**Platelet activation and neutrophil extracellular trap (NET) formation in immune thrombocytopenia: is there an association?**

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*Supplementary material*

**Supplementary Table 1.** Patients and controls clinical characteristics, and patient therapies.

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**Supplementary Figure 1.** Flow cytometric representative histograms of platelet size (FSC), CD62, CD63 binding, and neutrophil CD11b expression of two ITP patients and one parallel control.

**Supplementary Figure 2.** Correlations between platelet count, size, activation, and neutrophil degranulation in ITP patients.

**Supplementary Table 1. Patients and controls clinical characteristics, and patient therapies.**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Patients (n=63)** | **Controls (n=30)** | *p* |
| Age (median; range) | 49; 16-89 | 46; 20-78 | p=0.585 |
| Women, n (%) | 37 (58.7%) | 12 (40%) | p=0.121 |
| Chronic ITP, n (%) | 50 (79.4%) | Ø  |  |
| Persistent ITP,n (%) | 6 (9.5%) | Ø  |  |
| Newly diagnosed ITP, n (%) | 7 (11.1%) | Ø  |  |
| **Cardiovascular riskfactors** |
| Smokers | 4 (6.3%) | 1 (3.3%) | 1.000 |
| Hypertension | 20 (31.7%) | 3 (10.0%)  | **0.045** |
| Hypercholesterolemia | 20 (31.7%) | 5 (16.7%) | 0.219 |
| Diabetes | 8 (12.7%) | Ø  | 0.056 |
| **Previous arterial ischemic events** |
| Myocardial infarction | 6 (9.5%) | Ø  |  |
| Central retinal artery occlusion | 1 (1.6%) | Ø |  |
| **Previoustherapies** |
| Glucocorticoids, n (%) | 62 (98.4%) | Ø  |  |
| IVIG, n (%) | 12 (19.0%) | Ø  |  |
| Splenectomy, n (%) | 16 (25.4%) | Ø  |  |
| Romiplostin, n (%) | 28 (44.4%) | Ø  |  |
| Eltrombopag, n (%) | 17 (27.0%) | Ø  |  |
| Immunosupresion, n (%)Rituximab, n (%) | 6 (9.5%)6 (9.5%) | Ø Ø  |  |
| **Active treatments** |
| Glucocorticoids, n (%)  | 11 (17.5%) | Ø  |  |
| TPO-RAs, n (%) | 27 (42.9%)* 14 Romiplostin
* 13 Eltrombopag
 | Ø  |  |
| Immunosupresion, n (%) | 1 (MMF) (1.6%) | Ø  |  |
| No therapies, n (%) | 24 (38.1%) | Ø  |  |

Results are given as median; range, or as percentages. Abbreviations: IVIG, intravenous immunoglobulins; MMF, mycophenolatemofetil; TPO-RAs, thrombopoietin receptor agonist

**Supplementary Table 2.Association between clinical parameters in patients and plasma nucleic acid levels.**

|  |  |  |
| --- | --- | --- |
|  | **cfDNA (g/ml)** | **CitH3-DNA (OD)** |
| Age* ≤45 years
* >45 years
* p
 | 0.146; 0.067-0.6790.168; 0.041-0.588**0.041** | 0.163; 0.062-0.3910.150; 0.092-0.4440.696 |
| Sex * Female
* Male
* p
 | 0.146; 0.041-0.2380.174; 0.072-0.679**0.029** | 0.160; 0.062-0.3910.153; 0.103-0.4440.973 |
| Hypertension* Yes
* No
* p
 | 0.168; 0.041-0.5880.155; 0.080-0.6790.116 | 0.145; 0.092-0.3540.162; 0.062-0.4440.307 |
| Smoking* Yes
* No
* p
 | 0.170; 0.072-0.5880.155; 0.041-0.6790.067 | 0.145; 0.092-0.3660.166; 0.062-0.444**0.026** |
| Hypercholesterolemia* Yes
* No
* p
 | 0.170; 0.072-0.5880.155; 0.041-0.6790.106 | 0.145; 0.092-0.3660.166; 0.062-0.4440.380 |
| Diabetes* Yes
* No
* p
 | 0.194; 0.072-0.5880.155; 0.041-0.6790.133 | 0.132; 0.103-0.3540.161; 0.062-0.4440.224 |
| Splenectomy* Yes
* No
* p
 | 0.167; 0.107-0.2160.155; 0.041-0.6790.695 | 0.151; 0.083-0.2250.158; 0.062-0.4440.712 |
| CurrentTPO-RA therapy* Yes
* No
* p
 | 0.155; 0.041-0.5880.157; 0.067-0.6790.833 | 0.154; 0.062-0.4440.161; 0.083-0.3910.678 |
| Currenttherapy* Yes
* No
* p
 | 0.155; 0.041-0.5880.163; 0.067-0.6790.881 | 0.158; 0.062-0.4440.150; 0.083-0.3660.761 |
| Phase of disease* Chronic
* Non chronic
* p
 | 0.159; 0.041-0.6790.147; 0.080-0.2550.624 | 0.156; 0.062-0.4440.160; 0.092-0.2300.817 |

Results are given as median; range. Abbreviations: cfDNA, cell free DNA; OD, optical density; TPO-RA, thrombopoietin receptor agonists

**Supplementary Table 3. Patients characteristics and percentage of P-selectin positive platelets according to cfDNA concentration (by ≤ or > 75th percentile)**

|  |  |  |  |
| --- | --- | --- | --- |
|  | > 75th Percentile (n=14) | ≤ 75th Percentile (n=49) | *p* |
| Age > 50 years, n (%) | 10 (71.4%) | 20 (40.8%) | **0.043** |
| Hypertension, n (%) | 7 (50.0%) | 13 (26.5%) | 0.096 |
| Hypercholesterolemia, n (%) | 8 (57.1%) | 12 (24.5%) | **0.031** |
| Diabetes, n (%) | 4 (28.6%) | 4 (8.2%) | **0.043** |
| Previous ischemic event, n (%) | 4 (28.6%) | 3 (6.1%) | **0.020** |
| CD62+ (% platelets), median; range | 22.4; 6.6-54.7 | 15.1; 5.2-57.9 | 0.086 |

Results are given as median; range, or as percentages



**Supplementary Figure 1.** **Flow cytometric representative histograms of platelet size (FSC), CD62, CD63 binding, and neutrophil CD11b expression of two ITP patients and one parallel control.** Below each dot plot mean fluorescence intensity (MFI) values are presented. The green panels illustrate the flow cytometry plots from a 34 yr old female patient under Romiplostim with thrombocytopenia (platelet count 31x109/l; cfDNA 0.133 g/ml, H3Cit-DNA OD 0.062). The blue panels show the flow cytometry plots from a 57 yr old male patient under Romiplostim without thrombocytopenia (platelet count 298x109/l; cfDNA 0.168 g/ml, H3Cit-DNA OD 0.290). The red panels illustrate the flow cytometry plots from a 35 yr old female healthy control (platelet count 217x109/l; cfDNA 0.067g/ml, H3Cit-DNA OD 0.113).

FSC-H



**Supplementary Figure 2. Correlations between platelet count, size, activation, and neutrophil degranulation in ITP patients.** (A) Correlation between platelet counts and size; (B) correlation between platelet count and CD62 antigen density; (C) correlation between platelet count and CD63 expression; (D) correlation between platelet count and percentage of platelets expressing P-selectin; (E) correlation between platelet alpha granule (CD62) and dense/lysosomal granule (CD63) release; (F) correlation between platelet (CD62) and neutrophil (CD11b) activation. rs values represent Spearman's rank correlation coefficients.