The Impact of the Eculizumab on the Thrombogenicity Induced by Extracellular Vesicles in Paroxysmal Nocturnal Hemoglobinuria Patients

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TABLE 1 Number of extracellular vesicles (EV), levels of D-dimer and CEA in venous blood of colorectal cancer patients before and during chemotherapy

	Before 1st ChTx (baseline)	Before 2nd ChTx	p-value 1st vs 2nd	Before 3rd ChTx	p-value 1st vs 3rd
n	41	40		35	
EV (x 10^3 mL^{-1})	429 (288; 664)	376 (225; 584)	0.13	255 (181; 474)	0.007
PLT+EV (x 10^3 mL^{-1})	216 (129; 287)	171 (104;248)	0.056	119 (74; 210)	0.001
% of EV	50	45		47	
TF+EV (x 10^3 mL^{-1})	19 (10; 38)	20 (9; 32)	0.58	15 (9; 29)	0.101
% of EV	6	7		6	
D-Dimer (µg mL^{-1})	0.99 (0.48; 2.52)	0.69 (0.50; 3.06)	0.95	0.99 (0.52; 2.17)	0.95
CEA (µg L^{-1})	9.4 (1.9; 42.8)	9.7 (2.6; 70.6)	0.086	9.4 (3.0; 54.9)	0.39

Methods: Advanced CRC patients receiving 5-fluorouracil based ChTx were eligible. The number of EV was assessed by flow cytometry in fresh platelet poor plasma obtained from venous blood collected immediately before ChTx. EV were defined by size (forward scatter, < 1 µm) and annexin V binding and labeled using antibodies (anti-CD41a: platelet positive EV [PLT+EV]; anti-CD142: TF positive EV [TF+EV]). D-Dimer was assessed by ELISA. The paired *t*-test was used to compare baseline (BL) levels with levels obtained before ChTx. Data are given in absolute numbers (median [quartiles]) if not otherwise stated.
Results: 41 patients (mean age 64 years, 68% men) were included and 35 patients completed 3 cycles of ChTx. Table 1 shows the number of EV, the levels of D-dimer and CEA at BL and before 2nd and 3rd ChTx, respectively. EV significantly decreased from 429 (288; 664)x10^3 mL^{-1} at BL to 255 (181; 474)x10^3 mL^{-1} before the 3rd cycle. PLT+EV significantly decreased from 216 (129; 287)x10^3 mL^{-1} at BL to 119 (74; 210) x10^3 mL^{-1} before the 3rd cycle. The proportion of PLT+EV ranged from 45%-50% throughout ChTx. Number and proportion of TF+EV were small at all time points. D-dimer levels were 0.99 (0.48; 2.52) µg mL^{-1} at BL and did not decrease over the course of ChTx. D-dimer levels did not correlate with the number of EV.
Conclusions: In patients with advanced CRC, ChTx attenuates coagulation activation as indicated by a decline of the number of EV.

PB 676 | The Impact of the Eculizumab on the Thrombogenicity Induced by Extracellular Vesicles in Paroxysmal Nocturnal Hemoglobinuria Patients

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is characterized by a complement-mediated hemolysis. Complement attack induces extracellular vesicles (EV) production. These EV can be implied in thrombus formation, the leading cause of death in PNH patients. Eculizumab, an anti-C5 monoclonal antibody, decreases the thrombosis frequency in PNH.
Aims: We wanted to assess the impact of eculizumab on the EV quantification and on their procoagulant activity. The purpose is to check, if the antithrombotic activity of the eculizumab could be, in part, explained by its interaction with the EV.
Methods: We recruited 6 PNH patients. Informed consents were obtained for each patient. The study was led according to the declaration of Helsinki and approved by the local Ethic Committee. We collected platelet free plasma (PFP) for each patient before the start of eculizumab, after 4 weeks and after 11 weeks of treatment. We assessed the amount of platelets EV (flow cytometry), the procoagulant activity on PFP (Thrombin generation-TGA), provided by phospholipids (STA®-Procoag-PPL) and by isolated EV (TGA). We used mixed-effects linear regression (R 3.1.2 with nlme package) with logarithmic transformation for flow cytometry results.
Results: We compared the results obtained after 4 weeks or after 11 weeks of treatment compared to the value before the start of the treatment. The results on the patient's PFP show a decrease in the amount of platelet EV with the treatment. About the procoagulant activity of the EV, we observed a decrease in the procoagulant profile (PL and EV-induced) with the eculizumab.
Conclusions: Eculizumab can play its anti-thrombotic role by an action on the EV. With a bigger cohort, we could estimate a threshold value of the procoagulant activity induced by the EV. The purpose will be to measure the EV activity by STA®-Procoag PPL or TGA on isolated EV in order to assess the thrombotic risk of PNH patients treated with eculizumab.

PB 677 | Magnetic Capture of Extracellular Vesicles: A Quantitative Flow Cytometry Study

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Background: Cells release small membrane vesicles, called microparticles, exosomes or extracellular vesicles (EV), which attract interest for their diverse properties and potential applications. However, EV found in body fluids are heterogeneous, and there is yet no reliable method allowing the isolation of selected EV populations.