Background: Previous studies in multiple myeloma (MM) showed clearly the suppressive role of CD8 T regulatory cells (Tregs). Recently, another regulatory cell expressing CD8 phenotype was identified and reported to be elevated and functionally active in solid tumor patients with prostate, colon and nasopharyngeal carcinoma (Kiniwa et al, 2007; Chaput et al, 2009; Li et al, 2011). These CD8 Tregs do suppress T cells through various mechanisms including release of regulatory and suppressive cytokines, interference with antigen-presenting cells and cell-cell contact. In this study, we examined number, functional activity and cytokine profile of CD8 Tregs from MM patients using flow cytometry. Further, we also studied the FoxP3 gene expression from CD8 Tregs.

Methods: Using multi-color flow cytometry we analyzed peripheral blood CD8 Tregs in 57 newly diagnosed MM patients. All patients and healthy volunteers (n=11, controls) gave informed consent according to Helsinki declaration. We analyzed CD8 Tregs and their associated markers with following fluorochrome conjugated monoclonal antibodies: FITC-FoxP3, PE-CD45RO, CD28, CD26L, CD127, PE-Cy7-CD25 and APC-CD8. CD8 Tregs from 5 MM patients and controls were functionally evaluated by CFSE (carboxyfluorescein succinimidyl ester) based autologous mixed lymphocyte reaction (MLR) proliferation assay. From proliferative assay, culture supernatant was collected and profiled for IL-10 and IFN-γ using FACS Array.

Results: CD8 T cells expressing high level of CD25 was considered as CD8 Tregs (CD8+CD25hi+) and also these cells expressed FoxP3 (regulatory molecule); CD45RO, CD28, CD26L but not CD127. CD8 Tregs (median±SD: 5.91 ± 0.43; P<0.001) and CD8 T cells co-expressing (CD25 and FoxP3) (median±SD: 0.41 ± 0.11; P<0.01) frequencies were significantly increased in MM patients compared to controls. Frequency of total lymphocytes (median±SD: 15.70 ± 40.61; P<0.001) but not CD8 T cells were significantly reduced in MM patients compared to controls. In functional activity, CD8 Tregs isolated from MM patients and controls inhibited the proliferation of naive CD4 T cells in concentration dependent manner but CD8 Tregs from MM patients possessed more suppressive function than controls (P<0.05). Cytokine profiling showed that level of IFN-γ but not IL-10 was decreased in both MM and controls according to the number of CD8 Tregs added to the mixed lymphocyte reaction assay. Evaluation of FoxP3 gene expression by real-time PCR demonstrated that CD8 Tregs from MM patients had increased FoxP3 expression compared to controls which corroborated with flow cytometry finding. More interestingly, in comparison to CD8 Tregs, FoxP3 expression was decreased in CD8 Tregs.

Conclusion: Inline with previous findings from solid tumors, we were also able to demonstrate that CD8 Tregs are elevated and functional in MM. Our results suggest that regulatory cells with CD8 phenotype might enhance tumor evasion and disease progression in MM.

245/B139
Identification and Characterization of Blood Microparticles from Light Scattering
Anastasiya Konokhova1,2, Maxim Yurkin1,2, Alexander Moskalensky1,2, Andrey Chernyshev1,2, Maxim Yurkin1,2, Valeri Maltsev1,2
1Cytophysics and Biokinetics Laboratory, Institute of Chemical Kinetics and Combustion, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, 630090, Russia, 2Department of Physics, Novosibirsk State University, Novosibirsk, 630090, Russia

Background: Microparticles (MPs) have high potential as diagnostic biomarkers. Standardization of their analysis by flow cytometry (FCM) is limited by preanalytical variables. One of the most important preanalytical variables is the centrifugation.

Aims: i) To develop and validate a new MV quantification method by FCM in whole blood (WB) to avoid the problems to the centrifugation process, and ii) to apply this method to healthy subjects and patients suffering from hereditary spherocytosis (HS).

Methods: The new quantification method in citrated 109mM WB was developed on a BD FACS ARIA I. Intraassay-, between- and interindividual variations were studied. Erythrocyte-derived MPs (EryMPs) and platelet-derived MPs (PMPs) were measured and compared in WB and derived platelet-free plasma (PFP) from healthy subjects (n=14), HS (n=6), and other haemolytic anaemias (n=14). The influence of the delay between sampling and analysis on PMP and EryMP concentrations, was also studied in PFP and WB (n=10).

Results: We developed and validated a new and stable quantification method in whole blood.