Candidate's full name: Karolina Magdalena Soroczyńska

Title of the doctoral dissertation: "Significance of the molecular profile of extracellular vesicles from plasma and peritoneal fluid in the development of endometriosis"

Advisor(s): Małgorzata Czystowska-Kuźmicz, Ph.D., D.Sc.

Co-advisor (if appointed): N/A

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1. Topic of the dissertation

The doctoral thesis entitled "Significance of the molecular profile of extracellular vesicles from plasma and peritoneal fluid in the development of endometriosis" addresses a relevant and timely research problem concerning the molecular characterisation of extracellular vesicles (EVs) in endometriosis. The study employs an integrated experimental strategy combining EV enrichment from plasma and peritoneal fluid, complementary vesicle characterisation, TMT-based quantitative proteomics, and imaging flow cytometry (IFCM). The comparison of plasma- and peritoneal fluid-derived EV-enriched preparations obtained from the same clinical cohort is an important strength of the work, as it enables investigation of both local and systemic molecular alterations associated with endometriosis. The study generated extensive proteomic profiles of EV-enriched fractions from both biological compartments and revealed more pronounced disease-associated molecular differences in peritoneal fluid than in plasma. This comparative design provides an opportunity to examine compartment-specific and overlapping molecular alterations associated with endometriosis. The dissertation addresses a highly topical area of contemporary extracellular vesicle and proteomic research. I therefore consider the subject of Karolina Magdalena Soroczyńska's doctoral dissertation to be scientifically relevant and well aligned with current challenges in biomedical research.

2. The candidate's knowledge

Karolina Magdalena Soroczyńska demonstrates substantial interdisciplinary knowledge of extracellular vesicle biology, endometriosis and contemporary analytical approaches, including size-exclusion chromatography, nanoparticle tracking analysis, Western blotting, cryo-electron microscopy, imaging flow cytometry and mass spectrometry-based proteomics. The integration of these complementary methods and the comparative analysis of EV-enriched preparations derived from peritoneal fluid and plasma demonstrate the Author's ability to investigate both local and systemic molecular alterations associated with endometriosis and to interpret complex proteomic data in a relevant biological context.

Overall, the dissertation demonstrates a sound and comprehensive understanding of the field. At the same time, several aspects of the statistical analysis and interpretation of the proteomic findings require further clarification, particularly with respect to multiple testing, the biological

interpretation of shared EV-associated proteins, disease severity, and the translational significance of candidate biomarkers. These issues are addressed in the questions raised below.

In conclusion, Karolina Magdalena Soroczyńska's doctoral dissertation demonstrates a high level of general theoretical knowledge appropriate for a doctoral candidate in the discipline of medical sciences. The scope and presentation of the theoretical background confirm the Candidate's sound theoretical and academic preparation for independent scientific work and fulfil the requirements expected of a doctoral candidate in this discipline.

3. The candidate's independence

The dissertation provides evidence that Karolina Magdalena Soroczyńska is capable of conducting scientific research with a substantial degree of independence. This is reflected in the coherent integration of clinical material, extracellular vesicle characterisation, quantitative proteomics, and imaging flow cytometry, as well as in the comparative analysis of plasma- and peritoneal fluid-derived EVs. The Author demonstrates the ability to formulate research questions, analyse complex multidimensional datasets, and interpret the results in the biological context of endometriosis. She also demonstrates an ability to critically assess the limitations of the study and to recognise the need for further validation of the findings. This supports a positive assessment of her ability to critically evaluate her own research.

Given the technically complex and multidisciplinary nature of the study, which combines several highly specialised methodological platforms with a multicentre clinical cohort, the dissertation does not always allow the Candidate's individual contribution to each experimental and analytical component to be distinguished with complete clarity. Nevertheless, the dissertation supports a positive assessment of her scientific independence, particularly in the integration and biological interpretation of complex experimental data. Clarification of her individual contribution to the experimental, analytical, bioinformatic, and statistical components during the defence would further strengthen this assessment.

4. Originality of the dissertation

The dissertation addresses a scientifically relevant research area and provides original data concerning extracellular vesicle-associated molecular alterations in endometriosis. The broader concept of analysing extracellular vesicles from plasma and peritoneal fluid in endometriosis has precedents in the literature. Nevertheless, the dissertation provides an original contribution through the integrated analysis of plasma and peritoneal fluid samples obtained from the same clinical cohort, combined with multiplexed TMT-based quantitative proteomics and IFCM-based EV phenotyping.

This experimental framework enables a direct comparison of local and systemic EV-enriched molecular profiles and provides an original solution to the specific scientific problem addressed in the dissertation. The originality of the work lies primarily in the integrated experimental design and comparative analytical strategy applied to the two biological compartments, rather than in the use of EV analysis in endometriosis per se.

5. Questions and/or critical comments to which the Reviewer expects the Candidate to respond during the dissertation defence:

The following questions concern the principal methodological and interpretative issues identified in the dissertation and are intended to clarify the rationale for selected analytical decisions and the strength of the conclusions drawn from the presented data.

Given the technically complex and multidisciplinary nature of the study, I would first ask the Author to clarify her individual contribution to the design and execution of the work, including EV isolation and characterisation, TMT-based proteomics, bioinformatic and statistical analyses, and imaging flow cytometry. Which elements were performed independently by the Author and which were carried out in collaboration with other members of the research team? I would also ask for clarification of the study population. The dissertation reports 52 samples, comprising 26 plasma and 26 peritoneal fluid samples. How many individual participants were included, what was the relationship between the plasma and peritoneal fluid specimens, and how was this study structure handled statistically? In addition, how should the control population used in this study be defined, and what implications might its clinical characteristics have for interpretation of the results?

Several aspects of the statistical analysis require clarification. The dissertation states that outlier results were excluded before statistical analysis. How were outliers defined, which statistical or quality-control criteria were applied, how many observations were excluded and from which groups or analyses, and did their exclusion materially affect the principal findings? Thousands of proteins were analysed, while some differentially abundant proteins were selected using nominal p-values together with a fold-change threshold. How does the Author distinguish between nominally significant findings and proteins remaining significant after FDR correction, and which results does she regard as the most robust basis for biological interpretation? How does the Author interpret the set of 24 overlapping candidate differentially abundant proteins identified in plasma- and peritoneal fluid-derived EV preparations, and how should differences in statistical significance and direction of change between the two compartments influence their interpretation as a common molecular signature of endometriosis?

I would also ask the Author to provide further detail on the TMT experimental design. How were endometriosis and control samples allocated across the individual TMT plexes, was channel assignment randomised or balanced, and how was potential confounding between biological group and TMT batch minimised? What variables were included in the batch-effect correction model, and how was it verified that this procedure removed technical variation without eliminating biologically meaningful differences? Similarly, what exact parameters were used for missing-value imputation, including the shift and width of the normal distribution and the maximum proportion of missing values permitted for a protein to be retained in the analysis? How sensitive are the differential-abundance results to the chosen imputation strategy?

The EV methodology raises several additional questions. How does the Author assess the degree of EV enrichment and the presence of non-vesicular components in material obtained by SEC from plasma and peritoneal fluid, and what evidence supports attribution of the proteomic signal specifically to EV-associated material? What was the rationale for selecting

the qEV 70 nm column, and how does the Author assess the balance between EV recovery and reduction of non-vesicular contaminants achieved with this approach? Cryo-EM characterisation was performed using an EV preparation from only one patient with endometriosis; what conclusions can legitimately be drawn from these images regarding the morphology and quality of EV preparations across the cohort?

The IFCM component also requires clarification. How does the Author define the term "validation" in relation to the perioperative IFCM cohort, and what conclusions can legitimately be drawn from this part of the study? How should the changes in EV populations observed 48 hours after surgery be interpreted in view of the biological consequences of the surgical procedure itself?

With regard to biological interpretation, the dissertation groups rASRM stages III and IV together as "severe disease", although only one patient had stage IV endometriosis. What was the rationale for this classification, and what conclusions regarding the relationship between EV-associated proteomic profiles and disease severity can be supported by this cohort? The term "disease progression" is also used in several parts of the dissertation. Given the cross-sectional design of the principal clinical study, to what extent can progression be distinguished from associations with disease status or stage?

The translational interpretation of the findings is another important issue. Which proteins or EV surface markers identified in the dissertation does the Author consider the strongest candidates for further diagnostic development, and what additional evidence would be required to establish their diagnostic utility? Finally, how do the proteomic findings presented in this dissertation compare with those reported by Khalaj et al. (2019), who previously analysed extracellular vesicles derived from both plasma and peritoneal fluid in endometriosis, and how would the Author explain the similarities and discrepancies between the two studies?

Several statements in the Abstract, Discussion, and Conclusions appear to extend beyond what can be directly supported by the exploratory and cross-sectional nature of the study. Which limitations does the Author consider most important for interpretation of the diagnostic and biological implications of the findings, and how do these limitations constrain the strength and generalisability of the conclusions?

Finally, I noted several editorial and presentational inconsistencies that warrant correction. These include the numerical inconsistency in the IFCM antibody panel, where the text refers to 25 markers whereas Table 5 lists 10; errors in chapter and subsection numbering, including duplicated section numbers and inconsistent numbering of the Discussion, Conclusions, and References; and several language and typographical errors, such as "the final analysis included a total of 52 samples were included", "sing size exclusion chromatography" instead of "using size exclusion chromatography", and "to identify potentially diagnostic biomarker" in the Abstract. These issues do not affect the overall scientific assessment of the dissertation but should be addressed during final editorial revision.

6. Final grade:

The doctoral dissertation submitted by Karolina Magdalena Soroczyńska addresses a relevant scientific problem, employs an ambitious multidisciplinary approach, and provides a substantial body of original experimental data. Despite the methodological and interpretative issues raised above, these concerns do not undermine the overall scientific value of the work.

In my opinion, the dissertation demonstrates the Candidate's general theoretical knowledge in the discipline of medical sciences and her ability to conduct scientific research independently. It also constitutes an original solution to the scientific problem addressed. I therefore conclude that the dissertation fulfils the requirements specified in Article 187 of the Act of 20 July 2018 – Law on Higher Education and Science, as amended, and recommend that Karolina Magdalena Soroczyńska's doctoral dissertation be admitted to public defence.

Podsumowanie – ocena końcowa

Rozprawa doktorska przedłożona przez Karolinę Magdalenę Soroczyńską dotyczy istotnego problemu naukowego, ma charakter interdyscyplinarny i opiera się na zastosowaniu komplementarnych metod badawczych, dostarczając obszernego zbioru oryginalnych danych eksperymentalnych. Zgłoszone w recenzji uwagi metodologiczne i interpretacyjne nie podważają jej ogólnej wartości naukowej.

Moim zdaniem rozprawa doktorska spełnia wymagania określone w art. 187 Ustawy z dnia 20 lipca 2018 r. – Prawo o szkolnictwie wyższym i nauce (t.j. Dz.U. z 2024 r., poz. 1571 z późn. zm.), ponieważ prezentuje ogólną wiedzę teoretyczną Kandydatki w dyscyplinie nauk medycznych, potwierdza jej umiejętność samodzielnego prowadzenia pracy naukowej oraz stanowi oryginalne rozwiązanie podjętego problemu naukowego. W związku z powyższym rekomenduję dopuszczenie rozprawy doktorskiej Karoliny Magdaleny Soroczyńskiej do publicznej obrony.

Jastrzębiec, 4 września 2026

Prof. dr hab. Monika Pietrowska