Rapid paper

Identification of two autoantigens recognised by circulating autoantibodies as potential biomarkers for diagnosing giant cell arteritis

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ABSTRACT

Objective. *Giant cell arteritis (GCA) is a common vasculitis affecting patients aged 50 and older. GCA leads to chronic inflammation of large/medium-sized vessel walls with complications such as permanent vision loss and risk of stroke and aortic aneurysms. Early diagnosis is crucial and relies on temporal artery biopsy (TAB) and ultrasound imaging of temporal and axillary arteries. However, these methods have limitations. Serum biomarkers as autoantibodies have been reported but with inconclusive data for their use in the clinical setting. Additionally, C-reactive protein and erythrocyte sedimentation rate are non-specific and limited in reflecting disease activity, particularly in patients treated with IL-6 inhibitors. This study aimed to identify serum autoantibodies as new diagnostic biomarkers for GCA using a human protein array.*

Methods. *One commercial and one proprietary human protein array were used for antibody profiling of sera from patients with GCA (n=55), Takayasu (TAK n=7), and Healthy Controls (HC n=28). The identified candidate autoantigens were purified and tested for specific autoantibodies by ELISA.*

Results. *Antibodies against two proteins, VSIG10L (V-Set and Immunoglobulin Domain Containing 10 Like) and DCBLD1 (discoidin), were identified and found to be associated with GCA, with an overall prevalence of 43–57%, respectively, and high specificity as individual antibodies. A control series of TAK sera tested negative.*

Conclusion. *Detecting GCA-specific autoantibodies may offer a new, non-invasive tool for improving our diagnostic power in GCA. Even though cell-mediated immune responses are crucial for GCA pathogenesis, this finding opens the way for investigating the additional role of humoral immune responses in the disease.*

Introduction

Giant cell arteritis (GCA) is the most frequent primary systemic vasculitis in patients aged ≥50 years, leading to chronic inflammation of the large- and medium-sized vessel walls. The most dreadful complication of GCA is permanent vision loss. The disease can cause other ischaemic complications, such as stroke, and increases the risk for aortic aneurysms and dissection, warranting long-term monitoring (1). Correct and early diagnosis of GCA is crucial due to its serious complications and the requirement of high-dose glucocorticoids or adjunctive immunosuppressive drugs to control relapses in up to 40–50% of patients (2).

The gold standard for diagnosing GCA has long been temporal artery biopsy (TAB). Nonetheless, this invasive procedure has limitations, including invasiveness, low sensitivity (as low as 40-60%), and operator-dependent results (3, 4). Recent advancements have led to the adoption of ultrasound imaging of temporal and axillary arteries as a reliable first-line tool for GCA diagnosis, offering high sensitivity and specificity. Ultrasound is non-invasive, cost-effective, and repeatable, and it extends diagnostic evaluation to extra-cranial arteries, enhancing diagnostic accuracy and allowing early diagnosis when incorporated into fast-track clinics (5, 6).

However, access to specialised clinics with expert sonographers and adequate equipment is limited in Europe. Moreover, the timing of imaging studies in relation to glucocorticoid treatment is crucial. As false positive ultrasound findings have been described, interpreting results in the context of clinical presentation and pre-test probability is required to avoid misinterpretation (5). Specific diagnostic biomarkers for GCA are lacking. C-reactive protein (CRP) and erythrocyte sedimentation rate are the only biomarkers currently used in clinical practice; however, they are non-specific, do not consistently reflect disease activity, and have limited value in assessing patients treated with IL-6 inhibitors such as tocilizumab (2). Research is ongoing to identify new biomarkers for GCA diagnosis and monitoring. While crucial in diagnosing other rheumatic conditions, autoantibodies have not been consistently found in GCA, and no specific autoantibody marker for GCA diagnosis has yet been identified (7-10).

The study aimed to identify serum autoantibodies detectable in GCA patients through human protein array analysis and explore their diagnostic value.

Materials and methods

Patients and study design

We included 55 patients with GCA classified according to the 2022 ACR/EULAR classification criteria for GCA (1). Seven Takayasu's arteritis (TAK) patients classified according to Grayson *et al*. (11) were included as a pathological control group of a different large vessel vasculitis. All the samples have been collected at the time of the diagnosis. Twenty-eight age/sex-matched healthy controls (HC) were also investigated.

Serum samples were collected according to standard operating procedures in 3 different structures: University of Milan, Italy, University of Bern, Switzerland, and University of Modena-Reggio Emilia, Reggio Emilia, Italy. Sera were aliquoted and stored at -80°C. The study was approved by the Ethics Committee at Istituto Auxologico Italiano, Milan, Italy (code 2019-_05_21_16).

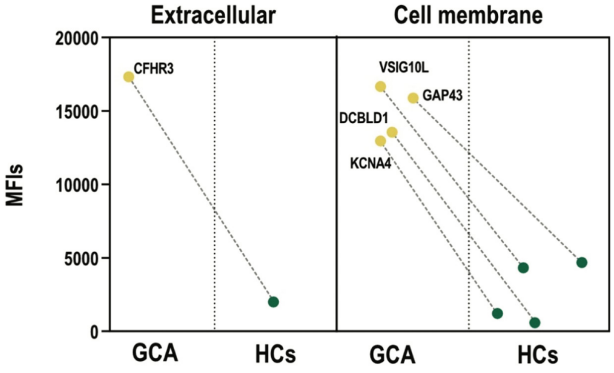

Fig. 1. Candidate autoantigens specifically recognised by GCA compared to healthy control (HC) sera. Correlation plot illustrating the different signal intensity between GCA and HC sera for the five selected proteins localised in the extracellular space and cell membrane.

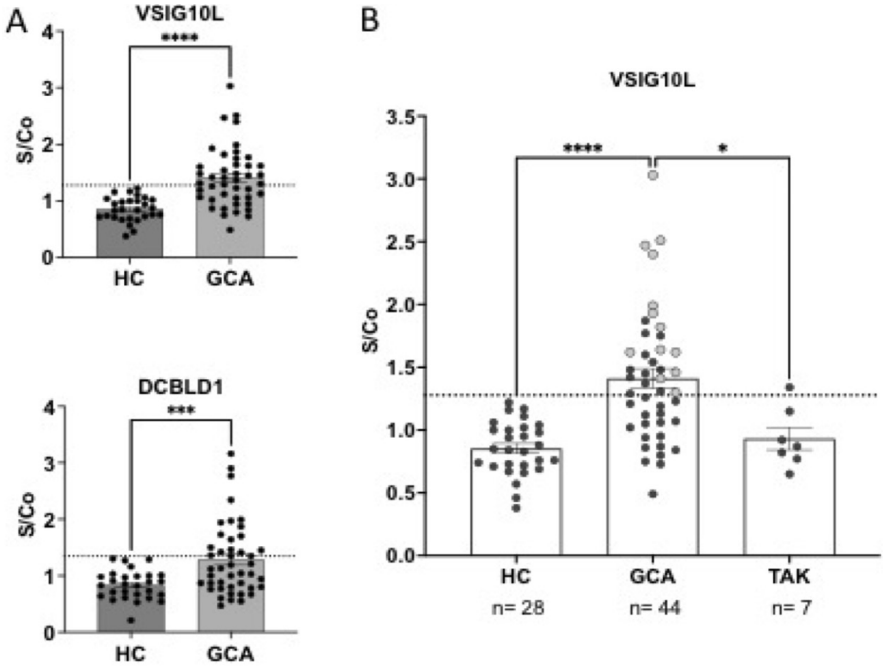

Fig. 2. A) VSIG10L and DCBLD1 display a higher immunoreactivity with sera of GCA patients than HC. Box plots show the reactivity of the recombinant proteins VSIG10L and DCBLD1 when incubated with individual sera from a total of 44 GCA patients and 28 HC by ELISA. B) Reactivity of GCA, TAK, and HC sera against VSIG10L alone (●) or both VSIG10L and DCBLD1 (●). The reactivity is expressed as Signal/Control ratio (S/Co). Dotted line represents the threshold. Unpaired Mann-Whitney test and Kruskal-Wallis test.
**p*<0.05, ****p*<0.001, *****p*<0.0001.

For the discovery phase, 20 sera were used (11 GCA and 9 age/sex-matched HC; three pools of 3-4 sera), whereas for the validation phase 79 individual sera were used (44 GCA, 7 TAK, 28 HC).

Autoantibody profiling by protein array

Sera of the discovery phase were tested in pools (3–4 sera each) on i) ProtoArray v. 5.0 Human Protein Microarray (Thermo Fisher Scientific), containing over 9,000 unique GST-fusion proteins, and ii) a proprietary human protein array including approximately 1,650 His-tagged proteins predicted to be secreted or membrane-associated. Further details on protein array, staining, detection and data analysis are provided in the Supplementary material.

Expression and purification of candidate autoantigens

Candidate autoantigens were expressed as His-tagged recombinant forms in *E. coli* system and purified using $Ni2^{+}$-NTA affinity chromatography by AktaPure (GE Life Sciences) (Supplementary material).

ELISA assays

Autoantigen validation was performed by ELISA on plates coated with the recombinant proteins, using sera of the validation phase (Suppl. material).

Statistical analysis

Data normalisation and statistical analysis of protein array data were performed using R software (v. 4.0.5, R Core Development Team). All statistical analyses and plotting were conducted with Prism 8 (GraphPad Software). This included frequency comparisons using the chi-square test or Fisher's exact test (when expected frequencies were less than five), and differences between serum groups were analysed with two-way ANOVA. Sensitivity, specificity, and Receiver operating characteristic (ROC) curve analysis were carried out using CombiROC (12). A two-sided *p*-value of less than 0.05 was considered statistically significant.

Results

Identification of new autoantigens recognised by GCA sera

In the discovery phase, we performed an autoantigen profiling analysis of pooled sera using the two human protein arrays (see *Materials and methods* section). We observed that GCA sera exhibited significantly higher reactivity than HC sera for the spotted proteins on both of the two arrays (positive thresholds: GCA sera, MFI>12,500; HC sera, MFI<5,000). Collectively, fifty-nine autoantigens recognised by at least 2 out of 3 of the pools of GCA sera but not by HC sera were selected (Suppl. Table S1). Specifically, we focused the analysis on five proteins that showed the highest MFI values, and were prioritised to be predicted as extracellular or associated with the plasma membrane (CFHR3 extracellular; KCNA4, GAP43, DCBLD1 and VISIG10L, cell membrane) (Fig. 1 and Suppl. Table S1), as assessed by the DeepLoc 2.0 algorithm.

VSIG10L and DCBLD1 were confirmed to be highly associated with GCA

We confirmed the reactivity of the five selected autoantigens by ELISA, using the validation set of sera (44 GCA patients and 28 HC). Among the five autoantigens tested, VSIG10L (V-Set and immunoglobulin domain containing protein 10 like) and DCBLD1 (discoidin) displayed significantly higher reactivity with GCA than HC sera (Fig. 2A). Specifically, 25 out of 44 GCA patients (57%) were positive for antibodies against VSIG10L, while 19 out of 44 patients (43%) were positive for anti-DCBLD1 IgG. We tested seven TAK sera as a further pathological control group. Only 1 out of 7 TAK sera showed borderline reactivity against these two proteins. Interestingly, among 25 GCA patients positive for VSIG10L, 13 (52%) also had antibodies against DCBLD1, suggesting that the combination of these autoantibodies may be more effective for diagnosis than either antibody alone (Fig. 2B).

Finally, we evaluated the sensitivity and specificity of antibodies against DCBLD1, VSIG10L, and their combination in discriminating patients with GCA from controls using ROC analysis. Overall, anti-DCBLD1 and anti-VSIG10L antibodies demonstrated very high specificity (SP) as individual autoantibodies (SP DCBLD1=1; SP VSIG10L=0.964). The combination of the two autoantibodies showed a slight improvement in performance, as assessed by the area under the curve (AUC: 0.73, 0.86, and 0.88 for DCBLD1, VSIG10L, and combination, respectively) (Fig. 3).

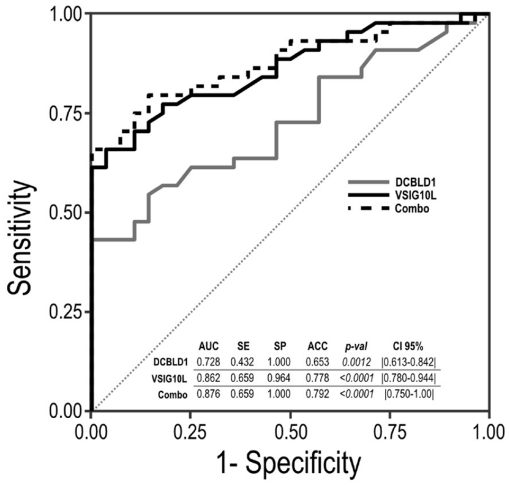

Fig. 3. ROC curves of DCBLD1 and VSIG10L antibodies predicting ELISA positivity against GCA. ROC curves were performed using data of the serology against the DCBLD1 and VSIG10L obtained from 44 GCA patients who exhibited anti-DCBLD1 and anti-VSIG10L antibodies and 28 who did not. The area under the curve (AUC), *p*-value of the ROC, sensitivity (SE) and specificity (SP) with 95% confidence intervals are reported. Accuracy (ACC) is also shown.

Discussion

We report the presence of circulating antibodies reacting against two self-proteins (DCBLD1 and VSIG10L) as new biomarkers in sera of active GCA patients, with an overall prevalence of 43-57% and a specificity >96%. More than half of the GCA patients positive for anti-VSIG10L antibodies also display anti-DCBLD1 IgG, suggesting that detecting both antibodies may increase their diagnostic accuracy. The findings support their diagnostic usefulness in addition to the gold standard tools, the TAB and imaging methods such as ultrasound of temporal and axillary arteries.

Anti-DCBLD1 and anti-VSIG10L IgG have been detected in GCA but not in TAK sera. Takayasu arteritis displays an immune-mediated granulomatous vascular wall inflammation of the aorta and its major branches. The pathogenesis shares many features with GCA, but TAK is a large-vessel vasculitis of adolescents and young adults (11). The lack of anti-DCBLD1 and anti-VSIG10L IgG in TAK strongly supports a diagnostic value in GCA. Moreover, these antibodies cannot be detectable in age-matched healthy controls, ruling out an association with age.

Even though cell-mediated immune responses are the pillar of GCA pathogenesis, there is also sound evidence that humoral immune responses may play a role. For example, effector B cells, but not IL-10-positive regulatory B cells, decrease in newly diagnosed GCA or polymyalgia rheumatica patients but normalise after treatment (8, 13). Moreover, artery tertiary lymphoid

organs (ATLOs) were found in 60% of GCA arterial biopsies accompanied by the expression of B cell growth factors and B cell attracting chemokine 1 (14). Circulating antibodies against ferritin, serum 14-3-3 proteins, or proteic lysates of human umbilical endothelial/vascular smooth muscle cells have been described in GCA but also in other vasculitides. This makes their diagnostic value less stringent (7, 9, 10, 15).

Despite the lack of information on the presence of VSIG10L and DCBLD1 in TAB from GCA patients, the characterisation of these molecules as cell membrane proteins is consistent with the hypothesis that they can be potential autoantigen targets for circulating autoantibodies. It is useful to speculate whether autoantibodies reacting with cell membrane antigens may directly damage the cell targets or alternatively may affect their function and contribute to vascular inflammation and remodelling in GCA (6).

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Competing interests

R. Caporali has received speaker's and consultation fees from Abbvie, AstraZeneca, Alfa-Sigma, Celltrion, Galapagos, GSK, Janssen, Lilly, MSD, Novartis, Pfizer, UCB. The other authors have declared no competing interests.

References

1. PONTE C, GRAYSON PC, ROBSON JC *et al*.: 2022 American College of Rheumatology/EULAR Classification Criteria for Giant Cell Arteritis. *Arthritis Rheumatol* 2022; 74(12): 1881-89. https://doi.org/10.1002/art.42325
2. HELLMICH B, AGUEDA A, MONTI S *et al*.: 2018 Update of the EULAR recommendations for the management of large vessel vasculitis. *Ann Rheum Dis* 2020; 79(1): 19-30. https://doi.org/10.1136/annrheumdis-2019-215672
3. LUQMANI R, LEE E, SINGH S *et al*.: The role of ultrasound compared to biopsy of temporal arteries in the diagnosis and treatment of giant cell arteritis (TABUL): a diagnostic accuracy and cost-effectiveness study. *Health Technol Assess* 2016; 20(90): 1-238. https://doi.org/10.3310/hta20900
4. RESTUCCIA G, BOIARDI L, CAVAZZA A *et al*.: Flares in biopsy-proven giant cell arteritis in northern Italy: characteristics and predictors in a long-term follow-up study. *Medicine* (Baltimore) 2016; 95(19): e3524. https://doi.org/10.1097/md.0000000000003524
5. DEJACO C, RAMIRO S, BOND M *et al*.: EULAR recommendations for the use of imaging in large vessel vasculitis in clinical practice: 2023 update. *Ann Rheum Dis* 2024; 83(6): 741-51. https://doi.org/10.1136/ard-2023-224543
6. CICCIA F, MACALUSO F, MAURO D, NICOLETTI GF, CROCI S, SALVARANI C: New insights into the pathogenesis of giant cell arteritis: are they relevant for precision medicine? *Lancet Rheumatol* 2021; 3(12): e874-85. https://doi.org/10.1016/S2665-9913(21)00253-8
7. RÉGENT A, DIB H, LY KH *et al*.: Identification of target antigens of anti-endothelial cell and anti-vascular smooth muscle cell antibodies in patients with giant cell arteritis: a proteomic approach. *Arthritis Res Ther* 2011; 13(3): R107. https://doi.org/10.1186/ar33889
8. SCHMITS R, KUBUSCHOK B, SCHUSTER S, PREUSS KD, PFREUNDSCHUH M: Analysis of the B cell repertoire against autoantigens in patients with giant cell arteritis and polymyalgia rheumatica. *Clin Exp Immunol* 2002; 127(2): 379-85. https://doi.org/10.1046/j.1365-2249.2002.01751.x
9. BAERLECKEN NT, LINNEMANN A, GROSS WL *et al*.: Association of ferritin autoantibodies with giant cell arteritis/polymyalgia rheumatica. *Ann Rheum Dis* 2012; 71(6): 943-7. https://doi.org/10.1136/annrheumdis-2011-200413
10. RÉGENT A, LY KH, BLET A *et al*.: Contribution of antiferritin antibodies to diagnosis of giant cell arteritis. *Ann Rheum Dis* 2013; 72(7): 1269-70. https://doi.org/10.1136/annrheumdis-2012-202963
11. GRAYSON PC, PONTE C, SUPPIAH R *et al*.: 2022 American College of Rheumatology/EULAR classification criteria for Takayasu arteritis. *Ann Rheum Dis* 2022; 81(12): 1654-60. https://doi.org/10.1136/ard-2022-223482
12. MAZZARA S, ROSSI RL, GRIFANTINI R, DONIZETTI S, ABRIGNANI S, BOMBACI M: (2017) CombiROC: an interactive web tool for selecting accurate marker combinations of omics data. *Sci Rep* 2017; 7: 45477. https://doi.org/10.1038/srep45477
13. VAN DER GEEST KS, ABDULAHAD WH, CHALAN P *et al*.: Disturbed B cell homeostasis in newly diagnosed giant cell arteritis and polymyalgia rheumatica. *Arthritis Rheumatol* 2014; 66(7): 1927-38. https://doi.org/ 10.1002/art.38625
14. CICCIA F, RIZZO A, MAUGERI R *et al*.: Ectopic expression of CXCL13, BAFF, APRIL and LT-β is associated with artery tertiary lymphoid organs in giant cell arteritis. *Ann Rheum Dis* 2017; 76(1): 235-43. https://doi.org/ 10.1136/annrheumdis-2016-209217
15. KISTNER A, BIGLER MB, GLATZ K *et al*.: Characteristics of autoantibodies targeting 14-3-3 proteins and their association with clinical features in newly diagnosed giant cell arteritis. *Rheumatology* (Oxford) 2017; 56(5): 829-34. https://doi.org/10.1093/rheumatology/kew469