Background:

Multiple myeloma (MM) is a hematological malignancy characterized by clonal plasma cells accumulation within the bone marrow. MM diagnosis and prognosis rely on bone marrow biopsy. Given clonal plasma cell patchy distribution, bone marrow biopsies may not reflect disease heterogeneity whilst submitting patients to invasive procedures. Liquid biopsies, such as extracellular vesicles (EV) from peripheral blood, may overcome these limitations. In MM, there is evidence that EV intervene in key processes such as tumor progression and drug resistance. Most studies analyzing EV from MM patients focus on genome, and real-world studies on EV proteome are scarce.

Aims:

We characterized blood and bone marrow samples from the precursor stage, MGUS and from MM patients to determine whether EV content can discriminate between diagnosis and predict patient prognosis. These results were compared to healthy donor samples.

Methods:

After informed consent signed, a cohort of 102 patients and 19 healthy donors were followed in median time of 25 months. Blood and BM samples were collected at diagnosis, response and relapse of patients. EV were isolated by ultracentrifugation. Protein and EV particle concentrations were quantified. Proteomic analysis was performed by mass spectrometry (LC-MS/MS). Diagnostic and prognostic impacts of EV characteristics were investigated. A multivariable longitudinal logistic regression model was built to determine the association with common myeloma-related blood parameters.

Results:

We report a set of peripheral blood EV-proteins (PDIA3, C4BPA, BTN1A1, APRIL, PSMB8 and PDE8B) that have the potential to be used as new diagnosis biomarker for myeloma patients. Functional enrichment analysis revealed that proteins differentially expressed in patient vs healthy donors are strongly related to immune response, supporting increased immune dysfunctions in patients with active multiple myeloma.

We identified that the level of EVcargo (protein/particle ratio) is significantly associated to patient overall survival. High EVcargo patients (≥0.6 µg/10^8 particles) had a shorter overall survival compared to low EVcargo patients (≤0.6 µg/10^8 particles). High EVcargo is associated with high sFLC lambda, IgA immunoparesis and shorter time in response. The downregulation of IGH1/IGHA confirmed patients immunoparesis. Upregulation of Ig lambda production proteins confirmed the increased presence of sFLC in the same patients. Also, we report an association
between the progression free survival time and expression level at diagnosis of SERPINA2, RPS26 and UBBP4, suggesting their potential as prognosis biomarkers.

**Image:**

**Summary/Conclusion:**

To our knowledge, this is the first report of EV protein content from a real-world MGUS and MM patient cohort followed for more than 2 years. Our findings show that PDIA3, BTN1A1, APRIL and complement proteins should be further explored in myeloma, as peripheral blood biomarkers of diagnosis. Circulating EVcargo is a promising prognostic biomarker for MM patients. Patients with high EVcargo had 12 times increased risk of dying and high EVcargo is associated with important prognostic features such as immunoparesis, sFLC or duration of treatment response. EVcargo is an indirect and more affordable measure to infer EV protein load compared to mass-spectrometry. Our results show that EV have the potential to be used as diagnosis biomarker in MGUS and MM patients and as an added approach to discriminate patients with poor survival. Our results substantiate the interest of EV as liquid biopsies in myeloma and future validation in independent clinical settings is urged.

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