Prevalence and Thrombotic Risk Assessment of Anti-β_2 Glycoprotein I Domain I Antibodies: A Systematic Review

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Abstract

Background To date, the exact prevalence of anti-β_2 glycoprotein I domain I (anti-β_2GPI-DI) antibodies in patients with antiphospholipid syndrome (APS) and their role when assessing thrombosis risk is uncertain.

Objectives To estimate the prevalence of anti-β_2GPI-DI in patients with APS and to determine whether anti-β_2GPI-DI-positive individuals are at greater risk of thrombosis, as compared with individuals without anti-β_2GPI-DI, by systematically reviewing the literature.

Methods A detailed literature search was applied a priori to Ovid MEDLINE In-Process and Other Non-Indexed Citation 1986 to present and to abstracts from the European League Against Rheumatism (EULAR) and American College of Rheumatology (ACR)/ Association for Rheumatology Health Professionals (ARHP) Annual Meetings (2011–2015).

Results A total of 11 studies, including 1,585 patients, were analyzed. Patients were distributed as follow: 1,218 patients APS (45.4% anti-β_2GPI-DI-positive; in more detail: 504 primary APS [55.4% anti-β_2GPI-DI-positive], 192 secondary APS [43.2% anti-β_2GPI-DI-positive], and 522 not specified), 318 with systemic lupus erythematosus (SLE; 26.7% anti-β_2GPI-DI-positive), 49 asymptomatic carriers of antiphospholipid antibodies (aPL) (30.6% anti-β_2GPI-DI-positive), and 1,859 healthy controls. When considering the five studies eligible for thrombotic risk assessment, four studies found a significant association of anti-β_2GPI-DI-positivity with thrombotic events, whereas one study found no predictive correlation with thrombosis (overall odds ratio [OR] for pooled data: 1.99; 95% confidence interval [CI]: 1.52–2.6; $p < 0.0001$).

Conclusion We report an overall estimated median prevalence of anti-β_2GPI-DI antibodies of 44.3% in patients with APS and/or SLE and a significantly higher prevalence among patients with APS compared with SLE alone. Anti-β_2GPI-DI antibodies might represent a promising tool when assessing thrombotic risk in patients with APS.

Keywords
- antiphospholipid syndrome
- antiphospholipid antibodies
- β_2 glycoprotein I domain
- non-criteria aPL
- thrombosis

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The antiphospholipid syndrome (APS) is an autoimmune disorder characterized by vascular thrombosis and/or pregnancy morbidity (miscarriages, fetal deaths, premature births, etc.) associated with a persistent positivity for antiphospholipid antibodies (aPL). The current classification criteria for APS include three laboratory tests: lupus anticoagulant (LA), anticardiolipin (aCL), and anti-β_2 glycoprotein-I (β_2GPI). To prevent detection of transient antibodies, tests must be positive on $\geq$2 occasions, at least 12 weeks apart.[1] β_2GPI is hypothesized to be the main antigenic target for aPL, especially in vascular tissues after conformational modification.[2]

Although some evidence is available supporting a role for anti-β_2GPI antibodies in contributing to thrombosis and pregnancy morbidity,[3,4] their pathogenicity remains a topic of heated discussion. In fact, not all patients positive for the presence of anti-β_2GPI antibodies develop clinical aPL-related manifestations.[3] Besides, anti-β_2GPI antibodies have also been detected in a large range of autoimmune diseases, such as multiple sclerosis,[5] and nonautoimmune diseases, such as leprosy and in children with atopic dermatitis,[6] with a vast heterogeneity in terms of titers and persistence.

This heterogeneity in the pathogenic potential of anti-β_2GPI antibodies might be ascribed to the molecular structure of β_2GPI, presenting multiple antigenic specificities that can be targeted by different autoantibodies.[7] β_2GPI has five homologous domains (DI–DV), and recently, several studies have focused on the epitope distribution of anti-β_2GPI antibodies to identify their clinical role.[8–10] The main epitope to have been found to be associated with APS involves regions of DI,[9,11] and growing evidence has resulted in the identification of DI as the "immunodominant epitope."[10,12,13] Both in vitro and in vivo preliminary data are in line with this hypothesis, supporting a role for anti-β_2GPI-DI antibodies in the development of APS-related clinical manifestations.[14,15]

However, the clinical role of such antibodies is still debated, and international criteria for derivation and confirmation of anti-β_2GPI-DI antibodies are still lacking.[16] To date, the exact prevalence of anti-β_2GPI-DI antibodies in patients with APS and the role of testing for anti-β_2GPI-DI when assessing the thrombosis risk are unclear.

In this study, we aim to estimate the prevalence of anti-β_2GPI-DI in patients with APS and to determine whether anti-β_2GPI-DI-positive individuals are at greater risk of thrombosis when compared with individuals without anti-β_2GPI-DI by systematically reviewing the literature.

Methods

A detailed literature search has been developed a priori to identify articles that reported findings from clinical and laboratory studies that tested anti-β_2GPI-DI antibodies. Key words and subject terms included are as follows: ("beta 2-glycoprotein i"[MeSH Terms] OR "beta 2-glycoprotein I" [All Fields] OR "beta 2 glycoprotein 1"[All Fields]) AND domain [All Fields]. The search strategy was applied to Ovid MEDLINE In-Process and Other Non-Indexed Citation 1986 to present. Abstracts from the European League Against Rheumatism (EULAR) and American College of Rheumatology (ACR)/Association for Rheumatology Health Professionals (ARHP) Annual Meetings (2011–2015) were screened and included in the analysis when meeting the inclusion criteria and not replicating studies published elsewhere.

Studies that met the criteria to evaluate the prevalence of anti-β_2GPI-DI antibodies and their association with the thrombotic risk in patients with APS and control populations were systematically analyzed by two independent reviewers (M. R. and I. C.). Disagreements were resolved by consensus; if consensus could not be achieved, a third party (S. S.) would provide an assessment of eligibility. As the data on eligibility were dichotomous (eligible: yes/no), interrater agreement at both the title and abstract review stage and the full article review stage was determined by calculation of Cohen's kappa coefficient ($k = 0.93$).[17]

Literature search strategy on the prevalence of positivity for anti-β_2GPI-DI antibodies is shown in ►**Fig. 1**. We included in our analysis only studies reporting: (1) clinical data referring to aPL-related manifestations, (2) laboratory data including aCL, LA, and/or anti-β_2GPI testing, and (3) anti-β_2GPI-DI antibodies testing with detailed assay methodology, isotype analyzed, defined cutoffs of positivity for anti-β_2GPI-DI antibodies. All published series including 10 or more patients meeting the aforementioned inclusion criteria were recorded. Methods of enrollment were also analyzed. Prevalence of anti-β_2GPI-DI antibodies was compared between populations by Fisher's exact test, two-tailed, whereas results for association of positivity and risk of thrombosis were compared using odds ratios reported in the studies analyzed.

This study has been performed according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines,[18] and the relative checklist can be supplied upon request.

Risk of Bias Assessment

Two reviewers (M. R. and S. S.) assessed the risk of bias of individual studies using the Newcastle–Ottawa Scale (NOS) for cohort studies, and the NOS for case–control studies. The NOS is a scoring tool used to assess quality of evidence and risk of bias for nonrandomized studies included in meta-analyses.[19]

Comment on Excluded Studies

A total of 13 studies,[20–32] including a total of 6,169 patients (comprising a total of 533 patients with APS and 68 with systemic lupus erythematosus [SLE]), which included testing of anti-β_2GPI-DI antibodies in diverse cohorts of patients, were excluded from the analysis of prevalence. The exclusion of these studies was based on (1) the impossibility of extrapolating clinical or laboratory data, in particular when reporting the results of anti-β_2GPI-DI positivity,[23,26] (2) lack of reported data on anti-β_2GPI-DI prevalence,[25] (3) no defined cutoffs of positivity,[27] and/or (4) testing results expressed only in relation to the control groups.[26]

Data summarizing the studies excluded from the analysis are illustrated in ►**Table 1**.

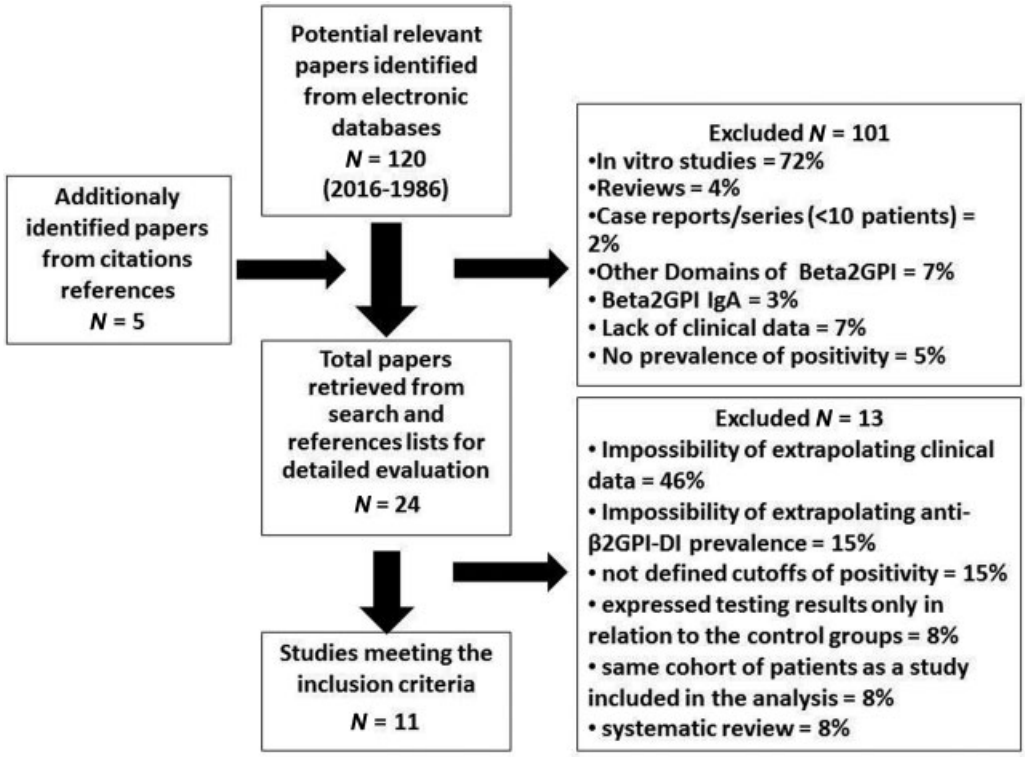

Fig. 1 Literature search strategy on prevalence of positivity for anti-β_2 glycoprotein I domain I antibodies.

Table 1 Studies not included into the prevalence analysis and reason of exclusion

Study	Year	Total number of patients	APS	SLE	Reason of exclusion
Müller-Calleja et al[21]	2016	4979	N/A	N/A	No clinical data of the patients analyzed
Manukyan et al[28]	2016	4979	N/A	N/A	No clinical data of the patients analyzed
Roggenbuck et al[29]	2016	61	61	N/A	Impossibility of extrapolating prevalence of β_2GPI-DI positivity
Pengo et al[22]	2015	65	N/A	N/A	No clinical data of the patients analyzed
Willis and Pierangeli[23]	2015	72	72	N/A	The study compared two assays for the detection of anti-β_2GPI-DI antibodies, without describing patients' positivity for the test
Rodríguez-García et al[30]	2015	1404	1,404	N/A	Systematic review
Meneghel et al[31]	2015	88	88	N/A	Impossibility of extrapolating prevalence of β_2GPI-DI positivity
Despierres et al[24]	2014	439	N/A	12	Clinical data were available for 371 patients, but only 12 patients with SLE were tested for β_2GPI-DI IgA
Zohoury et al[26]	2013	273	273	N/A	In this study, β_2GPI-DI positivity was only expressed as sensitivity, specificity, and likelihood ratios compared with normal controls
Andreoli et al[25]	2013	154	86	28	The results were considered as OD values, and the study used the same cohort of patients as in a study included in the analysis
Andreoli et al[20]	2011	154	86	28	The study used the same cohort of patients as a study included in the analysis
de Laat et al[27]	2007	33	16	N/A	No clinical data of the patients analyzed, no clear cutoffs of positivity
de Laat et al[32]	2005	198	6	176	Impossibility of extrapolating prevalence of β_2GPI-DI positivity

Abbreviations: β_2GPI-DI; -β_2 glycoprotein I domain I; APS, antiphospholipid syndrome; N/A, not available; OD, optical density; SLE, systemic lupus erythematosus.

Statistical Analysis

A detailed statistical analysis has been developed *a priori*. Odds ratios with 95% CI (OR [95%CI]) for arterial and/or venous thrombosis were recorded. If not available, they were calculated, whenever possible, by means of contingency tables. In case–control and cross-sectional studies, contingency tables were used to compare the proportion of anti-β_2GPI-DI antibodies in patients with and without thrombosis. In prospective studies, contingency tables were established as previously reported.[33,34] Briefly, if SLE was the enrolment criterion, the OR [95%CI] was calculated by comparing the proportion of anti-β_2GPI-DI antibodies positivity in patients who did or did not develop thrombosis. If thrombosis was the enrolment criterion, the OR [95%CI] was calculated by comparing the proportion of anti-β_2GPI-DI antibodies in patients with or without recurrent thrombosis during follow-up. If positivity for aPL was the enrolment criterion, the OR [95%CI] was calculated by comparing the rates of thrombosis during follow-up of patients grouped according to different antibody types and titers.

Results

A total of 11 studies,[9,35–44] including a total of 1,585 patients, met the inclusion criteria. Patients were distributed as follow: 1,218 patients with APS (504 with primary APS [PAPS], 192 with secondary APS [SAPS], and 522 not specified), 318 with SLE, 49 aPL asymptomatic carriers, and 1,859 healthy controls (HCs).

Of 11 studies, 9 differentiated patients with APS between PAPS and SAPS, and only 5 out of 11 studies specified the results of anti-β_2GPI-DI antibodies positivity stratifying for the presence of concomitant autoimmune diseases. Two studies out of 11 did not specify if the patients with APS were PAPS or SAPS.

Detection of anti-β_2GPI-DI antibodies was performed with an enzyme-linked immunosorbent assay (ELISA) in 5 out of 11 studies,[9,37,41,42,44] whereas 6 out of 11 studies used a chemiluminescent immunoassay (CIA).[35,36,38–40,43] All studies investigated the presence of immunoglobulin G (IgG) isotype, and one study[44] investigated the presence of IgM and IgA anti-β_2GPI-DI also.

The six studies that performed testing with CIA used the same cutoff of positivity (99th percentile: 20 chemiluminescence units [CU]). Regarding the studies that used ELISA, three studies[9,37,41] expressed the cutoff of positivity as mean + 3 standard deviation (SD) of the HC as reference, one study[44] used a cutoff at the 99th percentile of the HC, and lastly one study[42] used a cutoff at the 95th percentile of the HC.

Data summarizing the main characteristics of the tests used to identify anti-β_2GPI-DI antibodies are provided in ►**Table 2**.

None of the studies investigated persistent positivity to anti-β_2GPI-DI antibodies.

Inclusion criteria for 6 out of 11 studies[35,38–40,43,44] was based on APS diagnosis according to Sydney revised Sapporo guidelines.[1] Two out of 11 studies, although basing their inclusion criteria on the APS diagnosis, did not specify if the APS diagnosis met the Sydney revised Sapporo guidelines.[36,41] One study out of 11 had as inclusion criteria the diagnosis of SLE that met the ACR revised criteria.[37] Two studies out of 11 had as inclusion criteria the presence of anti-β_2GPI antibodies detected at least twice at least 12 weeks apart.[9,42]

Results of the critical appraisal of the included studies according to NOS are shown in ►**Table 3**.

The overall estimated median prevalence of anti-β_2GPI-DI antibodies in patients with APS and/or SLE was 44.3% (range: 26.7–55.4%). When focusing on patients with APS (either PAPS or SAPS), the estimated overall prevalence was high at 45.4%. Stratifying for diagnosis, a significantly higher prevalence of anti-β_2GPI-DI antibodies was observed in patients with APS (either PAPS or SAPS) than those with SLE without previous history of thrombosis (45.4 vs. 26.7%; $p < 0.0001$).

Data regarding the prevalence analysis are summarized in ►**Table 4**.

When stratifying patients for inclusion criteria used to enroll subjects in each study, a frequency of anti-β_2GPI-DI positivity of 38.6% was found in those studies enrolling patients with APS according to the Sapporo criteria,[35,38–40,43,44] 25.1% in studies including patients with SLE (according to ACR revised criteria),[37] 40.3% in patients with APS (without any details about Sydney or Sapporo criteria),[36,41] and 57.9% in those studies that aimed to assess the frequency of anti-β_2GPI-DI positivity in presently anti-β_2GPI positive patients.[9,42] As would be expected, and due to selection bias, an overall statistically significant higher anti-β_2GPI-DI positivity was seen in the two studies that used as inclusion criteria the presence of anti-β_2GPI positivity detected at least twice at least 12 weeks apart[9,42] when compared with the other studies (►**Table 5**).

Five out of 11 studies[9,35,36,38,42] were eligible for thrombotic risk assessment analysis, including a total of 1,014 patients. Patients were distributed as follow: 821 patients with APS (358 with PAPS, 179 with SAPS, and 284 not specified), 163 with SLE, 30 aPL asymptomatic carriers, and 139 HC. Andreoli et al[42] included 100 HC based on a previous analysis to set the cutoffs at the 95th percentile values for anti-β_2GPI-DI IgG.[20]

Three out of 5 studies analyzed IgG isotype with QUANTA Flash β_2GPI-DI CLIA assay and used the same cutoff of positivity that yielded a 99.5% specificity.[35,36,38] Two out of 5 studies analyzed IgG isotype with ELISA.[9,42] Four studies reported a significant association between thrombotic events and positive testing for anti-β_2GPI-DI antibodies.[9,19,20,38] In detail, Zhang et al[35] when investigating the association between thrombotic events and anti-β_2GPI-DI antibodies, reported an odds ratio (OR) of 3.27 (95%, CI 1.59–6.71), Agmon-Levin et al[36] reported an OR of 2.54 (95%, CI 1.05–6.15) and de Laat et al[9] reported an OR of 3.5 (95%, CI 2.3–5.4). Mahler et al[38] reported an OR of 4 (95%, CI 1.26–12.6) with the original cutoff of 20 CU used in the other studies as well. Furthermore, by optimizing the cutoff of positivity to increase the likelihood ratio (LR)+ and OR

Table 2 Characteristics of the test used to identify anti-β_2GPI-DI antibodies

Study	Methodology	Cutoff	Notes
Zhang et al[35]	CIA (QUANTA Flash assay, Inova Diagnostics)	20 CU	Recombinant DI coupled to paramagnetic beads by the use of BIO-FLASH technology
Mahler et al[38]	CIA (QUANTA Flash assay, Inova Diagnostics)	20 CU	Recombinant DI coupled to paramagnetic beads by the use of BIO-FLASH technology
de Craemer and Devreese[43]	CIA (QUANTA Flash assay, Inova Diagnostics)	20 CU	Recombinant DI coupled to paramagnetic beads by the use of BIO-FLASH technology
Cieśla et al[39]	CIA (QUANTA Flash assay, Inova Diagnostics)	19.9 CU	Recombinant DI coupled to paramagnetic beads by the use of BIO-FLASH technology
Mondejar et al[40]	CIA (QUANTA Flash assay, Inova Diagnostics)	20 CU	Recombinant DI coupled to paramagnetic beads by the use of BIO-FLASH technology
Agmon-Levin et al[36]	CIA (QUANTA Flash assay, Inova Diagnostics)	20 CU	Recombinant DI coupled to paramagnetic beads by the use of BIO-FLASH technology
Wahezi et al[37]	ELISA in house	Mean + 3 SD (HC)	β_2GPI coated on a hydrophobic plate and a hydrophilic plate[a]
Hunt et al[41]	ELISA in house	Mean + 3 SD (HC)	β_2GPI coated on a hydrophobic plate and a hydrophilic plate[b]
de Laat et al[9]	ELISA in house	OD (blank + three times SD	β_2GPI coated on a hydrophobic plate and a hydrophilic plate[c]
Andreoli et al[42]	ELISA developed by Inova Diagnostics	95th percentile (HC)	Recombinant DI coupled to ELISA plates
Cousins et al[44]	ELISA in house	99th percentile (HC)	Binding to purified wild-type and mutant recombinant human DI; cCoated on Nickel chelate-coated microwell plates; epitope R39–R43

Abbreviations: CU, chemioluminiscence units; DI, domain I; HC, healthy controls; ELISA, enzyme-linked immunosorbent assay; OD, optical density; SD, standard deviation; β_2GPI, β_2 glycoprotein 1.
[a]Epitope R39–R43.
[b]Coated on hydrophobic microtiter plates, goat antihuman IgG alkaline phosphatase labeled antibody.
[c]Coated on hydrophobic and hydrophilic plates, epitope R39–R43, monoclonal mouse anti-domain I antibody (mAb 3B7).

Table 3 Results of the critical appraisal of the included studies according to Newcastle–Ottawa Scale[19]

Study	Newcastle–Ottawa quality assessment scale		
	Selection	Comparability	Outcome
Zhang et al[35]	★★★	★	★
Mahler et al[38]	★★★	★	★
de Craemer and Devreese[43]	★★★	★	
Andreoli et al[42]	★★	★	★
Cieśla et al[39]	★★		
Mondejar et al[40]	★★★	★	
Cousins et al[44]	★★		
Agmon-Levin et al[36]	★★★	★	★
Wahezi et al[37]	★★		
Hunt et al[41]	★★	★	
B. de Laat et al[9]	★★		★

Table 4 Results of the prevalence study of antibodies directed against β_2GPI-DI (IgG) organized by diagnosis

Diagnosis	No. of patients eligible for prevalence study	No. of patients positive for anti-β_2GPI-DI IgG antibodies	%
APS[a]	1,218	553	45.4
PAPS	504	279	55.4
SAPS	192	83	43.2
SLE	318	85	26.7
aPL+, asymptomatic	49	15	30.6

Abbreviations: aPL, antiphospholipid antibodies; APS, antiphospholipid syndrome; IgG, immunoglobulin G; PAPS, primary antiphospholipid syndrome; SAPS, secondary antiphospholipid syndrome; SLE, systemic lupus erythematosus; β_2GPI-DI, β_2 glycoprotein 1 domain I.
[a]APS group includes 504 with primary APS, 192 with secondary APS, and 522 not specified.

(cutoff of positivity: 190.2 CU) for correlation with thrombosis, they reported an OR of 8. Conversely, Andreoli e al[42] found no significant predictive correlation with thrombosis, but reported that patients with recurrent thrombosis showed higher titers of anti-β_2GPI-DI antibodies. When pooling together the results of the 5 studies we calculated a significant association between anti-β_2GPI-DI positivity and thrombotic events (mean OR 1.99; 95% CI 1.52–2.60; $p < 0.0001$).

The forest plot describing data on thrombotic risk association with anti-β_2GPI-DI positivity is shown in ►**Fig. 2**.

Interestingly, three studies out of 5 reported a significant association between triple aPL positivity and anti-β_2GPI-DI positivity.[35,36,42]

Discussion

In this study, we report an overall median prevalence of anti-β_2GPI-DI antibodies in APS/SLE patients of 44.3%. We observed a significant higher frequency of anti-β_2GPI-DI in patients with APS (45.4%) when compared with SLE patients (26.7%). We noted an even higher prevalence of anti-β_2GPI-DI positivity in patients with PAPS (55.4%), compared with that of patients with SAPS (43,2%). We should acknowledge that this observation might be biased by the small sample size of included patients with SAPS.

When separating data for inclusion criteria, we observed an overall significantly higher anti-β_2GPI-DI positivity in the two studies that used as inclusion criteria the persistent positivity of anti-β_2GPI antibodies.[9,42] This observation is in line with the concept that being positive for anti-β_2GPI antibodies seems to increase the likelihood of also being positive for anti-β_2GPI-DI antibodies. However, Andreoli et al, in a cohort of 159 subjects with persistently positive, medium, or high-titer anti-β_2GPI IgG, found that 105 (66%) were positive for anti-β_2GPI-DI and 35 (22%) were positive for anti-DIV/V IgG. These observations might suggest that approximately a third of subjects positive for anti-β_2GPI antibodies could be negative for anti-β_2GPI-DI antibodies and positive for anti-β_2GPI-D-IV–V antibodies.[42]

In our analysis, when pooling data together, we observed that anti-β_2GPI-DI antibodies positivity doubled the risk for thrombotic events. In detail, four out of five studies found a significant association of anti-β_2GPI-DI antibodies positivity

Table 5 Data of the two studies that used persistent anti-β_2GPI positivity as inclusion criteria compared with data excluding the two studies

DIAGNOSIS	No. of patients eligible for prevalence study		No. of patients positive for anti-β_2GPI-DI IgG antibodies		%		*P* (Fisher's exact test)
	A	B	A	B	A	B	
APS_a	451	767	279	274	61.9	35.7	<0,0001
PAPS	323	181	225	54	69.7	29.8	<0,0001
SAPS	128	64	54	29	42.2	45.3	0.7577
SLE	93	190	36	31	38.7	16.3	<0,0001
aPL+, asymptomatic	30	19	13	2	43.3	10.5	0.0955

Abbreviations: aPL, antiphospholipid antibodies; APS, antiphospholipid syndrome; IgG, immunoglobulin G; PAPS, primary antiphospholipid syndrome; SAPS, secondary antiphospholipid syndrome; SLE, systemic lupus erythematosus; β_2GPI-DI, β_2 glycoprotein 1.
A: Data only of the two studies that used persistent anti-β_2GPI positivity as inclusion criteria.
B: Data excluding the two studies that used persistent anti-β_2GPI positivity as inclusion criteria.
[a]APS group includes 504 with primary APS, 192 with secondary APS, and 522 not specified.

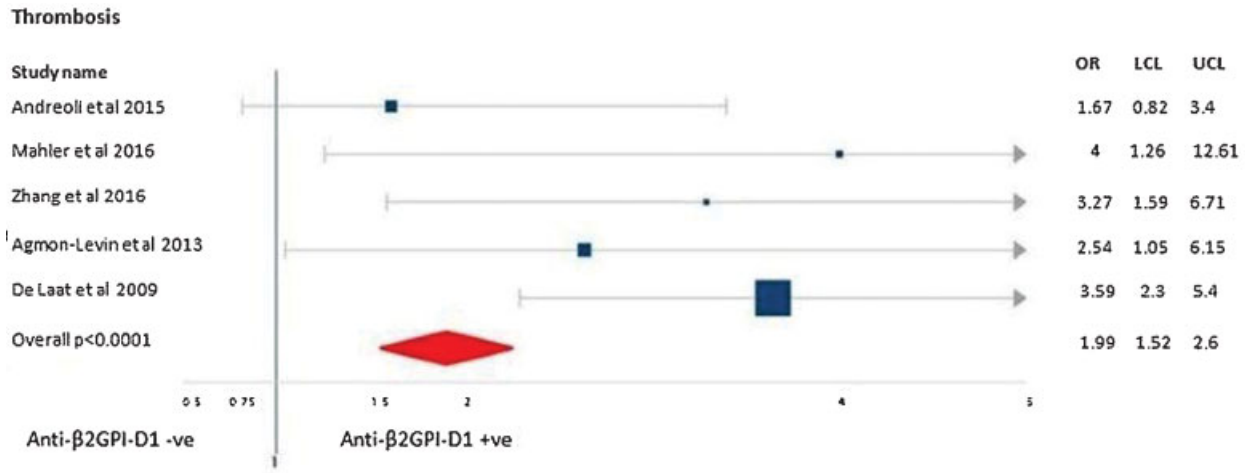

Fig. 2 Forest plot of thrombotic risk association with anti-domain I positivity.

with thrombotic events, whereas one study found no predictive correlation with thrombosis. However, one could speculate that these findings might be at least in part explained by the fact that anti-β_2GPI-DI antibodies levels have been shown to be significantly higher in individuals with a triple-positive aPL profile, a well-identified category at increased risk for thromboembolic events.[22]

A common limitation of the studies that analyze non-criteria aPL is the small sample size. However, we found that 7 out of 11 studies eligible for prevalence analysis included a large cohort of patients (>100), supporting the strength of the observation. Similarly, our analysis included a total of 1,809 (range: 30–200) HC.

In the studies analyzed, two different laboratory tests were used to identify anti-β_2GPI-DI antibodies (6 out of 11 studies used CLIA and 5 used ELISA). Some intrinsic differences due to the techniques might be speculated. However, recently, Willis and Pierangeli[23] reported a good qualitative ($k = 0.6$) and quantitative (*Spearman's rho* $= 0.76$) agreement between two laboratory tests: ELISA in house University College London and QUANTA Flash CIA (Inova Diagnostics).

Nevertheless, despite the reported good agreement between the two test types, some additional considerations are worth mentioning. First, it is reasonable to suspect that the antigenic preparations (conformational peptide versus whole DI) could influence the test's ability to detected anti-β_2GPI-DI antibodies. This difference might intrinsically influence the level of agreement between the studies considered in our analysis. Second, anti-β_2GPI-DI testing might be influenced by the conformational changes of β_2GPI that directly modify the exposure to the surface of DI.[45] In fact, anti-β_2GPI-DI antibodies have reactivity toward their target epitope only when DI is coated onto hydrophobic, but not hydrophilic plates.[32] Unfortunately, there is no study available that has specifically compared the concordance among techniques using different antigen preparations.

Clinical manifestations of APS include vascular thrombosis and/or pregnancy morbidity. In our review, we analyzed the thrombotic risk assessment in relation to anti-β_2GPI-DI antibodies positivity; however, no studies have reported a predictive association between pregnant morbidity and anti-β_2GPI-DI positivity to our knowledge.

Recently, Müller-Calleja et al[21] investigated IgG anti-β_2GPI-DI antibodies positivity with CIA in a large cohort of individuals (4,979 and 1,049 HC) to estimate the overall positivity in the general population. Although no significant difference was reported between the whole sample of individuals of the general population and the HCs, no detailed clinical data of the individuals included in the study was provided. Thus, although the general population is unlikely to benefit from indiscriminate screening for these antibodies, their detection in patients with SLE, connective tissue diseases, and/or previous thrombosis is justified by the high thrombotic risk associated with these clinical conditions.[42]

Strengths and Limitation of the Analysis

The strengths of this analysis lie on a priori designed search strategy, and the inclusion of gray literature searches and manual review of reference lists minimized the risk of missing eligible studies. We performed independent and duplicate review for study selection and data extraction.

However, there are also limitations. All of the included studies were observational studies, subject to the biases inherent in such study designs.

Our research strategy did not include Scopus as per a priori designed protocol. Additionally, there was heterogeneity in the data in terms of inclusion criteria, assay heterogeneity, cutoff values definition, detected Ig isotypes, clinical details, control groups, and site of thrombosis. Moreover, none of the studies reported data comparing persistent versus transient anti-β_2GPI-DI positivity. Also, only a minority of studies confirmed their findings by multivariate analysis. Furthermore, when analyzing the control groups used in the studies included in the analysis, no study included a control group consisted of event-free aPL carriers, limiting the generalizability of the role of anti-β_2GPI-DI

positivity in the different subgroups (e.g., thrombotic APS, obstetric APS, isolated aPL carries, aPL carriers in patients with SLE). Finally, while significant international improvements have been achieved in the standardization of IgG and IgM anti-β_2GPI antibody measurement,[46,47] one could not exclude that some potential bias related to the availability of existing reference materials might still exist.

Conclusions

Despite the limitations of the studies included, we report an overall estimated median prevalence of 44.3% in patients with APS and/or SLE and a higher prevalence of anti-β_2GPI-DI antibodies among patients with APS compared with SLE alone. Furthermore, when pooling data together, we observed that anti-β_2GPI-DI antibodies positivity doubles the thrombotic risk compared with patients negative for anti-β_2GPI-DI antibodies.

Thus, anti-β_2GPI-DI antibodies seem to be a potential candidate as a laboratory tool for the diagnosis of APS, in particular regarding thrombotic risk assessment. The inclusion of anti-β_2GPI-DI antibodies as laboratory criteria for the APS should be indubitably explored.

Author Contributions

M.R. and I.C. searched the literature, assisted with the organization of the manuscript, interpreted and collected data, and wrote and edited the review. P.L.M., D.R., and S.S. interpreted and collected data, helped to design the figures and panel, and wrote and edited the review.

Conflict of Interest

None.

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None.

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