II) were used. Systolic blood pressure was measured in the brachial artery, posterior tibial artery, and dorsalis pedis artery in both limbs. The ratios between tibial and dorsalis pedis systolic blood pressure in each leg, and the higher brachial blood pressure were calculated. The final value for each limb was the lower value of those obtained between tibial and pedis blood pressure[17]. An ABI value ≤0.9 was considered suggestive of stenosis, whereas a value >1.4 was considered to be suggestive of vascular stiffness.

2. SUPPLEMENTARY RESULTS

Supplementary table 1. Lifestyle paramaters

	ILERVAS Cohort			
	All	Males	Females	*P*-value
Physical activity, minutes/week				
Vigorous	135.86 (714.08)	201.12 (850.39)	78.95 (563.11)	<.001
Moderate	227.97 (612.5)	252.45 (663.95)	206.62 (563.03)	.026
Walk	531.75 (673.27)	509.01 (728.37)	551.65 (620.42)	.096
Total	893.87 (1231.68)	961.58 (1395.74)	834.67 (1064.44)	.003
Physical activity, n (%)				<.001
Low	5088 (60.95%)	2656 (64.37%)	2432 (57.96%)	
Moderate	2643 (33.78%)	1103 (28.65%)	1540 (38.26%)	
Vigorous	448 (5.27%)	274 (6.98%)	174 (3.78%)	
Adherence to Mediterranean diet, n (%)				<.001
Adherence	520 (7.47%)	215 (6.57%)	305 (8.26%)	
Low adherence	4367 (52.76%)	1917 (47.56%)	2450 (57.29%)	
Very low adherence	3331 (39.77%)	1917 (45.87%)	1414 (34.46%)	

Values are shown as relative frequencies. Sample weights were applied in the analysis. Detailed types of physical activity (walking, moderate, and vigorous intensities) and the metabolic equivalent of task (METs)-minute per week were assessed. Participants were classified as low, moderate or vigorous physical activity according to IPAQ guidelines. Adherence to Mediterranean diet was defined as ≥10 points in the MEDAS questionnaire, low adherence as 7-9 points, and very low adherence <7 points. IPAQ: International Physical Activity Questionnaire; MEDAS: 14-item Mediterranean Diet Adherence Screener.

Supplementary table 2. Respiratory parameters

	ILERVAS Cohort			
	All	Males	Females	p-value
Spirometry				
FEV1, %	95.27 (18.58)	91.93 (17.82)	98.24 (18.74)	<.001
FVC, %	94.82 (17.61)	91.57 (16.66)	97.72 (17.93)	<.001
FEV1/FVC, %	77.65 (7.65)	77.63 (7.76)	77.67 (7.54)	.891
Ventilatory function, n (%)				<.001
Normal	4522 (72.51%)	2133 (68.48%)	2389 (76.11%)	
Obstructive, n (%)	836 (12.8%)	424 (12.94%)	412 (12.67%)	
Restrictive, n (%)	822 (14.69%)	486 (18.58%)	336 (11.22%)	
Daytime sleepiness scale, n (%)				.004
Normal	6194 (97.9%)	3035 (97.15%)	3159 (98.56%)	
Excessive	155 (2.1%)	90 (2.85%)	65 (1.44%)	
Risk of OSA, n (%)				<.001
Low risk	2939 (52.0%)	1452 (49.62%)	1487 (54.18%)	
High risk	2433 (45.2%)	1219 (45.46%)	1214 (44.96%)	
CPAP users	142 (2.8%)	114 (4.92%)	28 (0.86%)	

Values are shown as relative frequencies of qualitative variables; and means and standard deviations for normally distributed quantitative variables. Sample weights were applied in the analysis. A restrictive spirometric pattern was defined by FVC <80% of the predicted value with a FEV1/FVC ratio ≥70%, with a flow-volume curve showing a convex pattern. An obstructive spirometric pattern was defined by a FEV1/FVC <70% according to the global Initiative for the Chronic Obstructive Lung disease (GOLD). An excessive daytime sleepiness was defined as a score ≥11 in the Epworth sleepiness questionnaire. High risk of OSA was defined as a positive score on ≥2 categories in the Berlin questionnaire. CPAP: Continuous Positive Airway Pressure; FEV1: Forced Expiratory Volume in the first second; FVC: Forced Vital Capacity; OSA: Obstructive Sleep Apnoea.

Supplementary table 3. Intraclass correlation agreement between the ILERVAS point-of-care methods and gold standard biochemical methods.

Determination	Sample size	ICC	95% IC
Creatinine	68	.87	.58 - .94
HbA1c	46	.81	.67 - .89
Uric acid	68	.95	.91 - .97
TC	68	.84	-.04 - .96
HDL-C	62	.94	.89 - .97
LDL-C	61	.85	-.04 - .96
TG	62	.98	.97 - .99
ACR	59	.55	.26 - .85

ACR: Albumin/Creatinine Ratio; Hb1Ac: Glycosylated hemoglobin; HDL-C: High-Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; TC: total Cholesterol; TG: Triglycerides.

3. SUPPLEMENTARY FIGURE 1

Figure S1

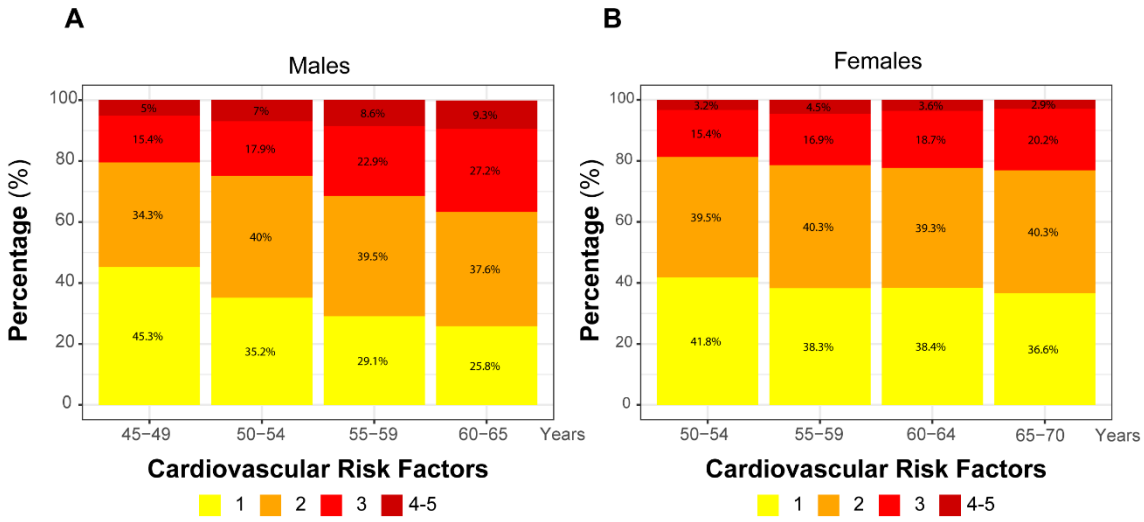

Figure S1 Presence of cardiovascular risk factors in the ILERVAS study. Cardiovascular risk factors (CRRFs) were stratified by age and sex.

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