SERUM S100 PROTEINS AS A MARKER OF DISEASE ACTIVITY IN LARGE VESSEL VASULITIS

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ABSTRACT

Objectives: Serum S100A8/S100A9 and S100A12 levels have been shown to be elevated in giant cell arteritis (GCA). This study aimed to determine if levels of serum S100 proteins perform as markers in a comparable fashion to standard markers of disease activity in large-vessel vasculitis.

Methods: Serum samples were obtained from the Vasculitis Clinical Research Consortium (VCRC) Longitudinal Study of GCA and Takayasu's arteritis (TAK). A mixed effects model compared S100 proteins during active and inactive disease states. Receiver operating characteristic curves compared models using S100 proteins to models using ESR and CRP.

Results: There were 106 samples (50 during active disease) from patients with GCA and 32 samples (16 during active disease) from patients with TAK. In GCA, S100A8/S100A9 and S100A12 were significantly elevated during active disease (2150 ng/mL vs. 2020 ng/mL, p = 0.003;150 ng/mL vs. 130ng/mL, p=0.016, respectively). There were weak correlations between levels of S100 proteins and ESR or CRP. A model including S100A8/S100A9, S100A12, ESR, and CRP was a better indicator of disease activity compared to ESR and CRP together. In TAK, there were no significant differences between active and inactive disease for either the S100 proteins or ESR/CRP.

Conclusions: Serum levels of S100A8/S100A9 and S100A12 are elevated during active disease and perform comparably to ESR and CRP as measures of disease activity in giant cell arteritis.

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INTRODUCTION

Giant cell arteritis (GCA) and Takayasu's arteritis (TAK) are forms of large-vessel vasculitis characterized by similarities in histological changes as well as the distribution of vessel involvement. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are widely used clinical inflammatory markers in the assessment of disease activity in both GCA and TAK. However, there are limitations to the clinical use of these biomarkers. In TAK, the ESR and CRP have been shown to be normal in up to 30% and 48%, respectively, of patients with clinically active disease (1, 2). In GCA, the sensitivity of ESR and CRP for predicting a positive temporal artery biopsy is 84% and 87% respectively (3). Furthermore, autopsy studies have suggested that smoldering large-vessel vasculitis can exist that is not recognized clinically (4). A search for improved biomarkers is warranted.

Calgranulins, including S100A8 (also known as calgranulin A, myeloid-related protein 8), S100A9 (also known as calgranulin B, myeloid-related protein 14) and S100A12 (also known as calgranulin C, myeloid-related protein 6), are calcium binding proteins expressed during myeloid differentiation. S100A8 and S100A9 are found in granulocytes, monocytes, and early differentiation stages of macrophages and form heterodimeric complexes in a calcium-dependent manner. In contrast, S100A12 is more restricted to granulocytes. Calgranulins are linked with the innate immune response, and upon release by myeloid cells serve as damage-associated molecular pattern molecules (DAMPs). S100A12 is able to bind to and activate the receptor of advanced glycation end products (RAGE) expressed on vascular smooth muscle cells, mononuclear cells and endothelial cells and initiate a proinflammatory danger signal (5). Previous studies have shown that S100A8/S100A9 are useful markers for disease activity for various rheumatic diseases including oligoarticular and polyarticular juvenile rheumatoid

arthritis (JRA), ulcerative colitis, systemic lupus erythematosus, reactive arthritis, and rheumatoid arthritis (6-11).

Prior studies have also demonstrated the presence of S100A8/A9 and S100A12 in biopsy specimens in GCA and TAK patients. In a Spanish cohort (12) of 35 temporal artery biopsies with active vasculitis it was demonstrated that S100A8/S100A9 complex can be identified in the adventitia and media of affected arteries, whereas S100A12 expression is found around the vasa vasorum of the adventitia. In the same cohort, serum concentrations by ELISA of both S100A8/S100A9 and S100A12 were significantly higher in patients with giant cell arteritis than in healthy non-matched controls (12). Moreover, gene expression profiles of temporal arteries from patients with GCA, revealed a significant increase in the gene expression of S100A8, S100A9, S100A12 as compared to control temporal arteries. Interestingly, the gene expression for these S100 proteins were significantly increased even when no inflammation was seen on temporal arteries of GCA patients suggesting that S100A8, S100A9 and S100A12 may play an early role in the inflammatory cascade (13). In addition, S100 positive cells have been found to be prominent in the inflammatory infiltrate of the aortic wall of patients with TAK (14).

This study is aimed to assess the association of S100 proteins with disease activity in two forms of large vessel vasculitis, GCA and TAK, and to determine the role of these markers in predicting relapse of disease activity compared to the standard inflammatory markers, ESR and CRP.

METHODS

Serum samples were obtained from the Vasculitis Clinical Research Consortium (VCRC) Longitudinal Study of GCA and TAK. The VCRC is an integrated group of academic medical centers, patient support organizations, and clinical research resources dedicated to conducting clinical research in different forms of vasculitis. This study is in compliance with the Declaration of Helsinki and has been approved by the Cleveland Clinic institutional review board.

All patients met either the modified American College of Rheumatology (ACR) criteria for GCA or the modified ACR criteria for TAK(15, 16). There were a total of 106 samples from 59 patients with GCA and 32 serum samples from 16 patients with TAK. Physicians completed a physician global assessment (PGA) and Birmingham Vasculitis Activity Score version 1 (BVAS1). Active disease was defined as BVAS1 score greater than then 0 and a physician's global assessment greater than 0. The serum concentrations of S100A8/S100A9 heterodimers and S100A12 were measured by ELISA as described previously (12).

A mixed effects model was used to compare average levels of S100A8/S100A9 or S100A12 in active and inactive disease states. Actual modeling was done using measurements on the log scale and then estimates and confidence intervals were converted back onto the original measurement scale. Estimated differences between active and inactive disease states therefore are represented by multiplicative factors. Logistic regression models were used to predict disease state from marker levels. Two separate models were fit, one involving the traditional measurements and the other the two S100 measurements. For each model, the

inclusion of the spline terms was investigated using bootstrap validation, and as a result ESR was included as a cubic spline with 4 knots.

The qualities of predictions of disease activity from these models using either the traditional measures (ESR and CRP) or the two new measurements (S100A8/S100A9 and S100A12) were assessed using receiver operating characteristic (ROC) curves and were based on the area under the curve (AUC). Confidence intervals for AUC were produced using the DeLong approach.

Correlations between the markers were described separately for the active disease and inactive disease states, to avoid potential effects on correlations due to repeated measurements on patients. The type of correlation used for this was Kendall's rank correlation tau. All analyses were done using R software (version 2.15.1, Vienna, Austria). A significance level of 5% was used for all testing.

RESULTS

Giant Cell Arteritis

Serum samples were obtained from 59 patients with GCA (**Table 1**). The majority of patients were female (74.6%) and Caucasian (98.3%). The average age at study registration was 71 years old. Forty-two patients (71%) had positive temporal artery biopsies diagnostic for GCA. Forty-six (78%) patients had samples drawn during both active and inactive disease.

Table 1: Baseline Patient Characteristics

		Giant Cell Arteritis		Takayasu's Arteritis	
Factor		N	Statistic	N	Statistic
		59		16	
Positive TAB[a]		42	71.2%		
Sex[a]	Female	44	74.6%	14	87.5%
	Male	15	25.4%	2	12.5%
Race[a]					
	Asian	1	1.7%	1	6.3%
	White	58	98.3%	15	93.8%
Age at enrollment (years)[b]		59	70.65±13.28		35.4±12.8
Age at visit	Active	50	70.9(67.5,74.4)		
(by sample,years)[bd]	Inactive	56	71.5(68.1,74.9)		
Observation times		59		16	
	Active and Inactive	46	78.0%	14	87.5%
	Both Active	1	1.7%		
	Just Active	2	3.4%	1	6.3%
	Just Inactive	10	16.9%	1	6.3%
Medications[a]		Active	Inactive		
	Prednisone	84.0%	92.7%	75.0%	68.8%
	Duration (months)[c]	2	3	15	3.5
	Aspirin	58.0%	66.4%	68.8%	68.8%
	Azathioprine	0.0%	0.0%	12.5%	18.8%
	Cyclophosphamide	2.0%	0.0%	0%	0%
	Infliximab	0.0%	0.0%	12.5%	18.8%
	Methotrexate	14.0%	23.6%	37.5%	50.0%
	Mycophenolate	0.0%	0.0%	6.3%	0.0%

[a]Percentage; [b]Mean±SD;[c]Median;[d]p<0.001, TAB = temporal artery biopsy

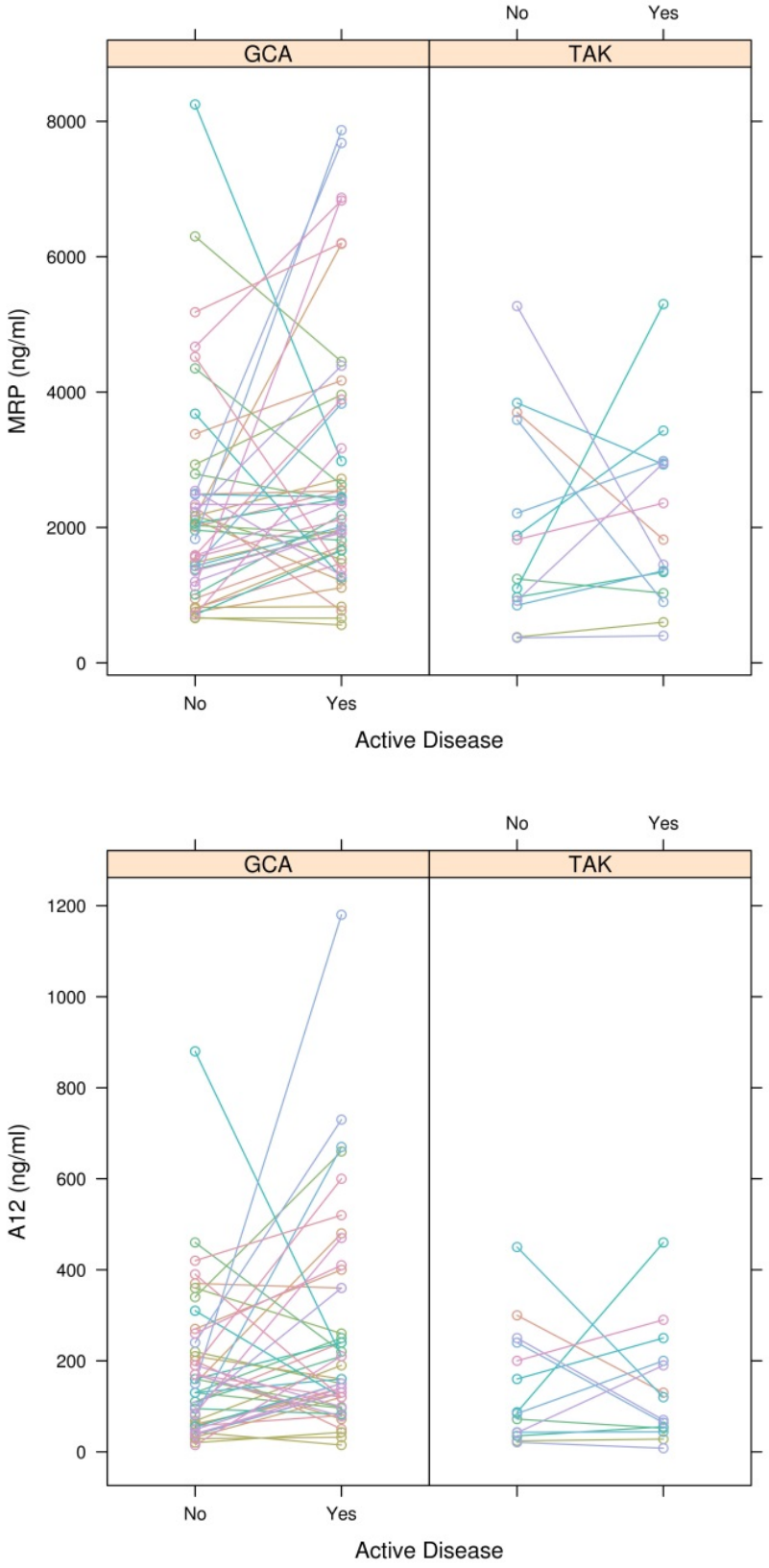
There were significantly higher CRP serum levels during active (7.8 mg/L [IQR 3.0-30.6]) compared with inactive disease (5.2 mg/L [IQR 3.0-8.5]) (p = 0.015) and there was a non-significant trend toward a high ESR during active disease (26.5 mm/h [12-44] versus 15.5 mm/h [IQR 10-25]; p = 0.066). Serum S100A8/A9 and S100A12 levels were measured on 50 samples during active disease and 56 samples during inactive disease. The mean serum levels of S100A8/9 and S100A12 were both significantly higher during active disease compared with inactive disease; S100A8/9: 2150 ng/mL [IQR 1563,3665] vs. 2020 ng/mL [IQR 1355,2493], p = 0.003; S100A12: 150 ng/mL [IQR 98,335] vs. 130ng/mL [IQR 60,213], p=0.016 (**Table 2, Figure 1**).

Table 2: Summary of serum levels[1] of tested biomarkers in inactive and active disease in patients with GCA

		Inactive Disease		Active Disease		
Biomarker	Total Samples	N	Median [IQR]	N	Median [IQR]	p-value
ESR (mm/hr)	100	54	15.5 [10.2, 25]	46	26.5 [12.2, 43.8]	0.066
CRP (mg/L)	94	50	5.2 [3, 8.5]	44	7.8 [3, 30.6]	0.015
S100A8/9 (ng/mL)	106	56	2020 [1355, 2492.5]	50	2150 [1562.5, 3665]	0.003
S100A12 (ng/mL)	106	56	130 [60, 212.5]	50	150 [98.2, 335]	0.016

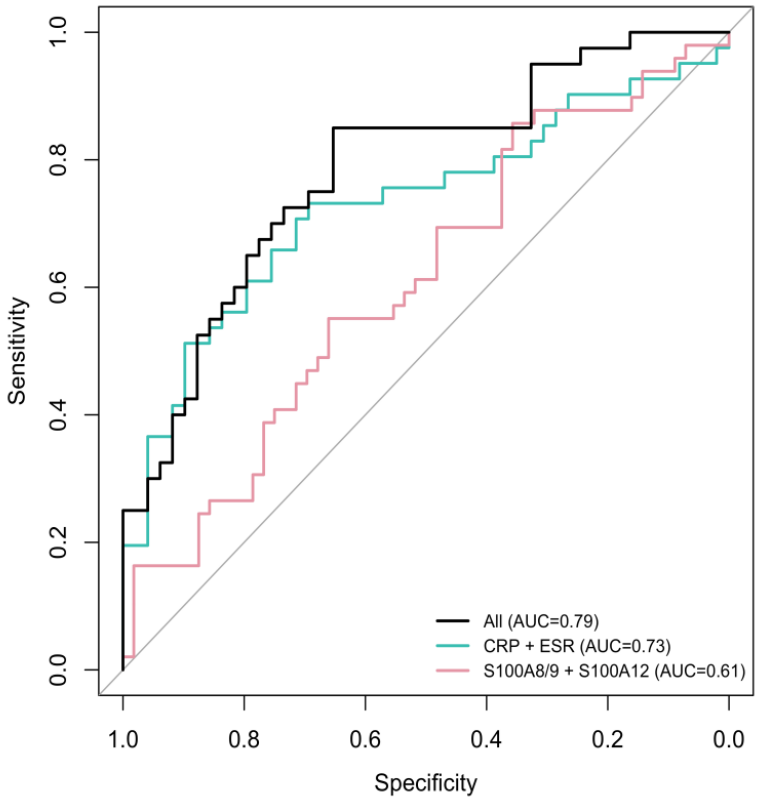
[1]Comparisons on log scale using linear mixed effects model. GCA=Giant cell arteritis, N=number of subjects, IQR=interquartile range, ESR=erythrocyte sedimentation rate, CRP=C-reactive protein.

Figure 1: Serum levels of S100A8/9 (MRP) (top) and S100A12 (A12) (bottom) during active and inactive disease in giant cell arteritis (GCA) and Takayasu's (TAK).



An AUC of 0.73 was obtained from an ROC curve using the combination of ESR and CRP for the association with disease activity (as defined earlier). Using a model with the combination of S100A8/9 and S100A12 an AUC of 0.61 was obtained. A model using all the markers (ESR, CRP, S100A8/9, and S100A12) produced an AUC of 0.79 which was not significantly greater than models using only S100 proteins or traditional biomarkers (p=0.13) (**Figure 2**). Accounting for repeated measurements, using a generalized estimating equations approach without splines, did not change results considerably (p=0.15).

Figure 2: ROC curves for tested biomarkers as measures of disease activity in patients with giant cell. AUC=Area under the curve; CRP=C-reactive protein; ESR=Erythrocyte sedimentation rate arteritis



There was a strong correlation between measurements of CRP and measurements of ESR during active disease. Similarly, there was a strong correlation between S100A8/9 and S100A12 measurements during active disease. However, there were weak correlations between both ESR and CRP, and the S100 proteins. Similarly, weak correlations were seen between the S100 proteins and traditional biomarkers during inactive disease (**Table 3**).

Table 3. Correlations between biomarkers in giant cell arteritis

Active Disease

	CRP	ESR	S100A8/A9	S100A12
CRP	-			
ESR	0.56	-		
S100A8/A9	0.03	0.01	-	
S100A12	0.01	-0.02	0.75	-

Inactive Disease

	CRP	ESR	S100A8/A9	S100A12
CRP	-			
ESR	0.35	-		
S100A8/A9	-0.03	0.10	-	
S100A12	0	0.11	0.66	-

CRP=C-reactive protein, ESR=erythrocyte sedimentation rate.

Takayasu's Arteritis

Serum samples were obtained from 16 patients with TAK (**Table 1**). The majority of patients were female (87.5%) and Caucasian (93.8%). The mean age when the serum was collected was 35 years old (range 19-65). The mean CRP during active disease was 1.2 times that of inactive disease (6.20mg/L [95%CI 2.20, 17.56] vs. 5.12mg/L [2.41, 10.86], p=0.69). The mean ESR during active disease was 1.3 times that of inactive disease (16.43mm/h [95% CI 8.62, 30.66] vs. 12.67mm/h [95% CI 7.41, 21.66], p=0.40). There was a non-statistically significant increase in S100A8/9 levels during active disease compared to levels during inactive disease: 1028 ng/mL [95%CI 640, 1652] vs 843ng/mL [95%CI 536, 1327], p=0.38. There were no differences in the S100A12 levels between active and inactive disease (86.8 ng/mL [95%CI 50.2, 150.6] vs 85.1 ng/mL [47.9, 151.1], p=0.93) (**Figure 1**). None of the biological markers (CRP, ESR, S100A8/9 nor S100A12) significantly correlated with disease activity in logistic regression models for each of these measurements.

DISCUSSION

Results of the present study demonstrate that circulating levels of S100A8/A9 and S100A12 are significantly higher in patients with GCA when the disease is active compared to when the disease is inactive. However, compared to the traditional biomarkers ESR and CRP, the S100 proteins had weaker correlations with disease activity. Furthermore, there was insufficient evidence that measurement of serum S100 proteins significantly provides additional clinical information when used in conjunction with traditional biomarkers.

In TAK there was no significant association between serum S100 proteins levels and disease activity. Similarly, there was a poor association between either ESR or CRP and disease activity, a finding consistent with prior reports (1, 2). Although assembling 16 paired samples of during active/inactive disease is a moderately sized group for a study of such a rare disease, each of the measurements was associated with wide confidence intervals and the study may well have been underpowered to detect a clinically meaningful difference between S100 proteins during active and inactive disease.

There are several strengths to this study. First, patient samples were provided from a large registry to which multiple centers across the United States and Canada contribute. Thus, the results of this study are likely generalizable to patients with GCA and TAK in North America. Second, patient assessments were conducted prospectively, using standardized forms. Third, the S100A8/A9 and S100A12 samples were all tested in the same laboratory using similar techniques.

There are limitations of this study to consider. The majority of patients in the study were on some dose of prednisone when the serum was obtained which may have suppressed levels of the S100 proteins, CRP, and ESRs. However, GCA is a sight threatening disease in which rapid onset of treatment with glucocorticoids is imperative to prevent permanent vision loss. Thus the majority of patients with a suspicion of GCA are started on glucocorticoids even prior to obtaining confirmatory tests such as temporal artery biopsies. The second limitation was that ESR and CRP are widely used markers for assisting in assessment of disease activity and each could have potentially biased the PGA score and resulted in overestimations of the association of these markers with disease activity. Conversely, this bias could have led to an underestimation of the additional utility of measuring levels of S100 proteins. The third limitation was that 29% of our GCA cohort was not biopsy proven; however, all met validated classification criteria for the diagnosis of GCA.

This study provides insufficient evidence supporting use of serum S100A8/A9 and S100A12 levels in the assessment of disease activity in large-vessel vasculitis. However, this is the first study to demonstrate a statistical difference between serum levels S100A8/A9 and S100A12 based on disease activity in GCA. Prior studies from out group have demonstrated that gene expression of S100A8, S100A9 and S100A12 is increased in the temporal arteries of patients with GCA compared to controls (13), even in temporal arteries that did not show signs of inflammation, suggesting a role of these S100A proteins early in the inflammatory cascade. The exact role of S100 proteins in the pathogenesis of vasculitis remains unclear. The role of S100A8/A9 and S100A12 as a diagnostic marker of GCA in “normal” temporal artery biopsies has yet to be explored. This study also emphasizes that both ESR and CRP have limited utility

for the assessment of disease activity in large-vessel vasculitis and the need for development of better biomarkers in the future.

REFERENCES

1. Kerr GS, Hallahan CW, Giordano J, Leavitt RY, Fauci AS, Rottem M, et al. Takayasu arteritis. Ann Intern Med. 1994;120(11):919-29.
2. Dagna L, Salvo F, Tiraboschi M, Bozzolo EP, Franchini S, Doglioni C, et al. Pentraxin-3 as a marker of disease activity in Takayasu arteritis. Ann Intern Med. 2011;155(7):425-33.
3. Kermani TA, Schmidt J, Crowson CS, Ytterberg SR, Hunder GG, Matteson EL, et al. Utility of erythrocyte sedimentation rate and C-reactive protein for the diagnosis of giant cell arteritis. Semin Arthritis Rheum. 2012;41(6):866-71.
4. Ostberg G**.** Morphological changes in the large arteries in polymyalgia arteritica. Acta Med Scand Suppl. 1972;533:135-59.
5. Foell D, Wittkowski H, Vogl T, Roth J**.** S100 proteins expressed in phagocytes: a novel group of damage-associated molecular pattern molecules. J Leukoc Biol. 2007;81(1):28-37.
6. Frosch M, Strey A, Vogl T, Wulffraat NM, Kuis W, Sunderkotter C, et al. Myeloid-related proteins 8 and 14 are specifically secreted during interaction of phagocytes and activated endothelium and are useful markers for monitoring disease activity in pauciarticular-onset juvenile rheumatoid arthritis. Arthritis Rheum. 2000;43(3):628-37.
7. Schulze zur Wiesch A, Foell D, Frosch M, Vogl T, Sorg C, Roth J**.** Myeloid related proteins MRP8/MRP14 may predict disease flares in juvenile idiopathic arthritis. Clin Exp Rheumatol. 2004;22(3):368-73.
8. Lugering N, Stoll R, Schmid KW, Kucharzik T, Stein H, Burmeister G, et al. The myeloic related protein MRP8/14 (27E10 antigen)--usefulness as a potential marker for disease activity in ulcerative colitis and putative biological function. Eur J Clin Invest. 1995;25(9):659-64.
9. Soyfoo MS, Roth J, Vogl T, Pochet R, Decaux G**.** Phagocyte-specific S100A8/A9 protein levels during disease exacerbations and infections in systemic lupus erythematosus. J Rheumatol. 2009;36(10):2190-4.
10. Foell D, Kane D, Bresnihan B, Vogl T, Nacken W, Sorg C, et al. Expression of the pro-inflammatory protein S100A12 (EN-RAGE) in rheumatoid and psoriatic arthritis. Rheumatology (Oxford). 2003;42(11):1383-9.
11. Chen YS, Yan W, Geczy CL, Brown MA, Thomas R**.** Serum levels of soluble receptor for advanced glycation end products and of S100 proteins are associated with inflammatory, autoantibody, and classical risk markers of joint and vascular damage in rheumatoid arthritis. Arthritis Res Ther. 2009;11(2):R39.
12. Foell D, Hernandez-Rodriguez J, Sanchez M, Vogl T, Cid MC, Roth J**.** Early recruitment of phagocytes contributes to the vascular inflammation of giant cell arteritis. J Pathol. 2004;204(3):311-6.
13. Hajj-Ali RH, Thomas; Lee, Michael; Li, Jianbo; Hernandez-Rodriguez, Jose; Cid, Maria; García-Martínez, Ana; Brown, Earl; Prayson, Prayson; Hoffman, Gary**.** Distinct patterns of gene expression in temporal artery biopsies associated with pathogenesis of giant cell arteritis. Arthritis Rheum. 2006;54(9):Supplemental:S 756.
14. Inder SJ, Bobryshev YV, Cherian SM, Wang AY, Lord RS, Masuda K, et al. Immunophenotypic analysis of the aortic wall in Takayasu's arteritis: involvement of

lymphocytes, dendritic cells and granulocytes in immuno-inflammatory reactions. Cardiovasc Surg. 2000;8(2):141-8.
15. Hunder GG, Bloch DA, Michel BA, Stevens MB, Arend WP, Calabrese LH, et al. The American College of Rheumatology 1990 criteria for the classification of giant cell arteritis. Arthritis Rheum. 1990;33(8):1122-8.
16. Arend WP, Michel BA, Bloch DA, Hunder GG, Calabrese LH, Edworthy SM, et al. The American College of Rheumatology 1990 criteria for the classification of Takayasu arteritis. Arthritis Rheum. 1990;33(8):1129-34.