SSC Subcommittee Project/Collaborative Project

NAME OF PROJECT: Assessment of impaired fibrinolysis in patients with antiphospholipid syndrome

Subcommittee: Lupus Anticoagulant/Antiphospholipid Antibodies of the Scientific and Standardization Committee of the International Society on Thrombosis and Hemostasis

Person responsible (Chair / Principal Investigator): Hannah Cohen (Chair ISTH-SSC on Lupus anticoagulant / antiphospholipid antibodies) / **Deepa J Arachchillage** (Principal Investigator)

Description Abstract:

Stroke and deep vein thrombosis (DVT) are major causes of morbidity and mortality and are the principal complications of thrombotic antiphospholipid syndrome (APS). Recurrent thrombosis despite anticoagulation is a serious concern in patients with APS as some studies demonstrate recurrent thrombosis can be as high as 30% in 10 years, especially those with triple positive APS (1). These patients have limited treatment options because further intensification of anticoagulation increases the risk of serious bleeding, including intracerebral hemorrhage. Impaired fibrinolytic response is an important determinant of developing stroke and recurrent thrombosis (2). Several studies demonstrated that patients with APS have impaired fibrinolysis (3-9). Impaired fibrinolysis may also explain why APS, unlike other factors that increase the risk of blood clots, is overrepresented in chronic thromboembolic pulmonary hypertension (CTEPH) (10).

Fibrinolysis follows enzymatic cleavage of circulating plasminogen to plasmin by tissue plasminogen activator (tPA) or Urokinase type Plasminogen Activator (uPA). It is regulated by plasminogen activator-1 (PAI-1) which inhibits tPA, and uPA and by alpha 2 anti-plasmin (α2AP), which is the principal inhibitor of plasmin. Thrombomodulin (TM) forms a complex with thrombin, activates Thrombin activatable fibrinolysis inhibitor (TAFI) and downregulates tPA-mediated plasminogen activation. Alterations in the levels of TM, tPA, PAI-1 and TAFI can affect the fibrinolytic response in APS. Plasma levels of fibrinolytic markers including D-dimer, global fibrinolytic assessment in plasma (turbidometry) and fibrinolytic potential in whole blood may help to identify mechanisms underpinning thrombotic risk in APS patients and potential therapeutic

strategies to improve the morbidity and mortality, not only of patients with thrombotic APS but also non-APS thrombosis, especially stroke and CTEPH.

Identifying patients at increased risk of developing recurrent thrombosis in APS is of high clinical importance, in order to tailor treatment to prevent recurrence. Data gathering from this project will aid in identifying novel treatment targets within fibrinolytic pathways. The global thrombosis test (GTT) (Thromboquest Ltd, London, UK) is an automated point of care test, which assesses platelet reactivity and endogenous fibrinolysis in native, non-anticoagulated blood (11). Impaired fibrinolysis detected by GTT is a strong independent predictor of recurrence in acute coronary syndrome and cardiovascular disease without APS (hazard ratio: 8.03; 95% CI: 4.28-15.03; P <0.001) (12, 13). GTT will be performed in fresh whole blood. Therefore, in this prospective observational study, we aim to use GTT to evaluate the formation of a shear-induced thrombus and its subsequent lysis and compare with a plasma based global fibrinolysis test (turbidometry) and plasma markers of fibrinolysis in APS patients with recurrent thrombosis vs single events and with healthy controls.

Design and methodology (Data expected to collect, sample size and statistical analysis):

This is a prospective observational study which initially starts at a single centre (ethical approval already in place) with the intention to expand into a multicenter study. The study population will comprise patients with thrombotic APS. Consecutive patients referred to recruiting centers, who consented for the study, will be included. We aim to recruit a minimum of 100 patients, with an equal number of those with single thrombotic events or recurrent events (on or off anticoagulation). 30 healthy controls will be recruited for comparison with the APS patients. Blood samples will be collected (whole blood) for the global thrombolysis test and plasma prepared for fibrinolytic markers including plasminogen activator inhibitor-1 (PAI-I), tissue plasminogen activator (tPA) (antigen and activity), soluble thrombomodulin and for the global fibrinolytic assay (turbidometry), TAFI and plasmin generation assay. Patient demographics, comorbidities, clinical events, medication history including anticoagulant, antiplatelet treatment, immunomodulatory treatment and statins, and antiphospholipid antibody (aPL) profile will be collected.

Continuous variables will be assessed for normality and appropriate parametric- or non–parametric tests applied. Categorical variables will be analysed using Chi-squared or Fisher's Exact Tests. Correlation and linear regression analyses will explore associations between

variables. Experiments on blood samples or material from APS patients' blood will be compared to single thrombotic event vs multiple events and with 30 thrombotic non-APS and 30 healthy controls with no aPL. The effect of aPL profile, venous vs arterial, primary vs secondary APS and the effect of comorbidities on fibrinolysis will be assessed by performing cluster analysis. When comparing groups, propensity matched (for age, sex, comorbidities, anticoagulant use, other treatment that may have effects on fibrinolysis) analysis will be performed. GTT results will be analyzed following adjustment for the anticoagulation and their intensity. p value <0.05 will be considered significant. Data will be analysed using GraphPad Prism, SPSS or R.

Study population (Inclusion, exclusion, eligibility criteria) (patient population; recruitment of participating institutions/physicians and subjects; minimum number needed; expected number):

Inclusion criteria:

Patients with confirmed thrombotic APS (objectively confirmed thrombosis either venous or arterial or both with persistently positive aPL).

Age ≥ 18 years.

Exclusion criteria:

Patients who are not able to provide informed written consent.

Patients with thrombosis without persistently positive aPL.

Minimum number: total 100 patients (50 single thrombosis, 50 recurrent thrombotic events) expected 100-150 patients and if the study expands into other centres, we will increase the number as appropriate depending on the number of centres.

30 healthy volunteers without aPL.

30 thrombotic non -APS

Expected timeline:

Project stage/set up: Jan 2024

Launch Feb 2024

Projected date for the extension to other centres – September 2024

Duration: 24 months

Finalization/analysis: first quartile of 2026

Reporting: April 2026

Expected outcomes (ie. publications): present at national and international meetings as abstracts; anticipated publication in the Journal of Thrombosis and Haemostasis.

Publication types: SSC Communication and original articles.

Description of project set/up and management, needed infrastructure and resources (summary):

Deepa J Arachchillage (co-chair of the SSC LAC/aPL) will lead the project, supported by the chair, and especially the colleagues with daily and practical lab experience. When the study expands to include other centres, we will have PI from other centres. Also, experienced recognized authorities in the field will be invited to contribute.

No resources are needed as a single centre study but if the study is expanded as a multicentre study, funding will be sought externally.

References:

1. 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.
2. Lisman T. Decreased Plasma Fibrinolytic Potential As a Risk for Venous and Arterial Thrombosis. Semin Thromb Hemost. 2017;43(2):178-84.
3. Espinosa G, Cervera R, Font J, Asherson RA. The lung in the antiphospholipid syndrome. Ann Rheum Dis. 2002;61(3):195-8.
4. Wolf M, Boyer-Neumann C, Parent F, Eschwege V, Jaillet H, Meyer D, et al. Thrombotic risk factors in pulmonary hypertension. Eur Respir J. 2000;15(2):395-9.
5. Krone KA, Allen KL, McCrae KR. Impaired fibrinolysis in the antiphospholipid syndrome. Curr Rheumatol Rep. 2010;12(1):53-7.
6. Cesarman-Maus G, Ríos-Luna NP, Deora AB, Huang B, Villa R, Cravioto Mdel C, et al. Autoantibodies against the fibrinolytic receptor, annexin 2, in antiphospholipid syndrome. Blood. 2006;107(11):4375-82.

7. Cugno M, Cabibbe M, Galli M, Meroni PL, Caccia S, Russo R, et al. Antibodies to tissue-type plasminogen activator (tPA) in patients with antiphospholipid syndrome: evidence of interaction between the antibodies and the catalytic domain of tPA in 2 patients. Blood. 2004;103(6):2121-6.
8. Takeuchi R, Atsumi T, Ieko M, Amasaki Y, Ichikawa K, Koike T. Suppressed intrinsic fibrinolytic activity by monoclonal anti-beta-2 glycoprotein I autoantibodies: possible mechanism for thrombosis in patients with antiphospholipid syndrome. Br J Haematol. 2002;119(3):781-8.
9. Bu C, Gao L, Xie W, Zhang J, He Y, Cai G, et al. beta2-glycoprotein i is a cofactor for tissue plasminogen activator-mediated plasminogen activation. Arthritis Rheum. 2009;60(2):559-68.
10. Jiang X, Du Y, Cheng CY, Denas G, Zhou YP, Wu T, et al. Antiphospholipid Syndrome in Chronic Thromboembolic Pulmonary Hypertension: A Well-Defined Subgroup of Patients. Thromb Haemost. 2019;119(9):1403-8.
11. Yamamoto J, Inoue N, Otsui K, Ishii H, Gorog DA. Global Thrombosis Test (GTT) can detect major determinants of haemostasis including platelet reactivity, endogenous fibrinolytic and thrombin generating potential. Thromb Res. 2014;133(5):919-26.
12. Kanji R, Gue YX, Farag MF, Spencer NH, Mutch NJ, Gorog DA. Determinants of Endogenous Fibrinolysis in Whole Blood Under High Shear in Patients With Myocardial Infarction. JACC Basic Transl Sci. 2022;7(11):1069-82.
13. Gorog DA, Lip GYH. Impaired Spontaneous/Endogenous Fibrinolytic Status as New Cardiovascular Risk Factor?: JACC Review Topic of the Week. J Am Coll Cardiol. 2019;74(10):1366-75.