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RESEARCH ARTICLE

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High mobility group box 1 levels in large vessel vasculitis are not associated with disease activity but are influenced by age and statins

Alexandre W. S. de Souza^{1,2*}, Kornelis S. M. van der Geest¹, Elisabeth Brouwer¹, Frederico A. G. Pinheiro², Ana Cecília Diniz Oliveira², Emília Inoue Sato², Luis Eduardo C. Andrade², Marc Bijl³, Johanna Westra¹ and Cees G. M. Kallenberg¹

Abstract

Introduction: Takayasu arteritis (TA) and giant cell arteritis (GCA) are large vessel vasculitides (LVV) that usually present as granulomatous inflammation in arterial walls. High mobility group box 1 (HMGB1) is a nuclear protein that acts as an alarmin when released by dying or activated cells. This study aims to evaluate whether serum HMGB1 can be used as a biomarker in LVV.

Methods: Twenty-nine consecutive TA patients with 29 healthy controls (HC) were evaluated in a cross-sectional study. Eighteen consecutive GCA patients with 16 HC were evaluated at the onset of disease and some of them during follow-up. Serum HMGB1 levels were measured by enzyme-linked immunosorbent assay.

Results: In GCA patients at disease onset mean serum HMGB1 levels did not differ from HC (5.74 ± 4.19 ng/ml vs. 4.17 ± 3.14 ng/ml; $p = 0.230$). No differences in HMGB1 levels were found between GCA patients with and without polymyalgia rheumatica ($p = 0.167$), ischemic manifestations ($p = 0.873$), systemic manifestations ($p = 0.474$) or relapsing disease ($p = 0.608$). During follow-up, no significant fluctuations on serum HMGB1 levels were observed from baseline to 3 months ($n = 13$) ($p = 0.075$), 12 months ($n = 6$) ($p = 0.093$) and at the first relapse ($n = 4$) ($p = 0.202$). Serum HMGB1 levels did not differ between TA patients and HC [1.19 (0.45–2.10) ng/ml vs. 1.46 (0.89–3.34) ng/ml; $p = 0.181$] and no difference was found between TA patients with active disease and in remission [1.31 (0.63–2.16) ng/ml vs. 0.75 (0.39–2.05) ng/ml; $p = 0.281$]. HMGB1 levels were significantly lower in 16 TA patients on statins compared with 13 patients without statins [0.59 (0.29–1.46) ng/ml vs. 1.93 (0.88–3.34) ng/ml; $p = 0.019$]. Age was independently associated with higher HMGB1 levels regardless of LVV or control status.

Conclusions: Patients with TA and GCA present similar serum HMGB1 levels compared with HC. Serum HMGB1 is not useful to discriminate between active disease and remission. In TA, use of statins was associated with lower HMGB1 levels. HMGB1 is not a biomarker for LVV.

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33 Introduction

34 Takayasu arteritis (TA) and giant cell arteritis (GCA) are
 35 large vessel vasculitides (LVV) characterized by granu-
 36 lomatous inflammation of the vessel wall [1]. Although
 37 both diseases present significant overlap in features and
 38 some similarities in the distribution of angiographic le-
 39 sions [2], TA predominantly affects young females and
 40 involves the aorta and its main branches whereas GCA
 41 affects predominantly branches of carotid and vertebral
 42 arteries in individuals older than 50 years [1].

43 Despite clinical symptoms, acute phase reactants and
 44 vascular imaging help to assess disease activity in LVV,
 45 there is a need for novel biomarkers for diagnosis, progn-
 46 nosis and to distinguish active disease from damage or infec-
 47 tion. In TA, active disease is associated with higher serum
 48 levels of pentraxin-3, matrix metalloproteinase 9 (MMP-9),
 49 interleukin (IL)-6, IL-8, IL-18, B cell-activating factor
 50 (BAFF), monocyte chemoattractant protein-1 (MCP-1)
 51 and regulated on activation, normal T cell expressed and
 52 secreted (RANTES) [3–9]. In GCA, high serum levels of
 53 tumor necrosis factor alpha (TNF- α), IL-6, IL-10, che-
 54 mokine (C-X-C motif) ligand 9 (CXCL9) and BAFF are
 55 associated with active disease while serum levels of CC
 56 chemokines CCL2 and CCL11 are decreased at disease on-
 57 set [10–14]. Moreover, adaptive immunity is triggered dur-
 58 ing GCA pathogenesis manifested by T helper (Th)1 and

Th17 responses with the production of interferon (IFN)- γ 59
 and IL-17A, which enhance arterial inflammation [15, 16]. 60

High mobility group box 1 (HMGB1) is a nuclear non- 61
 histone protein that acts as an alarmin when released 62
 into the extracellular milieu either by cellular death or 63
 upon activation of inflammatory cells, e.g. macrophages 64
 by lipopolysaccharide (LPS) or IFN- γ [17, 18]. High 65
 serum HMGB1 levels have been observed in infectious 66
 diseases, atherosclerosis, mechanical trauma, cancer, and 67
 in systemic autoimmune diseases such as systemic lupus 68
 erythematosus (SLE) [19–23]. In systemic vasculitis, high 69
 serum HMGB1 levels were observed in Kawasaki dis- 70
 ease, immunoglobulin (Ig)A vasculitis, and in patients 71
 with antineutrophil cytoplasmic antibody (ANCA)- 72
 associated vasculitis, especially in granulomatosis with 73
 polyangiitis (GPA) with granulomatous manifestations 74
 [24–27]. Serum HMGB1 levels have not been evalu- 75
 ated in patients with LVV. This study aims to evaluate 76
 serum HMGB1 levels as a surrogate marker of disease 77
 activity in patients with LVV and associations between 78
 serum HMGB1 and acute phase reactants, disease 79
 manifestations and therapy in patients with TA and 80
 GCA. Due to epidemiological differences in the preva- 81
 lence of both diseases, patients with TA were recruited 82
 from Brazil whereas GCA patients were recruited 83
 from The Netherlands. 84

t1.1 **Table 1** Demographic, disease features and therapy of patients with giant cell arteritis at disease onset and Takayasu arteritis

t1.2 Variables	GCA	HC	<i>p</i>	Variables	TA	HC	<i>p</i>
t1.3	(<i>n</i> = 18)	(<i>n</i> = 16)			(<i>n</i> = 29)	(<i>n</i> = 29)	
t1.4 Demographic features							
t1.5 Age, years	72.0 (63.7–75.0)	68.5 (63.0–72.0)	0.643	Age, years	38.0 (34.5–48.5)	38.0 (27.5–48.5)	0.392
t1.6 Females, <i>n</i> (%)	14 (77.8)	11 (68.8)	0.551	Females, <i>n</i> (%)	28 (96.6)	27 (93.1)	0.553
t1.7 Disease features and therapy							
t1.8 GCA	Results			TA	Results		
t1.9 Headache, <i>n</i> (%)	12 (66.7)			Disease duration, months	108 (60–186)		
t1.10 Constitutional symptoms, <i>n</i> (%)	8 (44.4)			Angiographic type V, <i>n</i> (%)	16 (55.2)		
t1.11 Cranial ischemic manifestations, <i>n</i> (%)	8 (44.4)			Previous ischemic events, <i>n</i> (%)	11 (37.9)		
t1.12 <i>n</i> (%)							
t1.13 Jaw claudication, <i>n</i> (%)	6 (33.3)			Active disease, <i>n</i> (%)	11 (37.9)		
t1.14 Visual symptoms, <i>n</i> (%)	4 (22.2)			Remission, <i>n</i> (%)	18 (62.1)		
t1.15 Polymyalgia rheumatica, <i>n</i> (%)	4 (22.2)			Statins, <i>n</i> (%)	16 (55.2)		
t1.16 Headache, <i>n</i> (%)	12 (66.7)			Prednisone, <i>n</i> (%)	16 (55.2)		
t1.17 ESR, mm/1 st hour	69.6 ± 28.7			Prednisone daily dose, mg	8.7 (5.0–28.7)		
t1.18 CRP, mg/l	40.0 (20.2–84.2)			Immunosuppressive agents, <i>n</i> (%)	19 (65.5)		
t1.19 Positive TAB, <i>n</i> /total	8/11			Biological agents, <i>n</i> (%)	9 (31.0)		
t1.20 Positive PET-CT scan, <i>n</i> /total	13/15						

t1.21 Continuous variables are presented as mean ± standard deviation or as median and interquartile range

t1.22 CRP C-reactive protein, ESR erythrocyte sedimentation rate, GCA giant cell arteritis, HC healthy controls, *n* number of patients, PET-CT positron emission computed tomography, TA Takayasu arteritis, TAB temporal artery biopsy

85 **Methods**

86 **Study population**

87 The study comprised 18 GCA patients with 16 healthy
88 controls (HC), both from the University Medical Center
T1 89 Groningen (UMCG), The Netherlands (Table 1), and 29
90 consecutive TA patients from Universidade Federal de São
91 Paulo (UNIFESP), Brazil with 29 HC from the same region
92 (Table 1). Inclusion criterion for TA patients was the ful-
93 fillment of the 1990 American College of Rheumatology
94 (ACR) classification criteria [28] while the exclusion cri-
95 teria were current chronic infectious disease, malignancy,
96 and pregnancy. GCA patients were included if they
97 fulfilled the 1990 ACR criteria [29] or when presenting
98 compatible manifestations associated with an enhanced
99 ^{18}F -fluorodeoxyglucose uptake in large vessels by positron
100 emission computed tomography (^{18}F FDG-PET/CT). Exclu-
101 sion criteria for GCA included current chronic infectious
102 disease and malignancy. The study was approved by the
103 Ethics Committee on Research from UNIFESP and by the
104 Medical Ethical Committee of UMCG and complied with
105 the Declaration of Helsinki. All necessary consent was
106 provided from all participants involved in this study.

107 Active disease in GCA was considered if patients pre-
108 sented manifestations of active disease (e.g. temporal
109 headache, optic neuritis, jaw claudication) not attributable
110 to other causes and/or polymyalgia rheumatica (PMR)
111 symptoms with an increase in ESR > 30 mm/hour whereas
112 remission was considered in the absence of GCA mani-
113 festations with normal ESR [30]. Kerr's criteria and the
114 Indian Takayasu activity score 2010 (ITAS2010) with
115 acute phase response (ITAS.A) using ESR or CRP were
116 employed to ascertain disease activity in TA [31, 32].

117 In the 18 GCA patients, blood samples were collected at
118 disease onset prior to glucocorticoid therapy and follow-up
119 samples were obtained from 13 patients at 3 months and
120 from six patients at 12 months. Blood samples were col-
121 lected from 29 TA patients as a cross-sectional evaluation.

122 **Serum HMGB1**

123 Serum HMGB1 levels were determined by enzyme-linked
124 immunosorbent assay (ELISA) using a commercial kit
125 (Shino Test Corp., Sagami, Kanagawa, Japan) according
126 to the manufacturer's instructions. Results were expressed
127 in nanograms per milliliter.

128 **Statistical analysis**

129 Statistical analysis was performed using IBM SPSS soft-
130 ware for Windows version 20.0 (IBM Corp, Armonk, NY,
131 USA) and graphs were created with GraphPad Prism ver-
132 sion 3.02 (GraphPad Software, La Jolla, CA, USA). Mean \pm
133 standard deviation or median and interquartile range were
134 used to present normally distributed and nonnormally
135 distributed continuous variables, respectively. Categorical
136 variables were presented as total number and percentage.

Comparisons between groups were performed using Stu- 137
dent's *t* test or Mann-Whitney *U* test for continuous data 138
or using chi-square test or Fisher's exact test for categorical 139
variables. Correlations between numerical data were per- 140
formed with Spearman's correlation coefficient. A linear 141
regression model was built to analyze whether age and 142
the diagnosis of LVV were independently associated with 143
serum HMGB1 levels. Receiver operating characteristic 144
(ROC) analysis was performed to find out the HMGB1 145
cutoff with the best sensitivity and specificity to differenti- 146
ate GCA from TA. The cutoff value was chosen from the 147
maximized sum of sensitivity and specificity. Paired *t* test 148
or Wilcoxon's test were used to analyze longitudinal data. 149
The significance level accepted was 5 % ($p < 0.05$). 150

151 **Results**

152 **Disease features and therapy of GCA and TA patients**

153 Disease features and therapy of GCA and TA patients
154 are described in Table 1. After the first evaluation, all
155 GCA patients were treated with high-dose prednisolone
156 (60 mg/day) with slow tapering after improvement of
157 disease symptoms and laboratory abnormalities. Disease
158 relapse was observed in four (22.2 %) GCA patients and
159 the median time to the first relapse after diagnosis was
160 6.0 months (6.0–15.0). Methotrexate 10–15 mg per week
161 was added to two patients (11.1 %) after the first relapse
162 during steroid tapering. Five GCA patients (27.8 %) were
163 on statins at disease onset.

164 Previous ischemic events in TA included unstable an-
165 gina (four patients), stroke (three patients), acute myo-
166 cardial infarction (two patients), transient ischemic
167 attacks and mesenteric ischemia in one patient each.
168 Two TA patients were treated only with prednisone
169 whereas the remainder used either an immunosuppres-
170 sive drug or a biologic agent. ESR, ITAS.A ESR and
171 ITAS.A C-reactive protein (CRP) values were signifi-
172 cantly higher in TA patients with active disease than in
173 those in remission, whereas there was a trend for higher
174 serum CRP levels in patients with active disease. No sig-
175 nificant differences could be found between patients
176 with active disease and remission regarding therapy
177 (Table 2).

178 **HMGB1 levels in giant cell arteritis**

179 In GCA patients with active disease at onset and prior to
180 therapy mean serum HMGB1 levels did not differ between
181 patients and HC (5.74 ± 4.19 ng/ml vs. 4.17 ± 3.14 ng/ml;
182 $p = 0.230$) (Fig. 1). Furthermore, among GCA patients
183 mean serum HMGB1 levels at onset were not higher in
184 patients with or without PMR [1.25 (0.21–10.50) ng/ml vs.
185 5.42 (2.94–8.92) ng/ml; $p = 0.167$], cranial ischemic mani-
186 festations (5.56 ± 3.31 ng/ml vs. 5.89 ± 4.95 ng/ml; $p =$
187 0.873), constitutional symptoms (4.92 ± 3.90 ng/ml vs.

T2

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t2.1 **Table 2** Comparison between patients with Takayasu arteritis with active disease and in remission

t2.2 Variables	Active disease (n = 11)	Remission (n = 18)	p
t2.3 HMGB1, ng/ml	1.31 (0.63–2.16)	0.75 (0.39–2.05)	0.281
t2.4 ESR, mm/1 st hour	39.0 (25.0–68.0)	17.5 (8.0–25.5)	0.017
t2.5 CRP, mg/l	6.0 (4.4–24.9)	2.0 (0.1–10.7)	0.053
t2.6 ITAS2010	3.0 (2.2–5.2)	–	–
t2.7 ITASA ESR	3.5 (2.0–6.2)	1.0 (1.0–1.7)	0.001
t2.8 ITASA CRP	5.1 ± 2.5	2.1 ± 0.9	0.012
t2.9 Statins, n (%)	7 (63.6)	9 (50.0)	0.702
t2.10 Prednisone, n (%)	6 (54.5)	10 (55.6)	0.958
t2.11 Prednisone daily dose, mg	20.0 (7.5–45.0)	5.0 (2.5–13.7)	0.055
t2.12 Immunosuppressive agents, n (%)	7 (63.6)	12 (66.7)	0.868
t2.13 Biological agents, n (%)	3 (27.3)	6 (33.3)	0.732

t2.14 Continuous variables are presented as median and interquartile range or as mean ± standard deviation

t2.15 CRP C-reactive protein, ESR erythrocyte sedimentation rate, ITAS Indian Takayasu activity score, ITASA Indian Takayasu activity score with acute phase response,

t2.16 HMGB1 high mobility group box 1, n number of patients

188 6.40 ± 4.50 ng/ml; $p = 0.474$) or relapsing disease (4.75 ±
 189 3.31 ng/ml vs. 6.02 ± 4.47 ng/ml; $p = 0.608$), respectively.

190 Mean serum HMGB1 levels in GCA patients were
 191 5.74 ± 4.19 ng/ml at baseline, 5.18 ± 3.98 ng/ml at 3
 192 months, 8.19 ± 6.80 ng/ml at 12 months, and 6.23 ± 2.48
 193 ng/ml at the first relapse. During follow-up, no signifi-
 194 cant fluctuations on serum HMGB1 levels were ob-

F2 195 served from baseline levels to 3 and 12 months (Fig. 2).

196 Moreover, serum HMGB1 levels in relapsing patients
 197 were not different from their levels at disease onset ($p =$
 198 0.825), at 3 months ($p = 0.629$), at 12 months ($p = 0.601$)

T3 199 and from HC ($p = 0.170$) (Table 3). In GCA patients

200 no correlation was present between HMGB1 and ESR
 201 ($\rho = -0.220$; $p = 0.380$) or between HMGB1 and CRP
 202 levels ($\rho = -0.258$; $p = 0.301$).

Serum HMGB1 in Takayasu arteritis

203 As depicted in Fig. 3, serum HMGB1 levels did not differ
 204 between TA patients with active disease [1.31 (0.63–2.16)
 205 ng/ml], patients in remission [0.75 (0.39–2.05) ng/ml] and
 206 HC [1.46 (0.89–3.34) ng/ml] ($p = 0.220$). Similar median
 207 serum HMGB1 levels were found in TA patients with and
 208 without previous ischemic events [1.53 (0.42–3.34) ng/ml
 209 vs. 0.97 (0.50–1.93) ng/ml; $p = 0.486$]. There was no dif-
 210 ference in serum HMGB1 levels in TA patients under
 211 prednisone therapy compared with those not receiving
 212 prednisone [1.13 (0.45–2.34) ng/ml vs. 1.31 (0.36–1.94)
 213 ng/ml; $p = 0.676$] or between TA patients receiving im-
 214 munosuppressive agents compared with those on bio-
 215 logical agents [1.59 (0.43–2.45) ng/ml vs. 0.59 (0.42–0.96);
 216 $p = 0.140$]. However, serum HMGB1 levels were signifi-
 217 cantly lower in TA patients on statins compared with
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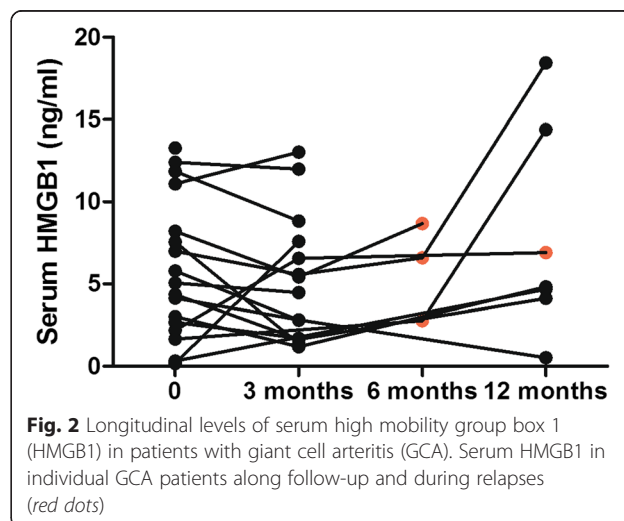
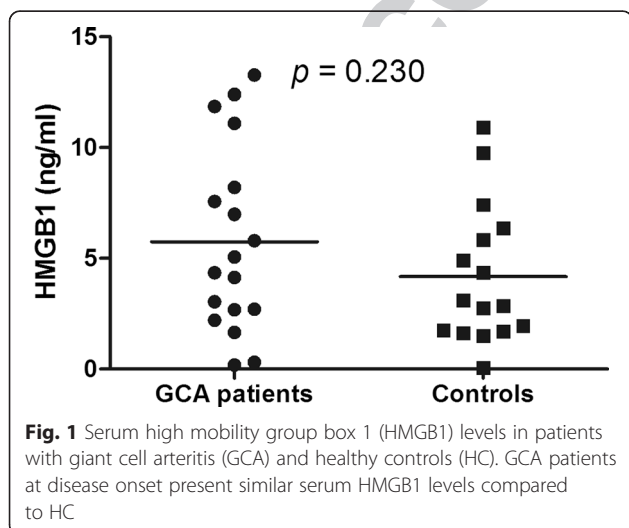
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t3.1 **Table 3** Longitudinal data on disease activity and serum HMGB1 levels in patients with giant cell arteritis

t3.2 Variables	Baseline (n = 18)	3 months (n = 13)	12 months (n = 6)	Relapse (n = 4)
t3.3 HMGB1, ng/ml	5.74 ± 4.19	5.18 ± 3.98	8.19 ± 6.80	6.23 ± 2.48
t3.4 ESR, mm/1 st hour	69.6 ± 28.7	15.1 ± 6.6	21.0 ± 4.9	57.5 ± 24.2
t3.5 CRP, mg/l	40.0 (20.2–84.2)	2.5 (2.5–7.0)	8.0 (5.1–14.7)	38.5 (12.0–82.2)
t3.6 Prednisolone, mg/day	–	20.0 (18.7–27.5)	18.7 (3.7–30.0)	6.2 (1.2–9.3)

t3.7 Continuous variables are presented as median and interquartile range or as mean ± standard deviation
 t3.8 CRP C-reactive protein, ESR erythrocyte sedimentation rate, HMGB1 high mobility group box 1

F4 219 patients not receiving these agents [0.59 (0.29–1.46) ng/ml
 220 vs. 1.93 (0.88–3.34) ng/ml; $p = 0.019$] (Fig. 4).

221 No correlation could be observed between serum
 222 HMGB1 levels and ESR ($\rho = 0.104$; $p = 0.590$), CRP
 223 ($\rho = 0.090$; $p = 0.642$), ITAS2010 ($\rho = 0.230$; $p = 0.231$),
 224 ITAS.A ESR ($\rho = 0.216$; $p = 0.261$) or ITAS.A CRP
 225 ($\rho = 0.070$; $p = 0.720$).

226 **Comparison between Takayasu arteritis and giant cell**
 227 **arteritis regarding serum HMGB1 levels**

228 GCA patients at disease onset presented significantly
 229 higher median serum HMGB1 levels compared with TA
 230 patients with active disease [4.70 (2.55–8.92) ng/ml vs.
 F5 231 1.31 (0.63–2.16) ng/ml; $p = 0.0075$] (Fig. 5). Even when
 232 GCA and TA patients without statins were analyzed sep-
 233 arately, serum HMGB1 levels were significantly higher
 234 in GCA patients compared to TA patients [5.06 (2.86–
 235 10.0) ng/ml vs. 1.80 (0.63–3.34); $p = 0.015$].

236 Higher serum HMGB1 levels observed in GCA com-
 237 pared with TA seems to be an effect of aging, since
 238 serum HMGB1 levels were also higher in GCA controls
 239 than in TA controls [2.98 (1.70–6.23) ng/ml vs. 1.46
 240 (0.89–3.34) ng/ml; $p = 0.019$]. A weak correlation was
 241 found between serum HMGB1 levels and age in all study
 242 participants ($\rho = 0.244$; $p = 0.019$) while in a linear re-
 243 gression model, age was independently associated with

244 serum HMGB1 levels ($\beta = 0.056$; $p = 0.003$; $R^2 = 0.099$),
 245 regardless of the diagnosis of LVV or control status.
 246 ROC analysis of GCA and TA patients showed that the
 247 best HMGB1 cutoff value for differentiating GCA from
 248 TA is 2.17 ng/ml with 83.3 % sensitivity and 79.3 %
 249 specificity.

250 **Discussion**

251 In this study, we observed that patients with active LVV
 252 present similar serum HMGB1 levels compared with pa-
 253 tients in remission and HC. TA patients in remission
 254 and those with relapsing disease were already under
 255 therapy and the use of statins was associated with lower
 256 serum HMGB1 levels. Furthermore, in GCA patients
 257 with active disease prior to therapy, serum HMGB1
 258 levels were not different from HC but were higher than
 259 HMGB1 levels found in TA patients with active disease.

260 The need for reliable biomarkers for disease activity is
 261 an issue of utmost importance in TA. The evaluation of
 262 disease activity is a challenge; since the disease course is
 263 protracted and silent relapses are common, occurring in
 264 up to 96 % of patients who attained remission. It is not
 265 easy to define when the disease is actually in remission
 266 and most patients develop new angiographic lesions over
 267 time usually without clear manifestations of disease

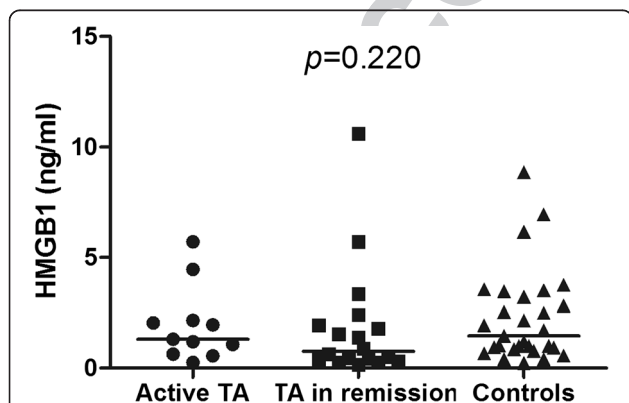


Fig. 3 Serum high mobility group box 1 (HMGB1) levels in patients with Takayasu arteritis (TA) and healthy controls (HC). TA patients with active disease and in remission present similar serum HMGB1 levels compared with HC

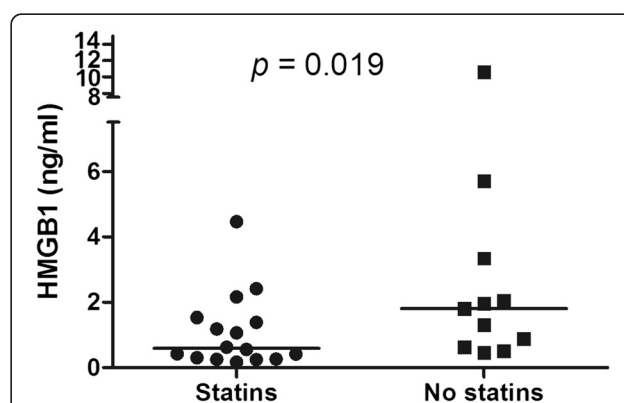


Fig. 4 Influence of statins use on serum high mobility group box 1 (HMGB1) levels in patients with Takayasu arteritis (TA). Statins use was associated with significantly lower serum HMGB1 levels in TA patients

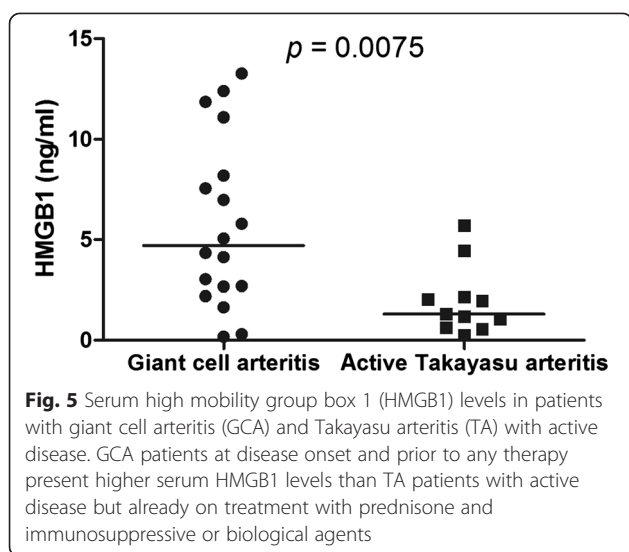


Fig. 5 Serum high mobility group box 1 (HMGB1) levels in patients with giant cell arteritis (GCA) and Takayasu arteritis (TA) with active disease. GCA patients at disease onset and prior to any therapy present higher serum HMGB1 levels than TA patients with active disease but already on treatment with prednisone and immunosuppressive or biological agents

268 activity [33]. In this context, a novel biomarker would
269 help medical decisions for TA.

270 Granulomatous inflammation and vessel wall necrosis
271 are well-known features of LVV [34]. Either necrosis or in-
272 filtrating macrophages are important sources of HMGB1
273 release into the extracellular milieu that in turn activate
274 innate and adaptive immunity [35]. Patients with GPA and
275 predominant granulomatous inflammation present higher
276 serum HMGB1 levels compared with GPA patients with
277 predominantly vasculitic manifestations [25]. Thus, we
278 evaluated associations between disease activity in LVV and
279 serum HMGB1 levels. Unfortunately, no difference could
280 be found between patients with active disease and remis-
281 sion or between patients with LVV and HC.

282 On the other hand, GCA patients at disease onset and
283 prior to therapy presented serum HMGB1 levels that
284 were similar to those of HC, and no association could be
285 found between HMGB1 and acute phase reactants, dis-
286 ease manifestations or disease relapse. Moreover, during
287 follow-up no significant fluctuations in serum HMGB1
288 levels were observed in GCA patients. Novel biomarkers
289 in GCA would help to recognize active disease in pa-
290 tients with signs and symptoms of GCA but normal
291 acute phase reactants. However, serum HMGB1 levels
292 were not increased in patients with active disease.

293 Serum HMGB1 levels were significantly higher in
294 GCA patients than in TA patients, and even though the
295 ROC analysis showed that a cutoff value of 2.17 ng/ml
296 in HMGB1 levels would help to differentiate GCA from
297 TA, we believe that it is unlikely that in clinical practice
298 it would replace the 50-year-old cutoff point used to dif-
299 ferentiate both entities [1]. Furthermore, GCA controls
300 had higher serum HMGB1 than TA controls. These
301 findings indicate that serum HMGB1 levels increase dur-
302 ing aging and may be influenced by the burden of

atherosclerosis in older individuals. In mice, the age- 303
dependent DNA double-strand break is associated with 304
a reduction of nuclear HMGB1 in neurons leading to an 305
increased release of extracellular HMGB1 [36]. However, 306
in a population study performed in Japan with 626 sub- 307
jects, aging did not seem to affect serum HMGB1 levels 308
in healthy subjects [37]. In the present study, although 309
only a weak correlation was found between age and 310
serum HMGB1 levels, age was independently associated 311
with serum HMGB1 levels regardless of the diagnosis of 312
LVV or control status. 313

We found a strong association between statins and 314
lower serum HMGB1 levels in 16 patients with TA (55.2 315
%). Recently, lower HMGB1 levels were observed in 316
hyperlipidemic patients and in GPA patients in remis- 317
sion both on statin therapy [38, 39]. Moreover, atorva- 318
statin was able to reduce *in vitro* the release of HMGB1 319
in stimulated human umbilical vein endothelial cell 320
(HUVEC) cultures. This indicates that the inhibition of 321
HMGB1 release by activated cells is one of the pleio- 322
tropic effects of statins [39]. Other drugs may also influ- 323
ence HMGB1 release from cells such as dexamethasone 324
and metformin [40, 41]. These findings may explain in 325
part why TA patients already under treatment presented 326
serum HMGB1 levels similar to HC. 327

The role of statins in GCA has still to be determined. 328
No impact on relapse rate or on the prevention of severe 329
ischemic events was observed in retrospective studies. 330
However, conflicting results were found regarding the 331
influence of statins on acute phase reactants and daily 332
glucocorticoid dose in GCA patients using statins [42–44]. 333
In TA patients, a retrospective study could not find any 334
difference in ischemic events between patients with and 335
without statins but associations with disease activity were 336
not analyzed [45]. In the present study, more TA patients 337
used statins than GCA patients at diagnosis although this 338
difference was not statistically significant (data not shown). 339
This could be due to the long disease course of our TA pa- 340
tients in comparison with the GCA patients who were eval- 341
uated at disease onset. 342

Limitations of this study are its mainly cross-sectional 343
nature and the inclusion of patients already on therapy 344
for TA, whereas the low number of patients and the 345
short-term follow-up period are limitations for the GCA 346
patients. Nevertheless, the data seem robust enough to 347
conclude that HMGB1 is not a suitable biomarker in 348
LVV in contrast to SLE [23]. 349

350 Conclusions

351 Serum HMGB1 levels were neither different between pa- 352
tients with LVV and HC, nor between patients with ac- 353
tive disease and those in remission. Therefore, serum 354
HMGB1 is not a useful biomarker for LVV. Moreover, 355
serum HMGB1 levels were not associated with any

356 disease phenotypes in LVV. In long-standing TA, ther-
357 apy with statins seems to lead to lower serum HMGB1
358 levels.

359 Abbreviations

360 18FDG-PET/CT: 18^F-fluorodeoxyglucose positron emission computed tomography;
361 ACR: American College of Rheumatology; ANCA: antineutrophil cytoplasmic
362 antibody; BAFF: B cell-activating factor; CRP: C-reactive protein; CXCL9: chemokine
363 (C-X-C motif) ligand 9; ELISA: enzyme-linked immunosorbent assay;
364 ESR: erythrocyte sedimentation rate; GCA: giant cell arteritis; GPA: granulomatosis
365 with polyangiitis; HC: healthy controls; HMGB1: high mobility group box 1;
366 HUVEC: human umbilical vein endothelial cell; IFN: interferon; Ig: immunoglobulin;
367 IL: interleukin; ITAS: Indian Takayasu activity score; ITAS-A: ITAS with acute phase
368 response; LPS: lipopolysaccharide; LVV: large vessel vasculitides; MCP-1: monocyte
369 chemoattractant protein-1; MMP-9: matrix metalloproteinase 9; PMR: polymyalgia
370 rheumatica; RANTES: regulated on activation, normal T cell expressed and
371 secreted; ROC: receiver operating characteristic; SLE: systemic lupus
372 erythematosus; TA: Takayasu arteritis; Th: T helper cell; TNF- α : tumor necrosis
373 factor alpha; UMCG: University Medical Center Groningen; UNIFESP: Universidade
374 Federal de São Paulo.

375 Competing interests

376 All authors declare that they have no competing interests.

377 Authors' contributions

378 AWSS contributed to the study design, performed laboratory tests,
379 conducted the statistical analysis, and drafted the manuscript. KSMG
380 contributed to the study design, evaluated the study participants, collected
381 data from medical records, and revised the manuscript. EB contributed to
382 the study design, collected data from patients' medical records, helped with
383 the interpretation of results, and revised the manuscript. FAGP evaluated the
384 study participants, collected data from medical records, helped with the
385 interpretation of data and revised the manuscript. ACDO evaluated the
386 study participants, collected data from medical records, helped with the
387 interpretation of data and revised the manuscript. EIS contributed to the
388 study design, helped with the interpretation of results, and revised the
389 manuscript. LECA contributed to the study design, helped with the
390 interpretation of results, and revised the manuscript. MB contributed to
391 the study design, interpretation of data and revised the manuscript. JW
392 contributed to the study design, performed laboratory tests, helped with
393 the interpretation of data and revised the manuscript. CGMK conceived the
394 study, contributed to the study design, interpretation of data and revised the
395 manuscript. All authors read and approved the manuscript.

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