Associations Among Antiphospholipid Antibody Types, Isotypes, and Titers: Results from the AntiPhospholipid Syndrome Alliance For Clinical Trials and InternatiOnal Networking (APS ACTION) Clinical Database and Repository ("Registry")

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Conflicts of Interest:

The authors declare that there is no conflict of interest for this work.

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Key Indexing Terms

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Abstract

Background/Purpose

Several antiphospholipid antibody (aPL) profiles ("triple" and lupus anticoagulant [LA] positivity) are associated with higher risk for clinical manifestations of antiphospholipid syndrome (APS). Further risk is correlated with higher levels of anticardiolipin (aCL) and anti-β_2 glycoprotein-I antibodies (aβ_2GPI), and with aPL persistence. Given that the three aPL tests detect partially overlapping sets of antibodies, the primary goal of this study was to characterize the associations among aPL tests using APS ACTION Core Laboratory data.

Methods

The APS ACTION Registry includes annually followed adult patients with positive aPL based on the Revised Sapporo Classification Criteria. We analyzed baseline and prospective Core Laboratory data of the Registry for associations among aPL tests, using Spearman's rank correlation with Bonferroni adjusted significance level for multiple comparisons. An aPL Load was calculated based on six tests (aCL IgG/M/A and aβ_2GPI IgG/M/A); a receiver operating characteristic (ROC) curve was used to evaluate the diagnostic performance of the aPL Load in predicting LA positivity.

Results

In 351 patients simultaneously tested for LA, aCL, and aβ_2GPI, the frequency of moderate-to-high (≥40 units) titers of aCL and aβ_2GPI IgG/M/A was higher in patients who were positive for LA versus negative. An aPL Load was calculated for each patient to assess their overall aPL

burden. For every one-point increase in aPL Load, the possibility of a positive LA test increased by 32% (OR 1.32, 95% CI 1.2, 1.5, p<.001).

Conclusion

Based on Core Laboratory data from a large international registry, most aPL ELISA ≥40U, and a high calculated aPL Load combining six aPL ELISAs were predictive of a positive LA. These data suggest that the combined quantitative burden of aPL may provide a mechanistic explanation of a positive LA.

Introduction

Antiphospholipid syndrome (APS) is characterized by thrombosis and/or pregnancy morbidity in the setting of antiphospholipid antibodies (aPL). Several aPL profiles are associated with a higher risk for the clinical manifestations of APS. These include aPL "triple positivity" (positive tests for lupus anticoagulant [LA], anticardiolipin antibodies [aCL], and anti-β_2 glycoprotein-I antibodies [aβ_2GPI]), and LA positivity itself. Further, risk is associated with higher levels of aCL and aβ_2GPI, and with aPL persistence over time.

The three aPL tests do not detect discrete antibody populations, but rather partially overlapping sets of antibodies. The two major antigenic targets of aPL are β_2GPI and prothrombin. The aβ_2GPI immunoassays detect isotype-specific antibodies to human β_2GPI. Among APS patients, aCL tests primarily detect isotype-specific antibodies to bovine β_2GPI present in the blocking buffer/sample diluent but can also detect antibodies with high propensity for binding cardiolipin alone or cardiolipin complexed with β_2GPI. Lupus anticoagulant tests can detect certain antibodies to β_2GPI and/or prothrombin of any isotype. Given that the LA test is a functional assay rather than a direct measurement of antibodies, its presence may indicate a relatively high aPL concentration that is sufficient to turn the functional assay positive[1].

The primary goal of this study was to describe the associations among aPL tests (LA, aCL, and aβ_2GPI) using APS ACTION (AntiPhospholipid Syndrome Alliance For Clinical Trials and InternatiOnal Networking) Core Laboratory data.

Methods

APS ACTION is an international network created to design and conduct large-scale, multi-center studies in persistently aPL-positive patients[2]. The APS ACTION Registry includes adult patients who have had positive aPL based on the laboratory component of the Revised Sapporo Classification Criteria[3] at least twice within one year prior to enrollment. The patients are followed up annually and blood is collected at enrollment and follow up visits. Through six APS ACTION Core laboratories samples of Registry patients are tested for aPL using standard validated protocols and reagents to confirm aPL positivity.

For this analysis, we used Core Laboratory aPL results: anticardiolipin and aβ_2GPI IgG/M/A were determined using ELISA tests (QUANTA Lite EIA, Inova Diagnostics); manufacturer recommended cut-offs were used after validation exercises in all core laboratories. Lupus anticoagulant was performed according to International Society on Thrombosis and Haemostasis (ISTH) guidelines[4] following validation, with a combination of silica clotting time (SCT), diluted Russell Viper Venom Time (dRVVT), (HemosIL, Instrumentation Laboratory – IL) and/or Taipan/Ecarin time (Diagnostic Reagents Ltd.) assays depending on the patient's anticoagulation status and using the aCL TOP Coagulation Analyzer Systems (IL). The aPL ELISA test titers were categorized as follows: 0 to <20 U (negative); 20 to <40 U (low-positive); 40 to <80 (moderate-positive); and ≥80 U (high-positive) to examine their association with LA positivity. Titers ≥ 40 U were characterized as moderate-to-high.

An aPL Load was calculated for each patient to assess their overall aPL burden. Results from each of the six aPL ELISA tests were assigned a score as follows: 0, if titer was between 0 to <20 U; 1, if 20 to <40 U; 2, if 40 to <80; and 3, if ≥80 U. The overall aPL Load for each patient was the sum of the scores for the six aPL ELISA tests. Thus, the aPL Load could range from 0 (all six ELISA tests with titers between 0 to <20 U) to 18 (all six ELISA tests with titers ≥80 U).

Spearman's rank correlation with Bonferroni adjusted significance level for multiple comparisons was used to assess correlation between all available aPL ELISA test results. Univariate logistic regression was used to assess laboratory predictors of positive LA. A receiver operating characteristic (ROC) curve was used to evaluate the diagnostic performance of the aPL Load in predicting a positive LA.

Results

As of January 2021, there were 854 patients enrolled in the Registry, of which 351 had their blood samples tested at APS ACTION Core Laboratories for all aPL tests (LA, aCL and aβ_2GPI IgG/M/A) at baseline visit. 567 patients had results for aPL ELISA tests (aCL and aβ_2GPI IgG/M/A) at baseline and follow up visits, amounting to a total of 1,008 sets of tests. Among the 351 LA tests at baseline, 206 were determined based on a combination of dRVVT and Taipan/Ecarin time (all patients on anticoagulation), 143 from SCT and dRVVT (none on anticoagulation), and two from SCT, dRVVT, and Taipan/Ecarin time (none on anticoagulation).

Based on the 1,008 sets of aPL ELISA tests, a strong correlation was found between aCL IgG and $a\beta_2$GPI IgG (r=0.74, p<.001), and between aCL IgM and $a\beta_2$GPI IgM (r=0.75, p<.001) (Figure 1). Moderate correlation was seen between aCL IgA and $a\beta_2$GPI IgG (r=0.44, p<.001), aCL IgA and $a\beta_2$GPI IgA (r=0.55, p<.001), $a\beta_2$GPI IgA and aCL IgG (r=0.43, p<.001), and $a\beta_2$GPI IgA and $a\beta_2$GPI IgG (r=0.4, p<.001), and very weak correlation among the rest of antibody combinations.

In 351 patients, who were simultaneously tested for LA, aCL, and $a\beta_2$GPI at baseline visit, LA positivity was present in 87.2% (306/351) and the frequency of moderate-to-high (≥40 units) titers of aCL and $a\beta_2$GPI IgG/M/A was uniformly higher in patients who were positive for LA (198/306, 65%) *versus* those who were negative for LA (10/45, 22%) (p<0.0001). The frequency of moderate-to-high titers of each aPL analyte in LA positive patients was greater by a factor of 2 to 12 times when compared to their LA negative counterparts (Figure 2). In univariate analysis, moderate-to-high titers of each of the aPL tests, except for $a\beta_2$GPI IgG and IgA, were associated with higher odds of a positive LA test compared to lower titers (Table 1).

Antiphospholipid Antibody Load

For patients with negative LA at baseline the median aPL Load was 1 (interquartile range: 0-2), while for patients with positive LA at baseline the median aPL Load was 4 (interquartile range: 1-6). For every one-point increase in aPL Load, the possibility of a positive LA test increased by 32% (OR 1.32, 95% CI 1.2, 1.5, p<.001) (Figure 3).

A ROC curve was used to assess whether the aPL Load could distinguish between patients with positive or negative LA at baseline. The area under the curve was 0.7 (95% CI 0.6, 0.8) and aPL Load with the highest discriminatory power was three (sensitivity 0.51, specificity 0.87, positive likelihood ratio of 3.8) (Figure 4).

Discussion

Core laboratory data from a large, international cohort of patients with persistently positive aPL with or without autoimmune diseases has shown a strong association between aCL IgG and aβ_2GPI IgG, as well as between aCL IgM and aβ_2GPI IgM, and moderate or low correlations between other isotypes of aPL. This finding is confirmatory of the overlapping nature of the aCL and aβ_2GPI antibodies. A similar correlation between aCL and aβ_2GPI IgG was reported in 168 patients with SLE with and without thrombotic events ($r=0.86$, $p<.0001$)[5].

Additionally, we showed that higher titers of each aPL ELISA test (except for aβ_2GPI IgG and IgA), as well as a higher aPL Load, were associated with a positive LA. A possible explanation to this association is the contribution of a higher aPL burden in turning the functional LA assay positive. Data from the Antiphospholipid Syndrome Collaborative Registry previously reported that patients with higher antibody titers of aCL IgG and IgM (separately and when summed) were exhibiting more frequently a positive LA[1]. As LA is a functional assay, its analytical sensitivity (lowest concentration of an analyte that can be reliably detected in an assay) is considerably lower compared to ELISA tests, requiring higher concentration of aPL to bind to phospholipids and prolong the coagulation assay[1]. Another interpretation of the association

between the higher aPL Load and LA positivity would be the presence of an autoantibody of unknown specificity, that is more likely to be present in patients with multiple aPL with identified specificities. Furthermore, when examining the aPL Load as a sum of the scores for the six aPL ELISA tests, a certain load may represent the contribution of one ELISA aPL test of higher titer, or the sum of multiple lower titer tests. Although the influence of such differences to LA positivity was not explicitly examined, we believe that the total aPL Load is of importance for LA positivity, irrespective of how it is reached. Future analysis may further address this question.

The strengths of this study include the large number of patients participating in the APS ACTION Registry, and the use of aPL test results performed at Core Laboratories, which decreases the degree of heterogeneity in determination of aPL assays, as seen across laboratories at different centers of the world. Study's weaknesses include lack of longitudinal LA data at follow up visits (LA data used from baseline visit), and lack of results for anti-prothrombin and anti-phosphatidylserine/prothrombin antibodies (aPS/PT). Certain aPS/PT have been shown to have LA activity[6,7] and are likely responsible for LA activity in some or most LA positive APS patients who are negative for aCL and aβ_2GPI. Future inclusion of aPS/PT in the aPL Load may improve the metric's predictive value for a positive LA. As above, autoantibodies of unknown specificities may explain isolated LA positivity in APS patients without aCL, aβ_2GPI, and/or aPS/PT. Additionally, in this study the aPL Load was calculated using equal weighting for aPL tests and their respective unit values. It is certainly possible that the type of test (aCL, aβ_2GPI, aPS/PT), isotype (IgG, IgM, and IgA), and antibody levels are associated with different levels of

risk for LA and APS clinical manifestations. Lastly, we did not correlate the aPL results with aPL-related clinical manifestations, which was beyond the scope of the study objective.

In conclusion, our study confirms and expands previously published data on contribution of aPL load in LA test positivity, which may provide a possible explanation on what constitutes and contributes to a positive LA.

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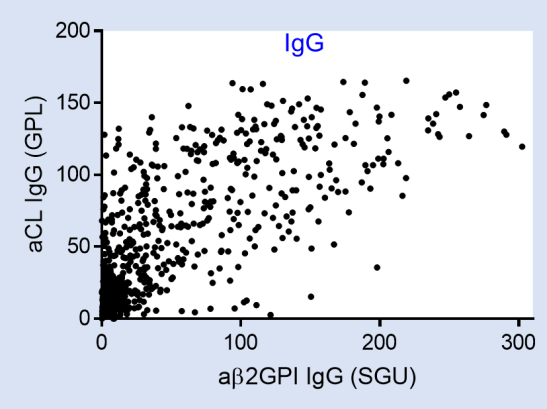
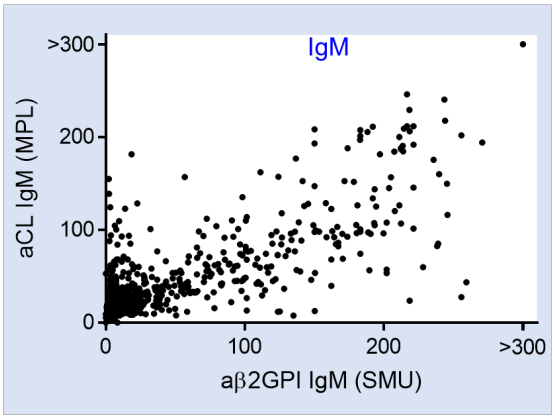
Tables and Figures

Figure 1: Correlation Between aCL IgG and aβ$_2$GPI IgG, and aCL IgM and aβ$_2$GPI IgM



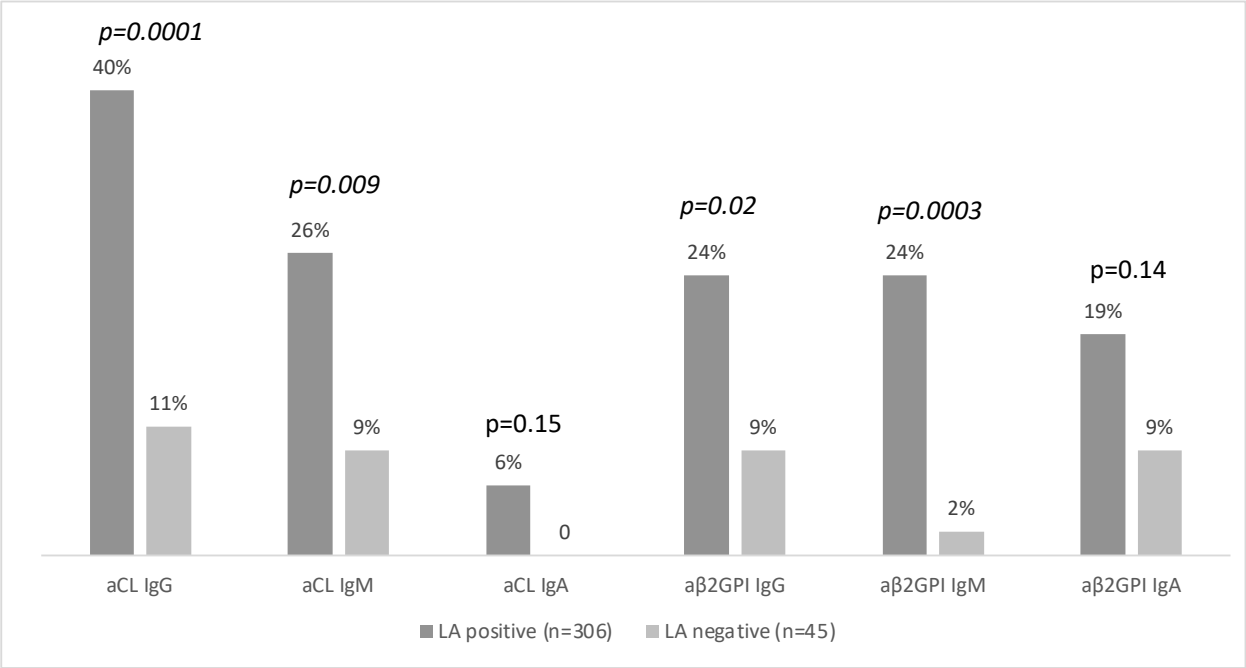
r = 0.74, p < 0.001



r = 0.75, p < 0.001

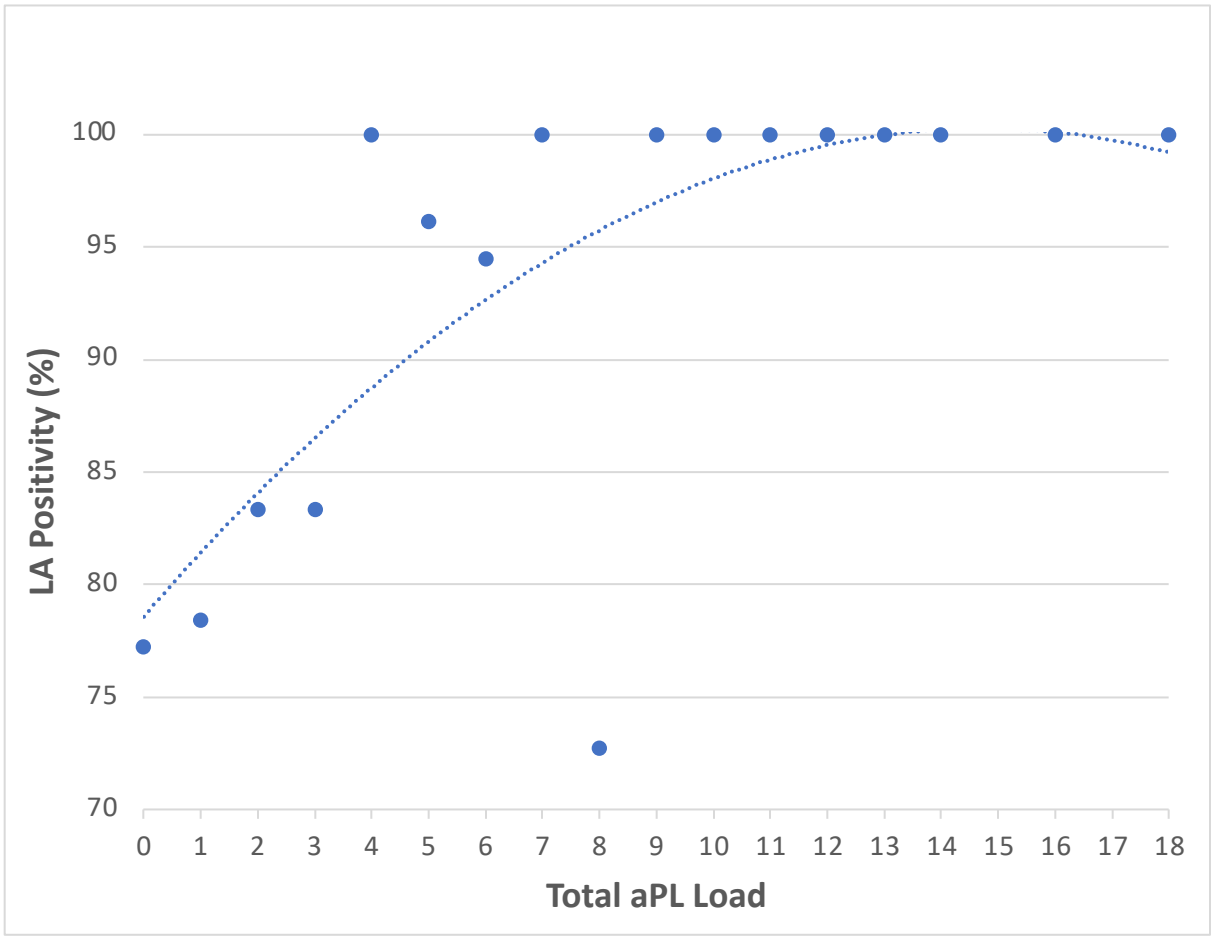
aCL: Anticardiolipin Antibody, aβ$_2$GPI: anti-β$_2$ Glycoprotein-I Antibody

Figure 2: Frequency of Moderate-to-High Titers of aCL and aβ$_2$GPI IgG/M/A (≥40U) Based on LA Result at Baseline



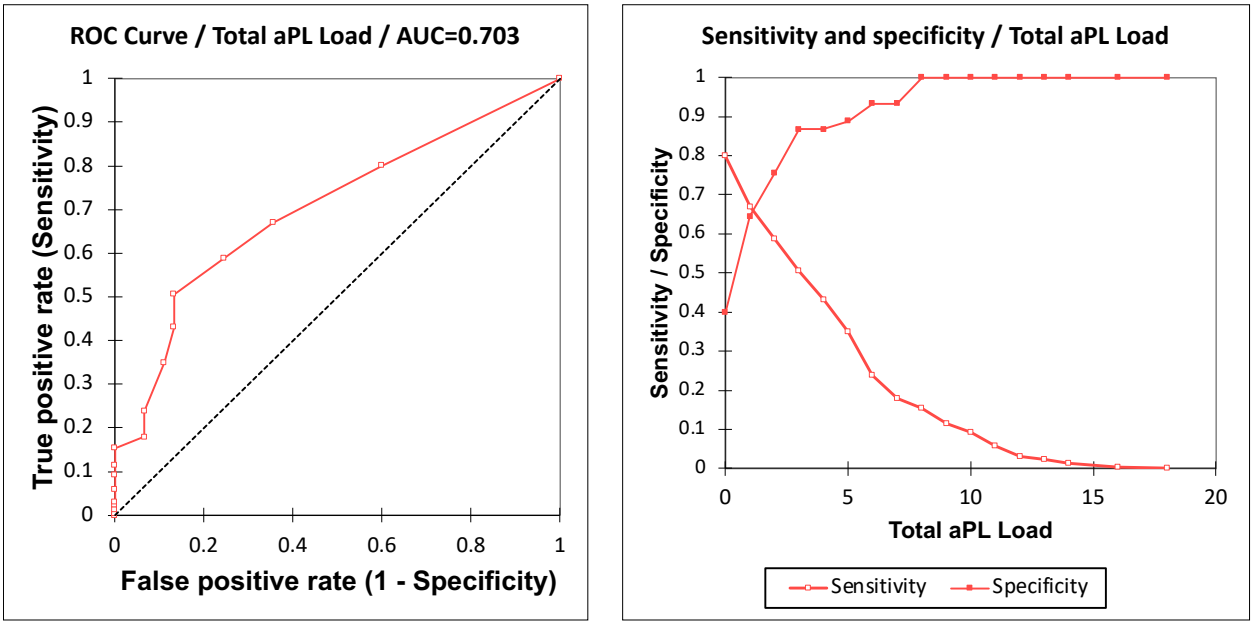
aCL: Anticardiolipin Antibody, aβ$_2$GPI: anti-β$_2$ Glycoprotein-I Antibody, U: Units, LA: Lupus Anticoagulant

Figure 3: Lupus Anticoagulant Positivity as a Function of Total aPL Load



aPL: Antiphospholipid Antibody, LA: Lupus Anticoagulant

Figure 4: Receiver Operating Characteristic (ROC) Curve of aPL Load as a Predictor of Positive Lupus Anticoagulant



aPL: Antiphospholipid Antibody, AUC: Area Under the Curve

Table 1: Prediction of Positive Lupus Anticoagulant (LA) Test by Anticardiolipin Antibody (aCL) and Anti-β_2-glycoprotein-I (aβ_2GPI) IgG/M/A Levels (n: 351)

	20 to <40 U	**40 to <80 U**	**≥ 80 U**
aCL IgG	OR 1.1 95% CI 0.5, 2.5, p=0.9	*OR 8.6 95% CI 1.1, 64.5, p=0.04*	*OR 4.5, 95% CI 1.5, 13.2, p=0.006*
aCL IgM	*OR 3.2 95% CI 1.2, 8.4, p=0.02*	*OR 4.7 95% CI 1.1, 20.3, p=0.04*	*OR 4.5 95% CI 1, 19.4, p=0.045*
aCL IgA	OR 1.3 95% CI 0.3, 5.7, p=0.76	*All LA positive (n=7)*	*All LA positive (n=10)*
aβ_2GPI IgG	OR 0.9 95% CI 0.3, 2.6, p=0.91	OR 3.1 95% CI 0.7, 13.3, p=0.14	OR 3.3 95% CI 0.8, 14.4, p=0.11
aβ_2GPI IgM	OR 0.8 95% CI 0.3, 2.2, p=0.6	*All LA positive*	*OR 10.4 95% CI 1.4, 77, p=0.02*
aβ_2GPI IgA	OR 1.3 95% CI 0.5, 3.4, p=0.7	OR 2.5 95% CI 0.6, 11, p=0.2	OR 2.4 95% CI 0.5, 10.4, p=0.3

Comparison group: 0 to <20 U; OR: Odds Ratio, CI: Confidence Interval, U: Units