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ORIGINAL RESEARCH

Influence of Homocysteine and Vertebral Fractures on prevalent Abdominal Aortic Calcification in Postmenopausal Women: A multicentric cross-sectional study.

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ABSTRACT

Introduction: osteoporosis and cardiovascular diseases are two major public health problems. Their relationship towards each other was often controversial. In this context, the objective of this study was to examine the influence of homocysteine (Hcy) and asymptomatic osteoporotic vertebral fractures (VFs) using vertebral fracture assessment (VFA) on prevalent abdominal aortic calcification (AAC) in Moroccan postmenopausal women.

Methods: the study cohort consisted of 188 consecutive postmenopausal women with no prior known diagnosis of osteoporosis or taking medication interfering with bone metabolism. Mean age, weight, height, body mass index and plasma homocysteine were determined. Lateral VFA images and scans of the lumbar spine and proximal femur were obtained using a Lunar Prodigy Vision densitometer (GE Healthcare Inc., Waukesha, WI). VFs were defined using a combination of Genant's semi quantitative approach and morphometry. VFA images were also scored for prevalent AAC using a validated 24 point scale.

Results: fifty-eight (30.9%) patients had densitometric osteoporosis. VFs were identified using VFA in 76 (40.4%) patients: 61 women had grade 1 VFs and 15 had grade 2 or 3 VFs. One hundred twenty nine women (68.6%) did not have any detectable AAC, whereas the prevalence of significant atherosclerotic burden defined as AAC score of 5 or higher, was 13.8%. A significant positive correlation between AAC score and homocysteine was observed. Women with extended AAC, were older, had a lower weight, BMI and BMD, higher homocysteine levels and more prevalent VFs than women without extended AAC. Multiple regression analysis showed that the presence of extended AAC was significantly associated with Age and grade 2/3 VFs and not independently associated with homocysteine levels.

Conclusion: this study does not confirm that homocysteine is an important determinant of extended AAC in postmenopausal women. However, this significant atherosclerotic marker is independently associated with VFs regardless of age.

KEY WORDS: Homocysteine, Vertebral Fracture, Abdominal Aortic Calcification, Postmenopausal Women.

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INTRODUCTION

Osteoporosis and cardiovascular diseases are two major public health problems. Both are associated with high morbidity, long-term hospitalization, mortality and loss of independence leading to institutionalization (1, 2). Vertebral fractures (VFs) are the most common osteoporotic fractures which are important to detect because they have been associated with reduced quality of life and increased risk of future vertebral and non-vertebral fractures (3). The costs of these fractures are also high for society (4). Moreover, drugs that are available for treating osteoporosis, such as bisphosphonates or strontium ranelate which should be used with restriction in patients at risk of stroke and ischemic cardiac events according the European Medicines Agency (5), are effective at reducing the risk of further VFs and are recommended for use in this

group of patients. The standard method to assess vertebral fracture is radiography of the thoraco-lumbar spine. However, there is no gold standard for the definition of osteoporotic vertebral fracture (6). A number of methods have been developed for interpretation of spinal X-rays, including the Genant semiquantitative method, which has been used as a surrogate gold standard in a number of key osteoporosis studies (7). This approach is more objective and reproducible than other qualitative methods (8). Vertebral morphometry using dual-energy X-ray absorptiometry (DXA) also known as vertebral fracture assessment (VFA) is a fast, low-radiation technique which produces images that are of sufficient quality to be used to diagnose the presence of vertebral deformity consistent with fracture (8, 9). VFA has demonstrated utility for vertebral visualization and thus is an important tool for fracture detection in women and men (9, 10). It has been shown also in many populations that this technique can simultaneously identify abdominal aortic calcification (AAC) (Fig. 1) and then improve the utility of this technology for this population even further (11).

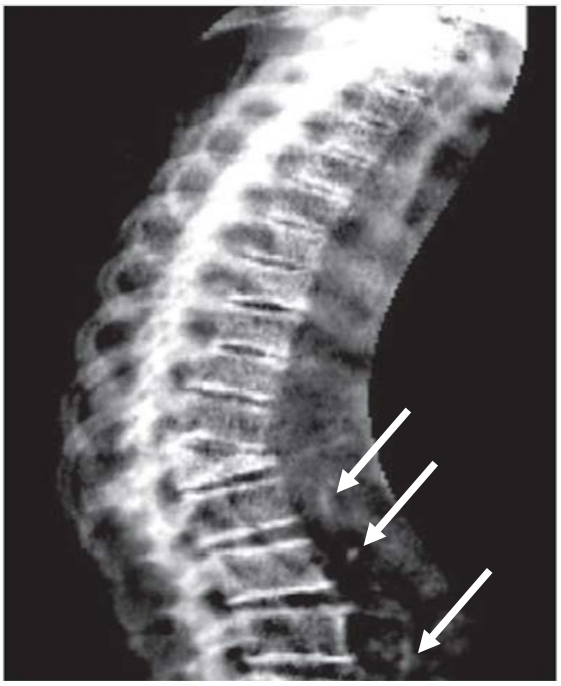

Figure 1: A VFA image showing multiple vertebral fractures (T12 grade 3, T9 and T8 grade 1) and abdominal aortic calcifications (arrows) scored 3.

Although the associations of age and bone mineral density (BMD) with AAC have been well examined (12, 13), whether osteoporotic vertebral fractures (VFs) and AAC are related to each other or are independent, age-related processes remain uncertain (14). Indeed, although bone mineral density (BMD) has been used to define osteoporosis, half of the fragility fractures occur in women with a BMD level more than the World Health Organization (WHO) threshold for osteoporosis (15). Some of the determinants of bone fragility are well known. These include age, body weight, prior fragility fracture, smoking, excess alcohol use, family history of hip fracture, rheumatoid arthritis, and the use of oral glucocorticoids (16, 17). Among the biological indices, biochemical markers of bone turnover, especially those reflecting bone resorption rate, serum levels of osteocalcin, insulin-like growth factor 1, and 17β-estradiol have been found to be associated with the risk of fractures independent of age and BMD (18). Recently, other biological markers to assess bone loss were studied such as homocysteine. It's a sulfur amino acid whose metabolism stands at the intersection of two pathways: remethylation to methionine, which requires folate and vitamin B12 (or betaine in an alternative reaction); and transsulfuration to cystathionine, which requires pyridoxal-5'-phosphate. The two pathways are coordinated by S-adenosylmethionine, which acts as an allosteric inhibitor of the methylenetetrahydrofolate reductase reaction and as an activator of cystathionine beta-synthase (19). Hyperhomocysteinemia, a condition that recent epidemiological studies have shown to be associated with increased risk of vascular disease as well as cognitive impairment, including that seen in Alzheimer disease (20, 21). A potential role of homocysteine in bone fragility has been considered from the observation of a high prevalence of osteoporosis in subjects with homocystinuria (22). A rare autosomal recessive disease and Hcy has recently been described to be an independent risk factor for osteoporosis and fractures in the elderly (23, 24).

In the present study, we examined the influence of homocysteine and asymptomatic osteoporotic vertebral fractures using VFA on prevalent abdominal aortic calcification in Moroccan postmenopausal women.

MATERIALS AND METHODS

Subjects

One hundred eighty-eight consecutive postmenopausal women who had no previous diagnosis of osteoporosis were entered in the study. Women were recruited prospectively through advertisements and "word of mouth" from June 2010 to March 2012. Original inclusion criteria were no previous osteoporotic fracture, 24 months of amenorrhea, and no previous hormone replacement therapy. Women with liver or renal disease, endocrine or metabolic abnormalities, and receiving medicine known to influence bone mineralization or levels of Hcy, such as corticosteroids, heparin, anticonvulsants, vitamin D, and bisphosphonates, were excluded. Our institutional review board approved this study. The procedures of the study were in accordance with the Declaration of Helsinki, and local ethics committee approval was obtained for the study. All the participants gave informed written consent. Each subject completed a standardized questionnaire designed to document the putative risk factors of osteoporosis. Height and weight in light indoor clothes without shoes were measured in our center before DXA. Body mass index (BMI) was calculated by dividing weight in kilograms by height in square meters.

Biological Measurement

Blood sample for plasma homocysteine, was taken from an antecubital vein between 8 and 9 AM, in the fasting state (overnight), placed on ice, centrifuged within 1 h, and the separated plasma was then immediately stored in 2 different tubes at -25°C until assayed. Plasma Hcy was analyzed by commercially available immunonephelometry kits with BN Prospec System Dimension RxL autoanalyzer (Dadee Behring, Liederbach, Germany). The assay had a sensitivity of 2 mmol/L and intra and inter assay CVs of 4.2% and 6.1%, respectively.

BMD Measurement

Bone mineral density was determined by a Lunar Prodigy Vision DXA system (Lunar Corp., Madison, WI). The DXA scans were obtained by standard procedures supplied by the manufacturer for scanning and analysis. All BMD measurements were carried out by 2 experienced

technicians. Daily quality control was carried out by measurement of a Lunar phantom. At the time of the study, phantom measurements showed stable results. The phantom precision expressed as the coefficient of variation was 0.08%. Moreover, reproducibility has been assessed in clinical practice and showed a smallest detectable difference of 0.04 g/cm (spine) and 0.02 (hips) (1, 25, 26). Patient BMD was measured at the lumbar spine (anteroposterior projection at L1–L4) and at the femurs (i.e., femoral neck, trochanter, and total hip). Using the Moroccan female normative data (1), the World Health Organization (WHO) classification system was applied, defining osteoporosis as T-score ≤ -2.5 and osteopenia as $-2.5 <$ T-score ≤ -1. Study participants were categorized by the lowest T-score of the L1–4 lumbar spine, femur neck, or total femur.

Vertebral fracture assessment

VFs was classified using a combination of Genant semi quantitative (SQ) approach (7) and morphometry in the following manner: each VFA image was inspected visually after training sessions by two trained clinicians (IG & AM) to decide whether it contained a fracture in any of the visualized vertebrae and assigned a grade based on Genant SQ scale, where grade 1 (mild) fracture is a reduction in vertebral height of 20–25%, grade 2 (moderate) a reduction of 26–40%, and grade 3 (severe) a reduction of over 40%. In case of doubt regarding fracture grade, the vertebrae in question was measured using built-in morphometry. Automatic vertebral recognition by the software was used. Positioning of the six morphometry points was modified by two experienced investigators (IG & AM) only when the software failed to correctly recognize vertebral heights. The intra-rater reproducibility was evaluated using the kappa score to 0.90 ($p < 0.0001$). Subjects with no fractures were included in the non-fracture group, whereas those with grade 1 or higher fractures were included in the fracture group. However, as many studies rarely report mild deformities as "fractures", and to realize comparisons with the literature, we performed a double analysis including and excluding grade 1 fractures from the fracture group.

Assessment of aortic calcifications

All VFA scans were studied on a separate occasion by the same readers (IG & AM) to assess the presence of prevalent AAC. To score the AAC extension, we used the score described by Kauppila et al. (27). The anterior and posterior aortic walls were divided into four segments, corresponding to the areas in front of the lumbar vertebrae L1–L4. Within each of these 8 segments, aortic calcification was recognized visually as either a diffuse white stippling of the aorta extending out to the anterior and/or posterior aortic walls, or as white linear calcification of the anterior and/or posterior walls. Aortic calcification scored as 0 if there was no calcification, as 1 if one-third or less of the length of the aortic wall in that segment was calcified, as 2 if more than one-third but two-thirds or less of the aortic wall was calcified, or as 3 if more than two-thirds of the aortic wall was calcified. The scores were obtained separately for the anterior and posterior aortic wall, resulting in a range from 0 to 6 for each vertebral level and 0 to 24 for the total score. The reproducibility of the assessment was evaluated in another study in 81 patients (intra-class correlation 0.93; $p < 0.0001$) (28).

Statistical Analysis

Results are presented as means (SD) and categorical variables are expressed as frequencies. Correlation between demographic characteristics, bone mineral density, abdominal aortic calcification score and homocysteine levels were assessed using the non parametric Spearman test. To compare patients with and without AAC, chi-square test and Student's t-test were used. Since a 24-point AAC scale score of ≥ 5 has been shown previously to be associated with a 2.4 fold increased risk of cardiovascular disease mortality (29), this cut-off was used to compare patients with and without extended AAC. To compare patients with and without vertebral fractures, chi-square test and analysis of variance ANOVA were used first. Potential risk factors for extended AAC were finally entered in a stepwise conditional binary logistic regression analysis and the resulting odds ratios with 95% confidence intervals were reported. The level for significance was taken as $p \leq 0.05$. Excel 2007 and SPSS 15.0 were used for statistical analysis.

RESULTS

Patient Demographics

In this cohort of 188 women, the mean ± SD (range) age, weight, and BMI were 57.9 ± 8.5 (50-91) yr, 74.4 ± 13.5 (38-150) kg, and 30.4 ± 5.2 (17.1 ± 50.7) kg/m2, respectively (Table 1).

Among the 188 women, 58 (30.9%) had densitometric osteoporosis (T-score ≤ -2.5 at the lumbar spine, femoral neck, or total hip site). VFs were identified using VFA in 76 patients (40.4%): 61 women had grade 1 VFs and 15 had grade 2 or 3 VFs.

AAC evaluation

Histogram of the AAC score on VFA images showed the AAC score distribution ranged between 2 and 18, whereas 68.6% of the evaluable participants did not have any detectable AAC (Fig. 2). The prevalence of significant atherosclerotic burden defined as a radiographic 24-point AAC score of 5 or higher was 13.8%. Mean AAC score increased with increasing age (Fig. 3).

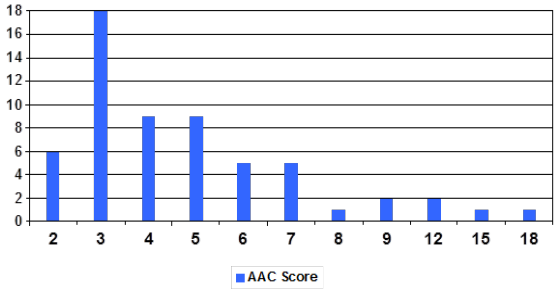

Figure 2: Frequency distributions of the abdominal aortic calcifications (AAC) 24-point score on VFA

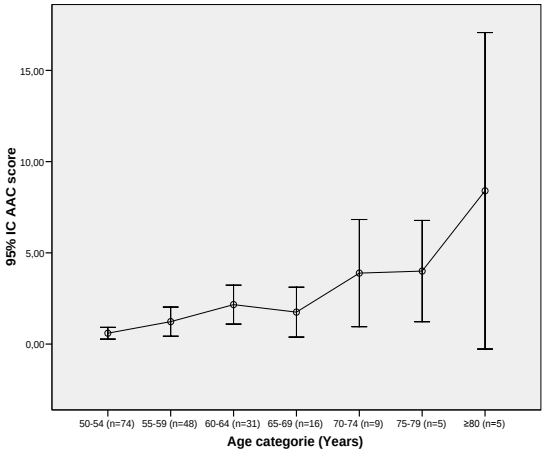

Figure 3: Mean abdominal aortic calcifications (AAC) score in our study population according to age categories.

Characteristics	Mean ± SD	Range
Age (yr)	57.9 ± 8.5	50-91
Weight (kg)	74.4 ± 13.5	38-150
Height (m)	1.56 ± 0.1	1.38-1.85
BMI (kg/m2)	30.4 ± 5.2	17.1-50.7
Years since menopause	5.2 ± 3.5	2-38
BMD lumbar spine (g/cm2)	0.971	0.758-1.726
BMD total hip (g/cm2)	0.918	0.409-1.273
T-score lumbar spine (SD)	-1.5 ± 0.3	-4.6 - 1.7
T-score total hip (SD)	-0.8 ± 0.2	-3.5 - 2.1
Homocysteine (mg/l)	12.4 ± 4.1	2.9-31.5
AAC score (0-24)	1.5 ± 0.9	0-18
Extended aortic calcifications (score ≥ 5): n (%)	26 (13.8)	-
Osteoporosis at any site: n (%)	58 (30.9)	-
Vertebral Fracture: n (%)	76 (40.4)	-

Table 1: Characteristics of the Study Population (N=188)
Abbr: SD, standard deviation; BMI, body mass index; BMD, bone mineral density.

	Age	BMI	LS BMD	TH BMD	AAC	Homocysteine
Age		0.02	-0.40*	-0.39*	0.45*	0.29*
BMI			0.24*	0.32*	-0.06	-0.02
LS BMD				0.66*	-0.24*	-0.18*
TH BMD					-0,27*	-0.23*
AAC						0.21*

Table 2: Correlation between demographic characteristics, bone mineral density, Homocysteine levels and abdominal aortic calcification score
AAC: abdominal aortic calcification, BMD: bone mineral density, BMI: body mass index, LS BMD: lumbar spine BMD, TH BMD: total hip BMD. Correlation was assessed using Spearman test. * Means correlation is significant at the 0.01 level (2-tailed).

Characteristic	Patients without extended AAC (n=159)	Patients with extended AAC (n=29)	*p*
Age (yr)	56.2 (7.1)	67 (9.8)	<0.001
Weight (kg)	75.6 (14)	67.7 (8.2)	<0.01
Height (m)	156.5 (0)	155 (0)	NS
BMI (kg/m2)	30.9 (5.3)	28.2 (3.6)	<0.01
Homocysteine (mg/L)	12.2 (4)	14 (4.3)	0.03
BMD total hip (g/cm2)	0.989 (0.17)	0.874 (0.13)	<0.001
T-score total hip (SD)	-1.4 (1.2)	-2.3 (1)	<0.001
BMD lumbar spine (g/cm2)	0.935 (0.14)	0.827 (0.13)	<0.001
T-score lumbar spine (SD)	-0.7 (1.16)	-1.6 (1.07)	<0.001
Osteoporosis at any site: n (%)	41 (25.8)	12 (58.6)	<0.001
Vertebral fractures grade 2/3: n (%)	9 (5.6)	9 (34.6)	<0.001

Table 3: Comparison between patients with and without abdominal aortic calcification (AAC)
Statistical analysis used chi-square test and Student's t-test.

	OR [95% CI]	p
grade 2/3 VFs	7.41 [1.88-29.21]	0.004
Age	1.16 [1.08-1.23]	<0.001

Table 4: Stepwise regression analysis for the presence of extended AAC
AAC: abdominal aortic calcification, VFs: vertebral fractures. Potential risk factors for AAC were entered in a stepwise conditional binary logistic regression analysis and the resulting odds ratios (OR) with 95% confidence intervals are reported.

Risk factors for Hcy, VFs and AAC

Table 2 showed a significant positive correlation between AAC score and Hcy and age, and a significant negative correlation between AAC score, Hcy and lumbar spine and total hip BMD.

Table 3 showed that, compared to women without AAC, women with AAC were older, had a lower weight, BMI and BMD, higher Hcy levels and prevalence of osteoporosis at any site and more prevalent VFs.

When all the variables significantly associated with high extended AAC prevalence in the univariate analysis were combined in a multiple stepwise conditional logistic regression analysis, it showed that the presence of extended AAC was associated significantly to grade 2/3 VFs (OR [95%CI] = 7.41 [1.88-29.21], p=0.004) and age (OR [95%CI] =1.16 [1.08-1.23], p<0.001) (Table 4).

DISCUSSION

Our study showed that vertebral fracture is indicator of the increased risk for extended aortic calcification regardless age in healthy postmenopausal women with a broad age range. We believe that the young age of our study population and the low number of patients with severe VFs did not reach the statistical power needed to show such an association. In our series, although significantly higher levels of Hcy were observed among patients with extended aortic calcification compared with those without extended aortic calcification, homocysteine is not an important determinant of this atherosclerotic marker. The epidemiology data linking Hcy with AAC is strong (30, 31) but the mediating cellular and molecular mechanisms for this association remain unclear. Experimentally Hcy stimulates a range of potentially pro-atherosclerotic changes, including inflammation and thrombosis and causes apoptosis of endothelial cells (32).

To our knowledge, this is the first study to evaluate the relationship between homocysteine, asymptomatic VFs and prevalent AAC in Postmenopausal Women. Indeed, Most of the previously published studies have assessed separately these parameters. In haemodialysis patients,

Jamal SA et al (33) found that serum homocysteine and aortic calcification were highly correlated (r=0.86) and were not included in the same regression models. ROC curves demonstrated that both serum homocysteine and the presence of lumbar aortic calcification were able to discriminate equally well between subjects with and without fractures.

Our study suggests that the association between severe AAC and VFs implies that DXA exam may provide opportunity to identify women for prevention of cardiovascular events and future fracture. Indeed, our results were agreed with several studies that concluded that low bone density and fragility fractures were strongly associated to aortic calcification in men and women from various populations (34). A longitudinal analysis of bone loss and vascular calcification over a 25-year period in the Framingham Heart Study showed that cortical bone loss measured at the metacarpal was associated with the progression of atherosclerotic aortic calcification in women (35). Another study (12) found that the group of menopausal women with moderate/severe vertebral fractures had a statistically significant higher AAC score and higher proportion of subjects with extended AAC, and lower lumbar spine and total hip BMD and T-scores than those without a VFA identified vertebral fracture. A series of publications (13, 36, 37) but not all (38) showed that low BMD at various skeletal sites is associated with severe AAC after adjustment for age. However, the associations between severe AAC and fracture risk were significant in multivariable models adjusted for age, BMD, prior falls and fractures. Thus, the association between severe AAC and fracture risk is not mediated by low BMD (39) . The mechanism underlying the link between AAC and fracture is not clear. Several common risk factors of severe AAC and fracture risk are possible: age, metabolic syndrome, vitamin D deficit, sex steroid deficit, poor renal function, and low grade systemic inflammation. However, in clinical studies, the link between fracture risk and severe AAC remained significant after adjustment for these factors (40, 41). The investigation into the mechanisms underlying the association between cardiovascular diseases, mainly AAC, and fracture risk are necessary for two reasons. On the one hand, better knowledge of these mechanisms would permit to develop biological markers which could improve identification of individuals who are both at higher risk of cardiovascular event and at high risk of osteoporotic fracture. On the other hand, it would permit to develop new medications which could prevent and/or treat both cardiovascular diseases and osteoporosis. Investigation into AAC is crucial given its central position in the association between these two pathologies (42).Our study has strengths and limitations. All of DXA measurements were conducted with a single bone densitometer and all of biochemical exams were done in a single biochemistry laboratory, with very careful quality controls in place. The assessment of fracture was carefully conducted using standard procedures of acquisition and standard reading of all VFAs. All the morphometric assessments were made by 2 experienced readers after training sessions and a previous global visualization. Before the diagnosis of fracture, a non-osteoporotic origin was considered for each deformity. However, although the subjects were asked about a history of trauma, we cannot exclude that some subjects did not report remote traumas. The main limitations lie in the cross-sectional nature of the study and the procedures used to select subjects, who were all volunteers and ambulatory. Our cohort may not be adequately representative of the whole population. However, because the recruited population is a balanced mixture of the various regions constitutive of the country, we believe that the impact on the prevalence estimate is limited.

Longitudinal studies are needed to explore the relationship between homocysteine, fractures and abdominal aortic calcification. Studies including more patients are also needed to determine whether interventions designed to lower Hcy levels, such as the administration of folic acid, either alone or combined with vitamin B6 or B12, result in a decreased incidence of vertebral fracture and atherosclerosis in postmenopausal women.

CONCLUSION

In summary, this study does not confirm that homocysteine is an important determinant of extended AAC in postmenopausal women. However, this significant atherosclerotic burden is independently associated with VFs regardless of age. VFA imaging with a bone densitometer permits detection of prevalent VFs and AAC, an important cardiovascular disease risk factor. The finding of vertebral fractures in relatively young postmenopausal women should be regarded as a sign for potential development of clinically atherosclerotic disease manifestations. An assessment of the patient's disease risk should be indicated.

ABBREVIATIONS

AAC	Abdominal Aortic Calcification
BMD	Bone Mineral Density
BMI	Body Mass Index
DXA	Dual-Energy X-Ray Absorptiometry
Hcy	Homocysteine
LS BMD	Lumbar Spine Bone Mineral Density
OR	Odds Ratios
SD	Standard Deviation
SQ	Semi Quantitative
TH BMD	Total Hip Bone Mineral Density
VFs	Vertebral Fractures
VFA	Vertebral Fracture Assessment
WHO	World Health Organization

AUTHORS' CONTRIBUTIONS

The participation of each author corresponds to the criteria of authorship and contributorship emphasized in the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly work in Medical Journals of the International Committee of Medical Journal Editors. Indeed, all the authors have actively participated in the redaction, the revision of the manuscript and provided approval for this final revised version.

PATIENTS' CONSENT

Written informed consent was obtained from each patient for publication of this study**.**

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SPONSORSHIP

Declared none.

COMPETING INTERESTS

The authors declare no competing interests.

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