**THROMBOCYTOPAENIA IN ANTIPHOSPHOLIPID SYNDROME:**

**A FREE RADICAL PERSPECTIVE**

Paul RJ Ames1,2, Tommaso Bucci3, Mira Merashli4, Alessia Arcaro5, Fabrizio Gentile5.

1Immune Response & Vascular Disease Unit, Nova University Lisbon, Rua Camara Pestana, Lisbon, Portugal; 2Department of Haematology, Dumfries Royal Infirmary, Cargenbridge, Dumfries, UK. 3Department of General Surgery, Surgical Specialties and Organ Transplantation “Paride Stefanini”, Sapienza University of Rome, Rome, Italy; 4Department of Rheumatology, American University of Beirut, Beirut, Lebanon; 5Department of Medicine and Health Sciences ‘V. Tiberio’, University of Molise, Campobasso, Italy.

**Corresponding author:** Paul RJ Ames, MD, MSc, PhD, FRCPAth, Dumfries & Galloway Royal Infirmary, Cargenbridge, DG2 8RX, Scotland, UK; phone +44 1387 246246; e-mail paxmes@aol.com

**Abstract**

Thrombosis associated with thrombocytopaenia is an apparent paradox that is present across a wide spectrum of disorders. While thrombocytopenia has been a controversial clinical classification criterion for antiphospholipid syndrome because initial reports failed to demonstrate a relation between low platelet count with other clinical or laboratory manifestations of the syndrome, recent data highlight the association between mild to moderate thrombocytopaenia and the subsequent risk of thrombosis. Although antiphospholipid antibodies may induce platelet activation *in vitro,* additional stimuli may contribute to their activation *in vivo*, amongst which reactive oxygen and nitrogen species and lipid peroxidation products, that are elevated in patients with the antiphospholipid syndrome: herein we provide a novel plausible framework involving free radicals that could add to the understanding of the thrombocytopaenia/thrombosis paradox in the antiphospholipid syndrome.

**Key words**: thrombocytopenia, antiphospholipid syndrome, reactive oxygen and nitrogen species, lipid peroxidation products.

**Key message**: free radicals may be implicated in the thrombocytopaenia/thrombosis paradox of the antiphospholipid syndrome

**Introduction**

In the first semester of this year two articles published in Rheumatology highlighted the negative impact of thrombocytopaenia in primary antiphospholipid syndrome (PAPS). The first study from Spain reported data from a longitudinal cohort of 114 antiphospholipid patients (APS) followed up for a mean of 19 years, 64% of which with PAPS: this demonstrated that low-moderate and persistent thrombocytopenia (platelet count 50-150 x 109/L), identified PAPS and secondary APS patients at a higher risk of thrombosis-related mortality (hazard ratios 2.8 and 4.4 respectively) (1). The second study from China reported data from a longitudinal cohort of 218 PAPS patients followed up for a median of 25 months, showing that mild thrombocytopenia (platelet count <100 x 109/L) was associated with a higher risk of thrombotic events (hazard ratio 2.9) with a recurrent thrombosis rate of 47.4% at six years (2)

These finding highlight a somewhat unexplored but not unexpected paradox in APS. In 2017, a longitudinal Japanese cohort of 953 consecutive antiphospholipid (aPL) positive patients assessed the predictive effect of platelet number on the risk of thrombosis via the antiphospholipid score (aPL-S) which combines different types and titres of aPL. Having discriminated low from high aPL-S, the authors found that within the low aPL-S group, patients with a platelet count ≤150x109/L were more likely to develop thrombosis than patients with a normal platelet count (HR 3.44), whereas patients with a high aPL-S developed thrombosis independently of the platelet count (3). Likewise, a platelet count ≤150x109/L was identified in 51 patients (26.8%) from another cohort consisting of 85 PAPS, 48 secondary APS patients and 52 asymptomatic aPL carriers: by propensity score analysis, thrombocytopenia (alongside hypertension), independently predicted the compound risk of thrombosis, obstetric morbidity and death (HR 10.95) (4).

The relation between thrombocytopaenia and thrombosis is not unique to APS and is found also in patients with immune thrombocytopaenia (ITP): a systematic review in adults with primary ITP concluded that ITP was associated with an increased risk of arterial thromboembolism (ATE) (RR 1.52) and a greater risk of venous thromboembolism (VTE) in splenectomised patients (RR 2.39) (5). A subsequent bi-national study from France and Sweden showed that the incidence rate of ATE was 15.0 and 14.7 per 1000 person-years respectively, whereas the incidence rate of VTE was 6.9 and 6.5, respectively: older age, male gender and a history of ATE were associated with a recurrent ATE in both countries (6).

In the ITP setting, but in the concomitant presence of aPL, the odds ratio of developing ATE in the presence of the lupus anticoagulant (LA) was 5.52 but it was lower at 2.12 in the presence of anticardiolipin antibodies (aCL) alone. Conversely, the odds ratio of developing VTE in the presence of LA or aCL was 5.13 and 2.00 respectively (7).

**Megakaryocytes, platelets, reactive oxygen and nitrogen species**

Platelets are small a-nucleated fragments deriving from megakaryocytes (MK): the latter originate from a progenitor stem cell that goes through several mitotic self-renewals before undergoing endomitosis into cells with progressive polyploidy; during this process platelet proteins are synthesized and the demarcation membrane system develops (8) giving rise to the long cytoplasmic branches that, once shed into the marrow sinusoids as pro-platelets (9), break up into platelets (10).

Reactive oxygen species (ROS), including superoxide anion (O·̄2) produced from different cell types residing in the bone marrow, are involved in endomitosis, polyploidization, maturation and platelet formation. By activating redox-sensitive transcriptional factors, ROS control the expression of target genes that code for enzymes endowed with antioxidant activity or for the biosynthesis of molecules with antioxidant activity that preserve the intracellular MK oxidant/antioxidant balance (11). An excess of ROS, differentially acting on anti-apoptotic BCL-xl and pro-apoptotic Bax and Bak, (12) decreases MK proliferation, favors cell cycle arrest and promotes senescence (13, 14).

In a simplified schematization, the interaction of O·̄2 with nitric oxide (•NO) deriving from endothelial and monocyte nitric oxide synthase generates reactive nitrogen species (RNS), amongst which peroxynitrite (ONOO-); although **•**NO, in isolation or in combination with thrombopoietin, allows the development of functional platelet-sized particles *in vitro*, the predominance of ONOO- over •NO induces premature apoptosis that limits platelet generation (15). Overproduction of ONOO-, detected as the 3-nitro-tyrosine adduct in plasma proteins, was documented in patients with PAPS (16) and systemic lupus erythematosus (SLE) (17). Interestingly, tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) enhanced endogenous RNS and indirectly suppressed MK maturation via the pathway mentioned above (18), whereas interferon- (IFN-) directly suppressed platelet production (19). Serum levels of TNF-α, IFN- and IFN-γ are raised in SLE (20, 21, 22, 23) and APS (24) (Figure 1).

**Megakaryocytes, platelets and lipid peroxidation products**

ROS can cause structural damage to DNA, proteins and lipids both directly and indirectly by fuelling the peroxidation of polyunsaturated fatty acids, such as arachidonic acid. Lipid peroxidation products, such as the reactive aldehydes acrolein, malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE), can form covalent electrophilic addition products with DNA and proteins (25); MDA and HNE adducts are present in SLE (17) and it is of particular interest that acrolein and 4-HNE can react with DNA via Michael addition to the *N2*-amino group of deoxyguanosine, resulting in the formation of 1,*N2*-exocyclic propane adducts (reviewed in 26). These can form inter-strand cross-links which impede DNA replication and transcription. Notably, in a murine model of Fanconi anaemia (FA), the knockout of the *Fanca* gene, encoding Fanconi anaemia group A protein (FANCA) which is required for DNA incision at sites of psoralen-mediated inter-strand cross links (27), caused defective megakaryopoiesis, accumulation of MKs with low ploidy, decreased platelet production, formation of nucleoplasmic bridges and chromosomal instability (28).

Lipid hydroxyperoxyl radicals also undergo, in addition to aldehyde formation, endocyclization to F2-isoprostanes (F2-IPs); in particular 8-*epi*-prostaglandin-F2 (8-*epi*-PG-F2), originating from arachidonic acid by the effect of O·̄2, independently of cyclo-oxygenase, caused a dose-dependent irreversible platelet aggregation in the presence of sub-threshold concentrations of collagen, adenosine diphosphate, arachidonic acid and analogues of thromboxane A2(29).

F2-IPs are increased in SLE and primary APS (reviewed in 30): urinary concentrations of 8-*ep*i-PGF2and IPF2-I were higher than normal in patients with SLE and correlated with serum levels of aCL (31) and of prothrombin fragment F1+2 (32).

**Megakaryocytes, platelets and thrombin**

It was known since the 1990s that thrombin could inhibit MK growth, once bound to its specific receptor on MK progenitors (33); indeed, thrombin causes G0/G1 arrest and down- regulation of BCL-2, inducing MK apoptosis and blocking platelet generation (34). Moreover, as little as 30 min of thrombin exposure *in vitro* may induce platelet fragmentation and exhaustion, accompanied by loss of platelet contractility, mitochondria depolarization and an increase in ROS; the resulting platelet-derived fragments have been defined “apoptotic cellular bodies” (35), whereas apoptotic platelets in plasma become smaller after shedding microparticles (Luff) and in both instances the membrane exposure of phosphatidylserine acts as an “eat me” signal for macrophage clearance (36).

**Free radicals, impaired megakaryocyte production and increased platelets destruction**

As mentioned earlier, *in vitro* experiments showed that free radicals reduce MK proliferation, favor cell cycle arrest and promote senescence (13,14). Similar *in vitro* experiments demonstrated that plasmas from SLE patients (but without APS), increased megakaryopoiesis but inhibited proplatelet formation; this effect was reverted by the same plasmas depleted of total antibodies containing the anti-platelet antibody fraction; additionally, platelet apoptosis, assessed by measuring the loss of mitochondrial membrane potential, the exposure of active caspase 3 and of phosphatidylserine, was increases in thrombocytopenic patients compared to patients with normal platelet counts. Both effects were maximal in patients with higher disease activity (Baroni Pietto), who were not necessarily harboring higher anti-platelet autoantibodies titers: it cannot be excluded that plasma free radicals mediate these effects, given that oxidative stress, measured as 8-*epi* PGF2, is greater in plasma of SLE patients with higher disease activity (Ames). Nevertheless, these data confirm premature MK apoptosis (15) that limits platelet production as well as increased platelet consumption that complements the effect of thrombin described in the previous paragraph. Although we have no data regarding *in vivo* survival of 111In-labeled or 51Cr-labelled autologous platelets in APS, there is indirect evidence for increased platelet turnover in some patients with APS, indicated by increased median CD63 expression compared to healthy controls (Joseph) and by reduced platelet size and increased platelet microparticles resulting from apoptosis in relation to APS (Lood).

**Coagulation and complement, reactive oxygen and nitrogen species**

While the occurrence of oxidative (30) and nitrative stress (16,17) in APS has been known for more than two decades, recent *in vitro* studies confirmed that aPL may induce ROS production from mitochondria (37) and from endothelial cells (38) and that aPL, either monoclonal or purified from APS patients, may activate endosomal NADPH-oxidase-2 that in turn releases O·̄2 and prompts inflammasome activation (39); the subsequent release of IL-1β, in a positive feedback loop, favors the uncoupling of superoxide dismutase, glutathione peroxidase and catalase that perpetuates unhindered intracellular ROS generation (40).

This oxidative and nitrative stress background promotes a procoagulant state characterized by enhanced coagulation (41,42,43) and complement activation (44, 45) processes known to be interlinked in APS (46) as well as fibrinolysis inhibition (47).

**Reversal of thrombocytopaenia and paradoxical thrombosis or re-thrombosis**

There have been reports where reduction of thrombin generation and/or quenching of oxidative stress led to reversal of thrombocytopenia; few cases occurred after the administration of acetylsalicylic acid (ASA) in PAPS (48-51): ASA may directly (52) or indirectly (53,54) increase the effect of antithrombin against thrombin (55) both at low (53,55) and at high dose (54); other cases occurred after the initiation of warfarin in some PAPS and SLE-related APS patients (56, 57), and by its very mechanism of action (58) warfarin prevents thrombin generation (59). Cloroquine led to the normalization of thrombocytopaenia in PAPS (60) and, interestingly, hydroxychloroquine improved platelet counts in paediatric (61) and adult (62) immune thrombocytopaenia. Hydroxychloroquine inhibits the NADPH oxidase system and dampens the generation of O·̄2 (63,64) and of isoprostane (65). Altogether, releasing the inhibitory effect of thrombin and ROS on MK and platelet apoptosis might restore megakaropoiesis and decrease platelet consumption.

**Concluding remarks**

In the light of the above information, persistent thrombocytopaenia in PAPS may be seen as a consequence of ongoing oxidative stress (30), nitrative stress (16, 17) and thrombin generation (66), key players in atherosclerosis (67, 68), that may exert different degrees of inhibition of megakaryocyte maturation; mechanistically, the ensuing thrombocytopaenia attempts to minimize the chance of platelet clot formation within atherosclerotic arterial vessels (69).

Of note, while the degree of thrombocytopenia reported by the previous authors does not need intervention, reversal of severe thrombocytopaenia, with a sudden rebound in the production of fully functional platelets, may prompt re-thrombosis or ex novo thrombosis in APS, mostly arterial; in particular, one myocardial infarction re-developed after commencement of warfarin (56), one after immune suppression (70) and two after thrombopoietic agents (Guitton), a recurrent ischemic stroke developed after starting warfarin (56) and another after starting a thrombopoietic agent (Guitton); the administration of thrombopoietic agents was followed by two ex-novo catastrophic APS (71, Guitton), one ex-novo pulmonary embolism and deep vein thrombosis each (Borrell, Guitton). It should be remembered that platelets constitute 31.3% of volume of arterial thrombi and 0.4% and 0.8% of venous thrombi and pulmonary embolism, respectively (Chernysh).

While the mechanisms leading to acute or chronic autoimmune thrombocytopaenia are not the scope of this article (73) the pathogenesis of thrombocytopaenia in APS may be more complex than anticipated: oxidative, nitrative and sulfhydryl stress, by inducing post-translational modifications of proteins and/or lipids, may conduce to the emergence of neoepitopes or favor epitope spreading in known and unknown platelet and MK targets, eliciting novel autoantibodies that add to those already known in autoimmune thrombocytopenia (74, 75).

The observation that different agents with antioxidant properties dampen oxidative stress (76, 77) inflammation (77), urinary thromboxane excretion (76), coagulation activation (76,77) and restore fibrinolysis (76) suggests that antioxidant administration represents an attractive therapeutic modality that could benefit patients with APS and should be explored in a randomized controlled trial.

**Funding**

No specific funding was received from any bodies in the public, commercial or not-for-profit sectors to carry out the work described in this article.

**Conflict of interest**

None of the authors have any conflicts of interest to declare.

**References**

1) Pardos-Gea J, Marques-Soares JR, Buján S, Ordi-Ros J, Alijotas-Reig J. Persistent thrombocytopenia predicts poor long-term survival in patients with antiphospholipid syndrome: a 38-year follow-up study. Rheumatology2022; 61: 1053-1061

2) Shi Y, Zhao J, Jiang H, Huang C, Qi W, Song Y et al. Thrombocytopenia in primary antiphospholipid syndrome: Association with prognosis and clinical implications. Rheumatology (Oxford). 2022 May 10: keac264. doi: 10.1093/rheumatology/keac264. Epub ahead of print. PMID: 35536236.

3) Hisada R, Kato M, Sugawara E, Fujieda Y, Oku K, Bohgaki T et al. Thrombotic risk stratification by platelet count in patients with antiphospholipid antibodies: a longitudinal study. J Thromb Haemost 2017; 15: 1782-1787

4) Moulinet T, Dufrost V, Clerc-Urmès I, Wahl D, Zuily S. Risk of thrombosis, pregnancy morbidity or death in antiphospholipid antibodies positive patients with or without thrombocytopenia. Eur J Intern Med 2021; 84: 101-103

5) Doobaree IU, Nandigam R, Bennett D, Newland A, Provan D. Thromboembolism in adults with primary immune thrombocytopenia: a systematic literature review and meta-analysis. Eur J Haematol 2016; 97: 321-330

6) Ekstrand C, Linder M, Baricault B, Lafaurie M, Sailler L, Lapeyre-Mestre M et al. Impact of risk factors on the occurrence of arterial thrombosis and venous thromboembolism in adults with primary immune thrombocytopenia - Results from two nationwide cohorts. Thromb Res 2019; 178: 124-131

7) Moulis G, Audemard-Verger A, Arnaud L, Luxembourger C, Montastruc F, Gaman AM et al. Risk of thrombosis in patients with primary immune thrombocytopenia and antiphospholipid antibodies: A systematic review and meta-analysis. Autoimmun Rev 2016; 15: 203-209

8) Mazzi S, Lordier L, Debili N, Raslova H, Vainchenker W. Megakaryocyte and polyploidization. Exp Hematol 2018; 57: 1-3

9) Potts KS, Farley A, Dawson CA, Rimes J, Biben C, de Graaf C et al. Membrane budding is a major mechanism of in vivo platelet biogenesis. J Exp Med 2020; 217: e20191206

10) Thon JN, Montalvo A, Patel-Hett S, Devine MT, Richardson JL, Ehrlicher A et al. Cytoskeletal mechanics of proplatelet maturation and platelet release. J. Cell Biol 2010; 191: 861–874

11) Eliades A, Matsuura S, Ravid K. Oxidases and reactive oxygen species during hematopoiesis: a focus on megakaryocytes. J Cell Physiol 2012; 227: 3355-3362

12) Josefsson EC, Vainchenker W, James C. Regulation of Platelet Production and Life Span: Role of Bcl-xL and Potential Implications for Human Platelet Diseases. Int J Mol Sci 2020; 21: 7591

13) Boonstra J, Post JA. Molecular events associated with reactive oxygen species and cell cycle progression in mammalian cells. Gene 2004; 337: 1-13.

14) Shao L, Li H, Pazhanisamy SK, Meng A, Wang Y, Zhou D. Reactive oxygen species and hematopoietic stem cell senescence. Int J Hematol 2011; 94: 24-32

15) Battinelli E, Loscalzo J. Nitric oxide induces apoptosis in megakaryocytic cell lines. Blood 2000; 95: 3451-3451

16) Ames PRJ, Batuca JR, Ciampa A, Iannaccone L, Delgado Alves J. Clinical relevance of nitric oxide metabolites and nitrative stress in thrombotic primary antiphospholipid syndrome. J Rheumatol 2010; 37: 2523-2530

17) Wang G, Pierangeli SS, Papalardo E, Ansari GA, Khan MF. Markers of oxidative and nitrosative stress in systemic lupus erythematosus: correlation with disease activity. Arthritis Rheum 2010; 62: 2064-2072

18) Schattner M, Pozner RG, Engelberger I, Gorostizaga A, Maugeri N, Gomez R et al. Effect of nitric oxide on megakaryocyte growth induced by thrombopoietin. J Lab Clin Med 2001; 137: 261-269

19) Yamane A, Nakamura T, Suzuki H, Ito M, Ohnishi Y, Ikeda Y et al. Interferon-alpha 2b-induced thrombocytopenia is caused by inhibition of platelet production but not proliferation and endomitosis in human megakaryocytes. Blood 2008; 112: 542-550

20) Idborg H, Eketjäll S, Pettersson S, Gustafsson JT, Zickert A, Kvarnström M et al. Svenungsson E. TNF-α and plasma albumin as biomarkers of disease activity in systemic lupus erythematosus. Lupus Sci Med 2018; 5: e000260

21) Oke V, Gunnarsson I, Dorschner J, Eketjäll S, Zickert A, Niewold TB et al. High levels of circulating interferons type I, type II and type III associate with distinct clinical features of active systemic lupus erythematosus. Arthritis Res Ther 2019; 21: 107

22) Barrat FJ, Crow MK, Ivashkiv LB. Interferon target-gene expression and epigenomic signatures in health and disease. Nat Immunol 2019; 20: 1574-1583

23) Labonte AC, Kegerreis B, Geraci NS, Bachali P, Madamanchi S, Robl R et al. Identification of alterations in macrophage activation associated with disease activity in systemic lupus erythematosus. PLoS One 2018; 13: e0208132

24) Swadzba J, Iwaniec T, Musial J. Increased level of tumor necrosis factor-α in patients with antiphospholipid syndrome: marker not only of inflammation but also of the prothrombotic state. Rheumatol Int 2011; 31: 307-313

25) Esterbauer H, Schaur RJ, Zollner H. Chemistry and Biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med 1991; 11: 81–128

26) Gentile F, Arcaro A, Pizzimenti S, Daga M, Cetrangolo GP, Dianzani C, Lepore A, Graf M, Ames PRJ, Barrera G. DNA damage by lipid peroxidation products: implications in cancer, inflammation and autoimmunity. AIMS Genetics 2017; 4(2): 103-137

27) Hinz JM, Nham PB, Urbin SS, Jones IM, Thompson LH. Disparate contributions of the Fanconi anemia pathway and homologous recombination in preventing spontaneous mutagenesis. Nucleic Acids Res 2007; 35: 3733–3740

28) Pawlikowska P, Fouchet P, Vainchenker W, Rosselli F, Naim V. Defective endomitosis during megakaryopoiesis leads to thrombocytopenia in Fanca-/- mice. Blood. 2014; 124(24): 3613-23. doi: 10.1182/blood-2014-01-551457

29) Praticò D, Smyth EM, Violi F, FitzGerald GA. Local amplification of platelet function by 8-epi-prostaglandin-F2-alpha is not mediated by thromboxane receptor isoforms. J Biol Chem 1996; 271: 14916-24

30) Merashli M, Bucci T, Pastori D, Pignatelli P, Arcaro A, Gentile F at al. Isoprostanes in systemic lupus erythematosus and antiphospholipid syndrome: A systematic review and meta-analysis. Autoimmun Rev 2021; 20: 102821

31) Iuliano L, Praticò D, Ferro D, Pittoni V, Valesini G, Lawson J, FitzGerald GA, Violi F. Enhanced lipid peroxidation in patients positive for antiphospholipid antibodies. Blood 1997; 90(10): 3931-3935

32) Praticò D, Ferro D, Iuliano L, Rokach J, Conti F, Valesini G, FitzGerald GA, Violi F. Ongoing prothrombotic state in patients with antiphospholipid antibodies: a role for increased lipid peroxidation. Blood 1999; 93(10): 3401-3407

33) Plantier JL, Berthier R, Rival Y, Schweitzer A, Rabiet MJ. Evidence for a selective inhibitory effect of thrombin on megakaryocyte progenitor growth mediated by the thrombin receptor. Br J Haematol 1994; 87: 755-762

34) Yang XL, Ge MK, Mao DK, Lv YT, Sun SY, Yu AP. Thrombin Maybe Plays an Important Role in MK Differentiation into Platelets. Biomed Res Int 2016; 2016: 9313269

35) Leytin V, Gyulkhandanyan AV, Freedman J. Platelet Apoptosis Can Be Triggered Bypassing the Death Receptors. Clin Appl Thromb Hemost 2019; 25: 1076029619853641

Luff SA, Kao CY, Papoutsakis ET. Role of p53 and transcription-independent p53-induced apoptosis in shear-stimulated megakaryocytic maturation, particle generation, and platelet biogenesis. PLoS One 2018; 13: e0203991

36) Kim OV, Nevzorova TA, Mordakhanova ER, Ponomareva AA, Andrianova IA, Le Minh G et al. Fatal dysfunction and disintegration of thrombin-stimulated platelets. Haematologica 2019; 104: 1866-1878

Baroni Pietto MC, Lev PR, Glembotsky AC, Marín Oyarzún CP, Gomez G, Collado V et al. Pathogenic mechanisms contributing to thrombocytopenia in patients with systemic lupus erythematosus. Platelets 2022; 33: 743-754.

Ames PRJ, Alves J, Murat I, Isenberg DA, Nourooz-Zadeh J. Oxidative stress in systemic lupus erythematosus and allied conditions with vascular involvement. Rheumatology. 1999; 38: 529-534.

Joseph JE, Donohoe S, Harrison P, Mackie IJ, Machin SJ. Platelet activation and turnover in the primary antiphospholipid syndrome. Lupus 1998; 7: 333-340

Lood C, Tydén H, Gullstrand B, Nielsen CT, Heegaard NH, Linge P et al. Decreased platelet size is associated with platelet activation and anti-phospholipid syndrome in systemic lupus erythematosus. Rheumatology 2017; 56: 408-416

37) Zussman R, Xu LY, Damani T, Groom KM, Chen Q, Seers B et al. Antiphospholipid antibodies can specifically target placental mitochondria and induce ROS production. J Autoimmun 2020; 111: 102437. doi: 10.1016/j.jaut.2020.102437

38) Velásquez M, Granada MA, Galvis JC, Álvarez ÁM, Cadavid Á. Oxidative stress in endothelial cells induced by the serum of women with different clinical manifestations of the antiphospholipid syndrome. Biomedica 2019; 39: 673-688

39) Müller-Calleja N, Köhler A, Siebald B, Canisius A, Orning C, Radsak M et al. Cofactor-independent antiphospholipid antibodies activate the NLRP3-inflammasome via endosomal NADPH-oxidase: implications for the antiphospholipid syndrome. Thromb Haemost 2015; 113: 1071-1083

40) Dominic A, Le NT, Takahashi M. Loop Between NLRP3 Inflammasome and Reactive Oxygen Species. Antioxid Redox Signal 2022; 36: 784-796

41) Zelaya H, Rothmeier AS, Ruf W. Tissue factor at the crossroad of coagulation and cell signaling. J Thromb Haemost 2018; 16: 1941-1952

42) Giannakopoulos B, Gao L, Qi M, Wong JW, Yu DM, Vlachoyiannopoulos PG et al. Factor XI is a substrate for oxidoreductases: enhanced activation of reduced FXI and its role in antiphospholipid syndrome thrombosis. J Autoimmun 2012; 39: 121-129

43) Ponczek MB. The susceptibility of plasma coagulation factor XI to nitration and peroxynitrite action. Int J Biol Macromol 2016 Oct; 91: 589-597

~~44~~) Hart ML, Walsh MC, Stahl GL. Initiation of complement activation following oxidative stress. In vitro and in vivo observations. Mol Immunol 2004; 41: 165-171

 45) Collard CD, Montalto MC, Reenstra WR, Buras JA, Stahl GL. Endothelial oxidative stress activates the lectin complement pathway: role of cytokeratin 1. Am J Pathol 2001; 159: 1045-1054

46) Ames PRJ, Alves JD, Gentile F. Coagulation and complement in antiphospholipid syndrome. Thromb Res 2017; 158: 149-151

47) Kolodziejczyk-Czepas J, Ponczek MB, Nowak P. Peroxynitrite and fibrinolytic system-The effects of peroxynitrite on t-PA-induced plasmin activity. Int J Biol Macromol 2015; 81: 212-219

48) Alarcón-Segovia D, [Sánchez-Guerrero](https://pubmed.ncbi.nlm.nih.gov/?sort=pubdate&size=50&show_snippets=off&term=S%25C3%25A1nchez-Guerrero+J&cauthor_id=2810261) J. Correction of thrombocytopenia with small dose aspirin in the primary antiphospholipid syndrome. J Rheumatol 1989; 16: 1359-1361

49) Alliot C, [Messouak](https://pubmed.ncbi.nlm.nih.gov/?sort=pubdate&size=50&show_snippets=off&term=Messouak+D&cauthor_id=11754406) D, [Albert](https://pubmed.ncbi.nlm.nih.gov/?sort=pubdate&size=50&show_snippets=off&term=Albert+F&cauthor_id=11754406) F, [Barrios](https://pubmed.ncbi.nlm.nih.gov/?sort=pubdate&size=50&show_snippets=off&term=Barrios+M&cauthor_id=11754406) M. Correction of thrombocytopenia with aspirin in the primary antiphospholipid syndrome. Am J Hematol 2001; 68: 215

56) Cohen MG, Lui SF. Multiple complications of the antiphospholipid syndrome with apparent response to aspirin therapy. J Rheumatol 1992; 19: 803-806

51) Durand JM, Narbonne H, Cretel E, Kaplanski G, Soubeyrand J. La thrombopenie du syndrome primaire des anticorps antiphospholipides est-elle una contre-indication a l'aspirine? Presse Med 1996; 25: 863

52) Villanueva GB, Allen N. Acetylation of antithrombin III by aspirin. Semin Thromb Hemost. 1986; 12: 213-215

53) Di Micco B, Colonna G, Di Micco P, Di Micco G, Russo BM, Macalello MA et al. Anti-thrombin action of low-dose acetylsalicylic acid. Eur J Pharmacol 2003; 460: 59-62

54) Wallén NH, Ladjevardi M. Influence of low- and high-dose aspirin treatment on thrombin generation in whole blood. Thromb Res 1998; 92: 189-194

55) Li XL, Wang Q, Yin HJ, Wang YH, Cao J, Fan L. Chronic Application of Low-Dose Aspirin Affects Multiple Parameters of Three Blood Cellular Types and Antithrombin Activity: A 1:1:1 Propensity Score Matching Analysis. J Cardiovasc Pharmacol 2021; 77: 115-121

56) Ames PRJ, Orefice G, Brancaccio V. Reversal of thrombocytopenia following oral anticoagulation in two patients with primary antiphospholipid syndrome. Lupus 1995; 4: 491-493

57) Wisbey HL, Klestov AC. Thrombocytopenia corrected by warfarin in antiphospholipid syndrome. J Rheumatol 1996; 23: 769-771

58) Hirsh J, Fuster V, Ansell J, Halperin JL; American Heart Association/American College of Cardiology Foundation. American Heart Association/American College of Cardiology Foundation guide to warfarin therapy. J Am Coll Cardiol 2003; 41: 1633-1652

59) Schmidt DE, Chaireti R, Bruzelius M, Holmström M, Antovic J, Ågren A. Correlation of thromboelastography and thrombin generation assays in warfarin-treated patients. Thromb Res 2019; 178: 34-40

60) Suarez IM,  [Diaz](https://pubmed.ncbi.nlm.nih.gov/?sort=pubdate&size=50&show_snippets=off&term=Diaz+RA&cauthor_id=8646233) RA, [Aguayo Canela](https://pubmed.ncbi.nlm.nih.gov/?sort=pubdate&size=50&show_snippets=off&term=Aguayo+Canela+D&cauthor_id=8646233) D, [Pujol de la Llave](https://pubmed.ncbi.nlm.nih.gov/?sort=pubdate&size=50&show_snippets=off&term=Pujol+de+la+Llave+E&cauthor_id=8646233) E. Correction of severe thrombocytopenia with chloroquine in the primary antiphospholipid syndrome. Lupus 1996; 5: 81-83

61) Roche O, Aladjidi N, Rakotonjanahary J, Leverger G, Leblanc T, Thomas C et al. Evaluation of the efficiency of hydroxychloroquine in treating children with immune thrombocytopenia (ITP). Am J Hematol 2017; 92: E79-E81

62) Khellaf M, Chabrol A, Mahevas M, Roudot-Thoraval F, Limal N, Languille L et al. Hydroxychloroquine is a good second-line treatment for adults with immune thrombocytopenia and positive antinuclear antibodies. Am J Hematol 2014; 89:194-198

63) Jančinová V, Pažoureková S, Lucová M, Perečko T, Mihalová D, Bauerová K et al. Selective inhibition of extracellular oxidants liberated from human neutrophils-A new mechanism potentially involved in the anti-inflammatory activity of hydroxychloroquine. Int Immunopharmacol 2015; 28: 175-181

64) Müller-Calleja N, Manukyan D, Canisius A, Strand D, Lackner KJ. Hydroxychloroquine inhibits proinflammatory signalling pathways by targeting endosomal NADPH oxidase. Ann Rheum Dis 2017; 76: 891-897

~~65~~) Rahman RA, Murthi P, Singh H, Gurungsinghe S, Leaw B, Mockler JC et al. Hydroxychloroquine Mitigates the Production of 8-Isoprostane and Improves Vascular Dysfunction: Implications for Treating Preeclampsia. Int J Mol Sci 2020; 21: 2504

66) Ames PRJ, Tommasino C, Iannaccone L, Brillante M, Cimino R, Brancaccio V. Coagulation activation and fibrinolytic imbalance in subjects with idiopathic antiphospholipid antibodies - A crucial role for acquired free protein S deficiency. Thromb Haemost 1996; 76: 190-194

67) Wang W, Kang PM. Oxidative Stress and Antioxidant Treatments in Cardiovascular Diseases. Antioxidants (Basel) 2020; 9: 1292

68) Ten Cate H, Hemker HC. Thrombin Generation and Atherothrombosis: What Does the Evidence Indicate? J Am Heart Assoc 2016; 5: e003553

69) Ames PRJ, Antinolfi I, Scenna G, Gaeta G, Margaglione M, Margarita A. Atherosclerosis in thrombotic primary antiphospholipid syndrome. J Thromb Haemost 2009; 7: 537-542

70) Tan S, Tambar S, Chohan S, Ramsey-Goldman R, Lee C. Acute myocardial infarction after treatment of thrombocytopenia in a young woman with systemic lupus erythematosus. J Clin Rheumatol 2008; 14: 350-352

71) LaMoreaux B, Barbar-Smiley F, Ardoin S, Madhoun H. Two cases of thrombosis in patients with antiphospholipid antibodies during treatment of immune thrombocytopenia with romiplostim, a thrombopoietin receptor agonist. Semin Arthritis Rheum 2016; 45: e10–e12

72) Boulon C, Vircoulon M, Constans J. Eltrombopag in systemic lupus erythematosus with antiphospholipid syndrome: thrombotic events. Lupus 2016; 25:331

Borrell H, Nolla JM, Narváez J. Letter to the editor. Commentary to the article: LaMoreaux B, Barbar-smiley F, Ardoin S, Madhoun H. Two cases of thrombosis in patients with antiphospholipid antibodies during treatment of immune thrombocytopenia with romiplostin, a thrombopoietin receptor agonist. Semin Arthritis Rheum 2015i: S0049-0172(15)00196-1.

Guitton Z, Terriou L, Lega JC, Nove-Josserand R, Hie M, Amoura Z et al. Risk of thrombosis with anti-phospholipid syndrome in systemic lupus erythematosus treated with thrombopoietin-receptor agonists. Rheumatology 2018; 57: 1432-1438

Chernysh IN, Nagaswami C, Kosolapova S, Peshkova AD, Cuker A, Cines DB et al. The distinctive structure and composition of arterial and venous thrombi and pulmonary emboli. Sci Rep. 2020; 10: 5112

73) Jaime-Pérez JC, Ramos-Dávila EM, Meléndez-Flores JD, Gómez-De León A, Gómez-Almaguer D. Insights on chronic immune thrombocytopenia pathogenesis: A bench to bedside update. Blood Rev 2021; 49: 100827

74) Morris G, Puri BK, Olive L, Carvalho AF, Berk M, Maes M. Emerging role of innate B1 cells in the pathophysiology of autoimmune and neuroimmune diseases: Association with inflammation, oxidative and nitrosative stress and autoimmune responses. Pharmacol Res 2019; 148: 104408

75) Bibli SI, Fleming I. Oxidative Post-Translational Modifications: A Focus on Cysteine *S-*Sulfhydration and the Regulation of Endothelial Fitness. Antioxid Redox Signal 2021; 35: 1494-1514

76) Ames PRJ, Tommasino C, Alves J, Morrow JD, Iannaccone L, Fossati G et al. Antioxidant susceptibility of pathogenic pathways in subjects with antiphospholipid antibodies: a pilot study. Lupus 2000; 9: 688-695

77) Pérez-Sánchez C, Aguirre MÁ, Ruiz-Limón P, Ábalos-Aguilera MC, Jiménez-Gómez Y, Arias-de la Rosa I et al. Ubiquinol Effects on Antiphospholipid Syndrome Prothrombotic Profile: A Randomized, Placebo-Controlled Trial. Arterioscler Thromb Vasc Biol 2017; 37: 1923-1932



Figure 1.

**Effects of oxidative and nitrative stress molecules on megakaryocyte and platelet production.**

Antiphospholipid antibodies stimulate the release of superoxide anion (O·̄2) and nitric oxide (•NO) from endothelial cells giving rise to peroxynitrite (ONOO-) and lipid peroxidation products (LPO) such as 4-hydroxynonenal (HNE) and isoprostanes; these may induce premature megakaryocyte (MK) and platelet apoptosis leading to thrombocytopaenia and platelet exhaustion. Image adapted from Chen et al. Cell Death and Disease 2013; 4: e722