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The Early Detection and Progression of Subclinical Atherosclerosis in Psoriasis (EDSAP) study: Rationale and design

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5 Protocol for an observational, prospective cohort study.
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ABSTRACT

Introduction: Life expectancy of patients with psoriasis is reduced by 4-5 years due to cardiovascular disease with an increased risk of myocardial infarction at an earlier age compared to general population. This increased risk is independent of traditional cardiovascular risk factors and higher in moderate-to-severe forms of psoriasis. Inflammation may play a key role in the development of atherosclerosis in these patients.

Methods and analysis: A prospective cohort study, Early Detection and Progression of Subclinical Atherosclerosis in Psoriasis (EDSAP), was initiated in January 2020 to investigate the presence and progression of subclinical atherosclerosis in patients with psoriasis. 120 patients aged 30-65 years and eligible for biological treatment have been recruited at Hospital Ramón y Cajal in Madrid, Spain. Patients undergo a baseline visit, and one-year follow up visit after starting biologic therapy. Each visit includes: assessment of cardiovascular risk factors, screening for subclinical atherosclerosis by 2D/3D ultrasound of carotid and femoral arteries, cardiac computed tomography of coronary arteries and blood sampling. All baseline visits were completed by December 2022, and the remaining follow-up visits will be concluded by the end of 2023. The EDSAP study aims to identify new molecular and imaging markers associated with the presence of atherosclerosis and its progression in a chronic inflammatory state such as psoriasis. This has the potential to: (1) help improve primary cardiovascular prevention strategies in these patients; (2) understand the effect of biologic drugs on the cardiovascular system; (3) serve as a model for understanding atherosclerosis in other chronic inflammatory diseases.

Ethics and dissemination: The study protocol has been approved by the Institutional Review Board of the Hospital Ramón y Cajal in Madrid. We will present our findings at national and international congresses, and peer-reviewed journals.

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56 73 **Strengths and limitations**
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- 8
9 74 • Strict application of protocols, the collection of biobank samples and the prospective data
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11 75 collection which allows to evaluate the impact of anti-inflammatory therapies on
12
13 76 atheroma plaque characterization and modulation.
- 14
15 77 • The application of state-of-the-art scientific techniques to measure the anatomical and
16
17 78 biological characteristics of subclinical atherosclerosis.
- 18
19 79 • Being an observational study, it is more vulnerable to potential confounding factors
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21 80 compared to randomized controlled trials.
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24 81 • The open-label, non-randomized use of psoriasis treatments in a small sample size and
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26 82 with a short follow-up duration.

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86 **INTRODUCTION**

87 Psoriasis is a complex chronic inflammatory and immune-mediated disease of the skin and joints
88 associated with multiple comorbidities (1,2). The life expectancy of patients with psoriasis is
89 reduced by 4-5 years due to cardiovascular (CV) disease, and there is an increased risk of
90 myocardial infarction at an earlier age compared to individuals without the disease (3,4). This
91 elevated CV risk could be due to systemic inflammation characteristic, especially in the moderate-
92 to-severe forms of the disease (5,6). Therefore, classical screening methods such as the
93 Framingham risk score, which is based on classical cardiovascular risk factors (CVRFs), do not
94 reliably assess the risk of coronary heart disease in patients with psoriasis (4,7).

95 Detection of atherosclerosis in its subclinical stage may help to identify strategies to halt the
96 development of the disease. Many imaging studies in patients with psoriasis assessed subclinical
97 atherosclerosis in individual vascular territories (4,8–10), but given the systemic nature of
98 atherosclerosis, a multi-territorial analysis has the potential to provide a more comprehensive
99 overview of the distribution and burden of atherosclerosis in these patients (11). The natural
100 history of atherosclerosis involves a prolonged subclinical phase, where the disease is usually
101 detected only at an advanced stage or after a CV event. Early detection of subclinical
102 atherosclerosis and adoption of primary prevention measures, including adequate control of
103 systemic inflammation, may minimise the risk of CV disease in patients with psoriasis. It has
104 therefore been proposed that these patients should undergo comprehensive screening for
105 subclinical atherosclerosis (4), a proposal that has arisen from the need to find a non-invasive,
106 simple and widely available biomarker for its early detection (12).

107 The most widely used and validated technique for screening subclinical atherosclerosis is vascular
108 ultrasound (VUS), a reproducible, non-invasive technique with no side effects. In the last years,
109 there has been a particular interest in the study of subclinical atherosclerosis in the femoral
110 arteries. Studies in healthy adults have shown that femoral plaques are more prevalent than carotid
111 plaques and are more associated with traditional CVRFs and coronary calcium (12,13), as well as
112 being an independent predictor of future CV events (11,14). Interestingly, in the PESA study

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3 113 (middle-aged participants from the general population), the presence of iliofemoral disease
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5 114 increases the risk of concurrent coronary calcium and is predictive of disease elsewhere (11). In
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7 115 fact, screening femoral arteries with vascular ultrasound has been introduced in current clinical
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9 116 practice guidelines as a risk modifier in individuals at low or moderate risk individuals (15). In
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11 117 this regard, our research group evaluated the usefulness of femoral artery ultrasound for the
12
13 118 detection of subclinical atherosclerosis in psoriasis. We observed that screening of femoral
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15 119 plaques improves the detection of subclinical atherosclerosis in these patients, whereas carotid
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17 120 artery scanning was not sufficiently accurate (12,16). Semiautomated 3-dimensional vascular
18
19 121 ultrasound (3DVUS) has been proposed as a better method for quantifying peripheral
20
21 122 atherosclerotic burden. 3DVUS is a feasible, reproducible and novel imaging technique to
22
23 123 quantify early carotid and femoral atherosclerotic burden in large populations. Furthermore, 3D-
24
25 124 VUS offers incremental value over the presence of plaque alone in its association with
26
27 125 cardiovascular risk (13,17). In the last decade, the advent of coronary computed tomography
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29 126 angiography (CCTA) has emerged as a promising non-invasive tool to assess coronary artery
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31 127 structure over time. It has been proposed that the ability of CCTA to identify and quantify the
32
33 128 morphology of high-risk plaques, together with therapy monitoring, will eventually become the
34
35 129 cornerstone of treatment personalisation (18).
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39 130 Several studies have shown the potential benefits of biologic therapies on CV disease risk in
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41 131 patients with psoriasis. Biologic therapy in severe psoriasis has been associated with a favourable
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43 132 modulation of coronary plaque indices by CCTA (16). These results support the need to expand
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45 133 our knowledge on the potential effects of biologic therapies in atherosclerosis.
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48 134 In addition to imaging techniques, there is a need to discover and validate new molecular markers
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50 135 that have practical value for clinical intervention as well as for identifying and elucidating CV
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52 136 disease processes at the individual level. Therefore, proteomic studies are needed to gain further
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54 137 insights in psoriasis-associated accelerated atherosclerosis in order to obtain a comprehensive
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56 138 overview of this high-risk population.
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3 139 This article describes the rationale, aims and methods of the EDSAP study protocol, a longitudinal
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5 140 cohort study to decipher the molecular, imaging and clinical characteristics of this accelerated
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7 141 atherosclerosis phenotype associated with psoriasis and to explore the effect of anti-inflammatory
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9 142 therapies on it.
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11 12 143 **STUDY OBJECTIVES** 13

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15 144 The objectives of the EDSAP study are: (1) to assess the prevalence, vascular distribution and
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17 145 burden of subclinical atherosclerosis in patients with psoriasis and its relationship with
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19 146 inflammatory biomarkers and CV risk algorithms using 2DVUS of carotid and femoral arteries,
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21 147 3DVUS of carotid and femoral arteries and CCTA; (2) to characterize the composition of
22
23 148 atherosclerotic plaques by CCTA and 3D-VUS of the carotid and femoral arteries; (3) to evaluate
24
25 149 the effect of different treatments used in psoriasis on the progression and characterisation of
26
27 150 subclinical atherosclerosis in different arterial territories assessed by non-invasive imaging
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29 151 techniques; and (4) to characterise the atherosclerosis process in patients with psoriasis using
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31 152 laboratory analysis and "-omics" technologies, as well as to evaluate changes at the molecular
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33 153 level after treatment of the skin disease.
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36 37 154 **STUDY DESIGN AND POPULATION** 38

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40 155 The EDSAP study is an observational, longitudinal, prospective cohort study that includes
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42 156 psoriasis patients who will undergo a one-year medical follow-up (Figure 1). Recruitment is
43
44 157 voluntary among patients attending dermatology consultations at the Hospital Ramón y Cajal,
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46 158 Madrid (Spain).
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49 159 The study includes participants aged between 30 to 65 years, diagnosed with psoriasis clinically
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51 160 by an expert physician and deemed suitable for biologic therapy by the investigator. Exclusion
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53 161 criteria are as follows: history of CV disease (myocardial infarction, angina pectoris, peripheral
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55 162 vascular disease, aortic aneurysm, angioplasty, cardiac surgery, atrial fibrillation or any other
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57 163 cardiological condition), current oncological treatment, history of transplantation with active
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59 164 immunosuppressive or immunomodulatory treatment, morbid obesity (body mass index ≥ 40
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3 165 kg/m²), diabetes mellitus, chronic liver disease, , chronic kidney disease (glomerular filtration rate
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5 166 <60 mL/min/1.73 m²), other chronic inflammatory disease, presence of any pathology that
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7 167 decreases life expectancy to less than 3 years, or any disease or condition that could affect
8
9 168 adherence to study procedures. In addition, participants will be excluded if they have had a chest
10
11 169 computed tomography (CT) scan in the previous year, are pregnant or breastfeeding.

14 170 **DATA COLLECTION**

17 171 Two study visits are scheduled for each participant: at baseline and 1-year follow up. Both of
18
19 172 them include a clinical interview, physical examination (height, weight, waist circumference and
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21 173 blood pressure), fasting blood draw and assessment of atherosclerotic disease by non-invasive
22
23 174 vascular imaging tests (2D/3DVUS and CCTA). Participants may undergo an unscheduled
24
25 175 clinical visit if the patient suffers a worsening of the psoriasis. This visit includes a clinical
26
27 176 interview, physical examination (height, weight, waist circumference and blood pressure) and
28
29 177 fasting blood draw. Imaging studies will not be repeated due to CCTA radiation. Training sessions
30
31 178 and certification of all personnel involved in data collection are repeated throughout the study.
32
33 179 Inclusion started in January 2020 with baseline visits completed for all 120 patients by the end of
34
35 180 2022. The remaining 1-year follow up visits are expected to be completed by the end of 2023
36
37 181 (Figure 2).

41 182 **CLINICAL INTERVIEW: CVRFS, DIET AND LIFESTYLE HABITS**

43 183 Regarding CVRFS, patients are assessed for diabetes mellitus, hypertension, hyperlipidemia
44
45 184 obesity, smoking, metabolic syndrome and sedentary lifestyle (19). To assess the impact of the
46
47 185 Mediterranean diet on the CV risk in patients with psoriasis, a questionnaire from the PREDIMED
48
49 186 (Prevention with Mediterranean Diet) study is used. This is a validated tool that assesses the
50
51 187 degree of adherence to the Mediterranean dietary pattern with 14 simple questions (20). In order
52
53 188 to measure the impact of psoriasis on patients' daily activities, the Dermatology Life Quality Index
54
55 189 (DLQI) questionnaire is employed (21). It is a unidimensional scale consisting of a short, simple
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57 190 and easy-to-complete 10-question self-administered questionnaire. The questions are related to
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3 191 the perception of the impact of the skin disease on quality of life in the last week. All
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5 192 questionnaires are provided at all visits.
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8 193 **VASCULAR IMAGING STUDIES**

9
10 194 Carotid and femoral arteries are explored using 2DVUS and 3DVUS (Figure 3). Staff performing
11
12 195 and interpreting the images are blinded to the variables of the study and any other imaging
13
14 196 procedures. A Philips iU22® ultrasound system (Philips Healthcare Andover, MA), using a 2D
15
16 197 L9-3 MHz high-resolution linear transducer is employed for 2VUS image acquisition. The study
17
18 198 protocol includes cross-sectional and longitudinal views of both carotid and femoral arteries to
19
20 199 detect plaques and standardized longitudinal views for intima-media thickness measurements.
21
22 200 2DVUS images will be analyzed using QLAB software (Intellispace Portal, Philips Healthcare).
23
24 201 2DVUS analysis includes assessment of carotid and femoral IMT (values >0.9 mm will be
25
26 202 considered abnormal), the presence and extension of atherosclerotic plaques in the carotid and
27
28 203 femoral territories (plaques are defined as a focal protrusion into the arterial lumen of thickness
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30 204 >0.5 mm or >50% of the surrounding IMT or a diffuse thickness >1.5 mm measured between the
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32 205 media-adventitia and intima-lumen interfaces), maximal plaque thickness (maximal distance
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34 206 between the plaque-lumen and the plaque-adventitia interfaces), and plaque stenosis severity
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36 207 (significant stenosis will be considered when luminal narrowing is >50%) (22).
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41 208 A Philips iU22® ultrasound system equipped with a VL13-5 2D/3D volume linear array
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43 209 transducer (Philips Healthcare) is used for the 3DVUS protocol. The acquisition protocol for the
44
45 210 carotid arteries consists of a 30° automatic sweep (explored vessel segment =6 cm long) centered
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47 211 at the carotid bulb to include the distal common carotid artery, the bulb, the bifurcation, and the
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49 212 proximal internal and external carotid artery segments. For the femoral arteries, acquisition is
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51 213 centered at the bifurcation and includes the mid-distal common femoral artery, the bifurcation,
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53 214 and the proximal superficial and deep femoral artery segments. Images will be analyzed using the
54
55 215 Vascular Plaque Quantification (VPQ) feature of QLAB software (Intellispace Portal, Philips).
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57 216 The 3D variables quantified include: plaque volume, which will be determined by measuring the
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59 217 volumes of each atherosclerotic plaque visualized in the standardized 3D acquisition of each
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3 218 carotid and femoral artery individually as well as their sum, number of plaques by vascular site
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5 219 and participant and 3D vessel wall volume or burden, as the plaque volume between the outer and
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7 220 inner wall boundaries (12).
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10 **CORONARY IMAGING STUDIES**

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13 222 CCTA is performed with a 320-detector CT scanner (Aquilion ONE VISION, Toshiba, Japan) at
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15 223 the Hospital Universitario HM Sanchinarro in Madrid, following the guidelines of the NIH
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17 224 Radiation Exposure Committee. Scans are performed with prospective or retrospective EKG
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19 225 gating according to heart rate, tube potential of 100 or 120 kV, tube current of 100-850mA
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21 226 adjusted to the patient's body size, with a gantry rotation time of 275 ms. Images are being
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23 227 acquired with a slice thickness of 0.5 mm and a slice increment of 0.25 mm. Patient
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25 228 characteristics, such as date of visit and treatment, will not be considered when reading the scans.
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27 229 Coronary plaque quantification and characteristics will be analyzed in each of the main coronary
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29 230 arteries (with a diameter >2 mm) using specific software (QAngio CT, Medis; The Netherlands)
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31 231 (4,23). Automated longitudinal contouring of the inner lumen and outer wall will be performed,
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33 232 manually adjusting the results when there are clear deviations. Results of the automated
34
35 233 contouring will also be reviewed on transverse reconstructed cross-sections of the artery on a
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37 234 section-by-section basis at 0.25-mm increments. Lumen attenuation will be adaptively corrected
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39 235 for each scan using gradient filters and intensity values within the artery.
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43 236 Plaque volume (in cubic millimeters) will be divided by the corresponding segment length (in
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45 237 millimeters), to obtain a plaque index to take into account variable coronary artery lengths. Total
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47 238 plaque burden is defined as the sum of calcified plaque burden and non-calcified plaque burden
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49 239 (NCB), assessed in square millimeters. Non-calcified plaque volume and subcomponents will be
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51 240 obtained after adaptively correcting for lumen attenuation and represented as a function of the
52
53 241 software-derived Hounsfield units as previously described (24). Also, high-risk plaque features,
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55 242 defined as positive remodeling (remodeling index > 1.10), low-attenuation (<30 HU), or spotty
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57 243 calcification will be evaluated.
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3 244 All CCTA images will be analyzed in the Advanced Cardiac Imaging Unit at HM Sanchinarro,
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5 245 Madrid, and in The Laboratory of Inflammation and Cardiometabolic Diseases, National Heart,
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7 246 Lung and Blood Institute (Maryland, United States) for specific analysis. Figure 4 shows an
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9 247 example of coronary arteries with (green arrow) and without subclinical atherosclerosis by CCTA.

11 12 248 **PHYSICAL EXAMINATION, LABORATORY ANALYSIS AND BIOBANKING**

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15 249 Patients undergo basic clinical studies in which they are weighed, measured, have their blood
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17 250 pressure taken and their waist circumference measured. Moreover, fasting blood samples are
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19 251 collected at baseline, 1-year follow-up and flare-up visits as part of the clinical process. We will
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21 252 collect data of the results of the analysis of serum glucose, uric acid, alkaline phosphatase,
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23 253 bilirubin, apolipoprotein A1, apolipoprotein B, triglycerides, GlycA, total cholesterol, high-
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25 254 density lipoprotein cholesterol, low-density lipoprotein cholesterol, C-reactive protein, C-reactive
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27 255 protein ultra, lipoprotein (a), haptoglobin, insulin, hemoglobin, lymphocytes, neutrophils,
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29 256 monocytes and leukocytes. In addition, blood samples are collected in each scheduled visit to be
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31 257 processed and stored at -80°C for high-throughput “omics” analysis and biobanking. Each aliquot
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33 258 is assigned a unique identifier by using a laboratory information management system (Bio-e-
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35 259 Bank), managed by the Ramón y Cajal Institute for Health Research (IRYCIS), to ensure adequate
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37 260 tracking of all procedures.

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41 261 The proteomic analysis will be performed by qualified personnel from the vascular
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43 262 physiopathology department of the Hospital Nacional de Paraplégicos in Toledo. Building on the
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45 263 previous experience of our group, atherosclerosis in patients with psoriasis will be studied at the
46
47 264 molecular level, trying to generate proteomic profiles that will allow us to obtain new data that
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49 265 shed light on this high-risk phenotype, incorporating the most recent advances in post-genomic
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51 266 sciences to identify panels of novel biomarkers that can serve as potential tools in the diagnosis
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53 267 and prognosis of the early phase of atherosclerosis, allowing to improve the primary, or even
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55 268 primordial, preventive strategies for the management of cardiovascular risk in these patients. To
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57 269 this end, the overall proteomic discovery strategy will consist of a discovery phase, a verification
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59 270 phase and a validation phase: (1) The discovery phase will be performed using TMT 10-plex

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3 271 reagents, followed by LC-MS/MS using a reverse-phase C-18 nanocolumn. Identification of
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5 272 selected peptides will be performed using the likelihood ratio method (25) and the false discovery
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7 273 rate (FDR) calculated using inverted databases and the refined method (26). Only peptides
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9 274 identified with FDR $\leq 1\%$ will be used to quantify the relative abundance of each protein from
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11 275 reporter ion intensities. For statistical analysis of quantitative data, the WSPP statistical model
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13 276 will be used (27). Finally, a functional analysis of the whole set of quantified proteins will be
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15 277 performed by analyzing the coordinated protein responses in quantitative proteomics experiments
16
17 278 - the systems biology triangle (SBT) (28), which correlates the performance of groups of proteins
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19 279 within a biological process with their quantitative behavior. (2) The verification phase will
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21 280 involve a targeted proteomics strategy to study the proteins and the mechanisms previously
22
23 281 described by our group, which possibly could be involved in psoriasis, focusing on their
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25 282 relationship with atherosclerosis and CV risk stratification. (3) The Validation phase will consist
26
27 283 of targeted proteomics and immunoassays. The results obtained in the discovery phase will be
28
29 284 validated in an independent cohort of patients and will be analyzed for a comprehensive
30
31 285 assessment of lipoprotein biomarkers and inflammatory biomarkers. These last two phases will
32
33 286 use the Selected reaction monitoring (SRM) methodology – SRM design.

37 **CLINICAL EVENTS**

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40 288 In addition to these examinations, patients will have their routine hospital visits, during which
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42 289 additional information will be collected on any clinical or health-related events affecting the
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44 290 participants during the study period. In any case, the continuity of the patient in the study will be
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46 291 assessed, as the patient's health status will always be prioritized.

49 **STATISTICAL ANALYSIS PLAN**

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52 293 EDSAP is a longitudinal cohort study, in which measurements are made of different variables
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54 294 related to psoriasis and cardiovascular imaging at baseline and after one year of biologic
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56 295 treatment. This type of design allows simultaneous testing of different hypotheses, which will
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58 296 involve a specific statistical analysis plan and the selection of the most appropriate data treatment
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3 297 and models to study associations and account for potential confounders. On the one hand, cross-
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5 298 sectional studies will be carried out at each visit between the independent variables and the
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7 299 different outcome variables. Analyses will be performed using linear regression models for
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9 300 continuous variables and logistic regression models for categorical variables. On the other hand,
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11 301 longitudinal studies of the progression of subclinical atherosclerosis based on different
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13 302 independent variables will be performed using the most appropriate models for longitudinal data.
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16 303 The non-calcified coronary burden after one year of treatment was considered as the reference
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18 304 variable for the sample size calculation, given its clinical relevance and the existence of previous
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20 305 literature providing reference values (4,16). Accepting an $\alpha=0.05$ and a $\beta=0.2$ in a bilateral
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22 306 contrast for repeated (paired) means, a total of 97 subjects are needed to detect a minimum
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24 307 difference of 0.03 mm, a more conservative value than that obtained in previous studies (4,16).
25
26 308 We assumed a loss to follow-up rate of 10%. To date, we have recruited a total of 120 patients,
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28 309 far exceeding the minimum sample size needed to detect differences in the primary endpoint.
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30 310 Study results will be reported in accordance with STROBE guidelines (29).
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311 **PATIENT AND PUBLIC INVOLVEMENT**

312 In this study we have taken into account some considerations of patients in order to understand
313 their needs and perspectives. Of the latter, we found that one of their main concerns was the
314 impact of diet on psoriasis. For this reason, we included a questionnaire on adherence to the
315 Mediterranean diet from the PREDIMED study.
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3 322 **DISCUSSION**
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6 323 The EDSAP study aims to provide information on the presence and progression of subclinical
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8 324 atherosclerosis in patients with psoriasis in order to help establish earlier and more personalized
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10 325 management of care for these patients, whose life expectancy is reduced due to the increased risk
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12 326 of cardiovascular disease at younger ages compared to the general population.

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15 327 Traditional prediction systems based on classical CVRFs, underestimate the actual CV risk of
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17 328 patients with psoriasis and other chronic inflammatory states (30). There is a lack of markers that
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19 329 allow us to adequately predict those patients who will develop cardiovascular disease. In this
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21 330 scenario, numerous publications have emerged in recent highlighting the value of detecting
22
23 331 subclinical atherosclerosis through various imaging tests as a tool to overcome this gap. The use
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25 332 of ultrasound techniques has shown to be an accurate biomarker of atherosclerosis presence (4).
26
27 333 Screening for subclinical atherosclerosis in patients with psoriasis with imaging techniques in
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29 334 more than one vascular territory, such as the carotid and especially the femoral arteries, has proven
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31 335 to be a reliable biomarker for cardiovascular risk assessment (12). In addition, 3DVUS is another
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33 336 well-established imaging technique for quantifying early carotid and femoral atherosclerotic
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35 337 burden, as well as being an accessible, novel and reliable technique (13). Recently, this technique
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37 338 has been further developed to obtain plaque characterization and quantification, enabling risk
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39 339 stratification based on atherosclerotic plaque burden (16). Regarding CCTA, some studies have
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41 340 shown how biologics can modulate coronary plaque indices in psoriasis favorably, supporting
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43 341 further studies to qualify these results (16,18). These results have been recently validated in a
44
45 342 systematic review and meta-analysis evaluating the impact of licensed biologic treatments on
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47 343 blood and imaging biomarkers of CV risk in adult patients with psoriasis. The results
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49 344 demonstrated how some biologic drugs were associated with a reduction in aortic vascular
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51 345 inflammation (31). The EDSAP study is the first study that aims to have a comprehensive CV
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53 346 overview of the psoriasis patient, using novel imaging techniques to study peripheral and coronary
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55 347 subclinical atherosclerosis to accurately assess individual CV risk and minimize this risk through
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57 348 prevention and early treatment. This global understanding of the disease, and the ability to see
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3 349 how antipsoriatic therapies modulate the patient's internal inflammation, may represent a
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5 350 breakthrough in the treatment of the psoriasis patient, as well as in the understanding of psoriasis
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7 351 as a human model of atherosclerosis in inflammatory states.
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10 352 As a complement to multiterritorial imaging studies, EDSAP incorporates the use of new
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12 353 molecular techniques such as proteomics that could be the starting point to identify those
13
14 354 individuals potentially predisposed to develop atherosclerosis, allowing us to find potential
15
16 355 predictive and therapeutic targets. At present, few studies have evaluated the usefulness of
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18 356 proteomic strategies to identify a biomarker of atherosclerosis in patients with psoriasis (32,33).
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20 357 This approach aims to identify early markers of subclinical atherosclerosis that can, alone or in
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22 358 combination with imaging techniques, identify individuals at increased risk. The biological
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24 359 resource generated will also be available for future use in longitudinal association studies of any
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26 360 parameter of interest (e.g. clinical events, response to treatment, development of risk factors,
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28 361 progression of atherosclerosis, etc.), which will advance the understanding of how CV disease
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30 362 initiates and progresses.
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34 363 The main methodological advantages of this study are the strict application of protocols, the
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36 364 collection of biobank samples and the prospective data collection which allows to evaluate the
37
38 365 impact of anti-inflammatory therapies on atheroma plaque presence and characterization. In
39
40 366 addition, state-of-the-art scientific techniques are being applied to measure the anatomical and
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42 367 biological characteristics of subclinical atherosclerosis. However, there are limitations to be kept
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44 368 in mind: this is an observational study and more vulnerable to potential confounding factors
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46 369 compared to randomized controlled trials, and the open, non-randomized use of psoriasis
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48 370 treatments in a small sample and with a short follow-up duration. However, this could be the
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50 371 largest consecutive sample of psoriasis patients followed over time using CCTA, 3D and 2DVUS.
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52 372 In addition, we will follow a consecutive sample to minimize any selection bias in longitudinal
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54 373 follow-up. Finally, we will use arterial plaque imaging technology to understand modulation in
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56 374 CV disease risk secondary to biological treatment.
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3 375 In conclusion, the EDSAP study is designed to study psoriasis as a model of atherosclerosis in
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5 376 inflammatory states, as well as to provide clinical, imaging and molecular biomarkers, using
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7 377 innovative imaging techniques as well as omics technologies for the prevention and treatment of
8
9 378 these high-risk patients for early CV events.
10

11
12 379 **Ethics and dissemination of results:**
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14
15 380 The study protocol has been approved by the Ethics Committee of Hospital Ramón y Cajal in
16
17 381 Madrid. All participants will provide written informed consent that explicitly includes consent for
18
19 382 biobanking of surplus biological materials, which will be provided for future research projects.
20
21 383 We will present our findings at national and international congresses, and peer-reviewed journals.
22

23
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25

26
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37
38 390 all the participants in the EDSAP study, without them it would not have been possible.
39

40
41 391 **Data Availability statement:**
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43
44 392 Our data will be published in a timely manner at the conclusion of the follow-up period and will
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46 393 be made available upon request.
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3 395 **REFERENCES**

- 4
5 396 1. Griffiths CE, Barker JN. Pathogenesis and clinical features of psoriasis. *Lancet Lond Engl*.
6
7 397 2007 Jul 21;370(9583):263–71.
- 8
9
10 398 2. Neimann AL, Shin DB, Wang X, Margolis DJ, Troxel AB, Gelfand JM. Prevalence of
11
12 399 cardiovascular risk factors in patients with psoriasis. *J Am Acad Dermatol*. 2006 Nov
13
14 400 1;55(5):829–35.
- 15
16
17 401 3. Risk of Myocardial Infarction in Patients With Psoriasis | Acute Coronary Syndromes |
18
19 402 JAMA | JAMA Network [Internet]. [cited 2022 Nov 30]. Available from:
20
21 403 <https://jamanetwork.com/journals/jama/fullarticle/203598>
- 22
23
24 404 4. Lerman JB, Joshi AA, Chaturvedi A, Aberra TM, Dey AK, Rodante JA, et al. Coronary
25
26 405 Plaque Characterization in Psoriasis Reveals High-Risk Features That Improve After
27
28 406 Treatment in a Prospective Observational Study. *Circulation*. 2017 Jul 18;136(3):263–76.
- 29
30
31 407 5. Garshick MS, Barrett TJ, Wechter T, Azarchi S, Scher JU, Neimann A, et al.
32
33 408 Inflammasome Signaling and Impaired Vascular Health in Psoriasis. *Arterioscler Thromb*
34
35 409 *Vasc Biol*. 2019 Apr;39(4):787–98.
- 36
37
38
39 410 6. Potential Immunological Links Between Psoriasis and Cardiovascular Disease - PubMed
40
41 411 [Internet]. [cited 2022 Dec 6]. Available from: <https://pubmed.ncbi.nlm.nih.gov/29910818/>
- 42
43
44 412 7. Mehta NN, Krishnamoorthy P, Yu Y, Khan O, Raper A, Van Voorhees A, et al. The impact
45
46 413 of psoriasis on 10-year Framingham risk. *J Am Acad Dermatol*. 2012 Oct;67(4):796–8.
- 47
48
49 414 8. Gonzalez-Cantero A, Gonzalez-Cantero J, Sanchez-Moya AI, Perez-Hortet C, Arias-
50
51 415 Santiago S, Schoendorff-Ortega C, et al. Subclinical atherosclerosis in psoriasis. Usefulness
52
53 416 of femoral artery ultrasound for the diagnosis, and analysis of its relationship with insulin
54
55 417 resistance. *PloS One*. 2019;14(2):e0211808.
- 56
57
58
59
60

- 1
2
3 418 9. Armstrong AW, Harskamp CT, Ledo L, Rogers JH, Armstrong EJ. Coronary artery disease
4
5 419 in patients with psoriasis referred for coronary angiography. *Am J Cardiol*. 2012 Apr
6
7 420 1;109(7):976–80.
8
9
10 421 10. Mansouri B, Kivelevitch D, Natarajan B, Joshi AA, Ryan C, Benjegerdes K, et al.
11
12 422 Comparison of Coronary Artery Calcium Scores Between Patients With Psoriasis and Type
13
14 423 2 Diabetes. *JAMA Dermatol*. 2016 Nov 1;152(11):1244–53.
15
16
17 424 11. Fernández-Friera L, Peñalvo JL, Fernández-Ortiz A, Ibañez B, López-Melgar B, Laclaustra
18
19 425 M, et al. Prevalence, Vascular Distribution, and Multiterritorial Extent of Subclinical
20
21 426 Atherosclerosis in a Middle-Aged Cohort: The PESA (Progression of Early Subclinical
22
23 427 Atherosclerosis) Study. *Circulation*. 2015 Jun 16;131(24):2104–13.
24
25
26
27 428 12. González-Cantero A, Gonzalez-Cantero J, Sanchez-Moya AI, Perez-Hortet C, Arias-
28
29 429 Santiago S, Martin-Rodriguez JL, et al. Femoral artery ultrasound for improving the
30
31 430 detection of atherosclerosis in psoriasis. *J Am Acad Dermatol*. 2019 Mar;80(3):784–6.
32
33
34
35 431 13. López-Melgar B, Fernández-Friera L, Oliva B, García-Ruiz JM, Peñalvo JL, Gómez-
36
37 432 Talavera S, et al. Subclinical Atherosclerosis Burden by 3D Ultrasound in Mid-Life: The
38
39 433 PESA Study. *J Am Coll Cardiol*. 2017 Jul 18;70(3):301–13.
40
41
42 434 14. Kaur S, Kingo K, Zilmer M. Psoriasis and Cardiovascular Risk—Do Promising New
43
44 435 Biomarkers Have Clinical Impact? *Mediators Inflamm*. 2017;2017:7279818.
45
46
47 436 15. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to
48
49 437 reduce cardiovascular risk | *European Heart Journal* | Oxford Academic [Internet]. [cited
50
51 438 2022 Dec 13]. Available from:
52
53 439 <https://academic.oup.com/eurheartj/article/41/1/111/5556353>
54
55
56
57
58
59
60

- 1
2
3 440 16. Elnabawi YA, Dey AK, Goyal A, Groenendyk JW, Chung JH, Belur AD, et al. Coronary
4
5 441 artery plaque characteristics and treatment with biologic therapy in severe psoriasis: results
6
7 442 from a prospective observational study. *Cardiovasc Res*. 2019 Mar 15;115(4):721–8.
8
9
10 443 17. López-Melgar B, Mass V, Nogales P, Sánchez-González J, Entrekin R, Collet-Billon A, et
11
12 444 al. New 3-Dimensional Volumetric Ultrasound Method for Accurate Quantification of
13
14 445 Atherosclerotic Plaque Volume. *JACC Cardiovasc Imaging*. 2022 Jun 1;15(6):1124–35.
15
16
17 446 18. Elnabawi YA, Dey AK, Mehta NN. Emerging Applications of Coronary CT Angiography
18
19 447 in Coronary Heart Disease: Getting Better with Time. *Eur Heart J*. 2018 Nov
20
21 448 1;39(41):3682–4.
22
23
24 449 19. Hu SCS, Lan CCE. Psoriasis and Cardiovascular Comorbidities: Focusing on Severe
25
26 450 Vascular Events, Cardiovascular Risk Factors and Implications for Treatment. *Int J Mol*
27
28 451 *Sci*. 2017 Oct 21;18(10):2211.
29
30
31
32 452 20. Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, et al. Primary Prevention
33
34 453 of Cardiovascular Disease with a Mediterranean Diet Supplemented with Extra-Virgin
35
36 454 Olive Oil or Nuts. *N Engl J Med*. 2018 Jun 21;378(25):e34.
37
38
39 455 21. Finlay AY, Khan GK. Dermatology Life Quality Index (DLQI)--a simple practical measure
40
41 456 for routine clinical use. *Clin Exp Dermatol*. 1994 May;19(3):210–6.
42
43
44 457 22. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Desvarieux M, et al.
45
46 458 Mannheim Intima-Media Thickness Consensus. *Cerebrovasc Dis*. 2004;18(4):346–9.
47
48
49 459 23. Kwan AC, May HT, Cater G, Sibley CT, Rosen BD, Lima JAC, et al. Coronary artery
50
51 460 plaque volume and obesity in patients with diabetes: the factor-64 study. *Radiology*. 2014
52
53 461 Sep;272(3):690–9.
54
55
56
57
58
59
60

- 1
2
3 462 24. Sorokin AV, Patel N, Abdelrahman KM, Ling C, Reimund M, Graziano G, et al. Complex
4
5 463 association of apolipoprotein E-containing HDL with coronary artery disease burden in
6
7 464 cardiovascular disease. *JCI Insight*. 7(10):e159577.
8
9
10 465 25. Martínez-Bartolomé S, Navarro P, Martín-Maroto F, López-Ferrer D, Ramos-Fernández A,
11
12 466 Villar M, et al. Properties of average score distributions of SEQUEST: the probability ratio
13
14 467 method. *Mol Cell Proteomics MCP*. 2008 Jun;7(6):1135–45.
15
16
17 468 26. Navarro P, Vázquez J. A refined method to calculate false discovery rates for peptide
18
19 469 identification using decoy databases. *J Proteome Res*. 2009 Apr;8(4):1792–6.
20
21
22
23 470 27. García-Marqués F, Trevisan-Herraz M, Martínez-Martínez S, Camafeita E, Jorge I, Lopez
24
25 471 JA, et al. A Novel Systems-Biology Algorithm for the Analysis of Coordinated Protein
26
27 472 Responses Using Quantitative Proteomics. *Mol Cell Proteomics MCP*. 2016
28
29 473 May;15(5):1740–60.
30
31
32 474 28. Isern J, Martín-Antonio B, Ghazanfari R, Martín AM, López JA, del Toro R, et al. Self-
33
34 475 renewing human bone marrow mesenspheres promote hematopoietic stem cell expansion.
35
36 476 *Cell Rep*. 2013 May 30;3(5):1714–24.
37
38
39 477 29. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, et al. The
40
41 478 Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)
42
43 479 Statement: guidelines for reporting observational studies. *Int J Surg Lond Engl*. 2014
44
45 480 Dec;12(12):1495–9.
46
47
48
49 481 30. Gonzalez-Cantero A, Reddy AS, Dey AK, Gonzalez-Cantero J, Munger E, Rodante J, et al.
50
51 482 Underperformance of clinical risk scores in identifying imaging-based high cardiovascular
52
53 483 risk in psoriasis: results from two observational cohorts. *Eur J Prev Cardiol*. 2022 Mar
54
55 484 30;29(4):591–8.
56
57
58
59
60

- 1
2
3 485 31. González-Cantero A, Ortega-Quijano D, Álvarez-Díaz N, Ballester MA, Jimenez-Gomez
4
5 486 N, Jaen P, et al. Impact of Biological Agents on Imaging and Biomarkers of Cardiovascular
6
7 487 Disease in Patients with Psoriasis: A Systematic Review and Meta-Analysis of Randomized
8
9 488 Placebo-Controlled Trials. *J Invest Dermatol.* 2021 Oct;141(10):2402–11.
10
11
12 489 32. Kaiser H, Wang X, Kvist-Hansen A, Krakauer M, Gørtz PM, McCauley BD, et al.
13
14 490 Biomarkers of subclinical atherosclerosis in patients with psoriasis. *Sci Rep.* 2021 Nov
15
16 491 2;11(1):21438.
17
18
19 492 33. Qi F, Tan Y, Yao A, Yang X, He Y. Psoriasis to Psoriatic Arthritis: The Application of
20
21 493 Proteomics Technologies. *Front Med.* 2021;8:681172.
22
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24

25 494 **Author contributions:**

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27 495 Carlota Abbad- Jaime de Aragón.: Investigation, Conceptualization, Methodology, Original draft
28
29 496 preparation, Visualization. Emilio Berna-Rico.: Conceptualization, Methodology, Visualization,
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31 497 Writing- Reviewing and Editing. María Asunción Ballester-Martinez.: Writing- Reviewing and
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33 498 Editing. Pedro Jaén.: Writing- Reviewing and Editing. Jorge Solís.: Writing- Reviewing and
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35 499 Editing. María G. Barderas.: Software, Validation. Leticia Fernández- Frieria.: Writing-
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37 500 Reviewing and Editing. Nehal N Mehta.: Writing- Reviewing and Editing. Joel M Gelfand.:
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39 501 Writing- Reviewing and Editing. Álvaro González-Cantero.: Conceptualization, Methodology,
40
41 502 Writing- Reviewing and Editing, Funding acquisition, Supervision.
42
43
44

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48
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50
51 506 studies (Leo-Pharma, Almirall and Amgen).
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3 509 **Competing interests statement:**
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6 510 NNM is a full- time US government employee and has served as a consultant for several
7
8 511 pharmaceutical companies, receiving grants and/or research funding; and as a principal
9
10 512 investigator for the NIH receiving grants and/or research funding. JMG served as a consultant for
11
12 513 several pharmaceutical companies, receiving honoraria and research grants (to the Trustees of the
13
14 514 University of Pennsylvania). JMG is a co-patent holder of resiquimod for treatment of cutaneous
15
16 515 T cell lymphoma. JMG receives honoraria from multiple organisms, for being a Deputy Editor
17
18 516 for the Journal of Investigative Dermatology, Chief Medical Editor for Healio Psoriatic Disease.
19
20 517 He is also a member of the Board of Directors for the International Psoriasis Council, receiving
21
22 518 no honoraria. ACG has served as a consultant for several pharmaceutical companies receiving
23
24 519 grants/other payments. MABM has served as a consultant for several pharmaceutical companies
25
26 520 receiving honoraria. LFF, JS, EBR, MGB, PJ, CAJ have no interests to disclose.
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Figure 1. EDSAP study flow. CCTA, coronary computed tomography angiography. 2D, 2-dimensional. 3D, 3-dimensional.

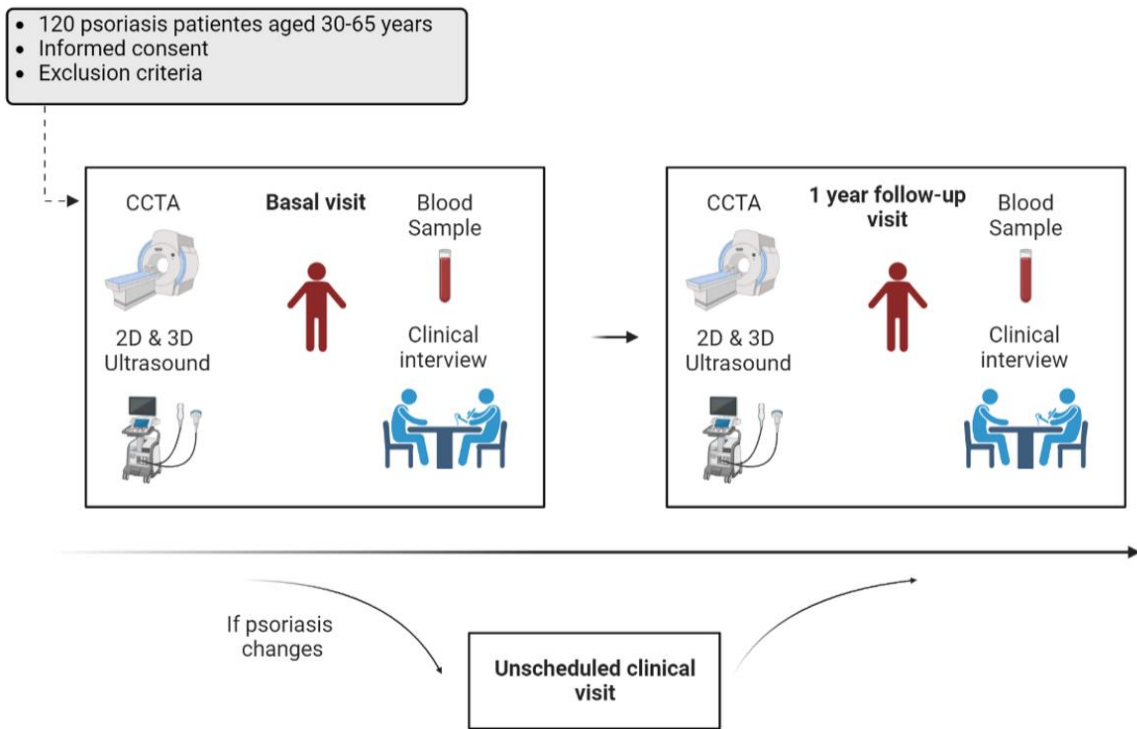
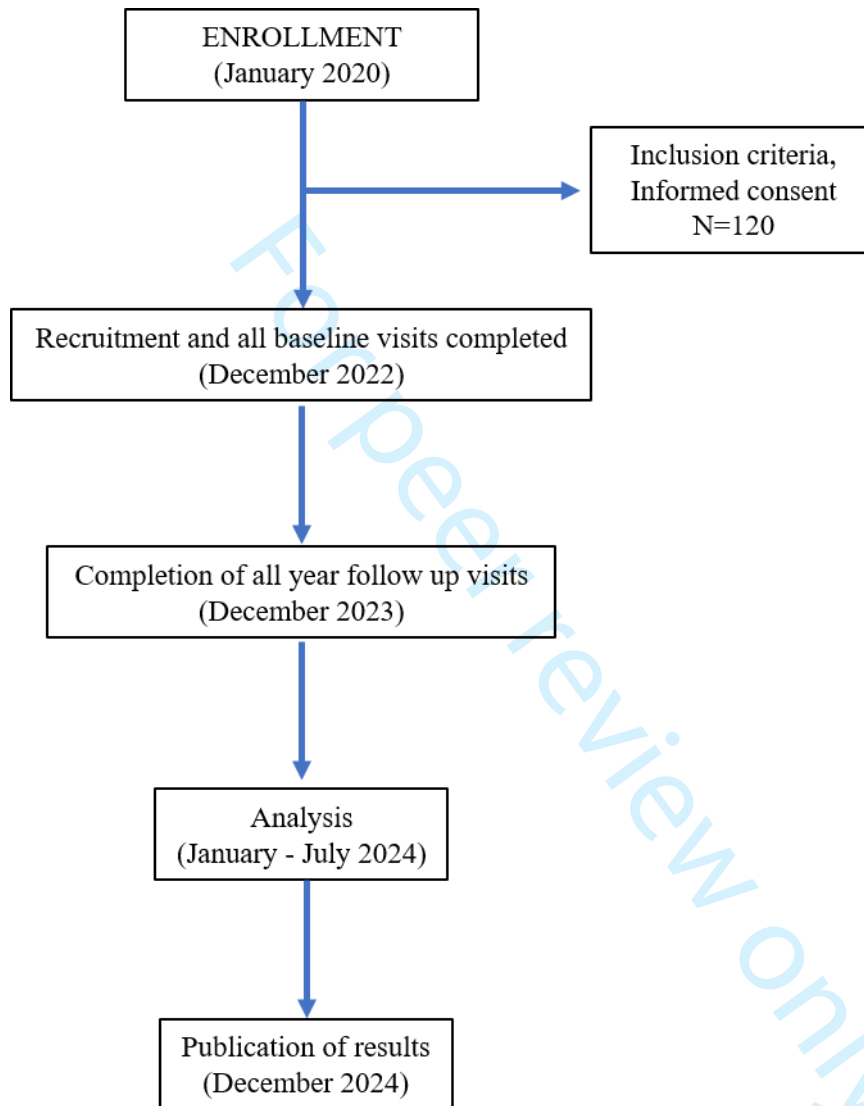
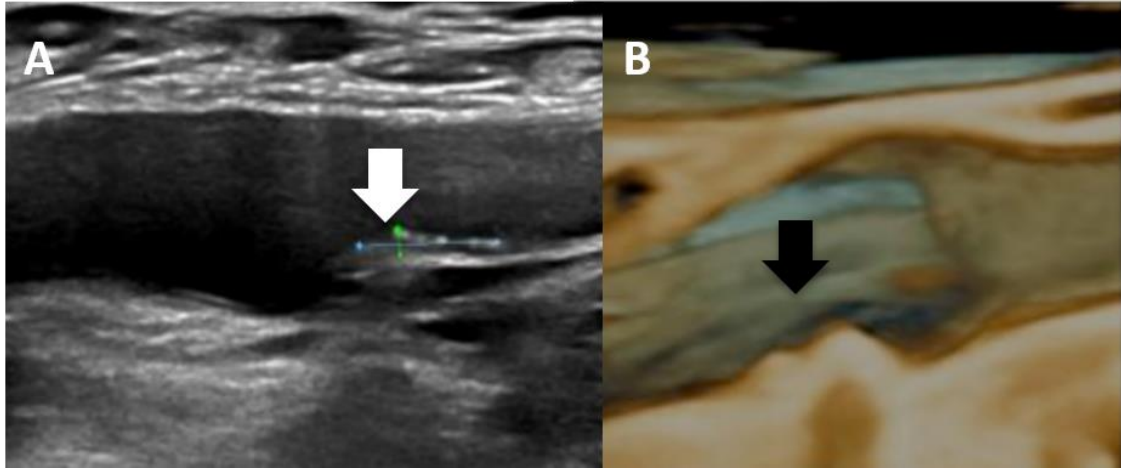


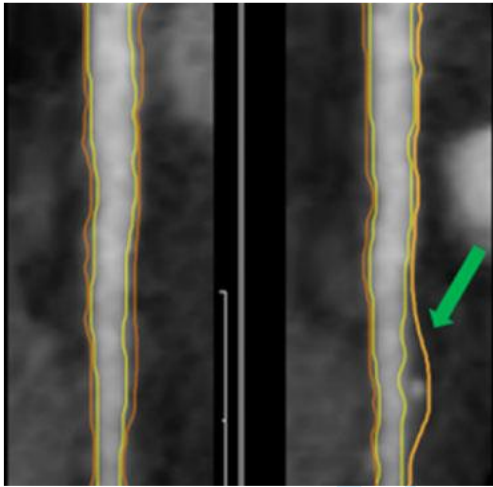
Figure 2. Participant timeline. This flow diagram illustrates the participant timeline including enrollment, baseline and 1-year follow up visits, the analysis of data and publication of results.



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3 **Figure 3. 2D vascular ultrasound of atherosclerotic plaque (white arrow) in the right**
4 **femoral artery and (A) and 3D vascular ultrasound of another atherosclerotic plaque (black**
5 **arrow).**
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3 **Figure 4. Left anterior descending coronary artery with (green arrow) and without**
4 **atheroma plaque by CCTA.**
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BMJ Open

The Early Detection and Progression of Subclinical Atherosclerosis in Psoriasis (EDSAP): Protocol for an observational, single-center, prospective cohort study.

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2023-072455.R1
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Date Submitted by the Author:	14-Jul-2023
Complete List of Authors:	<p>Abbad- Jaime de Aragón, Carlota; Hospital Universitario Ramon y Cajal, Dermatology Berna-Rico, Emilio; Hospital Universitario Ramon y Cajal, Dermatology Ballester-Martinez, María Asunción; Hospital Universitario Ramon y Cajal, Dermatology Jaén, Pedro; Hospital Universitario Ramon y Cajal, Dermatology Solís, Jorge; Hospital Universitario 12 de Octubre, Cardiology; Atria Clinic G. Barderas, María; Hospital Nacional de Paraplégicos, IDISCAM. Toledo, Spain, Department of Vascular Physiopathology Fernández- Frieria, Leticia ; Atria Clinic; HM Hospitales, Centro Integral de Enfermedades Cardiovasculares HM CIEC N Mehta, Nehal; Department of Cardiology,, George Washington Medical Center, Washington D.C, USA Gelfand, Joel ; University of Pennsylvania González-Cantero, Álvaro; Hospital Universitario Ramon y Cajal, Dermatology; Universidad Francisco de Vitoria, Facultad de Medicina</p>
Primary Subject Heading:	Dermatology
Secondary Subject Heading:	Cardiovascular medicine
Keywords:	Psoriasis < DERMATOLOGY, Coronary heart disease < CARDIOLOGY, Computed tomography < RADIOTHERAPY, Ultrasound < RADIOLOGY & IMAGING

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3 **Title:** The Early Detection and Progression of Subclinical Atherosclerosis in Psoriasis (EDSAP):
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5 Protocol for an observational, single-center, prospective cohort study.
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19 31 **Keywords:**

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22 32 Psoriasis; inflammation; cardiovascular disease; vascular imaging; coronary CTA; proteomics.
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31 35 **Number of figures:** 4
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3 46 **ABSTRACT**
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6 47 **Introduction:** Life expectancy of patients with psoriasis is reduced by 4-5 years due to
7
8 48 cardiovascular disease with an increased risk of myocardial infarction at an earlier age compared
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10 49 to general population. This increased risk is independent of traditional cardiovascular risk factors
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12 50 and higher in moderate-to-severe forms of psoriasis. Inflammation may play a key role in the
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14 51 development of atherosclerosis in these patients.
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17 52 **Methods and analysis:** A prospective cohort study, Early Detection and Progression of
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19 53 Subclinical Atherosclerosis in Psoriasis (EDSAP), was initiated in January 2020 to investigate
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21 54 the presence and progression of subclinical atherosclerosis in patients with psoriasis. 120 patients
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23 55 aged 30-65 years and eligible for biological treatment have been recruited at Hospital Ramón y
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25 56 Cajal in Madrid, Spain. Patients undergo a baseline visit, and one-year follow up visit after
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27 57 starting biologic therapy. Each visit includes: assessment of cardiovascular risk factors, screening
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29 58 for subclinical atherosclerosis by 2D/3D ultrasound of carotid and femoral arteries, cardiac
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31 59 computed tomography of coronary arteries and blood sampling. All baseline visits were
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33 60 completed by December 2022, and the remaining follow-up visits will be concluded by the end
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35 61 of 2023. The EDSAP study aims to identify new molecular and imaging markers associated with
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37 62 the presence of atherosclerosis and its progression in a chronic inflammatory state such as
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39 63 psoriasis. This has the potential to: (1) help improve primary cardiovascular prevention strategies
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41 64 in these patients; (2) understand the effect of biologic drugs on the cardiovascular system; (3)
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43 65 serve as a model for understanding atherosclerosis in other chronic inflammatory diseases.
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47 66 **Ethics and dissemination:** The study protocol has been approved by the Institutional Review
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49 67 Board of the Hospital Ramón y Cajal in Madrid (HIP/CI-BIOB-058-01). We will present our
50
51 68 findings at national and international congresses, and peer-reviewed journals.
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3 72 **Strengths and limitations**
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- 6 73 • Strict application of protocols, the collection of biobank samples and the prospective data
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8 74 collection which allows to evaluate the impact of anti-inflammatory therapies on
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10 75 atheroma plaque characterization and modulation.
11
12 76 • The application of state-of-the-art scientific techniques to measure the anatomical and
13
14 77 biological characteristics of subclinical atherosclerosis.
15
16 78 • Being an observational study, it is more vulnerable to potential confounding factors
17
18 79 compared to randomized controlled trials.
19
20 80 • The open-label, non-randomized use of psoriasis treatments in a small sample size and
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22 81 with a short follow-up duration.
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85 **INTRODUCTION**

86 Psoriasis is a complex chronic inflammatory and immune-mediated disease of the skin and joints
87 associated with multiple comorbidities (1,2). The life expectancy of patients with psoriasis is
88 reduced by 4-5 years due to cardiovascular (CV) disease, and there is an increased risk of
89 myocardial infarction at an earlier age compared to individuals without the disease (3,4). This
90 elevated CV risk could be due to systemic inflammation characteristic, especially in the moderate-
91 to-severe forms of the disease (5,6). Therefore, classical screening methods such as the
92 Framingham risk score, which is based on classical cardiovascular risk factors (CVRFs), do not
93 reliably assess the risk of coronary heart disease in patients with psoriasis (4,7,8).

94 Detection of atherosclerosis in its subclinical stage may help to identify strategies to halt the
95 development of the disease. Many imaging studies in patients with psoriasis assessed subclinical
96 atherosclerosis in individual vascular territories (4,9–13), but given the systemic nature of
97 atherosclerosis, a multi-territorial analysis has the potential to provide a more comprehensive
98 overview of the distribution and burden of atherosclerosis in these patients (14). The natural
99 history of atherosclerosis involves a prolonged subclinical phase, where the disease is usually
100 detected only at an advanced stage or after a CV event. Early detection of subclinical
101 atherosclerosis and adoption of primary prevention measures, including adequate control of
102 systemic inflammation, may minimise the risk of CV disease in patients with psoriasis. It has
103 therefore been proposed that these patients should undergo comprehensive screening for
104 subclinical atherosclerosis (4), a proposal that has arisen from the need to find a non-invasive,
105 simple and widely available biomarker for its early detection (15).

106 The most widely used and validated technique for screening subclinical atherosclerosis is vascular
107 ultrasound (VUS), a reproducible, non-invasive technique with no side effects. In the last years,
108 there has been a particular interest in the study of subclinical atherosclerosis in the femoral
109 arteries. Studies in healthy adults have shown that femoral plaques are more prevalent than carotid
110 plaques and are more associated with traditional CVRFs and coronary calcium (15,16), as well as
111 being an independent predictor of future CV events (14,17). Interestingly, in the PESA study

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3 112 (middle-aged participants from the general population), the presence of iliofemoral disease
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5 113 increases the risk of concurrent coronary calcium and is predictive of disease elsewhere (14). In
6
7 114 fact, screening femoral arteries with vascular ultrasound has been introduced in current clinical
8
9 115 practice guidelines as a risk modifier in individuals at low or moderate risk individuals (18). In
10
11 116 this regard, our research group evaluated the usefulness of femoral artery ultrasound for the
12
13 117 detection of subclinical atherosclerosis in psoriasis. We observed that screening of femoral
14
15 118 plaques improves the detection of subclinical atherosclerosis in these patients, whereas carotid
16
17 119 artery scanning was not sufficiently accurate (15,19). Semiautomated 3-dimensional vascular
18
19 120 ultrasound (3DVUS) has been proposed as a better method for quantifying peripheral
20
21 121 atherosclerotic burden. 3DVUS is a feasible, reproducible and novel imaging technique to
22
23 122 quantify early carotid and femoral atherosclerotic burden in large populations. Furthermore, 3D-
24
25 123 VUS offers incremental value over the presence of plaque alone in its association with
26
27 124 cardiovascular risk (16,20). In the last decade, the advent of coronary computed tomography
28
29 125 angiography (CCTA) has emerged as a promising non-invasive tool to assess coronary artery
30
31 126 structure over time. It has been proposed that the ability of CCTA to identify and quantify the
32
33 127 morphology of high-risk plaques, together with therapy monitoring, will eventually become the
34
35 128 cornerstone of treatment personalisation (21).
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39 129 Several studies have shown the potential benefits of biologic therapies on CV disease risk in
40
41 130 patients with psoriasis. Biologic therapy in severe psoriasis has been associated with a favourable
42
43 131 modulation of coronary plaque indices by CCTA (19,22). These results support the need to
44
45 132 expand our knowledge on the potential effects of biologic therapies in atherosclerosis.
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49 133 In addition to imaging techniques, there is a need to discover and validate new molecular markers
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51 134 that have practical value for clinical intervention as well as for identifying and elucidating CV
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53 135 disease processes at the individual level. Therefore, proteomic studies are needed to gain further
54
55 136 insights in psoriasis-associated accelerated atherosclerosis in order to obtain a comprehensive
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57 137 overview of this high-risk population.
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3 138 This article describes the rationale, aims and methods of the EDSAP study protocol, a longitudinal
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5 139 cohort study to decipher the molecular, imaging and clinical characteristics of this accelerated
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7 140 atherosclerosis phenotype associated with psoriasis and to explore the effect of anti-inflammatory
8
9 141 therapies on it.
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11 12 142 **STUDY OBJECTIVES**

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15 143 The objectives of the EDSAP study are: (1) to assess the prevalence, vascular distribution and
16
17 144 burden of subclinical atherosclerosis in patients with psoriasis and its relationship with
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19 145 inflammatory biomarkers and CV risk algorithms using 2DVUS of carotid and femoral arteries,
20
21 146 3DVUS of carotid and femoral arteries and CCTA; (2) to characterize the composition of
22
23 147 atherosclerotic plaques by CCTA and 3D-VUS of the carotid and femoral arteries; (3) to evaluate
24
25 148 the effect of different treatments used in psoriasis on the progression and characterisation of
26
27 149 subclinical atherosclerosis in different arterial territories assessed by non-invasive imaging
28
29 150 techniques; and (4) to characterise the atherosclerosis process in patients with psoriasis using
30
31 151 laboratory analysis and "-omics" technologies, as well as to evaluate changes at the molecular
32
33 152 level after treatment of the skin disease.
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36 37 153 **STUDY DESIGN AND POPULATION**

38
39 154 The EDSAP study is an observational, longitudinal, prospective cohort study that includes
40
41 155 psoriasis patients who will undergo a one-year medical follow-up (Figure 1). Recruitment is
42
43 156 voluntary among patients attending dermatology consultations at the Hospital Ramón y Cajal,
44
45 157 Madrid (Spain).
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48 158 The study includes participants aged between 30 to 65 years, diagnosed with psoriasis clinically
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50 159 by an expert physician and deemed suitable for biologic therapy by the investigator. Exclusion
51
52 160 criteria are as follows: history of CV disease (myocardial infarction, angina pectoris, peripheral
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54 161 vascular disease, aortic aneurysm, angioplasty, cardiac surgery, atrial fibrillation or any other
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56 162 cardiological condition), current oncological treatment, history of transplantation with active
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58 163 immunosuppressive or immunomodulatory treatment, morbid obesity (body mass index ≥ 40
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3 164 kg/m²), diabetes mellitus, chronic liver disease, chronic kidney disease (glomerular filtration rate
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5 165 <60 mL/min/1.73 m²), other chronic inflammatory disease, presence of any pathology that
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7 166 decreases life expectancy to less than 3 years, or any disease or condition that could affect
8
9 167 adherence to study procedures. In addition, participants will be excluded if they have had a chest
10
11 168 computed tomography (CT) scan in the previous year, are pregnant or breastfeeding.

14 169 **DATA COLLECTION**

17 170 Two study visits are scheduled for each participant: at baseline and 1-year follow up. Both of
18
19 171 them include a clinical interview, physical examination (height, weight, waist circumference,
20
21 172 blood pressure and psoriasis severity through psoriasis area severity index -PASI-), fasting blood
22
23 173 draw and assessment of atherosclerotic disease by non-invasive vascular imaging tests
24
25 174 (2D/3DVUS and CCTA). Participants may undergo an unscheduled clinical visit if the patient
26
27 175 suffers a worsening of the psoriasis. This visit includes a clinical interview, physical examination
28
29 176 (height, weight, waist circumference and blood pressure) and fasting blood draw. Imaging studies
30
31 177 will not be repeated due to CCTA radiation. Training sessions and certification of all personnel
32
33 178 involved in data collection are repeated throughout the study. Inclusion started in January 2020
34
35 179 with baseline visits completed for all 120 patients by the end of 2022. The remaining 1-year follow
36
37 180 up visits are expected to be completed by the end of 2023 (Figure 2).

41 181 **CLINICAL INTERVIEW: PSORIASIS PAST MEDICAL HISTORY, CVRFS, DIET AND**

43 182 **LIFESTYLE HABITS**

45 183 Regarding CVRFS, patients are assessed for diabetes mellitus, hypertension, hyperlipidemia
46
47 184 obesity, smoking, metabolic syndrome and sedentary lifestyle (23). To assess the impact of the
48
49 185 Mediterranean diet on the CV risk in patients with psoriasis, a questionnaire from the PREDIMED
50
51 186 (Prevention with Mediterranean Diet) study is used. This is a validated tool that assesses the
52
53 187 degree of adherence to the Mediterranean dietary pattern with 14 simple questions (24). In order
54
55 188 to measure the impact of psoriasis on patients' daily activities, the Dermatology Life Quality Index
56
57 189 (DLQI) questionnaire is employed (25). It is a unidimensional scale consisting of a short, simple
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1
2
3 190 and easy-to-complete 10-question self-administered questionnaire. The questions are related to
4
5 191 the perception of the impact of the skin disease on quality of life in the last week. All
6
7 192 questionnaires are provided at all visits. Patients will also be asked about psoriasis duration,
8
9 193 previous disease treatments and psoriasis comorbidities including psoriasis arthritis and intestinal
10
11 194 bowel disease.

14 195 **VASCULAR IMAGING STUDIES**

17 196 Carotid and femoral arteries are explored using 2DVUS and 3DVUS (Figure 3). Staff performing
18
19 197 and interpreting the images are blinded to the variables of the study and any other imaging
20
21 198 procedures. A Philips iU22® ultrasound system (Philips Healthcare Andover, MA), using a 2D
22
23 199 L9-3 MHz high-resolution linear transducer is employed for 2VUS image acquisition. The study
24
25 200 protocol includes cross-sectional and longitudinal views of both carotid and femoral arteries to
26
27 201 detect plaques and standardized longitudinal views for intima-media thickness measurements.
28
29 202 2DVUS images will be analyzed using QLAB software (Intellispace Portal, Philips Healthcare).
30
31 203 2DVUS analysis includes assessment of carotid and femoral IMT (values >0.9 mm will be
32
33 204 considered abnormal), the presence and extension of atherosclerotic plaques in the carotid and
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35 205 femoral territories (plaques are defined as a focal protrusion into the arterial lumen of thickness
36
37 206 >0.5 mm or >50% of the surrounding IMT or a diffuse thickness >1.5 mm measured between the
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39 207 media-adventitia and intima-lumen interfaces), maximal plaque thickness (maximal distance
40
41 208 between the plaque-lumen and the plaque-adventitia interfaces), and plaque stenosis severity
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43 209 (significant stenosis will be considered when luminal narrowing is >50%) (26).

46
47 210 A Philips iU22® ultrasound system equipped with a VL13-5 2D/3D volume linear array
48
49 211 transducer (Philips Healthcare) is used for the 3DVUS protocol. The acquisition protocol for the
50
51 212 carotid arteries consists of a 30° automatic sweep (explored vessel segment =6 cm long) centered
52
53 213 at the carotid bulb to include the distal common carotid artery, the bulb, the bifurcation, and the
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55 214 proximal internal and external carotid artery segments. For the femoral arteries, acquisition is
56
57 215 centered at the bifurcation and includes the mid-distal common femoral artery, the bifurcation,
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59 216 and the proximal superficial and deep femoral artery segments. Images will be analyzed using the

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2
3 217 Vascular Plaque Quantification (VPQ) feature of QLAB software (Intellispace Portal, Philips).
4
5 218 The 3D variables quantified include: plaque volume, which will be determined by measuring the
6
7 219 volumes of each atherosclerotic plaque visualized in the standardized 3D acquisition of each
8
9 220 carotid and femoral artery individually as well as their sum, number of plaques by vascular site
10
11 221 and participant and 3D vessel wall volume or burden, as the plaque volume between the outer and
12
13 222 inner wall boundaries (12).

16 223 **CORONARY IMAGING STUDIES**

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19 224 CCTA is performed with a 320-detector CT scanner (Aquilion ONE VISION, Toshiba, Japan) at
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21 225 the Hospital Universitario HM Sanchinarro in Madrid, following the guidelines of the NIH
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23 226 Radiation Exposure Committee. Scans are performed with prospective or retrospective EKG
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25 227 gating according to heart rate, tube potential of 100 or 120 kV, tube current of 100-850mA
26
27 228 adjusted to the patient's body size, with a gantry rotation time of 275 ms. Images are being
28
29 229 acquired with a slice thickness of 0.5 mm and a slice increment of 0.25 mm. Patient
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31 230 characteristics, such as date of visit and treatment, will not be considered when reading the scans.
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33 231 Coronary plaque quantification and characteristics will be analyzed in each of the main coronary
34
35 232 arteries (with a diameter >2 mm) using specific software (QAngio CT, Medis; The Netherlands)
36
37 233 (4,27). Automated longitudinal contouring of the inner lumen and outer wall will be performed,
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39 234 manually adjusting the results when there are clear deviations. Results of the automated
40
41 235 contouring will also be reviewed on transverse reconstructed cross-sections of the artery on a
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43 236 section-by-section basis at 0.25-mm increments. Lumen attenuation will be adaptively corrected
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45 237 for each scan using gradient filters and intensity values within the artery.
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49 238 Plaque volume (in cubic millimeters) will be divided by the corresponding segment length (in
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51 239 millimeters), to obtain a plaque index to take into account variable coronary artery lengths. Total
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53 240 plaque burden is defined as the sum of calcified plaque burden and non-calcified plaque burden
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55 241 (NCB), assessed in square millimeters. Non-calcified plaque volume and subcomponents will be
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57 242 obtained after adaptively correcting for lumen attenuation and represented as a function of the
58
59 243 software-derived Hounsfield units as previously described (28). Also, high-risk plaque features,

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3 244 defined as positive remodeling (remodeling index > 1.10), low-attenuation (<30 HU), or spotty
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5 245 calcification will be evaluated.
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8 246 All CCTA images will be analyzed in the Advanced Cardiac Imaging Unit at HM Sanchinarro,
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10 247 Madrid, and in The Laboratory of Inflammation and Cardiometabolic Diseases, National Heart,
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12 248 Lung and Blood Institute (Maryland, United States) for specific analysis. Figure 4 shows an
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14 249 example of coronary arteries with (green arrow) and without subclinical atherosclerosis by CCTA.
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17 250 **PHYSICAL EXAMINATION, LABORATORY ANALYSIS AND BIOBANKING**

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20 251 Patients undergo basic clinical studies in which they are weighed, measured, have their blood
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22 252 pressure taken, their waist circumference measured and their PASI evaluated. Moreover, fasting
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24 253 blood samples are collected at baseline, 1-year follow-up and flare-up visits as part of the clinical
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26 254 process. We will collect data of the results of the analysis of serum glucose, uric acid, alkaline
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28 255 phosphatase, bilirubin, apolipoprotein A1, apolipoprotein B, triglycerides, GlycA, total
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30 256 cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, C-reactive
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32 257 protein, C-reactive protein ultra, lipoprotein (a), haptoglobin, insulin, hemoglobin, lymphocytes,
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34 258 neutrophils, monocytes and leukocytes. In addition, blood samples are collected in each scheduled
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36 259 visit to be processed and stored at -80°C for high-throughput “omics” analysis and biobanking.
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38 260 Each aliquot is assigned a unique identifier by using a laboratory information management system
39
40 261 (Bio-e-Bank), managed by the Ramón y Cajal Institute for Health Research (IRYCIS), to ensure
41
42 262 adequate tracking of all procedures.
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46 263 The proteomic analysis will be performed by qualified personnel from the vascular
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48 264 physiopathology department of the Hospital Nacional de Paraplégicos in Toledo. Building on the
49
50 265 previous experience of our group, atherosclerosis in patients with psoriasis will be studied at the
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52 266 molecular level, trying to generate proteomic profiles that will allow us to obtain new data that
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54 267 shed light on this high-risk phenotype, incorporating the most recent advances in post-genomic
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56 268 sciences to identify panels of novel biomarkers that can serve as potential tools in the diagnosis
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58 269 and prognosis of the early phase of atherosclerosis, allowing to improve the primary, or even
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3 270 primordial, preventive strategies for the management of cardiovascular risk in these patients. To
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5 271 this end, the overall proteomic discovery strategy will consist of a discovery phase, a verification
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7 272 phase and a validation phase: (1) The discovery phase will be performed using TMT 10-plex
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9 273 reagents, followed by LC-MS/MS using a reverse-phase C-18 nanocolumn. Identification of
10
11 274 selected peptides will be performed using the likelihood ratio method (29) and the false discovery
12
13 275 rate (FDR) calculated using inverted databases and the refined method (30). Only peptides
14
15 276 identified with $FDR \leq 1\%$ will be used to quantify the relative abundance of each protein from
16
17 277 reporter ion intensities. For statistical analysis of quantitative data, the WSPP statistical model
18
19 278 will be used (31). Finally, a functional analysis of the whole set of quantified proteins will be
20
21 279 performed by analyzing the coordinated protein responses in quantitative proteomics experiments
22
23 280 - the systems biology triangle (SBT) (32), which correlates the performance of groups of proteins
24
25 281 within a biological process with their quantitative behavior. (2) The verification phase will
26
27 282 involve a targeted proteomics strategy to study the proteins and the mechanisms previously
28
29 283 described by our group, which possibly could be involved in psoriasis, focusing on their
30
31 284 relationship with atherosclerosis and CV risk stratification. (3) The Validation phase will consist
32
33 285 of targeted proteomics and immunoassays. The results obtained in the discovery phase will be
34
35 286 validated in an independent cohort of patients and will be analyzed for a comprehensive
36
37 287 assessment of lipoprotein biomarkers and inflammatory biomarkers. These last two phases will
38
39 288 use the Selected reaction monitoring (SRM) methodology – SRM design.

289 **CLINICAL EVENTS**

290 In addition to these examinations, patients will have their routine hospital visits, during which
291 additional information will be collected on any clinical or health-related events affecting the
292 participants during the study period. In any case, the continuity of the patient in the study will be
293 assessed, as the patient's health status will always be prioritized.

294 **STATISTICAL ANALYSIS PLAN**

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3 295 EDSAP is a longitudinal cohort study, in which measurements are made of different variables
4
5 296 related to psoriasis and cardiovascular imaging at baseline and after one year of biologic
6
7 297 treatment. This type of design allows simultaneous testing of different hypotheses, which will
8
9 298 involve a specific statistical analysis plan and the selection of the most appropriate data treatment
10
11 299 and models to study associations and account for potential confounders. On the one hand, cross-
12
13 300 sectional studies will be carried out at each visit between the independent variables and the
14
15 301 different outcome variables. Analyses will be performed using linear regression models for
16
17 302 continuous variables and logistic regression models for categorical variables. On the other hand,
18
19 303 longitudinal studies of the progression of subclinical atherosclerosis based on different
20
21 304 independent variables will be performed using the most appropriate models for longitudinal data.
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24
25 305 The non-calcified coronary burden after one year of treatment was considered as the reference
26
27 306 variable for the sample size calculation, given its clinical relevance and the existence of previous
28
29 307 literature providing reference values (4,19). Accepting an $\alpha=0.05$ and a $\beta=0.2$ in a bilateral
30
31 308 contrast for repeated (paired) means, a total of 97 subjects are needed to detect a minimum
32
33 309 difference of 0.03 mm, a more conservative value than that obtained in previous studies (4,19).
34
35 310 We assumed a loss to follow-up rate of 10%. To date, we have recruited a total of 120 patients,
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37 311 far exceeding the minimum sample size needed to detect differences in the primary endpoint.
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39 312 Study results will be reported in accordance with STROBE guidelines (33).
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42 **PATIENT AND PUBLIC INVOLVEMENT**

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44
45 314 None.
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54 55 318 **DISCUSSION**

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3 319 The EDSAP study aims to provide information on the presence and progression of subclinical
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5 320 atherosclerosis in patients with psoriasis in order to help establish earlier and more personalized
6
7 321 management of care for these patients, whose life expectancy is reduced due to the increased risk
8
9 322 of cardiovascular disease at younger ages compared to the general population.
10

11
12 323 Traditional prediction systems based on classical CVRFs, underestimate the actual CV risk of
13
14 324 patients with psoriasis and other chronic inflammatory states (8,34). There is a lack of markers
15
16 325 that allow us to adequately predict those patients who will develop cardiovascular disease. In this
17
18 326 scenario, numerous publications have emerged in recent highlighting the value of detecting
19
20 327 subclinical atherosclerosis through various imaging tests as a tool to overcome this gap. The use
21
22 328 of ultrasound techniques has shown to be an accurate biomarker of atherosclerosis presence (4).
23
24 329 Screening for subclinical atherosclerosis in patients with psoriasis with imaging techniques in
25
26 330 more than one vascular territory, such as the carotid and especially the femoral arteries, has proven
27
28 331 to be a reliable biomarker for cardiovascular risk assessment (15). In addition, 3DVUS is another
29
30 332 well-established imaging technique for quantifying early carotid and femoral atherosclerotic
31
32 333 burden, as well as being an accessible, novel and reliable technique (16). Recently, this technique
33
34 334 has been further developed to obtain plaque characterization and quantification, enabling risk
35
36 335 stratification based on atherosclerotic plaque burden (16). Regarding CCTA, some studies have
37
38 336 shown how biologics can modulate coronary plaque indices in psoriasis favorably, supporting
39
40 337 further studies to qualify these results (19,21,22). These results have been recently validated in a
41
42 338 systematic review and meta-analysis evaluating the impact of licensed biologic treatments on
43
44 339 blood and imaging biomarkers of CV risk in adult patients with psoriasis. The results
45
46 340 demonstrated how some biologic drugs were associated with a reduction in aortic vascular
47
48 341 inflammation (35). In this study, one of the interesting aspects to explore, and which could provide
49
50 342 more data on the complex relationship between cutaneous and vascular inflammation in these
51
52 343 patients, is whether this modulation of coronary plaques by antipsoriatic therapies is parallel to or
53
54 344 independent of the improvement in PASI, which would provide a more global view of the
55
56 345 systemic inflammation affecting the patient with psoriasis. The EDSAP study is the first study
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2
3 346 that aims to have a comprehensive CV overview of the psoriasis patient, using novel imaging
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5 347 techniques to study peripheral and coronary subclinical atherosclerosis to accurately assess
6
7 348 individual CV risk and minimize this risk through prevention and early treatment. This global
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9 349 understanding of the disease, and the ability to see how antipsoriatic therapies modulate the
10
11 350 patient's internal inflammation, may represent a breakthrough in the treatment of the psoriasis
12
13 351 patient, as well as in the understanding of psoriasis as a human model of atherosclerosis in
14
15 352 inflammatory states.

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18 353 As a complement to multiterritorial imaging studies, EDSAP incorporates the use of new
19
20 354 molecular techniques such as proteomics that could be the starting point to identify those
21
22 355 individuals potentially predisposed to develop atherosclerosis, allowing us to find potential
23
24 356 predictive and therapeutic targets. At present, few studies have evaluated the usefulness of
25
26 357 proteomic strategies to identify a biomarker of atherosclerosis in patients with psoriasis (36,37).
27
28 358 This approach aims to identify early markers of subclinical atherosclerosis that can, alone or in
29
30 359 combination with imaging techniques, identify individuals at increased risk. The biological
31
32 360 resource generated will also be available for future use in longitudinal association studies of any
33
34 361 parameter of interest (e.g. clinical events, response to treatment, development of risk factors,
35
36 362 progression of atherosclerosis, etc.), which will advance the understanding of how CV disease
37
38 363 initiates and progresses.

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42 364 The main methodological advantages of this study are the strict application of protocols, the
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44 365 collection of biobank samples and the prospective data collection which allows to evaluate the
45
46 366 impact of anti-inflammatory therapies on atheroma plaque presence and characterization. In
47
48 367 addition, state-of-the-art scientific techniques are being applied to measure the anatomical and
49
50 368 biological characteristics of subclinical atherosclerosis. However, there are limitations to be kept
51
52 369 in mind: this is an observational study and more vulnerable to potential confounding factors
53
54 370 compared to randomized controlled trials, and the open, non-randomized use of psoriasis
55
56 371 treatments in a small sample and with a short follow-up duration. However, this could be the
57
58 372 largest consecutive sample of psoriasis patients followed over time using CCTA, 3D and 2DVUS.
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3 373 In addition, we will follow a consecutive sample to minimize any selection bias in longitudinal
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5 374 follow-up. Finally, we will use arterial plaque imaging technology to understand modulation in
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7 375 CV disease risk secondary to biological treatment.
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10 376 In conclusion, the EDSAP study is designed to study psoriasis as a model of atherosclerosis in
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12 377 inflammatory states, as well as to provide clinical, imaging and molecular biomarkers, using
13
14 378 innovative imaging techniques as well as omics technologies for the prevention and treatment of
15
16 379 these high-risk patients for early CV events.
17
18

19 380 **Ethics and dissemination of results:**

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21
22 381 The study protocol has been approved by the Ethics Committee of Hospital Ramón y Cajal in
23
24 382 Madrid. All participants will provide written informed consent that explicitly includes consent for
25
26 383 biobanking of surplus biological materials, which will be provided for future research projects.
27
28 384 We will present our findings at national and international congresses, and peer-reviewed journals.
29
30

31 385 **Acknowledgements:**

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33
34 386 We thank all the participants in the study and gratefully acknowledge the collaboration and
35
36 387 assistance of the staff at Hospital Universitario Ramón y Cajal, Hospital HM Sanchinarro,
37
38 388 National Institute of Health, Hospital 12 de Octubre and Atria Clinic.
39

40
41 389 We would also like to thank the dedication and impeccable management of the biological samples
42
43 390 to all the members of the Biobank of the Hospital Ramón y Cajal. Finally, we are very grateful to
44
45 391 all the participants in the EDSAP study, without them it would not have been possible.
46
47

48 392 **Data Availability statement:**

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50 393 Our data will be published in a timely manner at the conclusion of the follow-up period and will
51
52 394 be made available upon request.
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REFERENCES

1. Griffiths CE, Barker JN. Pathogenesis and clinical features of psoriasis. *Lancet Lond Engl*. 2007 Jul 21;370(9583):263–71.
2. Neimann AL, Shin DB, Wang X, Margolis DJ, Troxel AB, Gelfand JM. Prevalence of cardiovascular risk factors in patients with psoriasis. *J Am Acad Dermatol*. 2006 Nov 1;55(5):829–35.
3. Risk of Myocardial Infarction in Patients With Psoriasis | Acute Coronary Syndromes | JAMA | JAMA Network [Internet]. [cited 2022 Nov 30]. Available from: <https://jamanetwork.com/journals/jama/fullarticle/203598>
4. Lerman JB, Joshi AA, Chaturvedi A, Aberra TM, Dey AK, Rodante JA, et al. Coronary Plaque Characterization in Psoriasis Reveals High-Risk Features That Improve After Treatment in a Prospective Observational Study. *Circulation*. 2017 Jul 18;136(3):263–76.
5. Garshick MS, Barrett TJ, Wechter T, Azarchi S, Scher JU, Neimann A, et al. Inflammasome Signaling and Impaired Vascular Health in Psoriasis. *Arterioscler Thromb Vasc Biol*. 2019 Apr;39(4):787–98.
6. Potential Immunological Links Between Psoriasis and Cardiovascular Disease - PubMed [Internet]. [cited 2022 Dec 6]. Available from: <https://pubmed.ncbi.nlm.nih.gov/29910818/>
7. Mehta NN, Krishnamoorthy P, Yu Y, Khan O, Raper A, Van Voorhees A, et al. The impact of psoriasis on 10-year Framingham risk. *J Am Acad Dermatol*. 2012 Oct;67(4):796–8.
8. Berna-Rico E, Abbad-Jaime de Aragon C, Garcia-Aparicio A, Palacios-Martinez D, Ballester-Martinez A, Carrascosa JM, et al. Cardiovascular Screening Practices and Statin Prescription Habits in Patients with Psoriasis among Dermatologists, Rheumatologists and Primary Care Physicians. *Acta Derm Venereol*. 2023 Mar 28;103:adv5087.
9. Gonzalez-Cantero A, Gonzalez-Cantero J, Sanchez-Moya AI, Perez-Hortet C, Arias-Santiago S, Schoendorff-Ortega C, et al. Subclinical atherosclerosis in psoriasis. Usefulness of femoral artery ultrasound for the diagnosis, and analysis of its relationship with insulin resistance. *PloS One*. 2019;14(2):e0211808.
10. Armstrong AW, Harskamp CT, Ledo L, Rogers JH, Armstrong EJ. Coronary artery disease in patients with psoriasis referred for coronary angiography. *Am J Cardiol*. 2012 Apr 1;109(7):976–80.
11. Mansouri B, Kivelevitch D, Natarajan B, Joshi AA, Ryan C, Benjegerdes K, et al. Comparison of Coronary Artery Calcium Scores Between Patients With Psoriasis and Type 2 Diabetes. *JAMA Dermatol*. 2016 Nov 1;152(11):1244–53.
12. Hjuler KF, Böttcher M, Vestergaard C, Deleuran M, Raaby L, Bøtker HE, et al. Increased Prevalence of Coronary Artery Disease in Severe Psoriasis and Severe Atopic Dermatitis. *Am J Med*. 2015 Dec;128(12):1325–1334.e2.
13. Tinggaard AB, Hjuler KF, Andersen IT, Winther S, Iversen L, Böttcher M. Prevalence and severity of coronary artery disease linked to prognosis in psoriasis and psoriatic arthritis patients: a multi-centre cohort study. *J Intern Med*. 2021 Sep;290(3):693–703.
14. Fernández-Friera L, Peñalvo JL, Fernández-Ortiz A, Ibañez B, López-Melgar B, Laclaustra M, et al. Prevalence, Vascular Distribution, and Multiterritorial Extent of Subclinical

- 1
2
3 437 Atherosclerosis in a Middle-Aged Cohort: The PESA (Progression of Early Subclinical
4 438 Atherosclerosis) Study. *Circulation*. 2015 Jun 16;131(24):2104–13.
5
6 439 15. González-Cantero A, Gonzalez-Cantero J, Sanchez-Moya AI, Perez-Hortet C, Arias-
7 440 Santiago S, Martin-Rodriguez JL, et al. Femoral artery ultrasound for improving the
8 441 detection of atherosclerosis in psoriasis. *J Am Acad Dermatol*. 2019 Mar;80(3):784–6.
9
10 442 16. López-Melgar B, Fernández-Friera L, Oliva B, García-Ruiz JM, Peñalvo JL, Gómez-
11 443 Talavera S, et al. Subclinical Atherosclerosis Burden by 3D Ultrasound in Mid-Life: The
12 444 PESA Study. *J Am Coll Cardiol*. 2017 Jul 18;70(3):301–13.
13
14 445 17. Kaur S, Kingo K, Zilmer M. Psoriasis and Cardiovascular Risk—Do Promising New
15 446 Biomarkers Have Clinical Impact? *Mediators Inflamm*. 2017;2017:7279818.
16
17 447 18. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to
18 448 reduce cardiovascular risk | *European Heart Journal* | Oxford Academic [Internet]. [cited
19 449 2022 Dec 13]. Available from:
20 450 <https://academic.oup.com/eurheartj/article/41/1/111/5556353>
21
22 451 19. Elnabawi YA, Dey AK, Goyal A, Groenendyk JW, Chung JH, Belur AD, et al. Coronary
23 452 artery plaque characteristics and treatment with biologic therapy in severe psoriasis: results
24 453 from a prospective observational study. *Cardiovasc Res*. 2019 Mar 15;115(4):721–8.
25
26 454 20. López-Melgar B, Mass V, Nogales P, Sánchez-González J, Entekin R, Collet-Billon A, et
27 455 al. New 3-Dimensional Volumetric Ultrasound Method for Accurate Quantification of
28 456 Atherosclerotic Plaque Volume. *JACC Cardiovasc Imaging*. 2022 Jun 1;15(6):1124–35.
29
30 457 21. Elnabawi YA, Dey AK, Mehta NN. Emerging Applications of Coronary CT Angiography
31 458 in Coronary Heart Disease: Getting Better with Time. *Eur Heart J*. 2018 Nov
32 459 1;39(41):3682–4.
33
34 460 22. Hjuler KF, Bøttcher M, Vestergaard C, Bøtker HE, Iversen L, Kragballe K. Association
35 461 Between Changes in Coronary Artery Disease Progression and Treatment With Biologic
36 462 Agents for Severe Psoriasis. *JAMA Dermatol*. 2016 Oct 1;152(10):1114–21.
37
38 463 23. Hu SCS, Lan CCE. Psoriasis and Cardiovascular Comorbidities: Focusing on Severe
39 464 Vascular Events, Cardiovascular Risk Factors and Implications for Treatment. *Int J Mol*
40 465 *Sci*. 2017 Oct 21;18(10):2211.
41
42 466 24. Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, et al. Primary Prevention
43 467 of Cardiovascular Disease with a Mediterranean Diet Supplemented with Extra-Virgin
44 468 Olive Oil or Nuts. *N Engl J Med*. 2018 Jun 21;378(25):e34.
45
46 469 25. Finlay AY, Khan GK. Dermatology Life Quality Index (DLQI)--a simple practical measure
47 470 for routine clinical use. *Clin Exp Dermatol*. 1994 May;19(3):210–6.
48
49 471 26. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Desvarieux M, et al.
50 472 Mannheim Intima-Media Thickness Consensus. *Cerebrovasc Dis*. 2004;18(4):346–9.
51
52 473 27. Kwan AC, May HT, Cater G, Sibley CT, Rosen BD, Lima JAC, et al. Coronary artery
53 474 plaque volume and obesity in patients with diabetes: the factor-64 study. *Radiology*. 2014
54 475 Sep;272(3):690–9.
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3 476 28. Sorokin AV, Patel N, Abdelrahman KM, Ling C, Reimund M, Graziano G, et al. Complex
4 477 association of apolipoprotein E-containing HDL with coronary artery disease burden in
5 478 cardiovascular disease. *JCI Insight*. 7(10):e159577.
- 7 479 29. Martínez-Bartolomé S, Navarro P, Martín-Maroto F, López-Ferrer D, Ramos-Fernández A,
8 480 Villar M, et al. Properties of average score distributions of SEQUEST: the probability ratio
9 481 method. *Mol Cell Proteomics MCP*. 2008 Jun;7(6):1135–45.
- 11 482 30. Navarro P, Vázquez J. A refined method to calculate false discovery rates for peptide
12 483 identification using decoy databases. *J Proteome Res*. 2009 Apr;8(4):1792–6.
- 14 484 31. García-Marqués F, Trevisan-Herraz M, Martínez-Martínez S, Camafeita E, Jorge I, Lopez
15 485 JA, et al. A Novel Systems-Biology Algorithm for the Analysis of Coordinated Protein
16 486 Responses Using Quantitative Proteomics. *Mol Cell Proteomics MCP*. 2016
17 487 May;15(5):1740–60.
- 19 488 32. Isern J, Martín-Antonio B, Ghazanfari R, Martín AM, López JA, del Toro R, et al. Self-
20 489 renewing human bone marrow mesospheres promote hematopoietic stem cell expansion.
21 490 *Cell Rep*. 2013 May 30;3(5):1714–24.
- 23 491 33. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, et al. The
24 492 Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)
25 493 Statement: guidelines for reporting observational studies. *Int J Surg Lond Engl*. 2014
26 494 Dec;12(12):1495–9.
- 28 495 34. Gonzalez-Cantero A, Reddy AS, Dey AK, Gonzalez-Cantero J, Munger E, Rodante J, et al.
29 496 Underperformance of clinical risk scores in identifying imaging-based high cardiovascular
30 497 risk in psoriasis: results from two observational cohorts. *Eur J Prev Cardiol*. 2022 Mar
31 498 30;29(4):591–8.
- 33 499 35. González-Cantero A, Ortega-Quijano D, Álvarez-Díaz N, Ballester MA, Jimenez-Gomez
34 500 N, Jaen P, et al. Impact of Biological Agents on Imaging and Biomarkers of Cardiovascular
35 501 Disease in Patients with Psoriasis: A Systematic Review and Meta-Analysis of Randomized
36 502 Placebo-Controlled Trials. *J Invest Dermatol*. 2021 Oct;141(10):2402–11.
- 38 503 36. Kaiser H, Wang X, Kvist-Hansen A, Krakauer M, Gørtz PM, McCauley BD, et al.
39 504 Biomarkers of subclinical atherosclerosis in patients with psoriasis. *Sci Rep*. 2021 Nov
40 505 2;11(1):21438.
- 42 506 37. Qi F, Tan Y, Yao A, Yang X, He Y. Psoriasis to Psoriatic Arthritis: The Application of
43 507 Proteomics Technologies. *Front Med*. 2021;8:681172.

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509 Carlota Abbad- Jaime de Aragón.: Investigation, Conceptualization, Methodology, Original draft
510 preparation, Visualization. Emilio Berna-Rico.: Conceptualization, Methodology, Visualization,
511 Writing- Reviewing and Editing. María Asunción Ballester-Martinez.: Writing- Reviewing and
512 Editing. Pedro Jaén.: Writing- Reviewing and Editing. Jorge Solís.: Writing- Reviewing and
513 Editing. María G. Barderas.: Software, Validation. Leticia Fernández- Frieria.: Writing-

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3 514 Reviewing and Editing. Nehal N Mehta.: Writing- Reviewing and Editing. Joel M Gelfand.:
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5 515 Writing- Reviewing and Editing. Álvaro González-Cantero.: Conceptualization, Methodology,
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7 516 Writing- Reviewing and Editing, Funding acquisition, Supervision.
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11
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20 521 **Competing interests statement:**

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22 522 NNM is a full- time US government employee and has served as a consultant for several
23
24 523 pharmaceutical companies, receiving grants and/or research funding; and as a principal
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26 524 investigator for the NIH receiving grants and/or research funding. JMG served as a consultant for
27
28 525 several pharmaceutical companies, receiving honoraria and research grants (to the Trustees of the
29
30 526 University of Pennsylvania). JMG is a co-patent holder of resiquimod for treatment of cutaneous
31
32 527 T cell lymphoma. JMG receives honoraria from multiple organisms, for being a Deputy Editor
33
34 528 for the Journal of Investigative Dermatology, Chief Medical Editor for Healio Psoriatic Disease.
35
36 529 He is also a member of the Board of Directors for the International Psoriasis Council, receiving
37
38 530 no honoraria. ACG has served as a consultant for several pharmaceutical companies receiving
39
40 531 grants/other payments. MABM has served as a consultant for several pharmaceutical companies
41
42 532 receiving honoraria. LFF, JS, EBR, MGB, PJ, CAJ have no interests to disclose.
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541 **Figure legends:**

542 **Figure 1:** EDSAP study flow. CCTA, coronary computed tomography angiography. 2D, 2-
543 dimensional. 3D, 3-dimensional.

544 **Figure 2.** Participant timeline. This flow diagram illustrates the participant timeline including
545 enrollment, baseline and 1-year follow up visits, the analysis of data and publication of results.

546 **Figure 3.** 2D vascular ultrasound of atherosclerotic plaque (white arrow) in the right femoral
547 artery and (A) and 3D vascular ultrasound of another atherosclerotic plaque (black arrow).

548 **Figure 4.** Left anterior descending coronary artery with (green arrow) and without atheroma
549 plaque by CCTA.

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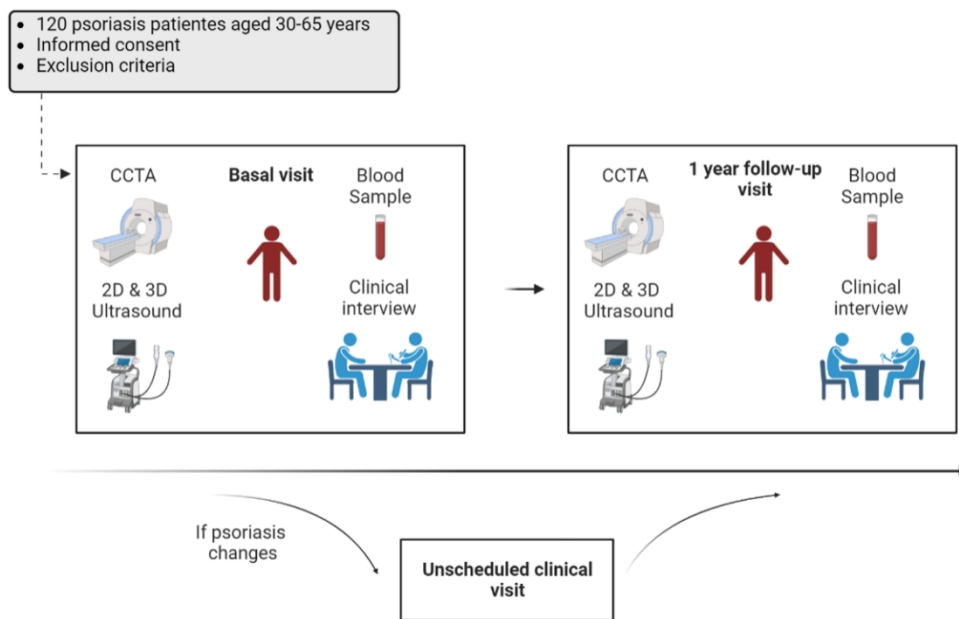
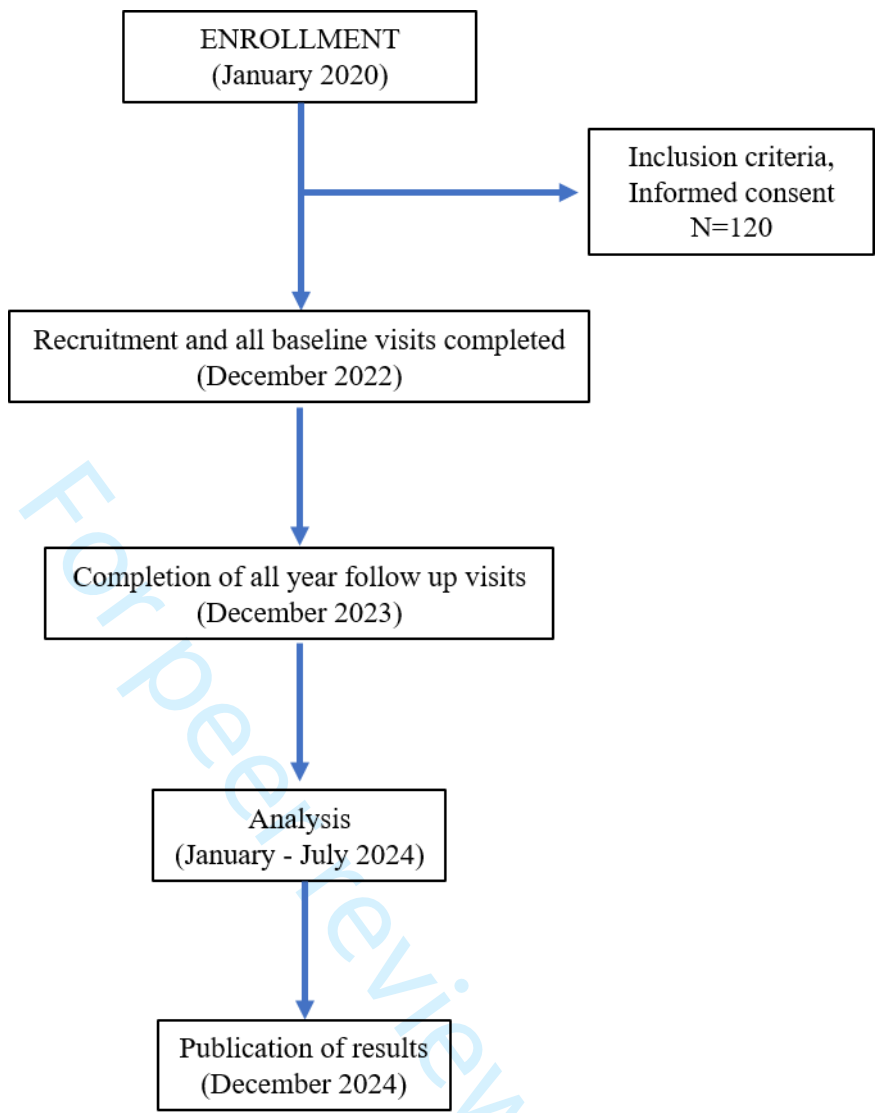


Figure 1. EDSAP study flow. CCTA, coronary computed tomography angiography. 2D, 2-dimensional. 3D, 3-dimensional.

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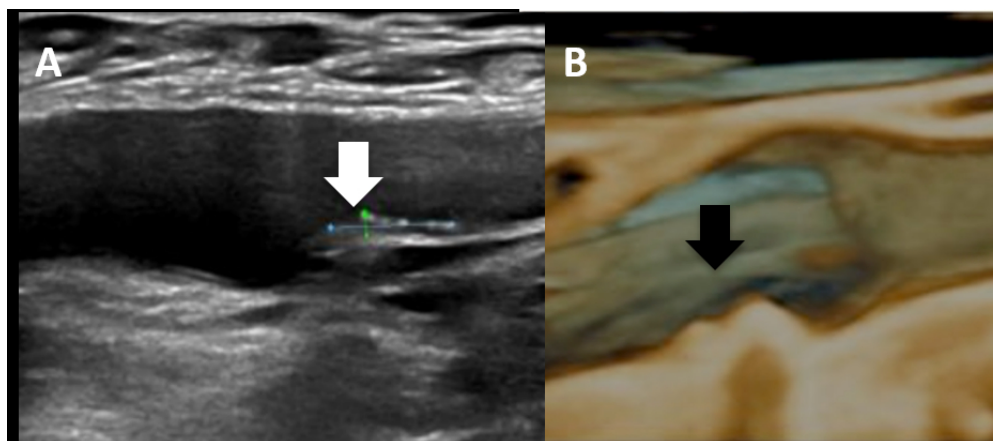


Figure 3. 2D vascular ultrasound of atherosclerotic plaque (white arrow) in the right femoral artery and (A) and 3D vascular ultrasound of another atherosclerotic plaque (black arrow).

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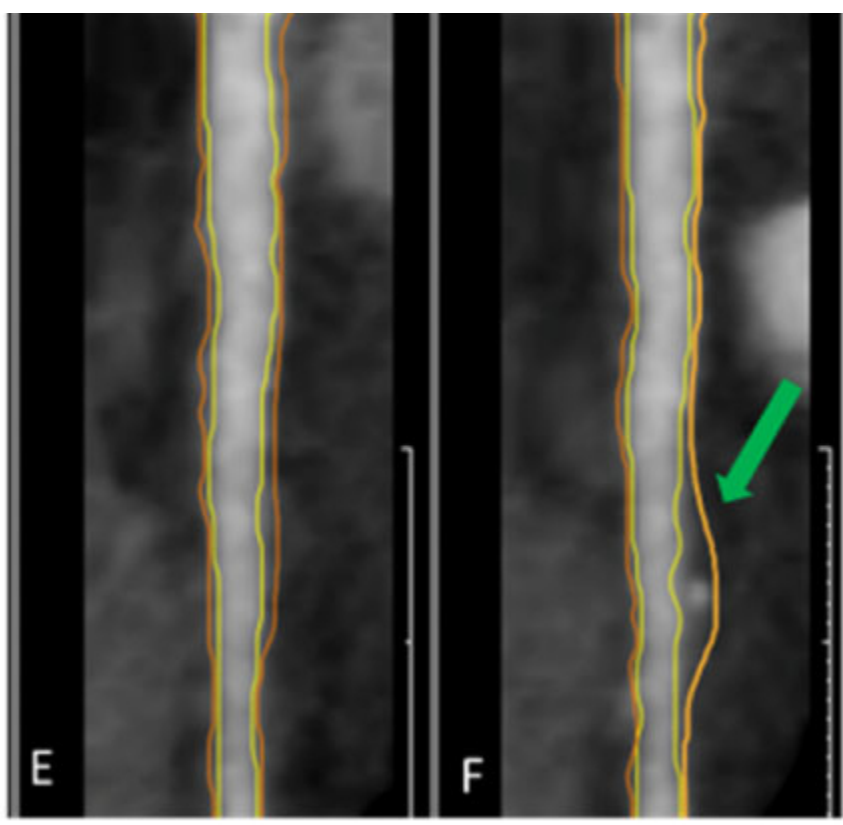


Figure 4. Left anterior descending coronary artery with (green arrow) and without atheroma plaque by CCTA
190x180mm (57 x 57 DPI)

BMJ Open

The Early Detection and Progression of Subclinical Atherosclerosis in Psoriasis (EDSAP): Protocol for an observational, single-center, prospective cohort study.

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Secondary Subject Heading:	Cardiovascular medicine
Keywords:	Psoriasis < DERMATOLOGY, Coronary heart disease < CARDIOLOGY, Computed tomography < RADIOTHERAPY, Ultrasound < RADIOLOGY & IMAGING

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Manuscripts

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3 **Title:** The Early Detection and Progression of Subclinical Atherosclerosis in Psoriasis (EDSAP):
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5 Protocol for an observational, single-center, prospective cohort study.
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8 **Authors:** Carlota Abbad-Jaime de Aragón, MSc^{a*}, Emilio Berna- Rico, MD^{a*}, María Asunción
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3 46 **ABSTRACT**
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6 47 **Introduction:** Life expectancy of patients with psoriasis is reduced by 4-5 years due to
7
8 48 cardiovascular disease with an increased risk of myocardial infarction at an earlier age compared
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10 49 to general population. This increased risk is independent of traditional cardiovascular risk factors
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12 50 and higher in moderate-to-severe forms of psoriasis. Inflammation may play a key role in the
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14 51 development of atherosclerosis in these patients.
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17 52 **Methods and analysis:** A prospective cohort study, Early Detection and Progression of
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19 53 Subclinical Atherosclerosis in Psoriasis (EDSAP), was initiated in January 2020 to investigate
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21 54 the presence and progression of subclinical atherosclerosis in patients with psoriasis. 120 patients
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23 55 aged 30-65 years and eligible for biological treatment have been recruited at Hospital Ramón y
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25 56 Cajal in Madrid, Spain. Patients undergo a baseline visit, and one-year follow up visit after
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27 57 starting biologic therapy. Each visit includes: assessment of cardiovascular risk factors, screening
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29 58 for subclinical atherosclerosis by 2D/3D ultrasound of carotid and femoral arteries, cardiac
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31 59 computed tomography of coronary arteries and blood sampling. All baseline visits were
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33 60 completed by December 2022, and the remaining follow-up visits will be concluded by the end
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35 61 of 2023. The EDSAP study aims to identify new molecular and imaging markers associated with
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37 62 the presence of atherosclerosis and its progression in a chronic inflammatory state such as
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39 63 psoriasis. This has the potential to: (1) help improve primary cardiovascular prevention strategies
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41 64 in these patients; (2) understand the effect of biologic drugs on the cardiovascular system; (3)
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43 65 serve as a model for understanding atherosclerosis in other chronic inflammatory diseases.
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47 66 **Ethics and dissemination:** The study protocol has been approved by the Institutional Review
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49 67 Board of the Hospital Ramón y Cajal in Madrid (HIP/CI-BIOB-058-01). We will present our
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51 68 findings at national and international congresses, and peer-reviewed journals.
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3 72 **Strengths and limitations**
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- 6 73 • Strict application of protocols, the collection of biobank samples and the prospective data
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8 74 collection which allows to evaluate the impact of anti-inflammatory therapies on
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10 75 atheroma plaque characterization and modulation.
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12 76 • The application of state-of-the-art scientific techniques to measure the anatomical and
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14 77 biological characteristics of subclinical atherosclerosis.
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16 78 • Being an observational study, it is more vulnerable to potential confounding factors
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18 79 compared to randomized controlled trials.
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20 80 • The open-label, non-randomized use of psoriasis treatments in a small sample size and
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22 81 with a short follow-up duration.
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85 INTRODUCTION

86 Psoriasis is a complex chronic inflammatory and immune-mediated disease of the skin and joints
87 associated with multiple comorbidities (1,2). The life expectancy of patients with psoriasis is
88 reduced by 4-5 years due to cardiovascular (CV) disease, and there is an increased risk of
89 myocardial infarction at an earlier age compared to individuals without the disease (3,4). This
90 elevated CV risk could be due to systemic inflammation characteristic, especially in the moderate-
91 to-severe forms of the disease (5,6). Therefore, classical screening methods such as the
92 Framingham risk score, which is based on classical cardiovascular risk factors (CVRFs), do not
93 reliably assess the risk of coronary heart disease in patients with psoriasis (4,7,8).

94 Detection of atherosclerosis in its subclinical stage may help to identify strategies to halt the
95 development of the disease. Many imaging studies in patients with psoriasis assessed subclinical
96 atherosclerosis in individual vascular territories (4,9–13), but given the systemic nature of
97 atherosclerosis, a multi-territorial analysis has the potential to provide a more comprehensive
98 overview of the distribution and burden of atherosclerosis in these patients (14). The natural
99 history of atherosclerosis involves a prolonged subclinical phase, where the disease is usually
100 detected only at an advanced stage or after a CV event. Early detection of subclinical
101 atherosclerosis and adoption of primary prevention measures, including adequate control of
102 systemic inflammation, may minimise the risk of CV disease in patients with psoriasis. It has
103 therefore been proposed that these patients should undergo comprehensive screening for
104 subclinical atherosclerosis (4), a proposal that has arisen from the need to find a non-invasive,
105 simple and widely available biomarker for its early detection (15).

106 The most widely used and validated technique for screening subclinical atherosclerosis is vascular
107 ultrasound (VUS), a reproducible, non-invasive technique with no side effects. In the last years,
108 there has been a particular interest in the study of subclinical atherosclerosis in the femoral
109 arteries. Studies in healthy adults have shown that femoral plaques are more prevalent than carotid
110 plaques and are more associated with traditional CVRFs and coronary calcium (15,16), as well as
111 being an independent predictor of future CV events (14,17). Interestingly, in the PESA study

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3 112 (middle-aged participants from the general population), the presence of iliofemoral disease
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5 113 increases the risk of concurrent coronary calcium and is predictive of disease elsewhere (14). In
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7 114 fact, screening femoral arteries with vascular ultrasound has been introduced in current clinical
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9 115 practice guidelines as a risk modifier in individuals at low or moderate risk individuals (18). In
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11 116 this regard, our research group evaluated the usefulness of femoral artery ultrasound for the
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13 117 detection of subclinical atherosclerosis in psoriasis. We observed that screening of femoral
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15 118 plaques improves the detection of subclinical atherosclerosis in these patients, whereas carotid
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17 119 artery scanning was not sufficiently accurate (15,19). Semiautomated 3-dimensional vascular
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19 120 ultrasound (3DVUS) has been proposed as a better method for quantifying peripheral
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21 121 atherosclerotic burden. 3DVUS is a feasible, reproducible and novel imaging technique to
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23 122 quantify early carotid and femoral atherosclerotic burden in large populations. Furthermore, 3D-
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25 123 VUS offers incremental value over the presence of plaque alone in its association with
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27 124 cardiovascular risk (16,20). In the last decade, the advent of coronary computed tomography
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29 125 angiography (CCTA) has emerged as a promising non-invasive tool to assess coronary artery
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31 126 structure over time. It has been proposed that the ability of CCTA to identify and quantify the
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33 127 morphology of high-risk plaques, together with therapy monitoring, will eventually become the
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35 128 cornerstone of treatment personalisation (21).
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39 129 Several studies have shown the potential benefits of biologic therapies on CV disease risk in
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41 130 patients with psoriasis. Biologic therapy in severe psoriasis has been associated with a favourable
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43 131 modulation of coronary plaque indices by CCTA (19,22). These results support the need to
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45 132 expand our knowledge on the potential effects of biologic therapies in atherosclerosis.
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49 133 In addition to imaging techniques, there is a need to discover and validate new molecular markers
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51 134 that have practical value for clinical intervention as well as for identifying and elucidating CV
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53 135 disease processes at the individual level. Therefore, proteomic studies are needed to gain further
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55 136 insights in psoriasis-associated accelerated atherosclerosis in order to obtain a comprehensive
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57 137 overview of this high-risk population.
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3 138 This article describes the rationale, aims and methods of the EDSAP study protocol, a longitudinal
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5 139 cohort study to decipher the molecular, imaging and clinical characteristics of this accelerated
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7 140 atherosclerosis phenotype associated with psoriasis and to explore the effect of anti-inflammatory
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9 141 therapies on it.
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11 12 142 **STUDY OBJECTIVES**

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14
15 143 The objectives of the EDSAP study are: (1) to assess the prevalence, vascular distribution and
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17 144 burden of subclinical atherosclerosis in patients with psoriasis and its relationship with
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19 145 inflammatory biomarkers and CV risk algorithms using 2DVUS of carotid and femoral arteries,
20
21 146 3DVUS of carotid and femoral arteries and CCTA; (2) to characterize the composition of
22
23 147 atherosclerotic plaques by CCTA and 3D-VUS of the carotid and femoral arteries; (3) to evaluate
24
25 148 the effect of different treatments used in psoriasis on the progression and characterisation of
26
27 149 subclinical atherosclerosis in different arterial territories assessed by non-invasive imaging
28
29 150 techniques; and (4) to characterise the atherosclerosis process in patients with psoriasis using
30
31 151 laboratory analysis and "-omics" technologies, as well as to evaluate changes at the molecular
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33 152 level after treatment of the skin disease.
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36 37 153 **STUDY DESIGN AND POPULATION**

38
39 154 The EDSAP study is an observational, longitudinal, prospective cohort study that includes
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41 155 psoriasis patients who will undergo a one-year medical follow-up (Figure 1). Recruitment is
42
43 156 voluntary among patients attending dermatology consultations at the Hospital Ramón y Cajal,
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45 157 Madrid (Spain).
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47

48 158 The study includes participants aged between 30 to 65 years, diagnosed with psoriasis clinically
49
50 159 by an expert physician and deemed suitable for biologic therapy by the investigator. Exclusion
51
52 160 criteria are as follows: history of CV disease (myocardial infarction, angina pectoris, peripheral
53
54 161 vascular disease, aortic aneurysm, angioplasty, cardiac surgery, atrial fibrillation or any other
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56 162 cardiological condition), current oncological treatment, history of transplantation with active
57
58 163 immunosuppressive or immunomodulatory treatment, morbid obesity (body mass index ≥ 40
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3 164 kg/m²), diabetes mellitus, chronic liver disease, chronic kidney disease (glomerular filtration rate
4
5 165 <60 mL/min/1.73 m²), other chronic inflammatory disease, presence of any pathology that
6
7 166 decreases life expectancy to less than 3 years, or any disease or condition that could affect
8
9 167 adherence to study procedures. In addition, participants will be excluded if they have had a chest
10
11 168 computed tomography (CT) scan in the previous year, are pregnant or breastfeeding.

14 169 **DATA COLLECTION**

17 170 Two study visits are scheduled for each participant: at baseline and 1-year follow up. Both of
18
19 171 them include a clinical interview, physical examination (height, weight, waist circumference,
20
21 172 blood pressure and psoriasis severity through psoriasis area severity index -PASI-), fasting blood
22
23 173 draw and assessment of atherosclerotic disease by non-invasive vascular imaging tests
24
25 174 (2D/3DVUS and CCTA). Participants who experience primary or secondary failure of the initial
26
27 175 treatment may undergo an unscheduled clinic visit as part of standard clinical practice, given the
28
29 176 observational nature of this study. The attending physician will make the decision to change
30
31 177 treatment based on the patient's previous medical history and clinical presentation. This visit will
32
33 178 include a clinical interview, a physical examination, and a fasting blood draw. Imaging studies
34
35 179 will not be repeated due to the radiation exposure associated with CCTA. Training sessions and
36
37 180 certification of all personnel involved in data collection are repeated throughout the study.
38
39 181 Inclusion started in January 2020 with baseline visits completed for all 120 patients by the end of
40
41 182 2022. The remaining 1-year follow up visits are expected to be completed by the end of 2023
42
43 183 (Figure 2).

47 184 **CLINICAL INTERVIEW: PSORIASIS PAST MEDICAL HISTORY, CVRFS, DIET AND** 48 49 185 **LIFESTYLE HABITS**

51
52 186 Regarding CVRFS, patients are assessed for diabetes mellitus, hypertension, hyperlipidemia
53
54 187 obesity, smoking, metabolic syndrome and sedentary lifestyle (23). To assess the impact of the
55
56 188 Mediterranean diet on the CV risk in patients with psoriasis, a questionnaire from the PREDIMED
57
58 189 (Prevention with Mediterranean Diet) study is used. This is a validated tool that assesses the
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3 190 degree of adherence to the Mediterranean dietary pattern with 14 simple questions (24). In order
4
5 191 to measure the impact of psoriasis on patients' daily activities, the Dermatology Life Quality Index
6
7 192 (DLQI) questionnaire is employed (25). It is a unidimensional scale consisting of a short, simple
8
9 193 and easy-to-complete 10-question self-administered questionnaire. The questions are related to
10
11 194 the perception of the impact of the skin disease on quality of life in the last week. All
12
13 195 questionnaires are provided at all visits. Patients will also be asked about psoriasis duration,
14
15 196 previous disease treatments and psoriasis comorbidities including psoriasis arthritis and intestinal
16
17 197 bowel disease.

18 19 20 21 198 **VASCULAR IMAGING STUDIES**

22
23 199 Carotid and femoral arteries are explored using 2DVUS and 3DVUS (Figure 3). Staff performing
24
25 200 and interpreting the images are blinded to the variables of the study and any other imaging
26
27 201 procedures. A Philips iU22® ultrasound system (Philips Healthcare Andover, MA), using a 2D
28
29 202 L9-3 MHz high-resolution linear transducer is employed for 2VUS image acquisition. The study
30
31 203 protocol includes cross-sectional and longitudinal views of both carotid and femoral arteries to
32
33 204 detect plaques and standardized longitudinal views for intima-media thickness measurements.
34
35 205 2DVUS images will be analyzed using QLAB software (Intellispace Portal, Philips Healthcare).
36
37 206 2DVUS analysis includes assessment of carotid and femoral IMT (values >0.9 mm will be
38
39 207 considered abnormal), the presence and extension of atherosclerotic plaques in the carotid and
40
41 208 femoral territories (plaques are defined as a focal protrusion into the arterial lumen of thickness
42
43 209 >0.5 mm or >50% of the surrounding IMT or a diffuse thickness >1.5 mm measured between the
44
45 210 media-adventitia and intima-lumen interfaces), maximal plaque thickness (maximal distance
46
47 211 between the plaque-lumen and the plaque-adventitia interfaces), and plaque stenosis severity
48
49 212 (significant stenosis will be considered when luminal narrowing is >50%) (26).

50
51
52
53 213 A Philips iU22® ultrasound system equipped with a VL13-5 2D/3D volume linear array
54
55 214 transducer (Philips Healthcare) is used for the 3DVUS protocol. The acquisition protocol for the
56
57 215 carotid arteries consists of a 30° automatic sweep (explored vessel segment =6 cm long) centered
58
59 216 at the carotid bulb to include the distal common carotid artery, the bulb, the bifurcation, and the

1
2
3 217 proximal internal and external carotid artery segments. For the femoral arteries, acquisition is
4
5 218 centered at the bifurcation and includes the mid-distal common femoral artery, the bifurcation,
6
7 219 and the proximal superficial and deep femoral artery segments. Images will be analyzed using the
8
9 220 Vascular Plaque Quantification (VPQ) feature of QLAB software (Intellispace Portal, Philips).
10
11 221 The 3D variables quantified include: plaque volume, which will be determined by measuring the
12
13 222 volumes of each atherosclerotic plaque visualized in the standardized 3D acquisition of each
14
15 223 carotid and femoral artery individually as well as their sum, number of plaques by vascular site
16
17 224 and participant and 3D vessel wall volume or burden, as the plaque volume between the outer and
18
19 225 inner wall boundaries (12).
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23 **CORONARY IMAGING STUDIES**

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25 227 CCTA is performed with a 320-detector CT scanner (Aquilion ONE VISION, Toshiba, Japan) at
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27 228 the Hospital Universitario HM Sanchinarro in Madrid, following the guidelines of the NIH
28
29 229 Radiation Exposure Committee. Scans are performed with prospective or retrospective EKG
30
31 230 gating according to heart rate, tube potential of 100 or 120 kV, tube current of 100-850mA
32
33 231 adjusted to the patient's body size, with a gantry rotation time of 275 ms. Images are being
34
35 232 acquired with a slice thickness of 0.5 mm and a slice increment of 0.25 mm. Patient
36
37 233 characteristics, such as date of visit and treatment, will not be considered when reading the scans.
38
39 234 Coronary plaque quantification and characteristics will be analyzed in each of the main coronary
40
41 235 arteries (with a diameter >2 mm) using specific software (QAngio CT, Medis; The Netherlands)
42
43 236 (4,27). Automated longitudinal contouring of the inner lumen and outer wall will be performed,
44
45 237 manually adjusting the results when there are clear deviations. Results of the automated
46
47 238 contouring will also be reviewed on transverse reconstructed cross-sections of the artery on a
48
49 239 section-by-section basis at 0.25-mm increments. Lumen attenuation will be adaptively corrected
50
51 240 for each scan using gradient filters and intensity values within the artery.
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54
55 241 Plaque volume (in cubic millimeters) will be divided by the corresponding segment length (in
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57 242 millimeters), to obtain a plaque index to take into account variable coronary artery lengths. Total
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59 243 plaque burden is defined as the sum of calcified plaque burden and non-calcified plaque burden
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3 244 (NCB), assessed in square millimeters. Non-calcified plaque volume and subcomponents will be
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5 245 obtained after adaptively correcting for lumen attenuation and represented as a function of the
6
7 246 software-derived Hounsfield units as previously described (28). Also, high-risk plaque features,
8
9 247 defined as positive remodeling (remodeling index > 1.10), low-attenuation (<30 HU), or spotty
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11 248 calcification will be evaluated.

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14 249 All CCTA images will be analyzed in the Advanced Cardiac Imaging Unit at HM Sanchinarro,
15
16 250 Madrid, and in The Laboratory of Inflammation and Cardiometabolic Diseases, National Heart,
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18 251 Lung and Blood Institute (Maryland, United States) for specific analysis. Figure 4 shows an
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20 252 example of coronary arteries with (green arrow) and without subclinical atherosclerosis by CCTA.

23 253 **PHYSICAL EXAMINATION, LABORATORY ANALYSIS AND BIOBANKING**

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26 254 Patients undergo basic clinical studies in which they are weighed, measured, have their blood
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28 255 pressure taken, their waist circumference measured and their PASI evaluated. Moreover, fasting
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30 256 blood samples are collected at baseline, 1-year follow-up and flare-up visits as part of the clinical
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32 257 process. We will collect data of the results of the analysis of serum glucose, uric acid, alkaline
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34 258 phosphatase, bilirubin, apolipoprotein A1, apolipoprotein B, triglycerides, GlycA, total
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36 259 cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, C-reactive
37
38 260 protein, C-reactive protein ultra, lipoprotein (a), haptoglobin, insulin, hemoglobin, lymphocytes,
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40 261 neutrophils, monocytes and leukocytes. In addition, blood samples are collected in each scheduled
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42 262 visit to be processed and stored at -80°C for high-throughput “omics” analysis and biobanking.
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44 263 Each aliquot is assigned a unique identifier by using a laboratory information management system
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46 264 (Bio-e-Bank), managed by the Ramón y Cajal Institute for Health Research (IRYCIS), to ensure
47
48 265 adequate tracking of all procedures.

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52 266 The proteomic analysis will be performed by qualified personnel from the vascular
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54 267 physiopathology department of the Hospital Nacional de Paraplégicos in Toledo. Building on the
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56 268 previous experience of our group, atherosclerosis in patients with psoriasis will be studied at the
57
58 269 molecular level, trying to generate proteomic profiles that will allow us to obtain new data that
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3 270 shed light on this high-risk phenotype, incorporating the most recent advances in post-genomic
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5 271 sciences to identify panels of novel biomarkers that can serve as potential tools in the diagnosis
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7 272 and prognosis of the early phase of atherosclerosis, allowing to improve the primary, or even
8
9 273 primordial, preventive strategies for the management of cardiovascular risk in these patients. To
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11 274 this end, the overall proteomic discovery strategy will consist of a discovery phase, a verification
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13 275 phase and a validation phase: (1) The discovery phase will be performed using TMT 10-plex
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15 276 reagents, followed by LC-MS/MS using a reverse-phase C-18 nanocolumn. Identification of
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17 277 selected peptides will be performed using the likelihood ratio method (29) and the false discovery
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19 278 rate (FDR) calculated using inverted databases and the refined method (30). Only peptides
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21 279 identified with $FDR \leq 1\%$ will be used to quantify the relative abundance of each protein from
22
23 280 reporter ion intensities. For statistical analysis of quantitative data, the WSPP statistical model
24
25 281 will be used (31). Finally, a functional analysis of the whole set of quantified proteins will be
26
27 282 performed by analyzing the coordinated protein responses in quantitative proteomics experiments
28
29 283 - the systems biology triangle (SBT) (32), which correlates the performance of groups of proteins
30
31 284 within a biological process with their quantitative behavior. (2) The verification phase will
32
33 285 involve a targeted proteomics strategy to study the proteins and the mechanisms previously
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35 286 described by our group, which possibly could be involved in psoriasis, focusing on their
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37 287 relationship with atherosclerosis and CV risk stratification. (3) The Validation phase will consist
38
39 288 of targeted proteomics and immunoassays. The results obtained in the discovery phase will be
40
41 289 validated in an independent cohort of patients and will be analyzed for a comprehensive
42
43 290 assessment of lipoprotein biomarkers and inflammatory biomarkers. These last two phases will
44
45 291 use the Selected reaction monitoring (SRM) methodology – SRM design.

292 **CLINICAL EVENTS**

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52
53 293 In addition to these examinations, patients will have their routine hospital visits, during which
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55 294 additional information will be collected on any clinical or health-related events affecting the
56
57 295 participants during the study period. In any case, the continuity of the patient in the study will be
58
59 296 assessed, as the patient's health status will always be prioritized.

297 **STATISTICAL ANALYSIS PLAN**

298 EDSAP is a longitudinal cohort study, in which measurements are made of different variables
299 related to psoriasis and cardiovascular imaging at baseline and after one year of biologic
300 treatment. This type of design allows simultaneous testing of different hypotheses, which will
301 involve a specific statistical analysis plan and the selection of the most appropriate data treatment
302 and models to study associations and account for potential confounders. On the one hand, cross-
303 sectional studies will be carried out at each visit between the independent variables and the
304 different outcome variables. Analyses will be performed using linear regression models for
305 continuous variables and logistic regression models for categorical variables. On the other hand,
306 longitudinal studies of the progression of subclinical atherosclerosis based on different
307 independent variables will be performed using the most appropriate models for longitudinal data.
308 The non-calcified coronary burden after one year of treatment was considered as the reference
309 variable for the sample size calculation, given its clinical relevance and the existence of previous
310 literature providing reference values (4,19). Accepting an $\alpha=0.05$ and a $\beta=0.2$ in a bilateral
311 contrast for repeated (paired) means, a total of 97 subjects are needed to detect a minimum
312 difference of 0.03 mm, a more conservative value than that obtained in previous studies (4,19).
313 We assumed a loss to follow-up rate of 10%. To date, we have recruited a total of 120 patients,
314 far exceeding the minimum sample size needed to detect differences in the primary endpoint.
315 Study results will be reported in accordance with STROBE guidelines (33).

316 **PATIENT AND PUBLIC INVOLVEMENT**

317 None.

318 **DISCUSSION**

319 The EDSAP study aims to provide information on the presence and progression of subclinical
320 atherosclerosis in patients with psoriasis in order to help establish earlier and more personalized
321 management of care for these patients, whose life expectancy is reduced due to the increased risk
322 of cardiovascular disease at younger ages compared to the general population.

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3 323 Traditional prediction systems based on classical CVRFs, underestimate the actual CV risk of
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5 324 patients with psoriasis and other chronic inflammatory states (8,34). There is a lack of markers
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7 325 that allow us to adequately predict those patients who will develop cardiovascular disease. In this
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9 326 scenario, numerous publications have emerged in recent highlighting the value of detecting
10
11 327 subclinical atherosclerosis through various imaging tests as a tool to overcome this gap. The use
12
13 328 of ultrasound techniques has shown to be an accurate biomarker of atherosclerosis presence (4).
14
15 329 Screening for subclinical atherosclerosis in patients with psoriasis with imaging techniques in
16
17 330 more than one vascular territory, such as the carotid and especially the femoral arteries, has proven
18
19 331 to be a reliable biomarker for cardiovascular risk assessment (15). In addition, 3DVUS is another
20
21 332 well-established imaging technique for quantifying early carotid and femoral atherosclerotic
22
23 333 burden, as well as being an accessible, novel and reliable technique (16). Recently, this technique
24
25 334 has been further developed to obtain plaque characterization and quantification, enabling risk
26
27 335 stratification based on atherosclerotic plaque burden (16). Regarding CCTA, some studies have
28
29 336 shown how biologics can modulate coronary plaque indices in psoriasis favorably, supporting
30
31 337 further studies to qualify these results (19,21,22). These results have been recently validated in a
32
33 338 systematic review and meta-analysis evaluating the impact of licensed biologic treatments on
34
35 339 blood and imaging biomarkers of CV risk in adult patients with psoriasis. The results
36
37 340 demonstrated how some biologic drugs were associated with a reduction in aortic vascular
38
39 341 inflammation (35). In this study, one of the interesting aspects to explore, and which could provide
40
41 342 more data on the complex relationship between cutaneous and vascular inflammation in these
42
43 343 patients, is whether this modulation of coronary plaques by antipsoriatic therapies is parallel to or
44
45 344 independent of the improvement in PASI, which would provide a more global view of the
46
47 345 systemic inflammation affecting the patient with psoriasis. The EDSAP study is the first study
48
49 346 that aims to have a comprehensive CV overview of the psoriasis patient, using novel imaging
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51 347 techniques to study peripheral and coronary subclinical atherosclerosis to accurately assess
52
53 348 individual CV risk and minimize this risk through prevention and early treatment. This global
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55 349 understanding of the disease, and the ability to see how antipsoriatic therapies modulate the
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57 350 patient's internal inflammation, may represent a breakthrough in the treatment of the psoriasis

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3 351 patient, as well as in the understanding of psoriasis as a human model of atherosclerosis in
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5 352 inflammatory states.

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8 353 As a complement to multiterritorial imaging studies, EDSAP incorporates the use of new
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10 354 molecular techniques such as proteomics that could be the starting point to identify those
11
12 355 individuals potentially predisposed to develop atherosclerosis, allowing us to find potential
13
14 356 predictive and therapeutic targets. At present, few studies have evaluated the usefulness of
15
16 357 proteomic strategies to identify a biomarker of atherosclerosis in patients with psoriasis (36,37).
17
18 358 This approach aims to identify early markers of subclinical atherosclerosis that can, alone or in
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20 359 combination with imaging techniques, identify individuals at increased risk. The biological
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22 360 resource generated will also be available for future use in longitudinal association studies of any
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24 361 parameter of interest (e.g. clinical events, response to treatment, development of risk factors,
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26 362 progression of atherosclerosis, etc.), which will advance the understanding of how CV disease
27
28 363 initiates and progresses.

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30
31 364 The main methodological advantages of this study are the strict application of protocols, the
32
33 365 collection of biobank samples and the prospective data collection which allows to evaluate the
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35 366 impact of anti-inflammatory therapies on atheroma plaque presence and characterization. In
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37 367 addition, state-of-the-art scientific techniques are being applied to measure the anatomical and
38
39 368 biological characteristics of subclinical atherosclerosis. However, there are limitations to be kept
40
41 369 in mind: this is an observational study and more vulnerable to potential confounding factors
42
43 370 compared to randomized controlled trials, and the open, non-randomized use of psoriasis
44
45 371 treatments in a small sample and with a short follow-up duration. However, this could be the
46
47 372 largest consecutive sample of psoriasis patients followed over time using CCTA, 3D and 2DVUS.
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49 373 In addition, we will follow a consecutive sample to minimize any selection bias in longitudinal
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51 374 follow-up. Finally, we will use arterial plaque imaging technology to understand modulation in
52
53 375 CV disease risk secondary to biological treatment.

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57 376 In conclusion, the EDSAP study is designed to study psoriasis as a model of atherosclerosis in
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59 377 inflammatory states, as well as to provide clinical, imaging and molecular biomarkers, using

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3 378 innovative imaging techniques as well as omics technologies for the prevention and treatment of
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5 379 these high-risk patients for early CV events.
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8 380 **Ethics and dissemination of results:**
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10 381 The study protocol has been approved by the Ethics Committee of Hospital Ramón y Cajal in
11
12 382 Madrid. All participants will provide written informed consent that explicitly includes consent for
13
14 383 biobanking of surplus biological materials, which will be provided for future research projects.
15
16 384 We will present our findings at national and international congresses, and peer-reviewed journals.
17
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19
20 385 **Acknowledgements:**
21

22 386 We thank all the participants in the study and gratefully acknowledge the collaboration and
23
24 387 assistance of the staff at Hospital Universitario Ramón y Cajal, Hospital HM Sanchinarro,
25
26 388 National Institute of Health, Hospital 12 de Octubre and Atria Clinic.
27
28

29 389 We would also like to thank the dedication and impeccable management of the biological samples
30
31 390 to all the members of the Biobank of the Hospital Ramón y Cajal. Finally, we are very grateful to
32
33 391 all the participants in the EDSAP study, without them it would not have been possible.
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36 392 **Data Availability statement:**
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39 393 Our data will be published in a timely manner at the conclusion of the follow-up period and will
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41 394 be made available upon request.
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REFERENCES

1. Griffiths CE, Barker JN. Pathogenesis and clinical features of psoriasis. *Lancet Lond Engl*. 2007 Jul 21;370(9583):263–71.
2. Neimann AL, Shin DB, Wang X, Margolis DJ, Troxel AB, Gelfand JM. Prevalence of cardiovascular risk factors in patients with psoriasis. *J Am Acad Dermatol*. 2006 Nov 1;55(5):829–35.
3. Risk of Myocardial Infarction in Patients With Psoriasis | Acute Coronary Syndromes | JAMA | JAMA Network [Internet]. [cited 2022 Nov 30]. Available from: <https://jamanetwork.com/journals/jama/fullarticle/203598>
4. Lerman JB, Joshi AA, Chaturvedi A, Aberra TM, Dey AK, Rodante JA, et al. Coronary Plaque Characterization in Psoriasis Reveals High-Risk Features That Improve After Treatment in a Prospective Observational Study. *Circulation*. 2017 Jul 18;136(3):263–76.
5. Garshick MS, Barrett TJ, Wechter T, Azarchi S, Scher JU, Neimann A, et al. Inflammasome Signaling and Impaired Vascular Health in Psoriasis. *Arterioscler Thromb Vasc Biol*. 2019 Apr;39(4):787–98.
6. Potential Immunological Links Between Psoriasis and Cardiovascular Disease - PubMed [Internet]. [cited 2022 Dec 6]. Available from: <https://pubmed.ncbi.nlm.nih.gov/29910818/>
7. Mehta NN, Krishnamoorthy P, Yu Y, Khan O, Raper A, Van Voorhees A, et al. The impact of psoriasis on 10-year Framingham risk. *J Am Acad Dermatol*. 2012 Oct;67(4):796–8.
8. Berna-Rico E, Abbad-Jaime de Aragon C, Garcia-Aparicio A, Palacios-Martinez D, Ballester-Martinez A, Carrascosa JM, et al. Cardiovascular Screening Practices and Statin Prescription Habits in Patients with Psoriasis among Dermatologists, Rheumatologists and Primary Care Physicians. *Acta Derm Venereol*. 2023 Mar 28;103:adv5087.
9. Gonzalez-Cantero A, Gonzalez-Cantero J, Sanchez-Moya AI, Perez-Hortet C, Arias-Santiago S, Schoendorff-Ortega C, et al. Subclinical atherosclerosis in psoriasis. Usefulness of femoral artery ultrasound for the diagnosis, and analysis of its relationship with insulin resistance. *PloS One*. 2019;14(2):e0211808.
10. Armstrong AW, Harskamp CT, Ledo L, Rogers JH, Armstrong EJ. Coronary artery disease in patients with psoriasis referred for coronary angiography. *Am J Cardiol*. 2012 Apr 1;109(7):976–80.
11. Mansouri B, Kivelevitch D, Natarajan B, Joshi AA, Ryan C, Benjegerdes K, et al. Comparison of Coronary Artery Calcium Scores Between Patients With Psoriasis and Type 2 Diabetes. *JAMA Dermatol*. 2016 Nov 1;152(11):1244–53.
12. Hjuler KF, Böttcher M, Vestergaard C, Deleuran M, Raaby L, Bøtker HE, et al. Increased Prevalence of Coronary Artery Disease in Severe Psoriasis and Severe Atopic Dermatitis. *Am J Med*. 2015 Dec;128(12):1325–1334.e2.
13. Tinggaard AB, Hjuler KF, Andersen IT, Winther S, Iversen L, Böttcher M. Prevalence and severity of coronary artery disease linked to prognosis in psoriasis and psoriatic arthritis patients: a multi-centre cohort study. *J Intern Med*. 2021 Sep;290(3):693–703.
14. Fernández-Friera L, Peñalvo JL, Fernández-Ortiz A, Ibañez B, López-Melgar B, Laclaustra M, et al. Prevalence, Vascular Distribution, and Multiterritorial Extent of Subclinical

- 1
2
3 437 Atherosclerosis in a Middle-Aged Cohort: The PESA (Progression of Early Subclinical
4 438 Atherosclerosis) Study. *Circulation*. 2015 Jun 16;131(24):2104–13.
5
6 439 15. González-Cantero A, Gonzalez-Cantero J, Sanchez-Moya AI, Perez-Hortet C, Arias-
7 440 Santiago S, Martin-Rodriguez JL, et al. Femoral artery ultrasound for improving the
8 441 detection of atherosclerosis in psoriasis. *J Am Acad Dermatol*. 2019 Mar;80(3):784–6.
9
10 442 16. López-Melgar B, Fernández-Friera L, Oliva B, García-Ruiz JM, Peñalvo JL, Gómez-
11 443 Talavera S, et al. Subclinical Atherosclerosis Burden by 3D Ultrasound in Mid-Life: The
12 444 PESA Study. *J Am Coll Cardiol*. 2017 Jul 18;70(3):301–13.
13
14 445 17. Kaur S, Kingo K, Zilmer M. Psoriasis and Cardiovascular Risk—Do Promising New
15 446 Biomarkers Have Clinical Impact? *Mediators Inflamm*. 2017;2017:7279818.
16
17 447 18. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to
18 448 reduce cardiovascular risk | *European Heart Journal* | Oxford Academic [Internet]. [cited
19 449 2022 Dec 13]. Available from:
20 450 <https://academic.oup.com/eurheartj/article/41/1/111/5556353>
21
22 451 19. Elnabawi YA, Dey AK, Goyal A, Groenendyk JW, Chung JH, Belur AD, et al. Coronary
23 452 artery plaque characteristics and treatment with biologic therapy in severe psoriasis: results
24 453 from a prospective observational study. *Cardiovasc Res*. 2019 Mar 15;115(4):721–8.
25
26 454 20. López-Melgar B, Mass V, Nogales P, Sánchez-González J, Entekin R, Collet-Billon A, et
27 455 al. New 3-Dimensional Volumetric Ultrasound Method for Accurate Quantification of
28 456 Atherosclerotic Plaque Volume. *JACC Cardiovasc Imaging*. 2022 Jun 1;15(6):1124–35.
29
30 457 21. Elnabawi YA, Dey AK, Mehta NN. Emerging Applications of Coronary CT Angiography
31 458 in Coronary Heart Disease: Getting Better with Time. *Eur Heart J*. 2018 Nov
32 459 1;39(41):3682–4.
33
34 460 22. Hjuler KF, Bøttcher M, Vestergaard C, Bøtcher HE, Iversen L, Kragballe K. Association
35 461 Between Changes in Coronary Artery Disease Progression and Treatment With Biologic
36 462 Agents for Severe Psoriasis. *JAMA Dermatol*. 2016 Oct 1;152(10):1114–21.
37
38 463 23. Hu SCS, Lan CCE. Psoriasis and Cardiovascular Comorbidities: Focusing on Severe
39 464 Vascular Events, Cardiovascular Risk Factors and Implications for Treatment. *Int J Mol*
40 465 *Sci*. 2017 Oct 21;18(10):2211.
41
42 466 24. Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, et al. Primary Prevention
43 467 of Cardiovascular Disease with a Mediterranean Diet Supplemented with Extra-Virgin
44 468 Olive Oil or Nuts. *N Engl J Med*. 2018 Jun 21;378(25):e34.
45
46 469 25. Finlay AY, Khan GK. Dermatology Life Quality Index (DLQI)--a simple practical measure
47 470 for routine clinical use. *Clin Exp Dermatol*. 1994 May;19(3):210–6.
48
49 471 26. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Desvarieux M, et al.
50 472 Mannheim Intima-Media Thickness Consensus. *Cerebrovasc Dis*. 2004;18(4):346–9.
51
52 473 27. Kwan AC, May HT, Cater G, Sibley CT, Rosen BD, Lima JAC, et al. Coronary artery
53 474 plaque volume and obesity in patients with diabetes: the factor-64 study. *Radiology*. 2014
54 475 Sep;272(3):690–9.
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3 476 28. Sorokin AV, Patel N, Abdelrahman KM, Ling C, Reimund M, Graziano G, et al. Complex
4 477 association of apolipoprotein E-containing HDL with coronary artery disease burden in
5 478 cardiovascular disease. *JCI Insight*. 7(10):e159577.
- 7 479 29. Martínez-Bartolomé S, Navarro P, Martín-Maroto F, López-Ferrer D, Ramos-Fernández A,
8 480 Villar M, et al. Properties of average score distributions of SEQUEST: the probability ratio
9 481 method. *Mol Cell Proteomics MCP*. 2008 Jun;7(6):1135–45.
- 11 482 30. Navarro P, Vázquez J. A refined method to calculate false discovery rates for peptide
12 483 identification using decoy databases. *J Proteome Res*. 2009 Apr;8(4):1792–6.
- 14 484 31. García-Marqués F, Trevisan-Herraz M, Martínez-Martínez S, Camafeita E, Jorge I, Lopez
15 485 JA, et al. A Novel Systems-Biology Algorithm for the Analysis of Coordinated Protein
16 486 Responses Using Quantitative Proteomics. *Mol Cell Proteomics MCP*. 2016
17 487 May;15(5):1740–60.
- 19 488 32. Isern J, Martín-Antonio B, Ghazanfari R, Martín AM, López JA, del Toro R, et al. Self-
20 489 renewing human bone marrow mesospheres promote hematopoietic stem cell expansion.
21 490 *Cell Rep*. 2013 May 30;3(5):1714–24.
- 23 491 33. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, et al. The
24 492 Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)
25 493 Statement: guidelines for reporting observational studies. *Int J Surg Lond Engl*. 2014
26 494 Dec;12(12):1495–9.
- 28 495 34. Gonzalez-Cantero A, Reddy AS, Dey AK, Gonzalez-Cantero J, Munger E, Rodante J, et al.
29 496 Underperformance of clinical risk scores in identifying imaging-based high cardiovascular
30 497 risk in psoriasis: results from two observational cohorts. *Eur J Prev Cardiol*. 2022 Mar
31 498 30;29(4):591–8.
- 33 499 35. González-Cantero A, Ortega-Quijano D, Álvarez-Díaz N, Ballester MA, Jimenez-Gomez
34 500 N, Jaen P, et al. Impact of Biological Agents on Imaging and Biomarkers of Cardiovascular
35 501 Disease in Patients with Psoriasis: A Systematic Review and Meta-Analysis of Randomized
36 502 Placebo-Controlled Trials. *J Invest Dermatol*. 2021 Oct;141(10):2402–11.
- 38 503 36. Kaiser H, Wang X, Kvist-Hansen A, Krakauer M, Gørtz PM, McCauley BD, et al.
39 504 Biomarkers of subclinical atherosclerosis in patients with psoriasis. *Sci Rep*. 2021 Nov
40 505 2;11(1):21438.
- 42 506 37. Qi F, Tan Y, Yao A, Yang X, He Y. Psoriasis to Psoriatic Arthritis: The Application of
43 507 Proteomics Technologies. *Front Med*. 2021;8:681172.

508 **Author contributions:**

509 Carlota Abbad- Jaime de Aragón.: Investigation, Conceptualization, Methodology, Original draft
510 preparation, Visualization. Emilio Berna-Rico.: Conceptualization, Methodology, Visualization,
511 Writing- Reviewing and Editing. María Asunción Ballester-Martinez.: Writing- Reviewing and
512 Editing. Pedro Jaén.: Writing- Reviewing and Editing. Jorge Solís.: Writing- Reviewing and
513 Editing. María G. Barderas.: Software, Validation. Leticia Fernández- Frieria.: Writing-

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3 514 Reviewing and Editing. Nehal N Mehta.: Writing- Reviewing and Editing. Joel M Gelfand.:
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5 515 Writing- Reviewing and Editing. Álvaro González-Cantero.: Conceptualization, Methodology,
6
7 516 Writing- Reviewing and Editing, Funding acquisition, Supervision.
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20 521 **Competing interests statement:**

21
22 522 NNM is a full- time US government employee and has served as a consultant for several
23
24 523 pharmaceutical companies, receiving grants and/or research funding; and as a principal
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26 524 investigator for the NIH receiving grants and/or research funding. JMG served as a consultant for
27
28 525 several pharmaceutical companies, receiving honoraria and research grants (to the Trustees of the
29
30 526 University of Pennsylvania). JMG is a co-patent holder of resiquimod for treatment of cutaneous
31
32 527 T cell lymphoma. JMG receives honoraria from multiple organisms, for being a Deputy Editor
33
34 528 for the Journal of Investigative Dermatology, Chief Medical Editor for Healio Psoriatic Disease.
35
36 529 He is also a member of the Board of Directors for the International Psoriasis Council, receiving
37
38 530 no honoraria. ACG has served as a consultant for several pharmaceutical companies receiving
39
40 531 grants/other payments. MABM has served as a consultant for several pharmaceutical companies
41
42 532 receiving honoraria. LFF, JS, EBR, MGB, PJ, CAJ have no interests to disclose.
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541 **Figure legends:**

542 **Figure 1:** EDSAP study flow. CCTA, coronary computed tomography angiography. 2D, 2-
543 dimensional. 3D, 3-dimensional.

544 **Figure 2.** Participant timeline. This flow diagram illustrates the participant timeline including
545 enrollment, baseline and 1-year follow up visits, the analysis of data and publication of results.

546 **Figure 3.** 2D vascular ultrasound of atherosclerotic plaque (white arrow) in the right femoral
547 artery and (A) and 3D vascular ultrasound of another atherosclerotic plaque (black arrow).

548 **Figure 4.** Left anterior descending coronary artery with (green arrow) and without atheroma
549 plaque by CCTA.

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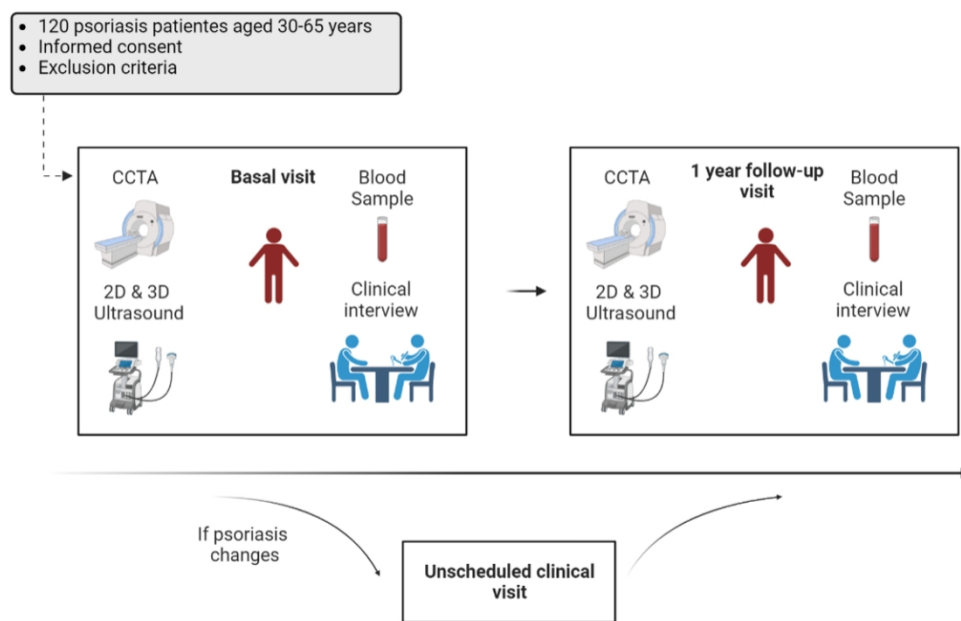
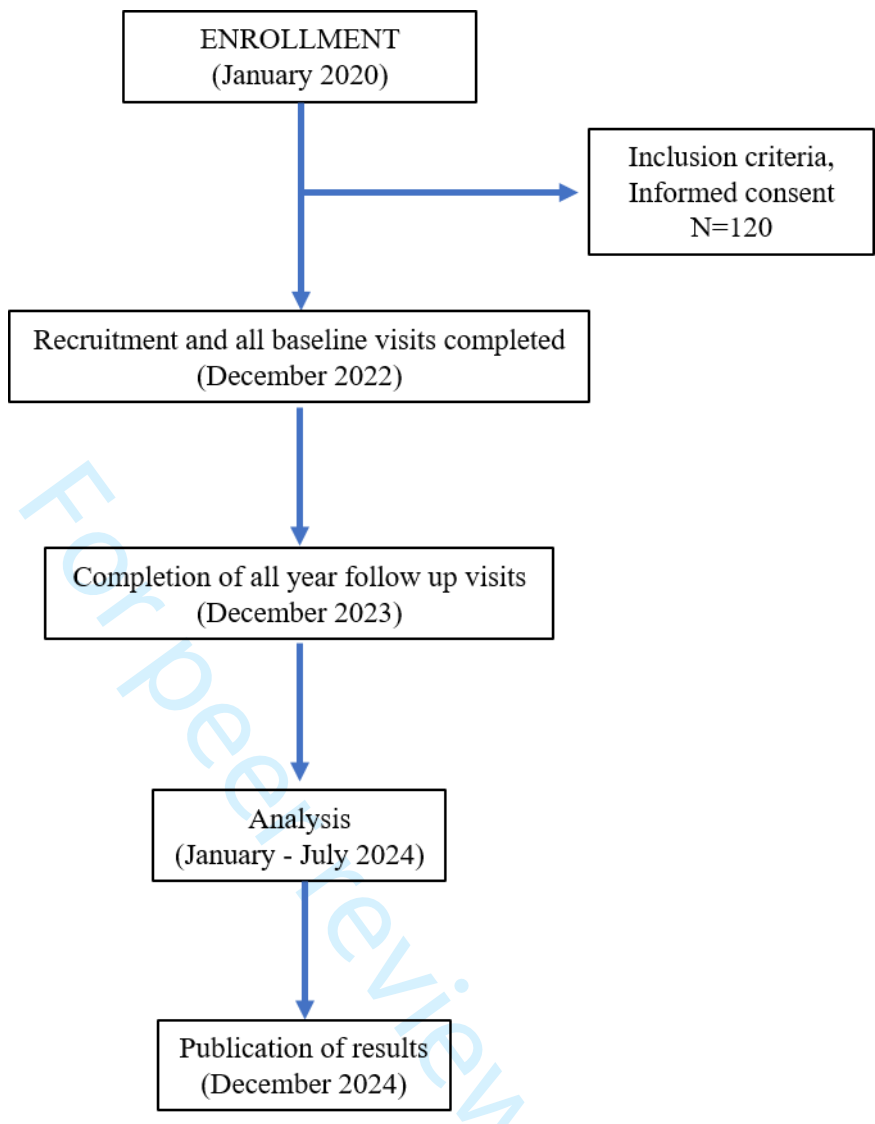


Figure 1. EDSAP study flow. CCTA, coronary computed tomography angiography. 2D, 2-dimensional. 3D, 3-dimensional.

100x64mm (300 x 300 DPI)

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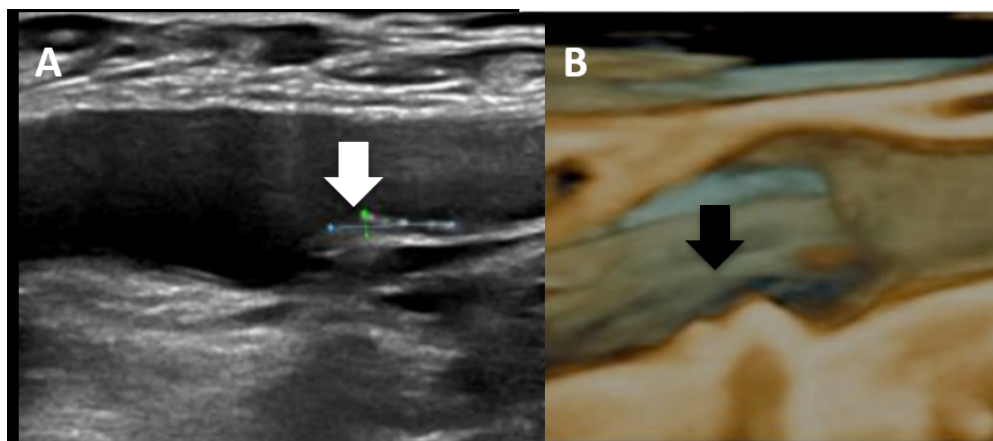


Figure 3. 2D vascular ultrasound of atherosclerotic plaque (white arrow) in the right femoral artery and (A) and 3D vascular ultrasound of another atherosclerotic plaque (black arrow).

79x34mm (300 x 300 DPI)

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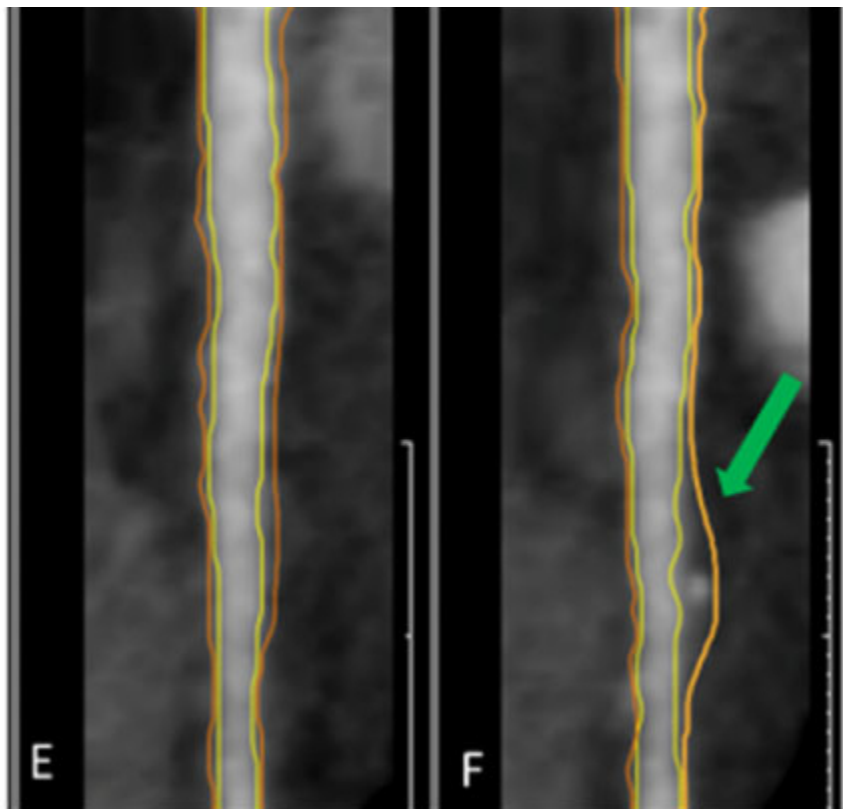


Figure 4. Left anterior descending coronary artery with (green arrow) and without atheroma plaque by CCTA

190x180mm (57 x 57 DPI)