Autoimmunity Reviews 23 (2024) 103510

Contents lists available at ScienceDirect

Autoimmunity Reviews

journal homepage: www.elsevier.com/locate/autrev

Review

Deciphering the clinical significance of longitudinal antiphospholipid antibody titers

Cecilia B. Chighizola [a,*], Rohan Willis [b], Gabriella Maioli [c], Savino Sciascia [d], Laura Andreoli [e], Olga Amengual [f], Massimo Radin [d], Maria Gerosa [c], Tatsuya Atsumi [f], Guilherme de Jesus [g], Laura Trespidi [h], D. Ware Branch [i], Roberto Caporali [a,c], Danieli Andrade [j], Robert Roubey [k], Michelle Petri [l], Maria Laura Bertolaccini [m]

[a] Department of Clinical Sciences and Community Health, University of Milan, Pediatric Rheumatology Unit, ASST G. Pini - CTO, Milan, Italy
[b] University of Texas Medical Branch, Internal Medicine, Galveston, USA
[c] Department of Clinical Sciences and Community Health, University of Milan, Clinical Rheumatology Unit, ASST G. Pini - CTO, Milan, Italy
[d] University of Turin, Department of Clinical and Biological Sciences, Turin, Italy
[e] Rheumatology and Clinical Immunology Unit - ERN ReCONNET, ASST Spedali Civili, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy
[f] Hokkaido University, Department of Rheumatology, Endocrinology and Nephrology Faculty of Medicine and Graduate School of Medicine, Sapporo, Japan
[g] Universidade do Estado do Rio de Janeiro, Department of Obstetrics, Rio de Janeiro, Brazil
[h] Department of Obstetrics and Gynaecology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[i] Department of Obstetrics and Gynecology, University of Utah Health, Salt Lake City, UT, USA
[j] University of Sao Paulo, Reumatologia, Sao Paulo, Brazil
[k] Department of Rheumatology, University of North Carolina, Chapel Hill, NC, USA
[l] Johns Hopkins University School of Medicine, Rheumatology, Baltimore, MD, USA
[m] Academic Department of Surgery, SCMMS, King's College London, London, United Kingdom

ARTICLE INFO

Keywords:
Antiphospholipid antibodies
Antibody titers
Seroconversion
Longitudinal
Follow-up
Thrombosis

ABSTRACT

In antiphospholipid syndrome (APS), the risk of clinical manifestations increases with higher titers of antiphospholipid antibodies (aPL). Despite the adoption of aPL titers in the classification approach to aPL-positive subjects, the value of longitudinal monitoring of those titers in the follow-up is still debated, being well studied only in systemic lupus erythematosus (SLE). The literature suggests that the rate of aPL positivity decreases during follow-up in primary APS, estimating that seroconversion occurs in between 8.9 and 59% of patients over time. Negativisation of aPL occurs more frequently in asymptomatic aPL carriers than in patients with full-blown APS as well as in subjects with single aPL positivity or low aPL antibody titers. In patients with SLE, aPL typically behave fluctuating from positive to negative and back again in the course of follow-up.

The few studies assessing the longitudinal course of aPL positivity with no associated systemic connective tissue disease reported a progressive decrement of aPL titers over time, in particular of antibodies against β2 glycoprotein I (antiβ2GPI) and cardiolipin (aCL) of IgG isotype. After a thrombotic event, aPL titers tend to decrease, as emerged from cohorts of both primary and secondary APS. Hydroxychloroquine has been identified as the most effective pharmacological agent to reduce aPL titers, with multiple studies demonstrating a parallel reduction in thrombosis rate. This review addresses available evidence on the significance of aPL titer fluctuation from clinical, therapeutic and pathogenic perspectives.

1. Introduction

The term titer is currently used to refer to antibody concentrations or levels. Antibody titers are of pivotal importance whenever dealing with antiphospholipid antibodies (aPL): aPL exert a pathogenic role in mediating both thrombotic and obstetric manifestations of

* Corresponding author at: Department of Clinical Sciences and Community Health, University of Milan, Pediatric Rheumatology Unit, ASST Pini – CTO, Piazza Cardinal Ferrari, 1 – 20122 Milan, Italy.
E-mail address: cecilia.chighizola@unimi.it (C.B. Chighizola).

https://doi.org/10.1016/j.autrev.2023.103510
Received 10 December 2023; Accepted 29 December 2023
Available online 1 January 2024

antiphospholipid syndrome (APS) (Figs. 1 and 2), and subjects with aPL are at risk of developing the manifestations included in the clinical spectrum of the syndrome [1,2]. Titers of aPL are known to be predictors of clinical events. Today, it is well accepted that the risk of developing aPL-mediated manifestations increases with the increase in antibody titers [2,3]. In addition, patients displaying high aPL titers are likely to be positive in all the 3 aPL laboratory detection tests routinely used for the diagnosis and classification of APS (anticardiolipin antibodies [aCL], antiβ2 glycoprotein I antibodies [antiβ2GPI] and the lupus anticoagulant [LA]) [4]. As a matter of fact, high titer antiβ2GPI antibodies may be detected in all three tests, which identify the same subset or overlapping subsets of antibodies: this so-called "triple positivity" is regarded as the highest risk profile for experiencing an aPL-related manifestation [5].

Despite the adoption of aPL titers and their persistence over the short term in the approach to a patient with clinical suspicion of APS, few studies have addressed the value of longitudinal monitoring during the follow-up of these individuals. It is still unclear if the titers are stable over time or rather fluctuate and, in the latter case, whether the fluctuation translates into a different risk of developing aPL-associated events. This issue has been addressed in the literature mainly in dichotomous terms, assessing "seroconversion" as the rate of patients in which aPL tests become negative during a short follow-up (Tables 1–3), while few studies have assessed the fluctuation of aCL and antiβ2GPI titers over time. It is even more complicated to monitor longitudinally LA activity. In the past, research efforts have been devoted to quantifying LA beyond the dichotomous determination. In general, those efforts have not been found to be useful: beyond the ratio adopted to define the threshold for LA positivity, higher ratios do not seem to confer greater risk. Scarcer data are available even about the potential effects of treatment on the titers of aPL: it is plausible that drugs acting on acquired immunity such as immunosuppressive agents, especially anti-B cell therapies, might affect antibody production thus resulting in a decrement of aPL titers [6].

The research quest for novel assays to detect circulating aPL is unceasing, and several second-line testing tools have been proposed by the scientific community [6,18]. Antibodies against phosphatidylserine/prothrombin (aPS/PT) represent the most promising additional tool to detect aPL, but no longitudinal study about the fluctuation of aPS/PT is available. Particular attention has been given to antibodies against domain 1 of β2GPI (antiD1), as it is being established as one of the true pathogenic antibody subsets in APS [18]. Despite their strong association with aPL-associated manifestations, there is a paucity of data on how antiD1 antibody titers fluctuate during follow-up.

2. Tests to detect antiphospholipid antibodies

aCL and antiβ2GPI tests are immunoassays that detect partially overlapping sets of antibodies to β2 glycoprotein I (β2GPI). ELISA is traditionally the most used technique to detect aCL and antiβ2GPI, as mirrored by the very recently issued APS classification criteria based as previously on measurement of aCL and anti-β2GPI by standardised ELISA [1,2]. In particular, aCL ELISA tests can detect antiβ2GPI as β2GPI present in the sample buffer/blocking buffer, and/or the patient serum specimen binds to the cardiolipin (CL)-coated test well. Additionally, aCL assays can also detect antibodies binding directly to CL (present in patients with syphilis and other conditions) and, potentially, antibodies to other CL-binding proteins present in the buffers/ patient serum specimens [19]. Thus, changes in aCL levels could be related to antibodies other than antiβ2GPI. At present, several different immunoassay techniques are being used in real life in lieu of ELISA to detect aCL and antiβ2GPI as well as non-criteria aPL. These include chemiluminescence, fluorescence enzyme and multiplex flow immunoassays assays. These assays exploit the same working principle: the antibodies from plasma or serum bind the antigen (*e.g.*, β2GPI) immobilized on a solid phase given by polystyrene cups, magnetic particles, microbeads or membranes, depending on the system. Anti-human IgG or IgM antibodies bound to a conjugate are then added; these antibodies can bind to the Fc part of those antibodies from the patient attached (and if) on the solid phase. Upon binding to the patient's antibodies, the conversion of a substrate through the conjugate results in a chemical reaction (color, chemiluminescent or fluorescent), which is measured by a detector. Quantification of the antibody titer consists in the comparison of the signal against a calibration curve. Assays differ in solid phase, detection principle, coating, source of antigens and antibodies, blocking agents to

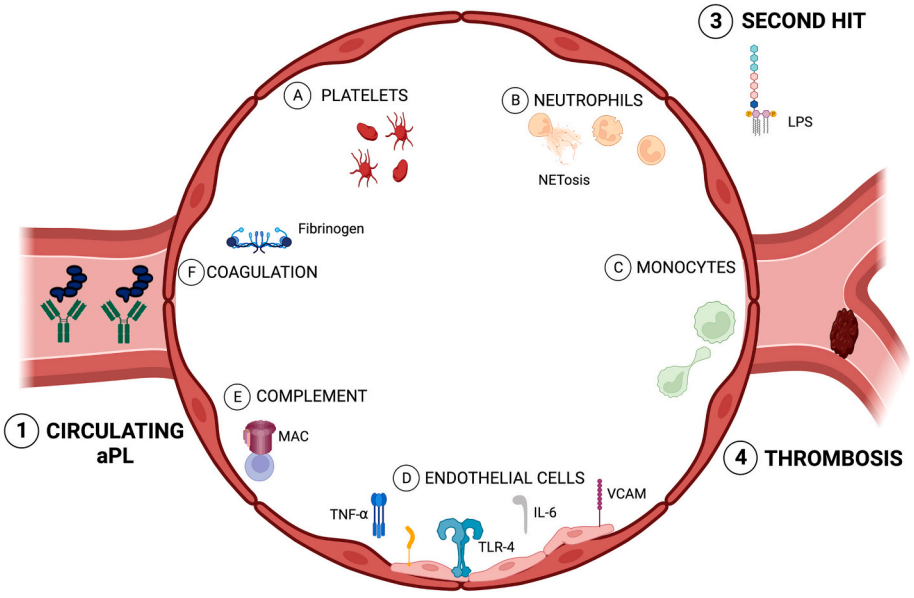

Fig. 1. Mechanisms of thrombosis induced by antiphospholipid antibodies. Antibodies against β2 glycoprotein I interact with endothelial cells, monocytes, platelets and neutrophils mediating cell activation. Antibodies also activate fluid-phase coagulation and complement cascades. These steps lead to a pro—thrombotic phenotype that, in the presence of an additional pro-inflammatory trigger, the so-called "second hit", ultimately results in vascular occlusion. Created with BioRender.com.

NET: neutrophil extra-cellular traps; LPS: lipopolysaccharide; TF: tissue factor; VCAM: vascular cell adhesion molecule; IL: interleukin; TLR: Toll-like receptor; TNF: tumour necrosis factor; MAC: membrane attack complex; aPL: antiphospholipid antibodies.

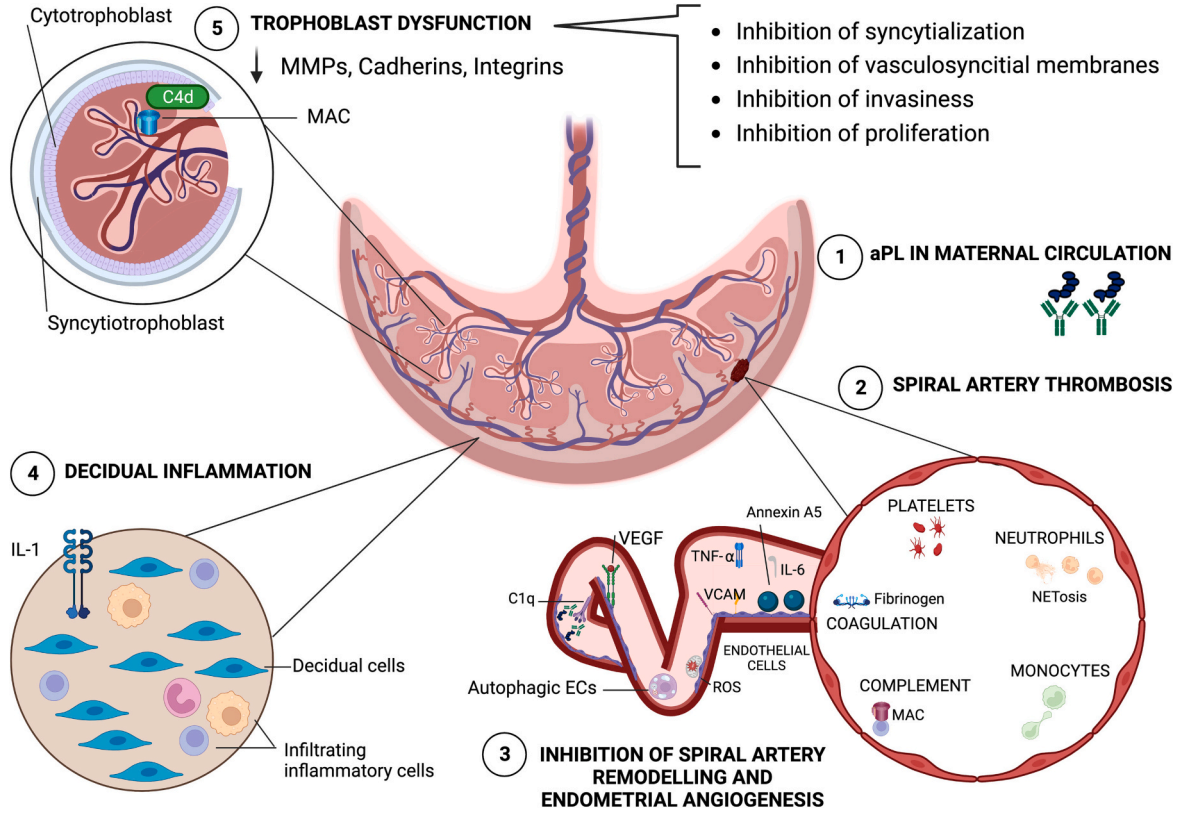

Fig. 2. Mechanisms of obstetric morbidity induced by antiphospholipid antibodies. Antibodies against β2 glycoprotein I interact with maternal vasculature inducing spiral artery thrombosis, maternal decidua to affect proliferation and fetal trophoblast to impair growth, syncytialization and invasiveness. Antibodies also activate fluid-phase coagulation and complement cascades. These steps are sufficient to prevent pregnancy progression. Created with BioRender.com.
aPL: antiphospholipid antibodies; NET: neutrophil extra-cellular traps; TF: tissue factor; VCAM: vascular cell adhesion molecule; IL: interleukin; TNF: tumour necrosis factor; MAC: membrane attack complex; VEGF: vascular endothelial growth factor; MMP: matrix metalloproteinase; EC: endothelial cells; ROS: Reactive oxygen species.

Table 1
Studies reporting rates of overall seroconversion of antiphospholipid antibodies not differentiating between tests.

Author, year [REF]	Follow-up	N of patients	Study cohort composition	Rate of seroconversion*
Giron-Gonzalez, 2004 [7]	36 months	404	226 APS 178 asymptomatic aPL carriers	15.4% in APS 22% in aPL carriers
Riancho-Zarrabeitia, 2017 [7]	114 months	105	49 obstetric PAPS 42 asymptomatic aPL carriers 14 aPL+ SLE	59%
Yelnik, 2017 [8]	13 years	98	aPL+	27%
Medina, 2017 [9]	60 months	70	PAPS	34%
Radin, 2019 [10]	14 years	259	APS	8.9%
Zen, 2021 [11]	19 years	16	APS-SLE	29.1%

N: number; APS: antiphospholipid syndrome; aPL: antiphospholipid antibodies; SLE: systemic lupus erythematosus; PAPS: primary antiphospholipid syndrome.
* If not otherwise detailed, the rate of seroconversion refers to aPL-positive patients turning negative.

prevent non-specific binding, dilution protocol, calibration, and units [19,20]. In addition, some assays exploit automated or semi-automated analyzers, which have been marketed since the 2010s and are still not universally available. Compared to traditional manual ELISA methods, newer techniques may offer several advantages: consistent protocols, lower inter-laboratory and inter-operator variation, and broader

Table 2
Rates of seroconversion of anticardiolipin antibodies in available studies.

Author, year [REF]	Follow-up	N of patients	Study cohort composition	Rate of seroconversion*
Out, 1989 [12]	6–47 months	53	aPL+ SLE	aCL IgG: 45% negative → positive aCL IgG: 58% positive → negative aCL IgM: 38% negative → positive aCL IgM: 44% negative → positive
Out, 1992 [13]	26 months	53	aPL+ SLE	aCL IgG: 49% aCL IgM: 30.2%
Levine, 2004 [14]	24 months	482 aCL +	aPL+	16.4%
Martinez-Berriotxoa, 2007 [15]	4 aPL tests	93 aCL +	aPL+ SLE	43%
Amory, 2016 [16]	24 months	485	aCL+	35.3%
Yelnik, 2017 [8]	13 years	72 aCL +	aPL+	43%
Frodlund, 2021 [17]	36 months	7 aCL IgG + 11 aCL IgM +	Recent-onset SLE	aCL IgG: 28.6% aCL IgM: 54.5%

N: number; aPL: antiphospholipid antibodies; SLE: systemic lupus erythematosus; aCL: anticardiolipin antibodies.
* If not otherwise detailed, the rate of seroconversion refers to aPL-positive patients turning negative.

Table 3
Rates of seroconversion of antiβ2 glycoprotein I antibodies in available studies.

Author, year [REF]	Follow-up	N of patients	Study cohort composition	Rate of seroconversion*
Amory, 2016 [16]	24 months	147	Anti-β2GPI +	16.3%
Yelnik, 2017 [8]	13 years	39	Anti-β2GPI +	23%
Frodlund, 2021 [17]	36 months	50	Recent-onset SLE	8%
Chighizola, 2023 [4]	4 years	170	aPL+	Antiβ2GPI IgG: 7.1% Positive ➔ negative: 6.5% Negative ➔ positive: 0.6%

N: number; aPL: antiphospholipid antibodies; SLE: systemic lupus erythematosus; antiβ2GPI: antiβ2 glycoprotein I antibodies.
* If not otherwise detailed, the rate of seroconversion refers to aPL-positive patients turning negative.

dynamic range [19–22]. On the other hand, newer methods may have greater analytical sensitivity, *i.e.*, detect lower antibody concentrations than standard ELISAs, which could lead to positive results that lack clinical significance.

LA assay allows detecting a heterogeneous group of immunoglobulins behaving as acquired *in vitro* inhibitors of coagulation due to the interference of antibodies with the assembly of coagulation factor complexes on anionic phospholipid (PL) membranes, thereby slowing down the reactions. LA phenomenon is mediated by antiβ2GPI antibodies and, in approximately one third of cases, anti-prothrombin antibodies (antiPT). It has been demonstrated that bivalent antiβ2GPI IgG mediate LA activity by inducing formation of multiple complexes of β2GPI on PL surfaces thanks to their bivalent property, thus hindering the lateral mobility and activation of clotting factors. Antibodies able to induce LA phenomenon are more often of IgG isotype and at high titers: the concentration of purified polyclonal or monoclonal antibodies required for LA activity is orders of magnitude greater than the concentration required for detection in immunoassays [23]. Antibodies mediating LA can usually also be detected in antiβ2GPI assays and traditional aCL assays, the already mentioned triple aPL positivity. In addition, these antiβ2GPI antibodies mediating LA phenomenon are likely to target the main β2GPI epitopes located in D1 of the molecule [24]. Not surprisingly, LA has emerged as the strongest predictor of aPL-related events and positivity for both antiβ2GPI antibodies and aCL is far more predictive of clinical manifestations when the autoantibodies induce the *in vitro* elongation of clotting time [25,26].

Besides the assays used, sources of heterogeneity in LA testing relates to the activator employed and the PL class, concentration and conformation. Several other variables can affect the reliability of LA testing, such as ongoing anticoagulant treatment and concomitant infectious or inflammatory event, since increased C reactive protein (CRP) levels can result in false-positive LA due to interference of CRP with PL [19,27].

3. How to determine titers of antiphospholipid antibodies

Levels of aCL and/or antiβ2GPI antibodies are determined based on a calibration curve, which should be performed in each run. Calibration curve should be rejected whenever not meeting the manufacturer's requirements or in case of correlation coefficient between test values and target values below 0.90 [28]. The dynamic range of the standard curve is the range of antigen concentrations that can be measured accurately by the assay. To be regarded as accurate, measurements within the dynamic range should present low standard deviation between replicates of the standards and strong correlation between the antibody concentration and the detection signal.

The biggest caveat in calibration is the lack of uniformity in reference material for assay calibration. For routine calibration procedures, manufacturers provide a variety of calibrators, not always traceable to a primary standard [28]. For aCL detection, polyclonal patient-derived calibrators, known as "Harris standards", are available after development by Harris et al. in the 1980s [29]. The concentration of a dilution series of affinity-purified aCL was determined by ELISA and expressed in IgG phospholipid units (GPL) and IgM phospholipid units (MPL) for aCL IgG and IgM, respectively, where 1 unit corresponds to the binding activity of 1 μg/ml of affinity purified aCL. Additional sets of calibrators were prepared by matching with the original calibrators, available on the market as lyophilized product [27,30]. Monoclonal antibody standards for aCL and antiβ2GPI IgG (HCAL) and IgM (EY2C9), also known as "Koike standards" or "Sapporo standards" were developed [31]. Monoclonals offer the advantage of higher reproducibility between batches and theoretical infinite production capacity, although not reflecting the polyclonality of circulating aPL in APS patients [32]. aCL results can only be reported in GPL/MPL units if validated against the original Harris standards; the conversion of the concentrations of monoclonal antibodies in GPL/MPL units is possible for aCL, although not always performed [32]. No international unit is available for antiβ2GPI testing and results can be expressed in a wide range of units: IU/mL, U/mL, SGU, SMU, g/mL, G units, M units, GAU/mL, and MAU/mL, depending on the manufacturer [32].

With regards to results interpretation of LA, current guidelines by the the International Society on Thrombosis and Haemostasis (ISTH) recommend converting screen and confirm clotting times to ratios *via* pooled normal plasma (PNP) values, in order to reduce the variability due to the operator and/or analyser performance, reagent quality and stability issues, and variation in PNP clotting time with different reagents [30]. The ratio is compared to a reference interval (RI) that should be generated locally and derived specifically to the reagent-analyser pairings. Conversely, guidelines issued in 2014 by the Clinical and Laboratory Standards Institute (CLSI) suggest normalizing against the RI mean clotting time, since PNP may not generate the same clotting times with different reagents [33].

4. How to define medium/high titer values for antiphospholipid antibody positivity

The setting of appropriate cut-off values to define medium/high titers of aPL is a relevant step in the laboratory diagnosis of APS; the greatest effort relies in the identification of a laboratory cut-off that corresponds to a clinically relevant threshold. Current APS classification criteria consider detection of aCL IgG or IgM to be clinically significant if moderate to high titer, measured with a standardised ELISA. The 99th percentile of the distribution of autoantibody titers in a cohort of healthy individuals is currently the most accepted approach to identify threshold values for solid-phase assays [1,27], but aCL moderate to high titers can also be defined as >40 GPL or MPL [1]. Notably the 99th percentile for aCL can be strikingly different from 40 GPL/MPL [34,35]; the wide inter-assay variability impacts the identification of a numeric threshold for classifying solid-phase aPL titers as "moderate to high". Consistently, the ISTH-SSC does not adopt 40 GPL/MPL as a cut-off but recommends solely to calculate a laboratory-specific cut-off value for medium/high titer positivity based on a non-parametric 99th percentile of at least 120 reference individuals [28]. Categorization of aCL titers as "negative" (lower than cut-off), "low" (between cut-off and 40 GPL/MPL), "medium/moderate" (between cut-off and 40 GPL/MPL), and "high" (above 80 GPL/MPL) has been suggested, but is currently not recommended [28,36].

The available guidelines are not homogenous in the identification of cut-offs for LA. Guidelines issued in 2020 by the ISTH recommend cut-offs derived from the 99th percentile, which equates to the RI mean + 2.3 standard deviations (SD) for normally distributed data [19]. Guidelines issued in 2012 by the British Committee for Standards in Haematology (BCSH) suggest deriving the cut-off from the RI mean plus

2SD, corresponding to the 97.5th percentile [37]. To accurately identify an adequate cut-off, the cohort of healthy individuals should be of appropriate size: the ISTH guidelines recommend a minimum of 40 healthy individuals, but other authors recommend a sample of at least 120 subjects [19,38].

5. The rates of antiphospholipid antibody seroconversion

Although limited and highly heterogeneous, available data allows extrapolating some considerations on seroconversion from positive to negative aPL status. First, there is unanimous consensus that patients with single aPL positivity are more prone to experiencing aPL negativization, as opposed to triple positive individuals [7–11,37–39]. When aCL or antiβ2GPI are positive at low titers, tests are also more likely to become negative in subsequent assays [7,9,40,41]. Furthermore, seroconversion is more likely seen in asymptomatic aPL carriers than in patients with full-blown APS [7,9,40,41].

Some studies have shown that the percentage of aPL positivity during follow-up, estimating that such seroconversion occurs in around 8.9–59% of patients with positive aPL over time (Table 1). The broad heterogeneity in the rates of aPL negativization reported across the literature could be ascribed to many variables such as the definition of seroconversion, the employed assays to detect circulating aPL, the initial aPL titers, the composition of the study cohort, the concurrent treatment (s), the study design and –most importantly- the length of follow-up. Interestingly, the rate of seroconversion appears to be higher for aCL (16.4–58%, Table 2) as compared to antiβ2GPI antibodies (7.1–23%, Table 3). Very few studies have addressed the stability of LA. Among 98 aPL carriers (59 with baseline positive LA) a repeat LA assay turned out negative in 22% of cases [8]. In a cohort of 53 patients, fluctuating LA was observed in 17 cases (32%) [13]. A study from APS ACTION on 472 aPL-positive subjects (35% with another associated systemic autoimmune disease, 258 subjects with previous vascular events and 136 women with pregnancy morbidity) defined aPL stability as the detection of positive LA and/or aCL and/or antiβ2GPI IgG/IgM $\geq$40 U in at least two thirds of follow-up measurements. In this work, the authors reported a stable aPL profile in 78% of patients over a median of 5.1 years of yearly follow-up samples [41]. To note, an isolated LA positivity increased the odds of an unstable aPL profile over time (odds ratio 3.3) [41].

6. The fluctuation of antiphospholipid antibody titers

The clarification of how frequently aPL positive individuals become negative during the follow up could have relevant implications on clinical management; however, it would be desirable for research purposes to get beyond the mere evaluation of the rate of aPL tests becoming negative.

In 2005, Erkan et al. evaluated the stability of aPL profile in a cohort of 204 aPL-positive subjects (81 with well-characterized APS). aCL and antiβ2GPI results were sub-classified into 4 categories: 0–19 U (negative), 20–39 U (low positive), 40–80 U (moderate positive) and > 80 U (high positive); results were defined as stable when remained in the same category of positivity. A stable aPL profile was reported in 87% of initially positive LA and 88% of those initially negative or low positive for aCL and in 75% of those with moderate-high positive aCL. For antiβ2GPI testing, results remained in the same category of positivity in 96% of cases when initially negative or low positivity and in 76% in case of moderate-high positivity [42].

More recently, APS ACTION assessed the longitudinal fluctuation of aPL titers yearly in 4 consecutive samples, each from 230 patients persistently positive for aPL. aPL were tested in APS ACTION core laboratories by chemiluminescence. Titers of antiβ2GPI and aCL IgG decreased progressively every year (median (interquartile range [IQR]) antiβ2GPI IgG titers [CU] in 170 patients with at least one positive antiβ2GPI IgG test over follow-up 702.7 [108.5–2255] at year (Y) 1, 573.2 [72.2–1860] at Y2, 501.8 [59.7–2445] at Y3, 415.9 [696–2628] at Y4, p: 0.010, Friedman statistic: 11.32); median [IQR] aCL IgG titers [CU] in 151 patients with at least one positive aCL IgG test over follow-up 247.6 [71.1–885.9] at Y1, 220.0 [62.1–825.9] at Y2, 156.5 [47.0–783.3] at Y3, 139.0 [42.1–763.3] at Y4, $p < 0.0001$, Friedman statistic: 34.64). The same decreasing trend could be reported for antiβ2GPI and aCL of IgM isotypes, but without a yearly progressive decrement. For aCL and antiβ2GPI IgG/IgM tests, antibody titers were significantly lower in the latest evaluation compared to baseline (median [IQR] antiβ2GPI IgM titers [CU] in 73 patients with at least one antiβ2GPI IgM test over follow-up 50.3 (25.5–185.3) at Y1, 41.8 (17.5–145.7) at Y2, 43.3 (16.6–132.1) at Y3, 42.3 (11.8–131.4) at Y4; p < 0.0001, Friedman statistic: 29.2); median [IQR] aCL IgM titers [CU] in 83 patients with at least one positive aCL IgM test over follow-up 56.1 [26.4–118.4] at Y1, 64.3 [26.8–121.7] at Y2, 50.5 (21.7–105.7) at Y3, 49.1 [21.2–147.5] at Y4; p: 0.0039, Friedman statistic: 13.4) [4].

7. The rates of antiphospholipid antibody seroconversion and fluctuation in systemic lupus erythematosus

Patients with SLE usually carry lower aPL titers compared to those with primary APS (PAPS) [4]. When a large sample size and longitudinal design were adopted, it has been shown that aPL typically fluctuate in SLE. Among lupus patients the aPL status tend to convert back and forth from positive to negative [43]. To capture this phenomenon, the number of LA positive tests during follow-up has been evaluated in one purely SLE cohort of 758 patients over 16 follow-up visits (quarterly for 4 years). To note, LA tested by diluted Russell viper venom time (dRVVT) was positive on 25% or less of the tests performed [43]. Smaller studies with shorter follow-up have observed seroconversion occurring among lupus patients at similar rates than those registered in PAPS subjects, ranging between 8 and 58% (Tables 1, 2 and 3) [11,13,12,15,17]. Despite the fluctuating pattern of antibody positivity, SLE patients that are aPL-negative at onset rarely develop aPL during follow-up [12,17]. Of note, in SLE patients, persistency of aPL positivity is shown to be more common among those who received an APS diagnosis prior to lupus onset [40].

It has been shown that antibody titers tend to decrease over time even among SLE patients. Out et al. evaluated aCL in 53 lupus patients during a 4-year timeframe, identifying 3 aCL categories (negative, low positive, high positive). A shift in these categories from baseline to latest follow-up was observed in 53% of patients for IgM and 60% for IgG [12]. In a cohort of 50 lupus patients, Frodlund et al. reported that the median levels of aCL and antiβ2GPI IgG and IgM antibodies decreased from inclusion to 36-month follow-up, although not significantly. A trend towards statistical significance was observed for antiβ2GPI IgG only [17].

An association between aPL titers and lupus disease activity had been proposed in the 80s, but this should be simply regarded as an intriguing hypothesis since there has been limited subsequent data to support it [13,44–48]. Besides the potential association with disease activity, aPL fluctuation in SLE might mirror the fluctuation of levels of total IgG and IgM and other autoantibody titers.

8. Titers of non-criteria aPL tests

Very few data are available on the longitudinal behaviour of non-criteria aPL tests. In the recent work by APS ACTION, antiD1 IgG were tested by chemiluminescence every year over 4 years in 135 individuals with at least one positive antiD1 test. AntiD1 seroconversion was reported in 19.3% of patients. More frequently, antiD1 results switched from positive to negative (20 individuals, 14.8%) and less often from negative to positive (6 individuals, 4.4%) [4]. AntiD1 antibody titers varied over time (median [IQR] anti-D1 IgG titers [CU] in 135 patients with at least one positive anti-D1 IgG test over follow-up 165.7 [56.9–680.9] at Y1, 140.0 [38.3–530.0] at Y2, 102.6 [30.60–512.1] at

Y3, 95.9 (28.60–468.8) at Y4, $p < 0.0001$, Friedman statistic: 508.5), significantly decreasing in 79% and increasing in 19% of samples. When antiD1 antibody titers were categorized into quartiles, throughout follow-up 46.7% of subjects remained at the same antD1 titer category, while 53.3% shifted titer categories, a change that was persistent in most cases. Shift in titer categories occurred less frequently in patients with previous thrombosis compared to subjects without a history of vascular events (5.9% *versus* 21.9%). In a multivariable mixed linear model adjusted for age and gender, antiD1 antibody titers decreased most markedly in the first year of monitoring (21% decrease, −1.3-fold). At year 4, adjusted antiD1 antibody titers were 1.5-fold lower compared to baseline (32% decrease) [4].

In the APS ACTION study, non-criteria antiβ2GPI and aCL IgA were found positive in 33% and 40.9% of patients, respectively. Antiβ2GPI IgA titers decreased at year 2 compared to baseline, to increase progressively at year 3 and 4 (median [IQR] antiβ2GPI IgA titers [CU] in 85 patients with at least one positive antiβ2GPI IgA test over follow-up 40.5 [24.0–95.0] at Y1, 35.7 [20.9–90.8] at Y2, 38.5 [18.3–95.6] at Y3, 39.9 [18.0–95.6] at Y4; p: 0.0074, Friedman statistic: 12.0). Titers of aCL IgA were higher at year 2 compared to baseline. At year 3, titers were lower but increased again at year 4 [4]. aPL of IgA isotype were also evaluated in a cohort of 50 lupus patients, observing a persistent positive result at 3 years in 25% for aCL and 71.4% for antiβ2GPI. The same pattern of fluctuation and negativization of non-criteria aPL was observed for antibodies against phosphatidylserine (antiPS) [17].

In a follow-up analysis of the WARSS-APASS study conducted in patients with stroke, stored sera with baseline antiPS positivity were retested for antiPS IgG/IgM. Of 115 patients initially positive for antiPS, 17.4% turned negative at 24-month follow-up [16].

9. Hydroxychloroquine and antiphospholipid antibody titers

It is well recognized that hydroxychloroquine (HCQ) exerts immunomodulatory effects: it alters antigen processing *in vitro*, by increasing the pH of intracellular vacuoles, resulting in dissociation of the invariant chain from the class II major histocompatibility complex and inhibition of antigen binding [49,50]. In 2005, Erkan et al. did not find any difference in HCQ prescription upon the stability of aPL profile in a cohort of 204 aPL positive subjects, including 81 patients with definite APS [42]. Conversely, more recent studies consistently suggest that HCQ treatment is associated with a decrease of aPL titers both in PAPS and SLE patients. In a retrospective cohort of 114 APS patients, Nuri et al. reported a significant reduction of aCL IgG and antiβ2GPI IgG/IgM titers in HCQ-exposed patients [47]. In a randomized prospective study, including 50 APS patients, long-term HCQ use was associated with a decrease of antiβ2GPI IgG and IgM titers [51]. In the recent APS ACTION study, ongoing HCQ treatment emerged as the only variable to significantly affect the fluctuation of both antiβ2GPI and antiD1 IgG antibody titers. Patients treated with HCQ at the time of blood sampling presented antiβ2GPI and antiD1 titers that were respectively 29% and 21% lower than those not on HCQ [4].

Less data are available in purely SLE cohorts of aPL-positive subjects. In a small case series of 12 SLE patients with aPL, individuals on HCQ presented lower levels of both aCL and antiβ2GPI antibodies when compared to those not receiving HCQ [52]. Similarly, Broder et al. observed lower odds of persistent LA, aCL and antiβ2GPI IgG/IgM positivity among 90 aPL positive lupus patients on HCQ even after adjusting for age, ethnicity and gender [53].

10. Immunosuppressive agents and antiphospholipid antibody titers

Due to their beneficial effects, immunosuppressants are employed to manage severe aPL-related manifestations, such as severe thrombocytopenia, kidney glomerulopathies, livedoid vasculopathy and chorea, among others. Immunosuppressants remain the therapeutic cornerstone in several rheumatic conditions in which positive aPL can be found. Thus, data about the effects of immunosuppressants on aPL titers can be extrapolated mainly from studies recruiting patients with secondary APS. Available studies grouped immunosuppressants as a single variable rather than considering them singularly. Evidence from studies recruiting exclusively lupus patients concordantly suggests that concurrent treatment with immunosuppressive agents does not affect aPL antibody titers [11,12,48], with only a single study identifying immunosuppressive therapy as an independent predictor of aPL negativization in SLE [53]. In the APS ACTION cohort of 230 aPL positive subjects, immunosuppressants did not exert any significant effect on antiβ2GPI and antiD1 antibody titers at any time point [4].

11. Glucocorticoids and antiphospholipid antibody titers

Glucocorticoids are powerful, broad-spectrum anti-inflammatory agents, whose exact pharmacological effects have yet to be dissected in terms of cellular mediators [54]. The use of high chronic dose of glucocorticoids in rheumatology is declining due to the many side-effects and the availability of drugs with better safety profile; this might account for the scarce and dated evidence about their potential effects on aPL titers. The single available study dates to 1992, when Derksen et al. assessed aPL fluctuation in 53 SLE patients. The authors reported an association between glucocorticoids treatment and fluctuating LA, most markedly when detected by the kaolin clotting time (KCT), in 4 patients. LA activity was reduced in all patients, disappearing in 2 [12].

12. Rituximab and antiphospholipid antibody titers

Rituximab is a chimeric monoclonal antibody which targets CD20, a surface protein expressed in the cytoplasmic membrane of B cells. B lymphocytes contribute to APS aetiopathogenesis not only by producing antibodies but also by acting as antigen presenting cells, regulating T helper cells, and releasing pro-inflammatory cytokines [6].

Loss of aPL and decreasing antibody titers have been both described after rituximab therapy, initially in few case reports [55–59]. Effects of rituximab on aPL titers have been assessed in heterogeneous populations of PAPS patients with contradictory results. In a cohort of 19 aPL positive patients receiving rituximab due to non-criteria APS manifestations, Erkan et al. observed that initial positivity for criteria aPL tests was invariably confirmed at 12 months after rituximab [60]. Berman treated 90 patients with catastrophic APS with rituximab, reporting a negativization of aPL tests (LA, aCL and antiβ2GPI IgG/IgM) in 50% of cases [61]. You et al. prescribed rituximab to 6 individuals with thrombotic APS, showing a significant decrease of aCL IgG but not of antiβ2GPI IgG/IgM titers [62] while Agmon-Levin et al. used rituximab in 23 subjects with refractory APS and documented a significant decrease of titers of aCL and/or antiβ2GPI IgG/IgM and LA ratios at 4–6 months only in the 13 subjects with complete response to rituximab [63]. The sub-optimal response to rituximab registered in these studies might be explained by the fact the stage of B cell differentiation with highest aPL production has not yet been identified.

13. Belimumab and antiphospholipid antibody titers

Belimumab is a fully human monoclonal antibody that specifically recognizes and inhibits the biological activity of B-lymphocyte stimulator (BLyS), also known as B cell activating factor (BAFF). Initial preclinical studies in NZW x BXSB mice showed that treatment with IgG against BAFF receptor did not impact the development of aCL even though it prevented aPL-related thrombotic vasculopathy prolonging survival [64].

Clinically, the use of belimumab in PAPS is limited to anecdotic cases [65,66], and results from the ongoing clinical trial BLAST (BeLimumab Antiphospholipid Syndrome Trial) are still awaited. Currently, the evidence on the potential effects of belimumab on aPL titers is available

exclusively from aPL-positive lupus patients. Most information comes from post-hoc analyses on data from randomized placebo-controlled trials, altogether pointing towards a net beneficial effect on antibody reduction even though with some discrepancies [67–69]. Real-world experience supports a progressive reduction of aPL titers during belimumab treatment, documenting a reduction of aCL (mainly IgG) and antiβ2GPI antibodies in 3 small case series [52,70,71]. The decrease of aPL titers seems to persist even when belimumab treatment is pursued at long-term. Indeed, aCL IgG titers decreased in an open-label continuation of a phase II study including 296 lupus patients through 7 years of belimumab treatment [72].

14. Combination therapy and antiphospholipid antibody titers

As *per* the data presented above, one could hypothesise that a polypharmacological approach could amplify the effects on aPL titer reduction. Unfortunately, current evidence is too limited to draw any definite conclusion. Data from a small case series of 12 SLE patients shows that the decrease in aPL titers following belimumab treatment was independent of HCQ [52], while the post-hoc analysis on data from BLISS-SC trial revealed an additive synergistic effect for these two pharmacological agents especially in case of longstanding treatment with antimalarials [69]. In the APS ACTION 2023 study, no significant interaction between HCQ and immunosuppressants emerged, but this could be due to the lower numbers of patients on immunosuppressants in the cohort [4].

15. Thrombotic events and antiphospholipid antibody titers

There is general agreement that patients with vascular thrombosis present with higher aPL titers at initial evaluation compared to those without any thrombosis [4]. Towards the end of the 90s, a group from Mexico described a decrease in aCL and antiβ2GPI antibody titers in 24 lupus patients who experienced a thrombovascular accident [73–75]. In the same study, antiβ2GPI antibodies became negative in 3 out of 24 patients (12.5%) [73]. A much higher rate of aPL negativization over follow-up after thrombosis was noted in the Hopkins Lupus cohort. Among 35 SLE subjects who had experienced a vascular event, a complete loss of positivity was shown to occur in 94% for aCL IgG, 94% for aCL IgM, 75% for aCL IgA and 86% for LA [76]. Within 5 years after the thrombotic event, 60% of initially aCL IgG-positive patients and 76% of those with a positive LA reacquired positivity [76]. It is well known that increased factor VIII levels at the time of thrombosis can lead to false-negative LA assessment in activated partial thromboplastin time (aPTT)-based LA but not those dRVVT-based [19]. Similar concerns apply to LA tested in case of surgery, inflammation, malignancy, and other conditions characterized by increased levels of factor VIII [77].

The behaviour of the titers of antibodies against D1 fine specificity and β2GPI whole molecule in case of vascular events has been recently explored in the APS ACTION cohort by the means of a case-crossover design. In subjects with incident thrombosis, both antiD1 and anti-β2GPI IgG antibody titers were significantly lower at the time of the vascular event with a subsequent increase thereafter [4]. A 1.6-fold decrease in antiD1 titers and a 2-fold decrease in antiβ2GPI titers conferred an odds ratio for incident thrombosis of 6.0 and 9.4, respectively [4].

In a cohort of 7 SLE subjects with thrombotic/neurological events, Inanc et al. reported a decrease of antiβ2GPI IgG titers in a single patient and aCL IgG in 5 and aCL IgM in 4 individuals, as compared to titers registered before the vascular event [48].

16. The fluctuation of antiphospholipid antibody titers during pregnancy

Several physiologic modifications occur during human gestation; in particular, the increased intravascular volume leads to hemodilution, which ultimately results in a lower plasma concentration of proteins, including antibodies. Almost all studies assessing the fluctuation of aPL in pregnant patients are concordant in confirming a change as the pregnancy progresses, although there is no consensus on the entity of such fluctuation. Unfortunately, most of these studies are flawed by important limitations, such as the small sample size, assaying one or two aPL tests and inclusion of patients with low titer aPL. Lynch et al. described wide fluctuations in aCL levels over 5 longitudinal testings, and the within subject variability was as high as 88–91% among 23 women with at least one positive aCL IgG test and 34 with at least one positive aCL IgM test [78]. This variability is mirrored in the high seroconversion rate registered in pregnant women: Donohoe et al. reported results turning negative in 54.5% of cases for LA, 42.8% for aCL IgG and 57% for aCL IgM, 60% for antiPT, 33.3% for antiβ2GPI IgM while seroconversion did not occur in case of antiβ2GPI IgG positivity. The study cohort was composed by 17 women with APS in which, before pregnancy, aCL IgG tested positive in 14 cases, IgM in 9; anti-β2GPI IgG were positive in 3 patients, IgM in 6; LA was positive in 11 patients; antiPT IgG tested positive in 5 patients, IgM were positive in 10 [79]. Salazar-Palamo et al. reported aPL seroconversion in 60% of 15 women with obstetric APS; aCL IgM remained stable during gestation while IgG aCL significantly decreased in the third trimester [80]. Topping et al. observed highest aCL IgG titers and dRVVT results in the first trimester in 32 pregnant women with APS [81]. In a group of 75 healthy pregnant women, levels of aCL and antiβ2GPI IgM and aCL IgG were higher after delivery as compared to gestation course, while antiβ2GPI IgG had an opposite fluctuation pattern [82]. The most solid data come from the prospective multicentre PROMISSE cohort, which recruited 152 pregnant aPL-positive women in the late first or early second trimester [83]. Approximately one quarter of LA-positive patients became negative in the second or third trimesters, to then return to baseline status three months after delivery. Among patients testing positive, aCL and anti-β2GPI IgG results remained in the positive range through pregnancy in 93% and 85% of patients, respectively. Even though aCL and antiβ2GPI decreased throughout pregnancy, aPL IgG levels were lower during the second and third trimesters compared to screening (before 18 gestational week) but with a small magnitude of change. Patients with high aPL titers in the beginning of pregnancy presented low fluctuation during the whole pregnancy: IgG titers remained in the high-positive range ($\geq$ 40 GPL units) and, by three months post-partum, aCL and antiβ2GPI IgG titers returned to baseline levels [83].

More recently, aPL titers have been longitudinally tested in 56 pregnant women with systemic autoimmune rheumatic conditions (19 APS, 7 aPL carriers, 8 aPL negative SLE, 10 connective tissue diseases other than SLE, 12 inflammatory arthritis). Titers of aCL and antiβ2GPI IgA and IgM and antiPS/PT IgG were shown to decrease in the second trimester of pregnancy to rise again in the third trimester and post-partum, with a pattern similar to albumin and serum Immunoglobulins. Conversely, aCL, antiβ2GPI and antiD1 IgG increased throughout gestation [84].

Consistently with the above-discussed burden of evidence, the updated ISTH guidelines concluded that aPL during pregnancy should be interpreted with caution. In particular, LA testing could generate false negative results due to increased levels of factor VIII [19,7], even though might be reliable if assessed in the first trimester of gestation. Pregnancy-induced increase in blood coagulation factors has been reported to resolve by 6 weeks postpartum, and LA results usually returns to baseline status by 3 months post-partum [85].

17. Obstetric complications and antiphospholipid antibody titers

Literature is highly inconsistent about the potential clinical relevance of changes in aPL titers in relation to obstetric outcome. In a pioneer study observing 7 pregnant women treated with low dose acetylsalicylic acid, aCL IgG –but not IgM- were observed to decrement

abruptly in patients with pregnancy morbidity while women with successful obstetric outcome presented a gradual decrease or stable levels of aCL titers [86]. Conversely, Lynch et al. described no relationship between fluctuating aCL levels and pregnancy complications in women with at least one positive aCL IgG/IgM test [78]. Accordingly, Donohoe et al. recruited 17 APS women and tested aPL on a median of 5 longitudinal samples per patient from antenatal visit throughout pregnancy. All patients received standard care with low molecular weight heparin and low dose acetil salicylic acid. The authors concluded for the lack of association between antibody fluctuation pattern and obstetric outcome [79]. Opposite findings emerged in a larger study on 123 women with recurrent abortions, since aCL and antiPS antibodies dramatically increased in women who miscarried the index pregnancy while remained stable or decreased in those who delivered a live infant [87]. Few years later, Topping et al. observed stable aPL levels in 4 out 12 APS women who miscarried, whereas in 8 out of the 12 women with uncomplicated pregnancies, aPL had become negative by the second trimester [81]. Consistent results were raised by Mexican authors, who observed that disappearance of aCL was associated with improved fetal survival in a cohort of 15 women with secondary APS, conveying a relative risk of 0.67 [80].

18. Evaluating antiphospholipid antibody fluctuation over time: technical considerations

As outlined above, testing for aPL is highly influenced by the methodology employed to perform the tests. The intra-assay and inter-assay variability translates into a poor reproducibility among different laboratories, accounting for the importance of longitudinally testing aPL by the same solid phase platform in the same laboratory as platforms cannot be used interchangeably. The wide heterogeneity in assay techniques, reagents, and calibrators results in a high inter-assay variability when looking at the quantitative (antibody titer) interpretation of aCL and antiβ2GPI results and, to a lower extent, to qualitative (positive/negative) interpretation [20]. Some authors have suggested a return to early days in APS when aCL and antiβ2GPI assays were expressed semi-quantitatively [77]. Adopting a semiquantitative report of aCL and antiβ2GPI tests (*i.e.*, negative/low positivity/moderate positivity/high positivity) instead of a quantitative numerical value would lead to a homogeneous interpretation of results across laboratories, allowing higher reproducibility. Surely reporting quantitative antibody levels offers prognostic details about both vascular and obstetric risk that would be otherwise missed.

In addition, studies that evaluate the longitudinal fluctuation of antibody levels should account for the phenomenon named regression to the mean, which consists in the tendency of high values to be lower on re-measurement in the absence of any intervention. Besides being partially ascribed to random measurement error, regression to the mean implies that variability narrows around the true mean over the course of many repeated observations. Thus, regression to the mean might potentially lead to inaccurate conclusions that the intervention resulted in a treatment effect [88]. To carefully account for the regression to the mean, future studies should optimize the design to envisage control groups and observations taken from time points in which no interventions were implemented, since different groups should be equally affected by the phenomenon [88].

19. Current understanding informs future perspectives

The debate in the scientific community about the significance of aPL titers is still vibrant. Some authors claim that the longitudinal evaluation of aPL titers is a mere exercise in style, while preliminary evidence suggests that aPL titers offer a prognostic value and should influence therapeutic management during follow-up.

Surely, in such a debate, laying the first stone would require the identification of the optimal time frame to retest aPL to appreciate a clinically meaningful fluctuation.

Nevertheless, the main issue relates to reaching consensus about a standardised definition of a clinical significant change in aPL titers. The entity of such relevant change might depend on the methodology exploited to test aPL, thus arduous work should be devoted to clarifying this issue. In addition, there is no standardised definition of seroconversion that is, in fact, very heterogeneous across literature. In a recent survey by the Italian Society of Rheumatology, APS experts could not reach a consensus on how to define aPL seroconversion, even though most of the experts in the panel agreed to refer to aPL negativization in case of at least two negative determinations 1 year apart [89]. Beyond the heterogeneity seen in available studies with the limitation of short-term follow-up, data from the literature suggest that aPL become negative in between 8.9% and 59% of aPL-positive patients, with estimates being higher in case of single aPL positivity, IgM isotype or low antibody titers. Even the specificities of each study cohort might impact the negative seroconversion rate: figures are highest among aPL positive women attending a rheumatology/obstetric joint clinic and followed up for 114 months [39]. Conversely, a concomitant diagnosis of systemic autoimmune condition, mainly SLE, does not impact the proportion of aPL becoming negative [11,13,15,12].

Efforts should be concentrated towards a standardised definition of seroconversion. Once consensus is reached, the key issue would be to clarify the clinical impact of loss of aPL positivity on future prognosis: i) are patients whose aPL have all turned negative still at risk of thrombosis? ii) do these individuals still require life-long anticoagulation? Indeed, according to international recommendations, anticoagulation could be discontinued in case of provoked thrombotic event whenever the underlying risk factors have resolved [90].

To tentatively answer the above questions, data could be extrapolated from studies, almost invariably with a limited sample size and follow-up time, but all assessing the rate of thrombosis after the discontinuation of anticoagulation when aPL become persistently negative. Some authors record no new thrombotic events after stopping anticoagulation [40,91], while other groups observed a higher incidence of thrombosis [9,92]. In a cohort of 105 women with positive aPL (49 with PAPS, 42 aPL carriers and 14 aPL-positive SLE; 16 on concomitant HCQ), low dose acetytilsalicylic acid was discontinued in 62 subjects whose aPL become negative without recording any clinical event at a median follow-up time of 40.95 months (range 9–135) [39].

To date, few studies have assessed the longitudinal fluctuation of aPL titers, reporting a progressive decrement of titers over time for antiβ2GPI and aCL of the IgG isotype [4]. Once ascertained this decrementing behaviour, it would be pivotal to identify clinical variables that might promote such a reduction. Indeed, drugs acting on the immune system might potentially trigger a reduction in immunoglobulin levels, comprising aPL. Unfortunately, any study has evaluated the effects of a given drug on aPL as compared to total immunoglobulin levels. Nevertheless, most evidence points towards the benefit of using HCQ. After an early report suggesting an inconclusive effect for HCQ, all the recent studies are concordant in identifying HCQ as the most effective pharmacological tool to reduce aPL titers to date [4,51–53,93]. When analysing these studies, it should also be considered that patients with chronic diseases tend to be poorly compliant with treatment, including the generally well-tolerated HCQ. Studies using high-performance liquid chromatography tandem mass spectrometry in serum samples of patients with lupus demonstrated that 16% of them were severely non-compliant with HCQ [94].

Again, the most relevant issue from a clinical perspective still remains to be clarified: does this reduction of aPL titers translate into a thromboprotective effect for HCQ? In a recent APS ACTION study, thrombotic events occurred even in patients on HCQ, only these subjects had lower aPL titers compared to those with incident vascular thrombosis not receiving HCQ [4]. Nevertheless, evidence about the thromboprotective role of HCQ is progressively accumulating. It was 1987 when, in a letter published in Arthritis & Rheumatism, Wallace

described for the first time an inverse relationship between thromboembolic recurrence and HCQ in lupus patients [95]. Since then, much support to this observation has been raised in several SLE cohorts, irrespective of aPL positivity [96–104], even though not in all studies [105–107]. Fewer data about the thromboprotective effects of HCQ are available from patients without a concomitant systemic autoimmune condition. A cross-sectional study including 56 asymptomatic aPL carriers suggested that HCQ might exert a protective role against thrombosis [108]. Its efficacy in the secondary prevention of venous events has been documented in a small prospective study on 40 PAPS patients: 20 treated with HCQ on top of vitamin K antagonists and 20 receiving only anticoagulants [109]. In a retrospective, propensity score-matched cohort study including patients with PAPS, HCQ with a mean exposure time of nearly 6 years appeared to reduce the annual incidences of recurrent thrombosis, even though not significantly, with a more prominent reduction of the rate of arterial events (0% *versus* 1.14%) [93]. More recently, a pilot open label randomized prospective study evaluated thrombosis prevention in 50 patients with PAPS allocated 1:1 to HCQ plus standard care *versus* standard care alone, revealing a lower incidence rate of thrombosis in those on dual treatment [51]. The HIBISCUS study, a multicentre study in patients with obstetric and thrombotic APS, might bring extra light on the role of HCQ for the secondary prevention of events [110].

Data from the literature show that aPL titers tend to decrease after a thrombotic event, as emerged in cohorts of both PAPS and secondary APS [4,48,73–76]. Unfortunately, mechanistic insights into such post-thrombosis reduction are lacking. One hypothesis is that antibodies are consumed *in situ* during the vascular event. While not confirmed, such hypothesis might urge us to reconsider the optimal timing for aPL testing in patients who had experienced vascular events: assaying aPL too early might carry a high rate of false negatives or transiently low aPL titers.

20. Conclusions

In summary, available evidence suggests that the positivity rates and titers of aPL can fluctuate over time. aPL seem to fluctuate during pregnancy but data are so conflicting that most experts responding to a survey by the ISTH recommended to confirm aPL positivity 6 weeks post-partum [19]. Though limited, available evidence also supports the notion that, in patients with PAPS, loss of aPL positivity might translate into a lower risk of thrombosis thus allowing to withdraw anticoagulation. The scenario is drastically different in SLE: the fluctuating pattern back and forth between positive and negative aPL means that the clinician can never be secure in considering the aPL risk factor as resolved. This observation implies that the “anticoagulation forever” rule is most appropriately applied to lupus patients with thrombosis [76]. If the observation that aPL titers drop at the time of thrombosis might shed light into the mechanistic steps that drive aPL-mediated thrombosis, further studies are warranted to decipher the significance of aPL titer fluctuation from both clinical and pathogenic perspectives.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

[1] Barbhaiya M, Zuily S, Naden R, Hendry A, Manneville F, Amigo MC, et al. 2023 ACR/EULAR antiphospholipid syndrome classification criteria. Ann Rheum Dis 2023;82(10):1258–70.

[2] Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 2006;4(2):295–306.

[3] Tektonidou MG, Andreoli L, Limper M, et al. EULAR recommendations for the management of antiphospholipid syndrome in adults. Ann Rheum Dis 2019;78(10):1296–304.

[4] Chighizola CB, Pregnolato F, Andrade D, Tektonidou M, Pengo V, Ruiz-Irastorza G, et al. Fluctuation of anti-domain 1 and anti-B2 glycoprotein I antibody titers over time in patients with persistently positive antiphospholipid antibodies. Arthritis Rheumatol 2023;75(6):984–95.

[5] Pengo V, Ruffatti A, Legnani C, Gresele P, Barcellona D, Erba N, et al. Clinical course of high-risk patients diagnosed with antiphospholipid syndrome. J Thromb Haemost 2010;8(2):237–42. https://doi.org/10.1111/j.1538-7836.2009.03674.x [PMID: 19874470].

[6] Chighizola CB, Andreoli L, Gerosa M, Tincani A, Ruffatti A, Meroni PL. The treatment of anti-phospholipid syndrome: A comprehensive clinical approach. J Autoimmun 2018;90:1–27. https://doi.org/10.1016/j.jaut.2018.02.003.

[7] Girón-González JA, García del Río E, Rodríguez C, Rodríguez-Martorell J, Serrano A. Antiphospholipid syndrome and asymptomatic carriers of antiphospholipid antibody: prospective analysis of 404 individuals. J Rheumatol 2004;31(8):1560–7 [PMID: 15290736].

[8] Yelnik CM, Urbanski G, Drumez E, Sobanski V, Maillard H, Lanteri A, et al. Persistent triple antiphospholipid antibody positivity as a strong risk factor of first thrombosis, in a long-term follow-up study of patients without history of thrombosis or obstetrical morbidity. Lupus. 2017;26(2):163–9. https://doi.org/10.1177/0961203316657433 [PMID: 27432808].

[9] Medina G, Briones-García E, Cruz-Domínguez MP, Flórez-Durante OI, Jara LJ. Antiphospholipid antibodies disappearance in primary antiphospholipid syndrome: thrombosis recurrence. Autoimmun Rev 2017;16(4):352–4. https://doi.org/10.1016/j.autrev.2017.02.004 [PMID: 28216071].

[10] Radin M, Schreiber K, Sciascia S, Roccatello D, Cecchi I, Aguirre Zamorano MÁ, et al. Prevalence of antiphospholipid antibodies negativisation in patients with antiphospholipid syndrome: A long-term follow-up multicentre study. Thromb Haemost 2019;119(12):1920–6. https://doi.org/10.1055/s-0039-1696687 [PMID: 31587246].

[11] Zen M, Loredo Martinez M, Benvenuti F, Gatto M, Saccon F, Larosa M, et al. Prevalence, outcome and management of patients with SLE and secondary antiphospholipid antibody syndrome after aPL seroconversion. Rheumatology (Oxford) 2021;60(3):1313–20. https://doi.org/10.1093/rheumatology/keaa463 [PMID: 32940703].

[12] Out HJ, de Groot PG, Hasselaar P, dan Vliet M, Derksen RH.. Fluctuations of anticardiolipin antibody levels in patients with systemic lupus erythematosus: a prospective study. Ann Rheum Dis 1989;48(12):1023–8. https://doi.org/10.1136/ard.48.12.1023 [PMID: 2619354; PMCID: PMC1003942].

[13] Out HJ, van Vliet M, de Groot PG, Derksen RH. Prospective study of fluctuations of lupus anticoagulant activity and anticardiolipin antibody titre in patients with systemic lupus erythematosus. Ann Rheum Dis 1992;51(3):353–7. https://doi.org/10.1136/ard.51.3.353. PMID: 1575581; PMCID: PMC1004660.

[14] Levine SR, Brey RL, Tilley BC, Thompson JL, Sacco RL, Sciacca RR, et al. Antiphospholipid antibodies and subsequent thrombo-occlusive events in patients with ischemic stroke. JAMA. 2004;291(5):576–84. https://doi.org/10.1001/jama.291.5.576 [PMID: 14762036].

[15] Martinez-Berriotxoa A, Ruiz-Irastorza G, Egurbide MV, Garmendia M, Gabriel Erdozain J, Villar I, et al. Transiently positive anticardiolipin antibodies and risk of thrombosis in patients with systemic lupus erythematosus. Lupus. 2007;16(10):810–6. https://doi.org/10.1177/0961203307083316 [PMID: 17895304].

[16] Amory CF, Levine SR, Brey RL, Gebregziabher M, Tuhrim S, Tilley BC, et al. Antiphospholipid antibodies and recurrent thrombotic events: persistence and portfolio. Cerebrovasc Dis 2015;40(5–6):293–300. https://doi.org/10.1159/000441362 [PMID: 26513489; PMCID: PMC4659726].

[17] Frodlund M, Walhelm T, Dahle C, Sjöwall C. Longitudinal analysis of anti-cardiolipin and anti-β2-glycoprotein-I antibodies in recent-onset systemic lupus erythematosus: a prospective study in swedish patients. Front Med (Lausanne) 2021;8:646846. https://doi.org/10.3389/fmed.2021.646846. PMID: 33732724; PMCID: PMC7959716.

[18] Chighizola CB, Gerosa M, Meroni PL. New tests to detect antiphospholipid antibodies: anti-domain I beta-2-glycoprotein-I antibodies. Curr Rheumatol Rep 2014;16(2):402. https://doi.org/10.1007/s11926-013-0402-7 [PMID: 24390756].

[19] Devreese KMJ. Testing for antiphospholipid antibodies: advances and best practices. Int J Lab Hematol 2020;42:49–58.

[20] Montaruli B, De Luna E, Erroi L, Marchese C, Mengozzi G, Napoli P, et al. Analytical and clinical comparison of different immunoassay systems for the detection of antiphospholipid antibodies. Int J Lab Hematol 2016;38:172–82. 115.

[21] Van Hoecke F, Persijn L, Decavele AS, Devreese K. Performance of two new, automated chemiluminescence assay panels for anticardiolipin and anti-beta2-glycoprotein I antibodies in the laboratory diagnosis of the antiphospholipid syndrome. Int J Lab Hematol 2012;34:630–40.

[22] Devreese KM, Poncet A, Lindhoff-Last E, Musial J, de Moerloose P, Fontana P. A multicenter study to assess the reproducibility of antiphospholipid antibody results produced by an automated system. J Thromb Haemost 2017;15:91–5.

[23] Gkrouzman E, Willis R, Andrade D, Tektonidou MG, Pengo V, Ruiz-Irastorza G, et al. Associations among antiphospholipid antibody types, isotypes, and titers: an antiphospholipid syndrome alliance for clinical trials and international networking (APS ACTION) study. Lab Invest 2023;103(6):100147. https://doi.org/10.1016/j.labinv.2023.100147.

[24] Chighizola CB, Raschi E, Banzato A, Borghi MO, Pengo V, Meroni PL. The challenges of lupus anticoagulants. Expert Rev Hematol 2016;9(4):389–400. https://doi.org/10.1586/17474086.2016.1140034.

[25] Yelnik CM, Laskin CA, Porter TF, Branch DW, Buyon JP, Guerra MM, et al. Lupus anticoagulant is the main predictor of adverse pregnancy outcomes in aPL-positive patients: validation of PROMISSE study results. Lupus Sci Med 2016. https://doi.org/10.1136/lupus-2015-000131.

[26] Pregnolato F, Gerosa M, Raimondo MG, Comerio C, Bartoli F, Lonati PA, et al. EUREKA algorithm predicts obstetric risk and response to treatment in women with different subsets of anti-phospholipid antibodies. Rheumatology (Oxford) 2021;60(3):1114–24. https://doi.org/10.1093/rheumatology/keaa203 [PMID: 32441742].

[27] Devreese KM. Antiphospholipid antibody testing and standardization. Int J Lab Hematol 2014;36:352–63.

[28] Devreese KM, Pierangeli SS, de Laat B, Tripodi A, Atsumi T, Ortel TL. Subcommittee on lupus anticoagulant/phospholipid/dependent antibodies. Testing for antiphospholipid antibodies with solid phase assays: guidance from the SSC of the ISTH. J Thromb Haemost J Thromb Hemost 2014;12:792–5.

[29] Harris EN, Gharavi AE, Patel SP, Hughes GR. Evaluation of the anti-cardiolipin antibody test: report of an international workshop held 4 April 1986. Clin Exp Immunol 1987;68:215–22.

[30] Willis R, Lakos G, Harris EN. Standardization of antiphospholipid antibody testing–historical perspectives and ongoing initiatives. Semin Thromb Hemost 2014;40:172–7.

[31] Ichikawa K, Tsutsumi A, Atsumi T, Matsuura E, Kobayashi S, Hughes GR, et al. A chimeric antibody with the human gamma1 constant region as a putative standard for assays to detect IgG beta2-glycoprotein I-dependent anticardiolipin and anti-beta2-glycoprotein I antibodies. Arthritis Rheum 1999;42:2461–70.

[32] Pierangeli SS, Favaloro EJ, Lakos G, Meroni PL, Tincani A, Wong RC, et al. Standards and reference materials for the anticardiolipin and anti-beta2glycoprotein I assays: A report of recommendations from the APL task force at the 13th international congress on antiphospholipid antibodies. Clin Chim Acta 2012;413:358–60.

[33] Ledford-Kraemer MR, Moore GW, Bottenus R, et al. Laboratory testing for the lupus anticoagulant; approved guideline. CLSI document H; 2014.

[34] Devreese KM, Van Hoecke F. Anticardiolipin and anti-beta2glycoprotein-I antibody cut-off values in the diagnosis of antiphospholipid syndrome: more than calculating the in-house 99th percentiles, even for new automated assays. Thromb Res 2011;128:598–600.

[35] Bor MV, Jacobsen IS, Gram JB, Sidelmann JJ. Revisiting the Phadia/EliA cut-off values for anticardiolipin and antibeta2-glycoprotein I antibodies: a systematic evaluation according to the guidelines. Lupus 2018;27:1446–54.

[36] Neville C, Rauch J, Kassis J, Chang ER, Joseph L, Le Comte M, et al. Thromboembolic risk in patients with high titre anticardiolipin and multiple antiphospholipid antibodies. Thromb Haemost 2003;90:108–15.

[37] Keeling D, Mackie I, Moore GW, Greer IA, Greaves M, British Committee for Standards in Haematology. Guidelines on the investigation and management of antiphospholipid syndrome. Br J Haematol 2012;157:47–58.

[38] Moore GW. Commonalities and contrasts in recent guidelines for lupus anticoagulant detection. Int J Lab Hematol 2014;36:364–73.

[39] Riancho-Zarrabeitia L, Daroca G, Muñoz P, López-Hoyos M, Haya A, Martínez-Taboada VM. Serological evolution in women with positive antiphospholipid antibodies. Semin Arthritis Rheum 2017;47(3):397–402. https://doi.org/10.1016/j.semarthrit.2017.05.001. PMID: 28576307.

[40] Criado-García J, Fernández-Puebla RA, Jiménez LL, Velasco F, Santamaría M, Blanco-Molina A. Retirada de la anticoagulación en el síndrome antifosfolípido primario cuando se negativizan los anticuerpos anticardiolipina [Anticoagulation treatment withdrawal in primary antiphospholipid syndrome when anticardiolipin antibodies become negative]. Rev Clin Esp 2008;208(3):135–7. https://doi.org/10.1157/13115821 [PMID: 18275767].

[41] Gkrouzman E, Sevim E, Finik J, Andrade D, Pengo V, Sciascia S, et al. Antiphospholipid antibody profile stability over time: prospective results from the APS ACTION clinical database and repository. J Rheumatol 2021;48(4):541–7. https://doi.org/10.3899/jrheum.200513. PMID: 33259328.

[42] Erkan D, Derksen WJ, Kaplan V, Sammaritano L, Pierangeli SS, Roubey R, et al. Real world experience with antiphospholipid antibody tests: how stable are results over time? Ann Rheum Dis 2005;64(9):1321–5. https://doi.org/10.1136/ard.2004.031856. PMID: 15731290; PMCID: PMC1755632.

[43] Petri MA, Avci M, Magder LS. Evaluation of different ways to identify persistent positivity of lupus anticoagulant in systemic lupus erythematosus. Lupus. Sci Med 2020;7:e000406.

[44] Sarabi ZS, Sahebari M, Rezaie AE, Norouzi MT, Hashemzadeh K, Mirfeizi Z. The relationship between systemic lupus erythematosus activity and persistent positive antiphospholipid antibodies. Curr Rheumatol Rev 2018;14(2):145–52. https://doi.org/10.2174/1573397113666161122152553 [PMID: 27881058].

[45] Isenberg DA. The relationship of anti-DNA antibody idiotypes and anti-cardiolipin antibodies to disease activity in systemic lupus erythematosus. Medicine (Baltimore) 1986;65(1):46–55.

[46] de Brandt M, Benali K, Guillevin L, Hachulla E, Job C, Fautrel B, et al. Longitudinal determination of antiphospholipid antibodies in lupus patients without previous manifestations of antiphospholipid syndrome. A prospective study. J Rheumatol 1999;26(1):91–6.

[47] Tsutsumi A, Matsuura E, Ichikawa K, Fujisaku A, Mukai M, Kobayashi S, et al. Antibodies to beta 2-glycoprotein I and clinical manifestations in patients with systemic lupus erythematosus. Arthritis Rheum 1996;39(9):1466–74. https://doi.org/10.1002/art.1780390905 [PMID: 8814057].

[48] Inanç M, Donohoe S, Ravirajan CT, Radway-Bright EL, Mackie I, Machin S, et al. Anti-beta2-glycoprotein I, anti-prothrombin and anticardiolipin antibodies in a longitudinal study of patients with systemic lupus erythematosus and the antiphospholipid syndrome. Br J Rheumatol 1998;37(10):1089–94. PMID: 9825748.

[49] Lombard-Platlet S, Bertolino P, Deng H, Gerlier D, Rabourdin-Combe C. Inhibition by chloroquine of the class II major histocompatibility complex-restricted presentation of endogenous antigens varies according to the cellular origin of the antigen-presenting cells, the nature of the T-cell epitope, and the responding T cell. Immunology. 1993;80(4):566–73 [PMID: 7508420; PMCID: PMC1422266].

[50] Lafyatis R, York M, Marshak-Rothstein A. Antimalarial agents: closing the gate on toll-like receptors? Arthritis Rheum 2006;54(10):3068–70. https://doi.org/10.1002/art.22157 [PMID: 17009223].

[51] Kravvariti E, Koutsogianni A, Samoli E, Sfikakis PP, Tektonidou MG. The effect of hydroxychloroquine on thrombosis prevention and antiphospholipid antibody levels in primary antiphospholipid syndrome: A pilot open label randomized prospective study. Autoimmun Rev 2020;19(4):102491. https://doi.org/10.1016/j.autrev.2020.102491 [PMID: 32084592].

[52] Emmi G, Bettiol A, Palterer B, Silvestri E, Vitiello G, Parronchi P, et al. Belimumab reduces antiphospholipid antibodies in SLE patients independently of hydroxychloroquine treatment. Autoimmun Rev 2019;18(3):312–4. https://doi.org/10.1016/j.autrev.2018.11.002 [PMID: 30639638].

[53] Broder A, Putterman C. Hydroxychloroquine use is associated with lower odds of persistently positive antiphospholipid antibodies and/or lupus anticoagulant in systemic lupus erythematosus. J Rheumatol 2013;40(1):30–3. https://doi.org/10.3899/jrheum.120157. PMID: 22859353; PMCID: PMC3768146.

[54] Hardy RS, Raza K, Cooper MS. Therapeutic glucocorticoids: mechanisms of actions in rheumatic diseases. Nat Rev Rheumatol 2020;16(3):133–44. https://doi.org/10.1038/s41584-020-0371-y [PMID: 32034322].

[55] Veneri D, Ambrosetti A, Franchini M, Mosna F, Poli G, Pizzolo G. Remission of severe antiphospholipid syndrome associated with non-Hodgkin's B-cell lymphoma after combined treatment with rituximab and chemotherapy. Haematologica 2005;90. Suppl:ECR37. PMID: 16266928.

[56] Bakshi J, Stevens R. Rituximab therapy for recurrent thromboembolic disease in antiphospholipid syndrome. Lupus. 2013;22(8):865–7. https://doi.org/10.1177/0961203313491023 [PMID: 23740031].

[57] Gkogkolou P, Ehrchen J, Goerge T. Severe antiphospholipid antibody syndrome - response to plasmapheresis and rituximab. J Dermatolog Treat 2017;28(6):564–6. https://doi.org/10.1080/09546634.2017.1282599 [PMID: 28084106].

[58] Gamoudi D, Cutajar M, Gamoudi N, Camilleri DJ, Gatt A. Achieving a satisfactory clinical and biochemical response in antiphospholipid syndrome and severe thrombocytopenia with rituximab: two case reports. Clin Case Rep 2017;5(6):845–8. https://doi.org/10.1002/ccr3.946. PMID: 28588823; PMCID: PMC5458029.

[59] Pons I, Espinosa G, Cervera R. Eficacia y seguridad de rituximab en el tratamiento del síndrome antifosfolipídico primario: análisis de 24 casos a partir de la revisión de la bibliografía [Efficacy and safety of rituximab in the treatment of primary antiphospholipid syndrome: analysis of 24 cases from the bibliography review]. Med Clin (Barc) 2015;144(3):97–104. Spanish, https://doi.org/10.1016/j.medcli.2014.01.034. 24698717.

[60] Erkan D, Vega J, Ramón G, Kozora E, Lockshin MD. A pilot open-label phase II trial of rituximab for non-criteria manifestations of antiphospholipid syndrome. Arthritis Rheum 2013;65(2):464–71. https://doi.org/10.1002/art.37759 [PMID: 23124321].

[61] Berman H, Rodríguez-Pintó I, Cervera R, Morel N, Costedoat-Chalumeau N, Erkan D, et al. Rituximab use in the catastrophic antiphospholipid syndrome: descriptive analysis of the CAPS registry patients receiving rituximab. Autoimmun Rev 2013;12(11):1085–90. https://doi.org/10.1016/j.autrev.2013.05.004 [PMID: 23777822].

[62] You Y, Shi C, Zhou Z, Wang F, Sun Y, Teng J, et al. Rituximab in thrombotic primary antiphospholipid syndrome: a pilot study from a single Centre in China. Ann Rheum Dis 2021;80(6):820–2. https://doi.org/10.1136/annrheumdis-2020-219303 [PMID: 33452001].

[63] Agmon-Levin N, Berman M, Harel L, Lidar M, Drori T, Hajyahia S, et al. Rituximab for refractory manifestations of the antiphospholipid syndrome: a multicentre Israeli experience. Clin Exp Rheumatol 2021;39(5):1049–55. https://doi.org/10.55563/clinexprheumatol/cc5taf [PMID: 33124581].

[64] Kahn P, Ramanujam M, Bethunaickan R, et al. Prevention of murine antiphospholipid syndrome by BAFF blockade. Arthritis Rheum 2008;58(9):2824–34.

[65] Yazici A, Yazirli B, Erkan D. Belimumab in primary antiphospholipid syndrome. Lupus. 2017;26(10):1123–4. https://doi.org/10.1177/0961203316682102 [PMID: 27913749].

[66] Klemm P, Müller-Ladner U, Tarner IH, Lange U, Hudowenz O. Belimumab reduces antiphospholipid antibodies in primary triple-positive antiphospholipid syndrome. Autoimmun Rev 2020;19(8):102594.

[67] Stohl W, Hiepe F, Latinis KM, Thomas M, Scheinberg MA, Clarke A, et al. Belimumab reduces autoantibodies, normalizes low complement levels, and reduces select B cell populations in patients with systemic lupus erythematosus. Arthritis Rheum 2012;64(7):2328–37. https://doi.org/10.1002/art.34400 [PMID: 22275291; PMCID: PMC3350827].

[68] Chatzidionysiou K, Samoli E, Sfikakis PP, Tektonidou MG. Effect of belimumab treatment on antiphospholipid antibody levels: post-hoc analysis based on two randomised placebo-controlled trials in systemic lupus erythematosus. Ann Rheum Dis 2020;79(2):304–7. https://doi.org/10.1136/annrheumdis-2019-216367 [PMID: 31712248].

[69] Bettiol A, Pregnolato F, Sciascia S, Emmi G, Prisco D, Meroni PL. Association of subcutaneous belimumab and long-term antimalarial treatment reduces antiphospholipid antibodies levels in systemic lupus erythematosus: post-hoc analysis of a randomised placebo-controlled trial-comment on: 'Effect of belimumab treatment on antiphospholipid antibody levels: post-hoc analysis based on two randomised placebo-controlled trials in systemic lupus erythematosus' by Chatzidionysiou *et al.* Ann Rheum Dis 2022;81(8):e140. https://doi.org/10.1136/annrheumdis-2020-218377 [PMID: 32753414].

[70] Sciascia S, Rubini E, Radin M, Cecchi I, Rossi D, Roccatello D. Anticardiolipin and anti-beta 2 glycoprotein-I antibodies disappearance in patients with systemic lupus erythematosus and antiphospholipid syndrome while on belimumab. Ann Rheum Dis 2018;77(11):1694–5. https://doi.org/10.1136/annrheumdis-2018-213496 [PMID: 29776976].

[71] Cavazzana I, Kumar R, Pozzari C, Ottaviani R, Fredi M, Piantoni S, et al. Autoantibodies' titre modulation by anti-BlyS treatment in systemic lupus erythematosus. Lupus. 2019;28(9):1074–81. https://doi.org/10.1177/0961203319860191 [PMID: 31296140].

[72] Ginzler EM, Wallace DJ, Merrill JT, Furie RA, Stohl W, Chatham WW, et al. Disease control and safety of belimumab plus standard therapy over 7 years in patients with systemic lupus erythematosus. J Rheumatol 2014;41(2):300–9. https://doi.org/10.3899/jrheum.121368 [PMID: 24187095].

[73] Gómez-Pacheco L, Villa AR, Drenkard C, Cabiedes J, Cabral AR, Alarcón-Segovia D. Serum anti-beta2-glycoprotein-I and anticardiolipin antibodies during thrombosis in systemic lupus erythematosus patients. Am J Med 1999;106(4):417–23.

[74] Drenkard C, Sánchez-Guerrero J, Alarcón-Segovia D. Fall in antiphospholipid antibody at time of thromboocclusive episodes in systemic lupus erythematosus. J Rheumatol 1989;16(5):614–7 [PMID: 2754665].

[75] Alarcón-Segovia D, Delezé M, Oria CV, Sánchez-Guerrero J, Gómez-Pacheco L, Cabiedes J, et al. Antiphospholipid antibodies and the antiphospholipid syndrome in systemic lupus erythematosus. A prospective analysis of 500 consecutive patients. Medicine (Baltimore) 1989;68(6):353–65. https://doi.org/10.1097/00005792-198911000-00003 [PMID: 2811661].

[76] Khawaja M, Magder L, Goldman D, Petri MA. Loss of antiphospholipid antibody positivity post-thrombosis in SLE. Lupus Sci Med 2020;7(1):e000423. https://doi.org/10.1136/lupus-2020-000423. PMID: 33023978; PMCID: PMC7539588.

[77] Vandevelde A, Devreese KMJ. Laboratory diagnosis of antiphospholipid syndrome: insights and hindrances. J Clin Med 2022;11(8):2164. https://doi.org/10.3390/jcm11082164. PMID: 35456258; PMCID: PMC9025581.

[78] Lynch AM, Rutledge JH, Stephens JK, Murphy JR, Marlar RA, Davila GH, et al. Longitudinal measurement of anticardiolipin antibodies during normal pregnancy: a prospective study. Lupus. 1995;4(5):365–9.

[79] Donohoe S, Quenby S, Mackie I, Panal G, Farquharson R, Malia R, et al. Fluctuations in levels of antiphospholipid antibodies and increased coagulation activation markers in normal and heparin- treated antiphospholipid syndrome pregnancies. Lupus. 2002;11(1):11–20.

[80] Salazar-Paramo M, Jara LJ, Ramos A, Barile L, Machado G, Garcia-De La Torre I. Longitudinal study of antinuclear and anticardiolipin antibodies in pregnant women with systemic lupus erythematosus and antiphospholipid syndrome. Rheumatol Int 2002;22(4):142–7.

[81] Topping J, Quenby S, Farquharson R, Malia R, Greaves M. Marked variation in antiphospholipid antibodies during pregnancy: relationships to pregnancy outcome. Hum Reprod 1999;14(1):224–8 [PubMed: 10374125].

[82] Al-Balushi MS, Hasson SS, Said EA, et al. Fluctuation in the levels of immunoglobulin M and immunoglobulin G antibodies for cardiolipin and beta2-glycoprotein among healthy pregnant women. Sultan Qaboos Univ Med J 2014;14:e478–85.

[83] Yelnik CM, Porter TF, Branch DW, et al. Brief report: changes in antiphospholipid antibody titers during pregnancy: effects on pregnancy outcomes. Arthritis Rheumatol 2016;68:1964–9. https://doi.org/10.1002/art.39668.

[84] Erra R, Lonati P, Trespidi L, Gerosa M, Borghi MO, Da Via A, et al. Complement activation products and anti-phospholipid antibodies in pregnancy. In: 12th International Conference on Reproduction, Pregnancy and Rheumatic Diseases London, UK - 8 -10 September; 2023 [Abstract].

[85] Hellgren M. Hemostasis during normal pregnancy and puerperium. Semin Thromb Hemost 2003;29(2):125–30.

[86] Sanchez-Guerrero J, Alarcon-Segovia D. Course of antiphospholipid antibodies in patients with primary antiphospholipid syndrome before, during and after pregnancy treated with low dose aspirin. Relationship of antibody levels to outcome in 7 patients. J Rheumatol 1992;19(7):1083–8 [PMID: 1512762].

[87] Kwak JY, Barini R, Gilman-Sachs A, Beaman KD, Beer AE. Down-regulation of maternal antiphospholipid antibodies during early pregnancy and pregnancy outcome. Am J Obstet Gynecol 1994;171(1):239–46. https://doi.org/10.1016/0002-9378(94)90476-6 [PMID: 8030706].

[88] Linden A. Assessing regression to the mean effects in health care initiatives. BMC Med Res Methodol 2013;13:119. https://doi.org/10.1186/1471-2288-13-119. PMID: 24073634; PMCID: PMC3849564.

[89] Sciascia S, Foddai SG, Alessandri C, Alunno A, Andreoli L, Barinotti A, et al. Clinical Delphi on aPL Negativization: report from the APS Study Group of the Italian Society for Rheumatology (SIR-APS). Thromb Haemost 2022;122(9):1612–20. https://doi.org/10.1055/a-1798-2400. PMID: 35292950.

[90] Radin M, Cecchi I, Rubini E, Foddai SG, Barinotti A, Menegatti E, et al. Treatment of antiphospholipid syndrome. Clin Immunol 2020;221:108597. https://doi.org/10.1016/j.clim.2020.108597 [PMID: 32961331].

[91] Coloma Bazán E, Donate López C, Moreno Lozano P, Cervera R, Espinosa G. Discontinuation of anticoagulation or antiaggregation treatment may be safe in patients with primary antiphospholipid syndrome when antiphospholipid antibodies became persistently negative. Immunol Res 2013;56(2–3):358–61. https://doi.org/10.1007/s12026-013-8407-x [PMID: 23568055].

[92] Comarmond C, Jego P, Veyssier-Belot C, Marie I, Mekinian A, Elmaleh-Sachs A, et al. Cessation of oral anticoagulants in antiphospholipid syndrome. Lupus. 2017;26(12):1291–6. https://doi.org/10.1177/0961203317699285 [PMID: 28355985].

[93] Nuri E, Taraborelli M, Andreoli L, Tonello M, Gerosa M, Calligaro A, et al. Long-term use of hydroxychloroquine reduces antiphospholipid antibodies levels in patients with primary antiphospholipid syndrome. Immunol Res 2017;65(1):17–24. https://doi.org/10.1007/s12026-016-8812-z [PMID: 27406736].

[94] Pedrosa TN, Pasoto SG, Aikawa NE, Yuki EF, Borba EF, Filho JCF, et al. Understanding the dynamics of hydroxychloroquine blood levels in lupus nephritis. Lupus. 2020;29:560–8. https://doi.org/10.1177/0961203320912832.

[95] Wallace DJ. Does hydroxychloroquine sulfate prevent clot formation in systemic lupus erythematosus? Arthritis Rheum 1987;30(12):1435–6. https://doi.org/10.1002/art.1780301219 [PMID: 3435574].

[96] Wallace DJ, Linker-Israeli M, Metzger AL, Stecher VJ. The relevance of antimalarial therapy with regard to thrombosis, hypercholesterolemia and cytokines in SLE. Lupus. 1993;2(Suppl. 1):S13–5 [PMID: 8485565].

[97] Jung H, Bobba R, Su J, Shariati-Sarabi Z, Gladman DD, Urowitz M, et al. The protective effect of antimalarial drugs on thrombovascular events in systemic lupus erythematosus. Arthritis Rheum 2010;62(3):863–8. https://doi.org/10.1002/art.27289 [PMID: 20131232].

[98] Kaiser R, Cleveland CM, Criswell LA. Risk and protective factors for thrombosis in systemic lupus erythematosus: results from a large, multi-ethnic cohort. Ann Rheum Dis 2009;68(2):238–41. https://doi.org/10.1136/ard.2008.093013. PMID: 18782792; PMCID: PMC2875136.

[99] Bessant R, Duncan R, Ambler G, Swanton J, Isenberg DA, Gordon C, et al. Prevalence of conventional and lupus-specific risk factors for cardiovascular disease in patients with systemic lupus erythematosus: A case-control study. Arthritis Rheum 2006;55(6):892–9. https://doi.org/10.1002/art.22343 [PMID: 17139666].

[100] Mok MY, Chan EY, Fong DY, Leung KF, Wong WS, Lau CS. Antiphospholipid antibody profiles and their clinical associations in Chinese patients with systemic lupus erythematosus. J Rheumatol 2005;32(4):622–8 [PMID: 15801016].

[101] Roman MJ, Shanker BA, Davis A, Lockshin MD, Sammaritano L, Simantov R, et al. Prevalence and correlates of accelerated atherosclerosis in systemic lupus erythematosus. N Engl J Med 2003;349(25):2399–406. https://doi.org/10.1056/NEJMoa035471. Erratum in: N Engl J Med. 2006;355(16):1746. PMID: 14681505.

[102] Petri M. Thrombosis and systemic lupus erythematosus: the Hopkins lupus cohort perspective. Scand J Rheumatol 1996;25(4):191–3. https://doi.org/10.3109/03009749609069986 [PMID: 8792794].

[103] Ruiz-Irastorza G, Egurbide MV, Pijoan JI, Garmendia M, Villar I, Martinez-Berriotxoa A, et al. Effect of antimalarials on thrombosis and survival in patients with systemic lupus erythematosus. Lupus. 2006;15(9):577–83. https://doi.org/10.1177/0961203306071872 [PMID: 17080912].

[104] Tektonidou MG, Laskari K, Panagiotakos DB, Moutsopoulos HM. Risk factors for thrombosis and primary thrombosis prevention in patients with systemic lupus erythematosus with or without antiphospholipid antibodies. Arthritis Rheum 2009;61(1):29–36. https://doi.org/10.1002/art.24232 [PMID: 19116963].

[105] Mok CC, Tang SS, To CH, Petri M. Incidence and risk factors of thromboembolism in systemic lupus erythematosus: a comparison of three ethnic groups. Arthritis Rheum 2005;52(9):2774–82. https://doi.org/10.1002/art.21224 [PMID: 16142761].

[106] Ho KT, Ahn CW, Alarcón GS, Baethge BA, Tan FK, Roseman J, et al. Systemic lupus erythematosus in a multiethnic cohort (LUMINA): XXVIII. Factors predictive of thrombotic events. Rheumatology (Oxford) 2005;44(10):1303–7. https://doi.org/10.1093/rheumatology/kei014 [PMID: 16030085].

[107] Hsu CY, Lin YS, Su YJ, Lin HF, Lin MS, Syu YJ, et al. Effect of long-term hydroxychloroquine on vascular events in patients with systemic lupus erythematosus: a database prospective cohort study. Rheumatology (Oxford) 2017;56(12):2212–21. https://doi.org/10.1093/rheumatology/kex357 [PMID: 29029334].

[108] Erkan D, Yazici Y, Peterson MG, Sammaritano L, Lockshin MD. A cross-sectional study of clinical thrombotic risk factors and preventive treatments in

antiphospholipid syndrome. Rheumatology (Oxford) 2002;41(8):924–9. https://doi.org/10.1093/rheumatology/41.8.924 [PMID: 12154210].

[109] Schmidt-Tanguy A, Voswinkel J, Henrion D, Subra JF, Loufrani L, Rohmer V, et al. Antithrombotic effects of hydroxychloroquine in primary antiphospholipid syndrome patients. J Thromb Haemost 2013;11(10):1927–9. https://doi.org/10.1111/jth.12363 [PMID: 23902281].

[110] Belizna C, Pregnolato F, Abad S, et al. HIBISCUS: hydroxychloroquine for the secondary prevention of thrombotic and obstetrical events in primary antiphospholipid syndrome. Autoimmun Rev 2018;17(12):1153–68. https://doi.org/10.1016/j.autrev.2018.05.012.