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Efficacy of an eradication protocol in case of *Pseudomonas aeruginosa* primo-infections in children with cystic fibrosis

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ABSTRACT

Introduction: Patients with cystic fibrosis (CF) are more susceptible to pathogens like *P. aeruginosa (PA)*. *PA* primo-infections require particular attention, as with failure in eradication, there is accelerated lung deterioration. The main aim of this study is to assess the rate of *PA* eradication according to our particular protocol with inhaled tobramycin and oral ciprofloxacin, as there is no consensus in the literature on what eradication protocol the best is.

Methods: Retrospective single centre study with data analysis from June 1st 2007 to June 1st 2011 of patients who had primo-infections exclusively treated by 3 x 28 days of inhaled tobramycin and oral ciprofloxacin for the first and last 21 days. Success in eradication is defined by ≥ 3 negative bacteriologies for 6 months after the beginning of the protocol. If ≥ 1 bacteriology is positive, we consider the eradication as a failure.

Results: Out of 41 patients, 18 were included in our analysis. 7 girls (38.9%) and 11 boys (61.1%) followed the eradication protocol. Boys had 12 primo-infections and girls had 8. Among these 20 primo-infections, 16 (80%) had an all-overall success in eradication and 4 (20%) a failure. No significant statistical difference for age between these groups (t-test = 0.07, p = 0.94), neither for FEV1% (t-test = 0.96, p = 0.41) nor BMI (t-test = 1.35, p = 0.27). Rate of success was 100% for girls and 66.6% for boys.

Conclusion: Our protocol succeeded in an overall eradication rate of 80%, without statistical significant impact on FEV1 % and BMI values. However, there is a sex difference with eradication rates in girls (100%) and boys (66.6%). A sex difference has not yet been reported in the literature. This should be evaluated in further studies.

Key words: Cystic fibrosis – P. aeruginosa – Primo-infection – Treatment – Eradication

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1 INTRODUCTION

1.1 Cystic fibrosis generalities

1.1.1 Genetic

Cystic fibrosis (CF) is the most common autosomal recessive disease (1). In Switzerland, the prevalence is around 1 to 2900 (2). The number of asymptomatic heterozygote carriers is approximately 4%, with a 25% probability that two asymptomatic heterozygote carriers give birth to a homozygote child (1).

CF is caused by a mutation on the long arm of chromosome 7. The concerned gene code for a protein: the cystic fibrosis transmembrane conductance regulator (CFTR). Since the discovery of the implicated gene in 1989 (1), over 1800 mutations have been described, with grouping the mutations in five classes (3):

- Class I: CFTR is not produced because of nonsense, frameshift or splice mutations
- Class II: CFTR is abnormally folded and is recognized to be destroyed
- Class III: CFTR has a regulation defect
- Class IV: CFTR has an abnormal conductance for chloride
- Class V: CFTR is correctly produced, but in reduced quantity

The most common mutation found in Switzerland, a deletion of phenylalanine in the amino acid position 508 (F508 deletion) forms a part of the class II (1,4).

1.1.2 General physiopathology

CFTR is located on the apical membrane of epithelial cells. In physiologic conditions, agonists (A) – like acetylcholine – bind to receptors (R) on the epithelial cells and increase the cyclic adenosine monophosphate (cAMP). cAMP activates the protein kinase A (PKA). PKA phosphorylates the CFTR. The phosphorylated CFTR permits the chloride transport (5). It regulates others ion channels (6).

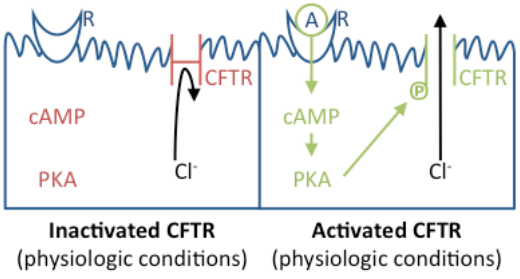

Figure 1: CFTR function in physiologic conditions, adapted from Cotran et al. (5)

In the sweat glands, chloride is less or not reabsorbed by defective CFTR from the lumen. Sodium stays in the lumen too. Therefore, the sweat has an increased concentration in chloride and sodium (5).

In the normal exocrine pancreas, the apical membrane of epithelial cells ejects chloride and absorbs bicarbonate. Then, chloride, sodium and water diffuse passively out the cells. Defective CFTR limits this and the pancreatic enzymes are retained in the cells too. Finally, these mechanisms destroy the pancreatic tissue and cause an exocrine pancreatic insufficiency responsible for malnutrition (6).

In the intestine, deficient CFTR decreases proximal liquid secretions and increases their distal absorption. Both anomalies lead to the desiccation of the intestinal content and to the

obstruction of the lumen. In newborns, they manifest in meconium ileus. In childhood or early adulthood, distal obstruction of the intestine is possible (6).

In the liver, abnormal CFTR causes bile retention, which is responsible for cirrhosis. In the gallbladder, water and NaCl are less secreted, causing cholecystitis and gallstones (6).

Chronic manifestations in the airways (see part 1.2.1) and malnutrition affect endocrine reproductive function. As a result, the puberty is often delayed and the menstrual cycle is sometimes slowed. As a consequence of inefficient CFTR, most of men suffer from azoospermia caused by the obliteration of vasa deferentia, some women are sterile because of abnormal mucus in the fallopian tubes and anomalies of liquids transport in the fallopian tubes and in the uterus (6).

1.1.3 Diagnosis of cystic fibrosis

Nowadays, the diagnosis of CF is based on newborn screening (NBS), introduced in Switzerland on January 1st 2011. NBS for CF was added to the Guthrie test, a blood test performed 72 to 96 hours after birth, which already screened for phenylketonuria, galactosaemia, biotinidase deficiency, medium-chain acyl-coenzyme A dehydrogenase deficiency, congenital hypothyroidism and congenital adrenal hyperplasia (7).

NBS for CF is based on the immunoreactive trypsinogen test (IRT), a test already available since 1980 (8). Children with an elevated IRT are considered as positive in NBS. To confirm respectively to rule out the diagnosis of CF, every country has its specific algorithm, including the sweat test and the genetic analysis (7).

In childhood, NBS prevents malnutrition with profits to growth, cognitive and pulmonary functions. It allows families a consented decision about performing an amniocentesis in following pregnancies. However, it is still less clear if there are significant benefits of NBS on pulmonary function later in life in adult patients (9).

The very first clinical manifestation, present at birth, is meconium ileus. Otherwise, the clinical diagnosis of CF in patients born prior to the introduction of NBS for CF is based on symptoms like recurrent cough, failure to thrive, steatorrhea, chronic abdominal pain, chronic rhinosinusitis and nasal polyps. Those symptoms can appear months or years after birth (7).

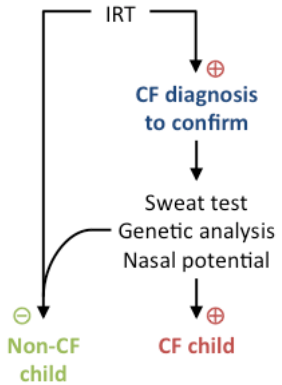

Figure 2: CF diagnosis algorithm, adapted from Barben et al. (7)

Three methods are available to confirm the diagnosis. The gold standard is the sweat test. It measures the concentration of chloride in sweat. The test is pathologic if the concentration is ≥ 60 mmol/L. The genetic analysis demonstrates the presence of two mutations on the CFTR gene. In some clinical situations it might lastly be necessary to measure the nasal potential difference, a method to analyse the CFTR function directly in the nasal epithelium (4).

1.2 Cystic fibrosis lung disease

1.2.1 Specific CFTR physiopathology in the airways

According to the mutation class, CFTR is either not produced or less functional (3). In the airways, it means that chloride is less or not secreted into the lumen. This is accompanied by an increased active transport of sodium. Both phenomena lead to an augmented reabsorption of water from the airway lumen to the epithelial cells. As a result, the airway surface liquid (ASL) is depleted and the mucociliary clearance decreases (5).

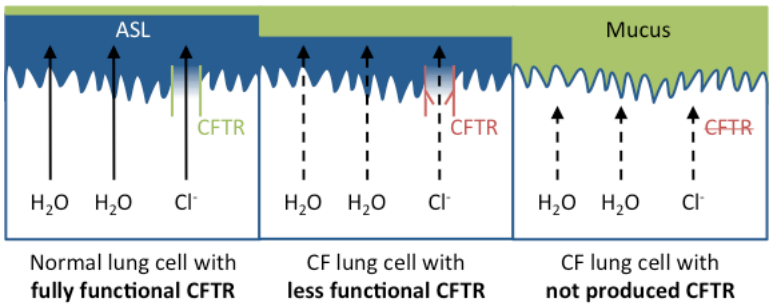

Figure 3: CFTR in lung cells, in physiologic and pathologic conditions, adapted from Cotran et al. (5)

1.2.2 Infections and inflammation in CF airways

1.2.2.1 Generalities on infections

CF lungs are more susceptible to infections. In early childhood, *S. aureus (SA)* and *H. influenzae (HI)* are frequently isolated. The presence of *P. aeruginosa (PA)* increases with age. Approximately 80% of patients at age of 20 years old are infected by *PA* (10). Even if CF is a systemic disease, airways manifestations, particularly aggravated by *PA* infections, weigh heavily on morbidity and mortality (11).

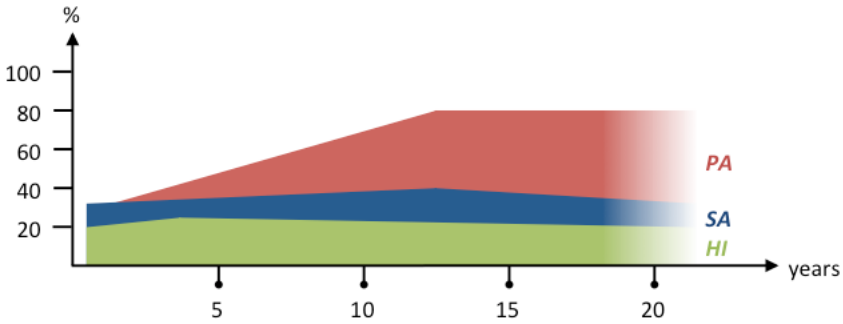

Figure 4: epidemiology of infections in CF lungs, adapted from Koch et al. (10)

1.2.2.2 Generalities on PA

PA is a motile, aerobe, non-fermentative and gram-negative rod. Even if this bacterium is ubiquitous, uses many sources of carbon and nitrogen and is highly virulent, it remains an opportunistic pathogen. Most of the time, it affects hosts with compromised defences, typically CF patients (12).

Many virulence factors, as structural components, toxins and enzymes, render *PA* resistant to most common antibiotics. This resistance is often caused by mutations of porins. These

mutations prevent antibiotics to penetrate into the cell. Moreover, *PA* β-lactamases inactivate many β-lactam antibiotics (12).

	Structural components
Capsule:	- protects the pathogen against phagocytosis and antibiotics - composed by polysaccharides for the docking
Adhesins and pili:	- necessary for the docking too
Lipopolysaccharides:	- have an endotoxin activity
Pyocyanin:	- blue pigment - stimulates inflammation by IL-8 release - catalyses the production of toxic oxygen radicals

Table 1a: PA virulence factors – structural components, adapted from Murray et al. (12)

	Toxins and enymes
Exotoxin A:	- inhibits proteins synthesis - is immunosuppressive
Exoenzymes S and T:	- damage epithelial cells - facilitate bacterial spread, tissue invasion and necrosis
Elastases:	- degrade elastin (contained in the lung parenchyma) - inactivate the complement and the neutrophils chemotaxis
Alkaline protease:	- destroys the tissue - contributes to the spread of *PA* - interferes with the immune response

Table 1b: PA virulence factors – toxins and enzymes, adapted from Murray et al. (12)

1.2.3 From *PA* primo-infection to chronic colonisation in CF airways

PA can sometimes be found in the oropharyngeal tract and from there, go intermittently into the tracheobronchial tract (13). The first identification of *PA* in CF patients is called primo-infection witch potentially have a response to the treatments (11). Depending on the defined free interval, patients can have a second, third or more *PA* primo-infections.

In the tracheobronchial tract, CFTR is deficient (3) and the mucociliary clearance is decreased (5). Both phenomena prevent from *PA* internalisation and destruction. *PA* affinity for epithelial cells receptors is increased (14). After *PA* bound to these cells, it proliferates. If it's not adequately treated, at a certain density, proliferation decreases with differentiation of *PA*. It forms a biofilm, which is a complex of several bacteria surrounded by a polymeric matrix (11). This matrix is constituted by alginate polysaccharide (13). Biofilm production is induced by the change of *PA* lateral chain from smooth lipopolysaccharide to rough, with appearance of *PA* on the agar plate as mucoid (14).

Moreover, *PA* induces the inflammation (15), which is more stimulated once *PA* becomes mucoid (14).

3 months of *PA* persistence could be enough to lead to the transformation of *PA* in its mucoid form, making an eradication by antibiotics more difficult (11), resulting in a chronic colonisation (14).

1.2.4 Diagnosis of *PA* in the airways

CF lung disease – worsened by *PA* colonisation – has the biggest influence on morbidity and mortality (11). Therefore, early detection of *PA* is fundamental, in order to eradicate it before reaching the mucoid stage (16). Samples are taken at least every 3 months during the routine follow-up of outpatient clinics, or in case of pulmonary exacerbations. Several methods to get a bacteriological sample exists (14):

- Throat swaps are used for children who can't expectorate (11).
- Sputum sample is the common choice for older children or adults who can expectorate (11). Induced expectoration is possible after inhalation of hypertonic saline solution or physiotherapy (13).
- Bronchoalveolar lavage (BAL) is an invasive method (11) that requires a general anaesthesia in children. It can be used in young children who can't expectorate (13). However, a recent study showed that this invasive method in case of pulmonary exacerbations is not better in detection of pathogens than a throat swap (17).

1.3 Objective

1.3.1 Primary outcome

The aim of our study is to retrospectively analyse the success rate of *PA* eradication in primo-infections treated by three months consecutive of inhaled tobramycin with twice a cycle of oral ciprofloxacin (see part 2.5.2).

1.3.2 Secondary outcomes

- Analysis of diagnostic methods, according to the age of our patients
- Analysis of type and frequency of concomitant pathogens
- Analysis of *PA* status one year before and one year after the eradication therapy
- Analyse of effects of the eradication protocol on forced expiratory volume in 1 second expressed in per cent (FEV1 %) and on body mass index (BMI)

2 METHODOLOGY

2.1 Study design

Retrospective single centre study

2.2 Study period

June 1st 2007 to June 1st 2011

2.3 Study population

Patients with a confirmed diagnosis of CF (clinical symptoms consistent with CF and two positive sweat tests [chloride ≥ 60mmol/L] or a genotype with two identifiable CF-causing mutations), followed in the paediatric CF outpatient clinic at Lausanne University Hospital in Switzerland were included in the study.

2.3.1 Included patients

Inclusion of all patients who fulfilled the criteria for primo-infection with *PA,* treated according the eradication protocol (see part 2.5).

2.3.2 Excluded patients

Exclusion of all patients:

- With uncompleted eradication protocol
- With adapted eradication protocol
- Who got intravenous (i.v.) antibiotics in addition to the eradication protocol
- Who suffered from chronic *PA* infection
- With uncompleted data
- With positive bacteriological culture for other *Pseudomonas* than *PA*

2.4 Ethic

Ethic committee approval was obtained on April 20th 2011.

2.5 Definitions

For analysis, we used the following definitions:

2.5.1 Primo-infection with *PA*

An infection with *PA* is considered as a primo-infection, if no *PA* was diagnosed for the last 6 months. *PA* was identified using standard bacterial culture methods.

2.5.2 Eradication protocol

Three consecutive cycles of inhaled tobramycin (TOBI®, Bramitob®), each cycle defined as twice daily inhalation of 300 mg for 28 days ("inhalation period"). Oral ciprofloxacin (30 to 40 mg/kg body weight in two doses with a maximum of 1500 mg/day) was added during the first 21 days and the last 21 days of the "inhalation period".

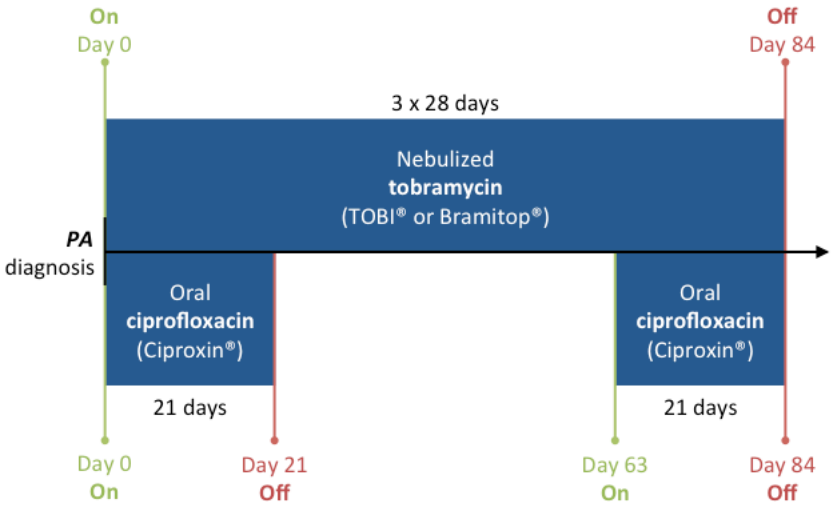

Figure 5: eradication protocol

2.5.3 Success in eradication

Success was defined when ≥ three sputum samples and/or throat swaps were negative during a 6 months period, counted from the first day of treatment to the last bacteriological sample at the end of the 6 months.

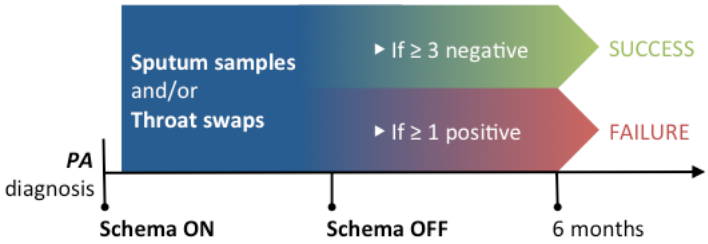

Figure 6: success and failure in eradication rules

2.5.4 Failure in eradication

Failure was defined when minimum at least one sputum sample and/or throat swap (out of three) was positive for *PA* during the period of 6 months, counted from the first day of treatment to the last bacteriological sample at the end of the 6 months.

2.6 Literature review

Literature review was performed throughout summer 2011, with these Mesh terms used in Pubmed:

1. ((cystic fibrosis) AND pseudomonas aeruginosa) AND (((antibiotic) OR therapy) OR treatment)
2. ((((cystic fibrosis) AND pseudomonas aeruginosa) AND early infection) AND tobramycin) AND administration, inhalation)

Application of first Mesh terms revealed 2121 citations; this research was useful to find general literature on CF. The second Mesh Terms (110 articles identified) helped us to focus our review on the context of our study.

2.7 Data collection

2.7.1 Data sources

Data collection was accomplished during autumn 2011 from three different sources:

1. Paper sheets (clinical files)
2. CF FileMaker® (in-hospital database)
3. *Archimed* database (hospital archives)

The gathered data were entered in a specific created FileMaker® database. Most data came from the paper sheets. The CF FileMaker® database was used to complete the rare missing or imprecise information. The *Archimed* database gave more information particularly on hospitalisations.

2.7.2 Collected data

- General information: name, age and sex of patients
- Primo-infection according definition
- Bacteriology (type of *PA* such as *non-mucoid* or *mucoid*, *Pseudomonas spp,* …), date of the positive result
- Eradication protocol according definition: doses of tobramycin and ciprofloxacin, dates of beginning of cycles
- Other infections: type of pathogens one year before and one year after the diagnosis
- Evolution of patients: clinical symptoms at the diagnosis, during the treatment period, hospitalisation, additional or i.v. treatments
- Anthropometry: weight, height, body mass index and dates of measures of these parameters, one year before and one year after each primo-infection
- Lung function parameter: FEV1 % and dates of measures, one year before and one year after each primo-infection

2.8 Methodology

We proceeded in that order:

1. First, all bacteriological results in the clinical file were reviewed in order to determine which patients were diagnosed for a primo-infection.
2. Second, still with the paper sheets, the consultations letters were analysed. This step was fundamental to determine – among the patients who had a primo-infection – who were treated by the protocol.
3. Finally, we gathered other information on patients corresponding to the criteria of the two first points, like symptoms, hospitalisations, FEV1 % and anthropometry data.

Data collection was completed from fall 2011 to the beginning of 2012. All patients meeting the inclusion criteria were classified in two groups: “success” and “failure”, according to our definitions (see parts 2.5.3 and 2.5.4).

2.9 Statistical analysis

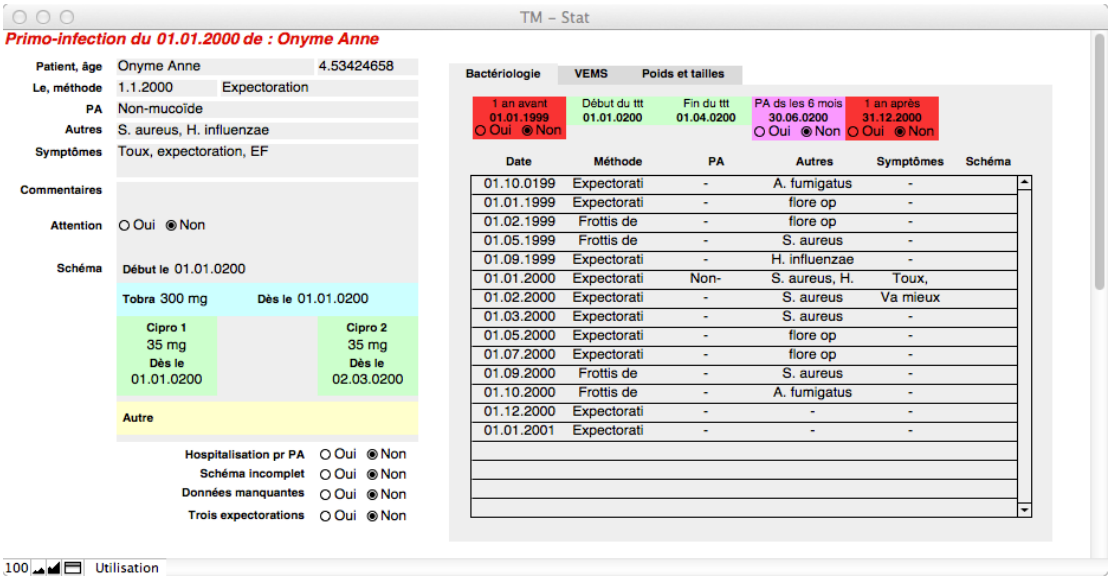

Figure 7: FileMaker® database screenshot

Data gathered in our FileMaker Pro 11® database were exported. Then, Microsoft Excel 2011® was used in order to analyse these data. P values were calculated with the help of http://www.stattools.net (last consult in August 2012).

2.9.1 Included data

2.9.1.1 Primary outcome

2.9.1.1.1 Successes or failures in eradication

We count the number of primo-infections and calculate the percentage of successful respectively of unsuccessful eradications. We determined the number of concerned patients with success or failure in eradication and calculated the mean ± standard deviation (SD), minimum and maximum ages. We calculated the percentage of successes or failures according to the sex.

2.9.1.2 Secondary outcomes

2.9.1.2.1 Diagnostic methods

We determined the number of primo-infections diagnosed by sputum samples or throat swabs. We calculated the mean ± SD, minimum and maximum ages of patients according to the diagnostic methods used.

2.9.1.2.2 Bacterial analysis

For each primo-infection, we determined the *PA* type (mucoid or not) and the diagnostic method.

We calculated the percentage of concomitant microbes at diagnosis. We determined the percentage of microbial species.

2.9.1.2.3 PA status one year before the protocol

We calculated the percentage of primo-infections preceded by a *PA* detection during the year before the diagnosis and the mean ± SD, minimum and maximum ages of concerned patients.

2.9.1.2.4 PA status one year after the protocol

We calculated the percentage of *PA* detection one year after the diagnosis and the mean ± SD, minimum and maximum ages of concerned patients.

2.9.1.2.5 FEV1 %

In order to determine if the FEV1 % values change after the primo-infection and the protocol, we used:

- A reference value, measured the last consultation before the *PA* diagnosis.
- Multiple comparison values, measured after the 6 months necessary to determine if the protocol is a success or failure.

We calculated the mean ± SD and the median of references values and of comparison values. P value was calculated with a t-test.

2.9.1.2.6 BMI

In order to determine if the BMI values change after the primo-infection and the protocol, we used:

- A reference value, measured the last consultation before the *PA* diagnosis.
- A comparison value, measured after the 6 months necessary to determine if the protocol is a success or failure.

We calculated the mean ± SD and the median of references values and of comparison values. P value was calculated with a t-test.

2.9.2 Excluded data

Excluded data were classified in five categories:

1. Non-*PA* or non-specified *Pseudomonas* infections treated by our eradication protocol
2. Infections not completely treated by the protocol
3. Infections with incomplete data
4. Infections treated by the protocol, however after a hospitalisation for i.v. antibiotics
5. Chronic infections treated with the protocol after

We count the number of primo-infections for these five categories. For the concerned patients, we put how many primo-infections they had in each category.

3 RESULTS

56 patients were followed from June 1st 2007 to June 1st 2011 in the paediatric CF outpatient clinic at Lausanne University Hospital in Switzerland.

41 patients (73.2%) suffered from one or more *Pseudomonas spp* infections during the study period.

18 patients fully followed our protocol (see part 2.3.1). They had 20 primo-infections. Data from them will be analysed. However, among these 18 patients, 10 had other primo-infections with gathered – but not used – data, due to non-*PA* or non-specified *Pseudomonas* infections, primo-infections not completely treated by the protocol, infections with incomplete data, infections treated by the protocol after an hospitalisation for i.v. antibiotics, chronic infections treated by the protocol.

3.1 Included data

18 patients fully followed our protocol according our definition (see part 2.3.1). These patients had 20 primo-infections.

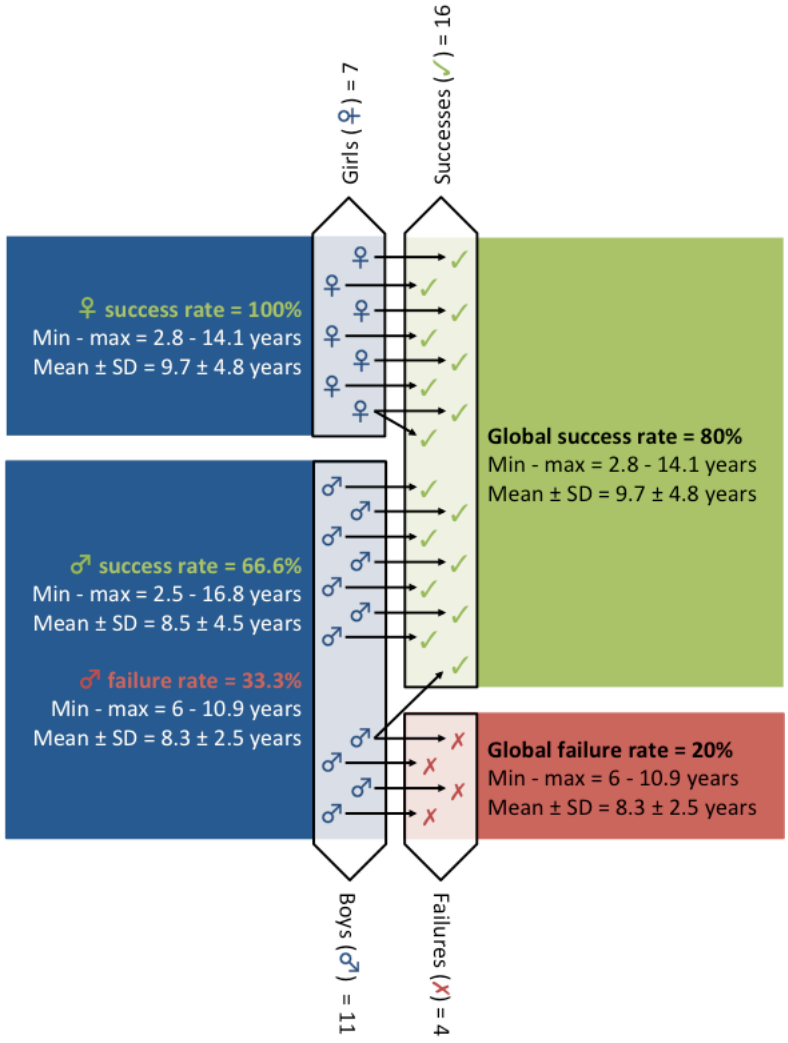

Figure 8: repartition of included patients and primo-infections

3.1.1 Primary outcome

3.1.1.1 Successes and failures in eradication

Success was accomplished in 16 primo-infections (all overall rate of 80%) in 15 patients (aged from 2.5 to 16.8 years, mean ± SD = 9.1 ± 4.6 years). One of these 15 patients had a further primo-infection with a failure in eradication (see bellow).

- 7 girls (aged from 2.8 to 14.1 years, mean ± SD = 9.7 ± 4.8 years) with 1 of them who had 2 primo-infections (at 11 and 12 years old), success rate of 100% among girls.
- 8 boys (aged from 2.5 to 16.8 years, mean ± SD = 8.5 ± 4.5 years), success rate of 66.6% for the boys.

4 boys (aged from 6 to 10.9 years, mean ± SD = 8.3 ± 2.5 years) had a failure in treatment. One of these four patients had a previous primo-infection, successfully treated (see bellow). The three others had 1 primo-infection during the study.

3.1.2 Secondary outcomes

3.1.2.1 Diagnostic methods

In the "success" group, 11 primo-infections were diagnosed by sputum samples (patients aged from 6.5 to 16.9 years, mean ± SD = 10.8 ± 3.3 years) and 5 by throat swaps (patients from 2.5 to 13.8 years, mean ± SD = 5.2 ± 4.9 years).

In the "failure" group, all primo-infections were diagnosed by sputum samples (patients aged from 6 to 10.9 years, mean ± SD = 8.3 ± 2.5 years).

3.1.2.2 Bacterial analysis

In the "success" group, bacterial analysis revealed 15 non-mucoid *PA* (10 by sputum samples, 5 by throat swaps), and 1 mucoid *PA* (by sputum sample). In the "failure" group, sputum samples showed initially 4 non-mucoid *PA*.

Number of associated microbes in successfully treated primo-infections:

- 1 microbe in 7 primo-infections (43.8%)
- 2 microbes in 4 primo-infections (25%)
- 3 microbes in 3 primo-infections (18.8%)
- 5 microbes in 2 primo-infections (12.5%)

In the "success" group, oropharyngeal flora is present in 75% of primo-infections. Other concomitant microbes and their presence percentage for successfully treated primo-infections are:

- *S. aureus*, 56.3%
- Others, 56.3%
- *H. influenzae*, 25%

50% of unsuccessfully primo-infections are associated with the presence of oropharyngeal flora. *A. fumigatus* is found in 50% of primo-infections with a failure in eradication.

3.1.2.3 PA status one year before the protocol

In the "success" group, 3 out of 15 patients (20%) had a primo-infection during the year before inclusion according to our protocol (aged from 4.3 to 13.8 years, mean ± SD = 10 ± 5.1 years):

- 1 girl had 2 primo-infections successfully treated, 11 months apart between positive specimens (both included in our results).
- 1 girl had a primo-infection successfully treated 6 months before, but not by the protocol (not included in our results).
- 1 boy were hospitalized and successfully treated for a primo-infection 11 months before (not included in our results).

In the "failure" group, none of the 4 boys had a primo-infection the year before.

3.1.2.4 PA status one year after the protocol

In the "success" group, 11 (73.3%) patients out of 15 remained free from *PA.* A new primo-infection during the first year after the treatment was experienced by 4 (26.7%) patients (exclusion of 6 months after the beginning of the protocol, see part 2.5.3). In these 4 patients (aged from 4.3 to 16.8 years, mean ± SD = 11.5 ± 5.4 years):

- 1 girl followed successfully a second eradication protocol 9 months after (included in our results). She had a third primo-infection with incomplete data 8 months after the beginning of the successful second eradication protocol (not included in our results).
- 1 girl was hospitalized for a new primo-infection 10 months after (not included in our results).
- 1 boy followed an incomplete protocol 10 months after (not included in our results).
- One year after, 1 boy was hospitalized with i.v. antibiotics for 3 *PA* that were finally eradicated (not included in our results). More than two years after, he followed the protocol with a failure in eradication (included in our results).

3 boys (75%) who had a failure in eradication were hospitalized for i.v. antibiotics. They were successfully treated and remain free from *PA* during the year after. Despite a hospitalisation, 1 (25%) other boy with a failure of the protocol still had positive bacteriologies for *PA* during the year after.

3.1.2.5 FEV1 %

In the "success" group, the mean baseline FEV1 % was 102.3 ± 12.8, with a median of 101.5. After the treatment, the mean FEV1 % is 100 ± 14.7, with a median of 100. T-test = 0.90. Data for FEV1 % were not available for 4 primo-infections.

In the "failure" group, the mean baseline FEV1 % was 99.8 ± 21.6, with a median of 100. After the treatment, the mean FEV1 % is 92.6 ± 15, with a median of 92.5. T-test = 0.96. Data were available for all primo-infections.

3.1.2.6 BMI

Among the successfully treated primo-infections, the mean baseline BMI was 16.6 ± 3, with a median of 15.8. After the protocol, the mean BMI is 16.9 ± 3.2, with a median of 15.9. T-test = 0.12. Data for BMI were not available for 2 primo-infections (3.3%).

Among the primo-infections with a failure in eradication, the mean baseline BMI was 16.1 ± 0.3, with a median of 16.1. After the protocol, the mean BMI is 16.5 ± 0.6, with a median of 16.4. T-test = 1.35. Data were available for all primo-infections.

3.2 Excluded data

Among the 18 included patients, 10 had other infections with gathered – but not analysed – data. 10 other patients had only gathered, but not used, data. These 20 patients had 30 infections concordant with our exclusion criteria (see part 2.3.2):

- 10 non-*PA* or non-specified *Pseudomonas* infections treated by our eradication protocol
- 9 infections not completely treated by the protocol
- 2 infections with incomplete data
- 3 infections treated by the protocol, however after a hospitalisation for i.v. antibiotics
- 6 chronic infections treated with the protocol after

13 patients had infections not treated by our protocol (for instance, by colistin). Data from these patients were not gathered and not analysed.

3.3 Results summary

	Successes	Failures
Primo-infections:	- 16	- 4
Ages:	- Min = 2.5 years - Max = 16.8 years - Mean ± SD = 9.1 ± 4.6 years	- Min = 6 years - Max = 10.9 years - Mean ± SD = 8.3 ± 2.5 years
Type of *PA*:	- 15 non mucoid - 1 mucoid	- 4 non mucoid
Diagnostic methods:	- 11 by sputum samples - 5 by throat swaps	- 4 by sputum samples

Table 2: repartition of successes and failures

		Girls	Boys
	Patients:	- 7	- 11
Successes	**Primo-infections:**	- 8	- 8
	Ages:	- Min = 2.8 - Max = 14.1 years - Mean ± SD = 9.7 ± 4.8 years	- Min = 2.5 - Max = 16.8 years - Mean ± SD = 8.5 ± 4.5 years
	Type of *PA*:	- 7 non-mucoïd - 1 mucoid	- 8 mucoid
	Diagnostic methods:	- 5 by sputum samples - 3 by throat swaps	- 6 by sputum samples - 2 by throat swaps
Failures	**Primo-infections:**		- 4
	Ages:		- Min = 6.0 - Max = 10.9 years - Mean ± SD = 8.3 ± 2.5 years
	Type of *PA*:		- 4 non-mucoid
	Diagnostic methods:		- 4 by sputum samples

Table 3: repartition of girls and boys

4 DISCUSSION

4.1 Included data

4.1.1 Primary outcome

4.1.1.1 Successes and failures in eradication

According our inclusion criteria, 7 girls (38.9% of our 18 patients) and 11 boys (61.1% of our 18 patients) followed our eradication protocol. Boys had 12 primo-infections and girls had 8. In applying our protocol, *PA* was eradicated all overall in 80% of primo-infections. However, our study shows a difference in eradication between girls and boys. The success rate was 100% for girls and 66.6% for boys. Mean ± SD age is 9.1 ± 4.6 years in the "success" group and 8.3 ± 2.5 years in the "failure" group. There is no statistical difference in ages between "success" and "failure" groups (t-test = 0.07 with p = 0.94).

In order to discuss these results, we focused our literature review on studies that used inhaled tobramycin to treat *PA* primo-infections. Several trials were identified (18-24) with two of these studies (18,19) referenced in 2009 in a Cochrane review, because they were randomised, with a placebo group and from similar design (25).

Our literature review permits us first to legitimate our study by the fact that treatment of *PA* primo-infections is necessary. Gibson et al. (2003) demonstrated it. They compared 28 days of inhaled tobramycin twice daily versus placebo. They randomized 98 patients. However, they stopped their trial after an interim analyse of 21 patients (8 in "treatment" group, 13 in "placebo" group). This interim analyse showed that *PA* was eradicated in lower airways in all patients in "treatment" group but only in 1 patient (7.4%) in "placebo" group (19).

Our protocol is started as soon as *PA* is diagnosed. We don't use preventative cycles of therapy without proof that *PA* is present, in concordance with Treggiari et al. (2011) who showed that inhaled tobramycin should be prescribed only when *PA* is detected to avoid pulmonary exacerbation, instead of giving preventative cycled therapies in patients not-known for primo-infections respectively chronic *PA* infections. Their trial compared 4 regimens based on 28 days of inhaled tobramycin and 14 days of oral ciprofloxacin or placebo: inhaled tobramycin once every 3 months ("cycled therapy" group) or when *PA* culture is positive ("culture-based" group), with oral ciprofloxacin ("ciprofloxacin" group) or placebo ("placebo" group). 16% patients in "cycled" group and 17% in "culture-based" group had a severe pulmonary exacerbation requiring i.v. antibiotics or hospitalization (21).

Apart from an abstract presented in 2012 during the European Cystic Fibrosis Conference in Dublin (26), we found no other study using our protocol. Moreover, only one of the trials used the same endpoint with definition of success of eradication already after 6 months without *PA* (22). There is no unified consensus about the duration of absence of *PA* to define it as a success, but in most studies 12 months are chosen. This is problematic from our clinical point of view as patients who received eradication treatment between one to three months (19-24) remain in the "*PA* positive" cohort for another 9 to 11 months – but without treatment. During that time period, there is an increased risk to be re-exposed to *PA* by cross-infection by contact with other *PA* positive patients (27) during outpatient clinic visits.

Taccetti et al. (2012) used the same definition of duration of success as we did, comparing 28 days of inhaled tobramycin and oral ciprofloxacin ("arm A") versus inhaled colistin and oral ciprofloxacin ("arm B"). Success in eradication was obtained in 66 on 105 patients (62.8%) in "arm A" and in 77 on 118 patients (65.2%) in "arm B" with conclusion that there was no statistical difference in these protocols (22).

Key points
Girls: - n = 7 - primo-infections = 8 - eradication rate = 100% **Boys:** - n = 11 - primo-infections = 12 - eradication rate = 66.6% **Overall:** - eradication rate = 80% Our eradication rate for boys is close to the global rate of Taccetti et al. (2012) (22).

Table 4: successes and failures key points

If we focus on "arm A", we see that our results are better (80% versus 62.8%). However, Taccetti et al. (2012) were aware that their results were not as good as in other studies probably due to differences in the design of studies, differences in the protocols, follow-up, … (22). We conducted a single centre and retrospective study, but able to analyse data for 18 patients only. Taccetti et al. (2012) involved 13 centres and randomised 223 patients. He used a 28 days protocol of inhaled tobramycin (22), which is shorter than ours (84 days). It could explain the difference in the results.

Wiesemann et al. (1998) compared 80 mg of aerosolized tobramycin twice a day for 12 months versus placebo. They reported that the mean time necessary for conversion from *PA* positive to *PA* negative in cultures was 1.89 month (roughly 56 days) in the aerosolized tobramycin group and not assessable in the placebo group (18). However, Ratjen et al. (2010) evaluated 28 days versus 56 days of inhaled tobramycin twice a day. They randomised 88 patients (45 in "28-days" group and 43 in "56-days" group). They analysed data from 65 of them (34 in "28-days" group and 31 in "56-days" group) and reported that the time to recurrence was 26.12 months (in "28-days" group) and 25.82 months (in "56-days" group), not statistically significant (20). Moreover, Gibson et al. (2007) compared 28 days of inhaled tobramycin with 56, 84 or 112 days of follow-up and 56 days of inhaled tobramycin with 112 days of follow-up. Their study showed a similar rate in eradication: 75% in 28 days of inhaled tobramycin and 56 days of follow-up, 63% in 28 days of inhaled tobramycin and 84 days of follow-up, 75% in 28 days of inhaled tobramycin and 112 days of follow-up, 82% in 56 days of inhaled tobramycin and 112 days of follow-up (23).

Our study and Taccetti et al. (2012) trial include oral ciprofloxacin (22). However, Treggiari et al. (2011) demonstrated that oral ciprofloxacin during 14 days give no benefice in addition to 28 days of inhaled tobramycin. They showed no statistical significant difference in pulmonary exacerbations for patient on inhaled tobramycin with oral ciprofloxacin (19%) or placebo (14%) (21).

Our success rate in eradication for girls was 100%. The success rate for boys is with 66.6% close to the global success rate in eradication in Taccetti et al. (2012) trial (62.8%) (22). However, neither this study (22) nor the others (18-21,23-24) reported differences in eradication according to the sex of patients. An explanation for our results might be the excess of boys (11 compared to 7 girls). There is a slight difference in the mean ± SD age according to the sex – for boys, 8.4 ± 3.8 years and for girls, 9.7 ± 4.8 years – however without significant statistical difference (t-test = 0.15 with $p < 0.05$). The number of previous primo-infections is neither an explanation too. Only one of the patients with a failure in eradication had a primo-infection before. Finally, all boys in the "failure" group are known to be either non-compliant or to have social familial difficulties.

Before inhaled tobramycin was available, colistin was used to treat *PA* primo-infections. Some studies evaluated the efficacy of colistin (28-31). Taccetti et al. (2012) used it in the "arm B" of their trial. They get 65.2% of successes in eradication in "arm B" versus 62.8% in "arm A", with no statistical difference between the two groups (22). Proesmans et al. (2012) compared 28 days inhaled tobramycin ("TOBI" group) versus inhaled colistin with oral ciprofloxacin for 3 months ("CC" group) too. At the end of the treatment, 6 on 29 (89.7%) were successfully treated in "CC" group and 23 on 29 primo-infections (79.3%) in "TOBI" group. 6 months after the inclusion in the study, 19 primo-infections (65.5%) in "CC" group remained free from *PA* and 13 (44.8%) in "TOBI" group. They concluded that both treatments are equivalent (24). There is no indication to considerate the use of colistin instead of tobramycin. Taccetti et al. (2012) and Proesmans et al. (2012) confirmed that tobramycin protocols based are as effective as colistin protocols based indeed (22,24). Moreover, Proesmans et al. (2012) demonstrated that a tobramycin protocol based of 28 days is as efficient as a colistin protocol based of 3 months (24).

4.1.2 Secondary outcomes

4.1.2.1 Diagnostic methods

To perform *PA* diagnosis, sputum samples and throat swaps were used. Sputum samples were positive in identification of 15 primo-infections (75%) and throat swaps in 5 (25%).

Patient s that expectorated were older (mean ± SD = 10.2 ± 3.2 years, min = 6 years, max = 16.8 years) than patients in whom throat swaps were performed (mean ± SD = 5.2 ± 4.9 years, min = 2.5 years, max = 13.8 years). The age difference was anticipated because young patients are not able to expectorate.

A recent trial demonstrated that bronchoalveolar lavage is not better than other technics such as throat swaps and sputum samples to diagnose bacterial aetiologies in case of a pulmonary exacerbation (17).

Key points
20 primo-infections, diagnosed by: - 15 sputum samples - 5 throat swaps Sputum samples and throat swaps are good diagnostic methods in pulmonary exacerbations (17).

Table 5: diagnostic methods key points

4.1.2.2 Bacterial analysis

All patients had minimum one concomitant microbe, independent of success or failure in eradication. With exception of *PA,* using standard bacteriological or fungal cultures, only one microbe was identified in 62.1% of all our primo-infections, 2 microbes in 22.2%, 3 microbes in 16.7% and lastly 5 microbes in 11.1%.

For both groups, *S. aureus* was the most often found concomitant microbe, in 45% of our primo-infections, followed by 20% of *H. influenzae*, 15% of *A. fumigatus*. Other pathogens were concomitant in 40% of our primo-infections. Oropharyngeal flora was present in 70% of all our primo-infections. All primo-infections in the "failure" group had one (100%) concomitant pathogen. Primo-infections in "success" group had one (43.8%) and more (56.3%) concomitant pathogens.

Wiesemann et al. (1998) reported *S. aureus* and *H. influenzae* as concomitant phatogens, *S. maltophilia* and *Enterobacteriaceae spp* were isolated as well (18). Ratjen et al. (2010)

isolated in their both groups *S. aureus* in 39.5%, *H. influenzae* in 14%, *C. albicans* in 11%, *Aspergillus spp* in 9%, S*. maltophilia* in 4.5% of their cases (20). Treggiari et al. (2012) described the following concomitant pathogens among their 4 groups: *S. aureus* 58%, *S. maltophilia* 3.5%, *A. xylosoxidans* 1%, *B. cepecia complex* 0.75% (21). Taccetti et al. (2012) isolated those pathogens in their two groups: *S. aureus* 30%, *H. influenzae* 9.8%, *S. pneumoniae* 0.8% (22). Gibson et al. (2007) had *S. aureus* (32%), *H. influenzae* (29%), and *S. pneumoniae* (18%) as concomitant pathogens (23).

In conclusion, most of these studies, as ours, often isolated *S. aureus* and *H. influenzae* as concomitant pathogens. If concomitant pathogens are a precursor for *PA* infections remains however a matter of debate at current time.

Key points	
Concomitant microbes: - 1 in 62.1% primo-infections - 2 in 22.3% primo-infections - 3 in 16.7% primo-infections - 5 in 11.1% primo-infections	**Microbial types:** - *S. aureus* in 45% primo-infections - *H. influenza* in 20% primo-infections - *A. fumigatus* in 15% primo-infections - Others in 40% primo-infections - Orophyngeal flora in 70% primo-infections
In other trials, *S. aureus* and *H. influenza* are the most frequently pathogens found too (18,20-23).	

Table 6: bacterial analysis key points

4.1.2.3 PA status 1 year before the treatment

20% of patients in the "success" group but none in the "failure" group had a *PA* primo-infection during the year before the beginning of the protocol. It concerns 15% of all overall patients (n = 18).

Proesmans et al. (2012) included patients with first ever or new primo-infections. A new primo-infection was defined after minimum 6 months *PA* free. 6 patients on 19 (31.6%) in the "CC" group and 3 on 21 (14.3%) in the "TOBI" group had a primo-infection during the year before (24), the latter 14.3% is close to our 20%.

Taccetti et al. (2012) recruited patients with first ever primo-infections or with new primo-infections successfully treated and with 3 negative cultures during 6 months. Rate in eradication was similar for patients with first ever primo-infections (66.1%) and with new primo-infections (61.7%), but they didn't gave detailed data on primo-infections the year before (22), therefore we can't compare our result (20%) to this study.

Wiesemann et al. (1998), Gibson et al. (2003 and 2007), Ratjen et al. (2010) and Treggiari et al. (2011) didn't have data for this particular point we can compare to ours because of their criteria of inclusion (18-21,23).

4.1.2.4 PA status 1 year after the treatment

73.3% of our patients remained *PA* free one year after the beginning of the successful treatment, in comparison to 80% after 6 months, leading to 4 patients who re-acquired *PA* between 6 to 12 months after the start of the eradication therapy. 75% of our patients with a failure of the protocol – and then successfully treated after a course of two weeks i.v. antibiotics – were free of *PA* one year after the beginning of the protocol.

Wiesemann et al. (1998) treated their cohort for one year. At the end of the treatment period, 8/9 patients (88.9%) in the "tobramycin" group were free of *PA*, but only 1/5 patients (20%) in "placebo" group. Their rate of patients free of *PA* (88.9%) is higher than ours (73.3%), most likely due to the fact that the microbiological analysis was made directly at the end of treatment, and not as in our study, 7 months after the end of the treatment (18).

Taccetti et al. (2012) reported data about *PA* status 6 months after the end of their treatments, with 66 patients out of 105 (62.9%) in "arm A" and 77 out of 118 (65.3%) in "arm B" remained free of *PA* in the first year (22).

Gibson et al. (2003 and 2007) had no data concerning the *PA* status after one year (19,23). Ratjen et al. (2010) demonstrated a time to recurrence of 26.12 months in the "28-days" group and of 25.82 months in the "56-days" group. They didn't present results on *PA* status after one year (20). Data for *PA* status after one year weren't available for Treggiari et al. (2011) trial (21).

Key points	
Patients with *PA* 1 year before the treatment: - 20% patients in "success" group - 0% patients in "failure" group	**Patients *PA* free 1 year after the primo-infections:** - 73.3% patients in "success" group - 75% patients in "failure" group
Proemans et al. (2012) had similar rate to ours (24).	**Taccetti et al. (2012) had between 62.9% to 65.3% patients *PA* free after 1 year, less than us (22).**

Table 7: PA status 1 year before and after the beginning of the treatment key points

4.1.2.5 FEV1 %

We found no significant changes in FEV1 % values after the treatment neither in "success" nor in "failure" groups ("Success" group: t-test = 0.9, p = 0.39, "failure" group: t-test = 0.96, p = 0.41).

Wiesemann et al. (1998) didn't present data concerning variation in FEV1 (18). Ratjen et al. (2010) didn't observe any changes in FEV1 values in their both groups of their study (20). Treggiari et al. (2011) reported no difference in FEV1 values across their 4 treatments groups (21).

Taccetti et al (2012) however observed an increase of FEV1 values in both "arm A" and "arm B" (22). During the year or their study, Proesmans et al. (2012) observed a median change of -1% in both groups (24).

Gibson et al. (2003 and 2007) reported nothing on FEV1 values (19,23).

4.1.2.6 BMI

We found no significant changes in BMI values before and after the treatment, in both groups. ("Success" group: t-test = 0.12, p = 0.91; "failure" group: t-test = 1.35, p = 0.27).

Ratjen et al. (2010) didn't observe any influence of theirs treatments (28 days tobramycin or 56 days) on BMI values (20). Treggiari et al. (2011) didn't note statistically significant

variation in weight and height among their 4 different groups (21). Proesmans et al. (2012) didn't observe a significant change in BMI values in their groups during the year of their trial (24).

Gibson et al. (2003) mentioned that differences in weight were similar in "tobramycin" group and in "placebo" group, but no BMI data were available (19). Studies from Wiesemann et al. (1998), Gibson et al. (2007) and Taccetti et al. (2012) didn't present any data on BMI (18,22-23).

Key points	
FEV1 %: No significant changes in FEV1 % values after the treatment. Ratjen et al. (2010) and Treggiari et al. (2011) didn't report changes too (20-21).	**BMI:** No significant changes in BMI values before and after the treatment. Ratjen et al. (2010), Treggiari et al. (2011) and Proesmans et al. (2012) didn't report changes too (20-21,24).

Table 8: FEV1 % and BMI values key points

4.2 Excluded data

Excluded data will not be discussed further in detail.

4.3 Limitations of our study

A clear limitation of our study is the very small sample size (18 patients, with 20 primo-infections). Other studies had much more participants (20-24). The design of the study itself is a limitation too. Our study is retrospective and focus only on a shorter time (4 years), than other trials (20,21,24). We have no control group in our study. All the trials we identified by literature review had more than one arm (18-24). Moreover, some of these trials were randomised and double blinded (18-19). In those patients who experienced some time ago a *PA* infection, to define a true new primo-infection, genotyping of *PA* might be helpful. This is however not available in our routine bacterial analysis.

5 CONCLUSION

We achieved an eradication rate of 100% in girls using our protocol, with an overall rate of 80% for *PA* eradication. Our results are difficult to compare to other studies, because we did not identify another trial using our eradication protocol (18-24), apart from the group from the Royal Children's Hospital in Melbourne, who presented their data with an abstract during the European Cystic Fibrosis Conference 2012 in Dublin (26). And just one had the same outcome of success in defining success of eradication six months after the start of treatment (22). But our 66.6% eradication rate for boys is similar to the 60% for the inhaled tobramycin arm in this study (22). However, none of the studies we focus on analysed the rate in eradication according to the sex (18-24). Why this success in girls? We can only speculate on this. Is it due to the fact that there were more boys in our study than girls (11 boys vs 7 girls)? Are pre-pubertal girls more compliant with therapy, or better supervised by caregivers than boys? Is there a difference in the microbiome in girls comparing to boys? Although our sample size was very small, the sex difference merits certainly attention in further prospective eradication studies, if not a data analysis according to sex could be done in the studies already published.

Our literature review permits us to assess the pertinence of our protocol. One of these trials confirmed that *PA* antibiotics treatment is better than placebo (19) and another one that the treatment should be started only once the pathogens is diagnosed by microbiology (21). These studies are the proof we need to continue to perform frequent microbiology analysis in our patients and keep on going our protocol only if *PA* is present, particularly in girls. An initial i.v. therapy of two weeks has probably to be considered in case of *PA* primo-infections in boys with already knowledge about possible problem of compliance.

Two other studies showed that there is no difference between 28 or 56 days of inhaled tobramycin (20,23). Moreover, a study found no difference in pulmonary exacerbations using ciprofloxacin or not (21). Both the points of duration of inhaled tobramycin and use of oral ciprofloxacin should be further analysed in future studies to avoid overuse of antibiotics and the emergence of resistances.

Two studies showed that inhaled colistin is as efficient as inhaled tobramycin (22,24), but with colistin available by inhalation for a longer time (28-31). As mentioned already, the fact we had no comparison group in our study was a clear limitation. In a further trial, we could envisage to treat patients by inhaled colistin in a potential comparison group.

To conclude, our overall eradication rate is 80%. However, we found a difference between girls and boys (100% vs 66.6%). This difference should be investigated in further studies, as the duration of inhaled tobramycin versus colistin and the addition of oral ciprofloxacin.

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