

Assessment of lupus anticoagulant in anti-cardiolipin and/ or anti- β 2GPI sero-positive patient: Clinicopathological perspective from a tertiary care lab in Pakistan

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ABSTRACT

Objective: To assess the frequency of Lupus anticoagulant in patients seropositive for anti-cardiolipin and/or anti Beta-2 glycoprotein (anti- β 2GPI) antibodies, and to explore potential clinicopathological correlations.

Material and Methods: This Observational Cross-sectional study was conducted at the Department of Immunology, AFIP Rawalpindi from January 2022 to October 2024. A total of 108 samples seropositive for anti-cardiolipin and/or anti- β 2GPI antibodies were selected and further investigated for Lupus anticoagulant. Patients were stratified into single, double and triple positivity based upon their aPL profile. Chi-square test were used to find significant association among these aPL profiles. Mann-Whitney U test was used to compare age in LA-positive and negative groups. p -value <0.05 was considered statistically significant.

Results: Out of 108 samples analyzed, the LA positivity was observed in 13 (12%) patients and was significantly associated with younger age (median: 29 vs. 37 years, $p = 0.041$), but not with gender. Among cases positive for aCL IgG and a β 2GPI IgG (including dual positive cases), 12 (11.1%) and 2 (1.9%) were LA-positive, respectively. No association was observed between individual antibody positivity and LA ($p = 0.457$ for aCL IgG; $p = 0.221$ for a β 2GPI IgG). Dual positivity for aCL IgG and a β 2GPI IgG did not significantly increase LA positivity, and triple positivity (aCL IgG + a β 2GPI IgG + LA) was rare (0.9%).

Conclusion: To conclude, Lupus anticoagulant was detected in a subset of anti-cardiolipin and/or anti- β 2GPI seropositive patients, with a significant association observed with younger age.

Keywords: Antiphospholipid antibodies, Antiphospholipid Syndrome, Autoimmune disorder, Lupus anticoagulant.

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INTRODUCTION

Autoantibodies targeting phospholipid-binding plasma proteins, collectively termed antiphospholipid (aPL) antibodies, play a central role in the pathogenesis and diagnosis of antiphospholipid syndrome (APS) [1]. These autoantibodies are primarily directed against protein-phospholipid complexes, with β 2-

glycoprotein I (β 2GPI) and prothrombin being key antigenic targets. Their identification is pivotal for diagnosing APS and is achieved through both immunoassays and functional coagulation tests.

The prevalence of APS is quite challenging to estimate due to non-criteria manifestation, changes in APS classification criteria and other co-morbidities [2,3]. Although previous studies estimate the incidence and prevalence ranged between 1 and 2 cases per 100,000 and 40 to 50 cases per 100,000 respectively [4]. Secondary APS is more common in patients with systemic lupus erythematosus (SLE), with approximately 30% testing positive for antiphospholipid antibodies (aPL). APS is typically diagnosed in individuals aged around 30 to 40, However

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ethnic and age-related disparities exist. Despite these challenges, previous studies report significant mortality rate 50 to 80% higher than the general population [4,5]. However, there is a scarcity of research on APS in Pakistan, particularly regarding its prevalence and the relationship between aPL antibodies and their clinical outcomes [6]. This underscores the need for region-specific studies to enhance understanding of the syndrome's burden and its distinct manifestations within local populations. The study aims to address the existing data gap on APS and the correlation between aPL antibodies, offering insights into disease severity and thrombotic risk stratification.

The two main immunoassays utilized for identification of APS are anticardiolipin (aCL) and anti- β 2-glycoprotein I (a β 2GPI) antibody assays. Additionally, Lupus anticoagulant (LA), A coagulation-based assay holds a distinct role in APS diagnosis and risk stratification. Unlike its name suggests, LA is associated with a two-fold increase in thrombotic events[7,8]. The presence of LA, along with aCL and anti- β 2GPI antibodies, exacerbates these risks by impairing placental function and promoting thrombosis within placental vasculature. Consequently, APS is a leading cause of maternal morbidity and adverse pregnancy outcomes

Patients with APS face significant long-term health challenges, with over 30% experiencing permanent organ damage and more than 20% developing severe disabilities [9]. Conditions such as catastrophic APS (CAPS), pulmonary hypertension, kidney disease, central nervous system (CNS) involvement, and gangrene of the extremities are associated with worse outcomes. The clinical manifestations range from deep vein thrombosis (DVT) and stroke to recurrent pregnancy loss, making APS a multifaceted disease with systemic implications. In some cases, elevated APLA levels are observed in individuals with underlying malignancies which linked to a poorer prognosis, regardless of whether thromboses are present, further complicating the clinical course of these patients [10,11]. Furthermore, aPL antibodies co-relate

well with APS upon moderate to high levels aCL or anti- β 2GPI antibodies. Among these, the IgG isotype has demonstrated a more robust association with aPL-related clinical events, such as thrombosis and pregnancy complications, than the IgM isotype[12].

The rationale of this study is to evaluate the frequency of positive Lupus anticoagulant in patients already seropositive for anti-cardiolipin and/or anti- β 2GPI antibodies, thereby emphasizing the importance of repeat aPL testing and generating locally relevant baseline data to support future research within the Pakistani population, rather than relying solely on international data.

MATERIAL AND METHODS

This cross-sectional observational study was carried out at Department of Immunology, Armed Forces Institute of Pathology (AFIP) / NUMS, Rawalpindi after obtaining approval from the Institutional Ethical Review Board (reference no. Cons-IMM-3/READ-IRB/24/3645 dated 07 August 2024). The sample size was estimated using the WHO calculator based on a reported double-positivity prevalence of 2% for aCL and LA.¹³ The adequate sample size was calculated to be 84, however 108 patients who met the inclusion criteria were included. Sampling was done by non-probability consecutive sampling technique.

Inclusion criteria: Participants from both genders with moderate to high titers of anti-cardiolipin and/or anti-B2GPI antibodies (>40 GPL or 99th percentile) confirmed on ELISA were included.

Exclusion criteria: Patients on long term anti-coagulant therapy and/or presented with co-existing disorder influencing test results and those receiving immunosuppression were excluded.

Demographic and clinical data were noted for all the participants. For each patient, two samples were collected: One in a 3.2% trisodium citrate tube and the other in a serum-separating tube. Serum samples were analyzed for IgG anti-cardiolipin (aCL) and anti- β 2 glycoprotein I

(aβ2GPI) antibodies using commercially available ELISA kits as per the manufacturer protocol. Anti-cardiolipin results were reported in GPL units (IgG phospholipid units), with a medium/high titer defined as ≥40 GPL. Anti-β2GPI results were expressed in arbitrary units (U/mL), and the cut-off value was determined based on the 99th percentile of a healthy control population. Lupus anticoagulant testing was performed using dilute Russell's viper venom time (dRVVT) and silica clotting time (SCT) as integrated assays, with results reported as normalized screen-to-confirm ratios in accordance with the recommendations of the British Society for Haematology (BSH).

Data was analyzed using SPSS version 27.0. Frequencies and percentages were calculated for qualitative variables such as Gender, positivity for aCL, anti-B2GPI and LA, while median and IQR was calculated for quantitative variables such as age. Depending on the non-normal distribution of the data as assessed by the Shapiro–Wilk test ($p < 0.05$), the Mann–Whitney U test was employed to evaluate the differences in continuous variables across disease groups. Categorical associations between LA-positive and LA-negative groups were analyzed using either the Chi-square test or Fisher's exact test, as appropriate. A p -value of less than 0.05 was considered indicative of statistical significance.

RESULTS

Out of 108 suspected APS patients 37 (34.3%) were males and 71 (65.7%) were females with a mean age of 38.1 ± 11.9 years. Among these lupus anticoagulants (LA) was found to be positive in 13 (12%) while 95 (88%) tested negative.

Patients testing positive for Lupus Anticoagulant (LA) were significantly younger than their LA-negative counterparts (median age 29 vs. 37 years, $p = 0.041$). However, no significant associations were observed between LA status and gender or the presence of antiphospholipid antibodies, including anticardiolipin IgG, anti-β2-glycoprotein I IgG, or their combined positivity ($p > 0.05$), Table – I. Since only patients seropositive for aCL IgG and/or aβ2GPI IgG were included as per the study criteria, no LA testing was performed in patients negative for both antibodies. Therefore, no conclusions can be drawn about LA occurrence in double-negative patients. Conversely, the majority of LA-positive cases were observed in individuals seropositive for aCL IgG only (84.6%), with few cases (9.1%) occurring in those with isolated aβ2GPI positivity or dual positivity, Figure 1. Despite these patterns, statistical significance was not achieved, indicating insufficient evidence to establish a definitive relationship between specific antibody profiles and LA status in this study population.

Table I: Demographics and antibody distribution in relation to lupus anticoagulant positivity

Variable	Lupus Anticoagulant		Total (n = 108)	p-value
	Positive (n = 13) 12%	Negative (n = 95) 88%		
Age in years, Median (Range)	29 (24 - 48)	37 (20 - 72)	-	0.041 ^a
Gender	Male	2 (1.9%)	32 (32.4%)	0.212 ^b
	Female	11 (10.1%)	60 (55.6%)	
aCL IgG positive (including dual-positive)	12 (11.1%)	77 (71.3%)	89 (82.4%)	0.457 ^b
aβ2GPI IgG positive (including dual-positive)	2 (1.9%)	32 (29.6%)	34 (31.4%)	0.221 ^b
Dual aCL IgG + aβ2GPI IgG positivity	1 (0.9%)	14 (12.9%)	15 (13.8%)	0.689 ^b

a. Independent Sample Mann-Whitney U test

b. Fisher's Exact test

Venn Diagram with Counts and Percentages Distribution of aCL IgG, aβ2GPI IgG and LA Positivity

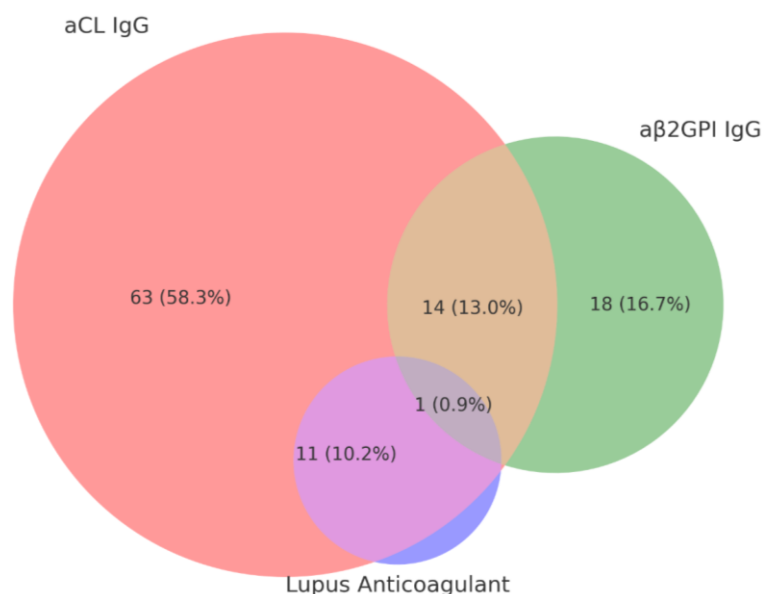


Figure-I: Venn diagram of anticardiolipin IgG (aCL IgG), anti Beta2 glycoprotein I IgG (aβ2GPI IgG) and Lupus Anticoagulant (LA) positivity among included seropositive patients. No aCL/β2GPI double-negative patients were tested in this study; therefore, the LA-only subset is not represented in the diagram.

DISCUSSION

This study investigated the association of lupus anticoagulant with anticardiolipin (aCL) and/or anti-β2- glycoprotein I (aβ2GPI) antibodies with focus on finding their individual and combined positivity. This study did not demonstrate any significant association between Lupus anticoagulant (LA) and the isolated presence of either anti-cardiolipin (aCL) or anti-β2 glycoprotein I (anti-β2GPI) antibodies. Among the 89 samples positive for aCL IgG, only 11 (13.5%) were also LA positive. Similarly, out of 34 anti-β2GPI IgG-positive samples, LA was detected in just 2 cases (5.9%). These findings suggest that LA may not consistently co-occur with individual antiphospholipid antibody profiles, indicating limited predictive value when these antibodies are considered in isolation. The chi-square analysis showed no statistically significant association between LA in either aCL IgG ($p = 0.457$) or aβ2GPI IgG ($p = 0.221$). These findings are in agreement with study by Neville C *et al*, which demonstrated that the non-persistence of aCL antibodies shows no to little significance in terms of lupus prevalence while persistence aCL antibodies linked to increased thrombotic risk [14]. Efthymiou M *et al* further

demonstrated that transient aPL positivity often lacks clinical significance, and that persistence over multiple tests is essential for accurate risk stratification [15]. This aligns with findings of this study, where aCL and aβ2GPI did not show significant associations with LA, suggesting that a single-time assessment may not be sufficient to establish clinical relevance.

One of the significant observations from this study is the younger age distribution of LA-positive individuals which was significantly lower than that of LA-negative individuals). This finding aligns with study from Pakistan by Anwar M *et al* which retrospectively analyzed the past seven years of data for Lupus shows a higher prevalence in younger populations presenting with thrombotic strokes or recurrent pregnancy loss with mean age of 28 years [16].

In terms of gender distribution, LA positivity was more frequently observed in females (11 cases) compared to males (2 cases) however chi-square test revealed no statistical significance. These findings align with the observations reported by Dabit JY *et al*, who also found no significant association between gender and Lupus anticoagulant positivity. Their study demonstrated a wide variation in the male-to-

female ratio, ranging from 1:1 to 10:1, further supporting the lack of consistent gender-based predisposition in such cases [4]. However, the slight female predominance remains noteworthy, as it may point to broader autoimmune and hormonal influences, as suggested in other autoimmune conditions [17]. The combined analysis of aCL IgG and anti- β 2GPI IgG positivity revealed that out of 89 double-positive samples, 13 were also positive for Lupus anticoagulant, indicating a potential additive effect of concurrent antibody positivity. Although this trend suggests a possible association, the lack of statistical significance may be attributed to the limited sample size and the relatively weak p-values, warranting further investigation in larger cohorts.

Contrary to the findings of the present study, previous research has demonstrated a higher clinical risk associated with triple positivity for antiphospholipid antibodies (Lupus anticoagulant, anti-cardiolipin, and anti- β 2GPI). Studies have shown that patients exhibiting multiple aPL positivity are more likely to experience severe clinical outcomes, including recurrent thrombotic events and pregnancy-related complications, compared to those with isolated antibody positivity [18,19].

This study has several limitations, including the lack of clinical data, which prevented the application of full diagnostic criteria for antiphospholipid syndrome (APS), limiting the findings to a laboratory-based, single-center perspective. The cross-sectional design also restricted longitudinal follow-up of LA-negative patients, thereby limiting insights into potential seroconversion. Additionally, temporal and inter-assay variability in antiphospholipid antibody expression may affect diagnostic accuracy, underscoring the need for larger, multicenter studies.

CONCLUSION

This study highlights the complex interplay between antiphospholipid antibodies, with no significant association found between aCL or anti- β 2GPI positivity and Lupus anticoagulant. LA was more prevalent in

younger patients, and the rarity of triple positivity highlights the importance of comprehensive aPL screening to better identify patients at risk.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Declared none

AUTHORS CONTRIBUTION

Mustajab Alam: Substantial contributions to study design, acquisition of data, manuscript drafting, final approval, accountable for all aspects of publication.

Muhammad Sulaiman: Study design, manuscript drafting, acquisition of data, data analysis final approval, accountable for all aspects of publication.

Muhammad Hussain: Manuscript drafting, data analysis, critical revision, has given, final approval of the version to be published.

Muhammad Aftab Hassan: Data analysis, data interpretation, final approval of the version to be published.

Muhammad Zain Ul Abdeen Javed: Data analysis, manuscript drafting, final approval of the version to be published.

Muhammad Shoaib Hassan: Data analysis, proofreading, final approval of the version to be published.

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