Immunothrombosis and the Role of Neutrophil Extracellular Traps (NETs) in the Pathogenesis of Antiphospholipid Syndrome

Thesis

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List of Abbreviations

aCL	: Anti-cardiolipin
Ann	: Annexin
β2GPI	: β2glycoprotein i
APC	: Activated protein c
aPE	: Anti-phosphatidyl ethanolamine
aPLs	: Anti-phospholipid antibodies
ApoER2	: Apoe receptor 2
APS	: Anti-phospholipid syndrome
aPS	: Anti-phosphatidylserine
APS ACTION	: The anti-phospholipid syndrome alliance for clinical trials and international networking
aPS/PT	: Anti-phosphatidylserine prothrombin
aPTT	: Activated partial thromboplastin time
C1-INH	: C1 esterase inhibitor
CAD	: Coronary artery disease
CCP	: Complement control protein
cfDNA	: Cell free dna
Cys	: Cysteine
DAMP	: Damage associated molecular pattern
DIC	: Disseminated intravascular coagulation
dRVVT	: Diluted russel viper venom time
DV	: Domain v
DVT	: Deep vein thrombosis
ECs	: Endothelial cells
GPIIb/IIIa	: Glycoprotein iib/iiia
H	: Histone
HK	: High molecular weight kininogen
HMGB1	: High mobility group box 1 protein
HNPs	: Human neutrophil peptide
ICA	: Index of circulating anticoagulant ()
ICAM	: Intracellular adhesion molecule
IFN	: Interferon
Ig	: Immunoglobulin
IL	: Interlukin
INR	: International normalized ratio
IP	: Induced protein
ISTH	: International society on thrombosis and haemostasis
KCT	: Kaolin clotting time
LA	: Lupus anticoagulant

LMWH	: Low molecular weight heparin
Lpa	: Lipoprotein a
MI	: Myocardial infarction
MPO	: Myeloperoxidase
NE	: Neutrophil elastase
NETs	: Neutrophil extracellular traps
NF-κB	: Nuclear factor-kappa beta
PAD4	: Peptidylarginine deiminase 4
PAMP	: Pathogen associated molecular patterns
PC	: Protein c
pDCs	: Plasmacytoid dendritic cells
PDI	: Protein disulfide isomerase
PDI	: Protein disulphide isomerase
PF4	: Platelet factor 4
PNP	: Pooled normal plasma
PS	: Phosphatidylserine
ROS	: Reactive oxygen species
SCT	: Silica test
SLE	: Systemic lupus erythematosus
ST	: Stent thrombosis
TF	: Tissue factor
TFPI	: Tissue factor pathway inhibitor
TLRs	: Tol-like receptors
TNF-α	: Tumor necrosis factor α
tPA	: Tissue plasminogen activator
tPA	: Tissue plasminogen activator
TXB2	: Thromboxane β2
VEGF	: Vascular endothelial growth factor
VKA	: Vitamin k antagonists
VTE	: Venous thromboembolism
VWF	: Von willebrand factor

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ABSTRACT

Background: Netosis is a complex process resulting in Neutrophil extracellular traps (NETs) release. It differs according to the stimulus and results in dramatic changes in the morphology of the cells. The exact stimulus that drives the cell in the direction of Netosis is still not clear. NETs have a procoagulant as well as an antifibriolytic effect, if unregulated will result in thrombotic disorders as VTE and DIC.A link between NETs and the process of thrombosis have been suggested and role of antiphospholipid antibodies (especially B2GPI) in activating the process of Netosis in APS patients have been proposed.

Purpose: to demonstrate the presence of NETs and Histones in APS patients and their relation to different laboratory and clinical criteria mentioned in Sydney classification criteria.

Patients and Methods: 41 patients diagnosed as primary APS according to the Sydney classification criteria (2004) were recruited from our outpatients clinics.40 normal age and sex matched controls were also recruited. Levels of NETs and Histones in sera of those subjects were measured and compared in patients and controls. Also NETs and Histones levels were compared against different laboratory criteria (Lupus anticoagulant, diluted Viper Venom Test, B2GPI and ACL) and clinical picture. Patients were divided into different groups according to positivity of different laboratory tests, levels of NETs and Histones were compared in those different groups.

Results: Both NETs and Histones levels were significantly higher in APS patients than in controls. Levels of NETs and Histones were significantly higher in triple positive patients compared to double and single positive patients.

Conclusion: NETs and histones were found to be correlated to APS suggesting a close relation to its pathogenesis as well as being related to laboratory positivity of those patients.

Keywords: *NETs – Histones- Antiphospholipid syndrome*

INTRODUCTION

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Immunothrombosis is a term coined by ***Engelmann and Massberg***; which describes an immune reaction induced by the formation of microthrombi; it is evident in both physiological and pathological settings in vivo ***(Engelmann and Massberg, 2013).***

Immunothrombosis mediates the recognition of pathogens and damaged cells, and inhibits pathogen dissemination and its survival. Immunothrombosis can be viewed as a novel element of intravascular immunity, which is a part of the immune system that helps the host detect and eliminate pathogens in the vasculature **(*Hickey and Kubes, 2009).***

Both arterial and venous thromboses involve hemostatic mechanisms that depend on active participation of cells of the innate immunity (mainly monocytes and neutrophils). These cells cause platelet activation, fibrin formation and propagation during the development of thrombosis ***(Engelmann and Massberg, 2013).***

Neutrophils are one of the major components of immunothrombosis through neutrophil extracellular traps

(NETs). NETs act as catalytic surfaces that promote and compartmentalize the coagulation system ***(Massberg et al., 2010).***

NETs were first described as a part of innate immunity that binds microorganisms, prevents them from spreading, and ensures a high local concentration of antimicrobial agents to degrade virulence factors and kill bacteria ***(Brinkmann et al., 2004).***

NETs contains proteins (elastase, cathepsin G, and myeloperoxidase) from azurophilic granules and proteins from specific granules and tertiary granules (lactoferrin and gelatinase) respectively. DNA and histones are also a major structural component of NETs ***(Brinkmann et al., 2004).***

NETs provide matrix for fibrin deposition, platelet entrapment and subsequent activation ***(Kambas et al., 2012).***

The components of NETs provide procoagulant activities: DNA initiates the intrinsic pathway via the activation of factor XII, histones are strong generators of thrombin ***(Von Bruhl et al., 2012; Iba et al., 2014)*** and granule proteins contribute to suppression of the anticoagulation system via degradation of tissue factor

pathway inhibitor (TFPI) and probably thrombomodulin ***(Iba et al., 2014).***

The antiphospholipid syndrome (APS) is an acquired autoimmune condition characterized by both arterial and venous thrombosis, gestational morbidity and presence of continuously elevated serum titers of antiphospholipid antibodies (aPL) ***(Saleem, 2014).***

Despite an abundance of clinical and basic research, pathophysiology of APS remains an enigma to this day ***(Chaturvedi and McCrae, 2015).***

Proposed pathogenic mechanisms include increased oxidative stress, impaired functions of nitrous oxide systems (NOS), activation of receptors by beta 2 glycoprotein -1 antibody (B2GP-1 Ab), increased expression and activation of tissue factor (TF), increased free thiol form of factor II, disruption of the annexin A5 shield, antibody mediated activation of complement C3 and C5 and increased expression of Toll like Receptor (TLR) 7 and TLR 8 **(*Giannakopoulos and Kirilis, 2014).***

Clinical presentation of APS is more polymorphic than it was thought ***(Öztürk et al., 2004)*** making its diagnostic criteria an ongoing process that is modified as