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► To cite this version:

V. Salle, A. Sagnier, M. Diouf, J. Schmidt, A. Smail, et al.. Prevalence of anti-S100A10 antibodies in antiphospholipid syndrome patients. *Thrombosis Research*, 2019, 179, pp.15 - 19. <10.1016/j.thromres.2019.04.027>. <hal-03485724>

HAL Id: hal-03485724

<https://hal.science/hal-03485724v1>

Submitted on 20 Dec 2021

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1 **Prevalence of anti- S100A10 antibodies in antiphospholipid syndrome**
2 **patients**

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16

17 Key words : antiphospholipid syndrome, S100A10, annexin A2

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19 HIGHLIGHTS

- 20
- S100A10 is a binding partner of annexin A2
 - S100A10 plays a pivotal role in regulation of fibrinolysis
 - S100A10 has been identified as a new autoantigen in antiphospholipid syndrome
 - The prevalence of anti-S100A10 in APS patients (11.9%) was statistically higher than in control group (1.7%)
 - Further studies in large cohort of APS patients are needed to confirm these preliminary data and to determine whether these antibodies could be pathogenic
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33 ABSTRACT

34 Introduction

35 Annexin A2 (ANXA2), an endothelial cell receptor for plasminogen and tissue plasminogen
36 activator, has been identified as a new autoantigen in antiphospholipid syndrome (APS).
37 ANXA2 can exist as a monomer or a heterotetrameric complex with S100A10 protein. This
38 S100A10 subunit also plays a pivotal role in the regulation of fibrinolysis. The aim of this
39 study was to evaluate the prevalence of autoantibodies directed against S100A10 protein in
40 patients with APS.

41 Methods. Patients with primary antiphospholipid syndrome (PAPS), patients with systemic
42 lupus erythematosus (SLE) and patients with unexplained thrombosis were retrospectively
43 included in this study. Patients were followed in the department of Internal Medicine of
44 Amiens University Hospital, Amiens, France. IgG and IgM anti-S100A10 antibodies were
45 detected in the serum of patients by enzyme-linked immunosorbent assay. The cut-off value
46 for positivity was defined as 3 standard deviations above the mean optical density (OD)
47 obtained in the sera of 116 healthy blood donors.

48 Results. The study group consisted of 116 healthy individuals and 106 patients: 42 APS
49 patients (26 patients with PAPS and 16 patients with secondary SLE-related APS), 43 SLE
50 patients without APS and 21 patients with unexplained thrombosis. The median age of APS
51 patients, SLE patients without APS, patients with unexplained thrombosis and healthy
52 individuals was 47, 38, 53 and 42 years, respectively. Anti-S100A10 antibodies were detected
53 in 11.9% of APS patients and this prevalence was statistically higher than that observed in
54 healthy individuals (1.7%) ($p=0.0148$). Highest levels of anti-S100A10 were observed in the
55 serum of one PAPS patient with venous thrombosis and one SLE patient with APS with a
56 history of stroke and recurrent miscarriage.

57 Conclusion. S100A10 protein, the binding partner of ANXA2, was identified as a target of
58 autoantibodies in sera from patients with APS. Further studies involving a large cohort of
59 APS patients are required to determine whether these antibodies could play a role in
60 thrombogenic mechanisms of APS and to determine their diagnostic value in discriminating
61 clinical subgroups of patients with APS, particularly those with seronegative APS.

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65 INTRODUCTION

66 Antiphospholipid syndrome (APS) is a systemic autoimmune disease characterized by
67 vascular thrombosis and/or pregnancy morbidity associated with persistent antiphospholipid
68 antibodies (1). Several pathogenic mechanisms are involved in APS, such as activation of
69 endothelial cells, platelets and monocytes, inhibition of the natural anticoagulant protein
70 C/protein S pathway, activation of the complement system and impairment of fibrinolysis (2,
71 3). Many components of the fibrinolytic system are targets for autoantibodies in APS, such as
72 tissue activator plasminogen (tPA), plasminogen (Plg), plasmin and annexin A2 (ANXA2)
73 (3). ANXA2, an endothelial cell surface receptor for tPA and Plg (4), was identified as a new
74 autoantigen in APS by several groups several years ago (5-7). ANXA2 belongs to the family
75 of annexins, which are Ca²⁺-regulated phospholipid-binding proteins (8). ANXA2 exists as a
76 monomer in cytoplasm or can form a heterotetrameric complex associated with the cell
77 membrane (9). This complex, comprising two ANXA2 subunits and a dimer of S100A10, is
78 involved in the assembly of tPA and Plg at the endothelial cell surface. S100A10 promotes
79 the translocation of ANXA2 to the cell surface (10) and ANXA2 protects S100A10 from
80 proteasomal degradation (11). Conflicting models have been proposed concerning the exact
81 role of these proteins (either ANXA2 or S100A10) individually (or as a complex) in the
82 plasmin activation process (12-14). S100A10 belongs to the S100 family of small dimeric
83 calcium-binding proteins (10-12 kD), which are involved in several cellular processes, such as
84 cell cycle regulation, cell growth, cell differentiation, motility, contraction, secretion and
85 transcription (15). S100 proteins contain two EF-hand motifs, which, except for S100A10, are
86 responsible for Ca²⁺-binding (16, 17). S100A10 has been observed in various cell types, such
87 as endothelial cells, macrophages, fibroblasts, epithelial cells and cancer cell lines (17).
88 S100A10 binds to tPA and Plg and plays a role in regulation of plasmin generation (17).
89 S100A10 has also been shown to play an important role in fibrinolysis and angiogenesis *in*
90 *vivo* (18). The aim of this study was to evaluate the prevalence of anti-S100A10
91 autoantibodies in APS.

92

93 PATIENTS AND METHODS

94 Patients

95 Patients with systemic lupus erythematosus (SLE), primary antiphospholipid
96 syndrome (PAPS) and unexplained thrombosis were retrospectively included in this study. All
97 SLE patients met the revised American College of Rheumatology classification criteria (19).

98 The majority of APS patients met the revised criteria for APS (1), but some patients presented
99 clinical features of APS with low positive anticardiolipin antibodies (ACL) and the diagnosis
100 of APS in these patients can be equivocal (20). Other patients also presented clinical features
101 of APS, but with negative "classical" antiphospholipid antibodies corresponding to a
102 diagnosis of seronegative APS (21). Patients with venous or arterial thrombosis with no
103 hereditary coagulation defects constituted the unexplained thrombosis group. None of these
104 patients had any underlying autoimmune or neoplastic disease. All patients were monitored at
105 the Amiens University Hospital department of Internal Medicine. Healthy individuals were
106 recruited at the Amiens branch of the French Blood Agency-North of France and from the
107 general population. Demographic data were collected for patients and healthy individuals.
108 APS subtypes were recorded for each APS patient, as follows: arterial, venous, microvascular,
109 obstetric and catastrophic (according to Asherson's criteria) (22). The following laboratory
110 data were collected for APS and SLE patients: IgG and IgM anticardiolipin antibodies (ACL),
111 IgG and IgM anti- β 2 glycoprotein I antibodies (aB2GPI). Blood samples for patients and
112 blood donors were stored at -80°C at the Picardy Biobank. Each patient and healthy
113 individual provided written informed consent to use these stored samples for research. Ethical
114 review and approval were not required for this study in accordance with the local legislation
115 and institutional requirements at the beginning of the study.

116

117 Detection of anti-S100A10 antibodies

118 An Enzyme-linked Immunosorbent assay (ELISA), performed in the biochemistry
119 laboratory, was used to detect IgG and IgM anti-S100A10 antibodies. The S100A10
120 recombinant protein (ABNOVA, Taiwan, RDC) was used as antigen. 96-well plates were
121 coated overnight at $+4^{\circ}\text{C}$ with S100A10 recombinant protein ($0.5\ \mu\text{g}/\text{ml}$) in even rows and
122 with phosphate buffered saline (PBS) in odd rows. After three washes with PBS/0.1% Tween
123 (PBST), wells were blocked with 3% bovine serum albumin in PBST for 90 min at room
124 temperature. Wells were then washed three times with PBST and serum (diluted to 1:200 in
125 blocking buffer) was added to both S100A10- and PBS-coated wells in duplicate for 30 min at
126 room temperature. Mouse monoclonal anti-S100A10 antibody (Abcam, Cambridge, UK) was
127 used as positive control. Sera from both patients and healthy individuals were used for each
128 ELISA assay. After three washes with PBST, wells were incubated with $100\ \mu\text{L}$ of
129 peroxidase-conjugated antibodies (goat anti-mouse IgG, goat anti-human IgG, goat anti-
130 human IgM (Sigma, St. Louis, MO, USA)) (diluted to 1:2000 in PBST) for 30 min at room

131 temperature. After three washes, 100 μ L of tetramethylbenzidine solution (Sigma) were added
132 and color development was stopped with 50 μ L of H₂SO₄. Absorbance was measured at 405
133 nm and the results were expressed as optical density (OD), calculated by subtracting the
134 background from wells without S100A10. The cutoff value for determining positivity of anti-
135 S100A10 IgG or IgM was defined by 3 standard deviations (SD) above the mean value
136 observed with a serum panel from 116 blood donors

137 Statistical analysis

138 The prevalence of anti-S100A10 antibodies in patients and controls was compared by Fisher's
139 exact test. 95% confidence intervals were calculated using the exact Klopfer- Pearson
140 method. A Kruskal Wallis test was used to compare the crude level of anti-S100A10 IgG and
141 IgM titers in healthy individuals and the groups of patients with SLE without APS, APS and
142 unexplained thrombosis. Post hoc analyses were performed using the Wilcoxon rank-sum test
143 with Hochberg adjustment. A pvalue<0.05 was considered for statistical significance.

144 All statistical analyses were performed with RStudio software Version 1.0.143 - © 2009-2016
145 RStudio (R.3.4.0 software).

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148 RESULTS

149 Patient characteristics

150 One hundred six patients were retrospectively included as follows: 42 APS patients, 43 SLE
151 patients without APS, 21 patients with unexplained thrombosis and 116 healthy individuals.
152 Five patients had seronegative APS, one of whom had seronegative obstetric APS with
153 positive anti-annexin A5 antibodies. Two patients had equivocal APS. Patient demographic
154 characteristics and biological profile of antiphospholipid antibodies of APS and SLE patients
155 are summarized in Table 1. APS patients presented a female predominance (78.5%) and the
156 sex ratio was almost the same as that reported in a large cohort of APS patients (23). Signs of
157 venous thrombosis were present in the majority of APS patients and arterial thrombosis was
158 observed in almost one-half of APS patients. Catastrophic APS was observed in 2 patients.

159 Prevalence of anti-S100A10 antibodies

160 The prevalence of anti-S100A10 antibodies among healthy individuals was 2 out of 116
161 patients (1.7%) (Table 2), similar to the reported prevalence of anti-annexin A2 in healthy

162 populations (5, 6). Anti-S100A10 antibodies were detected in almost 12% of APS patients
163 and this prevalence was statistically higher than in the control group ($p = 0.0148$) (Table 2).
164 The prevalence of anti-S100A10 antibodies in SLE without APS was low (2.3%) and similar
165 to that observed in controls (Table 2). No patient had both IgM and IgG anti-S100A10. Anti-
166 S100A10 antibodies were not detected in patients with unexplained thrombosis. We found a
167 statistically significant difference in IgG anti-S100A10 titers between the four groups studied
168 (Fig. 1) ($p = 0.0002$) and this difference was more specifically observed between unexplained
169 thrombosis and SLE without APS ($p=0.0034$) and between APS patients and SLE without
170 APS ($p=0.0049$).

171 The control group differed from all the other three groups regarding IgG ($p=0.0356$, $p=0.0399$
172 and $p=0.0399$ for APS, unexplained thrombosis and SLE respectively). IgG levels were not
173 different between APS and unexplained thrombosis ($p=0.7154$). No statistically significant
174 difference was observed when we compared APS patients with and without anti-S100A10
175 antibodies in terms of clinical phenotype such as venous thrombosis, arterial thrombosis,
176 microvascular thrombosis and obstetric morbidity (data not shown). We did not find any
177 positive results for anti-S100A10 antibodies in patients with catastrophic APS, the most
178 severe form of APS. The highest anti-S100A10 antibody levels were observed in serum from
179 two patients, a woman with SLE and APS with a history of thrombotic events, such as stroke
180 and recurrent pregnancy losses, and a man with PAPS and deep vein thrombosis (Table 3).

181

182 DISCUSSION

183 This present study identified, for the first time, S100A10 as a target antigen for autoantibodies
184 in APS. Anti-S100A10 antibodies were significantly more prevalent in patients with APS
185 than in healthy individuals. S100A10 is a binding partner of ANXA2 (24) and plays an
186 important role in fibrinolysis (18). At the cell surface, S100A10 is present predominantly in a
187 heterotetrameric complex consisting of two ANXA2 subunits and a S100A10 homodimer
188 (17). ANXA2 stabilizes intracellular S100A10 and endothelial translocation of ANXA2 to the
189 cell surface requires S100A10 expression (10). The prevalence of anti-S100A10 antibodies
190 was lower than that of anti-ANXA2 antibodies reported in APS by Cesarman-Maus (22.6%)
191 (5). The amino-terminal tail domain of ANXA2 contains the binding site for S100A10 and the
192 S100A10 subunits of the heterotetrameric complex are positioned in the center of the complex
193 in the classical “closed” conformation (25). However, three different molecular arrangements

194 of the tetrameric complex are observed depending on the presence of calcium and S100A10 is
195 exposed away from the phospholipid bilayer in the “open” conformation (25). The
196 conformation of the tetrameric complex may therefore mask target epitopes present on
197 S100A10 protein. Furthermore, in a recent study evaluating the vascular expression of
198 ANXA2 and its S100A10 subunit in lupus nephritis, the vascular expression of S100A10 in
199 patients with lupus nephritis was less intense than that observed for ANXA2 (26). However,
200 the study involves a relatively small number of patients and further studies in a large cohort of
201 APS patients are needed to confirm these preliminary data. Among positive patients for anti-
202 S100A10 antibodies, three APS patients were also positive for anti-ANXA2 antibodies with
203 the same isotype (data not shown). The co-occurrence of antibodies against S100A10 and to
204 ANXA2 could be explained by epitope spreading to distinct proteins within the same protein
205 complex. One of the two APS- positive patients with the highest levels of anti-S100A10
206 antibodies was a 51-year-old female patient with SLE and APS with a history of stroke and
207 recurrent pregnancy losses. S100A10 is present in the core transcriptome of 115 genes that are
208 critical for placental function across species (27). In a recent study investigating the cellular
209 localization of ANXA2/S100A10 complex, expression of S100A10, ANXA2 and the
210 ANXA2/S100A10 complex was observed in amniotic membrane, blood vessels, the brush
211 border area of the syncytium and trophoblasts (28). S100A10 may also play a crucial role in
212 embryo implantation (29). In the light of these data, we hypothesize that anti-S100A10
213 antibodies could have a pathogenic role in patients with obstetric morbidity. Further studies
214 are needed to determine whether anti-S100A10 antibodies are associated with obstetric
215 morbidity. S100A10 has a widespread distribution in body tissues, particularly in the brain.
216 An interaction has been observed between S100A10 and the serotonin receptor, 5-HT1B, and
217 S100A10 increases the localization of 5-HT1B receptors at the cell surface (30). S100A10
218 knockout mice exhibit a depression-like phenotype (30). APS patients may present several
219 psychiatric manifestations, such as depression (31). S100A10 also plays a crucial role in
220 endothelial cell function by regulating plasmin generation. S100A10 knockout mice present
221 increased vascular fibrin deposition. Cerebral involvement in APS involves an APL-induced
222 “prothrombic state” and an immune-mediated pathogenesis with direct binding of APL to
223 central nervous system antigens. Further studies are needed to evaluate whether anti-S100A10
224 antibodies are associated with thrombotic and non-thrombotic neurological features of APS.
225 The prevalence of anti-S100A10 antibodies should also be evaluated in patients with
226 cryptogenic stroke or depression.

227 In conclusion, we identified S100A10 protein, the binding partner of ANXA2, as a new
228 autoantigen in APS. Further studies involving a large cohort of APS patients are required to
229 determine whether anti-S100A10 antibodies could play a role in thrombogenic mechanisms of
230 APS and to determine their diagnostic value in discriminating clinical subgroups of patients
231 with APS, particularly those with seronegative APS.

232

233 Acknowledgments

234 We thank Dr. Agnès Brulé and Dr. Nathalie Becquet (French Blood Agency) for providing
235 blood samples from healthy individuals, Mrs Lucie Desjardins of the Division of Clinical
236 Research and Innovation of Amiens University Hospital, Dr. Clément Gourguechon of the
237 department of Internal Medicine of Amiens University Hospital and Dr Ericka Berthe of the
238 Immunology Laboratory of Amiens University Hospital, for their participation in this study.

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345 Tables

346 **Table 1**

Demographic characteristics and biological profile of antiphospholipid antibodies of APS and SLE patients					
	n	Median age, years (range)	Female/Male	Positive ACL (%)	Positive aB2GPI (%)
Healthy individuals	116	42 (18-69)	61/55	-	-
APS	42	47 (17-75)	33/9	61.9	45.2
- Primary APS	26	47.5 (24-75)	18/8	57.6	38.4
- Secondary APS related to SLE	16	35 (17-56)	15/1	68.7	56.2
SLE without APS	43	38 (19-78)	42/1	22.5	10
Thrombosis of unknown etiology	21	53 (32-74)	13/8	-	-

347 ACL: anticardiolipin antibodies, aB2GPI: anti-β2 glycoprotein I antibodies

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349 **Table 2**

Prevalence of anti-S100 antibodies				
	APS patients (n = 42)	SLE patients without APS (n = 43)	Unexplained thrombosis (n = 21)	Controls (n = 116)
Positive anti-S100A10 IgG	1/42=2.4% [0.06% ; 12.6%]	0/43=0% [0% ; 8.2%]	0/21=0% [0% ; 16.1%]	1/116=0.9% [0.02% ; 4.7%]
Positive anti-S100A10 IgM	4/42=9.5% [0% ; 8.2%]	1/43=2.3% [0.06% ; 12.3%]	0/21=0% [0% ; 16.1%]	1/116=0.9% [0.02% ; 4.7%]
Positive anti-S100A10 IgG and/or IgM	5/42=11.9% [4.0% ; 25.6%]	1/43=2.3% [0.06% ; 12.3%]	0/21=0% [0% ; 16.1%]	1/116=1.7% [0.2% ; 6.1%]

350 Data are presented as the number and percentages, with 95% confidence intervals.

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358 **Table 3**

Clinical and Laboratory data of healthy individuals and patients with positive anti-S100A10 antibodies						
	Gender	Age (years)	Clinical manifestations	LA	ACL	aB2GPI
Healthy*	F	20	Any	Negative	Negative	Negative
Healthy	M	50	Any	Negative	Negative	Negative
SLE	F	48	Discoid lupus, Raynaud's phenomena, serositis	NA	Positive	Positive
PAPS	F	31	Deep vein thrombosis, pulmonary embolism	Positive	Positive	Positive
PAPS**	F	36	Pulmonary embolism, recurrent pregnancy losses, retinal ischemia	Negative	Negative	Negative
PAPS*	H	68	Deep vein thrombosis	Positive	Negative	Negative
SLE + APS	F	28	Superior vena cava thrombosis, ischemic colitis	Negative	Positive	Positive
SLE + APS*	F	51	Stroke, recurrent pregnancy losses	Positive	Positive	Positive

359 * Patients with highest levels of anti-S100A10 antibodies. ** Seronegative APS. LA: lupus
 360 anticoagulant, ACL: anticardiolipin antibodies, aB2GPI: anti- β 2 glycoprotein I antibodies, NA: not
 361 available.

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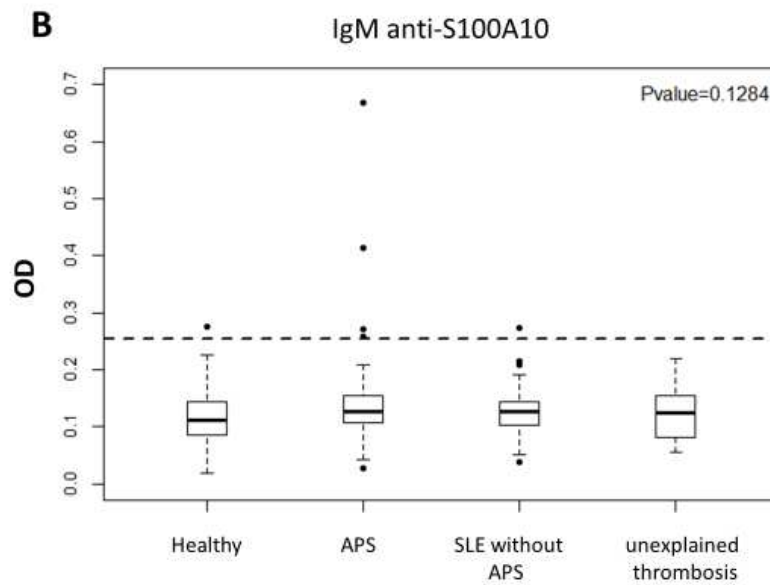
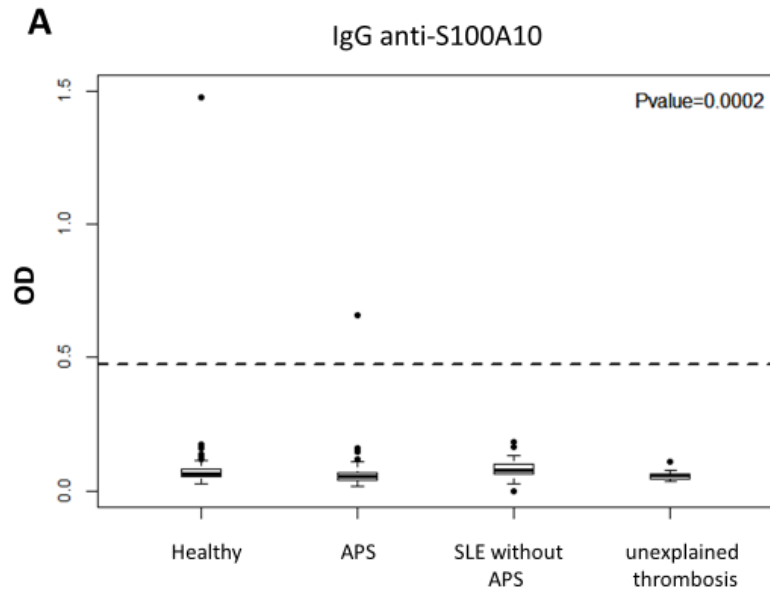
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372 **Figure 1.** Levels of IgG (A) and IgM (B) anti-S100A10 antibodies in patients and controls.

373 The dashed line represents the cutoff, which is the mean OD + three standard deviations of
 374 the 116 normal controls.

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