Peer reviewed article

Sweat testing practice in Swiss hospitals

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Summary

Aims: To determine whether the current practice of sweat testing in Swiss hospitals is consistent with the current international guidelines.

Methods: A questionnaire was mailed to all children's hospitals (n = 8), regional paediatric sections of general hospitals (n = 28), and all adult pulmonology centres (n = 8) in Switzerland which care for patients with cystic fibrosis (CF). The results were compared with published "guidelines 2000" of the American National Committee for Clinical Laboratory Standards (NCCLS) and the UK guidelines of 2003.

Results: The response rate was 89%. All 8 children's hospitals and 18 out of 23 answering paediatric sections performed sweat tests but none of the adult pulmonology centres. In total, 1560 sweat tests (range: 5–200 tests/centre/year, median 40) per year were done. 88% (23/26) were using Wescor® systems, 73% (19/26) the Macroduct® system for collecting sweat and 31% (8/26) the Nanoduct® system. Sweat chloride was determined by only 62% (16/26) of all centres; of these, only 63% (10/16) indicated to use the recommended diagnostic chloride-CF-reference value of >60 mmol/l. Osmolality was measured in 35%, sodium in 42% and conductivity in 62% of the hospitals. Sweat was collected for maximal 30–120 (median 55) minutes; only three centres used the maximal 30 minutes sample time recommended by the international guidelines.

Conclusions: Sweat testing practice in Swiss hospitals was inconsistent and seldom followed the current international guidelines for sweat collection, analyzing method and reference values. Only 62% were used the chloride concentration as a diagnostic reference, the only accepted diagnostic measurement by the NCCLS or UK guidelines.

Key words: sweat test; quality control; guidelines; reference values; sweat chloride; osmolality; conductivity; cystic fibrosis

Introduction

Cystic fibrosis (CF) is the most common lethal autosomal recessive disorder with an approximate incidence of 1:2000 births in Switzerland [1]. Patients with CF have an increased concentration of chloride and sodium in their sweat as a consequence of reduced sweat reabsorption in the distal sweat glands due to dysfunctional CF transmembrane regulator (CFTR). The quantitative measurement of chloride in sweat is still the current gold standard test for cystic fibrosis [2]. Even in countries, where newborn screening using immune reactive trypsinogen (IRT) has already been introduced [3, 4], sweat testing is not replaced by genetic mutation analysis. Especially in children with non-classic or atypical CF, a correctly performed sweat test is of utmost importance [5–7].

Since the discovery of the sweat electrolyte defect in CF patients 50 years ago by di Sant'Agnese [8], and the introduction of the quantitative pilocarpine iontophoresis (QPIT) into clinical practice by Gibson and Cooke,[9] there have been many improvements in methods for sweat collection [2, 10–12]. These days, the Wescor Macroduct® collection system is widely used as a quantitative pi-

There is no conflict of interest.

Abbreviations

CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane regulator
DC	Direct current
IRT	Immune reactive trypsinogen
mA	milliamperes
NCCLS	National Committee for Clinical Laboratory Standards
QPIT	Quantitative pilocarpine iontophoresis test
UK	United Kingdom

locarpine iontophoresis sweat test that is internationally accepted. Conductivity has been shown to be as effective as sweat chloride in its ability to discriminate diagnostically between patients with CF and non-CF subjects [12–15]. However, conductivity is still not accepted by the American CF Foundation as a diagnostic test [16]. The pad method using QPIT by Gibson & Cooke is even now believed to be the most accurate sweat test [11].

Performing a correct sweat test is time consuming and involves several steps, all of which are error prone [17]. Sweat testing requires qualified technicians and strict adherence to guidelines [18, 19]. For instance, the collection of a sufficient amount of sweat using various stimulation and different collecting systems is a well known difficulty in sweat testing procedures. Especially in infants, it is sometimes difficult to collect the minimum weight of sweat (75 mg for the Gibson-Cooke® procedure) or the minimum acceptable volume (15 µL for the Macroduct® collection system) within 30 minutes to ensure an average sweat rate of more than 1 g/m^2/min.[20] The sweat test has been reported to have unacceptably high false-positive (up to 15%) and false-negative (up to 12%) rates, attributable to inaccurate methodology, technical error, and patient physiology [1, 19, 21].

In this study, we wanted to know how sweat tests are performed in Switzerland, and we compared the reported procedures with the current guidelines used in the USA [19, 22] and United Kingdom (UK) [18].

Methods

A questionnaire (see appendix) was mailed in German or French (according to the language used in the region) to all tertiary children's hospitals (n = 8) and regional paediatric sections of adult hospitals (n = 28) as well as to all adult pulmonology centres (n = 8) in Switzerland which care for patients with CF. If a returned questionnaire was not completely filled out, an enquiry by phone was performed. The results were compared with the guidelines of the American National Committee for Clinical Laboratory Standards 1994 and 2000 (www.nccls.org) [19, 22] and the UK guidelines 2003 (www.acb.org.uk) [18].

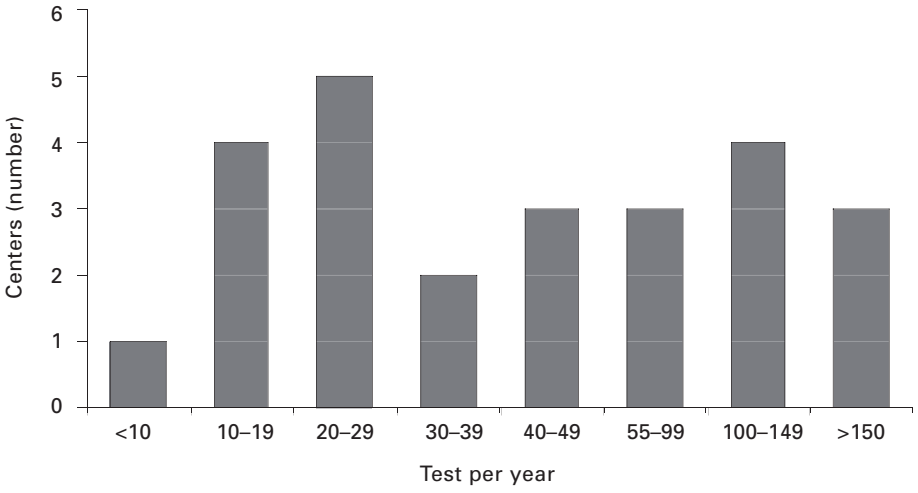


Figure 1
Tests per year per centre.

Results

The overall response rate to the questionnaire was 89% (39/44). All 8 children's hospitals and 78% (18/23) of the answering paediatric sections of general hospitals in Switzerland did sweat tests but none of the adult pulmonology centres. To our knowledge, no other private clinics or laboratories are performing sweat test in Switzerland.

Table 1
Sweat test analysis – normal values [1, 7, 10, 13, 25, 29].

	Chloride (mmol/L)	Conductivity (mmol/L)	Osmolality (mosmol/L)
Normal	<40	<60	<170
Borderline	40–60	60–80	170–200
CF	>60	>80	>200

In total 1560 sweat tests are performed per year (range, 5–200; median, 40; figure 1). Only 42% of the centres do more than 50 sweat test per year as requested by the UK guidelines, whereby all 8 tertiary paediatric centres perform hundred or more tests per year. In the reporting centres 1 to 15 persons perform sweat tests (median 3); in 31% (8/26) by laboratory technicians, in 58% (15/26) by nurses, and in 15% (4/26) by medical assistants. In four hospitals, all nurses of a paediatric ward have to do sweat testing. On an annual basis more than 56% of all nurses and technicians, who do sweat tests, perform less than 10 sweat tests

Figure 2

Methods of sweat test analysis.

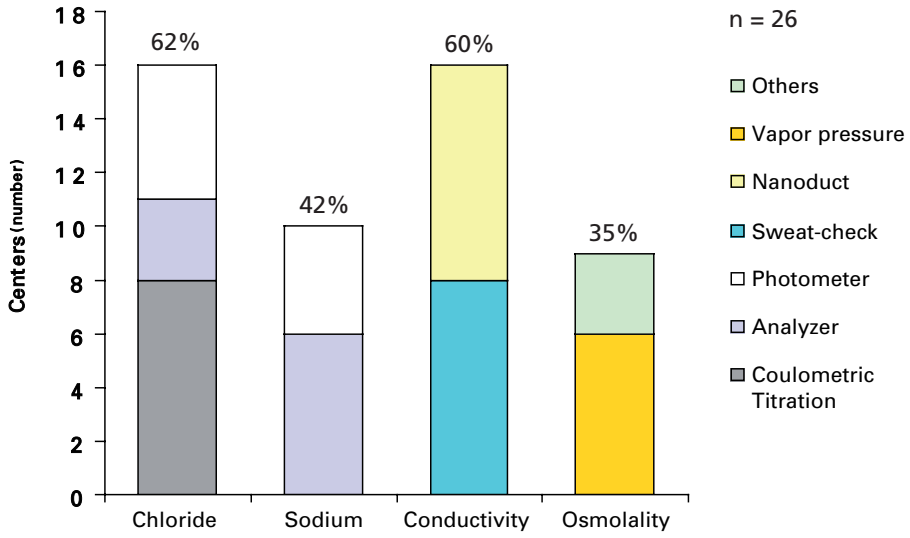
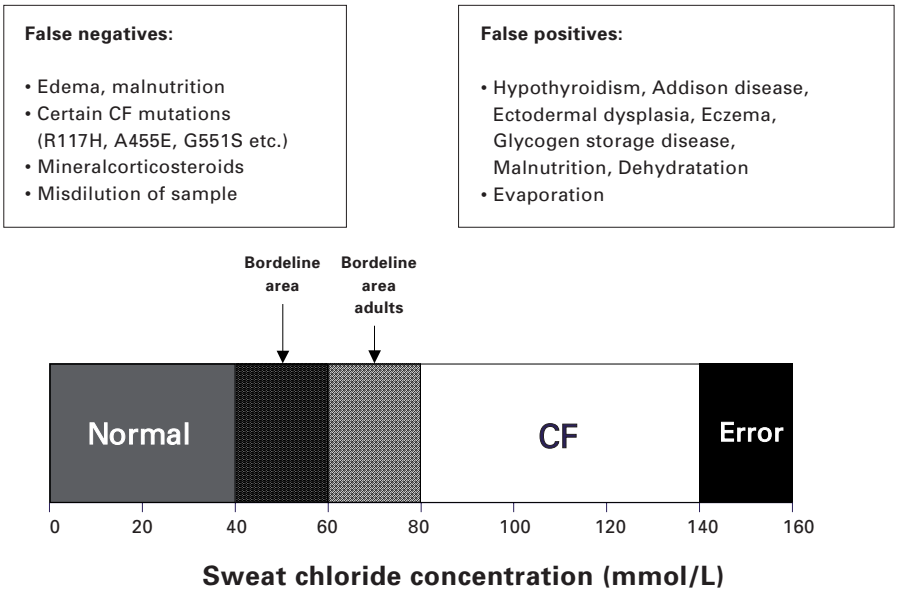


Figure 3

Sweat test – chloride values.



per year; 20% perform 10–24 tests per year, and 24% perform more than 25 tests per year.

Wescor® company systems are used by 88% (23/26) of the hospitals, the Macroduct® coil system for sweat collection is used by 73% (19/26) and the Nanoduct® sweat analyzing system is used by 31% (8/26), half of them as a screening method, the rest as diagnostic tool. Only three centres are using the classic pilocarpine iontophoresis and filter paper method by Gibson & Cooke.

The median sample time reported was 55 minutes (range: 30–120 minutes) and only three hospitals (12%) used the maximal time of 30 minutes recommended by the NCCLS or UK guidelines. Only 62% (16/26) of the centres indicated to use chloride in the sweat analysis, the only accepted diagnostic method by the NCCLS (figure 2). Osmolality was measured in 35% (9/26), sodium in 42% (11/26) and conductivity in 62% (16/26). One centre is using osmolality as an exclusive diagnostic tool for CF and eight centres are using osmolality as a screening test. Conductivity is used by 8 centres as a diagnostic tool and the others are using it as a screening method. The new Nanoduct® system is already used in 8 centres, however in half of them as a quick bedside screening method. A variety of different methods of sweat testing analysis are used (figure 2). Only 63% (10/16) of the centres, which are determining chloride in the sweat, used the recommended chloride value of >60 mmol/L for the diagnosis of CF; two centres used a chloride value of 70 mmol/L, one 50 mmol/L and three 45 mmol/L. Eighty-one percent (21/26) of the hospitals had no age (ie 2 weeks) or weight (ie 3 kg) limits for performing a sweat test in newborns.

Conclusion

In this study we could demonstrate, that sweat testing practice in Swiss hospitals is inconsistent and seldom follows the current NCCLS [19] or the UK guidelines [18] for sweat collection, analyzing methods and reference values. This is not surprising, as so far, since the inception of the sweat test by di Sant'Agnese in 1954 [8], no guidelines regarding sweat testing have ever been published in Switzerland. Similar reports with regard to the disturbing quality of sweat testing have been published from Australia and New Zealand, where newborn screening for CF has already been introduced for many years and confirmation for the diagnosis in unclear cases was made by sweat testing [3, 23]. In Switzerland, a country without specific newborn screening, the diagnosis of CF is made when clinical symptoms and the patients' history is suspicious. The putative diagnosis is then confirmed with two (properly performed) positive sweat tests. If genotyping is performed, a second sweat test is not always necessary [2].

Recommendations

Sweat testing is technically demanding, and requires special skills to avoid false-positive or false-negative results by timing, evaporation and contamination and it needs strict adherence to guidelines [17–19]. Sweat collection should only be performed by fully trained and experienced personnel which should do a minimum number of 10 collection procedures per person per year. For quality purposes, a minimum number of 50 sweat test per year should be performed in any centre as recommended by the UK guidelines [18].

Sweat testing contains usually three steps: sweat induction, collection and analysis – all of which are error-prone.

Sweat induction

For a correct sweat induction, the skin needs to be properly cleaned with distilled or deionised water which removes dead surface skin cells and any contaminating lotions, and hydrates the top skin layer [19]. Subsequently, the chemical pilocarpine gel is applied to two small areas on an arm or leg. Pilocarpine nitrate at 2–5 g/L (0.2–0.5%) is recommended for use at both electrodes. After attaching two electrodes, a weak electrical direct current (DC) is applied to stimulate sweating. For safety reasons, the current source should be battery-powered. As burn potential increases with the magnitude and duration of iontophoretic current, the recommended procedure for the iontophoresis is to start with a current of 0.5 milliamperes (mA) with a slow increase to a maximum of 4 mA and maintaining this for three to five minutes [18, 19]. This procedure avoids burns to the patient's skin.

Sweat collection

The minimum acceptable sweat volume depends on the size of the electrode used, the type of the collection material (filter paper, gauze, or microbore tubing), the sweat collection time, and the subsequent analytical method [19]. An accurate sweat test requires the determination of electrolytes from maximally stimulated sweat glands, as sweat chloride concentration decreases at low sweat rates which could lead to false-negative results [24]. Sweat secretion is low immediately after iontophoresis, increases to a maximum between 10 and 30 minutes, and then decreases rapidly [18]. In addition, evaporation can influence the results of sweat testing and becomes a more significant problem with smaller samples. Therefore, the sweat rate should exceed 1 g/m^2/min, which corresponds to a minimum sample weight of approximately 75 mg of sweat collected on 5×5 cm, electrolyte free gauze or filter paper or approximately 15 µL of sweat collected by the microbore tubing (eg Macroduct®) in 30 minutes. Extending the collection time will not significantly increase the sweat yield and may lead to sample evaporation. The proportion of inadequate technical collection failures should not exceed 5% unless many of the patients that are tested are <1 month of age [18, 19].

Sweat analysis

Determination of chloride concentration in sweat is the actual still only accepted diagnostic measurement for the diagnosis of CF by the current NCCLS or UK guidelines [18, 19]. Colorimetry, coulometry and ion-selective electrodes are satisfactory methods of analysis of sweat chloride in the laboratory [22]. In general sweat chloride concentrations <40 mmol/L are considered normal, values between 40–60 mmol/L are borderline, and concentrations >60 mmol/L are consistent with the diagnosis of CF (figure 3, table 1) [1, 2, 11, 25, 26]. Sweat chloride should always be interpreted with regard to age: data from a newborn screening program have shown that chloride concentrations >40 mmol/L in young infants are suggestive of a CF diagnosis [27]. On the contrary, a very small proportion of adults with other pulmonary disease than CF have sweat chloride values between 60–70 mmol/L [28]. In the light of the great heterogeneity in the clinical manifestations and atypical cases with CF respectively, the European Cystic Fibrosis Diagnostic Working Group has recently suggested to use values between 30–60 mmol/L as new borderline values [6].-

Sweat sodium is elevated in CF but does not discriminate as well as chloride between CF and healthy subjects. Some laboratories determine sodium in addition to chloride for quality control purposes and some are using the sodium: chloride ratio to distinguish CF (ratio <1) from other gastroenteral diseases, eg coeliac disease (ratio >1).

The value of sodium: chloride ratio as a discriminating test is currently unclear [18].

Osmolality of sweat reflects the total sweat concentration of cations and anions per kg of sweat, including uncharged molecules as urea and amino acids [29]. Osmolality correlates well with sodium but it has a poor discriminatory power compared to sweat chloride concentration [15]. The reference ranges for normal sweat osmolality are 50–150 mmol/kg, and values greater than 200 mmol/kg are consistent with CF [25]. Borderline values are considered to be between 150–200 mmol/kg by some authors [25], others are considering values <170 mmol/kg as normal [29]. Measuring osmolality can help as a screening method but it is not recommended for the diagnosis of CF [13, 18, 25].

Conductivity is the property of a solution that allows it to conduct a current. It depends on the concentration and mobility of the ions in the solution, and is therefore an indirect measurement of ions [30, 31]. Sweat conductivity is not equivalent to sweat chloride concentration because of other ions in sweat such as bicarbonate and lactate, therefore sweat conductivity is approximately 15 mmol/L higher than the sweat chloride concentration [14, 19]. Several authors have demonstrated that conductivity using the Macroduct® collection system and Sweat-Check® analyzer is as effective as sweat chloride in its ability to discriminate diagnostically between CF and non-CF subjects [12, 14, 15, 32]. The novel diagnostic system Nanoduct®, which induces and analyzes sweat conductivity in situ while attached to the patients requires only 3 µl of sweat, and reliable results are available within 30 minutes [13, 33]. But so far, only the Sweat-check® from Wescor has been accepted as screening method by the NCCLS [11]. According to the American CF Foundation, a conductivity value less than 50 mmol/L is considered normal and patients with values >50 mmol/L should be referred for a quantitative pilocarpine iontophoresis test (QPIT) [16, 25]. A conductivity level >80 mmol/L is believed to be diagnostic in addition to its screening value by some authors [13, 33], while others suggest values >90 mmol/L to support a diagnosis of CF [18, 32]. Until now, the NCCLS does not accept conductivity as a definitive diagnostic tool [19].

Limits for sweat testing

A sweat test can be performed at any age, however, current clinical practice showed that it can be difficult to collect adequate quantities of sweat from very young infants [20, 34]. Preterm infants do not sweat in the first 2 weeks, but most term infants are able to sweat as of day 1 [35]. The UK guidelines recommend that a sweat test can be performed after 2 weeks of age in infants >3 kg [18], whereby others suppose that sweat collection can be reliably performed in infants >36 weeks postmenstrual age, >2 kg, and >3 days postnatal age [20]. Sweat testing should not be performed before a child is at least 48 hours old, as in the first 24 hours after birth, a baby's sweat electrolyte concentrations are known to rise transiently [17]. A sweat test should always be delayed in children who are dehydrated, systemically unwell or who have eczema affecting the potential stimulation sites, or who are oedematous and/or on systemic corticosteroids [18]. In addition, many other diseases can lead to false-positive and false-negative results (figure 3) which is described in detail elsewhere [1, 17].

Part of this work has been presented at the European Cystic Fibrosis Conference in Copenhagen, June 14–18, 2006 [36].

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Appendix 1

Umfrage zur Durchführung des Schweisstests (Iontophorese)

1. Schweißstimulation:

Pilocarpine-Iontophorese: Ja (Wescor-System , EMS) Nein Unbekannt

2. Schweißsammlung:

Macroduct-System (Wescor) Filterpapier Gaze Anderes Verfahren .....
Nanoduct (Wescor)

3. Schweissanalyse: (bitte alle angewendeten Methoden angeben)

	Methode	*Normalbereich*	*Grauzone*	*CF-Diagnose*	
Na^+	Flammenphotometer Analyser Anderes Verfahren	.....	.....	.....	mmol/l
Cl^-	Photometrie Colorometrische Titration Direkte Hautelektrode Analyser	.....	.....	.....	mmol/l
Leitfähigkeit:	Sweat-Check (Wescor) Nanoduct (Wescor) Anderes Verfahren	.....	.....	.....	mmol/l
Osmolarität:	Dampfdruck Gefrierpunkterniedrigung Anderes Verfahren	.....	.....	.....	mmol/kg

Welche minimale Schweißmenge akzeptieren Sie ? ..... mg ..... µl ..... Technik
..... mg ..... µl ..... Technik

Geben Sie eine maximale Schweißsammelzeit vor ? Ja = ... min. Nein

Haben Sie ein Mindestalter oder -gewicht für die Untersuchung? Ja = ... Wochen/Monate, ... Kg Nein

Wann wiederholen Sie den Test ? .....

Beurteilen Sie Ergebnisse auch bei insuffizienter Schweißmengen ? Ja Nein

Wieviele Personen führen bei Ihnen den Schweißtest durch ? .....

Wer führt die Schweißtests bei Ihnen in der Klinik durch ?
Labor-Assistentin Krankenschwester Arztgehilfin Andere: .....

4. Angaben zur Klinik:

Universitäts-Kinderklinik Kinderklinik in Kantonsspital Kinderabteilung in Regionalspital
Universitätsklinik für Erwachsene (Abteilung für Pneumologie) Kantons/Stadt-Spital (Abteilung für Pneumologie)

Anzahl betreuter CF-Patienten in Ihrer Klinik ? ..... Kinder ..... Erwachsene

Anzahl Schweisstests in Ihrer Klinik: ..... pro Jahr
keine, Überweisung an: .....

Name für allfällige Rückfragen (fakultativ): .....

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