THE IMPACT OF THE STAPHYLOCOCCUS AUREUS INFECTION ON THE EVOLUTION OF CHILDREN WITH CYSTIC FIBROSIS FROM A REGIONAL CENTRE IN NORTH-EASTERN ROMANIA

Dana-Teodora Anton-Paduraru[1], Mihaela Moscalu[2], Gabriela Coman[3], Laura Florescu[4], Laura-Mihaela Trandafir[5]

[1,5]3rd Clinic of Pediatrics, "Grigore T. Popa" University of Medicine and Pharmacy, Iasi
[2]Department of Medical Informatics and Biostatistics, "Grigore T. Popa" University of Medicine and Pharmacy, Iasi
[3]Laboratory, "Sf. Maria" Children Emergency Hospital, "Grigore T. Popa" University of Medicine and Pharmacy, Iasi
[4]Pediatric care, "Grigore T. Popa" University of Medicine and Pharmacy, Iasi

ABSTRACT

Introduction. Chronic bacterial infections and – often – acute infectious exacerbations are characteristic to the lung disease of the cystic fibrosis (CF). The objective of this study was to identify children with CF and Staphylococcus aureus infection and quantify the impact of this infection on their clinical status.
Material and methods. A prospective study was carried out during 3 years on a batch of 37 children with CF registered in the records of the Regional Monitoring Centre of the 3rd Clinic of Pediatrics of Iasi, Romania. In these patients, we searched for the presence of S. aureus, the prevalence of methicillin-resistant S. aureus (MRSA) strains, the impact of the chronic infection with MRSA on the lung function concomitant with the presence of severity elements in CF.
Results. 22 of the 37 patients with CF had positive culture for S. Aureus; at 9 of them was isolated MRSA and 5 had chronic infection. The MRSA positive group of patients presented significantly lower values of the FEV1, a more poor nutritional status, severe bronchiectases, a larger number of pulmonary exacerbations and implicitly hospitalizations, pancreatic insufficiency in most cases and a higher degree of association with the F508del genotype.
Conclusions. S. aureus remains an important pathogen in CF. Due to its high pathogeny, S. aureus and especially the MRSA strains can contribute to an unfavourable clinical evolution.

Keywords: cystic fibrosis, children, Staphylococcus aureus

INTRODUCTION

Cystic fibrosis (CF) – the most frequent autosomal recessive disorder of the Caucasian population, is a complex disease, Often, the respiratory tract is the most affected and the lung disease is characterized by chronic infections (positive cultures after 6 months of germ isolation) or recurrent infections, inflammation and development of brochiectases (1).

The lungs of the patients with CF are the home of numerous bacterial infections, *Staphylococcus aureus* being one of the first bacteria detected in the respiratory tract of these children (2). Increased prevalence of methicillin-resistant *S. aureus* (MRSA during the past decade was associated with the worsening of the evolution of these disease (3). If in 1996 only approximately 2% of the patients included in the patients records of the CF Foundation had one or more positive MRSA cultures, in 2005 the prevalence increased to 17.2% and in 2007 to 21% (1,4). Recent studies reported that the persistent MRSA infection affects the lung function and survival (5,6).

Corresponding author:
Mihaela Moscalu, University of Medicine and Pharmacy "Grigore T. Popa", 16 Universitatii St., 700115 Iasi
E-mail: mmoscalu@yahoo.com

The objective of this study was to identify children with CF and *S.aureus* infection and to quantify the impact of this infection on their clinical status, the MRSA prevalence in these children, as well as the chronic MRSA infection impact on the lung function and the presence of severity elements in CF (bronchiectasis, severe lung disease, poor nutritional status).

MATERIAL AND METHODS

Patients

The prospective study between January 2010 and December 2012 was carried out on a lot of 37 children suffering from CF, registered in the records of the Regional CF Monitoring Centre of the 3rd Clinic of Pediatrics – "Sf. Maria" Children Clinical Emergency Hospital of Iasi.

All patients with CF were submitted to the micro-bacteriological test of the hypopharyngeal aspirate or the sputum for *S. aureus*. 3 study groups were made up, depending on their methicillin resistance: group 1 – patients with MRSA; group 2 – patients with methicillin-sensitive *S. aureus* (MSSA); group 3 (control) – patients in whom *S. aureus* was not isolated. The presence of minimum three positive cultures a year was considered a chronic MRSA infection. The association of the infection with *Pseudomonas aeruginosa* and the presence of bronchiectasis were also monitored (periodically patients were submitted to a lung CT scan).

All the parents of the children included in the study signed written informed consent.

Clinical data

In the three study groups made up, we monitored annually: clinical manifestations, nutritional status, pancreatic and lung function, associated infections (especially the association with *P. aeruginosa*).

The nutritional status was assessed by measuring weight, height and the Z score. We considered it necessary to use the Z-score for weight and height in assessing the nutritional status, taking into account that the patients' age was not homogenous, as the study included infants (0-12 months), small children (12-36 months) as well as children between 3 and 18 years. For the calculation of the Z score we used LMS values from the 2000 CDC Growth Charts (National Center for HealthStatistisc/Centers for Disease Control and Prevention) (7).

The pancreatic insufficiency was defined by the fecal elastase value under 200 μg/g fecal matter.

The lung function was assessed periodically (at the time of the patients' inclusion in the study – initially, after 6 months, after 12 months) by spirometry in patients over 6 years old, the pathological value being considered the forced expiratory volume/1 second (FEV_1) under 80%. All patients received antibiotherapy according to the antibiogram and substitutive treatment with pancreatic enzymes.

The CF evolution was assessed based on the nutritional status, the FEV_1 evolution and the presence of bronchiectasis. At early age (0-36 months) and at those who are in the acute phase, unable to perform CT did not allow confirmation or refutation of bronchiectasis in patients at inclusion in the study.

Bacteriological tests

The sputum or hypopharyngeal aspirate samples were examined microscopically and by insemination on ram blood agar, chocolate agar, selective Chapman, MacConkey medium and for the isolation of the Burkholderia cepacia complex. The *S. aureus* colonies were identified by the Coagulase test (latex-agglutination) and the methicillin resistance was determined by testing sensitiveness to cefoxitin (30 μg) and oxacilin (1 μg) micro-caplets on the Mueller-Hinton medium at 35ºC.

Statistical analysis

The statistics was achieved by means of the SPSS 20.0 (SPSS Inc., Chicago, IL, USA, 2011). The statistical study approached two aspects: descriptive statistics and analytical statistics. The descriptive statistics presented the classification and synthesis of the observation data. It concentrates the information existent in the said data by means of certain statistic indicators expressing characteristics and tendencies of the analyzed parameter. Continuous variables were expressed as the mean ± standard deviation (SD) in tables and figures.

Data were compared by means of the Independent Samples T test or T-Test for Dependent Samples (t test pairs). The difference was significant when the value p was < 0.05 (95% – confidence interval, 5% – maximum error probability). In the analysis of the correlation of category variables the Spearman Rank non-parametric correlation test was applied.

The multiple regression analysis was applied in assessing the effect of certain characteristics on an event (a variable defining the patients' evolution).

This test evaluated the odd ratio (exp(β) → OR – oddratio) and the Chi-squared (Wald) statistics testing the relation between the dependent variable and all the co-variables in the model.

RESULTS

Between 2010 and 2012, 22 (59.46%) of the 37 patients diagnosed with CF presented an *S. aureus* infection. The rest of the participants (15 cases – 40.54%) represented the control group.

MRSA was identified in 9 cases (24.32%), and MSSA in 13 cases (35.14%). The patients' characteristics corresponding to the three groups are described in Table 1.

The medium age of the patients in the three groups was 87.27 months ± 59.3 SD, with minimum values of 4 months and maximum values of 216 months. No significant differences were registered between the age values (p = 0.173512, 95%CI) and the gender of the children (p = 0.423) corresponding to the three analyzed groups. The chronic *S.aureus* infection was identified in 21.62% (8 cases), of which 13.51% had MRSA and 8.11% had MSSA. Therefore, the prevalence of the chronic MRSA infection in patients with CF was of 13.51%. The Z-score for weight at the time of the inclusion in the study presented negative values significantly lower in children with MRSA(-2.34 ± 1.31 DS) in comparison with those with MSSA (−0.61 ± 2.03 DS) (p = 0.0.35599, p < 0.05, 95%CI) and the control group (0.33 ± 2.2SD) (p = 0.021091). No significant differences were noticed between the values of Z-score for weight between the MSSA group and the control group (p = 0.72874). The values for Z-score for the height were similar to Z-score for weight: the values of the MRSA group (-1.82 ± 1.08 DS) were significantly lower (p = 0.01293) than the values of the control group (-0.81 ± 1.78 DS), and than those of the MSSA patients (-0.07 ± 1.8 DS) (p = 0.01563).

The association of S. *aureus* (MRSA, MSSA) with *P. aeruginosa* was not statistically significant (χ^2 = 3.780261, p = 0.15106). Other microbial species (*Haemophillus influenzae, Streptococcus pneumoniae, Branhamella catarrhallis, Stenotrophomonas maltophilia, Aspergillus fumigatus*) were present in all studied groups in relatively equal proportions (MRSA – 13.51%, MSSA – 10.81%, control – 8.11%) (χ^2 = 3.27, p = 0.19492). The spirometry performed at the beginning of the study revealed significantly lower FEV_1 values (p = 0.012086) in patients with MRSA (72.8 ± 5.26 SD) in comparison to the values registered in the control group (84.45 ± 8.23 SD). In patients with MSSA, the FEV_1 values were slightly lower than the pathological values (78.64 ± 6.53 SD), but they were not significantly different from the statistical point of view in comparison to the FEV_1 values of the control group (p = 0.08109).

The evolutive aspects of patients with CF after 12 months and 24 months are presented in Table 2.

FEV_1 values in the group of patients with MRSA presented significant decreases (p = 0.03486) in comparison with the initial time (72.8 ± 5.26 SD). The mean values of FEV_1 after 12 months were 65.2 ± 9.88 SD, and they remained low after 24 months (63 ± 13.27 SD) under eradication treatment according to the antibiogram. In patients with MSSA the FEV_1 values remained at the same level, the variations being insignificant (p = 0.6568): initially: 78.64 ± 6.53 SD, after 12 months: 77.73 ± 6.37 SD, 24 months: 77.54 ± 7.89 SD. Significant improvement of the FEV_1 (p = 0.000454) was registered by patients in the control group (initial: 84.45 ± 8.23 SD, after 12 months: 89 ± 5.81 SD, 24 months: 93.39 ± 4.17 SD).

TABLE 1. *Patients' characteristics at the begining of the study*

Characteristics	All patients (n = 37)	MRSA (n = 9-24.32%)	MSSA (n = 13-35.14%)	Control (n = 15-40.54%)	*p-value t test-pairs*
Age (month)	87.27 ± 59.3SDS	71.78 ± 65.41SD	112.08 ± 63.69SD	75.07 ± 47.29SD	0.17351
Gender (male/female)	24/13 (64.9%/35.1%)	6/3 (66.7%/33.3%)	10/3 (76.9%/23.1%)	8/7 (53.3%/46.7%)	0.4237
Z Score Weight-for-age	-0.9±2.08SD	-2.34 ± 1.31SD	-0.61 ± 2.03SD	-0.33 ± 2.2SD	0.0392
Z Score Length-for-age	-0.80 ± 1.74SD	-1.85 ± 1.08SD	-0.07 ± 1.8SD	-0.81 ± 1.78SD	0.0178
Chronic *S. aureus*	8 (21.62%)	5 (13.51%)	3 (8.11%)	-	0.00589
Pseudomonas aeruginosa	8 (21.62%)	3 (8.11%)	4 (10.81%)	1 (2.7%)	0.15106
Associated infections n (%)	12 (32.43%)	5 (13.51%)	4 (10.81%)	3 (8.11%)	0.1949
FEV_1 (% pred)	79.93 ± 8.11SD	72.8 ± 5.26SD	78.64 ± 6.53SD	84.45 ± 8.23SD	0.01619
*Mean ± SD (standard deviation)					

TABLE 2. *Evolutive aspects in patients with CF per groups: MRSA/MSSA/control*

Characteristics	Group	initial	12months	24months	*p-value T test-pairs*
FEV_1 (% pred)*	MRSA	72.8 ± 5.26SD	65.2 ± 9.88SD	63 ± 13.27SD	***0.0348634***
	MSSA	78.64 ± 6.53SD	77.73 ± 6.37SD	77.54 ± 7.89SD	***0.6568177***
	Control	84.45 ± 8.23SD	89 ± 5.81SD	93.39 ± 4.17SD	***0.0004544***
p-value (Independent Samples T test)		***0.016194***	***0.000004***	***0.00000061***	
Z Score* Weight-for-age	MRSA	-2.34 ± 1.30SD	-1.98 ± 1.13SD	-1.80 ± 1.30SD	***0.342155***
	MSSA	-0.60 ± 2.02SD	0.11 ± 2.25SD	0.37 ± 2.36SD	***0.003203***
	Control	-0.32 ± 2.2SD	0.14 ± 1.62SD	0.57 ± 1.70SD	***0.000533***
p-value (Independent Samples T test)		***0.052484***	***0.014574***	***0.018117***	
Z Score* Length-for-age	MRSA	-1.85 ± 1.08SD	-1.46 ± 1.12SD	-1.19 ± 1.17SD	***0.06248***
	MSSA	-0.52 ± 1.80SD	0.14 ± 1.67SD	0.68 ± 1.70SD	***0.00014***
	Control	0.07 ± 1.78SD	0.41 ± 1.57SD	0.72 ± 1.45SD	***0.05134***
p-value (Independent Samples T test)		***0.058041***	***0.046988***	***0.051252***	
Bronchiectasis n(%)	MRSA (n = 9)		6 (16.22%)	8 (21.62%)	
	MSSA (n = 13)		5 (13.51%)	7 (18.92%)	
	Control (n = 15)		3(8.11%)	6 (16.22%)	
Spearman correlation: p-value			**0.02503**	***0.04503***	
Pulmonary exacerbation* Allgroup: 4.72±2.24SD	MRSA (n = 9)			6.33 ± 1.87SD	
	MSSA (n = 13)			4.67 ± 2.5SD	
	Control (n = 15)			3.8 ± 1.74SD	
p-value (Independent Samples T test)				***0.02207***	
Death (3 cases/8.11%)	MRSA			3 (8.11%)	
	MSSA			-	
	Control			-	
Spearman correlation: p-value				***0.01042***	
*Mean ± SD (standard deviation)					

The nutritional status assessed depending on the values of Z-score for weight and age improved in the MSSA group (p = 0.003203): initially: -0.60 ± 2.02 SD, 12 months: 0.11 ± 2.25 SD, 24 months: 0.37 ± 2.36 SD and the control group (p = 0.000533): initially: -0.32 ± 2.2 SD, 12 months: 0.14 ± 1.62 SD, 24 months: 0.57 ± 1.70 SD. In the MRSA group, the values of the Z-score for weight and age increased insignificantly (p = 0.342155): initially: -2.34 ± 1.30 SD, 12 months: -1.98 ± 1.13 SD, 24 months: −1.80 ± 1.30 SD.

The values of the Z-score for height and age in the MRSA group did not improve significantly (p = 0.6248). Moreover, these presented negative means (small height for their age): initially: -1.85 ± 1.08 SD, after 12 months: -1.46 ± 1.12 SD, 24 months: -1.19 ± 1.17 SD. In exchange, a significant increase in the mean values registered in the MSSA group (p = 0.00014): initially: -0.52 ± 1.80 SD, after 12 months: 0.14 ± 1.67 SD, 24 months: 0.68 ± 1.70 SD, and in the control group the changes in the mean values were insignificant (p = 0.05134): initially: 0.07 ± 1.78 SD, after 12 months: 0.41 ± 1.57 SD, 24 months: -0.72 ± 1.45 SD – Fig. 1.

The prevalence of bronchiectasis after 12 months from the study initiation in the MRSA group was of 16.22% (6 cases), in the MSSA group of 13.51% (5 cases), and in the control group of 8.11% (3 cases). These results demonstrated the significant association between the presence of brochiectasis and MRSA ($\chi^2 = 5.289019$, p = 0.2503). After 24 months there was an increase in the prevalence of brochiectasis in all analyzed groups (MRSA: 21.62%, MSSA: 18.92%, control: 16.22%), the significant inter-group differences remaining the same ($\chi^2 = 6.200989$, p = 0.04503).

At the end of the study period, the number of pulmonary exacerbations and the number of hospitalizations were assessed. In the MRSA group this number (mean: 6.33 ± 1.87 DS) was significantly higher in comparison with the MSSA group (mean: 4.67 ± 2.5 DS) and in comparison to the control group (mean: 3.8 ± 1.74 DS).

During the past 24 months of monitoring, there were 3 deaths (8.11%) due to acute respiratory distress, all of them belonging to the MRSA group.

The CF evolution depending on the impact of the *S. aureus* infection was assessed by means of the multivariate test, using a multiple regression (stepwise regression) analysis model offering a useful means for the modelling of the dependence of a response variable (dependent variable) to sev-

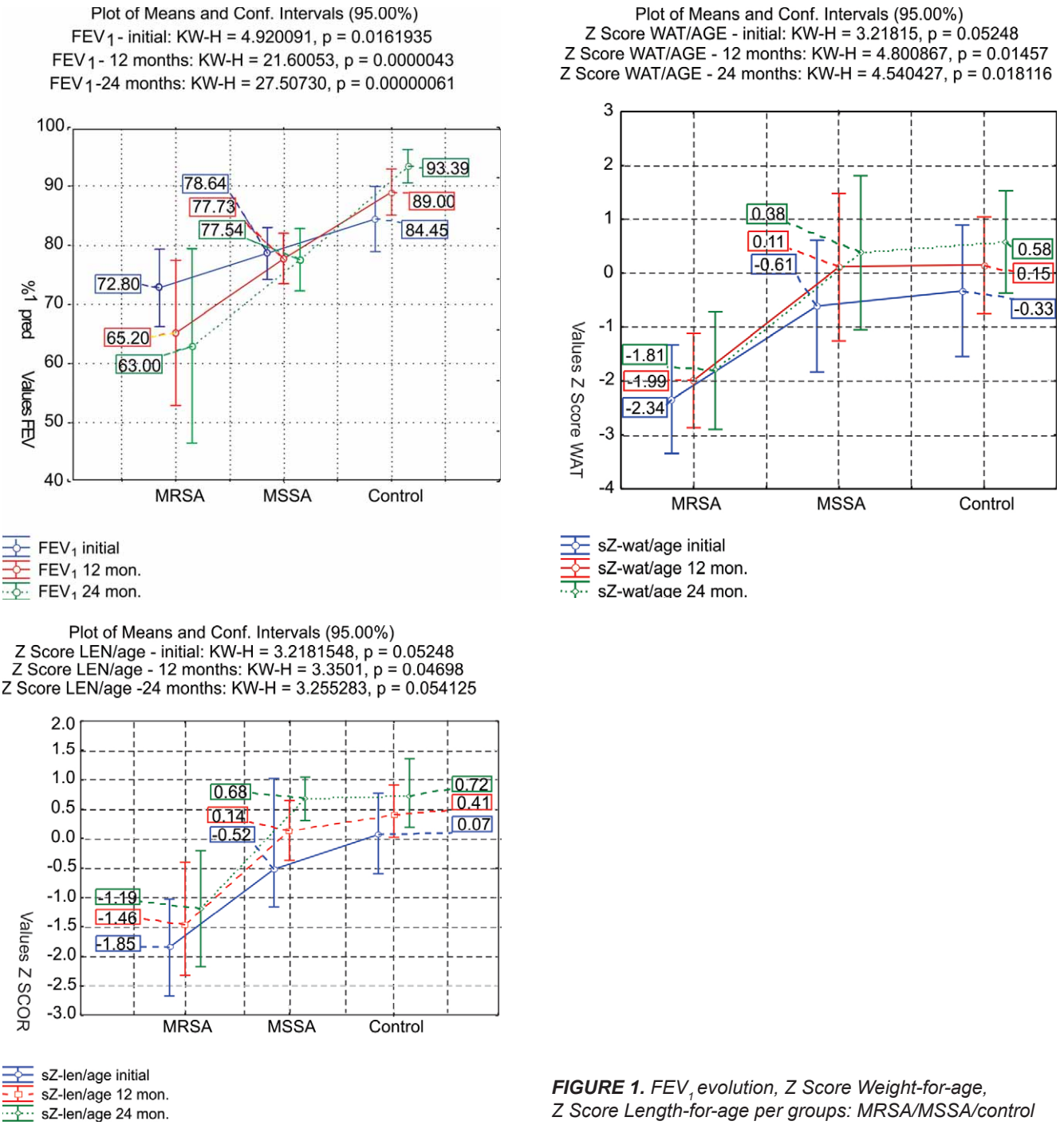

FIGURE 1. *FEV_1 evolution, Z Score Weight-for-age, Z Score Length-for-age per groups: MRSA/MSSA/control*

eral independent-explicative variables, called predictors. The independent variables (predictors) can be categoric or continuous variables. In our study, the number of exacerbations was considered a dependent variable and the independent variables were MRSA/MSSA, chronic *S. aureus* infection, associated infections.

Odd ratio calculated for the number of exacerbations Exp(β) = 2.76 (95%CI: 1.18 ÷ 4.34) proves that in chronic *S. aureus* infections the number of exacerbations is 2.76 times higher. The results of the multiple correlation indicated the absence of a significant correlation between the MRSA and the high number of exacerbations (OR = -0.23, β = -0.64, p = 0.187) as well as between the presence of associated infections and the number of exacerbations (OR = -0.05, β = 0.27, p = 0.724) Table 3.

DISCUSSIONS

CF is responsible for the chronic obstruction of the respiratory tract – a favourable situation for pulmonary infections during childhood.

Unlike the patients without CF, in which *S.aureus* is an unusual cause for upper respiratory tract infections, in patients with CF *S.aureus* is the

TABLE 3. *Multiple regression analysis regarding the influence of the S. aureus infection on the evolution of patients with CF*

R^2 = 0.314984 p = 0.006463 (95%CI)	Beta	SE	Wald	Sig. p	OddRatio Exp(β)	95% CI for Exp(B)	
						Lower	Upper
Intercept	70.47875	49.31697	1.42910	0.162666			
MRSA/MSSA	-0.64251	0.47687	-1.34733	0.187336	-0.232781	-0.584706	0.119143
Chronical *S. aureus*	0.370449	0.99401	3.56459	0.001105	2.767857	1.189846	4.345868
Associated infections	0.27631	0.77831	0.35501	0.724913	0.059039	-0.279712	0.397791

most frequent pathogen (8). It is to be found in 30% of the children under 3 months, in the bronchoalveolar lavage fluid, and in some clinics the prevalence is of approximately 50% in children under 10 years (9). In our study, 59.6% of the patients had *S. aureus* infection.

The MRSA strains represent the most threatening challenge, being frequently multi-resistant to antibiotics. Patients with CF have a particular risk for pulmonary colonization with MRSA, both due to the difficulty in evacuating the mucus, and due to their repeated hospitalizations, which increase the exposure to them. MRSA was identified for the first time in 1960 and was admitted as a major pathogen in 1980 (10). The prevalence of the MRSA infection in the lot studied was of 24.32%, the chronic infection being confirmed in 13,51% of the cases.

The methicillin-sensitive strains identified in 35,14% cases also represent a risk for patients with CF due to the existence of the biofilm in the infected lung, where antibiotics cannot penetrate.

S.aureus itself is not an ordinary cause of morbidity and mortality significant in CF, but it can determine tissue destruction and predispose to acute *P. aeruginosa* infection, which is not confirmed in the lot studied.

Among the factors associated with a high risk of lapse of the FEV_1 measured lung function, we quote the following: pancreatic insufficiency, poor nutritional status, *P. aeruginosa* infection, and as an additional factor – the number of pulmonary exacerbations (5).

In the studied lot, low values of the pancreatic elastase was observed in 6 of 9 patients with MRSA concomitant with an unfavourable clinical evolution.

MRSA infection in children with CF has a negative effect on growth (11,12,13). In comparison to the patients in the MSSA group, patients with MRSA had an unfavourable evolution of weight and height to which inappetence, increased catabolism and repeated admissions contributed (14). There were no significant differences between the two genders, in comparison to the study done by Murphy AJ, where a decrease of the Z-score for weight and height was observed with age, especially in males (15).

Recent epidemiological studies reveal contradictory data regarding the impact of the MRSA infection on the clinical evolution of patients with CF. According to some authors, MRSA infection as well as the *S.aureus – P. aeruginosa* co-infection can be associated with a faster decline of the lung function (16,17,18,19,20). Furthermore, the MRSA infection determined the decrease of the lung function in comparison to the MSSA infection (21,22). Dasenbrook et. al (5) showed that the chronic MRSA infection in patients with CF with ages between 8 and 21 years was associated with a 0.5% decrease of the FEV_1 a year.

The chronic infection with MRSA in CF is associated with severe bronchiectasis (23); in study presented, the presence of bronchiectasis was confirmed in 8 of the 9 patients with MRSA. The presence of bronchiectasis is a marker of severe pulmonary destruction and can favour the colonization with resistant bacteria (24).

During the study we observed higher decrease of the FEV_1 in the group of patients infected with MRSA, in agreement with the literature data according to which a MRSA infectious episode can determine a lapse twice as high of the FEV_1 in comparison to patients with no MRSA (25).

Patients with MRSA have an increase frequency of the number of admissions and need oral, inhaled and intravenous antibiotherapy. At the same time, hospital admissions are a major risk factor for the acquisition of MRSA (26).

In the group of patients with MRSA, the most frequent mutation was F508del (6 cases). We can argue that this genotype is associated with the severe lung disease (27). All 3 patients that died by respiratory distress had chronic MRSA infection and none of them benefited from a lung transplant.

CONCLUSION

S.aureus infection was present in 50% of patients with CF with predominantly pulmonary manifestations, reflecting the unfavorable clinical outcome.

The chronic MRSA colonization was associated with the precarious nutritional status, the presence of bronchiectasis, pancreatic insufficiency, the F508del genotype, and with a long hospitalization period. Due to the high pathogenity potential, the early treatment and, if possible, the eradication of the *S.aureus* infection represent the most adequate measures for increasing the life quality at these patients.

Conflict of interests

The authors state that they have no conflict of interest.

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