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Standardized clinical criteria and sweat test combined as a tool to diagnose Cystic Fibrosis

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Abstract

Context: CF is under-diagnosed in Ecuador; one out of every 11,252 live births born in Ecuador could have CF.

Aim: To analyze the clinical findings, based on previously established criteria, with the results of the sweat test, in circumstances where we do not have the routine molecular study.

Methods: Epidemiological, observational, analytic, cross-sectional study. It analyzed 180 patients clinically suspected of CF. Inclusion criteria: children of both sexes older than 30 days and younger than 12 years, who meet at least three clinical criteria suggestive for CF, outpatient and referred by a specialist physician who made a preliminary diagnosis. This is a pilot study.

Results: The combination of criteria pneumonia, chronic cough and chronic obstructive bronchial syndrome is the most frequent, with not a significant relationship with a positive sweat test. On the contrary, a significant relationship was found between the clinical combinations of pneumonia with cough and rhinosinusitis; pneumonia with cough; presence of *Pseudomonas aeruginosa*; and pneumonia with digital cough and clubbing, so it is recommended to perform the test in all these associations. The most frequent clinical criterion for the reference and performance of the electrolyte test in sweat is pneumonia to repeat for two or more episodes.

Conclusion: Clinical combinations of pneumonia with cough and rhinosinusitis; pneumonia with cough; presence of *Pseudomonas aeruginosa*; and pneumonia

with digital cough and clubbing are pathognomonic for CF and indication for the sweat test. The predictive performance in CF diagnosis, defined as compatible clinical presence plus high values of chloride in sweat test, was 91.1%.

Keywords: Paediatrics, Internal medicine, Evidence-based medicine

1. Introduction

1.1. Context and fundamentals

Cystic Fibrosis (CF) is under-diagnosed in Ecuador and the few patients that exist at this moment do not access treatment early, only in advanced stages of the disease can reach proper healthcare, which increases morbidity, complications and reduces the overall survival of these patients [1]. In Ecuador, it is estimate that one of every 11,252 live births born (RNV) can have CF [2], and the prevalence according to ethnic groups has not been establish, considering what the American and Ecuadorian population are genetically diverse [3]. According to this estimated prevalence, in Ecuador there should be at least 29 new cases each year of patients with CF, which means that at least 290 new cases should have been diagnose in the last decade. Currently this does not happen, only a few patients are report in pediatric hospitals. In the other hand, there is not a National Registry of patients with CF to obtain real data [1]. Some studies in Latin America suggest that CF affects to 1 in 1600 to 1 in 14,000 live newborns. The sub-diagnosis could reach 50% in some countries, and it remains an important challenge [1, 4].

Despite the improvement of public health systems [5], this situation exists in our country and is due to three basic problems: lack of diagnosis, late diagnosis, and difficult diagnosis of the disease [6]. The lack of diagnosis is mainly due to there is no awareness of rare diseases in the country, nor public policies aimed at improving diagnosis and timely access to treatment. Many of the patients with clinical suspicion of CF spend a long time in medical consultations, and/or emergency services without a precise diagnosis, which increases their complications, decreases the quality of life, and it saturates the hospital services [7, 8].

The late diagnosis, in turn, is due to three main reasons: 1) there is no qualified and sufficient medical attention; 2) there is no official program for patient recruitment, and 3) laboratory tests are not routinely available [9]. First, in relation to the Ecuadorian health system, it must be said that there are few specialists in pediatric pulmonology available in the country, which are concentrated in the three largest cities in the country, and in tertiary or specialized hospitals. On the other hand, general pediatricians, family doctors or general practitioners who examine the majority of patients in primary care, do not have enough training on this disease to diagnose it early. Second, there is no official and national program that requires the search and

recruitment of patients, this disease has not a mandatory notification which makes many patients lose their health system, or take a long time to reach a specialist. In addition, currently a neonatal screening program for CF in Ecuador is not performed even, although there is a national screening program for four other pathologies. Third, there is not a center that offers routinely sweat and molecular tests. The offer of diagnostic services is very limited, disperse, occasional and incomplete, since no center offers comprehensive diagnosis [10]. In addition, due to the low prevalence of the disease, there is little medical interest of physicians to consult these patients; they prefer to devote their efforts to more frequent pathologies. In addition, the molecular tests, especially genomic sequencing, are not available in the country and is limited by the cost, and in some the cases, is paid by pocket money of the relatives of a patient with suspected CF, when they have the funds [11, 12].

Diagnosis of CF is sometimes difficult by the general practitioner due mainly to: 1) imprecision in the clinical diagnosis [13, 14]. 2) Sweat test, when is available, generates confusion when the results shows a low value, or is doubtful [13, 14, 15, 16, 17] and; 3) the molecular diagnosis, when it can be done, does not cover all the minimum mutations that are the most frequent in our population. In the first instance, the great inter-professional clinical variability generates imprecision in the clinical diagnosis, this despite having adequate clinical guidelines. A multidisciplinary diagnosis is not made, which also creates confusion among specialists. In addition, the differential diagnosis is complex because of the large number of pathologies that can be differentiated, and because of the large amount of resources that must be invested for it, resources that are not always available in a country like ours. Second, the sweat test could not be performed with complete certainty in children less than 30 days old, and in children of more than 12 years, where the disease is very likely not present. Despite of that in nowadays, it could confirm the presence of less severe mutations and the CF diagnosis until adult age [17, 18, 19]. Third, in relation to current molecular tests, these are designed for a few mutations only, leaving a group of patients with diagnostic suspicion without complete diagnosis. Several studies showed that with the mutations described until now in Ecuadorian patients, only 70% patients could be cover to diagnosis, these mutations are F508del, G85E, G330E, A455E, G970S, W1098X, R1162X, and N1303K [2, 20, 21, 22, 23]. One study reported by second time the detection of the H609R mutation in Ecuadorian population [24]. It is worth to say that Ecuador is a multi-ethnic and multicultural country, with three major groups such as native Amerindians, mestizos, and Afro-Ecuadorians; these last two with a significant degree of genetic admixture that could change the genetic characterization of the pathology [25, 26].

For all the reasons previously described, a research project was initiated by our research team to improve the early diagnosis, and timely access to the treatment for the patients with CF. Situation appears complicated because several scenarios that can be present. In effect, these situations involve patients with higher values

in sweat test and clinical criteria, with higher values in sweat test but who do not meet the clinical criteria, with lower values in sweat test but with one or two mutations in the molecular test.

According with the state of the art, diagnosis standards are a combination of clinical findings, the measurement of chloride through a sweat test by two times, and the presence of two or more known mutations. The presence of salty sweat is still the gold standard test for CF diagnosis. This test consists in the measurement of sweat chloride and sodium concentrations [27, 28, 29]. Indeed, the analysis of electrolytes in sweat by a quantitative method has an accuracy superior to 90% [30, 31]. In the last years, a new method has been develop, the quantitative coulometric test that measure conductivity, especially to neonatal screening of CF [32].

Indeed, international recommendations suggest that after neonatal screening, newborns with two mutations of the CFTR gene could be diagnose with CF; but should also undergo a sweat test with chloride to confirm the diagnosis. Newborns with an identified CFTR only mutation require a chloride sweat test to distinguish healthy carriers from affected newborns with a second, unidentified mutation [48].

1.2. Objective

The purpose of this research is to analyze the clinical findings, based on previously established criteria, with the results of the sweat test, in circumstances where we do not have the routine molecular study. This research it could be applied in countries of low and middle resources.

2. Methods

2.1. Research design

Epidemiological, observational, analytic, and cross-sectional with one cohort of patients.

2.2. Context

This project had three phases: 1) Identification of patients with clinical criteria and suspicion of CF, in the largest pediatric hospitals in the country, being two hospitals that concentrate the majority of patients, the Carlos Andrade Marín Hospital and the Baca Ortiz Hospital but there are patients from other different medical centers also through the country. First contact physician diagnosed the patient only with regular clinical criteria; previously they were train. 2) The selected patients were send to the Translational Medicine Unit of the Faculty of Medical Sciences of the Central University, where a new clinical screening was carried out, through a survey which includes 25 diagnostic criteria, and those that meet at least three of the criteria are sent

to the sweat test. This second clinical screening is only confirmatory. 3) Patients with a positive test twice were send back to the hospital, where they enter a CF care program, and receive the proper treatment.

2.3. Subjects

It analyzed 180 (**n = 180**). Patients clinically suspected of CF. Inclusion criteria were: children of both sexes, older than 30 days and younger than 12 years, who meet at least three clinical criteria suggestive for the disease, ambulatory and that were referred by a specialist doctor who made a preliminary diagnosis, at major pediatric hospitals of Quito. Exclusion criteria were children under 30 days or older than 12 years, who were not under steroidal treatment, who have an acute illness or are hospitalized. Elimination criteria were deceased in the study period. Exposed: patients diagnosed by clinical criteria and high values of sweat test, Non-exposed: patients diagnosed only by clinical criteria.

2.4. Variables

The patient had to meet at least three (3) of 25 clinical criteria to perform the sweat test, and/or one of the major criteria. The clinical diagnostic criteria were: recurrent pneumonia with two or more episodes with hospitalization in one year, hyponatremic dehydration, brother deceased due to an unidentified respiratory cause, distal intestinal obstruction, digital clubbing, bronchial obstructive syndrome refractory to treatment, anemia, edema, hypoproteinemia in the infant, persistent or reticular radiological images, hepatomegaly and/or liver disease, nasal polyps, chronic cough of unknown cause, bilateral congenital absence of vas deferens, presence of *Pseudomonas aeruginosa*, brother with CF diagnosis, exocrine pancreatic insufficiency, chronic diarrhea, steatorrhea, asthma, bronchiectasis, hyponatremic dehydration with hypochloremic metabolic alkalosis, severe lower respiratory tract infections, chronic malnutrition, meconium ileus, rectal prolapse, chronic rhinosinusitis s in a certain etiology, prolonged neonatal illness. The major criteria were recurrent pneumonia (two or more episodes with hospitalization in a year); presence of *Pseudomonas aeruginosa aeruginosa* infection; exocrine pancreatic insufficiency; and digital clubbing. Survey also included a socio-demographic data, family history and past pathological history. The criteria were evaluated as presence or absence of them.

2.5. Laboratory method

Iontophoresis was perform through sweat test using sweat (Macroduct®, Wescor Inc., Logan, UT, USA) and analysis of osmolarity of sodium chloride through Sweat Check®. The sweat test system is composed of the sweat inducer (Webster

Sweat Inducer) that produces iontophoresis with sweat, the sweat collector (Macroduct® Sweat collector), and the conductive analyzer (Sweat Check Analyzer). Pilogel® iontophoretic discs are sweat ion gel deposits that bring simplicity and safety to the iontophoretic stimulation of sweat. A pilogel disc is simply inserted into each of the stainless steel recessed electrodes. The Webster Sweat Inducer Model 3700 performs sweat iontophoresis; it automatically provides the amount of sweat for the stimulation of the glands (equivalent to five minutes of iontophoresis at 1.5 mA). Some authors think that the Gibson and Cooke test could be a better choice than Macroduct® sweat collection system but Macroduct shows be easily performed, accurate method, and it can run in small quantity of samples, with shorter performance time and lower cost [18, 19].

2.6. Data source

Previous application of a patient survey with clinical criteria, for performance of the electrolyte test in sweat. The test is performed only in patients with clinical suspicion of CF. Prior to the test, anamnesis and physical examination of the patient by a physician trained in this pathology was performed.

2.7. Sampling

All the available patients, with a clinical suspicious, at the pediatric hospitals through the country were included, during a period of two years.

2.8. Avoided biases

It was always the same doctor who performed the physical examination, the operators of the equipment were always the same and received training to avoid variability; the equipment was calibrated before each sampling.

2.9. Quantitative variables

A test with less 60 mmol/L, it was a negative result; between 60 to 80 mmol/L, a doubtful result, and more than 80 mmol/L a positive result.

2.10. Statistical analysis

The statistical program R was used, and the packets knitr, epiR, Hmisc, caTools and ROCR. Patients with positive sweat test plus presence of three diagnostic clinical criteria were consider as “cases”. It performed bivariate and multivariate analysis.

3. Results

3.1. Subjects

It studied 180 patients, 88 (48.9%) of who were men. According to the ethnic distribution, most of the patients were self-define as mestizos, 173 (96.1%). The majority of patients were born and reside in the province of Pichincha (65.6%, n = 118); 159 patients (88.3%) were referred by health facilities belonging to the Integrated Public Health Network (RPIS); the remaining 11.7% were from private hospitals.

3.2. Descriptive data

Of the 180 patients, 30 (16.7%) presented a combination of clinical criteria suggestive of CF with positive sweat test; 158 (87.8%) only completed the clinical criteria, and the sweat test was negative.

3.2.1. Main results

Table 1 shows distribution of clinical findings according to standardized clinical criteria (n = 180). Most common findings were repeated pneumonia with more than two episodes (61.7%), chronic cough of unspecified cause (58.9%), bronchial obstructive syndrome refractory to treatment (36.7%) and asthma (36.7%). The most frequent clinical criterion for the reference and performance of sweat test is persistent pneumonia for two or more episodes with hospitalization; this means that the respiratory cause is predominant in some patients with CF.

Table 2 shows the distribution of the most frequent combinations of clinical criteria, with at least three findings in the same patient (n = 98). First combination was pneumonia + cough + digital clubbing (62.5%), followed by pneumonia + cough + asthma (43.8%), and, pneumonia + cough + *Pseudomonas aeruginosa* (60.0%). However, they do not have a statistically significant relationship with higher values in the sweat test. On contrary, we found a statistically significant relationship between the clinical combinations of pneumonia + cough + rhinosinusitis; pneumonia + cough + *Pseudomonas aeruginosa*; and pneumonia + cough + digital clubbing, in those cases the sweat test is indicated.

Table 3 showed the distribution of other related factors and use of medications in positive cases. They were growth retardation (53.3%), acropachies characterized by swelling and clubbing of fingers and toes and sometimes periostitis of the hands and feet (13.3%), inhaled corticosteroid (38.0%), oxygen saturation $\leq 90\%$ (14.7%) and, bronchodilators use (7.3%). The delay in growth, hypoxemia with oxygen saturation less than 90% and digital clubbing are statistically significant findings for perform the sweat test. The history of use of diuretic medications, systemic corticosteroids, bronchodilators, antibiotics and antihistamines had no statistical

Table 1. Distribution of clinical findings according to standardized clinical criteria (n = 180), descriptive analysis.

Ítem	Criteria	n=	%
1	Repeated pneumonia (more than 2 episodes)	111	61.7
2	Chronic cough of unspecified cause	106	58.9
3	Bronchial obstructive syndrome refractory to treatment	66	36.7
4	Asthma	66	36.7
5	Bronchiectasis	51	28.3
6	Chronic rhinosinusitis	49	27.2
7	Chronic malnutrition	41	22.8
8	Persistent or reticular radiological images	40	22.2
9	Prolonged neonatal jaundice	29	16.1
10	Anemia, edema and hypoproteinemia in the infant	24	13.3
11	Digital hypocrisy	23	12.8
12	Severe lower respiratory tract infections	22	12.2
13	Chronic diarrhea, steatorrhea	21	11.7
14	Presence of <i>Pseudomonas aeruginosa</i>	20	11.1
15	Hyponatremic dehydration	12	6.7
16	Meconial ileus	11	6.1
17	Distal intestinal obstruction	11	6.1
18	Brother with CF diagnosis	11	6.1
19	Brother deceased due to respiratory cause	9	5.0
20	Hepatomegaly and/or liver disease	8	4.4
21	Exocrine pancreatic insufficiency	7	3.9
22	Nasal polyps	6	3.3
23	Hyponatremic dehydration with hypochloremic metabolic alkalosis	2	1.1

Source: database of the patients analyzed. Elaboration: author.

relationship with the result of the sweat test. The use of topical corticosteroid medications is significantly associated with a negative result in the sweat test.

Table 4 shows the distribution of results by risk analysis according to bivariate analysis using simple 2×2 tables, where infection with *Pseudomonas aeruginosa* has 26.91 times more risk of presentation than others, digital clubbing 8.42 times, and 54.5 times more meconium ileus.

In Table 5, it saw the distribution of the results of the risk analysis according to bivariate analysis considering the presence or absence of CF. Patients with positive sweat test plus presence of three diagnostic clinical criteria were considered as cases. Infection with *Pseudomonas aeruginosa* were 26.91 times more frequent than other

Table 2. Distribution of the most frequent combinations of clinical criteria (n = 98).

Criteria combination	Cases with 3 or more criteria n = 37	%	Cases with 1 or 2 criteria only n = 61	%	Total n = 98	Chi-square	IC 95%
1 pneumonia + cough + digital clubbing	5	62.5	3	37.5	8	0.009	6.667 (1.431–31.07)
2 pneumonia + cough + asthma	7	43.8	9	56.3	16	0.02	3.565 (1.108–11.47)
3 pneumonia + cough + <i>Pseudomonas aeruginosa</i>	3	60.0	2	40.0	5	0.04	5.382 (0.8311–34.86)
4 pneumonia + cough + rhinosinusitis	5	45.5	6	54.5	11	0.05	1.15 (0.84–11.81)
5 pneumonia + cough + persistent or reticular Rx images	6	33.3	12	66.7	18	0.16	1.395 (0.39–4.56)
6 pneumonia + cough + bronchiectasis	5	27.8	13	72.2	18	0.29	1.401 (0.4267, 4.6)
7 pneumonia + cough + obstructive bronchial syndrome	6	27.3	16	72.7	22	0.39	1.15 (0.38–3.47)

Source: database of the patients analyzed. Elaboration: author.

Table 3. Distribution of other associated factors and use of medications.

	Non- exposed n = 150	%	Exposed n = 30	%	Total with finding	%	X ²	p < 0.05	CI (95%)
1 Growth retardation	80	53.3	23	76.7	103	57	16.43	0.001	9.29 (2.89–40.67)
2 Acropachies	20	13.3	10	33.3	30	17	17.77	0.001	7.16 (2.54–20.48)
3 Inhaled corticosteroid	57	38.0	2	6.7	59	33	11.71	0.001	0.11 (0.01–0.042)
4 Oxygen saturation $\leq 90\%$	22	14.7	8	26.7	30	17	6.83	0.005	3.54 (1.25–9.72)
5 Bronchodilators	11	7.3	4	13.3	15	8	3.15	0.030	3.08 (0.73–11.59)
6 Antibiotics	9	6.0	3	10.0	12	7	1.81	0.080	2.6 (0.5–11.28)
7 Systemic corticoid	6	4.0	0	0.0	6	3	1.27	0.120	0
8 Diuretics	5	3.3	0	0.0	5	3	1.05	0.150	0
9 Productive cough	42	28.0	9	30.0	51	28	0.8	0.180	1.5 (0.58–3.69)
10 Antihistamines	7	4.7	1	3.3	8	4	0.03	0.400	0.8 (0.093–6.99)

Source: database of the patients analyzed. Elaboration: author.

findings, digital clubbing 8.42 times, and meconium ileus 7.25 times. The bivariate analysis showed an association between the positive sweat test with the bronchial obstructive syndrome; chronic malnutrition; and very importantly with the presence of infection by *Pseudomonas aeruginosa* in sputum cultures; and also, with the presence of digital clubbing, meconium ileus, the development of severe lower respiratory infections, and the presence of nasal polyposis. Borderline association was observed between the positive sweat test with a sibling with CF diagnosis, and diagnosis of exocrine pancreatic insufficiency also. NO significant association were found with presence of rhino-sinusitis, images of reticular alterations in X rays, pneumonia, chronic diarrhea, with hyponatremic dehydration, anemia report, edema

Table 4. Distribution of results by risk analysis according to bivariate analysis using simple 2×2 tables.

Diagnostic criteria	Exposed n=	%	Non exposed n=	%	OR	IC (95%)	p < 0.05
1 Infection with <i>Pseudomonas aeruginosa</i>	8	80.0	22	12.90	26.91	5.36–135.01	0.0001
2 Digital clubbing	12	52.2	18	11.50	8.42	3.24–21.87	0.0001
3 Meconial ileus	6	54.5	24	14.20	7.25	2.05–25.64	0.0010
4 Obstructive bronchial syndrome	18	27.3	12	10.50	3.19	1.42–7.14	0.0040
5 Malnutrition	15	29.4	15	11.60	3.17	1.41–7.10	0.0040
6 severe lower respiratory infections	8	36.4	22	13.90	3.53	1.33–9.40	0.0080
7 Nasal polyps	3	50.0	27	15.50	5.44	1.04–28.41	0.0300
8 Pancreatic insufficiency	3	42.9	27	15.60	4.06	0.86–19.15	0.0580
9 Brother with CF	4	36.4	26	15.40	3.14	0.86–11.50	0.0700
10 Rhinosinusitis	12	24.5	18	13.70	2.04	0.90–4.62	0.0850
11 Reticular images in X rays	12	24.0	18	13.80	1.96	0.87–4.45	0.1000
12 Dehydration	4	33.3	26	15.50	2.73	0.77–9.73	0.1100
13 Hepatomegaly	3	37.5	27	15.70	3.22	0.73–14.29	0.1100
14 Bronchiectasis	11	21.6	19	14.70	1.59	0.70–3.64	0.2700
15 Neonatal jaundice	3	10.3	27	17.90	0.53	0.15–1.88	0.3200
16 Intestinal obstruction	1	9.1	29	17.16	0.48	0.06–3.92	0.4900
17 Anemia	3	12.5	27	17.30	0.68	0.19–2.45	0.5600
18 Chronic cough	19	17.9	11	14.90	1.25	0.56–2.81	0.5900
19 Asthma	12	18.2	18	15.80	1.19	0.53–2.65	0.6800
20 Pneumonia	19	17.1	11	15.90	1.09	0.48–2.45	0.8400
21 Chronic diarrhea	5	16.1	25	16.80	0.95	0.33–2.72	0.9300

Source: database of the patients analyzed. Elaboration: author.

and hypoproteinemia in the infant, the presence of bronchiectasis, asthma, a history of neonatal jaundice and hepatomegaly.

The distribution of risk factors included in the predictive model and associated with the diagnosis of CF according to multivariate models, performed with binary logistic regression, it was adjusted for age and the presence of allergies as confounding factors, was showed in Table 6. Hyponatremic dehydration, exocrine pancreatic insufficiency, meconium ileus, brother with CF diagnosis, and infection with *Pseudomonas aeruginosa* had a statistical difference with the other factors. The OR for hyponatremic dehydration was 191 times more and for exocrine pancreatic insufficiency 152 times more.

Table 5. Distribution of the results of the risk analysis according to bivariate analysis. Patients with high values in sweat test plus presence of three diagnostic clinical criteria were considered as cases.

	Exposed		Non-exposed		OR	IC (95%)	p < 0.05
	n = 30	%	n = 150	%			
1 Infection with <i>Pseudomonas aeruginosa</i>	8	26.7	2	1.3	26.91	5.36–135.01	0.0001
2 Digital clubbing	12	40.0	11	7.3	8.42	3.24–21.87	0.0001
3 Meconial ileus	6	20.0	5	3.3	7.25	2.05–25.64	0.0010
4 Bronchial obstructive syndrome	18	60.0	48	32.0	3.19	1.42–7.14	0.0040
5 Malnutrition	15	50.0	36	24.0	3.17	1.41–7.10	0.0040
6 Severe lower respiratory infections	8	26.7	14	9.3	3.53	1.33–9.40	0.0080
7 Nasal polyps	3	10.0	3	2.0	5.44	1.04–28.41	0.0300
8 Pancreatic insufficiency	3	10.0	4	2.7	4.06	0.86–19.15	0.0580
9 Brother with CF	4	13.3	7	4.7	3.14	0.86–11.50	0.0700
10 Rhinosinusitis	12	40.0	37	24.7	2.04	0.90–4.62	0.0850
11 Reticular images in X rays	12	40.0	38	25.3	1.96	0.87–4.45	0.1000
12 Dehydration	4	13.3	8	5.3	2.73	0.77–9.73	0.1100
13 Hepatomegaly	3	10.0	5	3.3	3.22	0.73–14.29	0.1100
14 Bronchiectasis	11	36.7	40	26.7	1.59	0.70–3.64	0.2700
15 Neonatal jaundice	3	10.0	26	17.3	0.53	0.15–1.88	0.3200
16 Intestinal obstruction	1	3.3	10	6.7	0.48	0.06–3.92	0.4900
17 Anemia	3	10.0	21	14.0	0.68	0.19–2.45	0.5600
18 Chronic cough	19	63.3	87	58.0	1.25	0.56–2.81	0.5900
19 Asthma	12	40.0	54	36.0	1.19	0.53–2.65	0.6800
20 Pneumonia	19	63.3	92	61.3	1.09	0.48–2.45	0.8400
21 Chronic diarrhea	5	16.7	26	17.3	0.95	0.33–2.72	0.9300

Source: database of the patients analyzed. Elaboration: author.

In the multivariate analysis with logistic regression, it found that factors associated with the diagnosis of clinical CF with higher values in sweat test and digital clubbing. At some time with *Pseudomonas aeruginosa* infection, with a history of having a brother diagnosed by CF, presence of meconium ileus, exocrine pancreatic insufficiency and hyponatremic dehydration. Factors that were inversely related with CF were the presence of chronic diarrhea or intestinal obstruction, all of which showed a statistically significant association and an adequate predictive power.

Fig. 1 showed a ROC curve for predictive performance in CF diagnosis, defined as compatible clinical presence plus positive sweat test, model evaluated; the total yield

Table 6. Distribution of risk factors included in the predictive model and associated with the diagnosis of CF according to multivariate models, performed with binary logistic regression. It was adjusted for age and the presence of allergies as confounding factors.

		OR	IC (95%)	p < 0.05
1	Hyponatremic dehydration	191.03	14.14–4505.61	0.0001
2	Exocrine pancreatic insufficiency	152.77	5.27–8064.36	0.006
3	Meconial ileus	65.73	6.16–1179.40	0.001
4	Brother with CF diagnosis	32.19	1.45–1401.39	0.041
5	Infection with <i>Pseudomonas aeruginosa</i>	24.37	2.08–487.07	0.019
6	Digital clubbing	5.46	1.24–26.46	0.027
7	Chronic rhinosinusitis	4.57	1.05–22.72	0.049
8	Chronic diarrhea	0.09	0.01–0.71	0.039
9	Intestinal obstruction	0.01	0.00–0.35	0.048

Source: database of the patients analyzed. Elaboration: author.

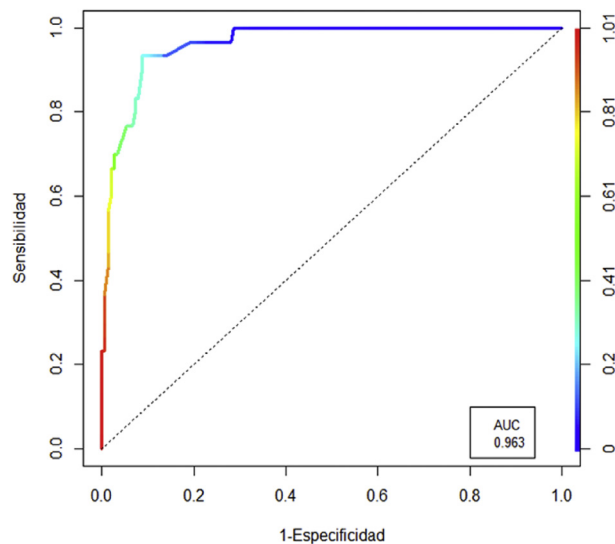


Fig. 1. ROC curve for predictive performance in CF diagnosis, defined as compatible clinical presence plus positive pilocarpine test, model evaluated; the total yield reached 91.1%, with an AUC: 0.963. The best cut point was reached with a probability $p \geq 0.25$, the prediction model reached a total yield of 91.1%, with a sensitivity of 93.3% (95% CI: 77.9% to 99.2%); specificity of 90.7% (95% CI: 84.8% to 94.8%); a positive predictive value (PPV) of 66.7% (95% CI: 54.6% to 76.9%) and a negative predictive value of 98.6% (95% CI: 94.7% a 99.6%), total area under the curve (AUC) was calculated at 0.963.

reached 91.1%, with an AUC: 0.963. The best cut point was reached with a probability $p \geq 0.25$, the prediction model reached a total yield of 91.1%, with a sensitivity of 93.3% (95% CI: 77.9% to 99.2%); specificity of 90.7% (95% CI: 84.8% to 94.8%); a positive predictive value (PPV) of 66.7% (95% CI: 54.6% to 76.9%) and a negative

predictive value of 98.6% (95% CI: 94.7% a 99.6%), the area under the curve (AUC) was calculated at 0.963.

4. Discussion

4.1. Clue results

According to [Table 1](#) the standardized clinical criteria was a very useful tool to guide diagnostic; these criteria are widely used [33]. All unspecific symptoms were eliminated of these criteria. Respiratory symptoms were the most common in early stages. Age of patients could vary the clinical manifestations age at presentation. Meconium ileus may be present in neonates, in children younger than one year may be observed wheezing, coughing, and/or recurring respiratory infections and pneumonias; in this study two or more hospitalizations were required as inclusion criteria. In early infancy gastrointestinal symptoms could be appear like steatorrhea, failure to thrive, and malnutrition as consequence [34]. In older children may diagnose pancreatic sufficiency and with chronic cough and sputum production. It is estimates that 10% of patients with CF show always meconium ileus [35]. At birth these patients could have abdominal distension, that progress to failure to pass meconium, bilious vomiting, and progressive abdominal distension. In children with complicated meconium ileus can present severe abdominal distention, abdominal wall erythema and edema occasional. Respiratory distress may be a consequence of abdominal. Differential diagnosis includes asthma, bronchiectasis, acute sinusitis, celiac disease, bronchiolitis, and short stature, failure to thrive, aspergillosis, and primary ciliary dyskinesia.

In [Table 2](#) it could see the most common combination with pneumonia plus cough and digital clubbing. The mechanism of digital clubbing is still unknown, with some theories proposed but none conclusive [36]. The combination of symptoms can be useful to identify patterns of disease, sometimes could be consider pathognomonic findings [29].

In [Table 3](#) it can see that delay in growth, hypoxemia with oxygen saturation less than 90% and digital clubbing most important related findings. The first one implies that it exist chronicity and general decay. The others two are related with the lack of tissues oxygen. Clubbing is due to distal digital vasodilation, and an in increased blood flow to the digits [37]. The only important related factor is the use of oral corticosteroid medications; which is significantly associated with low values result in the sweat test in this study. Oral corticosteroids at a dose equivalent to prednisolone of 1 to 2 mg/kg every other day seem to slow the progression of lung disease in CF [38].

[Table 4](#) shows that infection with *Pseudomonas aeruginosa*, digital clubbing, and meconium ileus is most likely diagnosis in all cases, without age difference.

Pseudomonas aeruginosa infections are due to chronicity caused by bacterial persistence, and some bacterial strains have a phenotypic change that carries a major production of a polysaccharide called alginate [39]. Prognosis is poor when *Pseudomonas aeruginosa* colonization is associated with *Staphylococcus aureus* [40].

The high values of sweat test was related with bronchial obstructive syndrome, chronic malnutrition, infection by *Pseudomonas aeruginosa*, digital clubbing, meconium ileus, the development of severe lower respiratory infections, and nasal polyposis, as it shown in Table 5. According with some authors [41, 42, 43], levels of sweat chloride could be higher adrenal insufficiency, atopic dermatitis, glycogen storage disease, hypothyroidism, type 1 fucosidosis, vasopressin-resistant diabetes insipidus, ectodermal dysplasia, malnutrition, mucopolysaccharidosis, pan hypopituitarism, familial cholestasis, familial hypoparathyroidism, and iatrogenic causes. CFTR gene mutations may be the cause of sweat test variability, with variations by time, environmental, residual factors, and individual factors mostly genetics [44].

Hyponatremic dehydration, exocrine pancreatic insufficiency, meconium ileus, brother with CF diagnosis, and infection with *Pseudomonas aeruginosa* were most important factors in clinical diagnosis. The cause of exocrine pancreatic insufficiency is accumulation of secretions within the pancreatic ducts that leads to interstitial fibrosis [45, 46].

In relation with Fig. 1 the ROC curve for predictive performance in CF diagnosis, defined as compatible clinical presence plus high values in sweat test, the total yield reached was 91.1%; with a sensitivity of 93.3% and a specificity of 90.7%. It means that one of the most common clinical combinations described before plus high values of chloride in sweat test, is sufficient to diagnose 91% of the patients.

It is important to say that sweat tests should be performed only in children older than two weeks and at least two kg of weight. Following standard recommendations [47, 48] sweat testing should be delayed in patients when conditions is acutely unwell, dehydrated, edematous, malnourished or does not have a suitable skin site free of eczema. This guideline also refers, that to chloridimetry, inductively coupled plasma mass spectrometry (ICP-MS) and ion chromatography/high performance liquid chromatography (IC-HPLC) are appropriate to perform analysis of sweat chloride analysis [48].

4.1.1. Limitations

Some limitations were present, it was not possible to correlate phenotypes with mutation because there was not availability of the molecular tests, only a very few patients has molecular results. Second, this study was a pilot study performed in patients of a few provinces.

4.1.2. Generalization

It is possible to generalize results to other countries with similar conditions, and no availability of routine molecular tests. For the author this project was very successful, and it received some petitions to move the idea to neighbors' countries.

4.1.3. Conclusion

Clinical combinations of pneumonia with cough and rhinosinusitis; pneumonia with cough; presence of *Pseudomonas aeruginosa*; and pneumonia with digital cough and clubbing are most common combinations of clinical presentation of CF that indicate sweat test analysis. Predictive performance of CF diagnosis, defined as compatible clinical presence plus high values of chloride in sweat test, was 91.1%.

Declarations

Author contribution statement

Fabricio Gonzalez-Andrade:conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools or data; wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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