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Clinical genetic, microbiological and functional parameters of mucoviscidosis patients in Moscow and the Moscow Region

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The study was aimed at assessing clinical genetic, microbiological and functional peculiarities of mucoviscidosis patients residing in Moscow and the Moscow Region by 31.12.2010. The average age of the patients was 12.1 ± 9.6 (0.2-43.2), median – 11.0 (16.0) years. The share of adults (patients over 18 years of age) was 30.6%. Age median at diagnosis establishment was 1.0 (5.0) years; diagnosis was established in adulthood to 3.8% of the patients (12.4% of the adult patients). The rate of the most widespread mutation – F508del – was 52.96%. The authors also observed a relatively high rate of mild genotypes among the adult patients. High infection rate of Burkholderia cepacia complex (8.7%) is of particular interest in the microbiological profile structure of the respiratory tract. The authors established considerable increase in the development rate of pneumothorax, hemoptysis, hypoxemic respiratory failure and pancreatic diabetes with fasting hyperglycemia, whereas the rate of hepatic cirrhosis with portal hypertension is comparable in the groups of adults and children.

Keywords: *mucoviscidosis, Moscow and the Moscow Region, register, age-specific peculiarities, dornase alfa.*

Introduction

Mucoviscidosis is one of the diseases considerably reducing patient life quality and expectancy. This nosology is caused by the *CFTR* gene mutation (mucoviscidosis transmembrane regulator), which leads to malfunction of the chloride channel localized in the apical membrane of exocrine glandular cells. This results in alteration of the generated secretion's physicochemical properties, which leads to the chain of pathologic reactions in various organs: tissue autolysis due to the glandular duct blockade with viscous secretion and development of fibrotic cavernous pancreatitis in the pancreas, early overlay of an infection with pattern development of purulent and, later, of purulent obstructive bronchitis and respiratory failure in the respiratory tract, development of intrahepatic tubular cholestasis and impaired bile lithogenicity in liver and obturation of vasa deferentia with the development of obstructive azoospermia and infertility.

There are National Registers of Mucoviscidosis Patients in the countries with high mucoviscidosis rate [1-6]. The need in keeping a Register on the national level is apparent: it is primarily the means of optimizing data and unifying the crucial clinical information on mucoviscidosis patients. The result of register creation and annual analysis of the data thereof

consists in understanding the following crucial epidemiological parameters: number of patients, including dead persons, in different regions of Russia, age of patients, gender distribution of patients. Such an appraisal yields the survivability (Kaplan-Meier median survival), which may be calculated for the country in whole or for each region in particular. Survivability indicates efficacy of the therapeutic and administrative measures conducted in a particular period of time. Appraisal of the median survival dynamics and analysis thereof for regions of Russia and other countries help to optimize long-term management tactics for mucoviscidosis patients [7]. It is equally important to appraise several clinical parameters reflected in the Register: the rate of various pulmonary and extrapulmonary complications, genetic status, social and family status, level of education, pernicious habits, amount of drug and rehabilitation measures.

The National Register of Mucoviscidosis Patients is only being developed in the Russian Federation (RF), which is why it appears relevant to analyze data of the mucoviscidosis patients residing in different RF subjects.

The study was aimed at determining clinical course peculiarities of the disease in different age periods on a sample of mucoviscidosis patients residing in Moscow and the Moscow Region.

Patients and methods

STUDY SUBJECTS

By 31.12.2010, 359 patients (183 men and 176 women) with confirmed diagnosis of mucoviscidosis established on the basis of clinical pattern data of the positive sweat test and/or genetic examination were registered in Moscow and the Moscow Region. The patients were observed at the Moscow Center for Mucoviscidosis at the premises of the Filatov Pediatric Municipal Clinical Hospital, at the Russian Pediatric Clinical Hospital, at the RAMS Scientific Center of Children's Health and at the Pulmonology Research Institute of the Russian FBMA (FSI).

STUDY METHODS

Extracts from medical cards and outpatient hospitals were subjected to retrospective analysis.

We appraised the following clinical data: age at diagnosis establishment, level of sweat chlorides (sweat test), pulmonary (pneumothorax, hypoxemic respiratory failure, hemoptysis / pulmonary hemorrhage) and extrapulmonary (pancreatic diabetes with fasting hyperglycemia, meconium ileus [anamnesis]) complications.

We appraised the respiratory function in the patients over 6 years of age according to the ERS/ATS criteria, forced expiratory volume in the first second (FEV1) and forced pulmonary vital capacity (FVC) as a percentage of the required values. If medical documents featured data of several spirometric tests, the analysis involved results of the test with the highest values.

Alteration or preservation of the pancreatic function was appraised on the basis of clinical symptoms (presence/absence of steatorrhea), coprogram and/or stool elastase-1. Pancreatic failure was established if a sufficient amount of neutral fat and/or elastase concentration of less than 200 mcg/g was identified with the help of a coprogram. Hemoglobin oxygen saturation was determined using pulse oximetry after sufficient rest and in the sitting position with the help of pulse oximeter Nonin (USA).

Most patients underwent a genetic examination concerning identification of mucoviscidosis mutations. DNA was extracted from peripheral blood leukocyte using a DNA extraction assay kit DNAPrep100 (DIAtomTM, Russia) according to the manufacturer's protocol. We used the method of multiplex amplification to study 11 insertion/deletion mutations (*CFTRdele2,3, F508del, I507del, 1677delTA, 2143delT, 2184insA, 394delTT, 3821delT, L138ins, 604insA, 3944delTG*). We used the method os allele-specific ligation with subsequent amplification in order to register 7 impact mutations (*G542X, W1282X, N1303K, R334W and 3849 + 10kbC > T,*

S1196X, 621 + 1G > T, E92K). Several patients underwent determination of nucleotide sequence with the method of direct automatic sequence analysis (apparatus manufactured by Applied Biosystems, Japan-USA) according to the manufacturer's protocol. We determined the allele frequency for all the detected mutations and identified genotype "severity": "severe" genotype – class I-III mutation, "mild" genotype – at least one class IV-V mutation.

We also examined microbiological profile of the lower respiratory tract. We considered the rate of pathological flora inoculation corresponding to the Leads (Great Britain) mucoviscidosis center criteria a chronic respiratory tract infection. We took into consideration the amount of therapy with the following drugs: dornase alfa, inhaled antibiotics, bronchial spasmolytics, pancreatic enzymes and ursodeoxycholic acid preparations.

We conducted comparative analysis of the aforementioned clinical genetic, functional and microbiological data and of the drug therapy amount between the group of adults and the group of children.

STATISTICAL DATA MANIPULATION

Results were analyzed using software package SPSS (SPSS Inc., USA). We analyzed correlation of the analyzed parameter's distribution values with the normal distribution law. Depending on the type of distribution, we used the mean value (M) ± the standard deviation (SD) or median (Me) (interquartile range) as the measures of central tendency and dispersion. We used the Student's *t*-test or the Mann-Whitney *U* test to compare mean values or medians. We used the χ^2 test or the Fisher's exact test to appraise differences between categorical variables in subgroups. The differences were considered statistically significant at $p < 0.05$.

Results

Age structure of the patients is given in pic. 1. The average age of the children was 12.1 ± 9.6 years (0.2-43.2), the age median – 11.0 (16.0) years. The share of adults (patients over 18 years of age) was 30.6%. Age median at diagnosis establishment was 1.0 (5.0) years; diagnosis was established in adulthood to 3.8% of the patients (12.4% of the adult patients) (pic. 2).

The average concentration of sweat chlorides (according to the sweat test conducted in order to establish diagnosis) was 88.8 ± 30.3 mmol/l (pic. 3).

Allele frequency of mucoviscidosis mutations with the rate of occurrence in the group over 0.5% is given in tb. 1 in the descending order. We also identified rarer mutations (with the rate of occurrence below 0.5%): *394delTT, 3821delT, Y569H, W1282R, 604insA, R1162X, S466X, K710X, D579Y, S1159P, 3677insTAAA, G461E, D572N, R553X, 2789+5G >A, Q493R, 4015delA, 3272-16T >A, G85E*. The total rate of the identified alleles was 86.5%. We revealed a significantly higher rate of occurrence of "mild" genotypes in adults (21.4%) in comparison with children (3.1%) ($p < 0.001$).

Appraisal of the respiratory tract's microbiological profile demonstrated deteriorative reduction, increase in contamination with *Staphylococcus aureus* and symmetrical increase in the inoculation rate of gram-negative flora. It has been revealed that a very high rate of a *B. cepacia complex*-induced infection development is observed in certain age periods (particularly, at the age of 18-24 years) (pic. 4). The total group rate of respiratory tract contamination with various flora genera is given in the descending order: *S. aureus* (monoculture) – 59.7%, *Pseudomonas aeruginosa* (monoculture or in combination with *S. aureus*) – 27.8%, *B. cepacia complex* (monoculture or in combination with *S. aureus* and/or any other gram-negative flora) – 8.7%, Achromobacter sp. (monoculture or in combination with *S. aureus* and/or *P. aeruginosa*) – 2.4%, Stenotrophomonas maltophilia (monoculture or in combination with *S. aureus* and/or *P. aeruginosa*) – 1.4%.

The average values of FEV1 and FVC in adults were significantly lower than in children: 57.3 ± 27.1 and 76.4 ± 23.9% (of the normal value); 81.8 ± 19.5 and 85.9 ± 17.6% (of the normal value), respectively ($p < 0.001$ and $p = 0.004$).
Comparative analysis of complication rates is given in tb. 2. Rates of different complications are given in the descending order: hepatic cirrhosis with portal hypertension – 5.6%, meconium ileus (anamnesis) – 5.3%, pancreatic diabetes with fasting hyperglycemia – 4.5%, hemoptysis / pulmonary hemorrhage – 3.6%, hypoxemic respiratory failure – 2.2% and pneumothorax in the last year – 2.0%.
The drug therapy amount is given in tb. 3.

Discussion

This study heralded the first analysis of clinical genetic, microbiological and functional data of a large sample of mucoviscidosis patients residing within one region conducted in Russia. I.K. Asherova had conducted a detailed study before: she analyzed mucoviscidosis patients residing in the Yaroslavl Region [8]. However, a huge difference in population density between the Moscow Region and the Yaroslavl Region predetermined a considerably lower number of patients fitting that study – only 53 persons. Territorial proximity of Moscow and Yaroslavl and close scientific and clinical cooperation between these cities predetermined a certain similarity of a range of the primary data, such as the group's average age, age at diagnosis establishment, *F508del* occurrence rate and the amount of patients infected with *P. aeruginosa* (in Yaroslavl – 43.4 and 30.2%, respectively). On the other hand, foundation of the Moscow centers before the centers in Yaroslavl, which predetermined accumulation of more extensive experience, and uninterrupted drug supply observed in Moscow in recent years resulted in slightly better results obtained in our study. E.g., the share of adult patients in the study by I.K. Asherova was 22.7%, which is lower by almost 1/3 than in our study. The lower share of adults predetermined (among other things) lower survivability of the patients at the Yaroslavl center (26.8 years) than of the patients residing in Moscow (35.7 years) [9].
Comparison of the obtained results with the data of Registers of Mucoviscidosis Patients of the Western countries demonstrated that the Moscow Region falls far behind the developed countries in terms of a range of parameters, primarily of the share of adult patients: the number of countries with prevalence of adult patients over children is increasing every year, whereas in the Moscow Region the share of adult patients is only 30.6%. The average age of the patients residing in a particular region is also closely associated with the share of adults: the values obtained by both our study and the study by I.K. Asherova are considerably worse, than the values indicated in Registers of the Western countries. Thus, the age median was 17 years in Great Britain (2010), 16 years in France (2009), 20 years in Canada (2010), 17.6 years in Australia (2009) and 19 years in Ireland (2010) [2-6]. Such a difference in the age parameters is caused by a considerably earlier beginning of mucoviscidosis treatment in those countries.
Determination of the allele *MBTP* mutation rate in the Moscow Region and the Yaroslavl Region demonstrated certain peculiarities in comparison with results of genetic examinations of patients in the Western countries. Lower *F508del* occurrence rate corresponds to the data obtained by the previous studies demonstrating lower occurrence rate of this mutation in Russia due to its distribution from North-West to South-East [10]. Other specific genetic profile peculiarities of Russian patients are a relatively high rate of extensive deletion affecting exons 2 and 3 (the so called Slavic mutation), the allele frequency whereof among the Yaroslavl and Moscow patients was 4.72 and 9.02%, respectively. Other mutations frequently occurring in the central Russia are mutations *2184insA* and *2143delT* (total allele frequency – more than 4%) with frameshift, which occur in Western and Central Europe far less often. Certain migrational processes resulted in a relatively high occurrence rate of mutation W1282X, which is considered ethnic for the Ashkenazi Jews. The most peculiar among the other mutations characteristic of certain nationalities and ethnic groups are *1677delTA*, which occurs only in the people residing in the

Caucasus region, and *E92K* – the so called Chuvash mutation. Discovery of mutation *E92K* was a considerable success of Russian geneticists. This discovery did not only unveil the mystery of the "latent" mutation observed in the Chuvash people [11], but also helped to improve identification of mutations in other regions; the allele frequency over 1% among the Moscow patients is a vivid example. It ought to be mentioned that this mutation is considerably less often observed in Tatars and other Turkic nationalities. Another important genetic profile peculiarity of mucoviscidosis patients in the RF is a relatively high occurrence of "mild" mutations; the total allele frequency thereof was 7.5% in our study. Taking into consideration a certain amount of patients without pancreatic failure, i.e. with developed "mild" phenotype and one or both undetected mutations, we may assume that the total allele frequency of "mild" mutations is close to exceed 10%. Such a high occurrence rate of these mutations may predetermine relatively frequent under-diagnosis of mucoviscidosis in the central Russia. Higher occurrence rate of these genotypes among adult patients is caused by late diagnosis (which often occurs in adulthood) and higher survivability of the patients with "mild" genotype.

Results of appraisal of occurrence rate of the primary respiratory tract pathogens are comparable with the data indicated in Registers of the Western countries, which demonstrate a steady increase in the rate of contamination with gram-negative flora with age and symmetrical decrease in the *S. aureus* colonization. Our study also revealed a high rate of the *B. cepacia complex*-induced infection, which is several times higher than in most countries of Europe, North America and Australia. This arouses a certain concern, as this very infection is associated with faster degradation of pulmonary function and, in some cases, with rapid deterioration in the condition and development of the so called cepacia syndrome, which is characterized by very high mortality.

Results of a comparative analysis of clinical functional data, the rate of complications and the drug therapy amount (the group of adults and the group of children) indicate steady deterioration of the general somatic condition. On the one hand, this involves lower respiratory function values and higher rate of pneumothorax, pulmonary hemoptysis and hypoxemic respiratory failure, on the other – a considerable increase in the rate of pancreatic diabetes. The lack of significant differences in the development rate of hepatic cirrhosis with portal hypertension between adults and children is noteworthy. Apparently, it means that in the vast majority of cases hepatic cirrhosis with portal hypertension do not develop in adults, but almost always results from its development in the children who have attained 18 years of age in the setting of this complication. Another important peculiarity is a significantly smaller number of patients, who had had meconium ileus (according to the anamnesis), among the adults. It may probably be explained by worse general somatic status of such patients and, therefore, lower survivability. On the other hand, professional treatment of this severe life-threatening complication occurring in the first days of life may be linked only with foundation of the first Moscow pediatric mucoviscidosis centers in 1991. Taking into account all the aforementioned, it is not surprising that the first time a patient attained adulthood and passed under observation by therapists was registered only in 2010.

A more pronounced pulmonary involvement and high rate of contamination with *P. aeruginosa* predetermined a more frequent use of bronchial spasmolytics and inhaled antibiotics in adult patients.

Dornase alfa (Pulmozyme by Hoffmann-La Roche Ltd., Switzerland) therapy is an inseparable part of complex treatment of the vast majority of both children and adults. An almost 100% use of this drug in patients of all age groups in our study was determined by its high clinical efficacy, high tolerability and wide availability (dornase alfa is included in federal program "7 nosologies"); this capacitated a range of patients to take this drug BID. High clinical efficacy of dornase alfa is defined not only by its unique mucolytic properties, but also by its anti-inflammatory action. According to clinical studies, dornase alfa therapy (OD or BID) resulted in decrease in the number of patients developing the protocol-provided respiratory infections requiring parenteral administration of antibiotics. The rate of respiratory infections in

the group of patients treated with Pulmozyme OD or BID reduced by 22 and 34%, respectively, in comparison with the placebo group (relative risks of Pulmozyme use OD or BID – 0.78 and 0.66, respectively, in comparison with the placebo) [17]. Use of dornase alfa reliably improves pulmonary function and nutritive status, reduces colonization of the bronchial tree by pathological microflora and rate of bronchopulmonary exacerbations. These data have been obtained in both short-term (2 weeks or 1 month) and long-term clinical studies (1-4 years). Efficacy for all age groups at different degrees of deterioration of spirometric parameters has been demonstrated [12-14]. Pulmonary function starts to improve very quickly when the patients begin the dornase alfa therapy. Thus, a study involving 181 patients (children ≥ 8 years of age and adults) conducted in the USA demonstrated improvement of such parameters as FEV1 and FVC as soon as after 10 days of the therapy using the drug under consideration. The average value of FEV1 increased from 10 to 15% ($p < 0.001$), of FVC – from 10 to 12% ($p < 0.001$) [15]. If only minimal or no improvement of FEV1 is observed in mucoviscidosis patients within the first 2 weeks of the dornase alfa therapy, it is recommended not to withdraw the treatment, as reduction in the risk of respiratory infections and the need in parenteral antibiotic therapy has been observed in the setting of the treatment using this drug [16]. It is important to mention that the dornase alfa therapy ought to be uninterruptedly observed on a daily basis in order to achieve sustained clinical improvement [17]. Most specialists associate the increase in the survivability of mucoviscidosis patients in the last 15-20 years with introduction of this very drug into the everyday therapy [7, 18].

Conclusion

Mucoviscidosis patients residing in Moscow and the Moscow Region have a range of clinical genetic and microbiological peculiarities in comparison with patients from other RF regions and a range of Western countries.

Ageing of mucoviscidosis patients is accompanied by reduction in respiratory function, change of the respiratory tract's microflora to a more aggressive one and increase in the rate of bronchopulmonary and gastrointestinal complications.

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Table 1. Allele frequency of mutations with the rate of occurrence > 0.5% (*n* = 338)

Mutation	Allele frequency, %
F508del	52.96
CFTRdele2,3	9.02
3849+10 kb C >T	3.25
2184insA	2.36
W1282X	2.22
2143delT	1.92
N1303K	1.48
1677delTA	1.33
G542X	1.18
E92K	1.18
L138ins	0.88
S1196X	0.88
R334W	0.59

Table 2. Rate of various complications of the disease (by age)

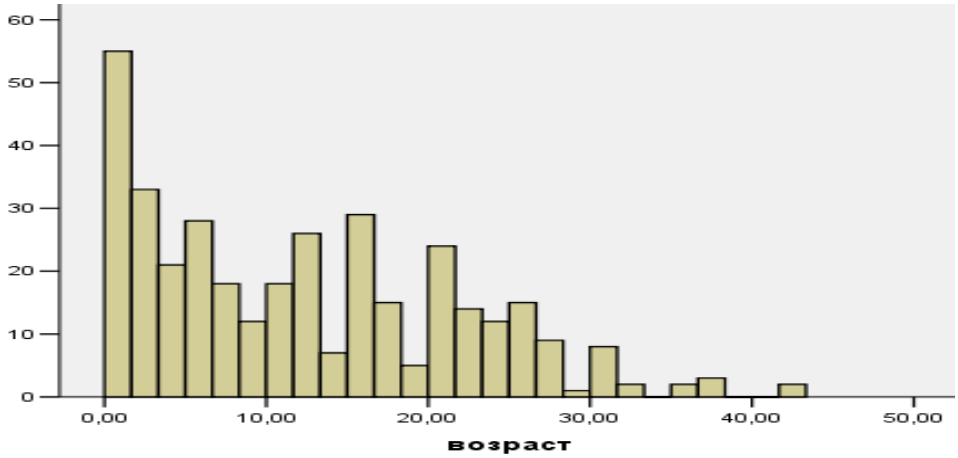
Complication	Under 18 years of age	Over 18 years of age	*p*
Pancreatic diabetes with fasting normoglycemia, %	1.2	11.8	< 0.001
Hepatic cirrhosis with portal hypertension, %	5.2	6.4	NS
Meconium ileus (anamnesis), %	7.2	0.9	< 0.001
Pneumothorax in 2010, %	0.8	4.5	< 0.001
Hemoptysis / pulmonary hemorrhage, %	1.2	9.1	< 0.001
Hypoxemic respiratory failure, %	0	7.3	< 0.001

Note. NS – not significant.

Table 3. Amount of drug therapy (by age)

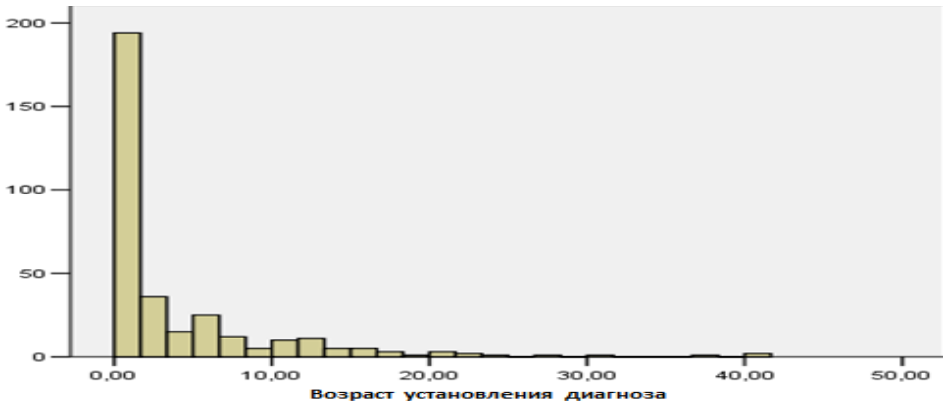
Drugs	Under 18 years of age	Over 18 years of age	*p*
Dornase alfa, %	96.4	95.4	NS
Bronchial spasmolytics, %	42.2	81.2	< 0.001
Inhaled antibiotics, %	32.4	52.7	< 0.001
Pancreatic enzymes, %	96.5	86.4	< 0.001
Ursodeoxycholic acid preparations, %	95.7	92.7	NS

Note. NS – not significant.



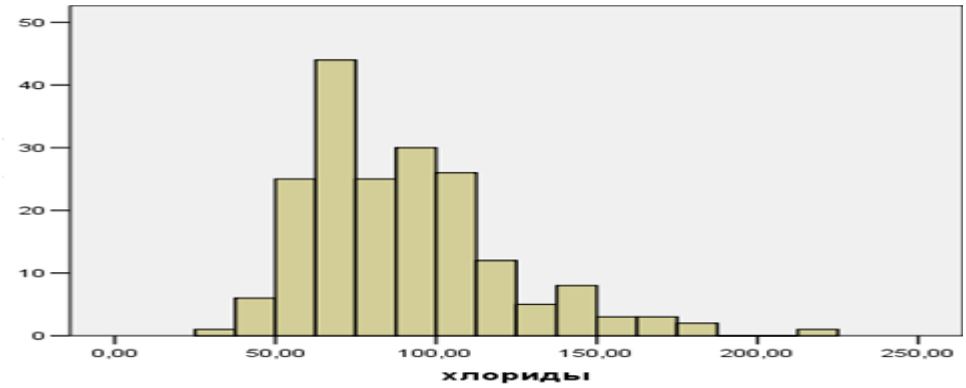
Pic. 1. Age structure of the patients

Hereinafter:	
возраст	*age*



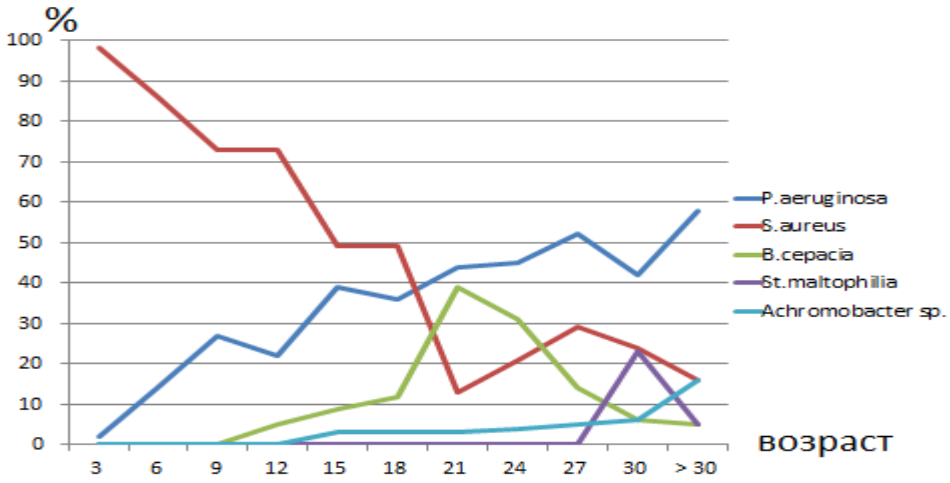
Pic. 2. Structure of age at diagnosis establishment

Возраст установления диагноза	*Age at diagnosis establishment*



Pic. 3. Sweat chlorides (diagnosis establishment)

хлориды	*chlorides*



Pic. 4. Microbiological profile (by age)