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Evaluation of the Tasnim Mohaissen PhD thesis titled "Novel mechanism of peripheral vascular endothelial dysfunction in heart failure in Tgαq*44 mice"

MPharm Tasnim Mohaissen's doctoral thesis was performed under the supervision of Prof. Stefan Chłopicki, MD, Ph.D., and Magdalena Sternak, Ph.D.. The dissertation was prepared in English and is affiliated with the Jagiellonian University, Collegium Medium, and Jagiellonian Center for Experimental Therapeutics.

Cardiovascular diseases (CVDs) according to the World Health Organization (WHO), are the leading cause of death. All the available statistics, present CVD as a significant global problem, which is projected to further increase due to multiple factors such as population growth, aging, and lifestyle changes. Therefore, it is important to **better understand the underlying causes of CVDs to improve the effectiveness of future treatment strategies**.

Prof Chlopicki is leading the recognized Center for study and understanding of endothelium physiology, dysfunction and their role in cardiovascular diseases which reflect the main focus of the evaluated dissertation.

Tasnim Mohaissen's Ph.D Thesis is presented as the dissertation, however, some results have been already published or are under review in high-impact scientific journals. In above mentioned papers Mrs Mohaissen is the first author. In one published manuscript, "*Temporal relationship between systemic endothelial dysfunction and alterations in erythrocyte function in a murine model of chronic heart failure.*" *Cardiovasc Res. 2022 Sep 20;118(12):2610-2624*, Tasnim Mohaissen conceived and designed the study, performed experiments, analyzed the data, prepared the figures and wrote the draft together the final version of the manuscript (as defined in the "Author's contribution" section of the manuscript).

The dissertation submitted for evaluation is comprised of 109 pages of typescript and is traditionally structured with separate paragraphs: Introduction, Methodology, Results, and Discussion. The work also includes a Summary, Aim of the Study, Conclusions, and relevant lists of Bibliography together with Mrs Mohaissen's publication list. The structural composition of the thesis is classical but in the reviewer's opinion, the addition of the abbreviation list used in the dissertation should be included for the reader's convenience.

The introduction paragraph focuses on the main subject of study, i.e., the role of endothelium in cardiovascular physiology and pathology. The author in a very elegant and comprehensive way presents the state-of-art regarding the importance of endothelial homeostasis and the main factors that could induce endothelial dysfunction. Moreover, the main technology behind the endothelial function assessment is presented. The last two paragraphs describe the red blood cell (RBC) parameters and their role in cardiovascular diseases and angiotensin pathway activation in endothelial dysfunction. From the reader's perspective, besides the undisputed meritorical value, the main drawbacks of this part, are the included figures. As an example, Fig.2. shows features/elements of endothelial dysfunction which will be very helpful to summarize the Introduction but due to the dark font used the text is barely readable. Moreover in Fig.3. the FDD term occurs without any explanation. I would appreciate the clarification of the term FDD in relation FMD definition.

In summary, the introduction paragraph provides basic knowledge and points out the urgent need for a comprehensive and detailed study of endothelium's role and mechanisms in heart failure. The introduction shows clearly that **Ph.D candidate is familiar with the scientific field and could with critical thinking skills present the current advances in endothelium-oriented research.**

The aim of the study was to decipher novel mechanism involving the RBC disfunction and Ang(1-12) pathway in endothelial dysfunction associated with heart failure. The aim is clearly stated and the designed experiments with the obtained results are in agreement with the presented thesis.

The Methodology paragraph starts with Tgαq*44 mouse model description. This model of non-ischemic chronic heart failure is extensively studied by Prof. Chlopicki Group. In my opinion, a more detailed description of the model should be given especially in the aspect of the altered signaling pathway, HF development, and phenotype consequences. Fig. 7. covering the above issues is present but unfortunately illegible due to the format applied. All applied in study techniques are sophisticated from the methodological point of view and comprehensive in the aspect of fitting in the scope of the tasks. Moreover, the variety of used approaches is impressive,

starting from the molecular evaluation (e. qPCR and WB) through physiological tests (e.g., aorta vasodilatation assays) and advanced EPR measurements and MRI imaging. The only critical comment from the reviewer side is that some of the method descriptions are not specific enough and are missing crucial details. To name a few: a method for qPCR result normalization/ primers and probe sequences are missing or RBC isolation protocol for evaluation of its influence on endothelial function is not described. Also, Reviewer could not find the idea behind the order of methods.

The results chapter is divided into three sections: i. Evaluation of endothelial function in peripheral circulation in Tgαq*44 mice, ii. Erythrocyte alternation in the studied model and iii. ANG-(1-12)/ANG II/TXA_2 pathway in peripheral circulation in Tgαq*44 mice.

In the first section Mrs Tasnim Mohaissen showed that Tgαq*44 mice aorta endothelium function is impaired in 10 and 12 months-old animals as shown by *ex vivo* evaluation and *in vivo* MRI measurements of AAA. This finding was associated with diminished bioavailability of NO and increased superoxide (O^{2-}) production. Moreover, observed changes in 12-month-old mice were concomitant with elevated production of proteinoids. The serum concentration of several cytokines (including IL-1beta, IL-2 or TNFalpha) was not altered in Tgαq*44 mice as compared to FVB control animals. Also, the changes in neurohormonal profile were noticed both in young and older animals. RBC dysfunction in HF and its impact on endothelium function is discussed in the second section. The main findings showed that RBC volume, size, and oxidative stress markers are altered in Tgαq*44 mice. A significant disturbance of endothelial function was noted after co-culture RBC obtained from Tgαq*44 mice with healthy, non-modified aortic segments isolated from FVB animals. The experiments with arginase inhibitors point out the possible mechanism of observed phenomena.

In the last section, Mrs Tasnim Mohaissen performed numerous experiments assessing the involvement of ANG-(1-12)/ANG II/TXA_2 pathway in HF-associated endothelial dysfunction.

Tgαq*44 mice aorta showed impaired vasorelaxation in response to Ang I, Ang II and Ang (1-12) as well as the eicosanoid profile including TXB_2 in 12 months old HF mice. As the following rescue experiments were performed and show that antagonists of TXA_2 receptor (SQ29548) and losartan could prevent the reduction of aorta vasodilatation. Ph.D. candidate demonstrated that she can conduct research independently and use a number of techniques to obtain high-quality results on the international level. The results are presented in a very logical and self-explanatory manner with clear given priority to the most significant findings. For a clearer distinction between HF vs healthy control results I would recommend choosing the more obvious color-coding strategy.

The discussion paragraph is a straightforward proof of Ph.D candidate's extensive knowledge concerning the subject of research and her ability to conclude and synthesize the conclusions based on performed work. I appreciate the summary of findings presented in Table (Fig. 35) and scheme (Fig.36.) form. As outlined the presented study identified the novel mechanism of endothelial dysfunction in the heart failure model which is based on observed erytropathy and involvement of ANG-(1-12)/ANG II/TXA_2 pathway. Moreover, the candidate can correctly assess the scientific significance of her results and place it in the context of existing knowledge in the cardiovascular disease field.

Out of duty as a reviewer, despite my very good assessment of the dissertation as a whole, I would appreciate discussing a few remarks and a request for clarification of some of the results.

Starting from the minor issue I would like to ask for an explanation of including the evaluation of nitrite, nitrate, and HBNO in RBC in the first results section instead of discussing this topic within the erytropathy devoted paragraph. Also, I was puzzled why the AFM evaluation of RBC shape was not included in the result section but only mentioned in the discussion.

I would also welcome further discussion regarding the animal model influence on obtained result. As mentioned before in the literature, the alpha-MHC promotor is widely used for cardiac-specific transgene expression but due to cardiac hypertrophy, its activity is progressively diminished. Is constitutively active Gαq protein decreased in 12-month-old Tgαq*44 mice? If yes, how this could influence obtained results? Also as showed by Protein Atlas the *MYH6* gene - human homolog for alpha-MHC murine gene is active in cardiovascular endothelial cells. Were the active Gαq protein present in endothelial cells and what kind of consequences could this expression generate?

To conclude, the evaluation presented by Tasnim Mohaissen thesis prepared under the supervision of prof. Chlopicki and dr Sternak, was a pleasure and an exceptional scientific experience. **The work is well-designed, performed with a tailored methodology and concluded with a meaningful result.**

In my opinion, the doctoral dissertation submitted for review meets all the requirements set out Regulation of the Minister of Science and Higher Education of 19 January 2018 on the detailed procedure and conditions for conducting activities in the doctoral procedure in the habilitation procedure and in the procedure for conferring the title of professor (D2.U.2018 item 261)

In view of the above, I hereby apply to the High Council of the Medical Sciences of the Jagiellonian University in Cracow to admit Tasnim Mohaissen M.Sc. to further stages of the doctoral procedure.

Uważam, że przedstawiona do recenzji rozprawa doktorska spełnia wszelkie wymagania określone w *Rozporządzeniu Ministra Nauki i Szkolnictwa Wyższego z dnia 19 stycznia 2018 r. w sprawie szczegółowego trybu i warunków przeprowadzania czynności w przewodzie doktorskim w postępowaniu habilitacyjnym oraz w postępowaniu o nadanie tytułu profesora (D2.U.2018 poz.261)*

W związku z powyższym, przedkładam Wysokiej Radzie Dyscypliny Nauk Medycznych Uniwersytetu Jagielońskiego w Krakowie wniosek o dopuszczenie mgr Tasnim Mohaissen do dalszych etapów przewodu doktorskiego.

Łącząc wyrazy szacunku

Signed by / Podpisano przez:
Natalia Rozwadowska
Date / Data: 2023-07-05 13:48

Prof. IGC PAN, dr hab. n.med. Natalia Rozwadowska