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Flow cytometry as a tool for extracellular vesicles phenotyping in immune diseases

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The aim of this study was to analyze extracellular vesicles (EVs) of blood plasma using high-sensitivity flow cytometry in patients with COVID-19 (15 patients), axial spondylitis (41), and after heart transplantation (23 with and 20 without acute rejection), to evaluate the diagnostic significance of the method. In patients with acute COVID-19, there was a significant increase in the levels of EVs expressing CD63+ (288 (220; 393) events/μL) and CD294+ (154 (113; 199) events/μL) compared to healthy donors (HD) (60 (55; 81) and 54 (37; 75) events/μL, respectively, $p < 0.01$), and a decrease in EVs expressing HLA-DR+ (74 (140; 328)) and CD45+ (96 (78; 111)) compared to HD (662 (444; 1084) and 560 (204; 1476) events/μL, respectively, $p < 0.01$). For axial spondylitis, there was a significant increase in the levels of CD3+ and CD19+ EVs compared to HD (282 events/μL (197; 1210) vs. 39 events/μL (31.75; 69.25) and 177 events/μL (55; 420) vs. 13 events/μL (4.750; 26.50), respectively, $p < 0.0001$). Additionally, there was an increase in EVs expressing HLA-A, B, C and HLA-DR in patients compared to the HD (4088 events/μL (1937; 11810) vs. 2340 events/μL (1820; 3708), $p = 0.0301$ and 4799 events/μL (1763; 20420) vs. 827 events/μL (265.3; 2721), $p = 0.002$, respectively). Patients with acute rejection after heart transplantation revealed a significant decrease ($p = 0.009$) in CD90+ EVs 3.4 (0.5; 181.1) vs. 2017.5 (8.0; 14225) and in CD81+ EVs 100 (40; 170) vs. 240 (75; 660) events/mL. These findings suggest that the analysis of extracellular vesicles using flow cytometry can provide valuable insights into different pathological conditions, potentially serving as diagnostic markers for immune-related diseases.

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