Sweat testing in newborns positive to neonatal screening for cystic fibrosis

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Sweat chloride concentrations above 40 mmol/l are unusual in newborns screened for cystic fibrosis and should be followed up. Centiles of sweat chloride concentrations in newborns positive to cystic fibrosis neonatal screening are presented. There are no significant correlations between age at sweat testing and sweat chloride concentration or quantity of sweat collected.

In this period, 82 cases of CF were diagnosed; seven of them had meconium ileus and IRT above the cut off. There were five false negatives. CF incidence in Tuscany was 1:3860. In 7.1% of the subjects, the quantity of sweat collected was insufficient at the first attempt, and the full sweat test was repeated. No adverse effects, apart from a slight reddening of the skin, were observed. A total of 78 (7.7%; 34 girls, 44 boys) of the 1003 newborns had chloride concentrations above the customary 60 mmol/l cut off and had clinical manifestations of the disease. In two other cases, blood spot genotyping alone allowed CF diagnosis: in one case, the sweat test was negative (genotype 2789+5G→H/ R117H), and in the other, the patient died and diagnosis was performed post mortem by DNA analysis.

Sweat testing was performed between 40 and 60 days of age. Ideally, hypertrypsinaemic newborns were tested when at least 30 days old and weighing at least 3000 g. Experience with sweat tests in the first few weeks of life and in small and preterm babies is limited. Considering the increasingly widespread use of neonatal screening in various countries, we wish to report our experience with quantitative measurement of sweat electrolytes in newborns positive to neonatal screening.

SUBJECTS AND METHODS
We screened all the newborns in Tuscany, a region with 3.5 million inhabitants and an annual birth rate of eight per thousand. We used the immunoreactive trypsinogen (IRT)/IRT protocol on dried blood samples from July 1991 to May 1992, and the IRT/IRT with meconium lactase complementary test from May 1992. In 2001, we introduced a protocol based on IRT, lactase, and DNA analysis (31 mutations) because the high allelic heterogeneity found in Tuscany made the classic IRT/DNA approach inapplicable.

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Sweat was stimulated by pilocarpine iontophoresis at 1.5 mA for five minutes on a surface measuring 19 cm², and then collected on dry filter paper on the same sized surface for 30 minutes. The minimum quantity was considered to be 60 mg (sweat rate 1.05 g/m²/min). Sample pooling was avoided, and the chloride was assayed by the Gibson and Cooke method.

RESULTS
We analysed the data from sweat tests performed on all 1003 newborns (557 girls, 446 boys) positive to neonatal screening out of the 335 903 children born in the study region from 1 July 1991 to 31 December 2003.
DISCUSSION

Hypertrypsinaemic subjects (at high risk of CF or carriers) should undergo sweat testing to complete diagnostic testing. As retesting is an important component of our screening procedure, it was only possible to carry out sweat testing in the first month in a limited number of cases. However, centres using the IRT/DNA protocol should be able to perform sweat testing at an early age. Although our data are numerically limited, they do not support the hypothesis that the quantity of sweat collected in the first month and the chloride concentration are different from those collected in the following three months. Furthermore, we found no correlation between age at sweat testing and chloride concentration or quantity of sweat collected. The first attempt at sweat testing was not satisfactory because of insufficient sweating in only 7.1% of cases. Sweat testing can be easily carried out by an experienced technician in the first few weeks of life without adverse effects. A local reddening of the skin is a normal reaction to pilocarpine iontophoresis.

The established concentration of 60 mmol/l helped us to identify 78/81 (96%) patients with CF. Chloride concentrations above 40 mmol/l are nevertheless unusual, and only 1% of newborns screened without CF had concentrations above this cut off point. Newborns with borderline sweat chloride concentrations (40–60 mmol/l) must be followed up with DNA analysis, investigation of exocrine pancreatic function, and repeated sweat tests until the diagnosis is clarified. The slight possibility that some hypertrypsinaemic newborns affected by CF may have negative results on the sweat test must be considered, but this underlines the need, where possible, for protocols based on DNA analysis.

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Table 1

| Centiles of chloride sweat concentrations in children positive to neonatal screening |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| 3rd            | 10th           | 25th           | 50th           | 75th           | 90th           | 95th           | 97th           | 99th           |
| 7.6            | 9.6            | 11.8           | 15             | 19.9           | 25.5           | 30.4           | 34.3           | 40.8           |

REFERENCES