

REVIEW

On the dissertation and abstract on

Feasibility of some echocardiographic techniques and microRNAs for the detection of subclinical myocardial injury in children and young adults with Beta Thalassemia Major

Presented for public defense before a scientific jury for the award of the degree of Doctor of Science and Education in the field of higher education 7 Health and Sport, professional field 7.1 Medicine in the doctoral program "Pediatrics"

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I have not found any omissions in the documentation attached by Dr. Kalina Binkova Ganeva. I have complied with the requirements of the Law on Academic Degrees and Academic Positions at MU-Varna. I have no conflict of interest from my participation in the scientific jury. I have no scientific works in common with the PhD student.

Significance of the topic. Beta thalassemia major (BTM) is one of the rare hemoglobinopathies in which the successes of modern medicine ensure normal growth

and development and, with the use of chelation therapy, largely prevent the long-term complications of regular haemo transfusions- the deposition of iron in internal organs, including the myocardium. Nevertheless, cardiovascular involvement is a leading cause of morbidity and mortality. Clinically manifested heart failure or dysrhythmias are most common with inadequate chelation therapy and in adults. Iron deposition in the heart that alters tissue characteristics of the myocardium and leads to impairment in its function has been found as early as childhood. Therefore, proactive management with the search for sensitive, specific, safe, maximally non-invasive, and reproducible methods to detect initial, discrete changes of cardiac damage are of utmost importance for the patient. The dissertation of Dr. Kalina Ganeva is devoted to the search for these methods to determine myocardial involvement as an expression of iron accumulation and to establish a protocol for monitoring the cardiac status of children and young adults.

No systematic, prospective studies have been performed in Bulgaria to assess diastolic function, myocardial deformation and microRNAi testing in children and young adults with BTM.

For this reason, Dr. Ganeva's dissertation is relevant and essential for the care of this group of patients in Bulgaria.

Structure. The thesis, written in 130 pages, is well balanced and includes an introduction and literature review (29 pages), aim and objectives (1 page), material and methods (12 pages), results and discussion (47 pages) summary with conclusions and contributions (5 pages). It is illustrated with 14 tables and 39 figures, and 2 appendices. The bibliography includes a total of 147 references, 6 in Cyrillic and 141 in Latin, with 45% of the cited publications from the last 10 years, and 29 (20%) from the last 5 years.

In the **literature review**, which occupies 20% of the thesis, the pathophysiology and clinical presentation of beta thalassemia major (BTM), iron metabolism, and monitoring of iron levels in the human body are fully, systematically, and consistently outlined. The role of ferritin as a key laboratory marker to assess iron stores in the body and the risk of future cardiovascular damage is highlighted. Current therapy of BTM is reviewed with emphasis on iron chelators, bone marrow transplantation, gene therapy, and monoclonal antibody administration. Special attention is given to cardiovascular

complications and their relationship to chelator therapy. Dr. Ganeva systematically and thoroughly describes the changes in hemodynamics of iron-induced cardiac injury and its complications. A major compensatory mechanism of chronic anemia and associated hypoxia is a hyperefficient state, which in the long term leads to ventricular remodeling and increased ejection indices. The molecular mechanism and cardiotoxicity of iron and the effects of oxidative damage on cardiomyocytes are reviewed in detail. The development of thalassemic cardiomyopathy (TCM) manifests phenotypically in two variants, dilated and restrictive TCM. In the evolution of iron accumulation, the diastolic function is impaired first, and the clinical manifestation of cardiac damage with systolic dysfunction and the image of DCMP is the final phase of TCMP. The dissociation between the amount of iron accumulation and the absence of symptoms is the rationale for searching for sensitive markers of early subclinical myocardial injury in childhood. The assessment of diastolic function in children with BTM, as well as the incidence and type of arrhythmias, as one of the complications of iron overload, are discussed in detail. The role of left atrial size as a prognostic factor for future arrhythmias is highlighted. Magnetic resonance imaging (MRI) and echocardiography are reviewed as contemporary noninvasive methods for the evaluation of iron deposition. The need to perform the first MRI at the age of 6-10 years is emphasized. The main focus is on modern echocardiographic techniques for determining systolic and diastolic left ventricular function and myocardial deformation, which are more sensitive than standard echocardiographic techniques for early assessment of ventricular dysfunction. Dr. Ganeva also discusses microRNAs as novel laboratory indexes and potential biomarkers of cardiac dysfunction such as cardiac hypertrophy, interstitial fibrosis, and cardiac remodeling, processes that are associated with BTM

Findings from the current scientific literature underscore the need for a comprehensive assessment of cardiac function in patients with BTM and provide a basis for building a working hypothesis that initial changes in cardiac function in asymptomatic young patients due to BTM and myocardial iron accumulation can be identified using modern noninvasive echocardiographic techniques and certain microRNAs.

The **aim** of the dissertation is to determine whether there are early cardiac function abnormalities in young patients with BTM and whether these can be identified using modern non-invasive echocardiographic techniques and some cardiac injury-specific microRNAs.

Seven well-formulated **tasks** were set that met the stated objective.

1. To assess the physical development of children and young adults with BTM including anthropometric indicators - height, weight, body surface area, as well as to clinically assess the cardiovascular system - heart rate, blood pressure. To be compared with healthy controls.
2. To perform echocardiographic assessment of left ventricular function in patients with BTM, and compare with healthy controls:
 - Measure and assess cardiac dimensions and left ventricular systolic function and myocardial strain by speckle tracking and calculation of global longitudinal strain (GLS).
 - Assessment of left ventricular diastolic function with of pulse and tissue Doppler
 - To analyze the diagnostic potential of tissue Doppler and GLS for the assessment of early cardiac dysfunction in asymptomatic children and young patients with BTM.
3. To investigate ferritin level as a laboratory marker of iron overload in children and young patients with BTM.
4. To investigate the expression of specific microRNAs as markers of heart failure, remodeling, and fibrosis in patients with BTM and healthy controls.
5. To correlate the results of microRNAs and echocardiographic parameters of patients.
6. To look for a correlation between echocardiographic assessment of cardiac function and myocardial iron accumulation using the MRI T2* technique by grade (mild, moderate, severe) in patients over 10 years of age.
7. To propose a protocol for echocardiographic examination and follow-up of cardiovascular status in patients with BTM.

Patients and methods. The study is prospective, a major merit of the dissertation, and has been approved by the Ethics Committee of MU-Varna. Seventy eight cases aged up to 25 years, 27 patients with proven BTM, and 51 healthy controls were included. Clinical methods, ECG, and modern Echocardiographic methods were used for detailed assessment of systolic and diastolic left ventricular function. Five microRNAs selected as markers of acute cardiac injury, cardiac remodeling and fibrosis were examined. Advanced statistical methods were used to ensure the reliability of the results

The results presented for each task are concluded with a discussion in which the results are critically and analytically compared with existing national and international studies.

For **Task 1**. There were no statistically significant differences in age, sex and body surface area between the study group and controls. Dr. Ganeva found higher heart rate, and lower systolic and diastolic pressures in the patients with BTM, which did not reach statistical significance with the controls and were explained by the chronic hyperefficient condition that BTM is.

Task 2. Dr. Ganeva demonstrated statistically significantly higher left ventricular muscle mass (in absolute and indexed values), which together with the increased left ventricular diameter is interpreted as a compensatory mechanism of the hyperefficient state due to chronic anemia. There was a statistically significant increase in indexed left atrial volume, an expression of diastolic dysfunction and iron deposition. Assessment of left ventricular systolic function by ejection fraction and GLS was within normal limits and showed no difference with controls. Detailed assessment of diastolic left ventricular function with pulse and tissue Doppler revealed no evidence of impaired left ventricular relaxation. However, the increased indexed left ventricular volume found was interpreted as an expression of incipient changes in diastolic left ventricular function that required follow-up.

On **Task 3**, Dr. Ganeva demonstrated mildly to moderately elevated ferritin levels in patients with BTM, which is an expression of adequate chelation therapy and low cardiovascular risk.

On **task 4**- five microRNAs were tested in 50 cases - 27 patients and 23 controls. Dr. Ganeva demonstrated expression of two of them in all patients with BTM and statistical significance with healthy controls - significantly lower levels at MIR 30a-5p and significantly higher levels at MIR 150-5p, which was interpreted as a sign of increased cardiovascular risk. An in-depth analysis of the expression of the remaining microRNAs was performed, which were not present in all patients, with no significant difference with healthy controls. It can be assumed that pathological expression of MIR -1-3 p and MIR-29 b-3p are potential for cardiac fibrosis.

On **task 5**, Dr. Ganeva found a moderate inverse relationship between MIR 150-5p and indexed left atrial volume ($r=0.44$)

On **task 6**, myocardial iron deposition was assessed by MRI in 19 patients. Severe iron deposition was evidenced in 2 patients and this was consistent with decreased systolic function values and an enlarged left atrium. The small number does not allow a correlation analysis, but the absence of iron deposition in the myocardium in the remaining 17 patients (90%) is an expression of adequate chelation treatment.

For **task 7**, Dr. Ganeva developed a protocol for detailed echocardiographic examination of patients with BTM.

The **conclusions**, 9 in number, logically and correctly formulated, follow the data of the results and the statistical analysis and meet the stated aim and objectives. I agree with the 5 contributions presented, two original and three confirmatory.

I have submitted 2 full text publications, 1 of them in a refereed international database journal and 2 contributions to national scientific forums, thus meeting the requirements of MU Varna.

The abstract meets the requirements of the Academic Staff Development Act.

In **conclusion**, the dissertation work of Dr. Kalina Ganeva, based on a prospective, in-depth evaluation of myocardial damage in children and young adults from northeastern Bulgaria with BTM, using affordable, non-invasive methods, demonstrates very good rehabilitation of patients up to 25 years of age. Adequate treatment, including

periodic blood transfusions and chelation therapy, demonstrates myocardial iron deposition by MRI in only 10% of the patients studied, and this correlates with standard methods of assessing left ventricular function, which are within reference values. Nevertheless, chronic anemia and associated hyper efficient state are the cause of increased left ventricular muscle mass and vital parameters. Using modern echocardiographic indices in these asymptomatic patients, find discrete changes in left ventricular diastolic function, an expression of impaired relaxation from the underlying pathological process. Of particular interest is the study of microRNAs, which was performed for the first time in the patients with BTM and demonstrated pathological values of MIR 30a-5p and MIR 150-5p, which could be associated with increased cardiovascular risk.

The dissertation work of Dr. Kalina Ganeva, a pediatrician and pediatric cardiologist, lays a solid foundation for cardiac involvement in asymptomatic children and young adults with BTM and allows for the expansion and deepening of studies on the evolution of cardiac involvement assessed with modern echocardiographic techniques and microRNAs

I confidently recommend the members of the scientific jury to positively evaluate the dissertation "**Possibilities of some echocardiographic techniques for the detection of subclinical myocardial damage in children and young adults with beta thalassemia major**" and to award Dr. Kalina Binkova Ganeva with the scientific and educational degree of "Doctor".

Sofia

04.05. 2023

Reviewed by:

(prof. Anna Kaneva-Nencheva MD)

