REVIEW

According to the order № P-109-133/05.04.24 of the Rector of MU-Varna

by Prof. Dimitrichka D. Bliznakova, MD, PhD

Subject: competition for the degree of Doctor of Science of Margarita Ivanova Nikolova, MD

Margarita Nikolova, MD was born on 08.01.1992 in Sofia. She graduated from High School 157 GICHE "Cesar Vallejo", Sofia in 2011.

Professional Development:

- In 2017 she graduated from Medical University "Prof. Dr. Paraskev Stoyanov" - Varna;
- Since 2018, she started specialization in pediatric pneumology and phthisiology at the Second Pediatric Clinic.

Academic Development:

- In 2019, after a competitive examination, she was enrolled as a full-time PhD student on the topic "Genotype-phenotype correlation in patients with cystic fibrosis";
- In 2023, she won a competition for a full-time assistant in the Department of Pediatrics.

Publications:

- In relation to the thesis: 5 ;
- Participation in international and national congresses in the field of paediatrics and paediatric pulmonology, as well as publications (with diverse topics) in peer-reviewed Bulgarian and foreign scientific journals;
- Her main scientific interests are in the field of pediatric pulmonology, chronic lung diseases - cystic fibrosis, bronchial asthma.

  Member of Bulgarian Medical Association (BMA), Bulgarian Paediatric Association (BPA), Bulgarian Society of Pulmonary Diseases, European Respiratory Society (ERS), European Cystic Fibrosis Society (ECFS).

She speaks fluently: Bulgarian, English, Spanish

Theme of the dissertation:

"Genotype-phenotype correlation in patients with cystic fibrosis"

The present dissertation highlights the role of knowledge of genotype-phenotype correlations in cystic fibrosis. This provides a basis to predict the course of the disease, prevention of complications, and diagnosis in the absence of neonatal screening at this stage.

The dissertation is presented in 147 pages and contains 16 tables, 32 figures and 2 annexes. 285 references are cited, of which 16 are in Cyrillic and 268 are in Latin.

Cystic fibrosis or cystic fibrosis (CF) is an inherited autosomal recessive disease belonging to the group of rare diseases. It is caused by a defect in a gene encoding a protein - CFTR. It is a chloride channel regulating mainly water and electrolyte transport. Its dysfunction leads to the accumulation of viscous secretions in all ductal structures, and the narrowing and obstruction of their lumen damages the corresponding organ and maintains a process of chronic infectivity. More than 2000 mutations have been described, and more than 300 of them are associated with disease. The phenotypic manifestation of CF ranges from affecting a single system to multisystem. The most significant changes are seen in the airways, GIT, sweat glands and urogenital system. Genotype-phenotype correlations in CF patients have been the subject of a number of studies and are relevant not only to the diagnosis but also to the prognosis of the disease. Class I and II mutations generally predispose to a more severe clinical picture, with genotype-phenotype correlations being more demonstrable for GIT symptoms. The variability of phenotypic manifestations is determined by the influence of genetic factors, environment and adherence to therapy and is difficult to predict.

Modern treatment and care of CF patients by multidisciplinary teams largely depend on their diagnosis and timely referral to a specialized center. For countries without an established mass NS, such as Bulgaria, phenotypic manifestations are the main indicator to search for the underlying genotype and confirm the diagnosis. This is the origin of the interest in the present work and the definition of its aim and objectives.

Prerequisites for its implementation are:

- Lack of developments on the topic in the territory of Northeast Bulgaria;
- Lack of opportunity for early diagnosis due to lack of mass NS;

- Concentration of multidisciplinary teams caring for CF patients in several regional cities of Bulgaria;
- Importance of early diagnosis for timely initiation of appropriate therapy;
- Importance of genotype for prognosis and timely prophylaxis and treatment;
- Availability of a suitable group for the study - patients with CF from North-Eastern Bulgaria who are followed up and treated at the University Hospital "St. Marina".

The purpose of the dissertation is clear, precise. It is based on the evaluation of genotype-phenotype correlation in CF patients from Northeastern Bulgaria who are followed and treated at the St. Marina University Hospital.

There are 5 tasks. They are properly structured. They include analysis of CFTR mutations in CF patients from Northeastern Bulgaria, analysis of age and phenotypic manifestations in confirmation of diagnosis in the absence of mass neonatal screening. Ambitious tasks are 4.1,4.2 and 4,3 to search for correlations between genotype and phenotypic manifestations on respiratory system, GIT, sweat test, CFRD, infertility. Task 5 - to propose an algorithm based on alerting symptoms for early diagnosis of CF, in the absence of mass NS, is extremely important and applicable in Bulgarian outpatient and hospital practice. The hypothesis is well thought out and meets the aim and objectives.

The study comprised 45 patients with proven CF who were enrolled in the ERPM and followed at the St. Marina University Hospital. The inclusion criteria were:

- Confirmed diagnosis of CF by detection of two pathological mutations in CFTR on genetic analysis and/or confirmed diagnosis of CF by two pathological sweat tests and presence of clinical manifestations;
- Signed informed consent to be included in the ERPM and to use the entered data for this dissertation;
- Availability of patient data for the last 5 years.

Methods:

Retrospective study: for the period 2019 to December 2023 in 45 patients aged 5 months to 37 years. Data included history, physical status, laboratory tests, microbiological, genetic and imaging studies.

Statistical methods:

- Method of statistical grouping of data
- Descriptive statistical analysis
- Statistical testing of hypotheses
- Correlation analysis
- Regression analysis

Data processing was performed using SPSS for Windows (ver. 22.0).

Results:

The retrospective study included 45 patients from Northeastern Bulgaria with a diagnosis of CF who were followed and treated at St. Marina University Hospital. A period of 5 years was covered, from January 2019 to December 2023. During this period, after signing informed consent, patients were enrolled in the EPM and the data from the EPM was used for this thesis. Three patients died and two patients left the country and continued their treatment and follow-up abroad.

Of the 45 patients enrolled, 28 were male and 17 were female. There was a male predominance.

The age of CF patients ranges from 5 months to 37 years. Of the patients included, 31 were younger than 18 years and 14 were older than 18 years. The mean age of the population was 14.94±9.80 years.

Two CFTR mutations were identified in 44 CF patients by genetic analysis. In 1 patient, a second mutation was not detected, and the diagnosis of CF was confirmed by two pathological sweat tests and the presence of clinical manifestations.

The F508del/F508del genotype had the highest frequency in 19 patients (42.22% of the genotypes included in the study. F508del/1220X genotype was detected in three patients, F508del/G542X genotype in two patients and F508del/574delA genotype in another two patients.

Ethnicity was tracked with the distribution of these genotypes, with the F508del/F508del genotype being the largest among the Bulgarian ethnicity, and double heterozygotes carrying one F508del and one other mutation predominating among the Turkish ethnicity.

The mean age at confirmation of CF diagnosis of the 45 included patients was 2.14±5.32 years. The earliest diagnosed patient was 2 months old and the latest diagnosed patient was 18 years old.

Clinical manifestations at diagnosis include MI, non-vomiting, chronic diarrhea, OS, anemia, hypoproteinemia, recurrent pneumonia, liver involvement, vomiting with alkalosis, pancreatitis.

During the 5-year follow-up period, there were three deceased patients, two males and one female.

As of 2020, CFTR-modulating therapy was initiated in 30 of the 42 CF patients enrolled, or 71.43%. The largest number of patients are on treatment with Elexacaftor/Tezacaftor/Ivacaftor, 20 patients or 47.62%. Ivacaftor/Lumikaftor treatment was conducted by 9 patients, 21.43%, and Ivacaftor treatment was conducted by only 1 patient, 2.38%. 12 patients or 28.57% of the CF patients from the Northeast did not undergo gene-modification therapy, four of them - 9.52% did not meet the inclusion criteria according to the type of mutations in their genotype, and the remaining 8 of them, or 19.05%, were not included because of other criteria - not reaching the minimum age for initiation, lack of interest on the part of the parents and/or the patient, or a health condition that did not allow starting the therapy.

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The results obtained are very well presented graphically and this enables the relevant analysis to be made.

The following correlations were found:

More severe affected by the class I and II mutations in terms of respiratory manifestations, GIT, and other manifestations - sweat test, CFRD, infertility. Progression of the disease with increasing age is also found.

In discussing the results and comparing them with the literature, it is known that the average life expectancy of CF patients has increased significantly worldwide, but remains low at around 30 years for both men and women. The prognosis in the European Union countries is better. For the included patients from the North-East, it is significantly lower - approximately 18 years, the reasons again being late diagnosis (lack of screening), socio-economic conditions, the functioning of the healthcare system and the centralisation of multidisciplinary teams.

It was confirmed that the most frequent mutation in the group of patients studied was F508del and the most frequent genotype was F508del/F508del.

The presence of genotype-phenotype correlations in respiratory, GIT, and other manifestations - sweat test, CFRD, infertility - has been confirmed.

The role of early confirmation of CF diagnosis in initiating CFTR-modulating therapy in the absence of mass NS is emphasized.

From the present work, it is known that 71.34% of CF patients from Northeastern Bulgaria have already started their treatment with CFTR modulators, with another 19.05% being suitable according to genotype. This percentage also matches the European average of 90% of CF patients having suitable mutations for CFRT modulating therapy.

On the basis of the study and the performance of 7 tasks, an algorithm for alarm symptoms was developed, with the aim of early referral to an expert centre for CF diagnosis and subsequent treatment. Symptoms are from respiratory system, GIT, and others like - anemia, edema, reproductive disorders, hypovitaminosis.

The conclusions of the dissertation are in line with the stated aim and objectives.

- Patients with CF from the Northeast who are followed up in the HPP are included in the EPM;
- We analyzed the distribution of CFTR mutations in CF patients from Northeastern Bulgaria, followed up in the VPP for the first time in our country;

- A more severe course of CF has been confirmed in patients with a genotype including class I and/or class II mutations compared to patients with a genotype including class III - VI or VUS mutations;
- Genotype-phenotype correlations have been found in CF patients from Northeastern Bulgaria regarding respiratory, GIT and other manifestations - sweat test, CFRD;
- An algorithm based on alerting symptoms for CF was created for early referral to a reference centre and subsequent diagnosis, treatment and follow-up.

Conclusion:

The dissertation of Margarita Nikolova, MD "Genotype - phenotype correlation in patients with cystic fibrosis" is extremely valuable for clinical practice in patients with one of the severe chronic polyorgan diseases. Gone are the years when children died at a young age. Advances in science and new technologies, especially in the field of genetics, have provided answers to many questions that give us the basis to make a timely diagnosis, to look for genotype-phenotype correlations related to the severity of the disease and to predict its course... And here we can quote the words of Jeff Fairlow (patient with CF) "Most importantly, cystic fibrosis has given me a good outlook on life, showing that life does not end with a medical diagnosis. It is a serious disease and requires a good psychological approach to patients, especially to parents and the sick child. Confidence, good cooperation in treatment, helps in the battle for survival. And everyone dreams of tomorrow being better than today."

When a clear goal is developed and the tasks are defined, the realization of the dissertation topic meets modern requirements. The main goal is to give light to the future of these patients to improve their quality of life, which requires the application of an individual approach in their care and treatment.

I propose the respected scientific jury to award the educational and scientific degree "DOCTOR" in the scientific specialty of “Pediatrics” to Margarita Nikolova, MD.

16.05.2024

Prof. Dimitrichka Bizmakova, MD, PhD