Background: The immunomodulatory cytokine IL-16 is increased in several inflammatory and autoimmune diseases¹. IL-16 recruits and activates CD4+ immune cells such as T cells, dendritic cells, or monocytes. IL-16 is produced by various immune and non-immune cells, but synthesis and storage of IL-16 is regulated differentially depending on the cell type and stimulation. For its biological activity, IL-16 cleavage by caspase-3 is required¹. Necrotizing granulomatous inflammation is a hallmark of granulomatosis with polyangiitis (GPA) with neutrophil dysregulation as a central driver of chronic inflammation and autoimmunity². Earlier studies showed a correlation between increased serum IL-16 and disease parameters in AAV, including GPA³, but functional evidence for a direct link between IL-16 and neutrophils in granulomatous inflammation is missing so far.

Objectives: In this study we aim to identify a functional link between increased IL-16, neutrophils, and the autoantigen proteinase 3 (PR3) with regard to chronic inflammation and autoimmunity in GPA.

Methods: IL-16 was measured in sera of GPA patients (n = 40) and healthy controls (HC, n = 50) by ELISA and correlated with clinical features, such as disease activity (BVAS), creatinine, GFR, VDI and PR3-ANCA status. IL-16 protein expression was analyzed in peripheral blood mononuclear cells (PBMC) and polymorphonuclear cells (PMN) from GPA patients and HC (n = 5, each) by SDS-PAGE and western blot. Binding affinity of recombinant pro-IL-16 to native human PR3 was assessed by microscale thermophoresis. Cleavage of pro-IL-16 by active human PR3 was performed at various time points at 37°C. Cleavage products were analyzed by SDS-PAGE and western blot.

Results: Circulating IL-16 was significantly increased in GPA patients compared to HC. Elevated IL-16 positively correlated with BVAS, creatinine, VDI and PR3-ANCA status and negatively correlated with GFR. In PMBC and PMN from GPA and HC we identified different expression patterns of precursor and active forms of IL-16. In healthy PBMC we found high amounts of precursor (80kD), pro-IL-16 (55kD) and active IL-16 (17kD). In contrast, PBMC from GPA patients had lower amounts of pro-IL-16 and no active IL-16, indicating activation and secretion of IL-16 due to inflammatory stimulation, as shown earlier². In GPA PMN we detected no precursor IL-16, but pro-IL-16 and its active form, in contrast to very low amounts of all IL-16 forms in healthy PMN. Processing and release of IL-16 in neutrophils has been linked to apoptosis and secondary necrosis⁵. By interaction studies we demonstrated direct binding of pro-IL-16 to PR3 with a Kd of 10nM. In a subsequent cleavage assay we confirmed IL-16 processing by PR3 in a time-dependent manner.

Conclusion: Correlation of serum IL-16 with clinical features of GPA suggests that IL-16 is associated with markers of disease activity, tissue damage and auto-reactivity. We showed that PBMC and PMN represent a source of IL-16 in GPA. By the identification of PR3 as an additional IL-16-activating enzyme we could demonstrate a potential link between excessive PR3 expression, cell death and IL-16-dependent mechanisms, contributing to chronic granulomatous inflammation and autoimmunity in GPA.

REFERENCES:

- [1] Glass, W. G. et al. Not-so-sweet sixteen: The role of IL-16 in infectious and immune-mediated inflammatory diseases. *J. Interf. Cytokine Res.* 26, 511–520 (2006).
- [2] Millet, A. et al. Proteinase 3 on apoptotic cells disrupts immune silencing in autoimmune vasculitis. *J. Clin. Invest.* 125, 4107–4121 (2015).
- [3] Yoon, T. et al. Serum interleukin-16 significantly correlates with the Vasculitis Damage Index in antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Res. Ther.* 22, 1–6 (2020).
- [4] Elssner, A. et al. IL-16 Is Constitutively Present in Peripheral Blood Monocytes and Spontaneously Released During Apoptosis. *J. Immunol.* 172, 7721–7725 (2004).
- [5] Roth, S. et al. Secondary necrotic neutrophils release interleukin-16C and macrophage migration inhibitory factor from stores in the cytosol. *Cell Death Discov.* 1, 15056 (2015).

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OP0055

ULTRASOUND HALO SIGN AS A POTENTIAL MONITORING TOOL FOR PATIENTS WITH GIANT CELL ARTERITIS: A PROSPECTIVE ANALYSIS

C. Ponte^{1,2}, S. Monti^{3,4}, C. A. Scire^{5,6}, P. Delvino^{3,4}, N. Khmelinski^{1,2}, A. Milanesi³, V. Teixeira^{1,7}, F. Brandolino³, F. M. Diamantino Saraiva¹, C. Montecucco³, J. E. Fonseca^{1,2}, W. A. Schmidt⁸, R. Luqmani⁹. ¹Hospital de Santa Maria - Centro Hospitalar Universitário Lisboa Norte, Department of Rheumatology, Lisboa, Portugal; ²Instituto de Medicina Molecular (IMM), Rheumatology Research Unit, Lisboa, Portugal; ³Fondazione I.R.C.C.S. Policlinico San Matteo, Department of Rheumatology, Pavia, Italy; ⁴University of Pavia, PhD in Experimental Medicine, Pavia, Italy; ⁵Italian Society for Rheumatology, Epidemiology Research Unit, Milan, Italy; ⁶University of Ferrara, Rheumatology Unit, Department of Medical Sciences, Ferrara, Italy; ⁷Centro Hospitalar Universitário do Algarve, Department of Rheumatology, Faro, Portugal; ⁸Immanuel Hospital Berlin, Medical Centre for Rheumatology Berlin-Buch, Berlin, Germany; ⁹University of Oxford, Nuffield Department of Orthopaedics Rheumatology and Musculoskeletal Sciences, Oxford, United Kingdom

Background: Ultrasound of the temporal ± axillary arteries showing a non-compressible halo sign is recommended for diagnosing patients with giant cell arteritis (GCA); however, its value for monitoring disease activity is still poorly understood.

Objectives: To assess the sensitivity to change of ultrasound halo features and their association with disease activity and glucocorticoid (GC) treatment in patients with newly diagnosed GCA.

Methods: Two centre prospective study of new patients with ultrasound confirmed-GCA who underwent serial ultrasound assessments of the temporal (TA) and axillary (AX) arteries at fixed time-points. The number of arterial segments with halo and the maximum halo intima-media thickness (IMT) per segment was recorded at each visit. Only time-points in which >80% of patients were assessed were considered for analysis. Sensitivity to change of the halo sign was calculated as standardised mean difference (SMD) for each time-point separately. Correlation between ultrasound findings and erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), Birmingham Vasculitis Activity Score (BVAS), and GC treatment was performed using Spearman's correlation coefficient. Logistic regression was used to determine the probability of being in remission for each unit increase (standardised) of the halo feature of interest. Remission was defined as the absence of relapse with a prednisolone dose <30 mg/day, and relapse as the recurrence of GCA-related symptoms or rise of CRP/ESR values not otherwise explained and requiring GC increase. Halo features at disease onset and first relapse were compared using Wilcoxon test.

Results: A total of 49 patients (73.5% females) with a mean age of 78.2 ± 7.4 years at baseline were assessed at 354 visits. TA involvement was reported in 47 (95.9%) patients and AX involvement in 11 (22.4%); 220 arterial segments with halo were recorded (201 TA, 19 AX). Halo sensitivity to change was calculated for weeks 1, 3, 6, 12 and 24, and showed a significant SMD between all time-points and baseline for the TA halo features, but only after week 6 for the AX halo features (Table and Figure). The number of TA segments with halo, sum of TA halo IMT and maximum TA halo IMT showed a significant correlation with ESR (0.41; 0.44; 0.48), CRP (0.34; 0.39; 0.41), BVAS (0.29; 0.36; 0.35) and GC cumulative dose (-0.34; -0.37; -0.32). The likelihood of achieving disease remission was lower in patients with a higher number of TA segments with halo (OR 0.39, p<0.05) and increased values of sum and maximum TA halo IMT (OR 0.34, p<0.05). By contrast, AX halo features showed no correlation with disease activity, nor any association with attaining clinical remission. During the study period, 32 relapses were observed (mean time for first relapse of 31.8 weeks ± 18.5 days). Halo sign was present in 16/17 (94.1%) cases of first disease relapse, all showing an increased halo IMT (sum and maximum) in relation to the previous ultrasound assessment performed. When compared to disease onset, a lower mean number of segments with halo and mean sum of halo IMT was reported for patients presenting with their first clinical relapse (2.93 ± 1.59 vs. 4.85 ± 1.51, p=0.0012; 2.01 ± 1.13 vs. 4.49 ± 1.95 mm, p=0.0012).

Table 1. SMD of halo features between baseline and different time-points

	Week 1	Week 3	Week 6	Week 12	Week 24
N of arterial segments					
Sum of all segments with halo (n=49)	-0.51	-0.78	-1.13	-1.69	-1.52
Sum of TA segments with halo (n=47)	-0.49	-0.78	-1.18	-1.87	-1.69
Sum of AX segments with halo (n=11)	-0.35	-	-0.62	-0.73	-0.91
Halo thickness (mm)					
Sum of all halo IMT (n=49)	-0.98	-1.44	-1.37	-1.60	-1.48
Sum of TA halo IMT (n=47)	-1.01	-1.55	-1.54	-1.81	-1.69
Sum of AX halo IMT (n=11)	-0.15	-0.45	-0.81	-0.84	-0.98
Max. TA halo IMT (n=47)	-1.07	-1.32	-1.47	-1.91	-2.19
Max. AX halo IMT (n=11)	-0.04	-0.29	-0.94	-1.13	-1.01

In bold p<0.05; n=n at baseline

Conclusion: Ultrasound is a reliable imaging tool to assess disease activity and response to treatment in patients with GCA. Future clinical trials in GCA should evaluate direct treatment effect on halo features as an outcome measure of interest.

Figure: Mean variation (not standardised) of halo features at different time-points

Eight arterial segments were evaluated at each time-point: bilateral common superficial temporal arteries, parietal and frontal branches, and bilateral axillary arteries

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