Indirect measurements of sweat electrolyte concentration in the laboratory diagnosis of cystic fibrosis

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Abstract

Aim—To investigate whether analytical methods based on the colligative physical chemical properties of ions or solutes in sweat are less effective than the specific measurement of electrolytes in the diagnosis of cystic fibrosis (CF).

Methods—A single sweat sample was collected (Macroad) from each of 211 infants and children, of whom 57 had CF, for the measurement of sodium, chloride, osmolality, and conductivity.

Results—The ranges within which CF and non-CF individual values overlapped (equivocal ranges), were wider for sodium and osmolality measurement than for chloride or conductivity. Neither of the latter two measurements provided a discriminatory advantage over the other. The utilisation of broadly based age related ranges for non-CF control subjects served to improve the discriminatory power of all four measurements to an extent that, in this cohort, both chloride and conductivity provided complete discrimination.

Conclusion—Sweat conductivity is as effective as chloride measurement in the laboratory diagnosis of CF.

Keywords: cystic fibrosis; sweat test; sodium; chloride; conductivity; osmolality

The laboratory diagnosis of cystic fibrosis (CF) remains largely dependent on the measurement of electrolytes in sweat, despite the clear benefits that CFTR mutation analysis has brought to the diagnostic process. In the USA the sweat test is one of those clinical laboratory procedures for which National Consensus Guidelines have been issued as an aid to good practice. The latter include advice that indirect physical chemical measurements, osmolality, and conductivity, provide only approximate estimates of sweat electrolyte concentration, to a degree that renders them unreliable for the purpose of diagnostic testing. This view has been endorsed by the American CF Foundation with the recommendation that the “indirect” methods should be confined to use as screening tests. In the absence of supporting laboratory data it is necessary to question the validity of such advice and recommendations.

In this study we have sought to obtain comparative data from these different methods of measurement. We consider that the issue is one of importance for clinical, and in particular, paediatric practice, not only because these recommendations are becoming more widely publicised, but also because in the UK, to our knowledge, a considerable number of hospital laboratories are using indirect methods to provide sweat test results for diagnostic purposes.

Subjects and methods

Sweat tests were carried out on 57 infants and children with CF (mean age 3 years 10 months, range 0.5 months to 15 years), and on 154 who did not have this disease and who served as controls (mean age 2 years 10 months, range 0.5 months to 14 years 8 months). The latter were mostly infants and children, under the clinical care of one of us (DAW), who had presented with one or more of the signs and symptoms commonly associated with CF. In view of the fact that virtually all (98%) CF cases born in East Anglia are detected by neonatal screening, and that most control subjects had been screened with negative results, a diagnosis of CF was considered unlikely in the vast majority of these cases. However, in a non-screening setting, it was considered that sweat testing would have been justified. A small minority of control subjects were well infants who underwent sweat testing either on the grounds of an existing family history of the disease or because of a positive neonatal screening test result (prolonged neonatal hypertrypsinemia), but who on clinical examination were found to have no discernible signs or symptoms; the sweat test results obtained were not considered diagnostic for CF by the generally accepted criteria, as defined below in Results. Table 1 shows the proportion of control subjects in these various clinical categories.

Table 1 Number (%) of control subjects according to clinical category

<table>
<thead>
<tr>
<th>Signs and symptoms</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Failure to thrive + GI obstruction, diarrhoea, rectal prolapse</td>
<td>70 45</td>
</tr>
<tr>
<td>Respiratory</td>
<td>46 30</td>
</tr>
<tr>
<td>Failure to thrive + respiratory</td>
<td>20 13</td>
</tr>
<tr>
<td>Family history of CF (well)</td>
<td>9 5</td>
</tr>
<tr>
<td>Positive screening test (well)</td>
<td>5 3</td>
</tr>
<tr>
<td>Prolonged jaundice</td>
<td>2 1.5</td>
</tr>
<tr>
<td>Failure to thrive + family history</td>
<td>2 1.5</td>
</tr>
</tbody>
</table>

The CF patients included those, who during the period of this study, had positive screening test results; diagnostic sweat tests were therefore performed. In most of these cases diagnosis was confirmed by mutation detection and, in the course of time, most infants developed some of the clinical signs and symptoms of the
Table 2 Results of direct and indirect electrolyte measurement in sweat, including equivocal ranges and number of subjects contributing to them

<table>
<thead>
<tr>
<th>Conductivity (mmol (NaCl eq)/l)</th>
<th>Osmolality (mmol/kg)</th>
<th>Sodium (mmol/l)</th>
<th>Chloride (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td><strong>CF</strong></td>
<td><strong>Control</strong></td>
<td><strong>CF</strong></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>37 (10)</td>
<td>110 (13)</td>
<td>23 (12)</td>
</tr>
<tr>
<td>Total no. subjects</td>
<td>3</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>CF (n)*</td>
<td>1 (B)</td>
<td>1 (B)</td>
<td>4 (A, B, C, D)</td>
</tr>
<tr>
<td>Control (n)*</td>
<td>2 (E, G)</td>
<td>6 (E, F, G, H, L, M)</td>
<td>8 (E, F, H, I, J, K, L, N)</td>
</tr>
</tbody>
</table>

*Individual patients A–N, in parentheses, as referred to in text.

Table 3 “Equivocal” results obtained in sweat of four CF patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (mth)</th>
<th>Genotype</th>
<th>Conductivity (NaCl eq/l)</th>
<th>Osmolality (mmol/kg)</th>
<th>Sodium (mmol/l)</th>
<th>Chloride (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>29</td>
<td>ΔF508/R117H</td>
<td>75</td>
<td>176</td>
<td>56</td>
<td>54</td>
</tr>
<tr>
<td>B</td>
<td>8</td>
<td>ΔF508</td>
<td>67</td>
<td>157</td>
<td>36</td>
<td>46</td>
</tr>
<tr>
<td>C</td>
<td>92</td>
<td>ΔF508</td>
<td>88</td>
<td>188</td>
<td>66</td>
<td>69</td>
</tr>
<tr>
<td>D</td>
<td>9</td>
<td>ΔF508</td>
<td>84</td>
<td>193</td>
<td>51</td>
<td>64</td>
</tr>
</tbody>
</table>

Results

Table 2 presents the results obtained from the four measurements on sweat specimens of both CF and control subjects. The data show that there are ranges of overlapping values observed for some CF and control subjects and that this occurs for both direct and indirect measurements (albeit with varying degrees of magnitude). In the diagnostic setting, these limits of overlap would constitute the observed equivocal ranges, and included in table 2 are the numbers of subjects of both categories, with individual patients designated A to N, whose values for each of the four measurements encompass the overlapping (equivocal) ranges.

From the data it appears that sweat conductivity and chloride measurements provide better discrimination between patient and controls than sodium and osmolality, with fewer data points overlapping within ranges of narrower limits. Only one of the CF patients (patient B) contributed “equivocal” values for all four measurements, the remaining three patients (A, C, and D) contributing only those of sodium. The results obtained for the control subjects were similar, with only two (E and G) providing “equivocal” values from each of three measurements, the remaining subjects contributing only to those of sodium and osmolality. Table 3 presents the individual results obtained for the four CF patients (A–D).

All four patients possessed one copy of the CFTR mutation ΔF508, and in one patient (A) R117H was identified as the mutation on the other chromosome. In spite of extensive testing the second mutation could not be identified in the three other CF patients. At the time these sweat tests were performed all four patients had developed clear signs and symptoms of the disease; all had increased blood immunoreactive trypsin (IRT) concentrations at birth.

It was observed that among the control subjects, whose sweat test results overlapped those of CF patients, there was an apparent over-representation of the relatively fewer older children (age 9–15 years), 30% versus 7% in the entire control population. Although there were too few subjects to establish reference values within narrowly defined age ranges, the increase in sweat electrolyte which occurs with age whether measured by direct or indirect methods is clearly discernible (see table 4). No such age related effect could be shown in the CF patients (data not shown). When taking age into account, in respect of non-CF control reference values, the number of subjects exhibit-
Conductivity measurement is as e
suggests that, for all practical purposes, sweat
to be found in the data presented here which
nostic value. Support for that assumption is not
chloride can provide analytical results of diag-
assertion that only direct measurements of
nating power of these indirect measurements.
The assumption that the latter, non-specific
chloride in distinguishing between CF and
measurement 1:57 CF, 1:154 non-CF; for conductivity
measurement 1:57 CF, 2:154 non-CF; for chloride
measurement 1:57 CF, 1:154 non-CF
(8) 12 (8)
18 (8) 12 (5)
7–42 6–25
34–35 24–46
34–35 24–46
9–51 5–30
51–56 30–54
63–66 16–42
67–73 42–73

Table 5 Sweat osmolality and sodium measurement in
neonates

Osmolality
(Sodium
(mmol/kg))
(mmol/l)

CF
Mean (SD) 241 (20) 91 (14)
Observed range 196–289 53–125
n 75 75
Control
Mean (SD) 103 (24) 22 (11)
Observed range 66–173 6–73
n 95 95

Discussion
On theoretical grounds osmolality and conductiv-
ity measurement might be expected to provide very good discrimination between nor-
ormal and CF sweat electrolyte concentrations.
Sodium, potassium, and chloride, the predomi-
nant electrolyte constituents of sweat, are all in-
creased in CF and the incremental contribu-
tion of each would be additive by virtue of the
colligative nature of the measurement. On the
other hand the contribution by other solutes
and electrolytes present in sweat, in variable
concentration and not specifically increased in
CF, would predictably diminish the discrimi-
nating power of these indirect measurements.
The assumption that the latter, non-specific
elements, predominate must underlie the
assertion that only direct measurements of
chloride can provide analytical results of diag-
nostic value. Support for that assumption is not
to be found in the data presented here which
suggests that, for all practical purposes, sweat
conductivity measurement is as effective as
chloride in distinguishing between CF and
non-CF subjects.
Confidence intervals for differences between
observed and expected proportions cannot be
estimated because this population generates
too few equivocal results; for conductivity
measurement 1:57 CF, 2:154 non-CF; for chloride
measurement 1:57 CF, 1:154 non-CF.
(table 2). Any generalisation about the prac-
tical implications of these findings must depend
primarily on the assumption that the CF popu-
lation sample studied was representative of
patients presenting in routine clinical practice.
Scrutiny of the data published by others, in
both prospective and retrospective studies, shows
that the proportion of cases and the range of values where CF and non-CF sweat
test results overlap, obtained either by direct or
indirect sweat electrolyte measurement, is very
similar to the results we report here. For exam-
ple, in two comparative case studies, the range
of values for sweat conductivity, chloride, sodium, and osmolality obtained from
“equivocal” cases have been cited as 51–79
mmol (NaCl eq)/l, which were predictive of CF. If, however, values for sweat chloride of greater than 60
mmol/l are considered predictive of CF then
the status of both these patients would be con-
cordantly judged by both sweat chloride and
conductivity measurement.
A recent survey has shown that the CF case
yield, from sweat testing undertaken in labora-
tories serving major paediatric centres, is
approximately one in 30 tests performed, from
an annual (median) workload of 150 tests. On
the basis of our findings, therefore, where sweat
post results by either chloride or conductivity
measurement were indistinguishable from “nor-
mal” in only one of 57 CF patients, such values
would be expected to occur in clinical practice
once in approximately 11 years. In fact such
cases might occur even more rarely in the dis-
11 years. In fact such
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11 years. In fact such
cases might occur even more rarely in the dis-
age range when compared with the subjects in other relevant studies. Our data show that a notable improvement in discriminatory power of the sweat test results, obtained either by direct or indirect measurement of electrolyte, occurs when the age of the subject is taken into account (table 4). Although age related changes in sweat electrolyte concentration have been recognised for some time, little attention seems to be given to this fact, and its potential for influencing the interpretation of a sweat result outside the traditional, but vague, concept of a demarcation between childhood and adulthood.

The results we have obtained in infants under 12 weeks of age (table 5), show that a precisely defined reference range enables osmometry to be used with diagnostic sensitivity and specificity. Our data also suggest that for older children, refining the reference ranges with respect to age can considerably improve the diagnostic efficiency of sweat osmolality measurement (table 4). Despite this improvement overall sweat osmolality and sodium measurements remain marginally inferior to conductivity and chloride.

The only CF patient (case B) whose sweat test results, for osmolality and sodium, remained equivocal, when age was taken into account, is of particular interest. He presented at a few weeks of age with respiratory symptoms that were immediately suggestive of CF. His sweat test, for chloride, was diagnostically inconclusive when performed in a specialist CF centre and the diagnosis was established by nasal epithelium chloride channel conductance measurement. Although born outside East Anglia where his neonatal biochemical screen test did not include IRT measurement, at the age of 8 months retrospective testing of his neonatal dried blood specimen showed increased IRT concentrations characteristic of an infant with CF. At the same time we carried out the four parameter sweat test; the results obtained were less equivocal for conductivity than they were for chloride (table 3). Another of the four CF patients detected by newborn screening (case A, table 3), repeatedly had unequivocally normal sweat chloride concentrations when tested elsewhere as an asymptomatic neonate.

There were no outstanding clinical features which served to distinguish control subjects (N, J, H; table 4) whose sweat test results, when age was taken into account, remained equivocal. However, one of the control subjects (subject G) included in the four parameter sweat test study deserves special note (table 2). He was 1 month old when reviewed clinically for a positive neonatal screening test result; he was a well thriving infant with no discernible signs or symptoms of CF. He was found to be heterozygous for ΔF508. His sweat test results were: conductivity 71 mmol (NaCl eq)/l, osmolality 169 mmol/kg, sodium 33 mmol/l, and chloride 47 mmol/l. On the grounds that none of these results were diagnostic of CF he was considered not to be affected. However, with the exception of sweat sodium, all of these results were three to five standard deviations greater than the control mean for his age (table 4). With hindsight, and in the light of the cumulative findings from this study, it seems reasonably likely that control patient G might be affected with a mild CF genotype with the probability that the clinical phenotype will develop later. Although it is well known that a mild biochemical phenotype can be expressed in some heterozygotes for ΔF508 and probably for other “severe” mutations, the magnitude of the deviation from “normal” observed in the sweat electrolyte concentrations of subject G makes it unlikely that he is merely heterozygous for the one mutation detected. Should his status need redefining in the event of clinical manifestation of CF, the inclusion of his sweat test results in the CF category would not alter the conclusions that we draw from this study.

At the present time it seems that CFTR mutation analysis is unlikely to provide the means whereby many of these “diagnostically equivocal” problems can be speedily resolved. Although all of our four cases (table 3) possessed the CFTR mutation ΔF508, in three of them (cases B, C, and D) the mutation on the other chromosome remained unidentified after tests which would have identified more than 90% of the CF mutations expected in our population. We anticipate that these three cases, like the fourth (case A, genotype, ΔF508/R117H), will possess one of the relatively rare “mild” mutations which, being dominant, will express as a mildly dysfunctional CFTR phenotype in the ductal epithelia of the sweat gland and probably in other tissues; in fact all four cases were pancreatic sufficient at the time of these sweat tests.

Indirect, physical chemical, measurement of sweat electrolytes, as described here, requires that the sample is collected directly as liquid from the iontophoresis site, in contrast with the absorbed sweat sampling technique as described by Gibson and Cooke for direct sodium and chloride measurement. Although the Macroduct collection coil greatly reduces the risks of specimen evaporation and contamination, and osmolality and conductivity measurement virtually eliminate the potential for volumetric and gravimetric error inherent in the Gibson–Cooke procedure, some investigators have reported an unacceptable failure rate in obtaining a sufficient amount of sweat for analysis compared with the traditional sweat absorption pad method. We have no comparison to offer but the 1.4% failure rate we experienced in this population sample is perfectly acceptable by any criteria. Furthermore, using the Macroduct collection system over a period of many years, during confirmatory sweat testing for positive neonatal screening test results, we have failed to obtain sufficient sweat for accurate analysis at first attempt in only one infant; he was in postoperative intensive care following gastrointestinal surgery.

Our findings and experience lead us to conclude that, in the absence of conflicting comparative data, there is no rational basis for the strongly held view, emanating from clinical
and laboratory practice in the USA, that indirect methods for the measurement of electrolytes are not as reliable as the traditional chemical analysis of chloride and sodium in the diagnosis of cystic fibrosis. We hope that the data presented here, and particularly that relating to sweat conductivity, will enable paediatricians and clinical biochemists to make an evidence based judgement about the scientific and clinical validity of the sweat test methods they choose to use in their practice. Clinical laboratories in the UK should welcome and benefit from current initiatives aimed at developing robust external quality assessment for all aspects of this important test.

We express our thanks to paediatrician and CF nurse specialist colleagues in East Anglia for their help in arranging the patient sweat tests, to Tony Mulville of CSP, Hornchurch, Essex for loan of a Wescor 5200 Osmometer and the supply of Macroducts for the study, and to Ms Dawn Anderson for the preparation of this manuscript.

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