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ORIGINAL ARTICLE

High prevalence of vitamin D deficiency and lack of association with subclinical atherosclerosis in asymptomatic patients with Type 1 Diabetes Mellitus from a Mediterranean area

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Abstract
Aims Several studies linked vitamin D deficiency with coronary artery disease (CAD). The aim of this study was to evaluate the relationship between the concentrations of 25-hydroxyvitamin D (25OHD) and the presence of early atherosclerosis in asymptomatic Type 1 Diabetes (T1D) patients with no previous history of ischemic heart disease.
Methods One hundred and forty-five patients with T1D (age 37.8 ± 8 years, 57 % male, all Caucasian, disease duration 20.6 ± 8.3 years, HbA1c 7.6 ± 1.4 % (60.2 ± 11.1 mmol/mol), body mass index (BMI) 25.2 ± 3.5 kg/m2, 52.4 % smokers, 23 % retinopathy, 10 % nephropathy) and 48 controls matched for age, sex, BMI and smoking habit were studied. 25OHD deficiency was defined for values ≤20 ng/mL. A sun exposure questionnaire, carotid ultrasonography to determine carotid intima-media thickness (CIMT) and the presence of atheroma plaques and cardiac computed tomography for evaluation of calcium artery calcification (CACS) were performed.
Results T1D subjects showed a high proportion of 25OHD deficiency (43.2 % vs. 21.7 %, $p = 0.032$). Of all, 82 % of T1D patients and 92 % of controls had a calcium score of 0. CIMT was greater in patients with T1D (0.55 ± 0.14 mm vs 0.48 ± 0.15, $p = 0.01$) compared with controls. T1D subjects showed no differences in the results of CACS or CIMT according to the vitamin D concentrations.
Conclusions T1D patients have lower concentrations and twice more prevalence of 25OHD deficiency than controls. There was no association between 25OHD concentrations and subclinical CAD.

Keywords Type 1 diabetes · Vitamin D deficiency · Cardiovascular disease · Subclinical atherosclerosis · Coronary artery calcification · Carotid intima-media thickness

Abbreviations

CAD	Coronary artery disease
T1D	Type 1 diabetes
hs CRP	High-sensitive C-reactive protein
BMI	Body mass index
25OHD	25-Hydroxyvitamin D
CT	Computed tomography
CU	Carotid ultrasound
CIMT	Carotid intima-media thickness
CVD	Cardiovascular disease
CACS	Coronary artery calcification score
CHD	Cardiac heart disease
HDL	High density lipoprotein cholesterol
LDL	Low density lipoprotein cholesterol
Ca	Calcium
Ph	Phosphate
PTH	Parathormone

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CV	Coefficient of variation
AU	Agatston units
SD	Standard desviation
EDTA	Ethylenediaminetetraacetic acid
HPLC	High-performance liquid chromatographic

Introduction

A billion people worldwide have vitamin D deficiency or insufficiency [1]. In the last decade, a series of studies have revealed the non-calciotropic actions of vitamin D [2–4]. A controversial issue is the possible link between cardiovascular disease (CVD) and vitamin D deficiency. Vitamin D deficiency may promote calcification, inflammation and endothelial dysfunction [5]. Different factors such as secondary hyperparathyroidism, chronic inflammatory state and oxidative stress could contribute to vascular damage in 25-hydroxyvitamin D (25OHD)-deficient patients [6]. Recently, some reports have shown lower vitamin D concentrations in T1D and T2D subjects compared with control population [7–11]. These vascular physiopathologic implications of 25OHD in diabetic population may act synergistically with classical cardiovascular risk factors as hypertension, dyslipidemia, smoking habit and poor glycemic control, leading to a higher degree of vascular damage.

Although a remarkable number of studies in general population, with and without diabetes, have demonstrated a relationship between 25OHD and a higher prevalence of cardiovascular risk [11, 12], not all the reports have confirmed this association [13, 14]. 25OHD deficiency has been linked to a greater prevalence of CVD [4, 15, 16], coronary artery calcification (CACS) [17, 18] and mortality [19, 20], thus postulating a cardioprotective effect of the reposition of this steroid.

There is scarce information concerning the possible relationship between 25OHD deficiency and subclinical coronary artery disease (CAD) in T1D patients. Two recent studies have been conducted in American T1D patients and have shown discordant results. Sachs et al. [13] did not find an association between 25OHD deficiency and subclinical atherosclerosis measured by CACS and carotid intima-media thickness (CIMT). Nevertheless, Young et al. [18] have found a significant association between 25OHD deficiency and an accelerated progression of vascular disease.

The aim of this cross-sectional study was to evaluate the possible relationship between the concentrations of vitamin D and the presence of early atherosclerosis assessed by CACS and CIMT in asymptomatic T1D patients with long disease evolution living in a Mediterranean country.

Methods

Patients and controls

An initial cohort of 150 asymptomatic T1D regularly followed at our outpatient clinic and a control group of 50 subjects matched by age, sex and smoke condition were consecutively recruited between 2010 and 2012 in Badalona (Barcelona, Spain; latitude 41° 25′). In this cohort, the presence of subclinical atherosclerosis was evaluated and previously reported elsewhere [21]. Inclusion criteria were being diagnosed of T1D (clinical and immunologic diagnosis), an age between 20 and 50 years and a disease evolution of more than 10 years. The exclusion criteria were a previous history of clinical macrovascular or cardiac heart disease (CHD), Type IV, V or VI skin phototype, or having T1D <10 years of evolution and current treatment with vitamin D or calcium. Five patients and two controls from the initial cohort were excluded because they were under vitamin D treatment for different reasons. The final sample studied included 145 T1D patients and 48 controls (all Caucasian).

Current smoking and previously smoking condition for less than 5 years were included in the same category. The control group of non-diabetic subjects was matched for age, sex, body mass index (BMI) and smoking condition and recruited from the staff of our hospital and their relatives. All patients were under intensive insulin treatment, and 15 % of them were using pump devices.

The study was approved by the local ethics committee, in accordance with the Declaration of Helsinki. All participants gave their written informed consent prior to inclusion.

Demographic and clinical data including age, sex, history of clinical macrovascular disease and microvascular diabetic complications, family history of early CHD in first degree relatives (defined as CHD occurring before age 55 years in men and before age 65 years in women) and medical treatment (antihypertensive agents, statins and acetylsalicylic acid) were recorded for all patients. Body mass index (BMI) was calculated as weight in kilograms divided by height per square meter.

Diabetic nephropathy was evaluated according to urinary albumin excretion. Thus, normal urinary albumin excretion was considered below 30 mg/24 h, microalbuminuria from 30 to 300 mg/24 h and proteinuria above 300 mg/24 h. These results were confirmed on at least two out of three consecutive determinations. Diabetic

retinopathy was defined by fundus oculi performed by a specialized ophthalmologist.

Trained personnel collected clinical parameters (age, sex, height, weight, BMI, blood pressure, smoking habit and family history of early CHD).

A sun exposure questionnaire was administered to all study participants in which the season of blood sample extraction whether obtained in winter (October–March) or summer (April–September) was considered for adjustment. This questionnaire has formerly validated in a Mediterranean Caucasian Italian population, in which a correlation between the sun exposure score and the concentrations of 25OHD was found [22]. Parameters as minutes spent outdoor ($\leq 5'$, 5–30′ or $\geq 30'$) and skin exposed zones (face and hands; face, hands and arms; face, hands, arms and legs; or "bathing suit") were categorized and registered in order to obtain an exposition score. The mean weekly exposition score was calculated (minimum = 0 and maximum = 56) for each participant.

Biochemical measurements

Blood samples were drawn by venipuncture at between 8.00 and 08.30 h. After an overnight fast, plasma glucose, total cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol, triglycerides, calcium (Ca) and phosphate (Ph) were measured by routine clinical chemistry immediately after extraction. HbA1c was measured in blood samples with ethylenediaminetetraacetic acid (EDTA) by high-performance liquid chromatography (HPLC) using a fully automated Adams Menarini HI-AUTO A1c 8160 analyzer manufactured by Arkray (Kyoto, Japan) with an inter-assay coefficient of variation of 1.8 and 1.5 % at HbA1c levels of 4.8 and 9.0 %, respectively (reference range: 4–5.8 %). This method is a cation-exchange HPLC method certified by the National Glycohemoglobin Standardization Program (NGSP) of traceability to the Diabetes Control and Complications Trial Reference (DCCT) method. Mean HbA1c was calculated as an average of three determinations in the previous year before the inclusion in the study.

Plasma 25OHD concentration was measured by the Liaison 25 OH Vitamin D total assay (Diasorin Inc, Stillwater, MN, USA), a competitive chemiluminescent immunoassay, with the Liaison Diasorin automated analyzer. Intra-assay and Inter-assay coefficient variation was <6.3 and 9.1 %, respectively; assay and sensitivity was 4 ng/mL.

Vitamin D status was considered as a categorical variable, with deficiency cutoff defined as a concentration of 25OHD $\leq$20 ng/mL, insufficiency as concentrations between 21 and 29 ng/mL and sufficiency $\geq$30 ng/mL [23]. These cutoffs were established in accordance with previous studies which assessed the elevation of parathormone (PTH) concentrations below the threshold of 20 ng/mL and decreased intestinal absorption of Ca with values of 25OHD less than 32 ng/mL [24]. As 25OHD concentrations are influenced by different factors such as the zenith angle, the hour of day, season and latitude, blood samples were classified in two groups depending on the season in which the sample was obtained: winter (October–March) and summer (April-September). In those collected during winter, 25OHD values were increased 20 % by convention and according to other authors [25], in order to correct the decreased cutaneous synthesis during this period of the year of decreased sunlight exposure.

Serum CRP concentrations were measured using an ultrasensitive CRP test (N High Sensitivity CRP) on a BN-ProSpec nephelometer (Dade Behring, GMBH, Marburg, Germany) with an inter-assay variation coefficient of 3.7 and 3.5 % for CRP concentrations of 2.38 and 52.2 mg/L, respectively.

Evaluation of subclinical atherosclerosis

A computed tomography (CT) to quantify CACS was performed using a 16-slice high-resolution CT ECG-gated, with retrospective reconstruction and with special attention to the coronary arteries (SOMATOM Sensation 16 and Syngo Calcium Scoring software for analysis and calcium calcification). CACS was identified as a dense area in the coronary artery exceeding the threshold of 130 Hounsfield units. A total Agatston score was determined for each patient. The results were expressed according to the classification previously described by Shaw et al. [26], and results were transformed as a categorical variable considering positive those greater than zero and negative those with negative results equal to zero.

A carotid ultrasound (CU) to measure the CIMT was performed in all participants. Ultrasonography images were acquired using high-resolution B-mode ultrasound (Siemens Acuson Sequoia 512) with an electric linear array 13–5 MHz transducer. The CIMT was the result of the median of the tunica intima and tunica media thickness in per protocol-defined carotid areas (left internal carotid, right internal carotid, common carotid and bifurcation). Plaque number and characteristics were recorded.

A single trained radiologist performed evaluation and acquisition of CT images and CU.

Statistical methods

Continuous variables were expressed as mean and standard deviations (SD) or median (interquartile range), and categorical variables as frequency and/or percentage. Differences between groups were tested by the Student's t test or the nonparametric Mann–Whitney U test, as appropriated.

A p value less than 0.05 was considered as statistically significant. Categorical variables were compared with a Chi-squared test. Correlation analyses between continuous variables were performed by using Spearman's correlation. All statistical analyses were performed using the Statistical Package for Social Science (SPSS, Chicago, IL, USA) for personal computers, version 12.0 (SPSS).

Results

The clinical and biochemical characteristics of the whole sample are shown in Table 1.

Vitamin D concentrations

There were no significant differences in the mean 25OHD concentrations in patients compared with controls (20.7 ± 7.7 vs 23.35 ± 8.3 ng/mL, $p = 0.06$). When comparisons were made using the corrected concentration of 25OHD, the differences were statistically significant, with lower values in patients in comparison with controls (22.72 ± 9.11 vs 26.63 ± 9.56 ng/mL, $p = 0.018$). Even with the application of winter correction, we found a high prevalence of 25OHD deficiency in T1D patients, which reached 43.2 versus 21.7 % in controls ($p = 0.032$) (Fig. 1a, b).

When very severe vitamin D deficiency was considered, defined as a 25OHD <15 ng/mL, the prevalence in T1D was 20.5 versus 3 % in controls ($p = 0.04$).

Moreover, considering the whole sample, lower 25OHD concentrations were found in males (22.1 ± 8.3 vs 26.0 ± 10.2 ng/mL, in females; $p = 0.01$) and in smokers (22.3 ± 9.7 vs 25.2 ± 8.9 ng/mL; $p = 0.04$). In the diabetic group, subjects with a longer evolution of diabetes (more than 20 years) had lower 25OHD concentrations (21.18 ± 7.9 vs 24.98 ± 9.5; $p = 0.04$) in relation to those patients with less than 20 years of evolution.

There were no differences in 25OHD concentrations according to BMI. T1D patients with a BMI over 25 kg/m^2 (48 %) displayed similar 25OHD concentrations to those patients with BMI under 25 kg/m^2. Nevertheless, this subgroup of patients with greater BMI showed significantly higher concentrations of hs CRP than lean T1D patients (3.1 ± 5.0 vs 1.3 ± 1.9 mg/L, $p = 0.009$). Moreover, the same between 25OHD and hs CRP was observed in obese (BMI > 30 kg/m^2) T1D patients (10 %).

Table 1 Clinical and biochemical characteristics of the cohort

	T1D	Controls	p value
n (n/%)	145 (75 %)	48 (25 %)	–
Age (years)	37.8 ± 8.0	38.5 ± 7.1	NS
Sex (M/W)	83/62 (57/43 %)	28/20 (58/42 %)	NS
BMI (kg/m2)	25.2 ± 3.5	25.4 ± 4.4	NS
Systolic tension (mmHg)	117.4 ± 13.5	–	–
Diastolic tension (mmHg)	71.0 ± 7.7	–	–
HbA1c (%) (mmol/mol)	7.6 ± 1.4/60.2 ± 11.1	–	–
Diabetes duration (years)	20.7 ± 8.3	–	–
Total cholesterol (mg/dL)	183.5 ± 24.6	191.6 ± 34.3	NS
HDL (mg/dL)	60.6 ± 15.1	61.8 ± 16.5	NS
LDL (mg/dL)	106.1 ± 21.8	112.2 ± 33.5	NS
Triglyceride (mg/dL)	63.5 (52.0–91.5)	64.0 (50.0–91.5)	NS
hs CRP (mg/L)	1.03 (0.7–2.4)	0.96 (0.6–3.1)	NS
Ca2+ (mg/dL)	9.2 ± 0.7	9.4 ± 0.3	$p < 0.01$
PO3− (mg/dL)	3.6 ± 0.8	3.7 ± 0.6	NS
25(OH)D (ng/mL)	20.4 ± 8.2	22.6 ± 7.4	NS
25(OH)D corrected (ng/mL)	22.7 ± 9.1	26.6 ± 9.6	$p < 0.02$
Sun exposure score	18.9 ± 15.9	23.5 ± 14.9	NS
Calcium score >1 (n/%)	24 (16.6 %)	4 (8.3 %)	NS
PTH (pg/mL)	39.2 ± 17.6	37.2 ± 17.9	NS
Smoking habit (n/%)	76 (52.4 %)	19 (39.6 %)	NS
Retinopathy (n/%)	25 (23.1 %)	–	–
Nephropathy	11 (10.2 %)	–	–
Statins treatment (n/%)	21 %	–	–
Antihipertensive therapy (n/%)	15 %	–	–
Salicylate treatment (n/%)	16 %	–	–

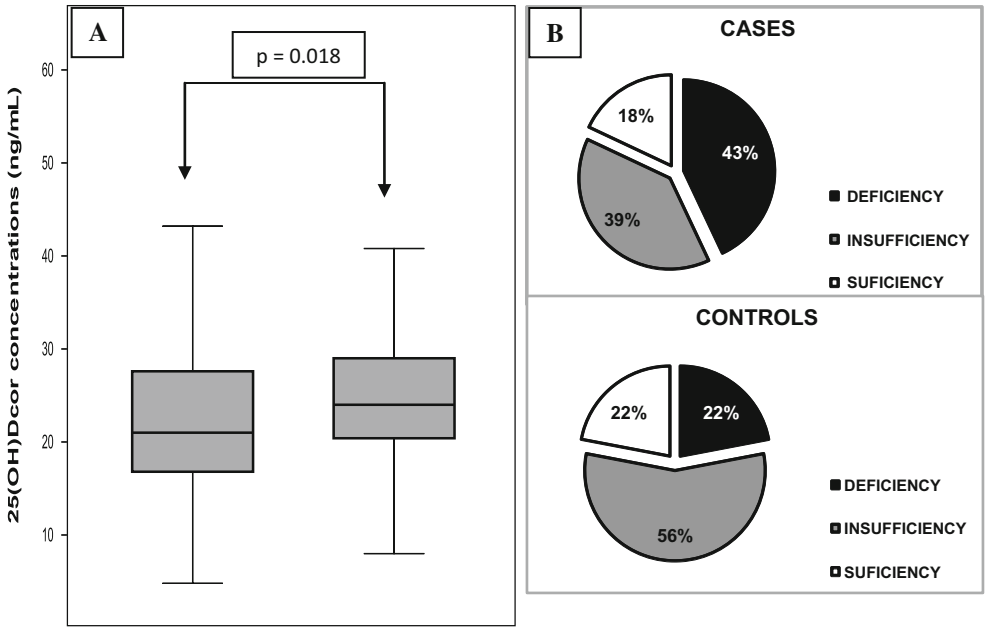

Fig. 1 **a** Difference between mean concentrations of corrected vitamin D in T1D versus controls. **b** Distribution of vitamin D categories in T1D and controls (deficiency, insufficiency and sufficiency)

In the diabetic group, lower concentrations in smokers were not found.

Sun exposure evaluation

As expected, the mean weekly sun exposure score was statistically significant and lower in those participants evaluated in winter than in those evaluated in summer (36.1 ± 12.2 vs 11.3 ± 4.9; $p = 0.0001$). Moreover, there was a correlation between the sun exposure score and 25OHD concentrations (rs = 0.152, $p = 0.023$). The mean weekly sun exposure score was lower in participants with deficient 25OHD concentrations (19.5 ± 13.1 vs 25.4 ± 16.7; $p = 0.01$). This finding was also observed in T1D patients and controls when evaluated separately (11.9 ± 8.5 vs 23.0 ± 17.8 in T1D patients; $p = 0.01$; and 21.2 ± 13.4 vs 26.5 ± 16.2; in controls; $p = 0.047$).

Furthermore, sun exposure score was also related to calcium metabolism parameters including PTH concentrations, so that sun exposure score was lower in participants with PTH ≥ 40 pg/mL (20.3 ± 14.8 vs 27.8 ± 15.4; $p = 0.05$). No differences were detected between the sun exposure score and gender, smoking habit, diabetic condition or years of diabetic evolution.

Subclinical atherosclerosis

Carotid ultrasonography

T1D patients showed a significantly higher CIMT compared with control group (0.55 ± 0.14 vs. 0.48 ± 0.14 mm, $p < 0.01$). There were no differences in the number of plaques between patients and controls. A low proportion of subjects in both groups presented atheroma plaques (16 patients and 4 control subjects, 11 vs. 8 %), in all cases conditioning stenosis of less than 50 %.

Carotid calcification

A high proportion of subjects in both groups, patients and controls, displayed a CACS of 0 (92 vs 82 %), and only 24 T1D patients and four controls presented a calcium score greater than 0. The differences between both groups were not significant (16.6 vs 8 % in controls, $p = 0.236$).

Correlations

When correlation analyses were performed, we did not find association between 25OHD concentrations and any cardiovascular results, neither CACS nor CIMT. In the group of T1D patients, there were no differences between the mean 25OHD of subjects with a CACS of 0 and those with

Table 2 Cardiovascular imaging outcomes in T1D patients according to vitamin D deficiency

	25OHD ≤ 20 (ng/mL)	25OHD > 20 (ng/mL)	*p* value
Calcium score ≥1 UA ($n = 24$)	8 (33.3 %)	16 (66.6 %)	NS
Carotid ecography CIMT (mm)	0.54 ± 0.14	0.55 ± 0.15	NS

a CACS greater than 0 (22.4 ± 9.5 vs 23.9 ± 7.3, $p = 0.97$). When T1D patients were categorized in those with and without 25OHD deficiency, no significant differences were found regarding CIMT between the two groups (0.54 ± 0.14 vs 0.55 ± 0.15, $p = 0.66$).

Table 2 summarizes the relationship between 25OHD and subclinical atherosclerosis imaging markers.

Discussion

In our population of T1D patients from a Mediterranean country, we did not find an association between vitamin D deficiency and subclinical atherosclerosis assessed by CACS and CIMT.

In our cohort of relatively young Caucasian Mediterranean T1D patients with a long disease duration, we found a high prevalence of vitamin D deficiency of about 40 %, doubling the one found for the control group. Moreover, considering even a lower cutoff < 15 ng/mL, we also found a higher percentage of T1D patients with these low concentrations in comparison with control group (20 vs 3 %), a surprising finding considering that our population live in a latitude much closer to the Equator than northern European countries. Other studies have also found a high prevalence of vitamin D deficiency [10, 20], but in most of the reports the prevalence was lower than in our population [9, 13, 18].

Evaluation of sun exposure score did not show differences between T1D patients and controls in the present study. These data do not explain the lower concentrations of 25OHD found in our T1D patients, although a good correlation was found between season of blood sample collection and 25OHD and PTH concentrations.

Compared with data from American non-Hispanic white patients participating in the Coronary Artery Calcification in Type 1 Diabetes (CACTI) study [18] with similar age, latitude and diabetes duration, our patients displayed fourfold higher prevalence of vitamin D deficiency (≤20 ng/mL). In comparison with the population of DCCT/EDIT study [13] (American, almost all Caucasian, younger, mean age of 32.4 ± 2.6 years) with slightly lower disease duration (7.5 ± 2.1 years), our patients presented 1.5-fold more 25OHD deficiency (≤20 ng/mL). In these later studies, 25OHD determination was performed by using high-performance liquid chromatography–tandem mass spectrometry, while in the present study, the determination was performed using a chemiluminescent immunoassay; this fact may have influenced the results, and in fact may explain differences with the aforementioned studies.

There was a low prevalence of subclinical atherosclerosis in our study. Only a 14.5 % of calcium score values were greater than 1 UA which is a low proportion considering other reports [27, 28].

Recently, low levels of 25OHD have been described in severe obese insulin-resistant subjects, with an inverse correlation with inflammatory markers [29]. In our T1D population, there were no differences in 25OHD levels according to BMI, despite that hs CRP levels were higher in overweight or obese. Only 10 % of our patients were obese, and none of them showed a BMI greater than 40 kg/m^2.

We observed greater values of CIMT in T1D patients without overt cardiovascular disease compared with controls, as other studies have described in patients with T1D, T2D and glucose intolerance [30, 31]. Although some authors suggested that impairment in glucose metabolism might have stronger association with early atherosclerosis in women that men [31], no sex-related CIMT differences were detected in our cohort.

Despite that some studies have found an association between vitamin D deficiency and a higher cardiovascular risk in T1D [18, 20], it was not the case in our cohort.In concordance with our findings, among the 1193 T1D patients from DCCT/EDIC [13], neither circulating 25OHD nor its metabolites were associated with changes in CIMT or CACS. In contrast with our study in which we performed simultaneous determinations of 25OHD concentrations and cardiovascular imaging tests, in the DCCT study, the cardiovascular tests were performed a median of 4–10 years after the determination of 25OHD. Despite these methodological differences, this study was unable to show a significant association between vitamin D deficiency and cardiovascular disease.

Young et al. [18] in CACTI study, with a similar population to ours, found a significant association between vitamin D deficiency and the risk of developing CACS.

Potential weaknesses of our study include the lack of data about calcium and vitamin D intake. On the other hand, the present study was initially designed to evaluate the subclinical atherosclerosis in T1D in comparison to controls.

In summary, in our population of Mediterranean T1D patients with quite long disease duration and with a low prevalence of subclinical atherosclerosis, despite a remarkable high prevalence of 25OHD deficiency, there was no association between 25OHD deficiency and subclinical cardiovascular disease.

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Conflict of interest Serra-Planas E, Aguilera E, Granada ML, Soldevila B, Salinas I, Reverter JL, Pizarro E, Pellitero S, Alonso N,

Mauricio D and Puig-Domingo M declare that there is no conflict of interest regarding to this work.

Human and animal rights disclosure All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Informed consent disclosure Informed consent was obtained from all participants for being included in the study.

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