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ABSTRACT N. PO06

EXTRACELLULAR VESICLES PROFILES IN PATIENTS WITH PORTO-SINUSOIDAL VASCULAR DISEASE.

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Background and aims: Porto-sinusoidal vascular disorder (PSVD) has recently been proposed to delineate a group of hepatic vascular diseases characterized by lesions involving the portal venules and sinusoids, irrespective of the presence/absence of portal hypertension. Although data is still limited, several hypotheses and emerging evidence suggest that extracellular vesicles (EVs) might exert a functional role in the pathogenesis of PSVD. The analysis of EVs in PSVD may unveil disease-specific alterations in intercellular communication, contributing to understand the prothrombotic mechanisms and to discover novel biomarkers for diagnosis and prognosis. The study aims: i) to compare plasma-derived EVs in PSVD patients *vs.* healthy controls by flow cytometry; ii) to evaluate their potential involvement in disease progression.

Methods: Twenty-nine PSVD patients (median age 58 yrs; 20 males, 9 females) were included and compared with 9 (4 males and 5 females) aged matched (± 3 yrs) healthy controls. Large extracellular vesicles (L-EVs) were isolated from platelet-poor plasma by centrifugation at 14,000 g for 30 min at 4 °C, immunolabeled with calcein-AM, annexin V, CD41, CD62P, CD45, CD14, CD62E, anti-human-tissue factor (TF), CD105 and CD147. For EV size calibration, fluorescent polystyrene beads Gigamix were used to set a gate between 0.2 and 0.9 µm bead populations, defined as L-EVs gate. EVs were expressed as events/µl (absolute count) with the volume measurement of the CytoFLEX S.

Results: All enrolled patients had histologically confirmed PSVD. Among them, 12 had unprovoked PSVD, 4 were drug-related, 3 were associated with gastrointestinal diseases, 3 with immune-mediated conditions, and 7 cases were linked to myeloproliferative disorders. Endothelium-derived L-EVs co-expressing calcein-AM, annexin V, CD62E, and TF were significantly increased in PSVD patients compared to healthy controls ($p<0.0001$), reflecting endothelial activation and prothrombotic predisposition (Table1). Patients also exhibited a marked increase in platelet-derived L-EVs, particularly calcein-AM$^+$/annexin V$^+$/CD41$^+$/CD62P$^+$ L-EVs ($p=0.004$), indicating enhanced platelet activation and a contribution to vascular and coagulative responses (Table1). Notably, endothelial and platelet L-EV significantly correlated with PSVD etiology ($r=0.38$, $p=0.04$), with patients affected by myeloproliferative disease–related PSVD showing significantly higher levels than those with other etiologies ($p=0.046$ and 0.032, respectively). Angiogenesis-related calcein-AM$^+$/annexin V$^+$/CD105$^+$/CD147$^+$ L-EVs were significantly elevated in patients compared to controls ($p<0.0001$), suggesting active vascular remodeling. Regarding the inflammatory panel, patients showed significantly lower levels of calcein-AM$^+$/annexin V$^+$/CD45$^+$/CD14$^+$ L-EVs compared to controls ($p=0.0002$), indicating reduced monocyte/leukocyte-driven inflammatory activation (Table1).

Conclusions: In conclusion, patients with PSVD display a L-EV profile characterized by vascular remodeling, endothelial and platelet activation. On the other hand, patients exhibited significantly lower levels of inflammatory L-EVs, suggesting limited systemic immune activation. Our preliminary findings support the hypothesis that PSVD is primarily driven by endothelial dysfunction and platelet activation, rather than by leukocyte-mediated inflammation. The etiology of PSVD appears to influence L-EV levels.

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	PSVD Patients (n. 29)	Healthy Controls (n. 9)	P value
Angiogenic Panel			
Calcein-AM+CD105+	4006 [1360]	1307 [667]	**<0.0001**
Calcein-AM+CD147+	2335 [634]	895 [419]	**<0.0001**
AnnexinV+CD105+	997 [301]	618 [133]	**0.002**
AnnexinV+CD147+	991 [302]	340 [92]	**<0.0001**
AnnexinV+CD105+CD147+	12 [9]	16 [8]	0.255
Calcein-AM+AnnexinV+CD105+CD147+	206 [100]	58 [24]	**<0.0001**
Inflammation Panel			
Calcein-AM+CD14+	722 [322]	1300 [756]	**0.003**
Calcein-AM+CD45+	681 [306]	1027 [669]	**0.026**
Annexin+CD14+	330 [119]	736 [149]	**0.0002**
Annexin+CD45+	387 [134]	729 [177]	**0.0008**
Annexin+CD45+CD14+	76 [31]	173 [37]	**<0.0001**
Calcein-AM+AnnexinV+CD45+CD14+	167 [65]	413 [78]	**0.0002**
Endothelial Panel			
Calcein-AM+CD62E+	517 [276]	560 [236]	0.731
Calcein-AM+TF+	234 [89]	43 [22]	**<0.0001**
Annexin+CD62E+	1023 [327]	58 [22]	**<0.0001**
AnnexinV+TF+	691 [242]	871 [332]	0.086
Annexin+CD62E+TF+	112 [42]	75 [42]	0.145
Calcein-AM+AnnexinV+CD62E+TF+	67 [29]	14 [11]	**<0.0001**
Platelet Panel			
Calcein-AM+CD41+	2026 [633]	965 [503]	**<0.0001**
Calcein-AM+CD62P+	579 [312]	436 [250]	0.084
AnnexinV+CD41+	1720 [690]	934 [467]	**<0.0001**
Annexin+CD62P+	463 [271]	339 [179]	0.058
AnnexinV+CD41+CD62P+	77 [44]	27 [9]	**<0.0001**
Calcein-AM+AnnexinV+CD41+CD62P+	1954 [583]	1388 [607]	**0.004**

L-EV profiles in cases and controls. Data (events/µL (V)) are expressed as Median [Dev.ST].