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Polymyalgia rheumatica (PMR) is a common, rheumatic inflammatory disease causing debilitating pain and stiffness of the shoulder and hip girdle.[1] Inflammation of bursae and tendon sheaths is a key finding in PMR. Glucocorticoids have remained the mainstay of treatment for 60 years. Alternative treatments for PMR are highly needed, since half of patients require prolonged treatment, which is associated with substantial toxicity.[1 2] A recent phase 2/3 trial has shown promising efficacy of anti-interleukin (IL)−6 receptor (antil-IL-6R) therapy for new-onset PMR[3]: 63% of patients treated with anti-IL-6R therapy reached glucocorticoid-free remission at week 16, whereas 12% of placebo-treated patients reached this primary end point. The biological rationale for targeting the IL-6 pathway stems from the observation that serum levels of IL-6 are increased in PMR.[2 4] However, it is unclear whether IL-6 is also expressed in the tissues affected by PMR.

We investigated the expression of IL-6 in synovial tissue obtained from six patients with new-onset, treatment-naïve PMR (four women; median age 72 years, range 58–79) showing subacromial-subdeltoid (SASD) bursitis on ultrasonography. The median erythrocyte sedimentation rate at the time of the biopsy collection was 67 mm/hour (range 39–89) and serum C reactive protein was 67 mg/L (range 3–118). All patients fulfilled the Chuang criteria for PMR.[1] Their diagnosis was confirmed after 6 months of follow-up and concomitant large-vessel giant cell arteritis was ruled out by vascular ultrasonography and/or FDG-PET/CT. Synovial tissue was obtained at diagnosis from the SASD bursa by ultrasound-guided biopsy with a 16G core needle (Argon Medical Devices). All bursae showed some level of synovial hypertrophy with power Doppler signal on ultrasound examination (online supplemental figure S1). The number of biopsies available from each patient was five (n=5 patients) or three (n=1 patient). More details are provided in online supplemental methods.

First, synovitis scores according to Krenn *et al* were determined on H&E-stained slides.[5] The median synovitis score was 2.5 (range 1–5.5), suggestive of low-grade synovitis. Next, immunohistochemistry was performed for IL-6 (figure 1A,B). A large number of IL-6$^+$ cells was observed throughout the synovial tissue, as indicated by semiquantitative scoring (figure 1C). A semiquantitative score of 4 was obtained for IL-6 expression in the patient in which three biopsies were obtained. Subsequently, immunofluorescence was applied on biopsies of all patients to identify potential cellular sources of IL-6. This cytokine was detected in substantial portions of CD34$^+$ endothelial cells, CD34$^+$ fibroblasts/stromal cells, CD90$^+$ fibroblasts and CD68$^+$ macrophages (figure 1D–F and online supplemental figure S2-4). Bearing in mind the limited number of patients, no correlation was found between semiquantitative IL-6 scores in the tissue and

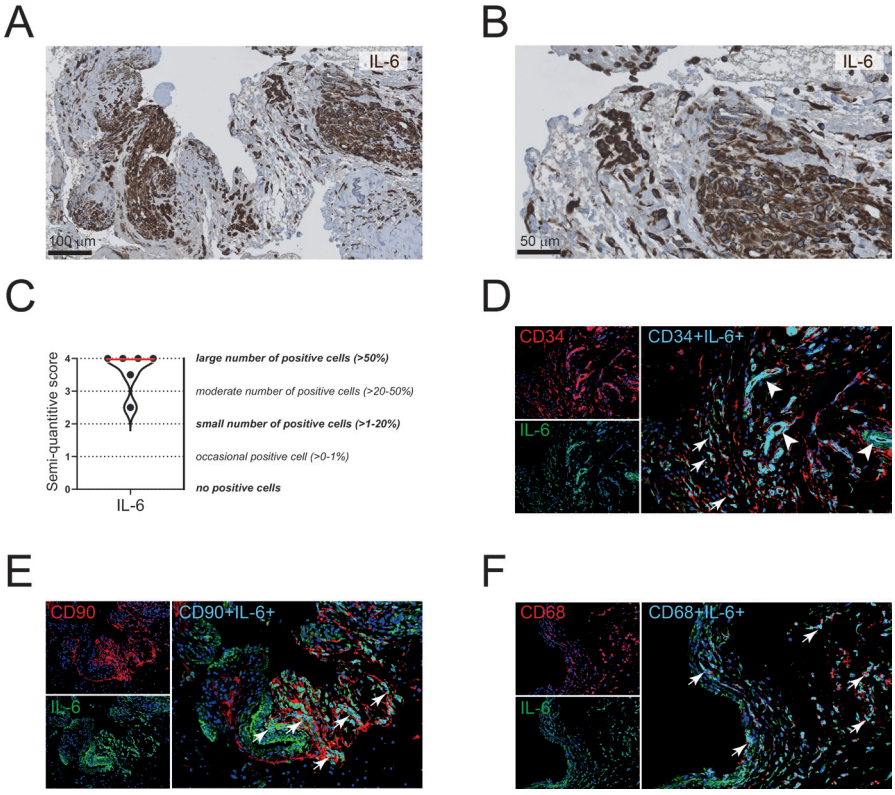

Figure 1 Expression of IL-6 in synovial tissue of patients with PMR. Ultrasound-guided synovial biopsies were obtained from the subacromial-subdeltoid bursa of six patients with PMR. (A) Representative immunohistochemical staining for IL-6 (brown) at 20× and (B) 40× magnification. (C) Semiquantitative scoring of immunohistochemical staining for IL-6. Scores of two independent investigators were averaged. (D) Immunofluorescence staining for IL-6 (green), CD34 (red) and co-localisation of IL-6/CD34 (cyan). Arrow heads point to endothelial cells and arrows to fibroblasts/stromal cells. (E) Immunofluorescence staining for IL-6 (green), CD90 (fibroblast marker; red), and co-localisation of IL-6/CD90 (cyan). Arrows highlight exemplary areas with CD90$^+$IL-6$^+$ cells. (F) Immunofluorescence staining for IL-6 (green), CD68 (macrophage marker; red) and co-localisation of IL-6/CD68 (cyan). Arrows highlight exemplary areas with CD68$^+$IL-6$^+$ cells. Nuclear staining for DAPI was also performed (blue). PMR, polymyalgia rheumatica.

other parameters such as serum levels of C reactive protein, the erythrocyte sedimentation rate, visual analogue scales for pain and stiffness of the biopsied shoulder or the synovitis score according to Krenn *et al* (online supplemental table S1). Furthermore, no clear relationship was observed for tissue IL-6 scores and the glucocorticoid dose needed to induce remission (online supplemental table S2).

This is the first report demonstrating expression of IL-6 in the synovial tissue of patients with PMR. Limitations include the small number of patients studied, and the absence of a control group (eg, patients with impingement syndrome or rheumatoid arthritis). A recent study reported only occasional IL-6$^+$ cells in the SASD bursa of patients undergoing shoulder surgery for non-inflammatory conditions.[6] It would be interesting to further evaluate IL-6 expression in a larger series of patients with PMR and to further characterise the macrophage and fibroblast subsets responsible for producing IL-6.

In conclusion, IL-6 is widely expressed by various cell populations in the SASD bursa of patients with PMR. Our study strengthens the rationale for anti-IL-6R therapy in PMR.

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Collaborators n/a.

Contributors WFJ, BD, EB and KSMvdG contributed to conception or design of the work. WFJ, AZ, AD, SH, KSMvdG contributed to acquisition of data. All authors contributed to analysis or interpretation of data. All authors were involved in drafting of the work or revising it critically for important intellectual content. All authors provided final approval of the version published. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Competing interests KSMvdG reports personal fees from Roche, outside the submitted work. BD reports consulting fees from Roche, Chugai, Sanofi, and sponsorship grants for international meetings and workshops with Roche, Sanofi, AbbVie and GlaxoSmithKline. EB reports personal fees from Roche, outside the submitted work. The other authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research. Advice on the feasibility and relevance of the study was obtained from patients through the Vasculitis Stichting and PMRGCAuk.

Patient consent for publication Not applicable.

Ethics approval The study was performed in accordance with the declaration of Helsinki. The study was approved by the Medical Ethical Committee of the UMCG (METc 2010/222, and all patients provided written informed consent. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

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Supplementary methods

Ultrasonography

Standard ultrasound images of the subacromial-subdeltoid (SASD) bursa were obtained prior to the ultrasound-guided biopsy using a eSaote MyLabTwice with a LA533 (3-13 MHz) or LA435 (6-18 MHz) transducer. Power Doppler was used at the lowest permissible pulse repetition frequency with maximum color gain without creating artifacts. Bursitis was defined as an enlargement (i.e. increase in diameter) of the bursa, with a well-defined, anechoic or hypoechoic area inside, with or without power Doppler signal [1]. Synovial hypertrophy of the bursa was defined as hypoechoic thickening of the bursa wall, with or without power Doppler signal, that was poorly compressible and non-displaceable. The latter definition is comparable to the definition of synovial hypertrophy for joints [2].

Ultrasound-guided biopsy

Ultrasound-guided biopsies of the SASD bursa were collected from 6 patients with a 16G core needle with a throw length of 13 or 23 mm (Argon Medical Devices) under local anesthesia with 1% lidocaine. Biopsies were dispersed in 10% formalin and paraffin embedded. Synovitis scores according to Krenn *et al*. were determined on hematoxylin and eosin (H&E)-stained sections [3].

Immunohistochemistry

Immunohistochemistry (IHC) was performed to detect IL-6. Sections were deparaffinized with xylene and dehydrated with alcohol before antigen retrieval was performed (Citrate buffer – pH6). Endogenous peroxidase was blocked following primary antibody incubation of 1 hour at room temperature. Slides were subsequently incubated with secondary antibody. Next, slides were incubated with DAB for 10 minutes and counterstained with hematoxylin. IHC sections were scanned with a Nanozoomer Digital Pathology Scanner (Hamamatsu Photonics). IHC staining was semi-quantitatively scored on a five point scale: 0= no positive cells, 1= occasional positive cells (0–1% estimated positive), 2= small numbers of positive cells (>1–20%), 3= moderate numbers of positive cells (>20–50%), 4= large numbers of positive cells (more than 50%). Scores of two independent investigators were averaged.

Marker	Antigen retrieval	Isotype	Clone	Dilution	Secondary antibody
IL-6	pH6	IgG2b	SC-130326 (Santa-Cruz)	1:25	Envision anti-mouse polymer-HRP (DAKO, K4006)

Immunofluorescence

Double labeling of IL-6/CD34, IL-6/CD90 and IL-6/CD68 was performed by immunofluorescence staining on biopsies of all six patients. After deparaffinization and antigen retrieval, the tissues were incubated for 90 minutes at room temperature (CD68+IL-6) or overnight at 4ºC (CD90+IL-6 and CD34+IL-6) with primary antibodies. Tissues were subsequently incubated with secondary and tertiary antibodies. Autofluorescence was blocked with Vector® TrueView® autofluorescence quenching kit according to the manufacturer's instruction. Nuclei were stained using DAPI. Nuance® Multispectral Imaging System (PerkinElmer) was used to take images of the biopsies. Image cubes were captured at 20x magnification using Nuance® FX software v3.0.1 (PerkinElmer). Multiple wavelengths were used; 440:460 for DAPI, 490:530 for Alexa 488, 570:600 for Alexa 568. Spectral unmixing was performed with spectral libraries of each fluorophore assigned different colors (DAPI = blue, AF488 = green, AF568 = red), subtracting the background autofluorescence. Colocalization of the two fluorophores was assigned the color cyan.

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CD68+IL-6

	Targets		
	IL-6	**CD68**	**Nucleus**
Isotype (primary ab)	Mouse IgG2b (Santa-Cruz, SC-130326) (1:25)	Mouse IgG3 (DAKO, M0876) (1:100)	
Secondary ab	Rat anti-mouse IgG2b (Biolegend, RMG2b-1) (1:20)	Goat anti-mouse IgG3 (Southern bio, 1101-01) (1:75)	
Tertiary ab	Donkey anti-rat IgG (abcam, ab150153) (1:50)	Donkey anti-goat IgG (abcam, ab175704) (1:75)	
Conjugate/dye	AF488	AF568	DAPI
Longpass filter	515LP	590LP	420LP

CD90+IL-6

	Targets		
	IL-6	**CD90**	**Nucleus**
Isotype (primary ab)	Mouse IgG2b (Santa-Cruz, SC-130326) (1:25)	Mouse IgG1 (Novus 7E1B11) (1:1000)	
Secondary ab	Rat anti-mouse IgG2b (Biolegend, RMG2b-1) (1:20)	Goat anti-mouse IgG1 (Southernbio, 10710-01) (1:100)	
Tertiary ab	Donkey anti-rat IgG (abcam, ab150153) (1:50)	Donkey anti-goat IgG (abcam, ab175704) (1:100)	
Conjugate/dye	AF488	AF568	DAPI
Longpass filter	515LP	590LP	420LP

CD34+IL-6

	Targets		
	IL-6	**CD34**	**Nucleus**
Isotype (primary ab)	Mouse IgG2b (Santa-Cruz, SC-130326) (1:25)	Mouse IgG1 (Abcam, Qbend10) (1:1000)	
Secondary ab	Rat anti-mouse IgG2b (Biolegend, RMG2b-1) (1:20)	Goat anti-mouse IgG1 (Southernbio, 10710-01) (1:100)	
Tertiary ab	Donkey anti-rat IgG (abcam, ab150153) (1:50)	Donkey anti-goat IgG (abcam, ab175704) (1:100)	
Conjugate/dye	AF488	AF568	DAPI
Longpass filter	515LP	590LP	420LP

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Statistics

Descriptive statistics were used. Median (range) were reported. Correlations were determined by Spearman's rank correlation coefficient. Data were analyzed with GraphPad Prism 9.2.0.

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Supplementary Tables

Supplementary Table S1. Relationship between IL-6 expression in bursa tissue and other parameters in patients with PMR. Semiquantitative score for IL-6 expression in tissue was determined. Data are shown for six patients with PMR. Correlations were determined by Spearman's rank correlation coefficient.

Correlation of IL-6 score in bursa tissue with other parameters	Spearman's rho	p value
Erythrocyte sedimentation rate	0.439	0.400
C-reactive protein	-0.257	0.733
Synovitis score according to Krenn *et al.*	0.051	0.933
Visual analogue scale pain in biopsied shoulder	0.676	0.200
Visual analogue scale stiffness in biopsied shoulder	0.507	0.333

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Supplementary Table S2. IL-6 expression in bursa tissue and glucocorticoid dose needed for induction of remission in patients with PMR. Semiquantitative score for IL-6 expression in tissue was determined. Data are shown for six patients with PMR. Five patients were initially treated with prednisolone 15 mg daily. If needed, the prednisolone dose was increased to 20 mg daily. One patient could be managed with repetitive intramuscular (IM) injections with methylprednisolone.

Patient	IL-6 IHC score	Glucocorticoid dose needed for induction of remission
A	4	Prednisolone 15 mg
B	4	Prednisolone 20 mg
C	2.5	Prednisolone 15 mg
D	3.5	Prednisolone 15 mg
E	4	Prednisolone 20 mg
F	4	Methylprednisolone 120 mg IM

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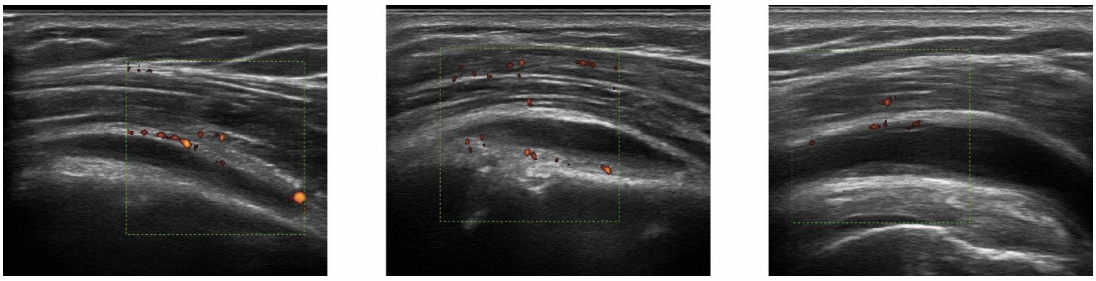
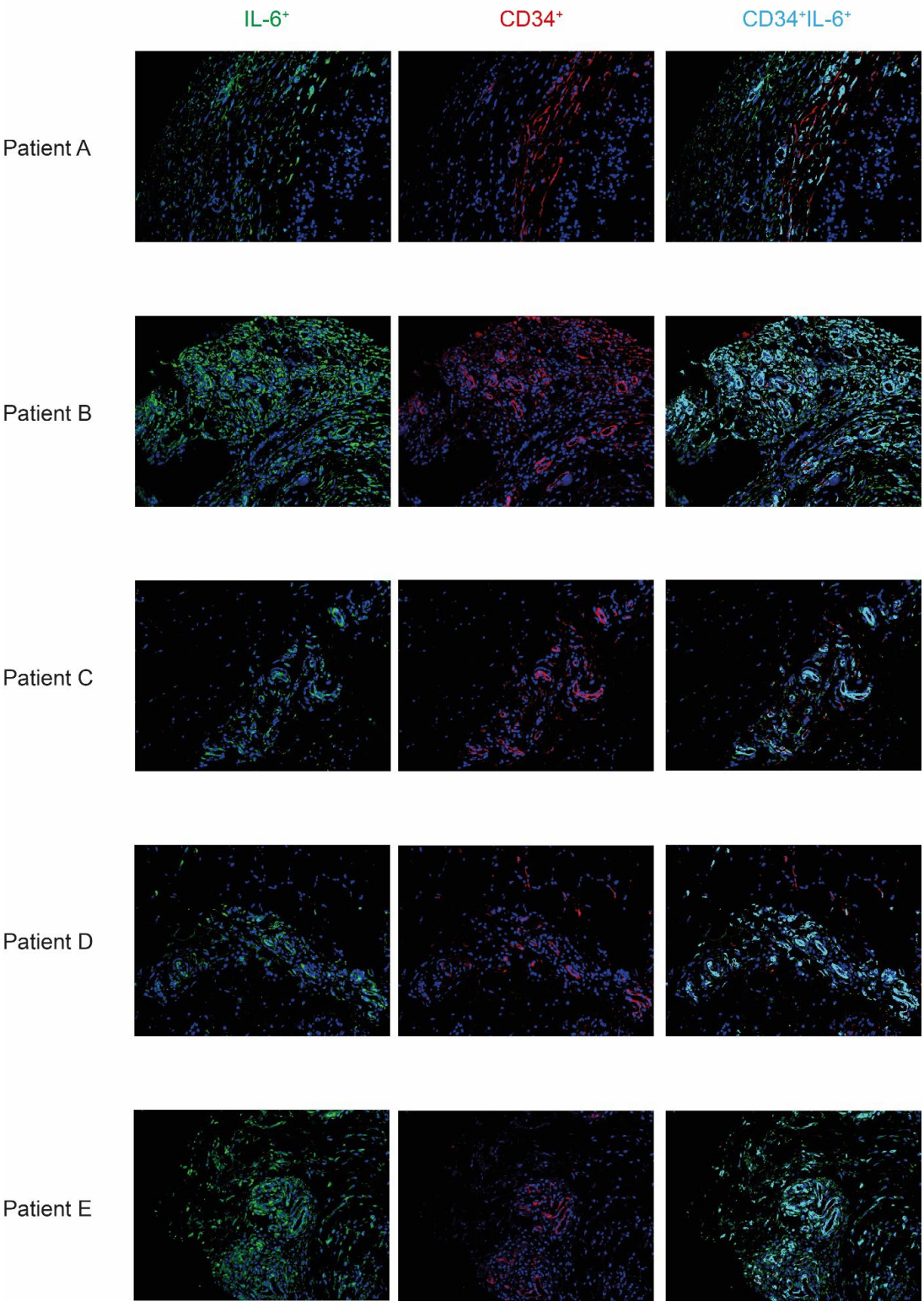
Supplementary figures

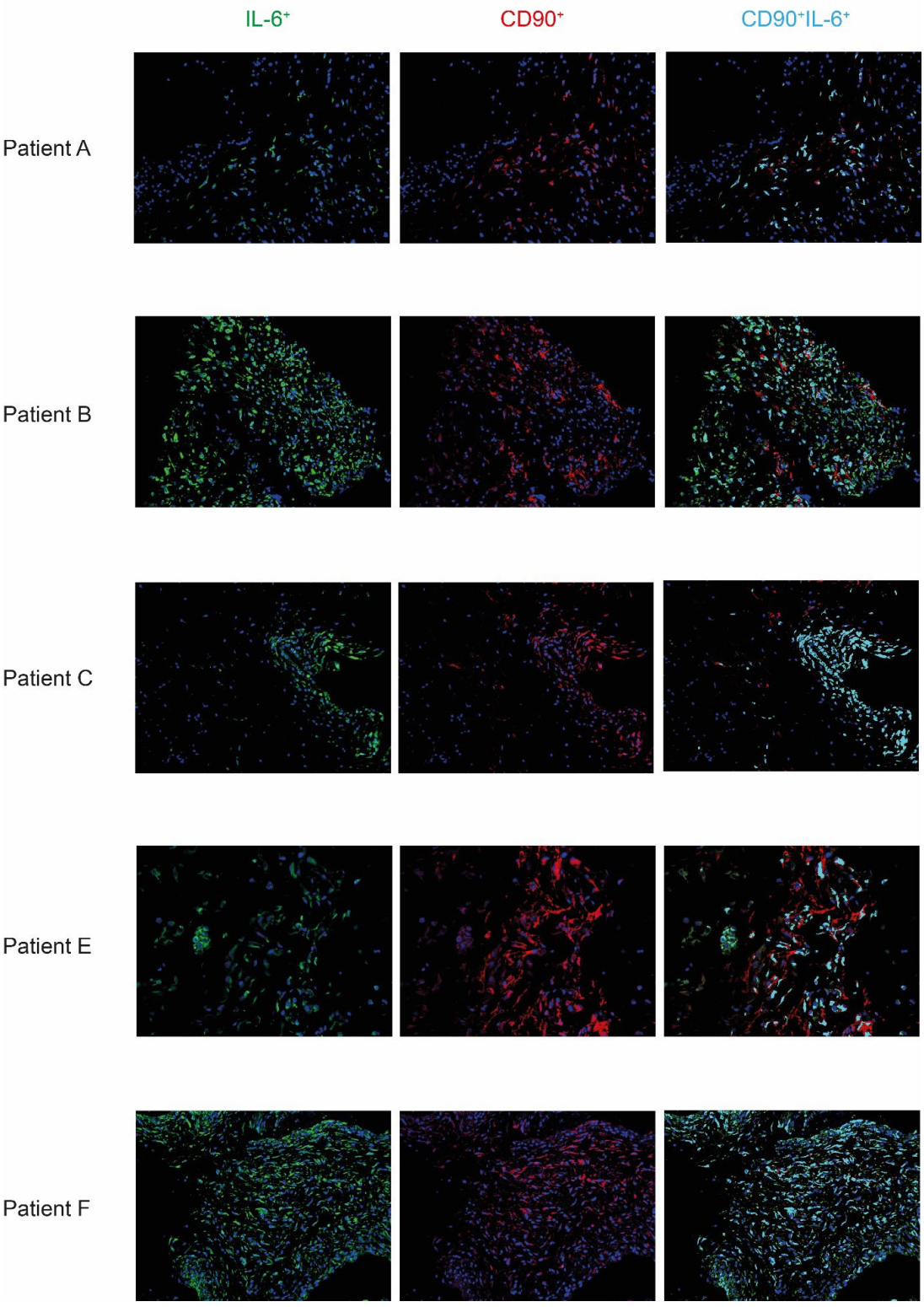
Supplementary Figure S1. Representative ultrasound images showing inflammation of the subacromial-subdeltoid bursa. Images of the biopsied bursae are shown for three different patients with PMR. The two images on the left show the bursa along the lateral edge of the greater tuberosity, where the bursa was forming a 'teardrop sign'. The image on the right depicts the bursa above the supraspinate tendon, as seen in the longitudinal view. Mild synovial hypertrophy and power Doppler signal are visible at the bursa wall.



Supplementary Figure S2. Co-expression of IL-6 and CD34 in the subacromial-subdeltoid bursa of patients with PMR. Immunofluorescence staining for IL-6 (green), CD34 (red) and co-localization of IL-6/CD34 (cyan) is depicted for the five remaining patients that were not shown in Figure 1.



Supplementary Figure S3. Co-expression of IL-6 and CD90 in the subacromial-subdeltoid bursa of patients with PMR. Immunofluorescence staining for IL-6 (green), CD90 (red) and co-localization of IL-6/CD90 (cyan) is depicted for the five remaining patients that were not shown in Figure 1.



Supplementary Figure S4. Co-expression of IL-6 and CD68 in the subacromial-subdeltoid bursa of patients with PMR. Immunofluorescence staining for IL-6 (green), CD68 (red) and co-localization of IL-6/CD68 (cyan) is depicted for the five remaining patients that were not shown in Figure 1.