**230. NETs and terminal pathway factors as potential biomarkers for complement overactivation assessment in ANCA-associated vasculitis**

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**Background:** Clinical, *in vitro*, and animal model-derived evidence has demonstrated a critical involvement of the alternative complement pathway (aCP) in the pathogenesis of ANCA-associated vasculitis (AAV). In this regard, neutrophil extracellular traps (NETs) have been suggested to be a key element between ANCA-induced neutrophil activation and aCP. However, the role of the terminal complement pathway (tCP) is less well studied.

**Methods:** A prospective, observational, multicenter study analyzing first episodes and relapses of patients with AAV, with a minimum follow-up of 6 months, was performed. Blood samples were collected at diagnosis (AAV-t1) and at remission (AAV-t2). Control population consisted of age and sex-matched individuals. Complement activation was assessed by analyzing the complement membrane attack complex (C5b-9) deposition on cultured endothelial cells (HMEC-1), by immunofluorescence, after exposing them to activated plasma (a-plasma: obtained by mixing patient’s citrated plasma with healthy subjects’ sera pool, 1:1). C5b-9 deposits induced by patients’ a-plasma were calculated as percentage of labeled area with respect to the total area analyzed. Results from patient and control samples were expressed as fold increase (mean±SEM) vs. those obtained with the pool of a-plasma from healthy subjects. Plasma levels of tCP and aCP soluble factors, such as sC5b-9 and sFBb (respectively), were also measured (mean±SEM). Circulating NETs were indirectly measured by quantifying circulant dsDNA plasmatic concentration (mean±SEM) as a NET surrogate.

**Results:** The present results were obtained with samples from 13 AAV-MPO patients who achieved complete remission (38% men, age 63±14 years) and 10 controls (45% men, age 66±6 years). At AAV-t1, there was a statistically significant increase (p<0.05) of C5b-9 deposition on HMEC-1 in response to patients’ a-plasma (fold increase of 5.3±1.3) compared to control samples (fold increase of 1.2±0.2). Samples obtained at AAV-t2 induced less C5b-9 deposition than at AAV-t1 (fold increase of 0.9±0.2; p<0.05), with values similar to controls. Regarding soluble factors, levels of both sC5b-9 and sFBb were significantly increased in AAV-t1 (1882±418 ng/mL and 3.2±0.4 µg/mL, respectively; p<0.05) vs. AAV-t2 (852±104 ng/mL and 1.9±0.2 µg/mL, respectively; p<0.05). At AAV-t2, levels were similar to controls (708±42 ng/mL for sC5b-9, and 2.4±0.2 µg/mL for sFBb). Circulating NETs were also increased in AAV-t1 (22.2±3.5 µg/mL) compared to both AAV-t2 and controls (13.6±1.1 µg/mL and 13.7±0.3 µg/mL, respectively; p<0.05). Moreover, NETs in AAV-t1 presented a significant correlation with sFBb levels, both in AAV-t1 (r=0,709; p<0.05) and in AAV-t2 (r=0,585; p<0.05).

**Conclusions:** There is a relationship between NETs, AAV activity and sFBb, which supports the role of NETs and cAP in AAV pathogenesis. Moreover, differences in C5b-9 deposition between the two stages of the disease suggest that tCP may be dysregulated in AAV. Further characterization of this dysregulation may lead to new diagnostic or disease activity biomarkers, as well as new therapeutic options for the management of patients with AAV.

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