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508.BONE MARROW FAILURE: ACQUIRED

Neutrophil Functionalities in Patients with Paroxysmal Nocturnal Haemoglobinuria Are Not Affected By Complement C5- Inhibition

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Paroxysmal nocturnal haemoglobinuria (PNH) is a rare, acquired haematological stem cell disorder characterized by haemolysis, thrombophilia, and cytopenia. PNH is caused by a somatic mutation in the phosphatidylinositol glycan class A (PIG-A) gene at the level of pluripotent, hematopoietic stem cells leading to a lack of GPI anchored cell surface proteins, such as complement-inactivating proteins, (e.g., CD55, CD59) on stem cells, polymorphonuclear neutrophil granulocytes (PMN), erythrocytes, and platelets. Subsequently, uncontrolled complement overactivity leads to the systemic complications. Phenotypic mosaicism is a distinct characteristic of PNH. For diagnostic purposes, the absence of the GPI-anchor on granulocytes and monocytes in the flow cytometry is considered the gold standard. However, the exact consequences of GPI deficiency in PMNs and monocytes have not been studied sufficiently yet. Since the implementation of complement inhibitors, the primary cause of morbidity and mortality in PNH has been significantly reduced. Despite this, the tendency towards thrombosis, as well as the consequences of the deficiency of GPI-linked proteins on PMNs and monocytes, remain largely unclear.
Therefore, the main objective of this project is to gain a deeper understanding of the impact of PNH-PMNs and monocytes on thrombo-inflammation in PNH. Specifically, the impact of complement inhibitors on these factors has been compared.
Blood from PNH patients (n=14) with a median clone size of 74 % were compared with an age- and sex-matched control group (n =20). Various cellular markers indicating a state of activation (CD11b, TREM1, CD64) or showing platelet adherence (CD42), were measured using flow cytometry. Unmutated PMNs and monocytes from PNH patients (PMN) served as intrinsic control, GPI positive PMNs (WT-PMN (PNH)) and monocytes from a control group served as extrinsic control. We compared unstimulated PNH and WT cells for their basal phenotype, and then functional changes upon stimulation (phorbol 12-myristate 13-acetate (PMA)).
Our data present a contrary perspective to our initial assumptions, indicating that PNH-PMNs have a direct impact on unmutated PMNs, resulting in the generation of a thrombogenic state and increased levels of activation. The unmutated GPI-positive PMNs and monocytes exhibit a significantly stronger direct aggregation with platelets compared to GPI-deficient clones.
It is hypothesized that the PNH clones influence the activity and aggregation propensity of the remaining unmutated granulocytes through additional cellular mechanisms.
In a subgroup analysis, the impact of complement factor 5 inhibitors (C5i) on the aforementioned factors was investigated detecting no significant differences in terms of PMN activation or platelet aggregation.
Thus, it can be hypothesized that thrombosis and hyper-inflammation are not solely driven by the PNH clones directly and modulated C5 inhibition. Additional cellular mechanisms and signaling pathways appear to play an important role, and these aspects have not been adequately addressed by the current therapy.

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