„The role of the granulocyte colony-stimulating factor receptor (CSF3R) polymorphism and expression in peripheral blood progenitor cells transplantation"

Haematopoietic stem cells (HSC) transplantation is a well-established method of tumour and non-tumour disease treatment. Currently, transplantations of haematopoietic cells mobilised from bone marrow into peripheral circulation are the most common. The above mentioned process of clinical mobilization is initiated by granulocyte colony-stimulating factor (G-CSF) application. It resembles the process of natural progenitor cells release into peripheral blood in response to inflammation and/or infection. However, molecular mechanisms directing this phenomena remain unclear.

Current study aimed to investigate in which way, if any, G-CSF receptor (CSF3R) gene polymorphism, mRNA expression of chosen G-CSF pathway genes (*CSF3R*, signal transducers and activators of transcription *STAT1, -3, -5* and suppressor of cytokine signalling *SOCS3*) and CSF3R protein expression on neutrophils and monocytes affects mobilization efficacy and haematological recovery in recipients of autologous haematopoietic progenitor cells transplantation.

For this purpose, peripheral blood was collected once from 279 healthy volunteers, serving as a control group, and from 105 patients at two time points (before and on the 5^{th} day of G-CSF treatment). All patients were characterised with haematological malignancies (multiple myeloma, non-Hodgkin's lymphoma, Hodgkin's lymphoma or acute myeloid leukaemia).

Mobilization yield was found to be greater in multiple myeloma (MM) and non-Hodgkin's lymphoma (NHL) patients when compared with Hodgkin's lymphoma (HL) group ($p=0.022$ for MM vs. HL and $p=0.013$ for NHL vs. HL). However, taking into account negative effect of previous intensive chemo- and/or radiotherapy on mobilization efficacy, the effect of diagnosis on HSC release reported herein remains unclear.

Five polymorphisms within the *CSF3R* gene (rs3917924, rs3918001, rs3918020, rs3918021 and rs146617729) were investigated using PCR-RFLP method or LightSNiP (Tib Molbiol) sets of primers and probes for PCR with melting curve analysis, while rs148916169 was analysed with DNA sequencing.

No significant correlation was observed between any studied polymorphisms and *CSF3R* expression, either on mRNA or protein level. Only a tendency between presence of the wild *C* allele or *CC* genotype of rs3917924 polymorphism seemed to correspond with higher *CSF3R* mRNA levels before G-CSF treatment ($p=0.093$ for *C*+ vs. *TT* genotype and $p=0.079$

for *CC* vs. *TT* genotype). None of the studied genetic variants seemed to affect either mobilization yield or haematological recovery after transplantation.

Relative mRNA expression of G-CSF pathway genes was assessed via performing quantitative PCR with reverse transcription. We found that changes in expression were not fixed: in some patients mRNA levels of investigated genes rose in response to G-CSF treatment, while in other patients they decreased or reminded on a constant level.

Importantly, mRNA levels of most studied genes were found to be correlated with each other, either before or on the 5th day of G-CSF treatment. However, those relations were not always strong. Before mobilization, *CSF3R* mRNA levels were associated the strongest with *STAT3* and *SOCS3* expression ($p<0.001$ for both *CSF3R-STAT3* and *CSF3R-SOCS3* correlations). On mobilization day 5, relationships between *CSF3R* expression and both *STAT3* and *SOCS3* mRNA levels reminded strong ($p<0.001$, as previously indicated). At this time point, also connections between *STAT3* and *STAT5A, STAT3* and *STAT5B, SOCS3* and *STAT5A,* as well as between *STAT3* and *SOCS3* rose to be the strongest of all ($p<0.001$ for all correlations mentioned).

Also, efficacy of haematopoietic stem cells release into peripheral circulation was negatively affected by high *STAT1* mRNA levels before G-CSF treatment ($p=0.033$) and by greater *STAT3* expression on mobilization day 5 ($p=0.025$).

Flow cytometry was employed to analyse expression of CSF3R receptor on neutrophils and monocytes. Numbers of those cell populations in patients' blood rose in response to G-CSF treatment ($p=0.033$ and $p=0.004$ for neutrophils and monocytes, respectively). However, the sole number of neutrophils or monocytes was not found to be related with HSC mobilization efficacy.

Percentage of neutrophils and monocytes expressing G-CSF receptor was significantly higher in patients than in the healthy control group ($p<0.001$ for CSF3R+ neutrophils and $p=0.017$ for CSF3R+ monocytes). CSF3R expression on neutrophils, both before ($p=0.064$) and on 5th day of mobilization ($p=0.075$), tended to negatively affect efficacy of progenitor cells release from the bone marrow.

In conclusion, our current results lead to better understanding of G-CSF-mediated clinical mobilization. They suggest a role of monocytic cell population, apart from neutrophils, in progenitor cells mobilization. Furthermore, they show an important role of STAT3, STAT5A, STAT5B and SOCS3 molecules in response to G-CSF treatment and imply that expression of G-CSF pathway genes affects mobilization efficacy.