

SISSME



SISN



GENCLI

Congresso Nazionale 2006
STRATEGIE DIAGNOSTICHE ED ASSISTENZIALI COME
GARANZIA DI QUALITA'

**È sempre corretto
che il neonato diventi paziente?**

SCREENING NEONATALE PER FIBROSI CISTICA: IL RICONOSCIMENTO DELLE FORME ATIPICHE DI MALATTIA

Rita Padoan

Centro di Supporto per la Fibrosi Cistica, Clinica Pediatrica, Brescia

Cystic Fibrosis newborn screening

The magnitude of the health benefits from screening for CF is sufficient that states should consider including routine newborn screening for CF in conjunction with systems to ensure access to high-quality care. (CDC, MMWR, Recommendations and Reports October 15, 2004 / 53(RR13):1-36)

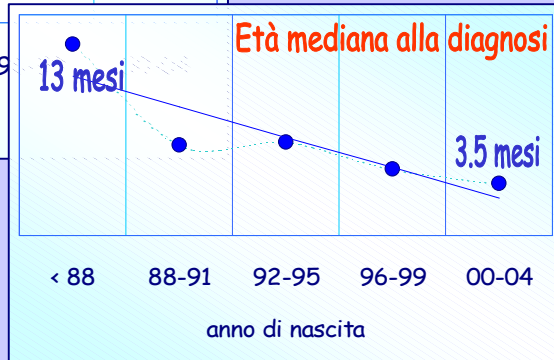
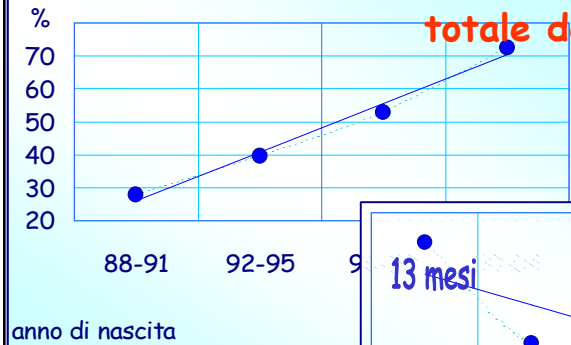
NBS is designed, by definition, to detect newborns who are affected by **“severe”** classical CF forms but have no symptoms.



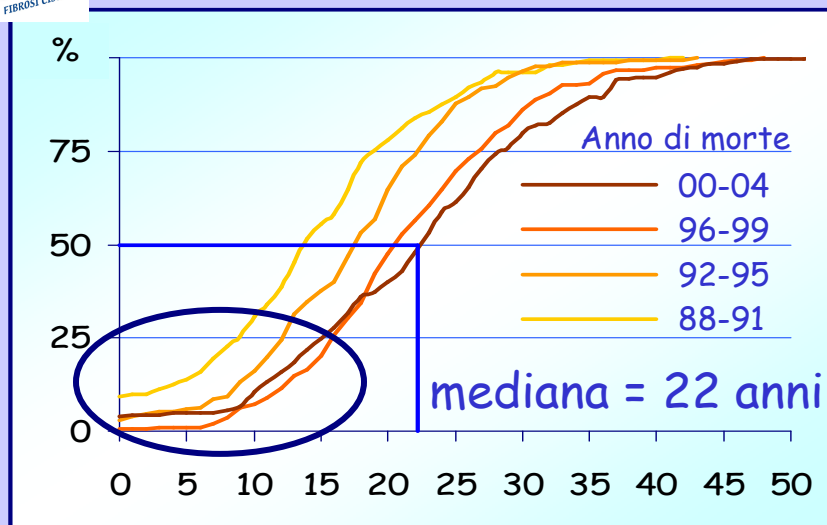
Andersen DH, Am J Dis Child 1938;56:344-399

Screening neonatale in Italia

% di neonati FC sottoposti a screening/
totale dei nati FC



Età alla morte (anni)



History of CF Newborn Screening (NBS) in Lombardia

<1980: CF NBS conducted with BM test

1983–92:IRT/IRT as pilot study (research protocol)

→ **1989: CFTR discovered on 7th chromosome**

1992: DNA analysis in CF NBS IRT/delF508/IRT
(in Italy delF508 = 54% of CF alleles)

Since October 1998 IRT/DNA (multi panel mutations)/IRT
(31-33 alleles = 75% of CF alleles)

Screening neonatale per FC – problematiche attuali

**Livelli di cut off per b-IRT
Scelta dei pannelli di mutazioni CFTR (DNA panels)**

**Protocolli di trattamento dei neonati identificati
dallo NBS
Possibilità di trials clinici**

**Formazione per gli operatori (neonatologi, pediatri)
Corretta informazione per le famiglie**

**È sempre corretto
che il neonato diventi paziente?**

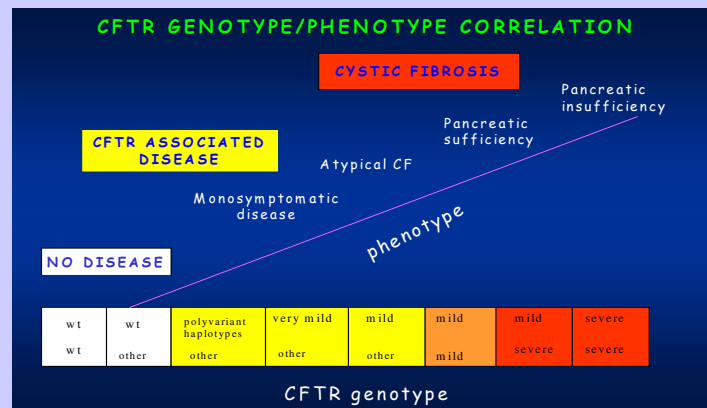
Screening neonatale per FC – problematiche attuali

- **NBS con l'uso di un'estesa analisi genetica**
- **Conferma diagnostica e riconoscimento dei portatori**
 - Valori normali di test del sudore <6 mesi
- **NBS e counselling: dilemmi diagnostici e forme atipiche**
- **Management delle forme atipiche**

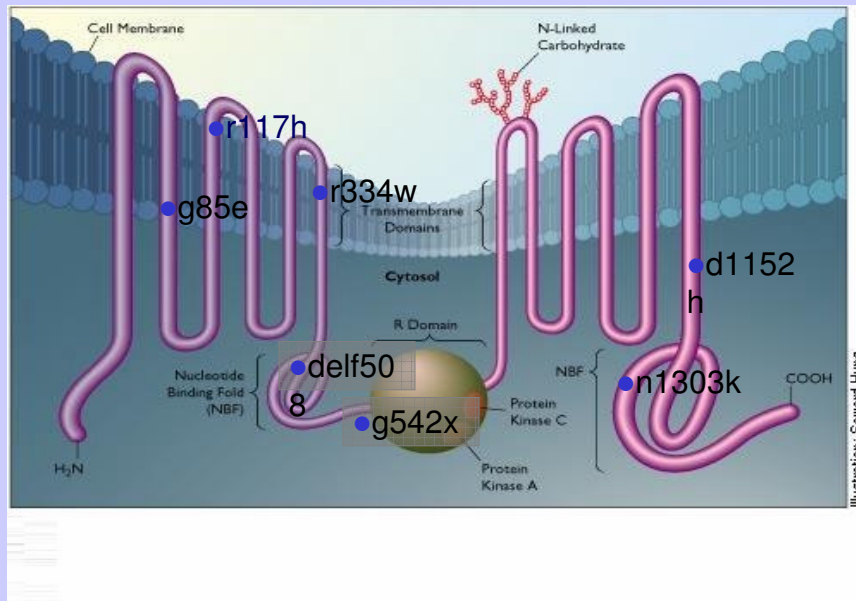
CYSTIC FIBROSIS DIAGNOSIS

Atypical CF:
sweat test >30- 60 mmol/l

Classical CF :
sweat test >60 mmol/l



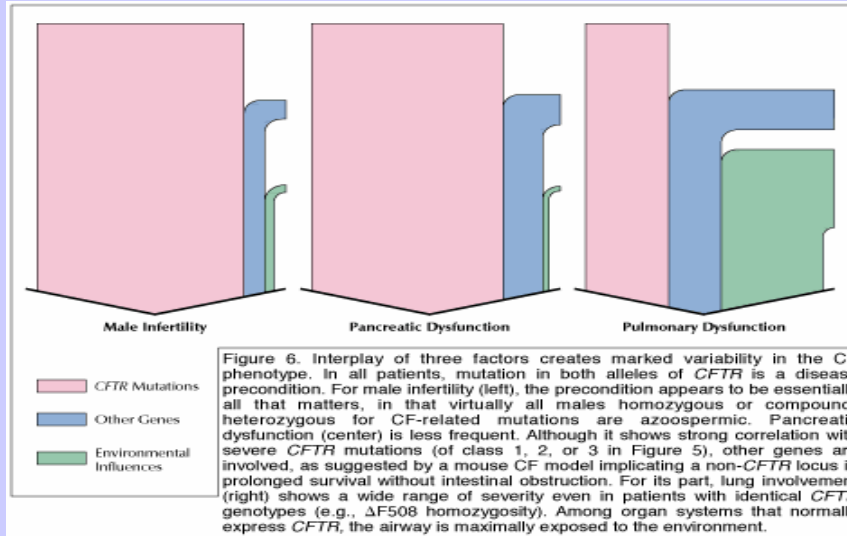
PROTEINA CFTR



MOLECULAR CONSEQUENCES OF CFTR MUTATIONS

Defect Classification	Normal	I	II	III	IV	V
Defect Result		No synthesis	Block in Processing	Block in Regulation	Altered Conductance	Reduced Synthesis
Types of Mutation		Nonsense; Frameshift	Missense; Amino Acid Deletion ($\Delta F508$)	Missense; Amino Acid Change (G551D)	Missense; Amino Acid Change (R117H) (R347P)	Missense; Amino Acid Change (A445E) Alternative Splicing
Potential Therapy		Gentamicin, Gene Transfer	Butyrates, Gene Transfer	Genistein, Gene Transfer	Milrinone, Gene Transfer	Gene Transfer

Genotype-phenotype correlation

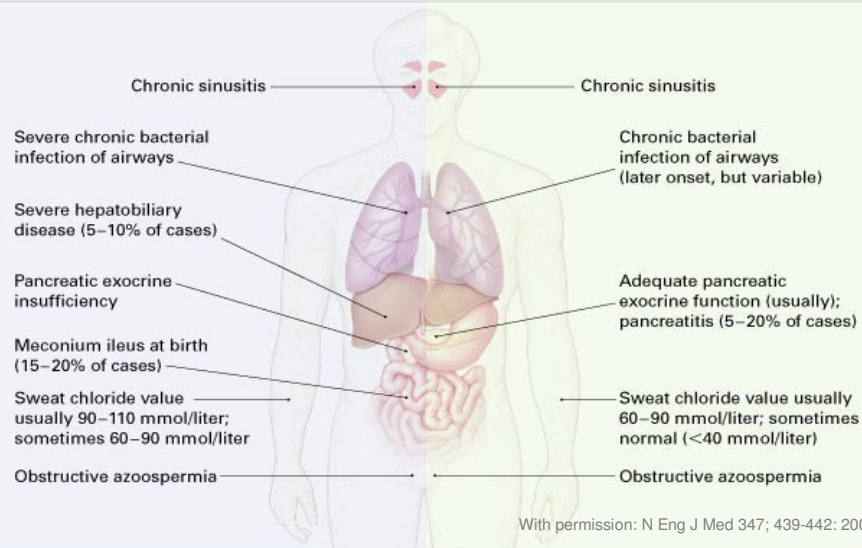


from Lap-Chee Tsui

Classic and Nonclassic Cystic Fibrosis

Classic cystic fibrosis
(no functional CFTR protein)

Nonclassic cystic fibrosis
(some functional CFTR protein,
providing survival advantage)



"Cystic Fibrosis and Related Disorders" :

- Classical CF Pancreatic-insufficient (PI).
- Classical CF Pancreatic-sufficient (PS).
- Atypical CF.
- CF other specified.
- CF not otherwise specified.
- Isolated obstructive azoospermia*.
- Chronic Pancreatitis*.
- Allergic bronchopulmonary aspergillosis (ABPA)*.
- Disseminated bronchiectasis*.
- Diffuse panbronchiolitis*.
- Sclerosing Cholangitis*.
- Neonatal hypertrypsinogenemia.

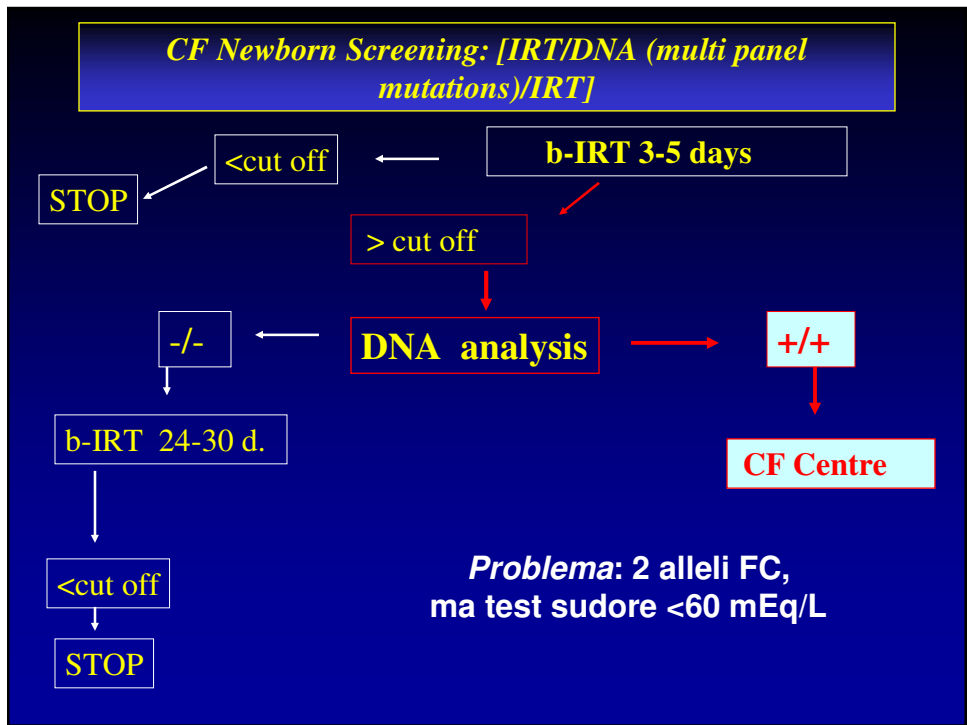
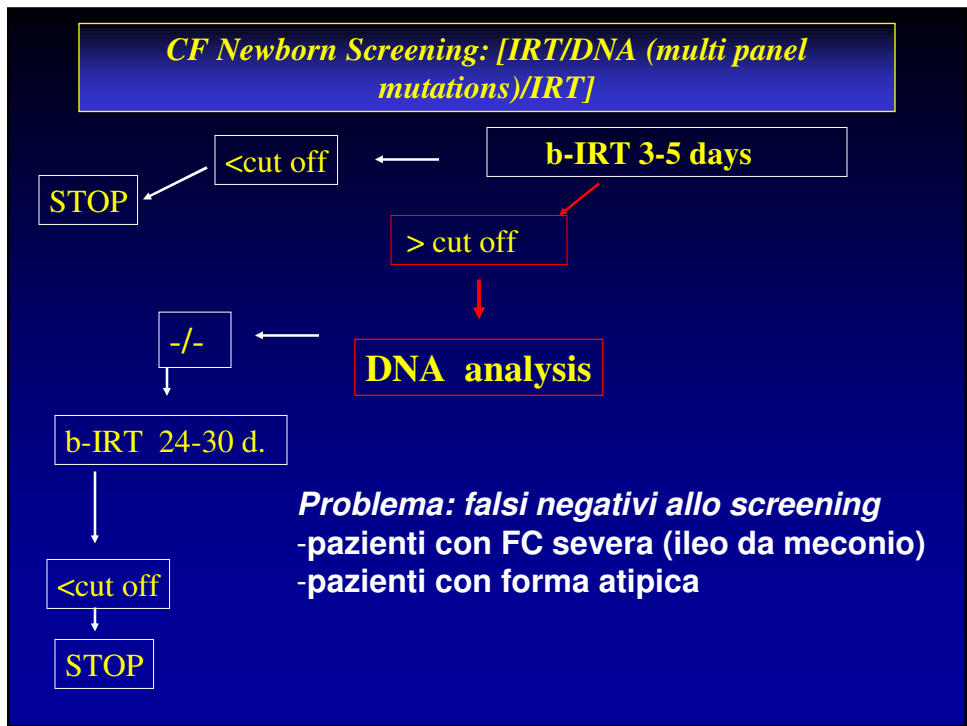
It is likely that this classification will need further revision in future as our knowledge and understanding of these conditions increase.

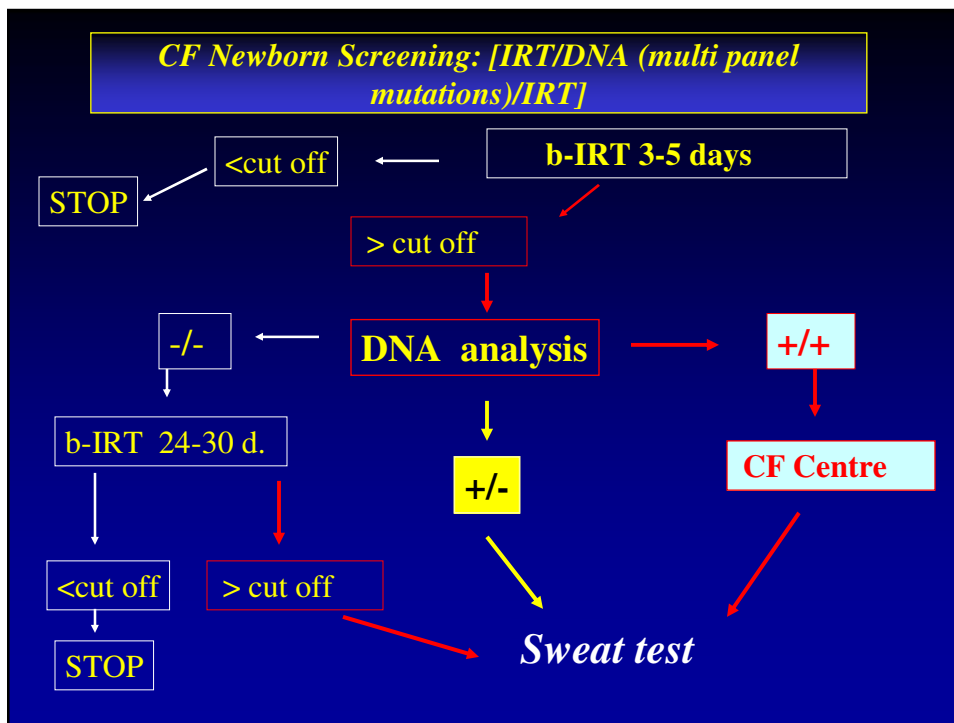
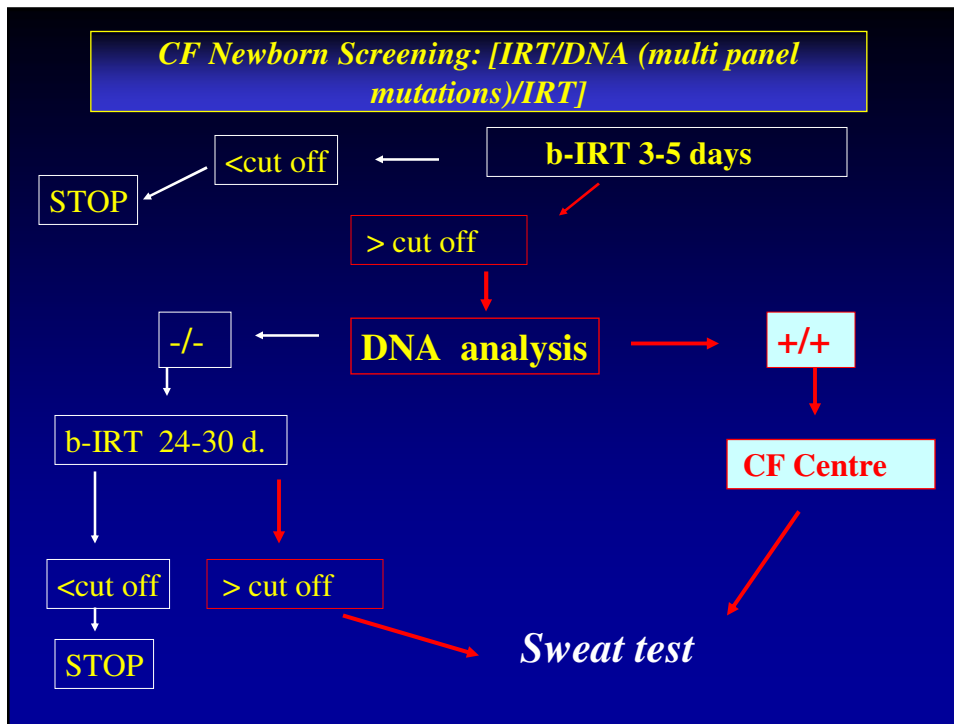
CF Newborn Screening: [IRT/DNA (multi panel mutations)/IRT]

b-IRT 3-5 days

<cut off

STOP





“Das Kind stirbt bald
wieder,
dessen Stirne bein
küssen salzig schmeckt”

“Morirà presto il
bambino, stregato, la cui
fronte sa di sale se
baciata”

Sweat test

Woe to that child which when kissed
on the forehead tastes salty.
They are bewitched and soon will die

PAEDIATRIC RESPIRATORY REVIEWS (2004) 5(Suppl A), S357–S359
Available online at www.sciencedirect.com

SCIENCE @ DIRECT®

Paediatric
Respiratory Reviews

Diagnosis of CF despite normal or borderline sweat chloride

Margaret W. Leigh*

Department of Pediatrics, University of North Carolina, Chapel Hill, NC, USA

© 1998 Oxford University Press

Human Molecular Genetics, 1998, Vol. 7, No. 4 729–735

A mutation in the cystic fibrosis transmembrane conductance regulator gene associated with elevated sweat chloride concentrations in the absence of cystic fibrosis

John E. Mickle¹, Milan Macek Jr^{1,†}, Stephanie B. Fulmer-Smentek¹, Michelle M. Egan¹, Erik Schwiebert^{2,§}, William Guggino², Richard Moss³ and Garry R. Cutting^{1,*}

Center for Medical Genetics and Department of ¹Pediatrics and ²Physiology, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA and ³Stanford University Medical Center, Stanford, CA, USA

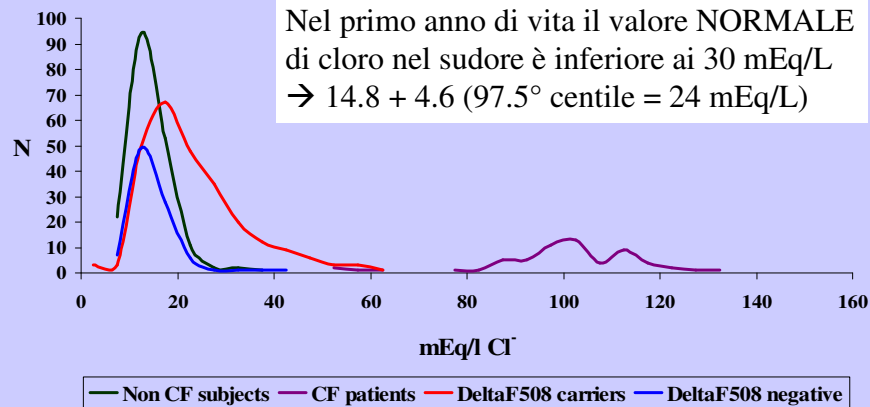
Eur J Pediatr 2002;161:212-5

"Negative sweat test in hypertrypsinaemic CF infants carrying rare CFTR mutations"

Padoan R., Bassotti A., Seia M., Corbetta C.,

YEARS of birth	Sex	Chloride mmol/l	Age at sweat tests	Age at re-evaluation	SYMPTOMS	GENOTYPE
93	M	43	4 mo	3y3m	respiratory	Δ F508/A309D
94	M	<60	4 mo	6y6m	Severe nasal polyposis	Δ F508/3849+10kbC→T
95	F	34	4 mo	5y4m	Familial hystory	Δ F508/R117H-7T
97	F	55	4 mo	2y	No symptoms	R117H-7T/L997F
97	F	37	3 mo	20m	Upper airways recurrent infections	Δ F508/R117H-7T
98	F	36	2 mo	18m	No symptoms	Δ F508/R117L

Sweat test: chloride values in non CF subjects (b-IRT-ve) and in b-IRT+ve infants (Δ F508 carriers, CF, Δ F508 negative)



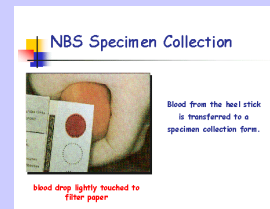
Test del sudore nei primi mesi di vita

Farrell P (USA)	<i>NEJM 1996</i>	IRT + ve No F508del	10.5 ± 5.3 mmol/L Cl-
Corbetta (Milano)	<i>IJP 1996</i>	IRT -ve No F508del	14.8 ± 4.6
"	"	IRT + ve No F508del	15.0 ± 5.4
Taccetti (Firenze)	<i>ADCFN 2004</i>	IRT +ve	16.1 ± 7.06
Massie J (Australia)	<i>J Ped 2000</i>	IRT+ F508del carriers	15.5 ± 6.2
Farrell P (USA)	<i>NEJM 1996</i>	IRT+ F508del carriers	14.9 ± 8.4



At the moment,
the incidence of atypical forms of CF
is **unknown**

In our region (N-W Italy).
all newborns undergo
a CF screening procedure



Aim of our study was to investigate the
capacity of the NS to recognize CF atypical
forms and to clarify their incidence

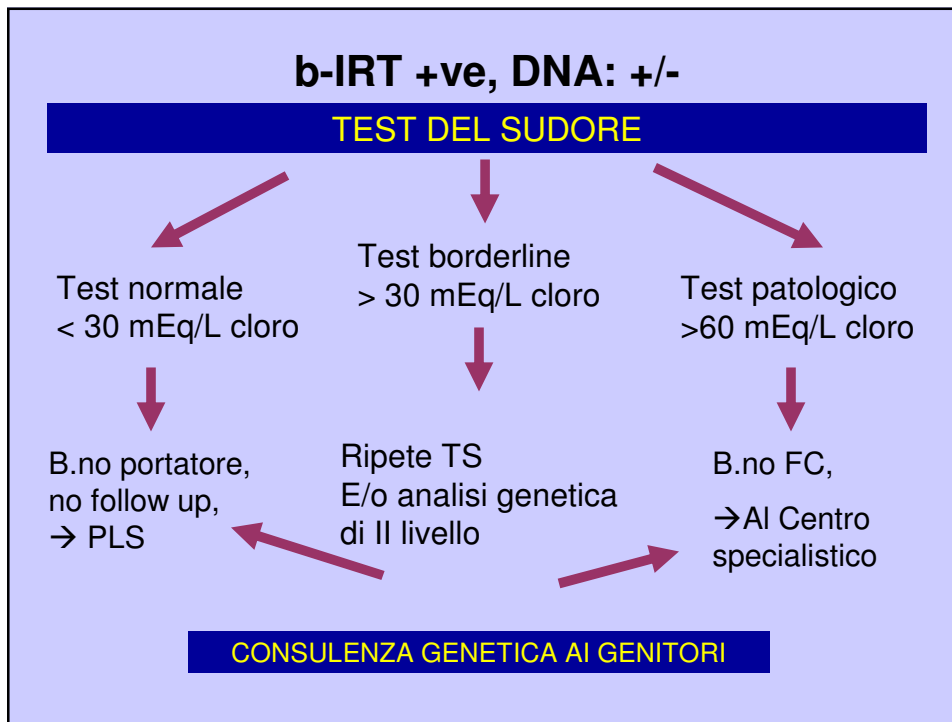
Screening neonatale e test del sudore

Test del sudore (cloro mEq/L)	Numero di mutazioni CFTR identificate con NBS		
	2	1	0
>60 (patologico)	Fibrosi Cistica	Fibrosi Cistica	Fibrosi Cistica
30-59 (borderline)	FC	Possibile FC	Possibile FC
<30 (normale)	FC ?	Carrier	Normale

2002: 89,108 screened newborns

1201 (1,35%) neo IRT+ve

Test del sudore (cloro mEq/L)	Numero di mutazioni CFTR identificate con NBS		
	2	1	0
	15	83	193 (recall+ve)
>60 (patologico)	14	12	0
30-59 (borderline)	1	15	1
<30 (normale)	0	56	192



Genetic analysis in infants with borderline sweat test (DHPLC and polyT)

<u>With CF symptoms</u>	<u>Without symptoms</u>
<i>R553X /R117H-5T*</i>	<i>ΔF508/ 5T</i>
<i>P5L/H199R (PI)*</i>	<i>ΔF508/ 5T</i>
<i>ΔF508/ D1152H*</i>	<i>G542X/ 5T</i>
<i>ΔF508/ D1152H*</i>	<i>ΔF508/ 5T</i>
<i>1717-1G-A/ D1152H*</i>	<i>2789+5G->A / 5T</i>
<i>3849+10KbC-->T/ 711+5G->A*</i>	<i>ΔF508/ 5T</i>
<i>R117H-7T/ unknown*</i>	<i>ΔF508/ - carrier</i>
<i>G542X/unknown (met. alkalosis)</i>	<i>G542X/ - carrier</i>
<i>* Recurrent respiratory infection</i>	<i>ΔF508/ - lost to follow up</i>

CF incidence in 2002

***27 classical CF (11 M.I.)
1:3,300 (27/89,108; IC 95% 1:2273-1:5006).***

***15 atypical CF
1:5,940 (15/89,108; IC 95% 1:3,593-1:10,608).***

*False negatives: ° meconium ileus (delF508/delF508) 121 mEq/l Cl;
prenatal diagnosis (2789+5G →A/Y89C) <30 mEq/L Cl;

Follow up diagnostico-terapeutico dei lattanti con test del sudore borderline

1. Test del sudore centralizzato (metodo quantitativo)
2. Analisi genetica di II livello (DGGE, DHPLC, delezioni)
3. Visita specialistica e consulenza genetica presso il Centro FC
4. Valutazione per malassorbimento (f- elastasi pancreatico)
5. Rx torace (o TAC ?) e valutazione microbiologica delle secrezioni bronchiali se sintomi respiratori

Follow up diagnostico-terapeutico dei lattanti con test del sudore borderline

6. Supplementazione salina nel periodo estivo
7. Stretto rapporto con il pediatra curante
8. Valutazione della differenza di potenziale nasale o differenza di potenziale intestinale
9. Rivalutazione del caso

Studio della funzione della proteina CFTR: differenza di potenziale nasale

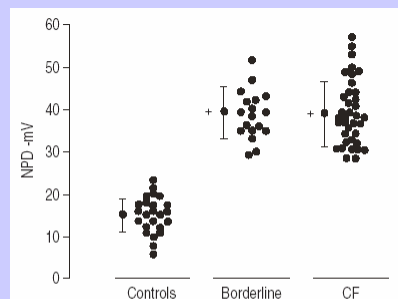
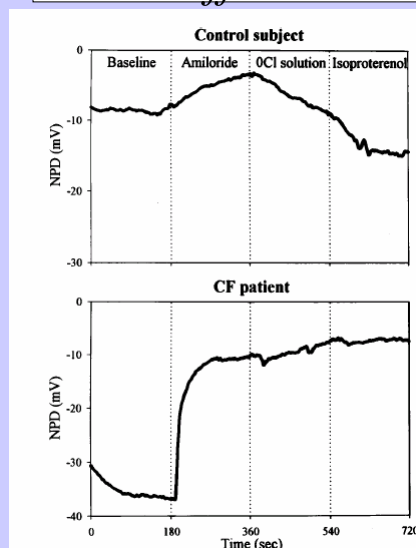


Fig. 1. - Values of nasal potential difference (NPD) obtained in non-CF control subjects (Controls), patients with cystic fibrosis and a borderline sweat test diagnosed by means of deoxyribonucleic acid (DNA) analysis (Borderline), and CF controls with abnormal sweat electrolytes (CF). Means±SD are represented beside the data for each group. +: $p < 0.0001$, with respect to non-CF controls. CF: cystic fibrosis.

Delmarco A. et al: ERJ 1997

Pradal U. et al: AJRCCM 1998

Conclusioni -1: NBS

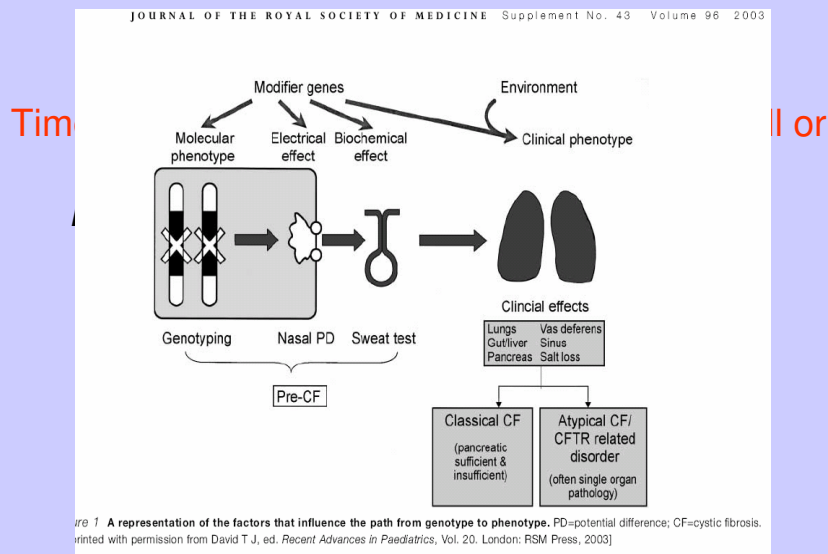
IRT/DNA
test del sudore
analisi molecolare

→

FC classiche
Carriers
FC atipiche

L'analisi molecolare di II livello può chiarire la diagnosi nei neonati ipertripsinemici con test del sudore borderline e senza sintomatologia nel primo anno di vita

Conclusioni -2: Fibrosi Cistica?



STRATEGIE DIAGNOSTICHE ED ASSISTENZIALI COME
GARANZIA DI QUALITA'

**Migliorare la qualità del programma di NBS:
interventi per gli Ospedali periferici
e i pediatri curanti**

Migliorare la conoscenza delle attuali procedure di
NBS → incontri per neonatologi, infermieri,
chirurghi pediatrici e pediatri

Raccomandazioni per migliorare l'esecuzione e la
valutazione del test del sudore → diffusione
informazioni sui limiti di normalità.

Centralizzazione del test del sudore per i carriers.

ringraziamenti



Dr. Carlo Corbetta,
Laboratorio di Riferimento Regionale per lo Screening Neonatale
AO Istituti Clinici di Perfezionamento, Milano

Dr.ssa Manuela Seia,
Laboratorio Genetica Molecolare,
Fondazione Ospedale Maggiore, Mangiagalli e Regina Elena

Prof.ssa Anna Bossi,
Istituto di Statistica Medica e Biometria,
Università degli Studi di Milano



Prof. L.D. Notarangelo
Dipartimento di Pediatria,
Università di Brescia

