A27 FLOW CYTOMETRIC DIAGNOSTIC ASSESSMENT OF CELL-DERIVED MICROPARTICLES IS SEVERELY CONFOUNDED BY IMMUNE COMPLEXES IN RHEUMATOID ARTHRITIS

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Background and objectives Microparticles/microvesicles are readily detectable in various biological fluids, and have recently been shown to be highly specific and sensitive biomarkers, for example, in tumours. Numerous autoimmune diseases including rheumatoid arthritis, systemic lupus erythematosus and systemic sclerosis, have also been reported to associate with elevated microparticle counts. On the other hand, the above autoimmune diseases have long been known to be characterised by accelerated immune complex formation as well. The goal of this study was to investigate the potential overlap between biophysical parameters of immune complexes and microparticles, which might perturb detection and/or isolation of microparticles.

Materials and methods We analysed microparticles from blood plasma of healthy individuals (n=12) and rheumatoid arthritis patients (n=12). Furthermore we also examined synovial fluid-derived microparticles from rheumatoid arthritis and osteoarthritis patients (n=11, respectively). Microparticles, as well as artificial and synovial fluid-derived immune complexes were characterised by using electron microscopy, atomic force microscopy, dynamic light scattering analysis and flow cytometry.

Results The used methods gave concordant results regarding the size distribution of the preparations. Very importantly, we found a significant size overlap between microparticles and immune complexes. As a consequence, immune complexes gave robust signals on flow cytometry within the conventional microparticle gate. This, in turn, affected microparticle quantification by flow cytometry in rheumatoid arthritis, where the immune complex content was high compared to osteoarthritis samples. We developed a novel method to differentiate between microparticles and immune complexes in flow cytometry. Using this method, we found that in rheumatoid arthritis, the total event count within the microparticle gate (earlier considered as microparticle count) was highly proportional with the amount of immune complexes, but did not correlate with microparticle count. Furthermore, immune complexes were found in the conventionally isolated microparticle pellets.

Conclusions For the first time, we drive attention to the fact that the presence of immune complexes in patient samples has significant impact on microparticle detection and isolation. These findings have general implications in diseases, where immune complex formation is common, including not only autoimmune diseases, but also haematological disorders, infections and cancer. These data may necessitate re-evaluation of certain published data on patient-derived microparticles, and contribute to correct the clinical laboratory assessment of the presence and biological functions of microparticles in health and disease.[1]

REFERENCES

1. **György B**, Módos K, Pállinger E *et al*. Detection and isolation of cell-derived microparticles are compromised by protein complexes due to shared biophysical parameters. *Blood*. 2010 Nov 1. [Epub ahead of print]