

Basic science

Immune complex-mediated neutrophil activation in patients with polymyalgia rheumatica

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Abstract

Objective: Neutrophils are important in host defence. However, neutrophils are also linked to inflammation and organ damage. The purpose of this study was to assess whether markers of neutrophil activation are increased in PMR.

Methods: Levels of immune complexes (IC), calprotectin and neutrophil extracellular traps (NETs) were measured in plasma of healthy individuals ($n=30$) and patients with PMR ($n=60$), at flare and upon treatment with glucocorticoids using ELISA. Plasma-mediated neutrophil activation was assessed in presence of an Fc γ RIIA inhibitory antibody (IV.3).

Results: Plasma levels of calprotectin and NETs were elevated in PMR ($P<0.001$). Mechanistically, neutrophil activation was driven by ICs, present in plasma, able to up-regulate neutrophil activation markers CD66b and CD11b ($P<0.0001$) in an Fc γ RIIA-dependent manner ($P<0.01$). Of note, circulating levels of IC correlated with plasma induced CD66b and CD11b ($r=0.51$, $P=0.004$, and $r=0.46$, $P=0.01$, respectively) and decreased after glucocorticoid therapy. In contrast to NETs, calprotectin significantly decreased after glucocorticoid therapy ($P<0.001$) and was higher in PMR without overlapping GCA compared with patients with overlapping disease ($P=0.014$). Interestingly, musculoskeletal involvement was associated with elevated levels of calprotectin before initiation of glucocorticoid therapy ($P=0.036$).

Conclusions: Neutrophil activation, including NET formation, is increased in PMR, through IC-mediated engagement of Fc γ RIIA. Clinically, neutrophil activation is associated with musculoskeletal involvement, with calprotectin, but not NETs, being a biomarker of treatment response in PMR patients. In all, IC-mediated neutrophil activation is a central process in PMR pathogenesis identifying potential novel therapeutic targets (Fc γ RIIA), as well as soluble markers for disease monitoring (calprotectin).

Keywords: neutrophils, PMR, calprotectin, neutrophil extracellular traps, immune complexes

Rheumatology key messages

- Neutrophil activation and neutrophil extracellular trap formation is increased in patients with polymyalgia rheumatica (PMR).
- Circulating immune complexes activate neutrophils through Fc γ RIIA in PMR patients.
- Inhibition of Fc γ RIIA signalling could be a novel therapeutic intervention for PMR.

Introduction

PMR is a chronic inflammatory disease of unknown aetiology that primarily affects elderly women [1]. Ageing of the immune system plays a key role in its pathophysiology [2]. PMR is often manifested with pain and stiffness in neck, shoulder and pelvic girdles and associated with elevated inflammatory markers at the onset of the disease [3]. Constitutional symptoms such as fever and weight loss are some of the clinical manifestations of PMR. Recent studies have suggested a role for neutrophils in PMR, with neutrophil to lymphocyte ratio (NLR) being associated with inflammation in these patients [4].

Further, *serum* levels of calprotectin (also known as S100A8/A9 or myeloid related protein 8/14 [MRP8/14]), a calcium-binding protein released by activated neutrophils [5], are elevated in patients with PMR and GCA and associated with acute phase reactants including ESR [6]. However, given the challenges with measuring calprotectin in serum, i.e. spontaneous release of calprotectin upon sample processing [7], it is important to determine whether neutrophil activation occurs in PMR by assessing calprotectin levels in *plasma*.

When activated, neutrophils can also release neutrophil extracellular traps (NETs), a meshwork of chromatin decorated

Received: 29 September 2022. Accepted: 21 December 2022

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with citrullinated histones, and granule-derived enzymes, such as proteinase 3, neutrophil elastase, calprotectin, myeloperoxidase (MPO) and LL37 [8]. Increased levels of NETs have been reported in several rheumatological conditions, including RA [9], SLE [10], SSc [11] and vasculitis [12]. Upon NET formation, several key auto-antigens, including dsDNA, MPO and proteinase 3, are exposed on the NETs [13]. NETs are prominent inducers of inflammation, signalling through DNA sensing TLR9 as well as the cyclic GMP-AMP synthase–stimulator of interferon genes pathway [10]. However, the role of NET formation in PMR has not been carefully addressed.

In this study, we investigated whether patients with PMR have elevated levels of calprotectin and NETs in their plasma. We also assessed whether levels of calprotectin and NETs changed significantly before and after glucocorticoid therapy in patients with PMR. Finally, we investigated mechanisms through which neutrophil activation may occur in PMR, with an emphasis on immune complex-mediated activation through engagement of neutrophil FcγRIIA.

Methods

Patient characteristics

Patients with PMR ($n = 60$) were recruited at time point of active disease and were followed up at remission at Umea University Hospital, Sweden. Demographic data, including sex, age at diagnosis, symptom duration before diagnosis and prednisolone dose, were recorded (Table 1). For all patients, laboratory data such as markers of systemic inflammation (CRP and ESR) were collected. For the PMR patients, EDTA plasma was used. At inclusion the concentration of total immunoglobulin G (IgG) levels in their plasma was also analysed and the mean (s.d.) levels were found to be 11.9 (3.4) g/l (Table 1). In the general population, the mean (s.d.) serum IgG levels have been reported to be 11.2 (2.5) g/l [14]. Additionally, for patients with PMR, overlap with GCA was noted.

EDTA plasma from healthy individuals (healthy controls [HCs], $n = 30$) was collected through the University of Washington, Seattle, USA. The study was approved by the

appropriate local institutional review boards at University of Washington, Seattle, WA (#3100) and the Ethic Committee at Umea University (§192/96, dnr 96-138), and informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

ELISAs

Plasma levels of calprotectin (R&D Systems, Minneapolis, MN, USA) and immune complexes (ICs) (MicroVue CIC-C1q EIA, Quidel, Athens, OH, USA) were measured by ELISA, following the manufacturers' instructions. The IC ELISA is based on the capacity of complement factor C1q, immobilized to the plate, to bind to circulating ICs. Quantification of circulating NETs was performed by utilizing myeloperoxidase (MPO)–DNA ELISA as described by us [10]. First, 96-well microtitre plates (Corning Inc., Corning, NY, USA) were coated with anti-MPO antibody (4 µg/ml; Bio-Rad Laboratories, Hercules, CA, USA) overnight at 4°C, and then blocked with 1% BSA in PBS for 2 h at room temperature (RT). Then, plasma samples diluted 1:100 (MPO–DNA ELISA) were added in 1% BSA in PBS with 2 mM EDTA, and incubated overnight at 4°C. Anti-DNA–HRP from the Cell Death Detection ELISA kit (clone MCA-33; Roche, Indianapolis, IN, USA) was added as detection antibody for 2 h at RT. The reaction was developed with 3,3',5,5'-tetramethylbenzidine (BD Biosciences, San Jose, CA, USA) for 20 min and stopped by the addition of 2 M sulphuric acid. Known concentrations of MPO–DNA complexes (rhMPO, R&D Systems, Minneapolis, MN, USA; calf thymus DNA, Trevigen, Gaithersburg, MD, USA) were utilized to construct a standard curve. Absorbance was measured by a plate reader at 450 nm (Synergy 2, BioTek, Winooski, VT, USA).

Neutrophil isolation

Heparinized blood from healthy individuals was layered on Polymorphprep (Axis-Shield, Dundee, UK) density gradient, according to the manufacturer's instructions, or as described previously [15–17]. Red blood cells were then lysed using RBC lysis buffer (BioLegend, San Diego, CA, USA). Neutrophils were re-suspended in serum-free RPMI-1640 medium (Thermo Fisher Scientific, Waltham, MA, USA) for *in vitro* assays.

Neutrophil activation assay

To assess neutrophil activation, neutrophils at a concentration of 3×10^5 cells/well were incubated in the presence or absence of the human FcγRIIA blocking antibody (IV.3) (5 µg/ml; Caprico Biotechnologies, Norcross, GA, USA) for 30 min before addition of stimuli, such as R848 (3.5 µg/ml), or plasma samples from patients with PMR ($n = 8$) and HCs ($n = 8$) (1:50 dilution) for an additional 120 min. Approximately 90–95% of neutrophils were viable after neutrophil stimulation with plasma samples. To assess neutrophil activation, neutrophils were labelled with phycoerythrin-conjugated anti-CD66b (clone G10F5, BioLegend) and APC-conjugated anti-CD11b antibodies (clone CBRM1/5, BioLegend) and analysed by flow cytometry (CytoFlex S, Beckman Coulter, Brea, CA, USA). Data were analysed by FlowJo (version 10.6.2, Tree Star Inc., Ashland, OR, USA), and results are presented as mean fluorescence intensity (MFI) of CD66b and CD11b.

Table 1. Baseline demographic characteristics of patients with PMR and HCs

Descriptive data	Patients ($n = 54$)	HCs ($n = 30$)	P-value ^a
Women, n (%)	44 (73.3)	21 (70)	0.667 ^b
Age at inclusion, mean (s.d.), years	73 (8)	40(14)	<0.001
Symptom duration before diagnosis, mean (s.d.), months	2 (2)	NA	NA
First follow-up, mean (s.d.), months	3.7(2)	NA	NA
MPO–DNA, mean (s.d.), nM	3.2 (1.25)	2.6 (1.5)	<0.001
Calprotectin, mean (s.d.), ng/ml	3002.9 (1812.6)	83.8 (95.0)	<0.001
Glucocorticoid dose, mean (s.d.), mg	22 (7)	NA	NA
ESR, mean (s.d.), mm/h	60(23)	NM	
CRP, mean (s.d.), mg/l	48 (37)	NM	
IgG, mean (s.d.), g/l	11.9 (3.4)	NM	
GCA, n (%)	14 (23.3)	NA	NA

^a Mann–Whitney.

^b Calculated using Pearson's χ^2 . HC: healthy control; NA: not applicable; NM: not measured.

Statistics

For statistical analyses, the Mann–Whitney *U*-test, the Wilcoxon signed-rank test and Spearman's correlation were appropriately applied. Comparisons of the levels of neutrophil activation markers between cases and controls were performed using linear regression modelling adjusting for age and sex. All analyses were performed in GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA) and SPSS Statistics software (v. 27.0 IBM Corp, Armonk, NY, USA) and considered statistically significant at $P < 0.05$.

Results

Demographic characteristics of the study population

The mean (s.d.) age of the patients with PMR at the time of diagnosis was 73 (8) years; 73.3% of the patients were female; the mean symptom duration before the diagnosis of PMR was 2 months; the mean (s.d.) daily dose of prednisolone at start was 22 (7) mg; and 23.3% of the patients with PMR had an overlap with GCA. The mean (s.d.) ESR was 60 (23) mm/h and the mean CRP was 48 (37) mg/l before the initiation of glucocorticoids (Table 1). The mean age of the PMR patients was significantly higher than that of the controls (Table 1). Further, the levels of MPO–DNA (nM) and of calprotectin (ng/ml) were significantly higher in the PMR cohort. After adjusting for sex and age, only calprotectin remained significantly elevated in the PMR patients ($P < 0.001$).

Neutrophil activation in patients with PMR

As depicted in Fig. 1A, and consistent with prior findings in serum [6], plasma levels of calprotectin were significantly elevated in patients with PMR with active disease and after glucocorticoid therapy as compared with HCs ($P < 0.001$). The mean concentration of calprotectin in 54 patients with active disease before glucocorticoid therapy was 3002.9 ng/ml with a s.d. of 1812.6 ng/ml, which was significantly higher ($P < 0.001$) compared with 30 HCs, where it was 83.8 ng/ml with a s.d. of 95 ng/ml. Patients with symptoms from shoulder, thighs and hips before initiation of glucocorticoid therapy had significantly elevated levels of calprotectin in comparison to patients having symptoms from cervical and lumbar spine (3125.3 ng/ml *vs* 1614.8 ng/ml, $P = 0.036$).

Of note, we made the novel observation that levels of NETs, as represented by MPO–DNA complexes, were similarly elevated in PMR with active disease and after treatment compared with healthy individuals ($P < 0.001$) (Fig. 1B). The mean (s.d.) concentration of MPO–DNA complexes in 54 patients with active disease before glucocorticoid therapy was 3.2 (1.3) nM, which was significantly higher than that of HCs (mean [s.d.]: 2.6 [1.5] nM, $P < 0.001$). Interestingly, for patients with active disease, patients with PMR without overlapping GCA had significantly elevated levels of calprotectin (mean [s.d.]: 3243.2 [1854.7] ng/ml) compared with patients with overlapping disease (mean [s.d.]: 1801.2 [947.3] ng/ml, $P = 0.01$). However, levels of MPO–DNA complexes, CRP and ESR were similar in PMR with or without overlapping GCA (data not shown).

Mechanisms of neutrophil activation in PMR

Given the elevated levels of neutrophil activation markers in PMR, we asked whether patients with PMR had circulating neutrophil-activating factors. To address that, neutrophils from healthy individuals were incubated with plasma from either PMR patients or HCs and assessed for up-regulation of degranulation markers CD66b and CD11b by flow cytometry. As compared with HCs, plasma from patients with PMR with active disease supported increased up-regulation of both CD66b ($P < 0.001$) and CD11b ($P < 0.001$) (Fig. 2A and B, respectively).

Given the extensive neutrophil activation observed in PMR in plasma, as well as the capacity of plasma to support *de novo* neutrophil activation, we next sought to determine which factor(s) could contribute to neutrophil activation in PMR. Prior work has demonstrated elevated levels of circulating immune complexes in patients with PMR [18, 19], with immune complexes known to activate neutrophils through FcγRIIA [20]. To address whether circulating immune complexes were driving neutrophil activation in PMR, neutrophils from healthy individuals were incubated with an FcγRIIA inhibitor, prior to addition of plasma from PMR patients, and assessed for neutrophil activation by flow cytometry. Addition of the FcγRIIA inhibitor markedly reduced the capacity of plasma from PMR to induce *in vitro* activation of neutrophils, as demonstrated by down-regulation of CD66b and CD11b (Fig. 3A and B, respectively). In all, these results

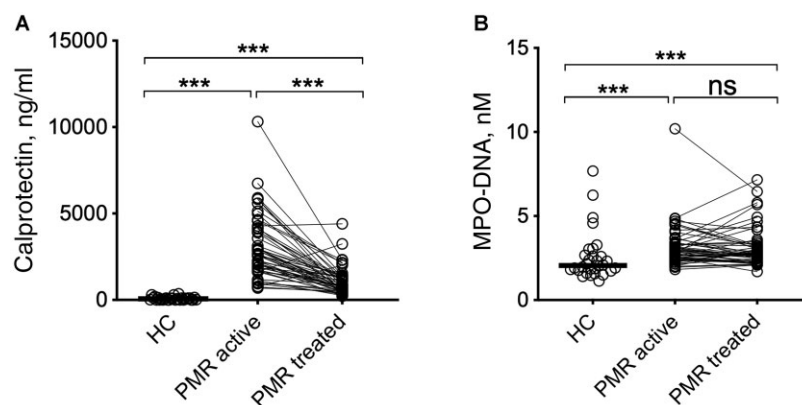


Figure 1. Levels of calprotectin and NETs in patients with PMR. Plasma levels of calprotectin (A) and MPO–DNA complexes (B) were measured by ELISA in HCs ($n = 30$) and patients with PMR with active disease ($n = 54$) and after treatment with glucocorticoid therapy ($n = 54$). Statistical analyses were performed using the Mann–Whitney *U*-test and the Wilcoxon signed rank test, respectively; *** $P < 0.001$. Each circle represents an individual sample, with the bar representing the median of the group. HC: healthy control; NET: neutrophil extracellular trap; ns: not significant

suggest that circulating immune complexes are involved in supporting neutrophil activation via Fc γ RIIA in PMR. Consistent with that interpretation, levels of circulating immune complexes were associated with the capacity of plasma to induce neutrophil activation *in vitro* (Fig. 4A and B) and decreased significantly after glucocorticoid therapy in patients with PMR (Fig. 4C). Levels of IC correlated with IgG levels ($r=0.332$, $P=0.03$) in patients with PMR. Similar correlations have been observed in our prior work, measuring IC levels in SLE patients, with levels of IgG correlating with IC as determined by C1q-ELISA ($r=0.19$, $P=0.03$) and our in-house flow cytometry-based IC assay ($r=0.20$, $P=0.02$) [21].

Role of glucocorticoid treatment on neutrophil activation markers

We then investigated whether levels of neutrophil markers, blood cell counts and acute phase reactants significantly changed after glucocorticoid treatment. As depicted in Table 2, plasma levels of calprotectin significantly decreased

after treatment with glucocorticoids, consistent with prior findings in PMR [6]. In contrast to calprotectin, levels of NETs were unaffected by treatment regimen. With regards to blood cell counts, there was a significant increase in lymphocytes, whereas there was a decrease in platelet count after treatment with glucocorticoids. All acute phase reactants (ESR, CRP and fibrinogen levels) significantly decreased after treatment with oral glucocorticoids. In all, calprotectin, lymphocytes, platelet count, and acute phase reactants such as ESR (Fig. 4D), CRP and fibrinogen could be used as surrogate markers of disease activity and monitoring of treatment response in patients with PMR (Table 2).

Discussion

NET formation is a neutrophil cell death process that plays a crucial role in the pathogenesis of many autoimmune diseases [22]. In the current study, we made the novel observation of elevated plasma levels of MPO–DNA complexes in patients with PMR in comparison with healthy individuals. High levels of circulating NETs in PMR could be due to exacerbated neutrophil cell death. There are reports of aberrant NET formation being related to ageing, as the key enzyme for NETs, protein arginine deiminase-4, has been reported to spontaneously generate more NETs with ageing [23, 24]. However, other studies demonstrated decreased capacity for NET formation in older individuals due to reduced reactive oxygen species production and impaired autophagy [25], suggesting that additional factors other than ageing might be responsible for the enhanced neutrophil activation. In our study, levels of NETs were not associated with age either in the PMR cohort or among healthy individuals (data not shown). Notably, treatment with glucocorticoids did not cause any significant change in the levels of MPO–DNA complexes. Thus, MPO–DNA complexes are not a sensitive biomarker for predicting reduction in systemic inflammation in patients with PMR.

In the subgroup analysis of patients with PMR with and without overlapping GCA, we did not find any significant difference in the plasma levels of MPO–DNA complexes either. Thus, propensity to undergo NET formation is not directly linked to disease activity or levels of inflammation, similar to what has been described in SLE [26]. Further studies are needed to determine the mechanism(s) leading to peripheral

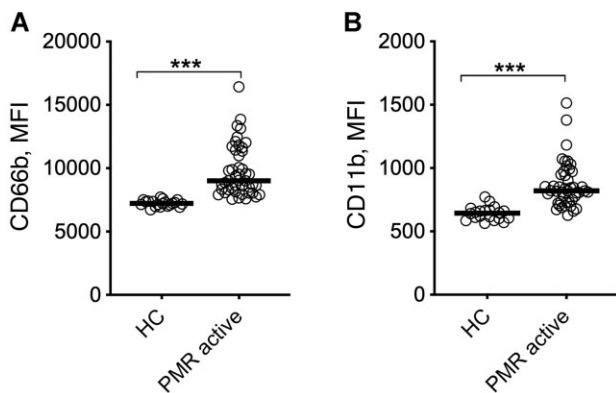


Figure 2. Neutrophil activation induced by plasma from patients with PMR. Neutrophils from a healthy individual were incubated with plasma from HCs ($n=20$) and patients with PMR ($n=45$) and analysed for the expression of the neutrophil cell surface degranulation markers CD66b (A) and CD11b (B) by flow cytometry. Results are presented as the mean fluorescence intensity (MFI). Statistical analyses were done using the Mann–Whitney U -test; *** $P<0.001$. Each circle represents an individual sample, with the bar representing the median of the group. HC: healthy control

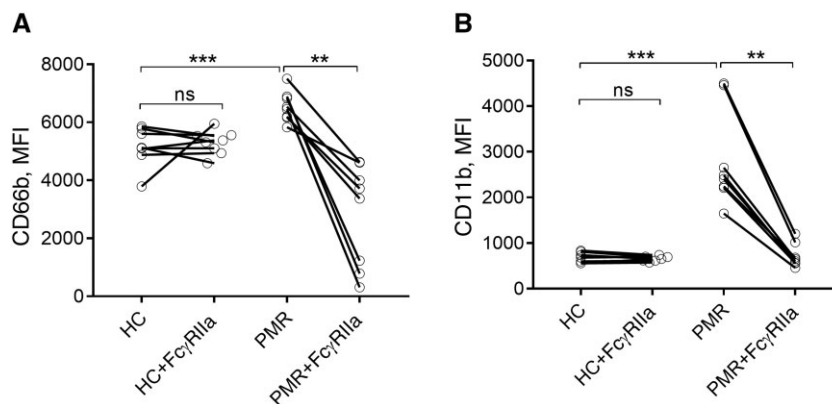


Figure 3. Fc γ RIIA-mediated neutrophil activation induced by plasma from patients with PMR. Plasma from HCs ($n=8$) and patients with PMR ($n=8$) were incubated *in vitro* with neutrophils isolated from a healthy object in the presence or absence of the Fc γ RIIA-blocking antibody (Clone IV.3) for 120 min and assessed for its capacity to up-regulate the neutrophil activation surface makers CD66b (A) and CD11b (B). Results are shown as the mean fluorescence intensity (MFI). Statistical analyses were done using the Mann–Whitney U -test as well as the Wilcoxon signed-rank test; ** $P<0.01$, *** $P<0.001$. HC: healthy control; ns: not significant

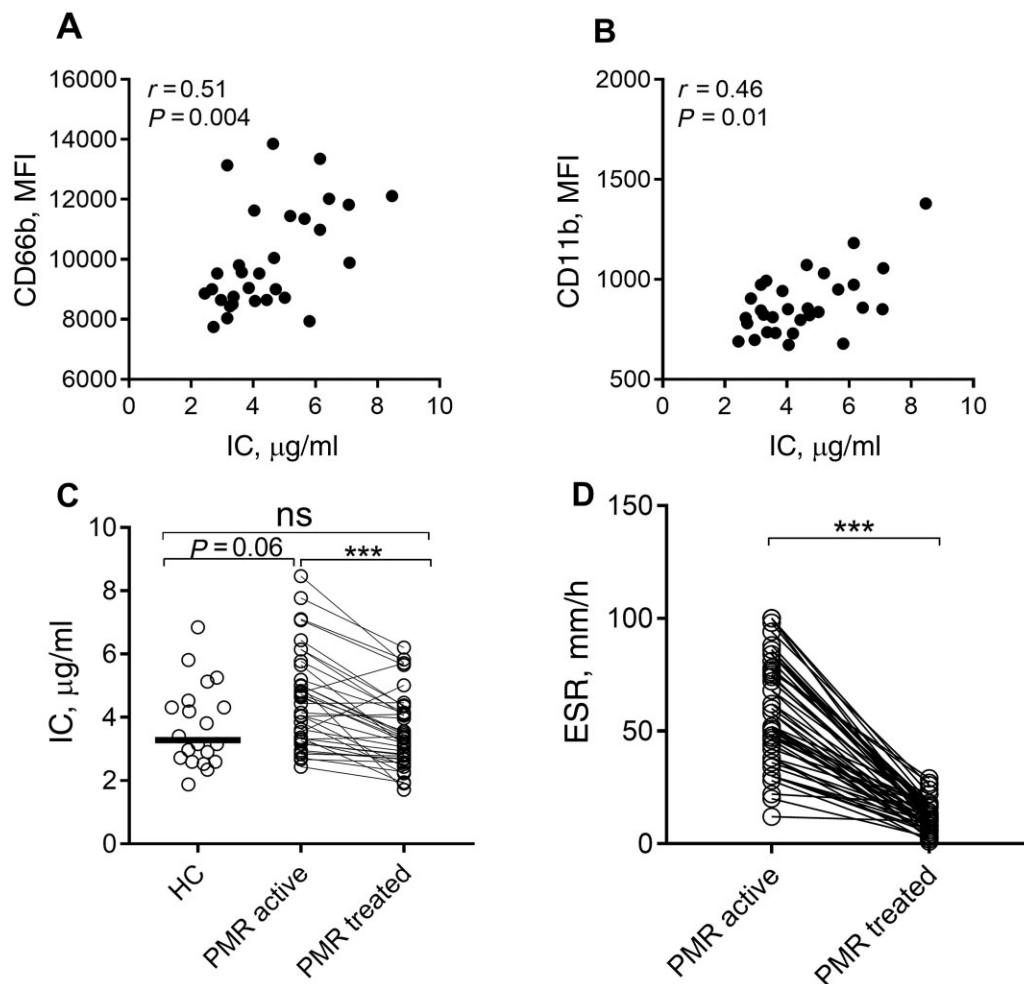


Figure 4. Association of circulating immune complexes with plasma-mediated neutrophil activation and treatment in patients with PMR. **(A, B)** Plasma-mediated neutrophil activation as assessed by CD66b **(A)** and CD11b **(B)** was correlated with plasma levels of immune complexes (IC) from patients with PMR ($n = 30$). Expression of neutrophil activation markers is presented as mean fluorescence intensity (MFI). **(C)** IC levels were measured by ELISA in HCs ($n = 20$) and patients with PMR with active disease ($n = 38$) and after treatment ($n = 38$). **(D)** Analysis of ESR levels before and after treatment in PMR patients. Statistical analysis was done using Spearman's correlation test, the Mann-Whitney U -test and the Wilcoxon signed rank test; *** $P < 0.001$. Each circle represents an individual sample, with the bar representing the median of the group. HC: healthy control; ns: not significant

Table 2. Mean concentration of neutrophil markers, blood cell counts and acute phase reactants in PMR

Biomarker	Before glucocorticoids ($n = 54$)	After glucocorticoids ($n = 54$)	P -value
MPO-DNA, nM	3.2 (1.25)	3.1 (1.13)	0.38
Calprotectin, ng/ml	3002.9 (1812.6)	1065.4 (767.8)	<0.001
Neutrophils, $\times 10^3/\mu\text{l}$	5.8 (1.4) ^a	5.9 (1.6)	0.63
Monocytes, $\times 10^3/\mu\text{l}$	0.80 (0.3) ^b	1.3 (1.5)	0.57
Lymphocytes, $\times 10^3/\mu\text{l}$	1.58 (0.4) ^b	2.0 (0.9)	0.02
Basophils, $\times 10^3/\mu\text{l}$	0.03 (0.02) ^b	0.4 (1.0)	0.19
Eosinophils, $\times 10^3/\mu\text{l}$	0.2 (0.9) ^b	0.3 (0.8)	0.30
Platelets, $\times 10^9/l$	357.2 (92.7) ^c	267.0 (61.7)	<0.001
Fibrinogen, g/l	4.9 (1.4) ^d	2.6 (1.1)	<0.001
ESR, mm/h	59.8 (23.3)	11.9 (6.6)	<0.001
CRP, mg/l	48.6 (37.9) ^e	11.1 (3.2)	<0.001

Data are means (s.d.). The biomarker was calculated for matched pairs and the P -values were determined by the Wilcoxon signed rank test.

^a $n = 23$ cases.

^b $n = 22$ cases.

^c $n = 51$ cases.

^d $n = 35$ cases.

^e $n = 50$ cases.

MPO–DNA complexes in PMR, and long-term consequences of elevated NET formation on organ damage, including thrombosis [26].

On the other hand, calprotectin not only differed between patients with PMR and HCs but was also reduced upon treatment with glucocorticoids. Our findings align with prior studies that assessed serum levels of calprotectin [6] and suggest that calprotectin in the plasma could be a reliable biomarker of disease activity and/or treatment response in patients with PMR. Levels of calprotectin were similarly found elevated in patients with other rheumatic diseases such as SLE, RA, systemic sclerosis and systemic vasculitides [10, 11, 27, 28].

We also found that patients with PMR without overlapping disease with GCA had significantly higher levels of calprotectin in their circulation, as compared with patients with overlapping disease, suggesting higher levels of neutrophil recruitment and release of calprotectin at sites of inflammation in patients with PMR without GCA. This observation could help in patient stratification and may reflect not only differences in levels of neutrophil activation, but also variability in circulating chemokines or cytokines, disease duration and burden, and absence or presence of vascular inflammation among patients with PMR and GCA.

Analysis of expression of calprotectin and NETs in tissues from synovial bursa of patients with PMR and association with disease activity and inflammatory markers might be helpful in identifying local tissue neutrophil infiltration and could be the focus of future prospective studies. For example, presence of neutrophils positive for expression of calprotectin has been identified in and around the vasa vasorum of biopsies from patients with GCA causing inflammation via activation of endothelial cells and production of reactive oxygen species [29].

The activated neutrophil phenotype observed in our patients with active disease has been demonstrated by another group but after 12 weeks of steroid therapy along with augmentation in the number of circulating neutrophils and a surge in the disease activity [30]. Interestingly, plasma-mediated neutrophil activation decreased in our patients with PMR upon blockade of Fc γ RIIA, a key neutrophil receptor for immune complexes [31]. Similarly, our group recently showed plasma-mediated neutrophil activation to be abolished by blocking Fc γ RIIA also in systemic sclerosis [11]. In the MRL/lpr lupus mouse model, administration of an immunoglobulin-binding peptide called TG19320 prevented glomerulonephritis by interfering with Fc γ R and IgG interaction [32]. In another murine lupus model, treatment with recombinant soluble Fc γ RII (CD32) successfully inhibited immune complex-mediated inflammation [33]. Notably, use of small chemical entities specific for the Fc γ RII dimer inhibited development of destructive autoimmune arthritis in Fc γ RIIA transgenic mice by blocking immune complex-mediated responses [34]. Our results suggest that presence of circulating immune complexes may partake in neutrophil-mediated inflammation via signalling through the Fc γ RIIA receptor in PMR. Thus, inhibition of Fc γ RIIA might be a potential attractive target for therapy in PMR. Of note, Fc γ RIIA is not only expressed on neutrophils, but also on several other important immune cells, including plasmacytoid dendritic cells, monocytes and platelets. Further studies are warranted to determine whether ICs can similarly result in activation of those cells in PMR.

Interestingly, while there was a significant increase in the lymphocyte cell count and a drop in the platelet count, there

was no significant change in the neutrophil cell count after glucocorticoid therapy in our cohort. Another study reported that neutrophils remained elevated whereas lymphoid subsets fluctuated in PMR patients during glucocorticoid therapy [35]. Differences in patient populations, study designs, sample size, clinical setting, treatment duration and follow-up times may possibly explain the discordant results in our study.

Our study has a few limitations. We recognize that this is a single-centre study susceptible to local influences. One additional limitation is the retrospective nature of our study. Another important consideration is that the source of calprotectin and MPO–DNA complexes could not be established in this study. MPO can be stored in azurophilic granules of both neutrophils and monocytes [36] and calprotectin can constitute up to 40–60% of the soluble cytosolic content in neutrophils, monocytes and activated macrophages [37, 38].

In conclusion, our data for the first time demonstrated that levels of neutrophil activation markers and NETs are elevated in the circulation of patients with PMR implicating an important role for neutrophil recruitment and cell death in PMR. Our data also support the use of calprotectin as a biomarker in monitoring disease activity in patients with PMR, which needs to be validated by larger multicentre studies. Finally, targeting Fc γ RIIA by interfering with its interaction with immune complexes could be a promising pharmaceutical strategy for treating patients with PMR.

Data availability

All data relevant to this study are included in the article or available upon reasonable request.

Contribution statement

D.M., S.R.D. and C.L. contributed to the conception and design of this study. D.M., L.J., S.R.D., R.K., T.W., P.H. and C.L. participated in experimental data collection and data analysis. S.R.D. and L.J. contributed with patient samples and clinical data. All authors contributed to data interpretation, critically reviewed and revised the manuscript and approved the final submission.

Funding

This work was supported by the NIH training grant award #5T32HL007028-44 (D.M.) and the NIH grants 1R21EY029391 (C.L.) and R21AR075129 (C.L.).

Disclosure statement: D.M. received Advisory Board fees from ChemoCentryx. C.L. received research funding from Exagen Inc., Pfizer, Gilead Sciences, Horizon Therapeutics, Amytryx, Redd Pharma and Eli Lilly. C.L. is a Scientific Advisory Board member at Redd Pharma.

Patient consent for publication: Informed written consent was obtained from all participants in accordance with the Helsinki Declaration.

Ethics approval: The study was approved by the regional ethics board at University of Washington, Seattle, WA (#3100) and Ethics Committee at Umea University, Sweden (§192/96, dnr 96-138).

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