3

A NOVEL FLOW CYTOMETRIC APPROACH REVEALS ABUNDANT CD8+ T CELL DERIVED MICROVESICLES IN RHEUMATOID ARTHRITIS SYNOVIAL FLUID SAMPLES

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Background and objectives Microvesicle (MV) secretion represents an evolutionally conserved feature of all living cells. The assessment of MVs may give insight into the pathomechanism of various disorders. Furthermore, it may serve as potential novel biomarker of disease. However, the characterisation of MVs in body fluids has not been fully standardised yet, and there are numerous pitfalls that hinder the correct assessment of MVs. Previously, the authors developed a 'differential detergent lysis' method to exclude MV-mimicking protein aggregates and immune complexes during flow cytometry. Using our novel method here the authors analysed synovial fluid (SF) derived MVs.

Materials and methods The authors tested plasma and SF samples of patients with osteoarthritis (OA), rheumatoid arthritis (RA) and juvenile idiopathic arthritis. The authors used electron microscopy and nanoparticle tracking analysis (NTA) to determine the particle size distributions in SF samples. The authors also applied mass spectrometry to determine the MV protein composition in SFs. To immune phenotype SF MVs, the authors applied flow cytometry using 'differential detergent lysis' method.

Results The different techniques gave concordant results regarding the size distribution of MVs in SF samples (80–400 nm). However, NTA analysis and MS revealed that most of the events were related to protein aggregates rather than cell-derived vesicles. Using our novel flow cytometric approach, the authors demonstrate for the first time that CD3+ and CD8+ T cell derived SF MVs are highly elevated in SFs of patients with RA compared to OA patients ($p=0.027$ and $p=0.009$, respectively after Bonferroni corrections). In contrast, T cell derived MVs were undetectable in the blood plasma of patients with OA or RA. B cell and T cell derived MV counts strongly correlated with rheumatoid factor positivity in RA ($r=0.912$, $p=0.002$ and $r=0.956$, $p=0.001$, respectively).

Conclusions The correct assessment of MVs in RA suggests local CD8+ T cell activation in the joints and may shed light on hidden immunopathological processes of this disease.