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Low-density granulocytes: A new marker of bone deterioration in patients on peritoneal dialysis

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Summary

Objetive: In kidney patients, bone-metabolic disease, systemic inflammation and malnutrition exacerbate the risk of vascular calcification (VC) and morbidity and mortality. Given the strong association between VC and fragility fractures, the objective of this study is to assess the contribution of the major determinants of VC to bone deterioration in patients on peritoneal dialysis (PD).

Methods: In 31 non-diabetic patients on PD (>6 months), markers of alterations in bone metabolism, vascular damage, inflammation and malnutrition, and their impact on bone deterioration (radiological osteopenia and/or history of fragility fracture) were studied.

Results: In these patients (20 men and 11 women; age=54±15 and 60±11 years, respectively (p=0.24)), the prevalence of fragility fractures was 5% in men and 27% in women. Bone deterioration was greater in older people, females, high Charlson and Kauppila indexes, lower muscle mass and with expansion of a highly inflammatory subpopulation of immature low-density granulocytes (iLDG). A logistic regression analysis showed that bone deterioration risk is more influenced by the female sex than by age and that, of the multiple factors associated with greater bone deterioration studied, only the expansion of iLDG estimates the risk of bone alterations in these patients regardless of age and sex.

Conclusion: The expansion of iLDG provides an accurate biomarker for the diagnosis of bone deterioration and to monitor strategies that attenuate its progression in PD patients of any age and sex.

Key words: Fragility fractures, bone metabolism, vascular calcification, inflammation, cardiovascular risk, malnutrition.

Introduction

The concept of metabolic bone disease associated with chronic kidney disease (CKD-MBD, Chronic Kidney Disease-Mineral and Bone Disorder) to refer to bone abnormalities (osteodystrophy), laboratory (calcium, P, vitamin D, parathyroid hormone –PTH–, fibroblast growth factor 23 –FGF23– and Klotho) and vascular (VC) or soft tissue calcification that occur in kidney patients has been established since 2007 and converge in an increase in cardiovascular risk (CVR), risk of fracture and, in short, an excess of morbidity and mortality[1,2].

Chronic inflammation plays a very important role in the development of vascular disorders in kidney patients and previous studies identified the importance of low-density granulocytes (LDG) in vascular calcification in dialysis patients compared to the control population[3], as well as in CVR of patients with lupus[4].

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The association between vascular calcifications and fragility fractures is established both in the general population and in kidney patients. Severe aortic calcification is associated with a greater number and severity of fractures[5]. This is a worrying fact since cardiovascular disease (CVD) is the primary cause of death in patients with CKD, this being 15 to 30 times higher than that of the general population when adjusted for age[6]. The pathophysiology of the so-called "bone-vessel axis" is complex and several factors are involved, including age, malnutrition and systemic inflammation, a non-traditional CVR factor aggravated in kidney patients due to accelerated aging, with an increase in cells senescent that promotes an inflammatory state that "synchronizes" the deterioration of multiple organs and systems, conditioning the grouping of various degenerative diseases[7].

In patients on peritoneal dialysis and hemodialysis, there are changes in leukocyte subpopulations such as decreases in angiogenic T cells (Tang), protectors of vascular homeostasis, increases in immuno-senescent cells (CD4+CD28null)[8], as well as an aberrant expansion of a subpopulation of immature low-density granulocytes (LDG CD14-, CD16-, CD15+) related to a greater propensity for VC in advanced CKD[3].

The increase in bone fragility observed with age (senile/postmenopausal osteoporosis) develops independently of CKD. Thus, it can be present in patients with CKD, with normal or slightly reduced renal function, and even coexist with CKD-MBD after being established. Added to this is the effect of CKD itself on bone quality and microstructure, previously defined as renal osteodystrophy, together with factors associated with uremia that some authors describe as uremic osteoporosis[9]. In addition, to the usual risk factors for osteoporosis in the general population, disorders in nutritional status, physical activity, the underlying disease itself and the taking of drugs that interfere with bone metabolism are added in CKD[10].

In renal patients, the three factors frequently coexist: malnutrition, inflammatory state and atherosclerosis, known as MIA syndrome, with a great negative impact on survival[11]. The evidence suggests that the decrease in food intake and the increase in acute phase reactants and inflammatory cytokines can be seen from early stages of CKD[12]. The same happens with atherosclerosis[13]. This combination results in marked cardiovascular damage that determines hospital admissions and mortality.

Our study is aimed at evaluating the contribution of changes in some cell populations of the immune system involved in vascular homeostasis and in other CV risk factors, such as malnutrition, to bone deterioration in a cohort of patients undergoing peritoneal dialysis.

Patients, material and methods

Participants

We recruited 31 patients with CKD stage 5 over 18 years of age, undergoing peritoneal dialysis (CKD5-PD) at the Nephrology Clinical Management Unit, Hospital Universitario Central de Asturias (HUCA), Spain) who gave their informed consent. Diabetic patients with a history of cardiovascular events (abdominal aneurysm, intermittent claudication and previous carotid surgery), immunosuppressive treatment, pregnancy, diagnosis of immune-mediated disease, cancer, diabetes mellitus, recent or current infections less than 3 months were excluded. These exclusion criteria limited the ability to recruit patients, but they were chosen with the aim of identifying early inflammatory mediators of vascular damage associated with PD and their possible impact on bone health.

Lab tests

Blood samples were obtained by venipuncture. Automated serum biochemical parameters, lipid analyzes and complete blood counts were performed in the Laboratory of Medicine (HUCA) with routine laboratory methods. Circulating Klotho levels were determined using an ELISA assay (Ref. 27998, human soluble a-Klotho; IBL Immuno-Biological Laboratories Co, JAPAN). For other determinations, serum or plasma samples were stored at -80°C until further analysis. Residual renal function (RRF) defined as mean urea and creatinine clearance ((CCr + CU)/2) in 24-h urine >1 mL/min) was also assessed.

Analysis of cell populations of the immune system in peripheral blood

Peripheral blood samples were immediately processed for peripheral blood mononuclear cells (PBMC) by centrifugation (1900 rpm, 20 min) on Ficoll TM density gradients (Lymphosep, Biowest, Germany) for identification of LDG. The CD4+CD28null population was analyzed directly in peripheral blood samples.

PBMC were treated with FcR blocking reagent (Milteny Biotech, Germany) for 20 minutes at 4°C to prevent nonspecific binding of antibodies to Fc receptors, followed by incubation with CD14 FITC (Immunostep, Spain), CD15 PE-Cy7 (Milteny Biotech), CD16 APC-Cy7 (BioLegend, Germany), CD4 PE (Immunostep) and CD28 APC-Cy7 (Thermo Fischer, Germany) or corresponding isotype control antibodies for 30 min at 4°C. After a wash with PBS. Cell types were analyzed by flow cytometry on a Canto II Flow Cytometer (BD Biosciences) equipped with FACS Diva 6.5 software. The identification of leukocyte cell subpopulations was carried out through the expression of their specific markers. Immature LDGs were detected by their CD14-, CD16-, CD15+ LDG phenotype and their values are expressed as % CD15+. Senescent CD4+ lymphocytes were identified by their CD4+ CD28null phenotype and their values are expressed as % of CD4+, according to the method described by Rodríguez-Carrio et al.[3].

Quantification of circulating cytokines

Circulating levels of IL-10, IL-6, IL-2, TNF-α, and IFN-γ were measured in serum samples using a multiplex assay (BiolegendPlex, BioLegend), following the protocol provided by the manufacturer. Detection limits were 1.2 pg/ml (IL-10 and IL-2) or 2.4 pg/ml (IL-6, TNF-α and IFN-γ).

Bone deterioration

Although only bone densitometry allows accurate quantification of bone mass, patients were stratified considering bone deterioration by virtue of the presence of radiological osteopenia and/or a history of fragility fracture. Plain scans of the dorsal and lumbar spine in lateral projection were evaluated and osteopenia was defined as the decrease in density of the vertebral body of the central trabecular bone and of the horizontal trabeculae, since the vertical ones are affected in more severe osteopenias, with the consequent highlighting of the

cortices. Vertebral fractures were identified following the Genant criteria. Peripheral fractures were confirmed by medical report or review of radiographs. An experienced radiologist carried out the scan readings.

Body composition

Body composition was assessed by electrical bioimpedance (BIA) using Fresenius Medical Care's BCM®, Body Composition Monitor. Measurements were obtained at a frequency of 50 Hz, with the patient in the supine position and after draining the PD fluid. The software returned the data of resistance ®, reactance (Xc), total body water (TBW), intracellular water (ICW), extracellular water (ECW), ECW/TBW, liters (OH), lean tissue mass (LTM Kg), lean tissue index (LTI Kg/m^2), adipose tissue mass (ATM, Kg), fat tissue index (FTI Kg/m^2), body mass index (BMI Kg/m^2) and phase angle (PhA) at 50Hz

PhA is a bioelectrical measure of cellular health that is calculated taking into account the state of hydration and the degree of tissue cellularity; and, it constitutes a marker of sarcopenia, oxidative stress, inflammation and vascular calcification in dialysis patients, and it is the BIA parameter that best predicts survival in CKD[14-19].

Ethical statements

This study has been approved by the Institutional Review Committee (Regional Clinical Research Ethics Committee, reference PI17/02181), in compliance with the Declaration of Helsinki. All participants gave their written informed consent before study inclusion.

Statistic analysis

The descriptive analysis is shown as percentages (%), means (X) and standard deviations (SD). For the analysis of the differences between the clinical and biochemical parameters, and their association with vascular calcification, the statistical tests of T-Student, Chi-square test, multiple logistic regression analysis and non-parametric tests (U-Mann Whitney) when necessary, with a confidence interval (CI) of 95% and considering a value of $p<0.05$ as statistically significant. Statistical analysis was carried out using SPSS 26.0 for Windows.

Results

Our study included 31 patients, 20 men and 11 women, aged 54±15 and 60±11 years, respectively (p=0.24). The prevalence of fragility fractures was 5% in men and 27% in women, all of them with vertebral fractures and, in one of the patients, also with two peripheral fractures.

Table 1 shows the clinical parameters, analytical determinations of bone metabolism related to CKD, as well as the nutritional and inflammatory parameters studied, the etiology of CKD, medical treatments with metabolic-bone repercussions and the differences between patients with or no bone loss. As expected, the patients with bone deterioration were older, with a significant predominance of women [26.32% men and 77.78% women (p=0.01)], with a slightly higher Charlson Index and with a lower LTI. No significant differences were found between patients with or without bone deterioration in the analytical parameters linked to CKD-MBD, including PTH levels, except for soluble Klotho, which was higher in patients with bone deterioration (p=0.03). This lack of linkage may be due to the categorical and inaccurate estimation of bone deterioration and, above all, to the sample size. In addition, until 2017 the relevance of DXA in the systematic evaluation of patients with CKD-MBD was not included and it was not part of the routine of clinical parameters, unlike the radiological evaluation[2].

Vitamin D deficiency/insufficiency is notable in both groups. The mean values of 25-hydroxy-vitamin D in patients supplemented with nutritional vitamin D (38.7%) were 8.4 [6.8, 18.3] ng/ml, compared to 9.3 [5.4, 15] ng/ml in those not supplemented, both levels in the range of vitamin D deficiency (<20 ng/ml). In 48.4% of the patients receiving active vitamin D (calcitriol or paricalcitol), the mean vitamin D values were 9.3 [5.4, 12.4] ng/ml, similar to that of the group of patients supplemented with nutritional vitamin D.

As for quantification of vascular calcifications, the Kauppila index was 6 times higher in patients with bone deterioration (p<0.01).

Regarding nutritional parameters, no significant differences were observed in the levels of total protein, albumin, PhA, phase angle or BMI. Only the LTI was lower in patients with bone deterioration.

Of all the leukocyte cell subpopulations studied, a significant elevation was only observed in the population of immature LDG CD14-, CD16-, CD15+ LDG in the group of patients with bone deterioration and, although there was a trend towards higher values in CD4+ CD28null lymphocytes , did not reach statistical significance.

Regarding the etiology of CKD and the medical treatments they were receiving at the time of the study, no differences were found between the patients associated with bone deterioration.

In the logistic regression analysis analyzing bone deterioration as a dependent variable with respect to age and sex, it was the female sex (OR 10.41; 95% CI: 1.52-119.30; p=0.03) variable with greater weight, while age was at the limit of significance (OR 1.09; 95% CI: 1.01-1.22; p=0.05).

When the independent contribution of each of the identified risk parameters to bone deterioration was analyzed, adjusting for age and sex, only immature LDG (LDG CD14-, CD16-, CD15+) turned out to be independent predictors both in raw data and adjusted for age, by sex and by both together (table 2). It should be noted that no differences in circulating neutrophils were observed between patients with or without bone deterioration. The CD14-, CD16-, CD15+ LDG immature LDG subset also did not correlate with absolute neutrophil counts (p>0.05, data not shown).

It is also important to note that all the changes in the differentially expressed parameters in patients with bone deterioration, such as the increases in the Charlson or Kauppila Index and in the density of immature LDG CD14-, CD16-, CD15+ LDG, as well as the LTI are correlated significantly with age (r=0.68, p=0.00; r=0.48, p=0.01; r=0.36, p=0.04; r=-0.40, p= 0.02) (figure 1).

Discussion

This study shows for the first time the association between the expansion of a subpopulation of immature LDG CD14-, CD16-, CD15+ LDG and bone deterioration in peritoneal dialysis patients. These increases had been previously linked to a greater propensity for vascular calcification.

The number of patients included, although it may seem small, was highly conditioned by the exclusion criteria aimed at selecting patients in whom early markers of in-

Table 1. Clinical, CKD-MBD, nutritional and immunological characteristics in patients with CKD5-DP, discriminated by the presence or absence of bone deterioration

	Yes N=14 (45.2%)	No N=17 (54.8%)	p value
Clinical parameters			
Gender (M/F)	5/9	15/2	**0.02**
Age (years) (mean (SD))	62.6 (9.5)	51.9 (15.3)	**0.02**
Charlson Index (median [IQR])	6 [3, 7]	4 [2, 6]	ns
Time on dialysis (months)(median [IQR])	18 [11, 32]	15 [9, 48]	ns
Residual renal function (Yes/No)	12/2	12/5	ns
CKD-MBD			
Calcium, mmol/L (mean (SD))	2.18 (0.18)	2.18 (0.17)	ns
Phosphorus, mmol/L (mean (SD))	1.69 (0.35)	1.64 (0.42)	ns
Magnesium, mmol/L (median [IQR])	0.81 [0.77, 0.95]	0.87 [0.67, 1.01]	ns
PTH, pg/mL (median [IQR])	386 [230, 453]	300 [214, 600]	ns
Vitamin D 25 OH, ng/mL (median [IQR])	10.1 [5,1, 18,6]	8 [6, 13]	ns
Alkaline phosphatase, U/L (median [IQR])	106 [81, 143]	89 [71, 127]	ns
Klotho, pg/mL (median [IQR])	1.79 [0.82, 2.83]	0.79 [0.64, 1.20]	**0.03**
Kauppila Index (median [IQR])	6 [1, 16]	0 [0, 4]	**<0.01**
Nutritional parameters			
Total protein, mg/dl (mean (SD))	63 (6)	63 (7)	ns
Albumin, mg/dl (mean (SD))	37 (3)	36 (5)	ns
LTI, Kg/m^2 (median [IQR])	13.1 [11.4, 17]	16.3 [14.5, 18.5]	**0.04**
FTI, Kg/m^2 (mean (SD))	14.8 (8.3)	10.1 (6.7)	ns
Phase angle, º (mean (SD))	4.9 (1.1)	5.6 (1.1)	ns
BMI Kg/m^2, (mean (SD))	28.6 (7.1)	27.1 (5.4)	ns
Inflammatory parameters			
CRP, mg/dl (median [IQR])	0.3 [0.1, 1.7]	0.3 [0.1, 0.7]	ns
IFN γ, pg/mL (median [IQR])	7.33 [3.80, 15.50]	7.82 [5.88, 11.19]	ns
TNFa, pg/mL (median [IQR])	8.13 [4.13, 13.07]	8.50 [7.76, 12.48]	ns
IL-2, pg/mL (median [IQR])	1.60 [1.32, 2.30]	1.52 [1.26, 1.91]	ns
IL-6, pg/mL (mean (SD))	11.26 (5.09)	11.31 (9.01)	ns
IL-10, pg/mL (median [IQR])	1.71 [1.36, 2.30]	1.91 [1.36, 4.78]	ns
CD4+CD28null, % CD4+ (mean (SD))	10.88 (4.53)	8.02 (2.97)	ns
Tang, % lymphocytes (mean (SD))	1.65 (0.69)	1.96 (0.71)	ns
Immature LDG[log], % CD15+ (mean (SD))	-0.44 (0.4)	-0.12 (0.4)	**<0.01**
DEFA3 [log] (mean (SD))	0.07 (0.41)	-0.12 (0.67)	ns
Etiology			
Glomerular, %	50%	23.5%	ns
Interstitial nephritis, %	0%	5.9%	ns
Unaffiliated, %	14.29%	35.29%	ns
Others, %	0%	11.76%	ns
Pyelonephritis, %	0%	5.88%	ns
PQHR, %	14.29%	11.76%	ns
Nephroangiosclerosis, %	21.43%	5.88%	ns
Treatments			
Nutritional vitamin D	38%	41.1%	ns
Active vitamin D	50%	47%	ns
Cinacalcet	35.7%	29.4%	ns
Phosphorus binders	64.28%	47%	ns
Corticosteroids	17.6%	18.75%	ns

The variables are expressed as mean±SD, %. The differences were analyzed using the Student's t, Mann-Whitney's U or chi 2 tests according to the normal or non-normal distribution in the analyzed variable. PTH: parathormone; LTI: lean tissue index; FTI: fat tissue index; BMI: body mass index; IFN γ: interferon gamma; TNFa: tumor necrosis factor alpha; IL: interleukins; CD4+ CD28null: senescent cells; Tang: angiogenic T cells; Immature LDG [log]: immature low-density granulocytes: logarithmic transformation; DEFA3[log]: defensin 3: logarithmic transformation; PQHR: hepatorenal polycystosis. Significant values are highlighted in bold.

Table 2. Risk factors for bone deterioration adjusted for age and sex in patients with CKD5-PD

	Raw analysis		Adjusted for age		Adjusted for sex		Adjusted for age and sex	
	Odds ratio [95% IC]	p	Odds ratio [95% IC]	p	Odds ratio [95% IC]	p	Odds ratio 95% IC]	p
Charlson Index	1.4 [0.97-2.01]	0.07	1.13 [0.71-1.82]	0.60	1.52 [0.96-2.41]	0.07	1.22 [0.68-2.21]	0.50
Kauppila score	1.52 [0.96-2.41]	0.07	1.05 [0.93-1.17]	0.44	1.11 [0.98-1.25]	0.10	1.06 [0.93-1.21]	0.39
LTI	1 [0.93-1.07]	0.92	1 [0.93-1.07]	0.94	0.99 [0.91-1.07]	0.78	0.99 [0.91-1.07]	0.78
LDGi [log]	8.57 [2.58-52.39]	**0.00**	10.5 [2.5-92.5]	**0.01**	13.21 [2.71-179.6]	**0.01**	15.6 [2.6-300.2]	**0.02**
CD4+ CD28null	1.27 [0.97-1.68]	0.08	1.14 [0.84-1.54]	0.39	1.31 [0.96-1.79]	0.09	1.18 [0.84-1.66]	0.33
Sex	13.5 [2.15-84.69]	**0.00**	15.63 [2.01-121.47]	**0.01**				
Age	1.07 [1-1.15]	**0.04**			1.09 [0.99-1.19]	0.07		

Logistic regression analysis between each of the risk factors for bone damage without adjustment or adjusted for age, sex or age and sex. CI: confidence interval; p: statistical significance; LTI: lean tissue index; LDG [log]: immature low-density granulocytes CD14-, CD16-, CD15+: logarithmic transformation; CD4+ CD28null: senescent T lymphocytes. Significant values are highlighted in bold.

flammation and their role in VC pathogenesis and bone deterioration could be determined, without interference from other factors processes or in advanced stages.

Regarding the general characteristics of the study population, the distribution by sex is similar to the general distribution of dialysis patients (2/3 men and 1/3 women)[20]. As might be expected, bone deterioration, despite being a qualitative variable, was predominant in females, as was the higher prevalence of fractures.

The risk of fracture increases as chronic kidney disease (CKD) progresses and the type of renal replacement therapy (RRT) influences its behavior. Patients with CKD G3-G5, those on dialysis (G5D) and those who are kidney transplant recipients have a fracture incidence of 2 to 100 times higher than the general population of the same age and sex[2]. Thus, the incidence of fractures triples in hemodialysis (HD) and doubles in peritoneal dialysis (PD) when compared to renal transplantation[21]. In fact, it has been seen that vertebral fractures are prevalent in up to a quarter of patients on HD[22] and a meta-analysis showed that HD increases the risk of hip fracture by 60% when compared to PD[23].

In our study, 13% of PD patients presented fragility fractures, being mostly women (75%). The same influence of sex was seen when assessing osteopenia. By grouping fractures and osteopenia under the term “bone deterioration”, we found that this was three times higher in women, although they represented only a third of the participants. Female sex is known to be a predictive factor for fracture[24]. In the study carried out by Naylor et al. During 3 years of follow-up, it was seen that up to 10% of women and 5% of men with CKD stage 5 had at least one fracture[25].

Along with gender, age was also different among patients with bone deterioration, 10 years more, similar to the DOPPS study data[26]. However, Maravic et al. showed that women on dialysis who present hip fracture are younger than the general population[24]. This fact reinforces the concept that CKD is a conditioning factor for developing early aging that includes the skeleton.

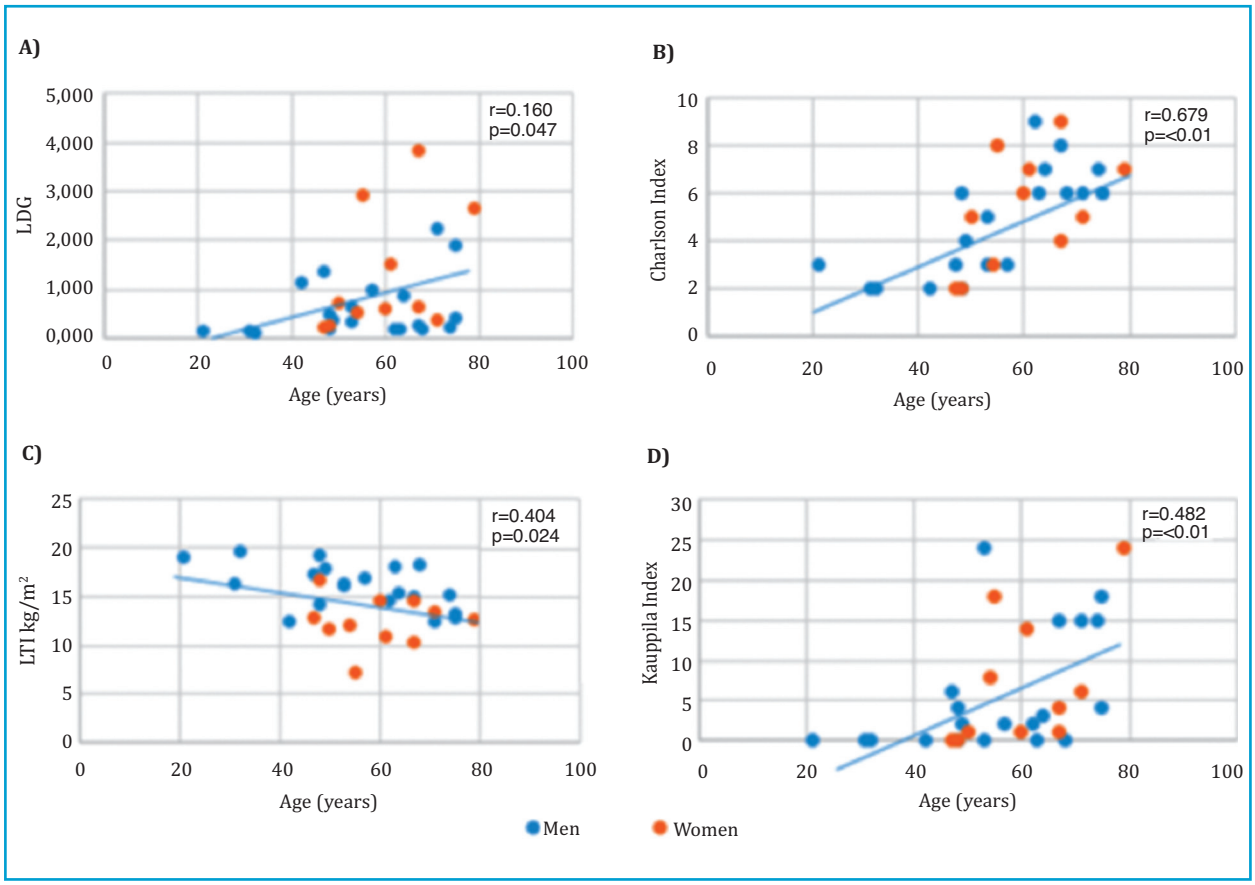
In the general population and in PD, comorbidity has been associated with the risk of fracture[27]. Patients who had bone deterioration presented a higher Charlson Index, these findings being prevalent in men.

On the other hand, the prevalence of malnutrition in PD patients is up to 56%[28]. It has been seen that protein malnutrition predominates in them as a result of low intake, protein loss through dialysis fluid and increased protein catabolism,which in turn leads to adynamic bone disease[29].

In dialysis units, BIA use is becoming more frequent to assess our patients’ nutritional status. We are mainly interested in lean mass, since the association between sarcopenia and osteoporosis, called "sarco-osteoporosis"[30], is known. The risk of fractures is greater due to the risk of falls that conditions the weakness induced by sarcopenia[31,32]. Our study concurs with research showing that the lower the lean tissue index, the greater bone deterioration, primarily in men. Verschueren et al. studied 679 men, among whom 11.9% had sarcopenia and a 3-fold higher risk of developing osteoporosis[33].Therefore, it would be worthwhile to encourage the more widespread use of BIA measurements in nephrology consultations from early stages of CKD.

In our study, the elevated Kauppila Index in patients with bone deterioration is consistent with the concept that bone demineralization is associated with vascular mineralization. However, the Kauppila Index did not have an independent contribution of age and sex as a possible determining factor of bone deterioration. There was also no evidence of an association between bone deterioration and markers of bone metabolic activity, in line with other studies where only PTH was weakly associated with the risk of fractures in dialysis patients[27,34]. Interestingly, soluble Klotho was higher in those with bone deterioration. Although the precise effect of Klotho on bone is unknown, it is known that deletion of the Klotho gene exclusively in osteocytes increases, rather than decreases, the rate of bone formation[35,36]. The impact of local actions of soluble Klotho is an area of great interest in nephrology, but still with many limitations due to the variability among commercial assays available to quantify circulating levels of Klotho. Given the anti-inflammatory and anti-aging actions of circulating Klotho, it would be important to confirm whether changes in blood levels have a favorable influence on attenuating leukocyte actions that affect the propensity for bone deterioration and CV. At this time, the favorable impact of increases in soluble Klotho on immune, renal, and vascular protection has been demonstrated only experimentally.

Figure 1. Association of age and sex with risk factors for bone deterioration in patients with CKD5-PD



Dispersion graphic. The correlation of age and sex with A) LDG: immature low-density granulocytes, B) Charlson Index, C) LTI, lean tissue index, and, D) Kauppila Index is shown. Each circle represents a study subject.

The clearly deficient vitamin D status and similar levels of 25-hydroxy-vitamin D among the patients who received or not supplementation, with respect to those treated with active metabolites, reflect the scant concern of nephrologists regarding the importance of correcting vitamin D deficiency in kidney patients, correction impossible to achieve with the administration of active vitamin D. The levels of 25-hydroxy-vitamin D in patients receiving these treatments rule out a possible induction of the degradation of nutritional vitamin D due to excessive doses of the active form[37] (table 1).

Of the most commonly used immune cell subpopulations to assess vascular damage, only exacerbated expansion of immature LDG CD14-, CD16-, CD15+ LDG was associated with bone deterioration. In the context of systemic inflammation, it has been shown that immature CD14-, CD16-, CD15+ LDGs are released early from the bone marrow through transcortical vessels when there are increases in osteoclast-mediated bone resorption[38]. Although CD14-, CD16-, CD15+ immature LDGs are a minor fraction of neutrophils, in a study of 2586 men, individuals with lower bone mineral density (BMD) had higher neutrophil counts,suggesting an inverse association between bone health and rate of granulopoiesis[39]. In fact, Terraciano et al found that postmenopausal women with low BMD had high concentrations in saliva of a type of defensin released by neutrophils called DEFA1[40].

The contribution of increases in circulating immature LDG CD14-, CD16-, CD15+ LDG to the degree of vascular calcification in patients on peritoneal dialysis and hemodialysis, also demonstrated the usefulness of measurements of the levels of messenger RNA of defensin 3 (DEFA3) in leukocytes circulating mononuclear cells, as an accurate marker of early granulopoiesis with expansion of immature LDGs LDG CD14-, CD16-, CD15+3. However, in the latter, defensin 3 messenger RNA levels are not associated with greater bone deterioration.

Limitations

Our study's greatest limitation is that bone deterioration was not evaluated with the gold standard, DXA, but by the presence of radiological osteopenia and/or fragility fractures. Thus, the result was a categorical variable, with less capacity to show a gradation of effects.

In addition, the small sample size due to the exclusion criteria, although it is appropriate to determine early effects and not interference due to other processes that alter immunity, may limit the real quantification of the influence of other variables.

Conclusions

The most important contribution of this study is the demonstration of the usefulness of measurements of the circulating density of immature LDG CD14-, CD16-, CD15+ in estimating bone deterioration regardless of the patient's age and sex. This makes circulating immature LDG CD14-, CD16-, CD15+ LDG an early marker of bone disorders in kidney patients that could allow a proactive attitude to be taken both in diagnosis and in decision-making to attenuate its progression.

Our study is in sync with current knowledge that bone deterioration is greater in elderly people, females, with a high Charlson Index, and in those with less muscle mass. On the other hand, we realize that it may open new avenues to deepen research into the pathogenic mechanisms of CKD-MBD in earlier stages of CKD, with a larger sample of patients and using DXA for an adequate validation of these findings.

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