

PTX-3 release is increased by monocytes from patients with primary fibromyalgia without major depression

Sirs,

In a recent issue of *Clinical and Experimental Rheumatology*, we were grateful to read an interesting article by Skare *et al.* (1) investigating plasmatic levels of pentraxin-3 (PTX3) in a total of 94 women with fibromyalgia (FM). They reported that PTX-3 levels were significantly higher in FM patients compared to healthy women group. We thank the authors for this contribution to the area of inflammation and its relationship with FM syndrome. However, we would like to indicate that 93% of patients in the study were treated with antidepressants (amitriptyline, fluoxetine, duloxetine and venlafaxine), and in our opinion, this fact is relevant. In many cases, FM patients are treated with antidepressant for pain, nevertheless fibromyalgia also occurs associated with major depression. We think that the authors should have revealed if FM patients were also suffering for major depression, since it can be an important confounding factor of results. Thus, it has been reported that PTX-3 expression is increased in subjects with major depression (2). Therefore, we think that Skare *et al.* should check this fact to conclude that the PTX-3 plasma levels are elevated in FM patients.

However, we have studied, in a pilot study, the production of PTX-3 by isolated monocytes and neutrophils of 10 primary FM women without depression (according to the FM classification of Müller *et al.* (3)) as well as healthy women. Blood samples were centrifuged in a density gradient (Histopaque, Sigma) obtaining a first halo containing monocytes and lymphocytes and a second one containing neutrophils. These two suspensions were washed in PBS. Monocytes were purified from the pool of mononuclear cells using the Monocyte Isolation Kit II (Miltenyi Biotec GmbH). Using this monocyte isolation technique, human monocytes were isolated by depletion of non-monocyte cells (negative selection). The magnetically labelled non-monocyte cells were depleted by retaining them on a MACS Column in the magnetic field of a Midi-MACS Separator (Miltenyi Biotec GmbH, Germany), while the unlabelled monocytes passed through the column. In this way, isolation of highly pure unlabelled monocytes was achieved by depletion of the magnetically labelled cells. Both monocytes and neutrophils were adjusted to 10^6 cells/ml of medium (Iscove Medium [GIBCO] supplemented with 10% foetal bovine serum, 1% penicillin/streptomycin, and 1% L-glutamine) in order to evaluate their PTX3 releasing capacity. Monocytes and neutrophils were cultured for 24 h at 37°C, 5% CO₂ and 100% RH in flat-bottom 48-well cell culture plates (Falcon, Becton Dickinson Labware). Supernatants were aliquoted in eppendorf tubes and stored at -80°C until assay.

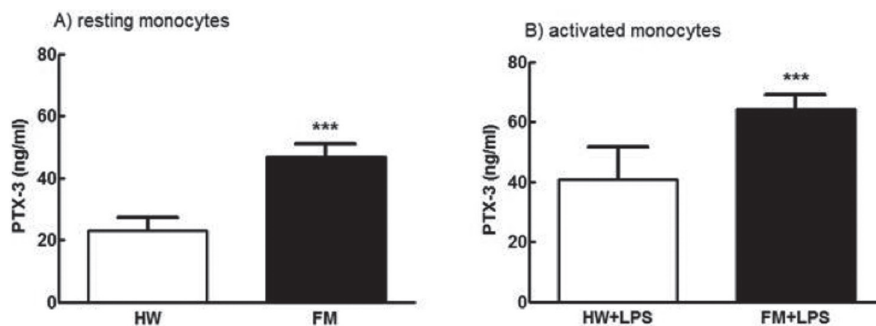


Fig. 1. PTX-3 released by monocytes ("resting monocytes": 1A) and by lipopolysaccharide (LPS)-activated monocytes ("activated monocytes": 1B) from healthy women (HW) and from FM patients. The results are present as the mean value \pm SEM of 10 experiments performed in duplicate (one experiment per volunteers). *** $p < 0.001$ with respect to HW.

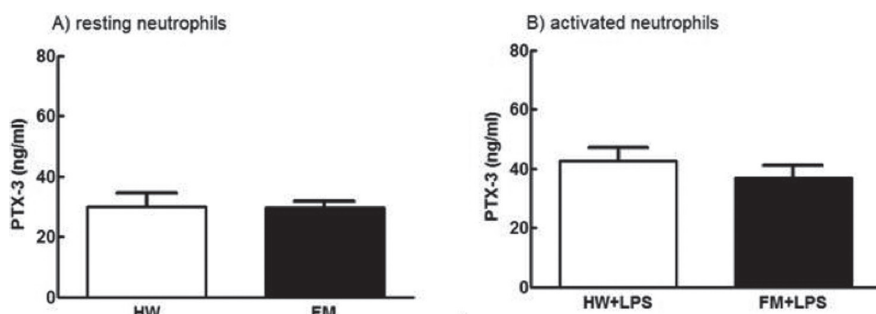


Fig. 2. PTX-3 released by neutrophils ("resting neutrophils": 2A) and by lipopolysaccharide (LPS)-activated neutrophils ("activated neutrophils": 2B) from healthy women (HW) and from FM patients. The results are present as the mean value \pm SEM of 10 experiments performed in duplicate (one experiment per volunteers).

Constitutive release by phagocytes (monocytes and neutrophils) of PTX3 was evaluated by ELISA, and compared between the FM and HW groups. This study was approved by the Ethics Committee of the University of Extremadura (Spain) and all participants signed consent.

Thus, monocytes from FM women (Fig. 1A) released constitutively ("resting monocytes") more PTX-3 ($p < 0.001$) than those from HW. In the same way, activated monocytes by LPS (Fig. 1B) from FM patients released also more PTX-3 ($p < 0.001$) than those from HW. However, as it shown in Fig. 2 the ability of neutrophils to release PTX-3 was similar ($p > 0.05$) both in FM patients and healthy women, this behaviour was similar in absence of LPS ("resting neutrophils" Fig. 2A) and in presence of LPS ("activated neutrophils": Fig. 2B).

Therefore, it seems possible that PTX-3 may be a biomarker for fibromyalgia whether the observed increased in plasma is independent of the depression. In addition, monocyte deregulation present in this disease is confirmed, since these cells of FM patients produce more proinflammatory cytokines (4, 5) and proinflammatory chemokines (6), together with increased local production of PTX-3 that the monocytes of healthy women. However, it does not occur in neutrophils from FM patients.

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References

- SKARE TL, PLAWIAK AC, VERONESE G, PAIVAS ES, MESSIAS-REASON I, NISHIHARA R: Pentraxin 3 levels in women with fibromyalgia. *Clin Exp Rheumatol* 2015; 33 (Suppl. 88): S142.
- SHELTON RC, LIANG S, LIANG P, CHAKRABARTI A, MANIER DH, SULSER F: Differential expression of pentraxin 3 in fibroblasts from patients with major depression. *Neuropsychopharmacology* 2004; 29: 126-2.
- MÜLLER W, SCHNEIDER EM, STRATZ T: The classification of fibromyalgia syndrome. *Rheumatol Int* 2007; 27: 1005-10.
- ORTEGA E, BOTE ME, GIRALDO E, GARCÍA JJ: Aquatic exercise improves the monocyte pro- and anti-inflammatory cytokine production balance in fibromyalgia patients. *Scand J Med Sci Sports* 2012; 22: 104-12.
- BOTE ME, GARCÍA JJ, HINCHADO MD, ORTEGA E: Inflammatory/stress feedback dysregulation in women with fibromyalgia. *Neuroimmunomodulation* 2012; 19: 343-51.
- GARCÍA JJ, CARVAJAL-GIL J, GUERRERO-BONMATTY R: Altered release of chemokines by phagocytes from fibromyalgia patients: a pilot study. *Innate Immun* 2015 Sep 3. pii: 1753425915602959.