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Diagnosis of cystic fibrosis

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Abstract

Applying the sweat-test as the first choice of test when a diagnosis of cystic fibrosis is suspected is still common practice and advisable. Since the cloning of the CFTR gene more than 400 different cystic fibrosis (CF) mutations have already been identified. The use of CF mutation analysis for diagnostic purposes in CF has therefore remained elusive so far. It is advised to perform sweat-tests as previously described by Gibson and Cooke. In this study we have re-evaluated the results of sweat-tests of 1905 subjects performed in our hospital over a period of 9 years (1983–1992). In 1825 subjects where the CF diagnosis was not made, the mean sodium value obtained was 15.5 ± 9.2 mmol/l. The upper limit of the normal range (2 SD above the mean) is 34 mmol/l. Re-examination of all 239 sweat sodium values (80.9 ± 19.5 mmol/l) in 80 newly diagnosed CF patients (all: $Na^+ > 70$ mmol/l) revealed that 5% of the values were below 50 mmol/l, the lowest sweat value obtained being 27 mmol/l. Based on these results, we recommend in case of clinical suspicion of CF and sweat values above 30 mmol/l to repeat the sweat-test and to determine both sodium and chloride for optimal discrimination.

Keywords: Cystic fibrosis; Genotype; Sweat-test; Diagnosis

1. Introduction

Classically, the diagnosis of cystic fibrosis (CF) is based on the high salt content in sweat due to a defect in reabsorbing chloride of the sweat duct [1]. The CF transmembrane conductance regulator may be held responsible for the defective chloride transport [2]. Since the cloning of the CFTR gene [3], more than 400 different cystic fibrosis (CF) mutations have already been identified. The use of CF mutation analysis for diagnostic purposes in CF has therefore remained elusive so far. In our centre (112 paediatric and 57 adult genotyped patients) no CF mutation was identified in 2.4% of patients while in 21.9% only one mutation could be found. Of all chromosomes tested, the mutations found are given in Table 1.

2. Clinical presentation of cystic fibrosis

Applying the sweat-test as a diagnostic procedure for CF originates from the clinical suspicion of the disease. It is thus crucial to have knowledge of the presenting symptoms of CF. In our hospital (118 paediatric CF patients) 68.6% were diagnosed within the first year of life. Twenty-six (22%) presented with meconium ileus while in all cases of meconium ileus studied in the last few years, 42 out of 54 (77.8%) appeared to have CF. Although the presence of meconium ileus is very

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suspicious of CF, it is not specific for this disease [4]. In 21% of the patients the mode of presentation was related to other intestinal manifestations such as steatorrhoea or failure to grow. In 19% respiratory symptoms raised the clinical suspicion of CF (chronic sinusitis, nasal polyps, chronic cough, recurrent lower airway infections, suspect sputum cultures with *Staphylococcus aureus* and *Pseudomonas aeruginosa*, or abnormal X-ray findings suggestive of bronchiectasis). Furthermore, respiratory as well as intestinal symptoms were present in 16.1% of patients. Finally, 8.5% of patients were detected due to a positive family history.

3. The sweat-test

For reliable sweat-testing it is advisable to use only the method as previously described by Gibson and Cooke [5]. Carefully wash the skin area with distilled water. Pilocarpine iontophoresis should be applied for 5 (max. 10) min. Use filter paper, cup or capillary tube for collection during 1 h. Determination should be performed of both chloride and sodium. It is advised that the amount of sweat produced is more than 100 mg. In our hospital (now > 400 tests yearly) false results due to a small production were not found when at least 30 mg of sweat is produced. However, we still advise to await a test result with more than 50 mg of sweat. It is important to perform the diagnostic sweat-test beyond the age of 6 weeks since early tests may give a false-positive result. This is due to the physiological low sodium reabsorption at young age. Here, a false-positive result may be suggested from the higher sodium content of the sweat compared to chloride.

Table 1
CF mutations found in CF Center Rotterdam (118 paediatric and 68 adult patients)

CF mutations	% of 338 chromosomes tested
dF508	73.3
A445E	4.1
1717 1G → A	2.7
G542X	2.1
N1303K	0.9
R553X	0.9
L927P	0.9
dL1260	0.6
R1162X	0.3
1078delT	0.3
R75Q	0.3

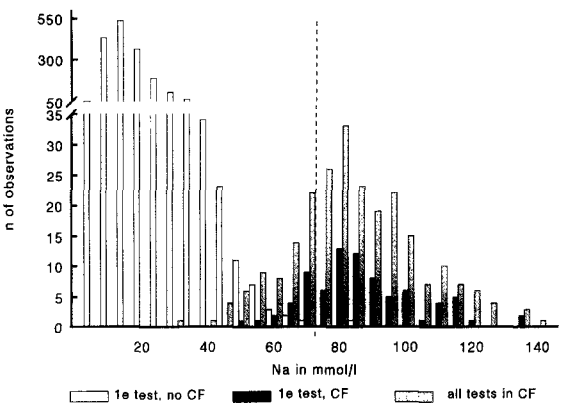

Fig. 1. All sweat sodium values of the *first* (adequately performed) sweat-test in the period from January 1983 to May 1992. In 1825 subjects (open bars) where the CF diagnosis was not made, the mean sweat-test result was 15.5 ± 9.2 mmol/l. From these subjects 144 had a first sweat sodium of more than 30 mmol/l in which the CF diagnosis may well be reconsidered. The mean of the first sweat-test results in 80 diagnosed CF patients (filled bars) was 83.7 ± 17.2 mmol/l. All CF patients fulfilled our previously used criterion of a positive sweat-test (> 70 mmol/l;vertical line). In addition, the variation of all 239 sweat sodium values in the 80 CF patients was considerable (stippled bars); 5% were below 50 mmol/l, and the lowest sweat value obtained was 27 mmol/l.

To be conclusive, a positive test result (chloride above 60 mmol/l or sodium above 70 mmol/l) should be obtained on two separate occasions. Sweat chloride levels provide a better discrimination between normal persons and CF patients than do sodium levels [6–8]. Using the above-mentioned criteria for a CF diagnosis, sweat-test results of some CF patients may be within the "normal" range [9,10]. In the following study an attempt was made to unravel the question of what really can be defined as the normal result of the sweat-test.

We re-examined all sweat-test results in the period from January 1983 to May 1992. Chloride measurements were not routinely performed in this period. From each of the 1905 subjects the result of the first adequately performed sweat-test

was used. These data were stratified for the known 80 CF patients diagnosed in this period. In 1825 subjects where the CF diagnosis was not made the mean sodium value obtained was 15.5 ± 9.2 mmol/l. The upper limit of the normal range (2 SD above the mean) is 34 mmol/l. All 80 CF patients fulfilled our previous criterion of a positive sweat-test result ($Na^+ > 70$ mmol/l). In addition, to investigate the variability of the sweat-test from the 80 CF patients all their 239 sweat-test results were obtained (Na^+ 80.9 ± 19.5 mmol/l). Five percent of the values were below 50 mmol/l and the lowest sweat value obtained was 27 mmol/l. This demonstrates that a CF diagnosis may not be excluded when sweat sodium is just around 30 mmol/l.

4. Intestinal current measurement

Studying intestinal current measurements (ICM) in rectal biopsies of CF patients [11], the magnitude of the chloride current (largely determined by the CF genotype) was found to correlate with the clinical phenotype [12]. Using the same technique for diagnostic purposes [13] revealed that it is very likely that CF can be diagnosed with ICM in those patients whose sweat sodium values are just above 30 mmol/l. Until now ICM has proved to be highly sensitive and specific since chloride channel and sodium channel activity can be studied separately. The sweat-test in itself is not very specific for CF since low sodium reabsorption (as found in several endocrinopathies) may also cause an abnormal result. Elevated sweat-tests (even with chloride in excess of sodium) may also be found in known CF carriers. Diagnostic evaluation of these carriers can be troublesome since they may well have one detectable CF mutation as do most "borderline" CF patients. Since sweat values tend to rise with age, difficulties in the interpretation of the test can also be found in adults. It is our experience that ICM is especially of value in these situations.

5. Different phenotypes of CF and their genotypes

Another fact pointing in the direction that the CF incidence may be higher than we presently presume, derives from the knowledge of males with congenital bilateral absence of vas deferens (CBAVD). This is likely to be a genital form of CF although some of these males may also suffer from other CF manifestations [14,15]. In males with CBAVD approximately 12% carry a R117H mutation [15]. This is suspected to be a CF mutation, occurring very rarely in CF patients. From Table 1 it can also be suggested that not all CF patients are recognized: The number of dF508 chromosomes found in CF patients (73.3%) is less than expected (77.3%) from the number of dF508 homozygous patients (59.8%).

6. Conclusion

It is strongly advised to change the lower limit of a borderline sweat-test from 50 to 30 mmol/l. Repeated, well-performed sweat-tests and in selected cases additional tests (i.e. CF mutation analysis, ICM, nasal PD measurements, CFTR antibodies) may be of help in the CF diagnosis.

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