Reviewer’s report

Title: A proteomic analysis of serum-derived exosomes in rheumatoid arthritis

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Reviewer: Mojca Frank-Bertoncelj

Reviewer’s report:

The manuscript presents interesting novel data and covers a rather under investigated field in RA. The paper is well written and easy to follow. However, I have some major concerns:

Major comments

- Purity and characterization of exosomes. ExoQuick can precipitate in addition to exosomes also other non-exosome protein components in serum, which could contaminate 2D-DIGE results. To determine to which extent DIGE results represent pure exosome proteins, I recommend additional characterization of exosome isolates and their purity e.g. by following the "Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles (Table 1, Lötvall J. et al. J Extracell Vesicles. 2014; 3: 10.3402/jev.v3.26913.) If possible, I recommend using a complementary method for exosome isolation, e.g. one of the gradient centrifugations, to confirm the major results.

- Expected and predicted molecular weights and isoelectric points of identified proteins do not match, the authors should comment on this observation (Table 3). I would recommend confirming major results with Western blot if possible. How the observed results fit into published evidence e.g. in Vesiclepedia (Kalra H. et al. Vesiclepedia: a compendium for extracellular vesicles with continuous community annotation. PLoS Biol. 2012;10(12): e1001450. doi: 10.1371/journal.pbio.1001450).

- 2D-DIGE: Could the authors provide more details about the characteristics of the spots (e.g. where there any changes in the horizontal/vertical positions of the spots, suggesting changes in the size/charge between the experimental group)s. Were the 204 spots identified in all 43 samples, can the authors provide the Suppl. Table with spot intensities in 43 samples? Were the overlapping spots (e.g. Fig 3A1) changed in the same direction (e.g. increased). There seem to be three predominant sizes of exosome proteins, can the authors comment on this. Could the authors mark the differentially enriched spots on Fig 2. for all 4 experimental groups (or provide additional supplementary Figure)?

Minor
- Pre-analytical variables: The authors should provide details on pre-analytical factors during blood withdrawal (needle gauge, tubes used, temperature of the centrifugation, time between blood withdrawal and centrifugation, any haemolysis present, were all the samples treated same way).

- The description of RA pathogenesis in the background is too simplified

- Table 1, please notify significant differences in parameters between iRA and aRA, I would suggest commenting briefly on patient characteristics in Results

- The authors suggest that the TLR3 fragment may be functional, which is speculative, it could be also a degradation product, can the authors comment on this. Is TLR3 fragment increased in exosomes from all patients with active RA?

- Recently, Poly(I:C), a TLR3 ligand, was shown to associate with extracellular vesicles with downstream effects on FLS (https://doi.org/10.3389/fimmu.2018.00028). How do the authors comment on this data with respect to their findings?

- In addition, OA vs healthy comparisons are interesting, why do the authors not comment on this. How do the authors comment on their finding that iRA and OA exosomes differ least? Can the authors comment all 6 differentially enriched spots in discussion?

- The electron microscopy image should be sharper.

- I would suggest using SD rather than SEM in Fig. 5, also dots would be more informative than bars.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

No

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

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If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.
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