THORAX/2009/125088

Derichs et al.: Intestinal current measurement for diagnostic classification of patients with questionable cystic fibrosis: validation and reference data

Online supplement

Intestinal Current Measurement

Ion transport properties in all patients and healthy volunteers were studied by means of intestinal current measurement (ICM). After risk minimisation by determination of capillary bleeding time and exclusion of a history of hemorrhoids, superficial rectal suction biopsies (2-3 mm in diameter) were taken with a rectal suction biopsy tool (Trewavis Surgical, Boronia, Australia) without sedation in a standardised procedure and defined suction pressure of 5 mmHg. Biopsies were preserved in phosphate-buffered saline on ice, mounted within 5 minutes in recirculating Ussing chambers with an exposed area of 1.13 mm² and incubated at 37° C with Meyler buffer solution (composition in mmol/l: Na^+ 126.2; Cl^- 114.3; K^+ 4.7; Ca^{2+} 1.3; Mg^{2+} 1.0; HCO_3^- 20.2; HPO_4^{2-} 0.3; $H_2PO_4^-$ 0.4; Glucose 10; Hepes 10; pH 7.4 when gassed with 95 % O_2, 5 % CO_2). The Ussing chambers were connected by KCl-agar bridges to calomel voltage electrodes (K401, Radiometer), and platinum current electrodes were used. Basal potential difference (PD_{basal}), short-circuit current ($I_{sc\ basal}$) and transepithelial resistance ($R_{t\ basal}$) were determined by an voltage clamp-amplifier (DVC-1000, WPI), the fluid resistance was taken into account. Subsequently, the tissue was short-circuited using voltage clamps and the I_{sc} as a direct measure for the net movement of ions across the epithelium was recorded. After equilibration for 20 minutes, the following specific compounds were added to the mucosal (M) and/or serosal (S) bathing solutions in a standardised sequence: amiloride (100µM, M) to inhibit amiloride-sensitive electrogenic sodium absorption, indomethacin (100µM, M+S) to reduce basal chloride secretion caused by endogenous production of prostaglandins, carbachol (100µM, S) to stimulate cholinergic calcium- and protein kinase C-mediated CFTR chloride secretion, 8-bromo-cAMP (1mM, M+S) and forskolin (10µM, S) to activate cAMP-dependent CFTR chloride secretion, 4,4´-diisothiocyanostilbene-2,2´-disulfonic acid (DIDS) (200µM, M) to inhibit DIDS-sensitive non-CFTR

chloride channels, histamine (500µM, S) to determine the DIDS-insensitive component of calcium dependent chloride secretion. All chemicals were obtained from Sigma Chemical Co., St. Louis, USA.

Typical I_{sc} responses after stimulation of CFTR-mediated chloride secretion are known to differ between CF patients and healthy control, representing a gradient of CFTR dysfunction with some residual CFTR function especially in PS-CF associated with “milder” *CFTR* mutations leading to some (subnormal) CFTR expression and activity.

Table A. Extensive *CFTR* genotype analysis in relation to clinical and chloride secretory phenotype of patients with questionable CF

ID	Age	Sex	Symptoms	PI	PA	Sweat Cl^-	$\Delta I_{sc\ carbachol}$	$\Delta I_{sc\ cAMP/forsk}$	$\Delta I_{sc\ histamine}$	$\Delta I_{sc\ carb+cAMP+hista}$	*CFTR* genotype	Intron 8 TGmTn	Exon 9	Classification
1	10.6	M	Bronchitis, penumonia	-	-	n.a.	84.1	6.2	46.9	137.2	c4075del8/-	n.a.	n.a.	CF unlikely
2	2.5	m	Diarrhoea	-	-	n.a.	66.0	15.9	35.4	117.4	-/-	T7/T7	15.8	CF unlikely
3	5.1	f	Bronchitis	-	-	6	75.1	23.9	68.3	167.3	R75Q/-	TG11T7/TG11T7	13.1	CF unlikely
4	4.1	m	Bronchitis	-	-	7	45.7	29.0	81.2	155.9	-/-	n.a.	n.a.	CF unlikely
5	8.2	f	Chronic cough, sputum	-	-	7	26.7	17.0	18.1	61.8	-/-	TG11T5/TG11T7	n.a.	CF unlikely
6	7.2	m	Bronchiectasis	-	-	9	17.4	52.4	13.5	83.2	-/-	n.a.	n.a.	CF unlikely
7	2.8	m	Bronchitis	-	-	9	97.2	44.6	82.1	223.9	F508del$^{\#}$/-	n.a.	n.a.	CF unlikely
8	15.8	f	Rectal prolapse	-	-	10	26.2	26.4	16.1	68.7	-/-	T7/T7	n.a.	CF unlikely
9	39.8	f	Pancreatitis (9x)	-	-	10	37.5	10.6	12.6	60.7	S1235R/-	n.a.	n.a.	CF unlikely
10	4.8	m	Bronchitis	-	-	12	25.8	8.0	9.2	43.0	2789+5G>A$^{\#}$/-	n.a.	n.a.	CF unlikely
11	3.6	m	Bronchitis, sinusitis	-	-	14	72.9	12.4	52.8	138.1	-/-	TG10/TG11	n.a.	CF unlikely
12	5.9	m	Failure to thrive	+	-	14	30.5	10.4	31.8	72.7	-/-	No T5	n.a.	CF unlikely
13	12.3	m	Bronchiectasis, failure to thrive	+	-	14	44.1	11.5	85.0	140.5	R75Q/-	T7/T7	n.a.	CF unlikely
14	2.6	f	Bronchitis	-	-	15	42.3	22.7	38.6	103.6	I177F/-	T5	n.a.	CF unlikely
15	3.2	f	Pneumonia, hyponatremia	-	-	16	44.8	5.3	27.4	77.5	F508del$^{\#}$/-	T7/T9	n.a.	CF unlikely
16	5.1	f	Sinusitis, cough	-	-	17	42.5	15.0	52.6	110.1	-/-	T7/T7	n.a.	CF unlikely
17	17.6	m	Bronchitis, failure to thrive	-	-	20	44.3	32.9	21.8	98.9	S1235R/-	T7/T7	n.a.	CF unlikely
18	5.0	f	Bronchiectasis	-	-	21	13.6	11.5	23.4	48.5	-/-	TG11T5/TG12T7	40.0	CF unlikely
19	7.6	m	Failure to thrive	-	-	22	62.7	12.4	56.8	131.9	-/-	TG11T7/TG12T7	9.8	CF unlikely
20	11.9	f	Bronchitis, asthma	-	-	23	33.5	14.2	32.4	80.0	F508del$^{\#}$/-	n.a.	n.a.	CF unlikely
21	8.9	m	Sister CF	-	+	23	28.3	23.4	62.0	113.6	R117H$^{\#}$/-	TG10T7/TG11T7	n.a.	CF unlikely
22	13.3	m	Asthma, diarrhoea	-	-	25	68.5	12.7	9.2	90.4	-/-	TG11T7/TG11T7	n.a.	CF unlikely
23	8.0	m	Chronic diarrhoea	-	-	25	20.7	22.5	12.4	55.6	-/-	TG11T7/TG11T7	8.2	CF unlikely
24	14.4	m	Chronic bronchitis	-	-	25	40.7	8.5	18.4	67.6	S1235R/-	TG12T7/TG10T9	13.7	CF unlikely
25	5.0	m	Chronic bronchitis	-	-	26	40.0	26.6	34.0	100.6	S1235R/-	n.a.	n.a.	CF unlikely
26	24.7	m	Chronic bronchitis, lobe resection	-	-	27	45.7	7.6	33.1	86.4	R334Q/-	no T5	n.a.	CF unlikely
27	3.6	f	Chronic bronchitis	-	-	29	52.0	18.6	24.6	95.2	G576A/-	n.a.	n.a.	CF unlikely
28	33.4	f	Sinusitis, bronchitis, pancreatitis	-	-	31	27.8	10.3	8.7	46.7	-/-	TG11T7/TG11T7	2.9	CF unlikely
29	7.8	f	Chronic bronchitis, cholelithiasis	-	-	32	34.5	33.3	41.2	109.0	F508del$^{\#}$/-	T5/T9	n.a.	CF unlikely
30	38.1	f	Rec.pancreatitis, nasal polyps	-	-	33	41.3	13.3	42.3	96.9	-/-	TG12T5/TG10T7	20.6	CF unlikely CFTR-RD ?
31	3.6	m	Bronchitis, diarrhoea	-	-	35	63.5	22.5	22.2	108.2	F508del$^{\#}$/D924N	n.a.	n.a.	CF unlikely
32	4.1	m	Pneumonia	-	-	37	41.9	6.2	10.7	58.8	-/-	n.a.	n.a.	CF unlikely
33	4.6	m	Pneumonia, failure to thrive	+	-	38	46.0	28.4	40.5	114.9	-/-	no T5	n.a.	CF unlikely
34	5.2	m	Chronic cough	-	-	39	27.1	7.3	15.0	49.4	G551D$^{\#}$/-	TG11T5/TG10T7	30.7	CF unlikely
35	11.1	f	Chronic cough	-	-	41	37.7	32.2	42.0	111.9	R117H$^{\#}$/-	T7/T7	n.a.	CF unlikely
36	17.4	m	Pancreatitis (3x), oligospermia	-	-	44	44.5	10.9	37.7	93.1	-/-	n.a.	n.a.	CF unlikely
37	6.3	f	Pneumonia, bronchiectasis	-	-	45	12.9	14.7	9.0	36.6	-/-	no T5	n.a.	CF unlikely
38	2.9	m	Asthma, failure to thrive	+	-	46	23.4	7.3	24.8	55.4	-/-	TG12T7/TG10T7	13.0	CF unlikely
39	9.4	m	Bronchitis, pneumonia	-	-	46	38.1	9.6	15.8	63.4	R75Q/-	n.a.	n.a.	CF unlikely
40	5.4	f	Bronchitis, chronic otitis	-	+	46	56.6	14.7	31.0	102.3	-/-	n.a.	n.a.	CF unlikely

41	56.9	f	Chronic cough, CF family history	-	+	50	26.7	31.7	31.2	89.6	-/-	n.a.	n.a.	CF unlikely
42	4.0	m	Pneumonia, nasal polyps	-	-	51	15.5	9.6	9.5	34.6	F508del[#]/-	no T5	n.a.	CF unlikely
43	18.0	m	Bronchitis, pansinusitis	-	-	52	25.7	59.3	23.4	108.3	-/-	T7/T7	n.a.	CF unlikely
44	15.9	m	Chronic bronchitis	-	-	53	35.4	10.3	28.0	73.6	-/-	T7/T9	n.a.	CF unlikely
45	4.7	f	Pneumonia, abdominal pain	-	-	55	15.8	7.0	12.4	35.2	-/-	TG11T5/TG10T9	12.2	CF unlikely
46	4.3	f	Bronchiectasis, asthma	-	+	57	37.2	18.4	13.0	68.6	-/-	n.a.	n.a.	CF unlikely
47	10.4	m	Pneumonia, asthma	-	-	60	12.6	17.7	11.3	41.5	-/-	TG12T5/TG12T7	41.6	CF unlikely CFTR-RD ?
48	22.7	m	Chronic pancreatitis	-	-	62	63.1	10.6	46.7	120.4	-/-	TG11T7/TG10T7	7.7	CF unlikely
49	14.8	m	Nasal polyps	-	-	63	37.2	59.5	51.9	148.5	-/-	TG11T7/TG11T7	10.0	CF unlikely
50	16.4	f	Pneumonia	-	-	63	37.7	8.7	16.5	62.8	-/-	T7/T7	n.a.	CF unlikely
51	13.5	m	Nasal polyps	-	-	64	49.4	51.3	50.0	150.7	-/-	TG11T5/TG12T7	32.4	CF unlikely
52	7.6	m	Nasal polyps, asthma	-	-	65	54.3	20.4	56.6	131.3	-/-	T7/T7	n.a.	CF unlikely
53	15.7	f	Chronic pancreatitis	-	-	69	33.6	22.5	35.9	92.0	Y122N/-	n.a.	n.a.	CF unlikely
54	34.7	m	Diabetes mellitus type I	+	-	90	32.4	23.7	28.1	84.3	F508del[#]/-	TG12T7/TG10T9	10.9	CF unlikely
55	8.4	m	Recurrent bronchitis	-	-	92	47.6	7.1	27.8	82.5	-/-	TG11T7/TG11T7	9.3	CF unlikely
56	15.5	f	Recurrent pancreatitis	-	-	42	9.5	7.0	16.0	32.5	D1152H/D1152H[#]	no T5	n.a.	PS-CF
57	6.5	f	Chronic bronchitis	-	-	49	12.7	2.8	1.6	17.2	1717-1G>A[#]/2789+2insA	TG10T7/TG10T7	n.a.	PS-CF
58	26.6	m	Bronchiectasis, sinusitis, azoospermia	-	+	50	4.4	12.0	11.2	27.6	F508del[#]/1874insT	TG12T7/TG10T9	n.a.	PS-CF
59	10.2	m	Chronic bronchitis	-	-	53	-0.5	9.1	12.6	21.2	F508del[#]/G576A	TG10T7/TG10T9	40.7	PS-CF
60	5.8	f	Chronic cough	-	-	70	20.2	3.9	8.5	32.6	G551D[#]/L206W[#]	TG10T7/TG9T9	n.a.	PS-CF
61	6.4	m	Salt loss, failure to thrive	-	-	107	1.6	2.0	1.4	5.0	W1098L/W1098L	TG11T7/TG11T7	8.5	PS-CF

Note. Age: age at ICM procedure (years); Sex: f female, m male; PI: Exocrine pancreatic insufficiency (verified by pancreatic stool elastase < 100 µg/g); PA: *P. aeruginosa* airway colonisation (determined by throat swab or sputum culture within the last 6 months); Sweat Cl^-: sweat Cl^- concentration (mmol/l), in individuals 1+2 no sufficient sweat amount could be collected; $\Delta I_{sc\ carbachol}$, $\Delta I_{sc\ cAMP/forsk}$, $\Delta I_{sc\ histamine}$, $\Delta I_{sc\ carb+cAMP+hista}$: short circuit current responses to carbachol, cAMP/forskolin and histamine (µA/cm²) and their cumulative value in intestinal current measurement; *CFTR* genotype: results of extensive genetic analysis by sequencing or SSCP/HD analysis and confirmation sequencing, [#] CF disease-causing mutation according to present consensus recommendations [3], [2]; Intron 8 TG_mT_n: Repeat sequences of thymidines/guanidines in intron 8 of the *CFTR* gene; Exon 9: quantitative transcript analysis (% of CFTR mRNA with skipped exon 9; CFTR total mRNA = 100%) in RNA derived from nasal epithelial cells; Classification: diagnostic interpretation based on all available phenotype and genotype results; CFTR-RD: CFTR-related disease; na: not available.

Figure S1. Flow diagram of study design

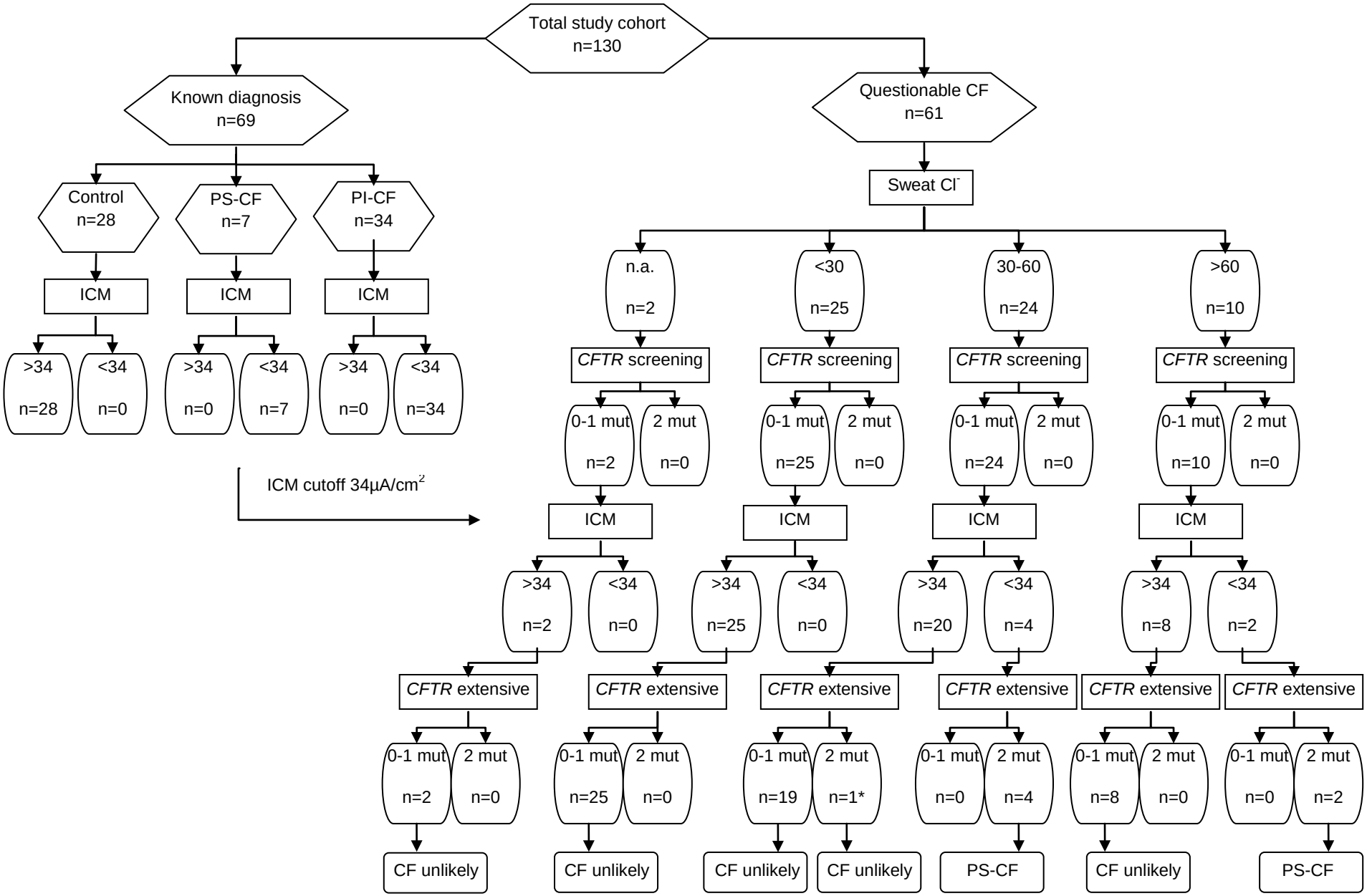

Figure legend:

Figure S1. Flow diagram of study design

ICM cutoff 34µA/cm^2 indicates the cumulative value of Isc carb+cAMP+hista, determined by ICM analysis of the group with known diagnosis. This cutoff value was subsequently applied to the diagnostic group with questionable CF and verified by extensive *CFTR* genotype analysis as the best available reference test (according to the STARD statement for reporting of studies of diagnostic accuracy; www.stard-statement.org). The reference test confirmed the diagnostic accuracy of the index test ICM. Sweat Cl^- concentration in mmol/l; *CFTR* screening: mutation screening for most common *CFTR* gene mutations; mut: number of detected *CFTR* mutations; ICM: Intestinal current measurement; *CFTR* extensive: extensive *CFTR* genotype analysis/sequencing (see Methods for details). *indicates patient with *CFTR* genotype F508del/D924N, in which the clinical relevance of the second mutation is unknown, and who is classified as CF unlikely according to sweat Cl^- and ICM.