Introduction:
Conditional on apheresis techniques, stem cell products contain a considerable amount of thrombocytes. Platelets are the major source of soluble CD40 ligand (sCD40L) (1) in the blood. It has been demonstrated that CD40L is cleaved from the surface of activated platelets: sCD40L is well known to show immunomodulatory functions and high concentrations in blood products (2). Therefore we examined sCD40L concentrations in stem cell apheresis.

Material and Methods:
In four patients suffering from multiple myeloma and undergoing autologous stem cell apheresis, sCD40L concentrations were measured in peripheral blood samples before, during and after apheresis procedure and in the respective stem cell product. sCD40L concentrations were determined by a commercially available ELISA Kit (R&D Systems). In an additional approach, platelet-rich plasma (PRP) from healthy volunteers (n=6) was incubated with different pharmacological inhibitors (MMP-2/MMP-9 Inhibitor I, MMP-9 Inhibitor I, MMP-2 Inhibitor I, recombinant ADAM 10, and recombinant ADAM-17) during platelet activation.

Results:
During stem cell apheresis, a decrease in platelet count could be observed from 94,822/µL ± 56,734/µL at the beginning to 55,007/µL ± 26,567/µL at the end of the procedure. The thrombocyte loss was accompanied by a significant lowering of sCD40L concentrations in peripheral blood samples from 241 pg/mL ± 137 pg/mL to 124 pg/mL ± 73 pg/mL (dependent on platelet count, linearly correlated, r = 0.95). In stem cell products, sCD40L concentrations were manifold elevated (range from 2189 to 3641 pg/mL) in comparison to concentrations of peripheral blood samples. Using the MMP-9 inhibitor (100 nM) and the MMP-2/9 inhibitor (3 µM) sCD40L release by platelets could be inhibited by >60%. Interestingly, the MMP-2
inhibitor (17 µM) completely prevented the shedding of sCD40L from activated platelets.

Conclusions:

During stem cell apheresis, sCD40L concentrations in peripheral blood were mainly influenced by alterations of platelet count. As known from platelet concentrates, an accumulation of sCD40L could also be observed in stem cell products pointing out the importance of sCD40L release by platelets. Additionally, these data support the hypothesis that MMP-2 might be the protease, primarily responsible for sCD40L cleavage from platelet surface.

References: