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Version of Record: https://www.sciencedirect.com/science/article/pii/S1297319X20302244
Manuscript_d6855979220834bb108594f8dfb75888

Tocilizumab controls bone turnover in early polymyalgia rheumatica

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Abstract

Objectives: This study explores changes in the bone homeostasis by testing the N-terminal collagen type I extension propeptide (PINP) marker for osteo-formation and the carboxy-terminal region of collagen type I (CTX-I) marker for osteo-resorption in patients taking tocilizumab for polymyalgia rheumatica (PMR).

Methods: Twenty patients were included in the prospective open-label TENOR study (Clinicaltrials.gov NCT01713842) and received three monthly tocilizumab infusions, followed by corticosteroids starting at week (W)12. PINP and CTX-I were tested at inclusion (W0), after tocilizumab but before steroid initiation (W12), at the end of the protocol (W24) and were compared to healthy controls. Information regarding disease activity, bone mineral density using scanographic bone attenuation correlation (SBAC), inflammatory parameters and interleukin (IL)-6 levels were collected during the follow-up of the patients.

Results: PMR patients were characterized by a reduction in bone mineral density and a higher levels of CTX-I relative to healthy controls matched in age and sex at baseline. PINP levels increased at W12 (p=0.0008, *versus* W0) following tocilizumab introduction and CTX-I levels decreased at W24 and after steroid initiation (p=0.001, *versus* W0). Such modifications explain the altered correlation observed between PINP and CTX-I at W0 (r=0.255 at W0 *versus* r=0.641 in healthy controls) and its correction after treatment (r=0.760 at W12 and r=0.767 at W24). Finally, greater changes in PINP were observed in patients whose circulating IL-6 levels decreased after tocilizumab therapy.

Conclusions: Control of bone turnover, in part through the inhibition of the IL-6 axis, is observed during tocilizumab and subsequent steroid treatment of PMR.

Key words: polymyalgia rheumatica, tocilizumab, PINP, CTX-I, IL-6, scanographic bone attenuation coefficient

1. Introduction

Polymyalgia rheumatica (PMR) is an inflammatory disorder affecting people after age 50. Cardinal symptoms are inflammatory pain of shoulder and hip girdles with pronounced stiffness lasting at least one hour. Acute phase reactants such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are usually elevated at disease onset, and inflammation has a direct impact in bone metabolism [1]. Blocking the interleukin (IL)-6 axis with tocilizumab has been demonstrated by our group and others to be effective in PMR [2–6], however its impact on bone turnover remains to be established since IL-6 possesses a dual role in bone remodeling and resorption. On one hand, IL-6 is involved in bone homeostasis via osteoclast formation [7], and, on the other hand, IL-6 is implicated in osteoclast differentiation by inducing the receptor activator of nuclear factor kappa-B ligand (RANK-L) [8]. Osteoporosis is a major adverse event induces by corticosteroids in PMR patients. Corticosteroids mainly affect bone formation. In healthy volunteers, 5 mg per days of prednisone decrease the N-terminal collagen type I extension propeptide (PINP) and osteocalcin, both markers of bone formation [9]. Corticosteroids decrease the number and the function of both osteoblasts and osteocytes. Moreover, corticosteroids decrease the muscle mass and the level of sex steroid hormones worsening the osteoporosis. There is a need for new effective treatment with less induced co morbidities.

Besides bone mineral density performed routinely by DXA, to screen patients at risk of bone fragility, the use of circulating bone markers provides an optimal monitoring to evaluate bone formation and bone resorption on a short period of time. Among these markers, bone osteo-formation can be evaluated by measuring PINP that is cleaved from the amino terminal part of type I collagen during bone formation [10]. For bone resorption, the carboxy-terminal region of collagen type I (CTX-I) is used since it is a result of the action of cathepsin

K on collagen. CTX-I exhibits important circadian rhythms, and as a consequence it's important to respect strict preanalytical conditions [11]. Several factors are also known to influence PINP and CTX-1 levels as reported by the National Bone Health Alliance [12]. Moreover, dual X-ray absorptiometry is not always available to evaluate bone mineral density and this can be appreciated using a scanographic bone attenuation coefficient as recently validated in several studies [13,14].

Accordingly, our objective was to describe the evolution of the two bone homeostasis markers PINP and CTX-I in patients with early PMR, treated with tocilizumab, an anti-IL-6 receptor monoclonal antibody, and then with steroids.

2. Method

2.1 Sample collection

Twenty untreated early PMR patients (median age 67 years old, range 55-79 years old), 13 males and 7 females, participating in a proof of concept study: the TENOR study (registered on Clinicaltrials.gov NCT01713842) at Brest and Nantes University Hospital, France, were included in this study. Inclusion and exclusion criteria have been described previously [2]. Tocilizumab was given as three 8 mg/kg intravenous infusions in monotherapy, at baseline and then at week (W) 4 and at W8. From W12 to W24, patients received low-dose prednisone (0.15 mg/kg/day with predefined tapering). Disease activity was measured by determining the PMR activity score (PMR-AS) [15,16]. Blood samples were collected at inclusion and before 1st tocilizumab infusion (W0), at W12 (under tocilizumab infusions only) and at W24 (under steroids). Samples for the TENOR study were collected in a referenced biocollection (Eudra-CT 2011-002730-39) and matched controls (median age 66 years old, range 55-79 years old;

63 males and 34 females) were obtained from a healthy Caucasian cohort (CHU Liege, Belgium) with normal calcium, phosphates, intact PTH (parathyroid hormone), and an estimated glomerular filtration rate >60 ml/min/1.73m^2. Blood samples were collected from overnight fasting. Plasma was then extracted and frozen at -80°C. Informed consent was obtained from all participants in accordance with the ethical committees and the study complied with the World Medical Association Declaration of Helsinki.

2.2 Automated assay and IL-6 measurement

The IDS-iSYS Automated Analyser (Immunodiagnostic Systems Limited, Pouilly-en-Auxois, France) was used to measure PINP, and CTX-I levels at each visit. IL-6 was quantified in 18/20 patients using the Human IL-6 ELISA Kit II (BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions. For IL-6, the detection limit threshold was 2.2 pg/mL.

2.3 Scanographic bone attenuation coefficient assessment

Patients included in Brest, in the TENOR study (17/20) underwent ^{18}F-fluorodesoxyglucose Positron Emission Tomography coupled to Computed Tomography (^{18}FDG-PET-CT) at inclusion and week 12 as it is performed in PMR protocols [17]. Scanographic bone attenuation coefficient (SBAC) was assessed as described by Pickhard et al. SBAC was measured as Hounsfield unit (HU) on a single oval region of interest placed on an axial slice of the first lumbar vertebra [Appendix A, Figure S1; See the supplementary material associated with this article online]. A 145 HU threshold was used to define patient with low bone mineralization and at higher risk of fracture [13].

2.4 Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM), analyzed by using a non-parametric ANOVA test (Friedman's test) and a post-hoc Dunn's test was used for multiple comparisons. Correlations between continuous variables were calculated using the Spearman's rho test. P values under 0.05 were considered significant. Statistical analyses were performed using GraphPad Prism 7.0a (La Jolla, CA).

3. Results

3.1 Bone turnover markers at baseline in PMR

Characteristics of the 20 patients with PMR included in the study were previously described [2,18] and they were matched in age and sex with 97 healthy controls (Table 1, Appendix A - Table S1). None of the patients was treated with anti-osteoporotic drug during the 24 weeks of the study. Data about alcohol intake, smoking, calcium intake, familial history of fracture, early menopause and amenorrhea was not available. Among the 17 patients with ^{18}FDG-PET-CT at inclusion, mean SABC was 118±10HU and among them 6/17 (35.3%) presented a high risk of fracture with an SABC ≥145 (Appendix A, Table S2). Only one patient had one vertebral fracture at inclusion and no fracture occurs during follow-up. The bone formation marker PINP and the bone resorption marker CTX-I were assayed. As reported in Figure 1A/B, an elevated level of CTX-I characterizes PMR patients at baseline (p=0.006), while PINP levels were unaffected. In addition, an altered balance between osteo-formation and osteo-resorption compared to healthy controls was reported at baseline in PMR due to the lack of correlation between PINP and CTX-I as observed in healthy controls (Fig 1C/D). Bone biomarkers (resorption and formation) are well correlated in healthy controls and fairly in

PMR reflecting more an altered balance between osteo-formation and osteo-resorption in this inflammatory disease.

3.2 Tocilizumab and bone turnover markers

To investigate whether tocilizumab as a first step and steroids as a second step, influence bone turnover markers, differences in PINP and CTX-I levels were tested at the time of the 1st treatment initiation (W0), after the 3rd tocilizumab infusion and before steroids were introduced (W12), and at the end of the protocol (W24). Higher levels of PINP were observed at W12 with a substantial decrease at W24 (p=0.0008 at W12 versus W0 and $p<10^{-4}$ at W24 versus W12; Fig 2A). When considering CTX-I, differences were reported at the end of the protocol (p=0.001; Fig 2B). Compared to W0, PINP and CTX-I at W12 were strongly correlated reflecting a more normal bone homeostasis obtained under tocilizumab (r=0.760, $p<10^{-4}$; Fig 2C) and this correlation was still preserved at W24 (r=0.760, $p<10^{-4}$; Fig 2D). Tocilizumab did not modify SBAC at week 12 (Appendix A, Table S2). But interestingly, bone formation markers (PINP) correlated with SBAC at inclusion and did not at week 12 whereas bone resorption (CTX-I) did not correlate with SBAC at inclusion and did at week 12 (Appendix A, Figure S2).

3.3 IL-6 responders

In response to IL-6 receptor blockade, IL-6 circulating levels significantly decreased at W12 in a subgroup of 9/18 (50%) PMR patients, which were referred as IL-6 responders (Fig 3A). Although not significant, there is a trend to have higher levels of CRP (whose production is stimulated by IL-6, Table 2) and CTX-I (Fig 3C) at basal level in the IL-6 responder subgroup. The role of IL-6 on bone homeostasis was further supported by the observation that

IL-6 responders have elevated levels of PINP at W12 (Fig 3B) following tocilizumab treatment. In contrast, PMR-AS and ESR were not associated with IL-6 response. Altogether, this supports that controlling the IL-6 pathway in PMR with tocilizumab firstly improved bone formation and secondly reduces bone-resorption when patients were treated by steroids.

4. Discussion

We demonstrated that tocilizumab therapy and subsequent steroid introduction influences and restores the bone homeostasis markers PINP and CTX-I in patients with early PMR. During the first weeks of therapy and in response to tocilizumab introduction, disease activity is corrected and an increase in the bone osteo-formation marker PINP is observed. Differences in kinetics are also described as PINP decreased after tocilizumab replacement with steroids, while CTX-I reduction was delayed at the end of the protocol.

PINP is commonly recognized as reflective of bone formation [19]. The tight control of bone turnover, and its restoration as observed in this study, is essential for bone health and to prevent osteoporosis that is exacerbated in PMR [20]. The uncoupling of bone formation and bone resorption in bone turnover has been associated with osteoporosis and with bone events such as fractures [21–23]. Reference values have been defined for serum intact PINP [24] but no threshold has been defined to determine an effective or ineffective bone turnover rate. In studies evaluating the effect of teriparatide – an analog of human parathormone – on bone formation markers an increase in PINP > 10 ng/ml is observed while not in patients treated with placebo [22]. In our study the PINP serum level increased from 39.5 ng/ml at W0 to 70.8 ng/ml at W12 suggesting an effect comparable to a 3-month treatment with teriparatide. After 12 weeks of tocilizumab therapy, patients were treated with corticosteroids. It is of great interest to note that corticosteroid therapy is associated with a decrease in PINP levels and a trend for decrease in CTX-I levels thus supporting a synergic action between tocilizumab and steroids in the control of the bone balance.

In PMR, data are scarce with regards to bone formation and resorption but a decrease in bone formation markers and an increase in bone resorption markers have been described in

early untreated PMR [25]. On one hand, bone formation was evaluated by the PINP serum level and was decreased compared to healthy controls. Such a difference was not observed in our study using age and sex matched healthy controls, which may be explained in part by differences in the selection of the control groups. On the other hand, bone resorption markers used were urinary free pyridinoline and deoxypyridinoline and an increase in these markers was suggested to be induced by inflammation as reported by another group [1]. In our study the serum level of CTX-I was higher at baseline, when compared to healthy controls and subsequently the CTX-I levels were further decreased at the end of the treatment. Although, CTX-I changes were evaluated early at W12 and W24 in our study and bone markers evaluated after 6 months of prolonged corticosteroid therapy in Dolan's study, both studies have concluded that there is an effect of the therapy on bone resorption markers. The data available in rheumatoid arthritis (RA) patients are heterogeneous and with some parallels with regards to our report in PMR. Indeed in RA, the use of tocilizumab was effective in decreasing the CTX-I serum level in several studies [26,27], PINP serum levels were either unmodified [27] or increased with tocilizumab therapy [26–28]. Baseline serum levels of CTX-I and PINP were concordant between studies and correspond to the concentrations measured in ours (0.3-0.4 ng/ml and 35-45 ng/ml respectively). The decrease of CTX-I in the studies performed in RA and not in our study could be explained by differences between patient selection and, in our study, we have selected an untreated and early PMR population. Indeed, it has been demonstrated that bone markers may fluctuate with disease evolution, treatment and activity [29]. Bone mineral density in RA was evaluated in a one-year prospective open study and was not modified by tocilizumab or corticosteroids therapy [28]. Authors concluded that, in RA, inflammation is a more important determinant for bone mineral density than treatment, even high dose of corticosteroids. Such a relation remains to be demonstrated in PMR. Moreover, in RA patients, an association between the CTX-

I/osteocalcin ratio and response to tocilizumab has been suggested [30]. We already reported an IL-6 serum responsive group in the TENOR study's patients [18], and now we provide new arguments supporting for this subgroup that IL-6 and inflammation might block bone formation and increase bone resorption during PMR and that the IL-6 receptor targeted therapy releases this blockade as suggested in RA.

Dual X-rays absorptiometry was not available in the TENOR study. Nevertheless, patients underwent ^{18}FDG-PET-CT. We were able to analyze bone mineral density using the scanographic bone attenuation coefficient (SBAC) as previously performed on conventional thoraco-abdomino-pelvic CT-scans. The SBAC values confirmed that a large majority of patients included in this pilot study were under the threshold of bone fragility (64.7 %). But among the 35.3% remaining, bone markers also suggest bone fragility and reinforce the idea to screen patients for osteoporosis and to treat them with more targeted therapy inhibiting the IL-6 axis. Thus, more options are mandatory for PMR treatment and we provide elements sustaining the use of tocilizumab in PMR.

The main limitation of our prospective study is related to the small statistical power given to the low population size.

We demonstrated that tocilizumab therapy has a positive impact on bone homeostasis increasing bone turnover via the activation of bone formation and bone resorption blockade. The underlying mechanisms regulating bone homeostasis and the advantages of tocilizumab in comparison to corticosteroid therapy are not clearly established yet. More data are needed to evaluate the consequences of tocilizumab therapy on bone mineral density and on bone events in particular on the incidence of fracture.

List of abbreviation:

CRP: C-reactive protein

CTX-I: carboxy-terminal region of collagen type I

ECR: erythrocyte sedimentation rate

HU: Hounsfield unit

IL: interleukin

PINP: N-terminal collagen type I extension propeptide

PMR: polymyalgia rheumatica

PMR-AS: polymyalgia rheumatica activity score

RA: rheumatoid arthritis

RANK-L: receptor activator of nuclear factor kappa-B ligand

SBAC: Scanographic bone attenuation coefficient

SEM: standard error of the mean

W: week

Declarations:

Ethics Approval and Consent to Participate

The TENOR was approved by an ethic committee "Comité de Protection des Personnes Ouest 6" on 2011 September, 8th. N° EudraCT 2011-002730-39, Trial code RB11.075. All patients gave an informed and written consent to participate and for biocollection for research pruposes.

Consent for publication

Not applicable

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interest: Roche-Chugai provided an unconditional grant for the Tolerance and Efficacy of tocilizumab iN pOlymyalgia Rheumatica (TENOR) study and for the ancillary biological analyses presented here. Tocilizumab was donated free of charge by Roche-Chugai. Roche-Chugai had no role in the design or conduct of the study; collection, management, analysis or interpretation of the data; or preparation, revision or approval of the manuscript. All authors have declared no conflict of interest.

Authors contribution: GCA, VDP and YR designed the study, analyzed the data, and prepared the initial draft. EB performed the experiments. GCA, AS, DC and VDP performed the clinical data gathering. The final manuscript was read and approved by all authors.

Acknowledgements: The authors thank the rheumatologists and general practitioners who referred their patients to the TENOR study. They are grateful to the Clinical Investigation Center at Brest University Medical School for centralizing the material. We are thankful to Dr. Wesley H. Brooks (University of South Florida, USA) for editorial assistance, to Valérie

Pedron (IDS, France) for technical assistance, and to Genevieve Michel and Simone Forest for their help typing of the paper.

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Table 1. Bone turnover markers in 97 healthy controls and 20 patients with polymyalgia rheumatic (PMR) treated with tocilizumab (Week (W)=0, W4 and W8), and from W12 to W24 patients received low-dose prednisone with tapering. Results are expressed as mean +/- SEM.

	Healthy controls	**PMR W0**	**PMR W12**	**PMR W24**
Age [range]	68 [56-79]	67 [56-79]	-	-
Sex (F:M)	34:63	7:13	-	-
PINP (ng/mL)	40.7±3.2	39.5±2.6	70.8±5.9***	37.5±3.9
CTX-I (ng/mL)	0.30±0.02	0.45±0.05*	0.46±0.07	0.34±0.05

Abbreviations: F: female; M: male; PINP: N-terminal collagen type I extension propeptide; CTX-I: carboxy-terminal region of collagen type I. Versus controls: ***$p<10^{-4}$ and *$p<0.05$

Table 2. Characteristics of the IL-6 responders within the patients with polymyalgia rheumatic (PMR) treated with tocilizumab.

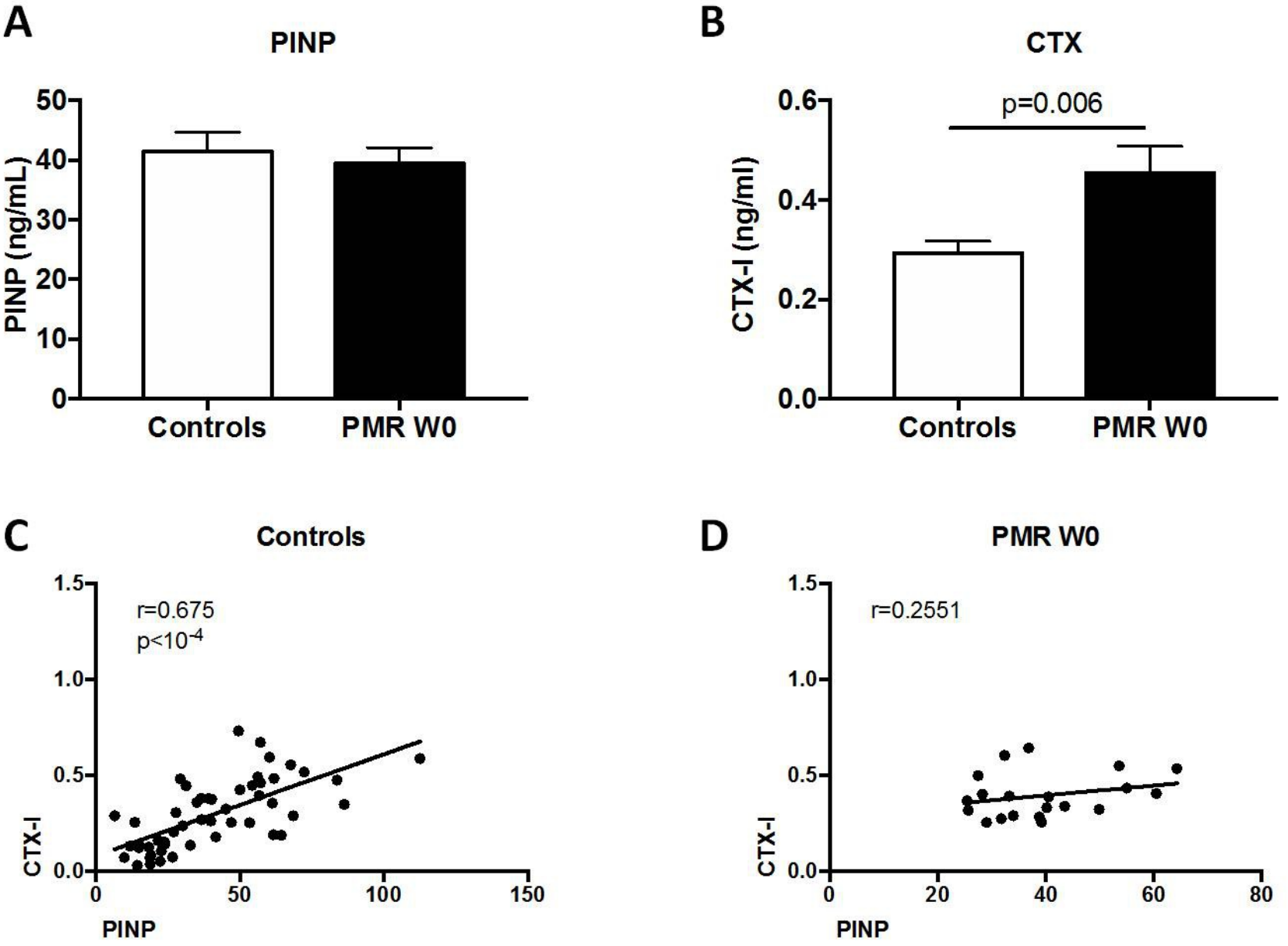
	IL-6 responders (n=9)			IL-6 non-responders (n=9)		
	W0	**W12**	**W24**	**W0**	**W12**	**W24**
PMR-AS	42±2	1.3±0.4	1.1±0.5	34±3	3.9±1.5	1.6±0.5
CRP (mg/L)	115±23	4.9±2.6	3.3±1.3	49±18	5.9±2.8	5.3±2.3
ESR (mm/h)	69±11	3.8±1.9	6.2±1.4	48±8	3.3±0.7	11.9±2.8
SBAC (HU)	113±13	109±10	-	116±17	120±17	-

Abbreviations: PMR-AS: PMR activity score; CRP: C reactive protein; ESR: erythrocyte sedimentation rate; SBAC: scanographic bone attenuation coefficient; HU: Hounsfield unit

Fig. 1 **Serum markers of bone-formation and bone-resorption.** A: PINP (N-terminal collagen type I extension propeptide) levels in 20 untreated patients with polymyalgia rheumatic (PMR) at week (W) 0 and in 97 healthy controls. **B:** CTX-I (C-terminal cross-linking telopeptide of type I collagen) levels. **C/D:** Correlation between PINP and CTX-I levels at basal level in healthy controls and PMR at W0. For Spearman's correlation rho (r) is indicated as well as *p* when significant ($p<0.05$).

Fig. 2 **Analysis of bone turnover markers in patients with polymyalgia rheumatic (PMR) treated with tocilizumab, followed by oral prednisolone from week (W)12 to W24. A:** PINP (N-terminal collagen type I extension propeptide) as bone-formation marker. **B:** CTX-I (C-terminal cross-linking telopeptide of type I collagen) as bone-resorption marker. **C/D:** Correlation between PINP and CTX-I levels at W12 and W24. For Spearman's correlation rho (r) is indicated as well as *p* when significant ($p<0.05$).

Fig. 3 **IL-6 response and bone turnover markers. A:** Serum IL-6 levels in patients at baseline (W0) and at W12 following tocilizumab introduction defines responders (R) from non-responders (NR). **B:** PINP (N-terminal collagen type I extension propeptide) levels at W0, W12 and W24 according to the IL-6 response subgroup. **C:** CTX-I (C-terminal cross-linking telopeptide of type I collagen) levels. For statistics p is indicated when significant ($p<0.05$).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PINP
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0.4
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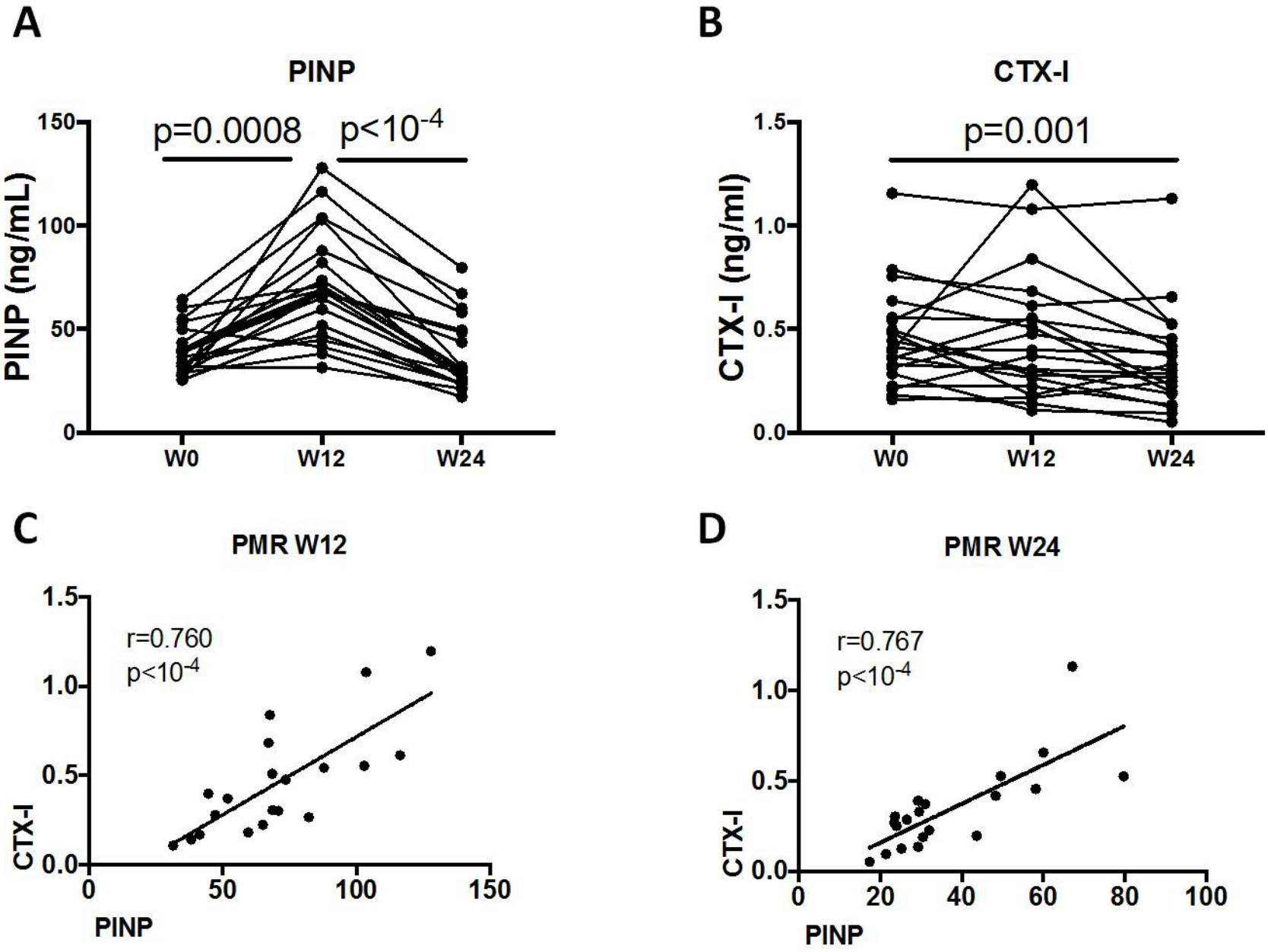
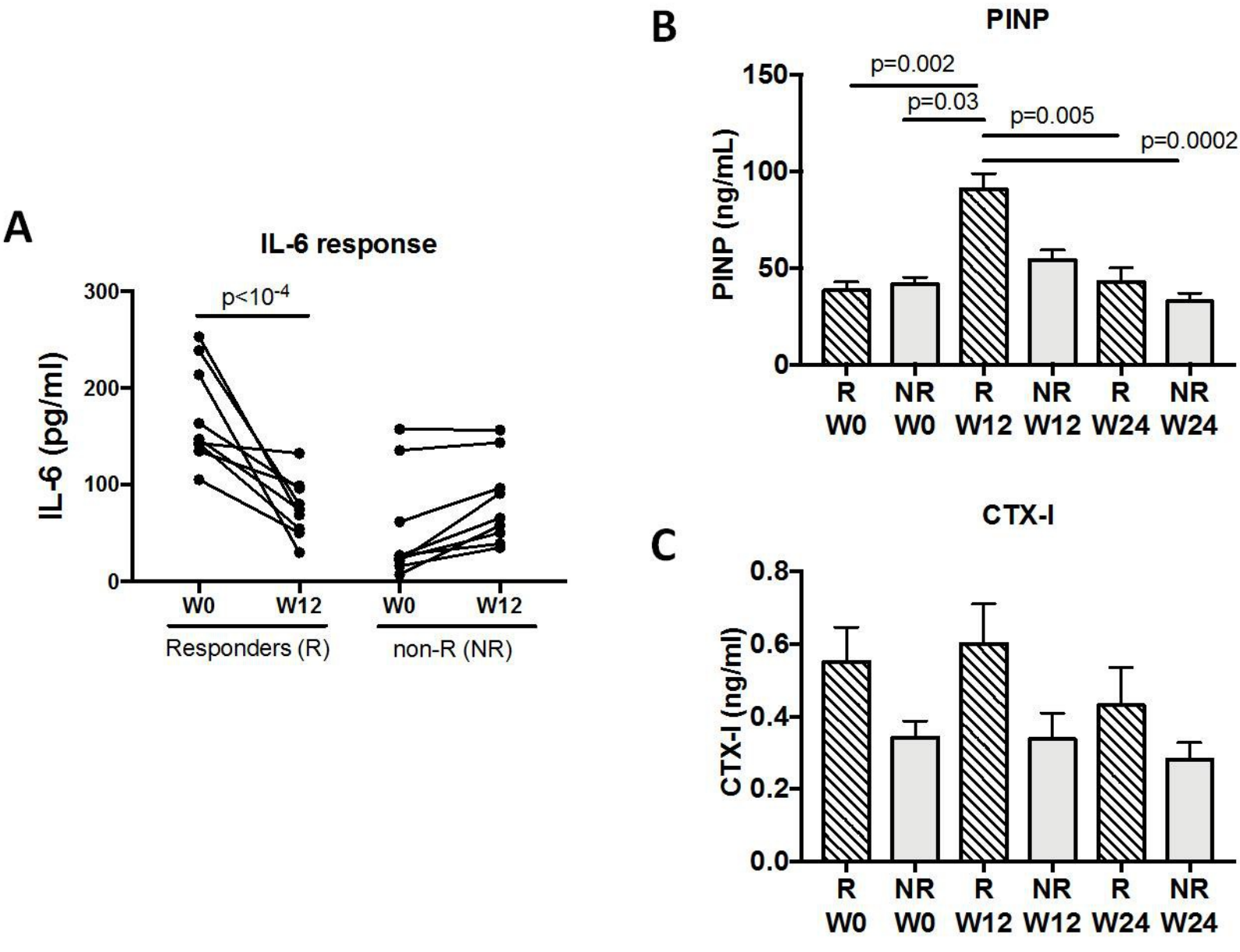
PMR W0
C
Controls
r=0.675
p<10-4
CTX-I
1.5
1.0
0.5
0.0
0
50
100
150
PINP
D
PMR W0
r=0.2551
CTX-I
1.5
1.0
0.5
0.0
0
20
40
60
80
PINP


A
PINP
p=0.0008
p<10-4
PINP (ng/mL)
150
100
50
0
W0
W12
W24
B
CTX-I
p=0.001
CTX-I (ng/ml)
1.5
1.0
0.5
0.0
W0
W12
W24
C
PMR W12
r=0.760
p<10-4
CTX-I
1.5
1.0
0.5
0.0
0
50
100
150
PINP
D
PMR W24
r=0.767
p<10-4
CTX-I
1.5
1.0
0.5
0.0
0
20
40
60
80
100
PINP


A
IL-6 response
IL-6 (pg/ml)
300
200
100
0
p<10-4
W0
W12
W0
W12
Responders (R)
non-R (NR)
B
PINP
PINP (ng/mL)
150
100
50
0
p=0.002
p=0.03
p=0.005
p=0.0002
R W0
NR W0
R W12
NR W12
R W24
NR W24
C
CTX-I
CTX-I (ng/ml)
0.8
0.6
0.4
0.2
0.0
R W0
NR W0
R W12
NR W12
R W24
NR W24