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POSTER ABSTRACTS

301.VASCULATURE, ENDOTHELIUM, THROMBOSIS AND PLATELETS: BASIC AND TRANSLATIONAL

Complement Activation Is Increased in Antiphospholipid Syndrome-Related Ischemic Stroke with Higher Risk Features

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Introduction: Antiphospholipid syndrome (APS) patients with arterial thrombosis, mostly ischemic stroke, have concerningly high rates of recurrent thrombosis despite antithrombotic treatment, with over 20% thrombosis recurrence within two years. Complement activation is increased in thrombotic APS patients, although its role has not been fully investigated in APS-related stroke. This study aimed to evaluate the degree of complement activation in APS-related ischemic stroke, transient ischemic attack (TIA) or other ischemic brain injury patients and whether complement activation correlated with APS severity and platelet hyperreactivity as a potential mechanism for increased thrombotic risk.

Method: Patients with confirmed APS by Sydney Criteria and imaging-proven ischemic stroke, TIA or other ischemic brain injury were recruited, subcategorized as high-risk APS (HR-APS, n=30) and lower-risk APS (LR-APS, n=41). HR-APS included triple antiphospholipid antibody (aPL) positive, anticoagulant refractory or both arterial and venous thromboembolism patients. LR-APS included patients with a single thrombotic event who were single or double aPL positive. Ischemic stroke patients without aPL (n=40) and healthy donors (n=40) were utilized as controls. Patients with active cancer, inflammation, infection or use of hydroxychloroquine, immunosuppression and heparins were excluded. Complement activation markers were measured as Bb fragment (Bb), C3a-desArg (C3a), C5a-des-Arg (C5a), and SC5b-9 using ELISA (MicroVue kits, Quidel Corp, Pathway Diagnostics Ltd). Complement-mediated cell killing was measured by the modified Ham (mHam) test, which determines the degree of cell lysis after adding patient serum to a paroxysmal nocturnal hemoglobinuria cell line (PIGA-null TF-1 cells) lacking cell surface complement regulators. A positive result was considered 20% or greater cell killing. Flow cytometry was used to evaluate whether complement activation correlated with platelet hyperreactivity. For this, plasma from mHam positive patients was added to healthy donor whole blood and procoagulant platelet response was measured as the absolute change in proportion of platelet events that were positive for annexin V and P-selectin following stimulation with thrombin 2U/mL.

Results: There was no significant difference in median age between the patient cohorts, ranging from 49 to 56 years, although the healthy controls were younger, with median age 36 (range 25-73, $p<0.001$). Circulating complement activation markers were all significantly increased in the APS cohorts compared to healthy controls ($p<0.01$). The most prominent increase was observed in C3a levels: HR-APS median (interquartile range - IQR) 215 ng/mL (137.1-297.4), LR-APS 290.8 ng/mL (120.3-450.3) and healthy controls 62 ng/mL (27.9-121.1)($p=0.004$ and $p<0.0001$ compared to healthy controls, respectively); and Bb fragment levels: HR-APS 1.2 μg/mL (IQR 1.0-1.4), LR-APS 1.3 μg/mL (IQR 1.0-1.5) and healthy controls 0.8 μg/mL (IQR 0.7-0.9)(both $p<0.0001$ compared to healthy controls). However, only C3a, SC5b-9 and Bb fragment levels in LR-APS remained significantly elevated when compared to stroke controls ($p=0.0009$, $p=0.01$ and $p=0.03$, respectively). There were no differences between HR-APS and LR-APS, nor between stroke controls and healthy controls. Complement-mediated cell killing was also significantly higher in APS patients overall, median (IQR) proportion of non-viable cells 18% (IQR 12-33), compared to stroke controls: 10% (IQR 8-15, $p<0.0001$) and healthy controls: 12.5% (IQR 6.3-17, $p=0.0009$). Notably, HR-APS had significantly higher proportion of positive mHam tests (60%), compared to LR-APS: 36.6%, stroke controls: 12.5%, and healthy controls: 17.5% ($p<0.0001$, Figure 1A). Importantly, HR-APS patients with positive mHam results (n=5) induced a substantially higher

increase in procoagulant platelets, median 17.2% (IQR 11.2-28.6), whereas healthy controls (n=4) only resulted in median change of -0.7% (IQR -7.0-1.4, p=0.02) (Figure 1B).
Conclusion: Preliminary data suggest that APS-related ischemic stroke, TIA or ischemic brain injury are associated with increased, functionally important, complement activation, more prominent in higher risk disease. Complement-mediated platelet hyperreactivity may have a contributory role that remains to be established.

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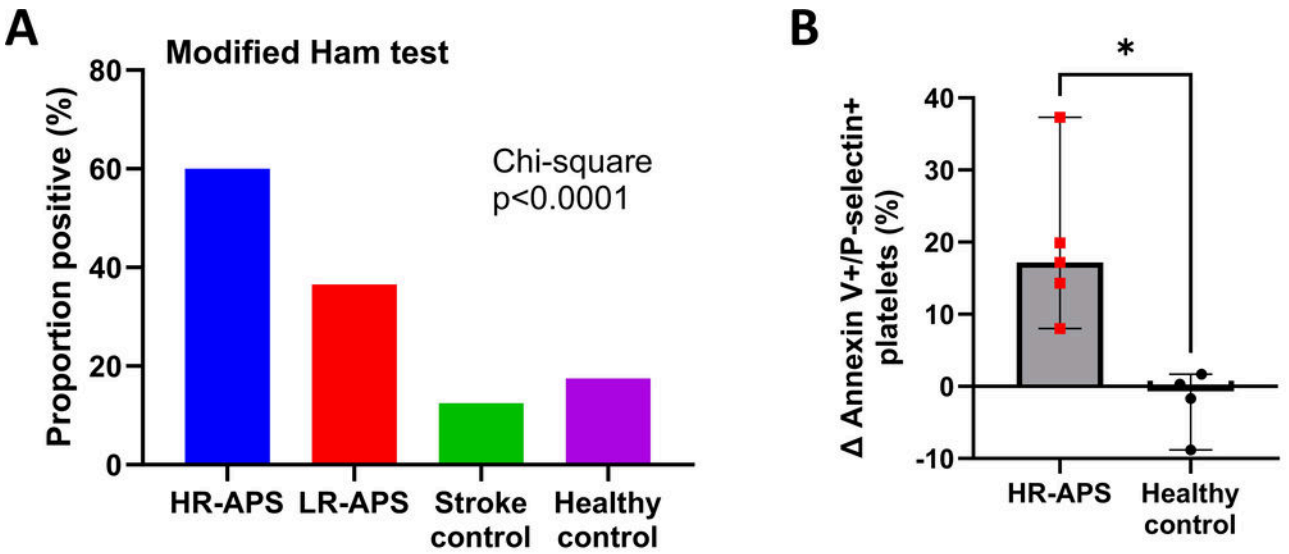

Figure 1. Complement activation is increased in high-risk antiphospholipid syndrome (APS)-related stroke. (A) Serum from high-risk APS (HR-APS, n=30), lower-risk APS (LR-APS, n=41), stroke controls (n=40) and healthy controls (n=40) was incubated with PIGA-null TF-1 cells susceptible to complement-mediated cell death. Non-viable cells were determined by lack of release of WST-1 dye reagent, quantified by a plate reader relative to heat-inactivated control. (B) Flow cytometry was used to measure procoagulant platelets (defined by annexin V^+/P-selectin$^+$) response after addition of HR-APS (n=5) or healthy control (n=4) plasma and thrombin 2U/mL to healthy donor whole blood, with absolute change in proportion of dual positive platelets in comparison to adding autologous plasma displayed (bar: median, error bars: 95% confidence interval). *p<0.05

Figure 1

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