

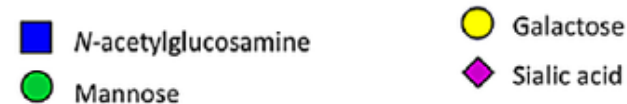
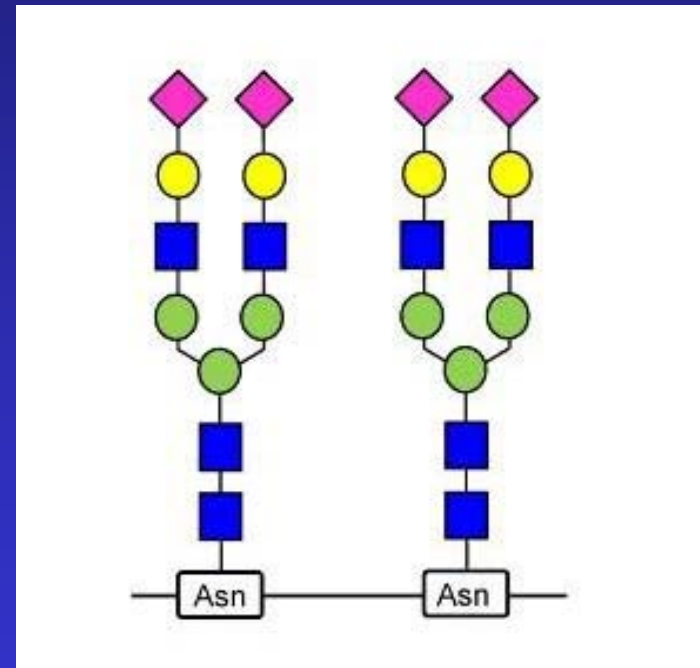
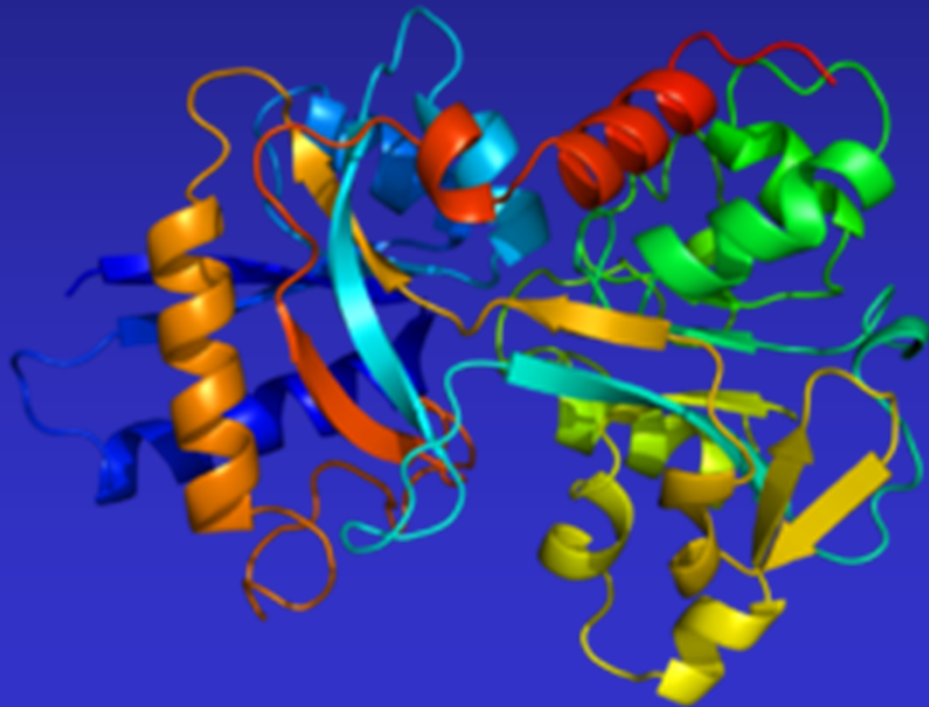
# **Clinical Diagnostics of Transferrin Glycoforms by High-resolution CE**

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Institute for Infectious Diseases  
University of Bern  
Bern, Switzerland**

**CE Pharm 2020 – September 28, 2020**

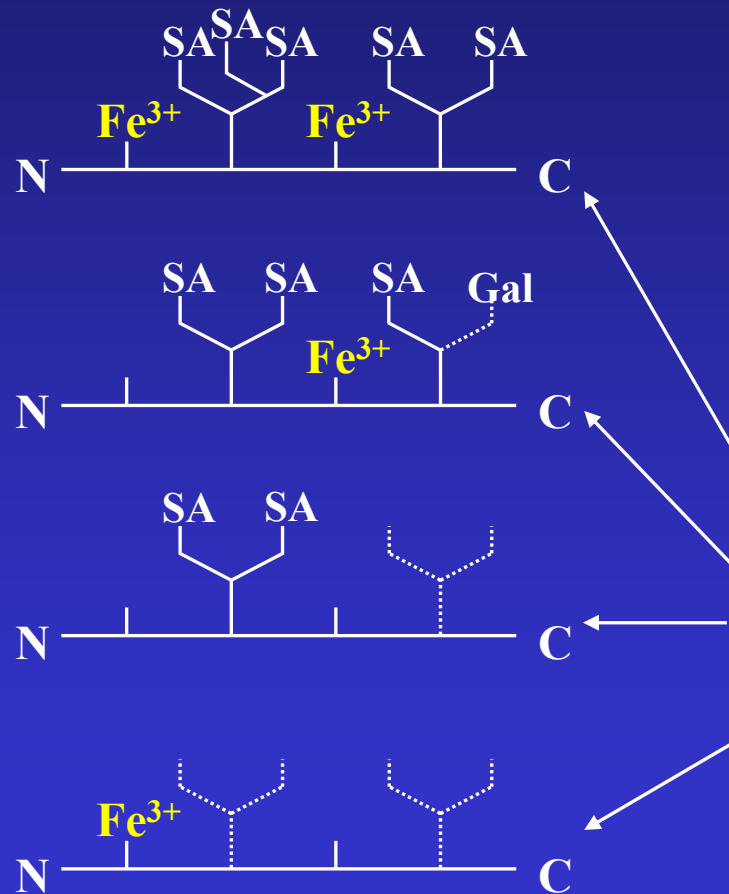
# Transferrin is a glycoprotein

- Single peptide chain with 679 amino acids
- Globular C- and in N-terminal domains with an Fe(III) binding site each
- Two N-linked glycan chains (bound to asn at positions 413 and 611)
- Molecular mass: about 80 kDa
- 2-4 g/L in human serum (~ 4 % of total serum proteins)



# Serum transferrin (Tf)

- Glycoforms with various Fe<sup>3+</sup> loads and N-glycan chains
- Carbohydrate-deficient transferrin (CDT, marker for chronic alcohol abuse)
  - Genetic variants, congenital disorders of glycosylation (CDG)



| Glycoform     | % of total Tf (healthy person) | pI *) |
|---------------|--------------------------------|-------|
| Octasialo-Tf  | ND                             | ≈ 5.0 |
| Heptasialo-Tf | < 1.5                          | ≈ 5.1 |
| Hexasialo-Tf  | 1-3                            | 5.2   |
| Pentasialo-Tf | 12-18                          | 5.3   |
| Tetrasialo-Tf | 64-80                          | 5.4   |
| Trisialo-Tf   | 4.5-9                          | 5.6   |
| Disialo-Tf    | < 1.7                          | 5.7   |
| Monosialo-Tf  | ND                             | ≈ 5.8 |
| Asialo-Tf     | ND                             | 5.9   |

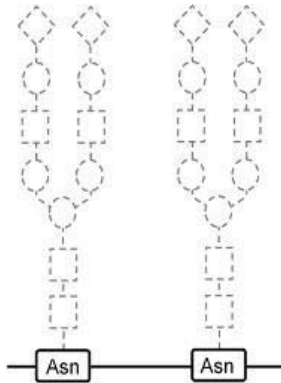
} **CDT**

\*) complete iron saturated of C1-Tf

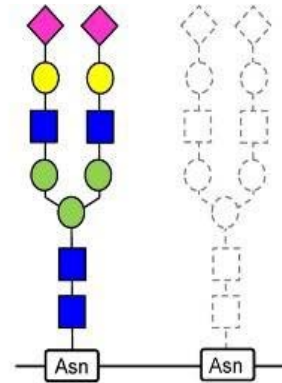
SA = sialic acid, Gal = galactose

Adapted from Clin. Chem. 47 (2001) 13.

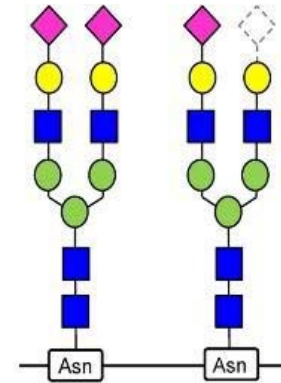
# Common transferrin glycoforms



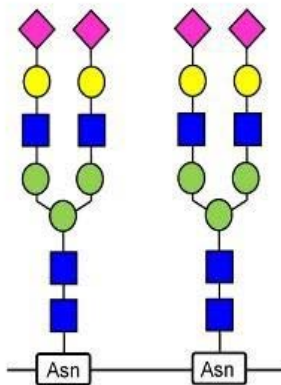
Asialo-transferrin



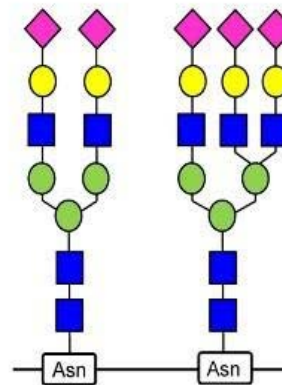
Disialo-transferrin



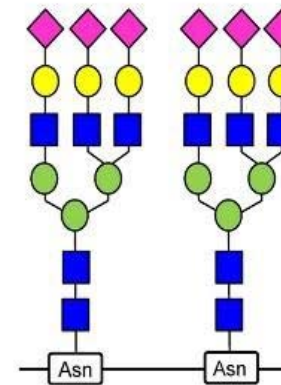
Trisialo-transferrin



Tetrasialo-transferrin



Pentasialo-transferrin



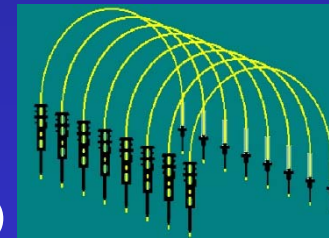
Hexasialo-transferrin

# CE analysis of Tf glycoforms

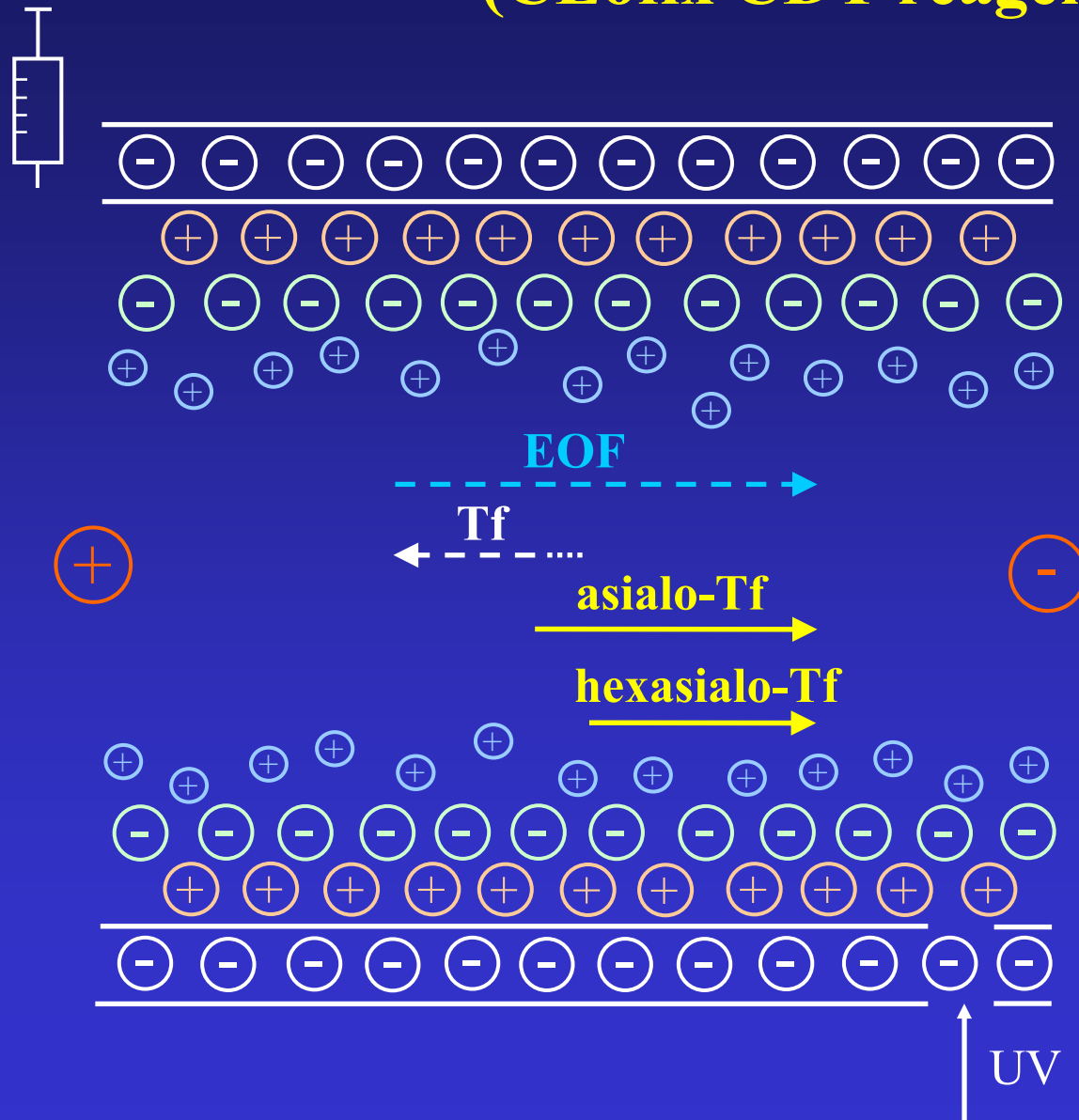
- Glycoforms differ in charge and mass
- Fe (III) saturation reduces number of molecules
- 20-50  $\mu\text{m}$  ID fused-silica capillary
- Separation at alkaline pH (pH about 8.5)
- Capillary wall conditioning
- Detection at 200 nm
- Immunoextraction (low Tf concentration and interferences)

## CE instruments and reagents

- CE instrument with laboratory made or commercial reagents (CEofix, Analis, Belgium)
- Multicapillary analyzers with reagent kits (walk away automation) with Capillarys (Sebia) or V8 CE system (Helena Biosciences Europe)

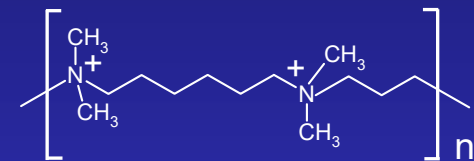


# Principle of dynamic double coating (CEofix CDT reagents)



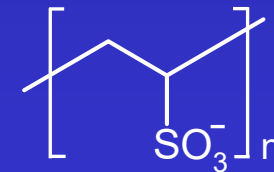
## Polycation:

e.g. polybrene

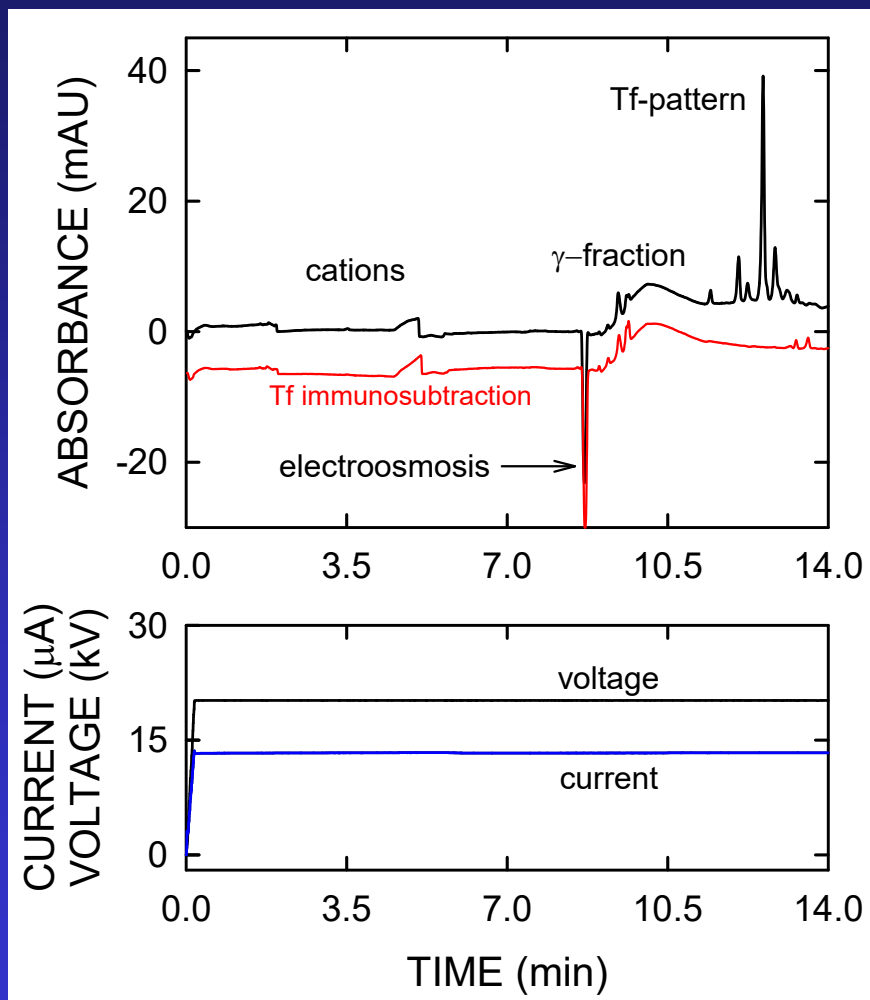


## Polyanion:

e.g. poly(vinylsulfonate)



# High-resolution analysis based on CEofix CDT reagents of Analis



J. Chromatogr. A 1130 (2006) 272.

## Sample preparation: serum + Fe<sup>3+</sup> solution (60 μL each)

- P/ACE MDQ (Beckman Coulter)
- Capillary: 50 μm ID x 60 cm total length
  - Conditioner: 0.2 M NaOH
- Initiator: polycation in Tris/phosphate, pH 2.0
- Buffer: polyanion in Tris/borate, pH 8.5
  - Injection: 0.5 psi x 12.0 s (vacuum)
  - Separation: 20 kV, 30 °C
  - Detection: 200 nm
  - Sample throughput: 2.7/h

### Assay of Analis:

- 50 cm capillary/28 kV/30 °C
- 5 samples/h - lower resolution

### Smaller amount of sample:

- NanoVials for 5 μL serum

# CEofix electropherograms

Valley to valley peak integration; area % in relation sum of all Tf peaks.

## Healthy person:

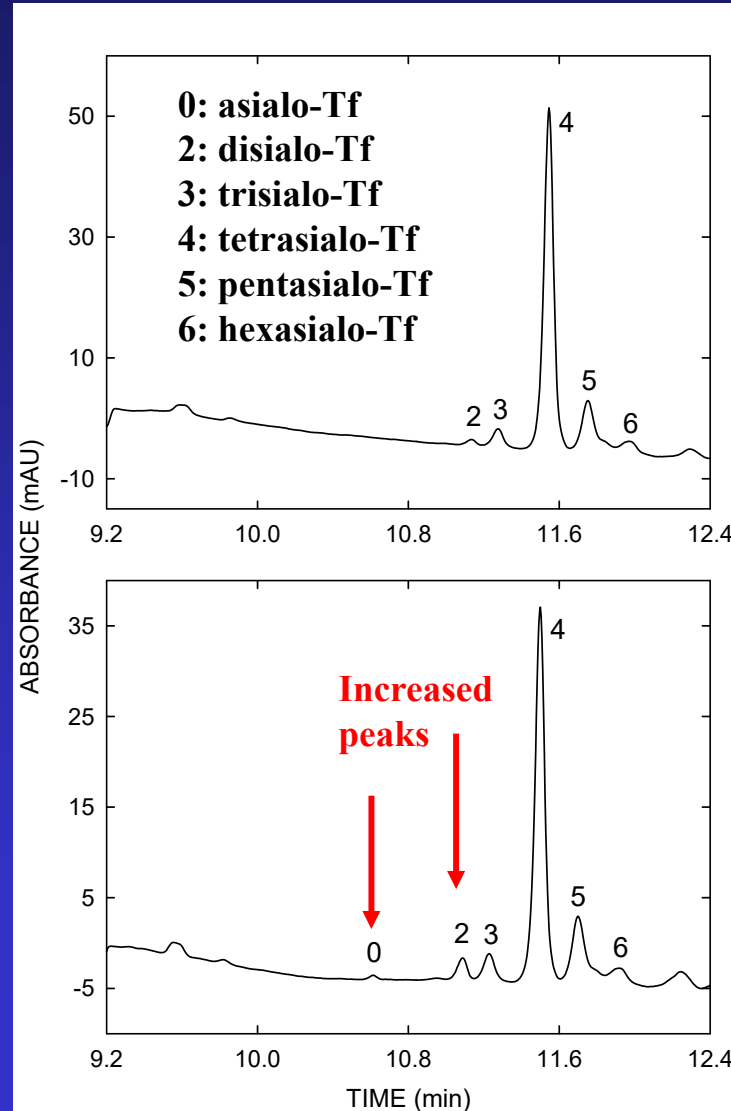
CDT 1.06 %

|    |         |
|----|---------|
| 2: | 1.06 %  |
| 3: | 4.32 %  |
| 4: | 78.37 % |
| 5: | 13.48 % |
| 6: | 2.72 %  |

## Alcohol abuser (CDT > 1.70 %):

CDT 4.56 %

|    |         |
|----|---------|
| 0: | 0.70 %  |
| 2: | 3.86 %  |
| 3: | 5.58 %  |
| 4: | 72.27 % |
| 5: | 14.73 % |
| 6: | 2.86 %  |



## Interday precision

(n = 8)

|    | time   | amount |
|----|--------|--------|
| 2: | 0.58 % | 2.07 % |
| 3: | 0.59 % | 1.14 % |
| 4: | 0.59 % | 0.22 % |
| 5: | 0.59 % | 0.79 % |
| 6: | 0.60 % | 3.11 % |

## Interday precision

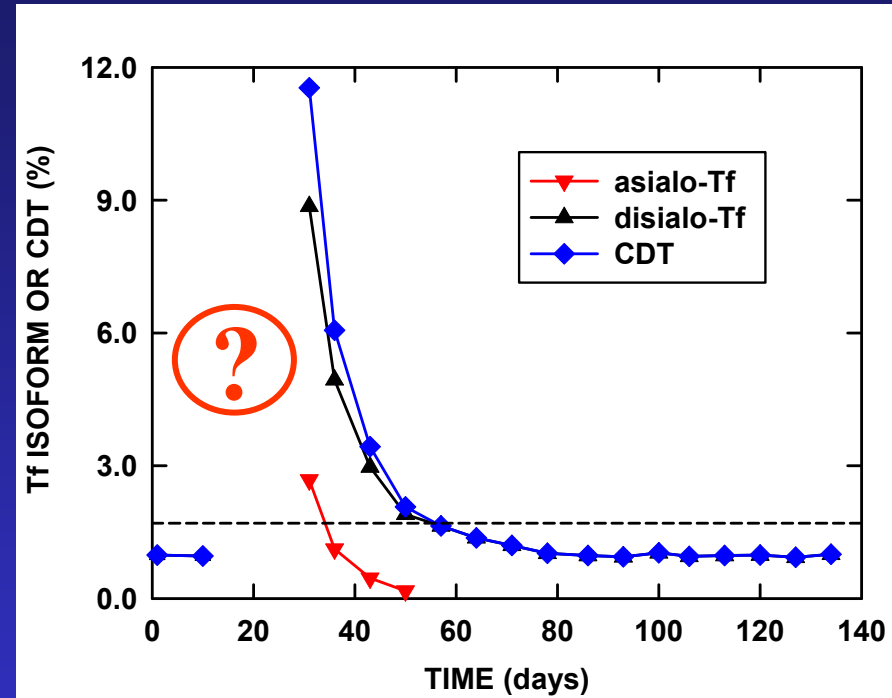
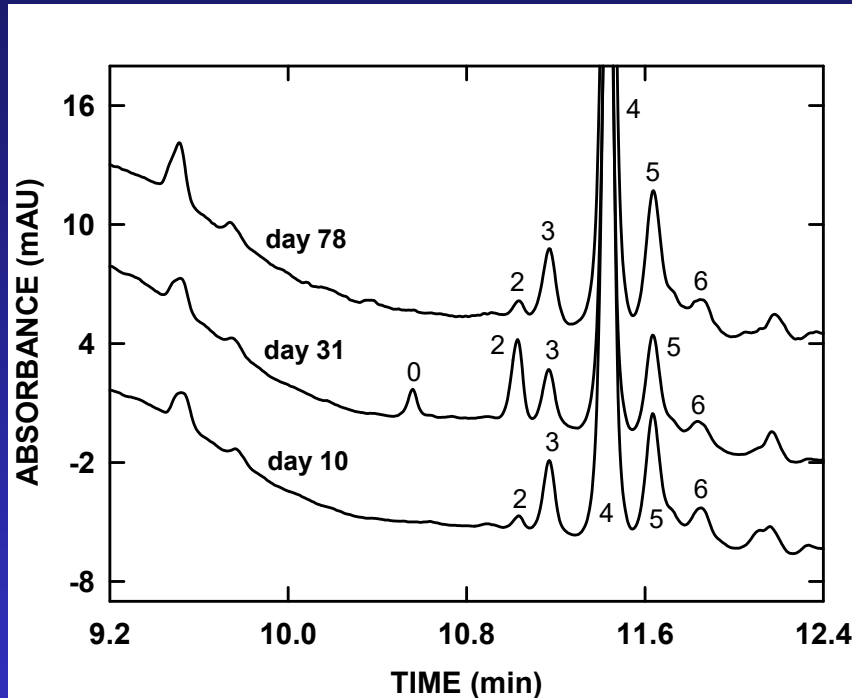
(n = 8)

|    | time   | amount |
|----|--------|--------|
| 0: | 0.60 % | 3.43 % |
| 2: | 0.63 % | 1.44 % |
| 3: | 0.63 % | 0.70 % |
| 4: | 0.64 % | 0.26 % |
| 5: | 0.65 % | 0.94 % |
| 6: | 0.66 % | 3.64 % |



# Patient monitoring

Monitoring of a patient during 19 weeks with a relapse drinking episode  
(no blood alcohol could be determined)



Upper reference  
value for CDT:  
1.70 %

Electrophoresis 25 (2004) 2309.

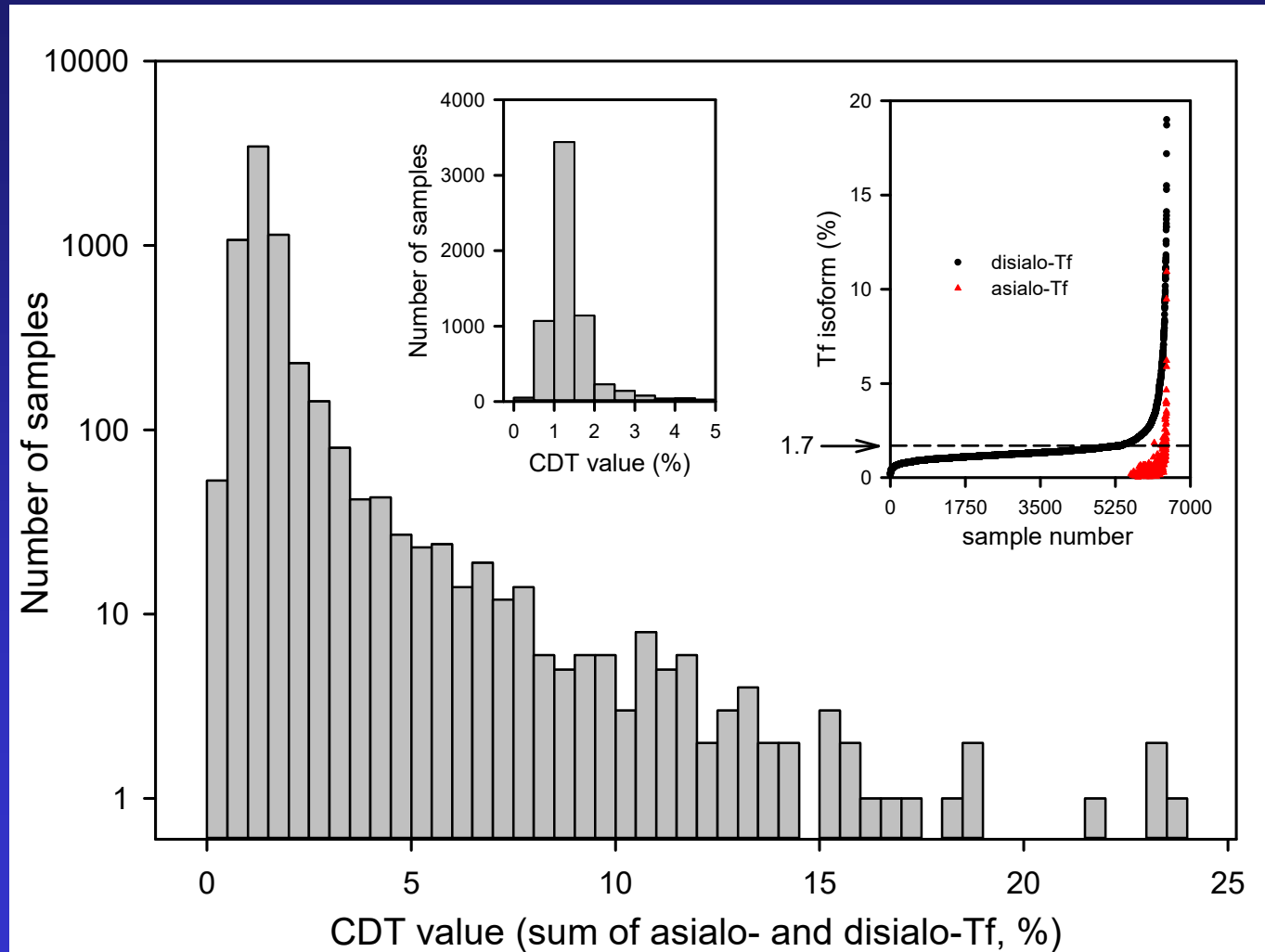


apparent half-lives:

|             |                      |
|-------------|----------------------|
| 0-sialo-Tf: | $T_{1/2} = 4.9$ days |
| 2-sialo-Tf: | $T_{1/2} = 7.2$ days |
| CDT:        | $T_{1/2} = 6.7$ days |

# Patient screening for CDT

CDT values measured during a 10-year period (6449 samples)



**CDT < 1.70 %:**  
**5375 samples**  
**(83.35 %)**

**CDT ≥ 1.70 %:**  
**1074 samples**  
**(16.65 %)**

**Asialo-Tf:**  
**414 samples**

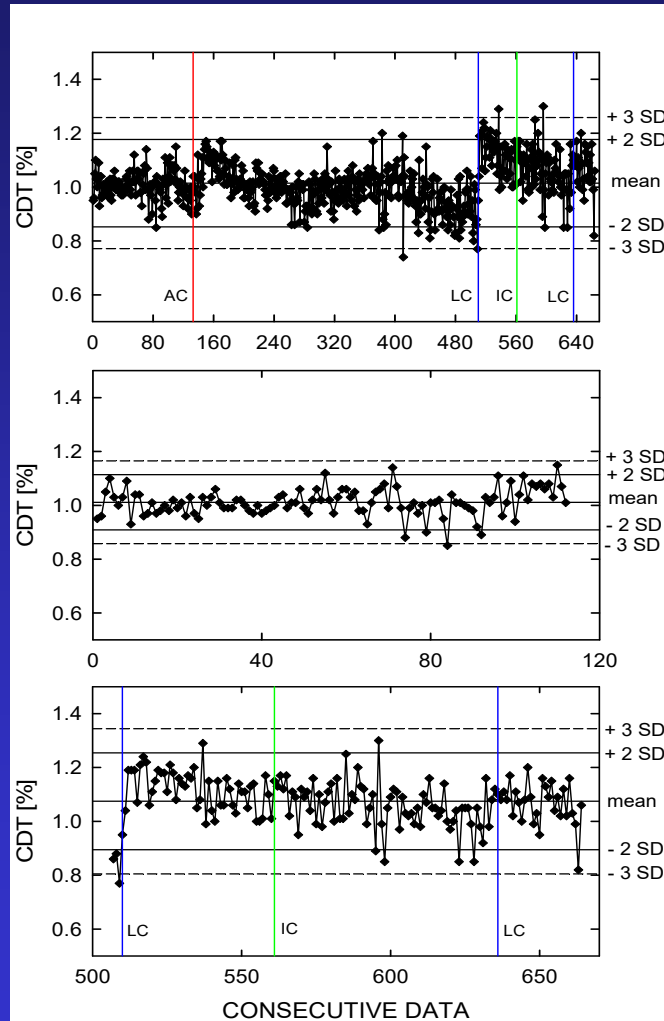
# Quality control

## Serum of a healthy person

**10-year period  
(664 samples)**  
Mean  $\pm$  SD:  
 **$1.02 \pm 0.08$  %**

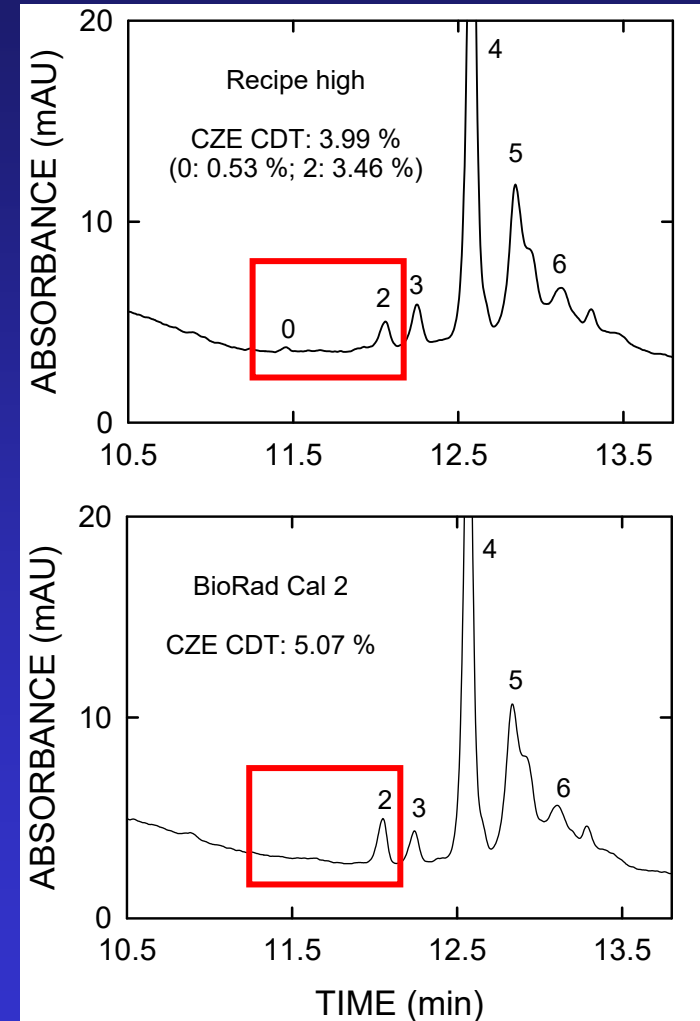
**Years 1 and 2  
(112 samples)**  
Mean  $\pm$  SD:  
 **$1.01 \pm 0.05$  %**

**Years 9 and 10  
(158 samples)**  
Mean  $\pm$  SD:  
 **$1.08 \pm 0.09$  %**



LC: lot change, AC: assay change, IC: instrument change

## Commercial controls

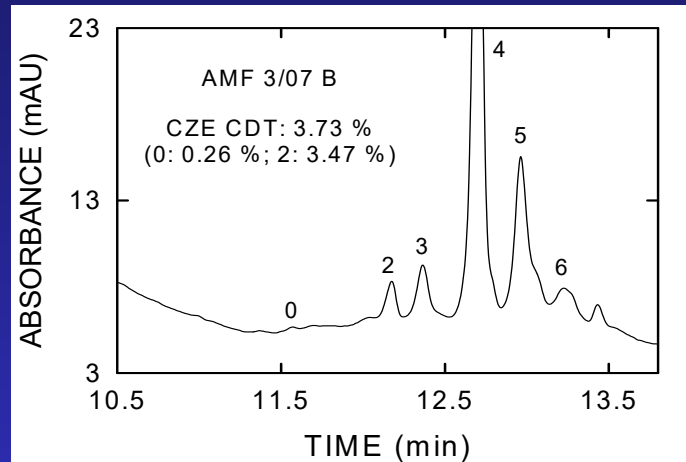


Electrophoresis 34 (2013) 1563.

# External quality control

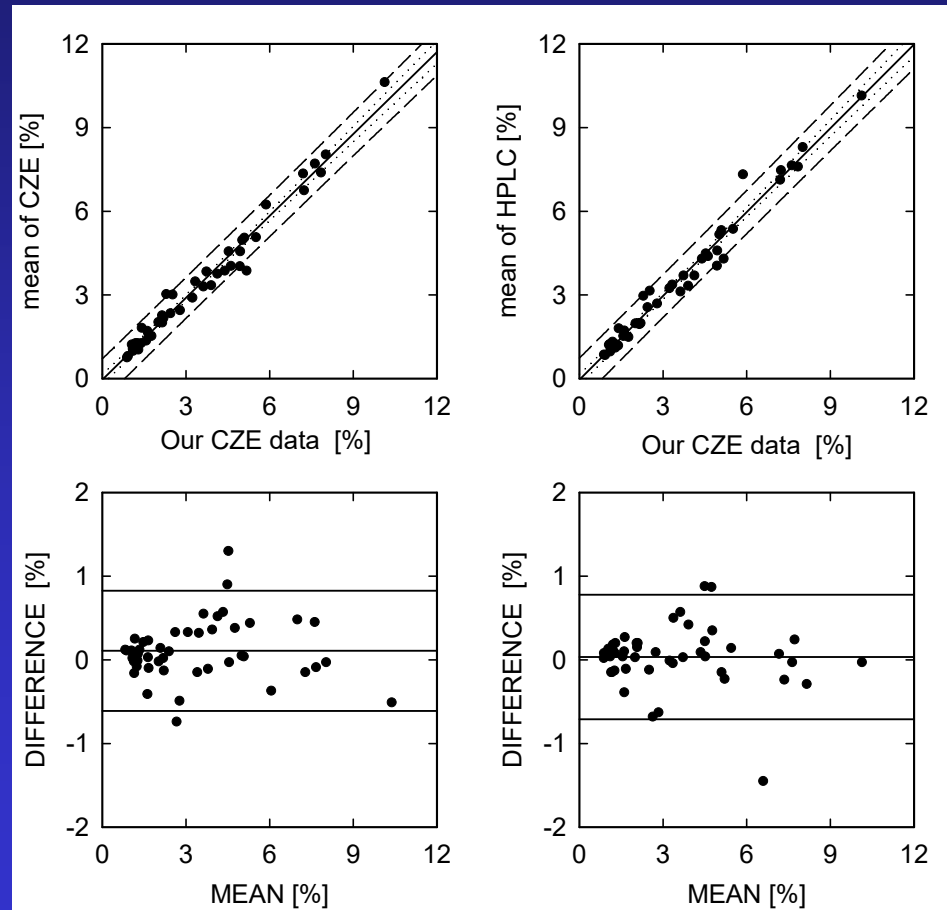
GTFC proficiency test, markers of alcoholism in serum:

AMF 3/07, sample B: positive



Data comparison (n=46)

Our data vs. CE (not Sebia) and HPLC

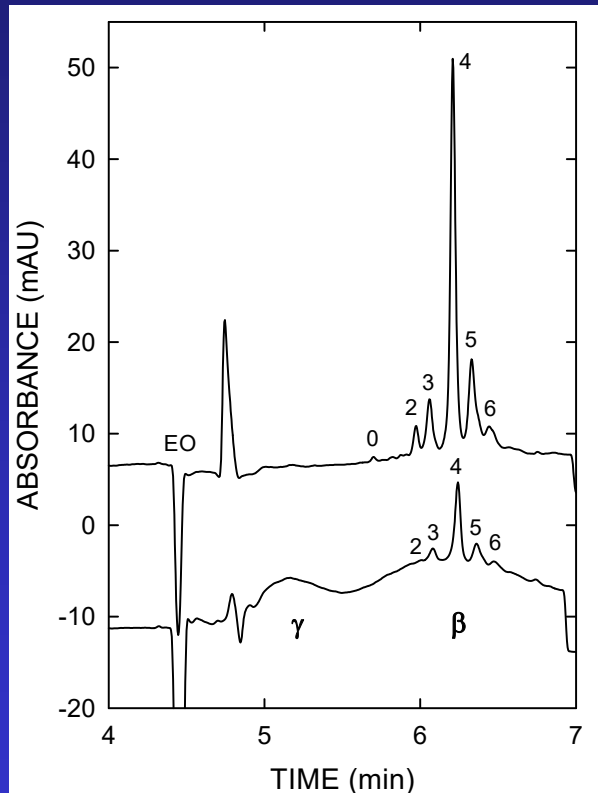


results of 57 participants:

| Assay | n  | Mean (%) | RSD (%) |
|-------|----|----------|---------|
| CE    | 10 | 3.84     | 10.4    |
| Sebia | 8  | 2.78     | 12.6    |
| HPLC  | 26 | 3.70     | 12.2    |

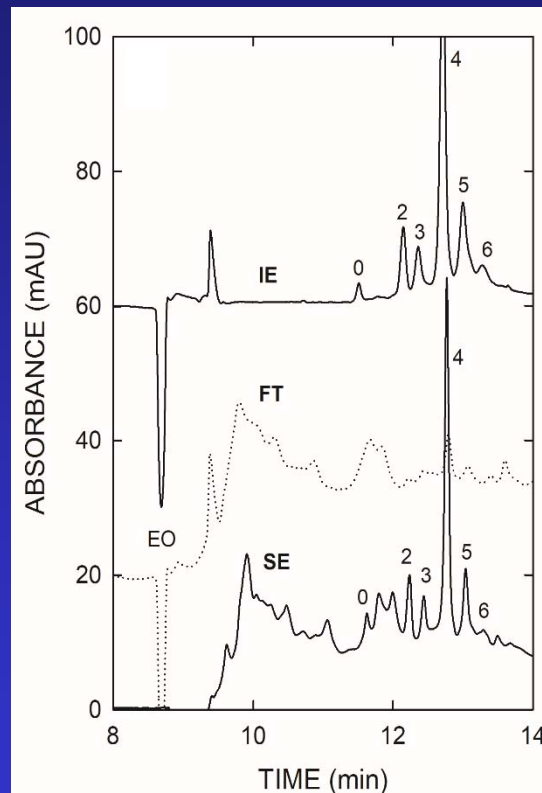
# Immunoextraction of transferrin (low amounts of transferrin / interferences)

Before and after IgY  
Tf purification



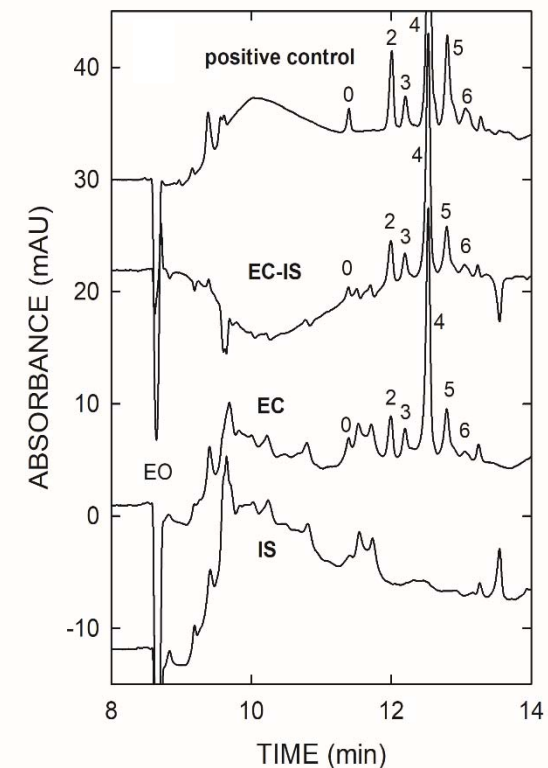
J. Chromatogr. A 1206 (2008) 33.

Purification with lab  
made anti-Tf column



SE: serum analysis  
FT: flow-through fraction  
IE: immunoextract

Immunosubtraction



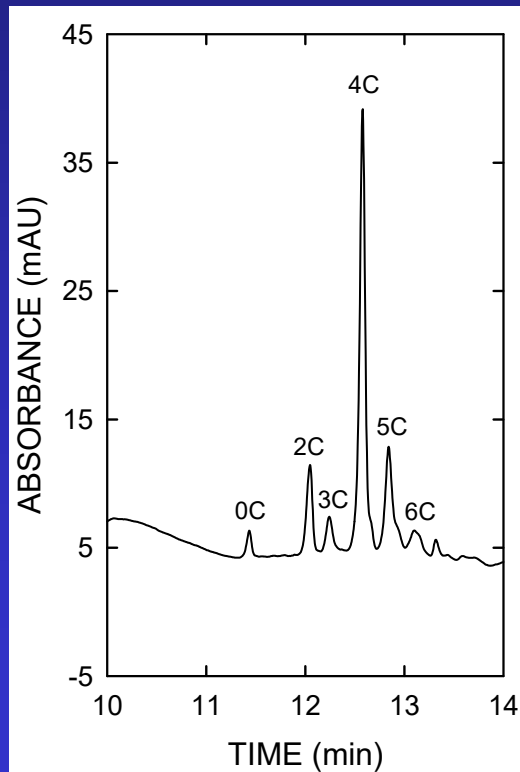
IS: immunosubtraction  
EC: 2.25-fold diluted serum  
EC-IS: difference data

J. Sep. Sci. 35 (2012) 3521.

# Genetic variants of transferrin

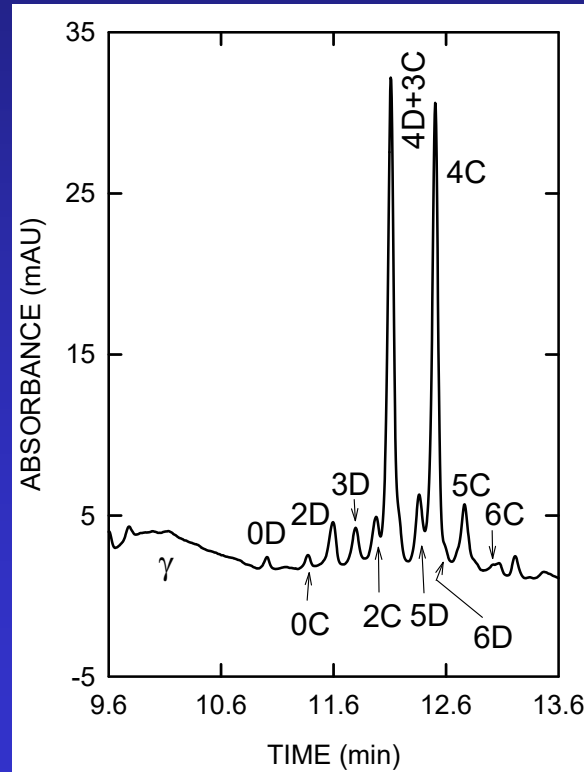
- Substitution of one or several amino acids in peptide chain
- Composite of two glycoform patterns (typically 1:1 distribution)

## Homozygote alcohol abuser



Tf-C

## Heterozygote alcohol abuser



Tf-CD  
variant;

pI (D) > pI(C)

# Genetic variants of transferrin

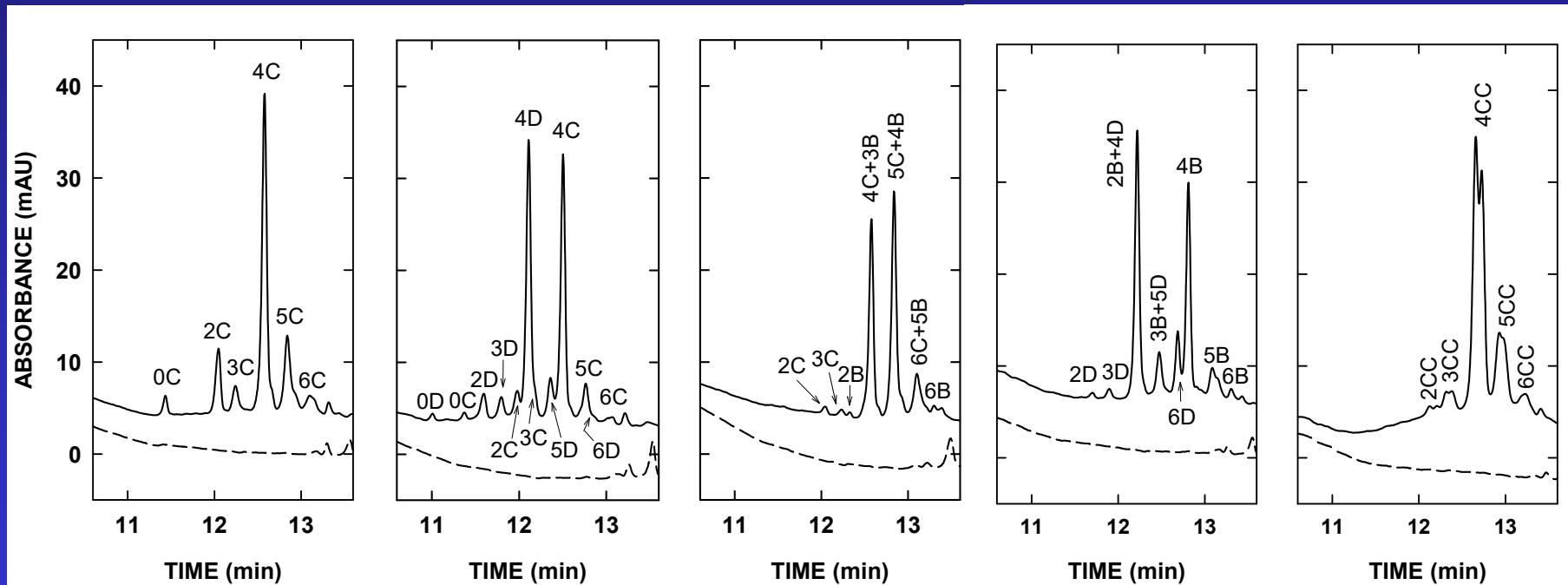
Homozygote

Tf-CD

Tf-BC

Tf-BD

Tf-CC

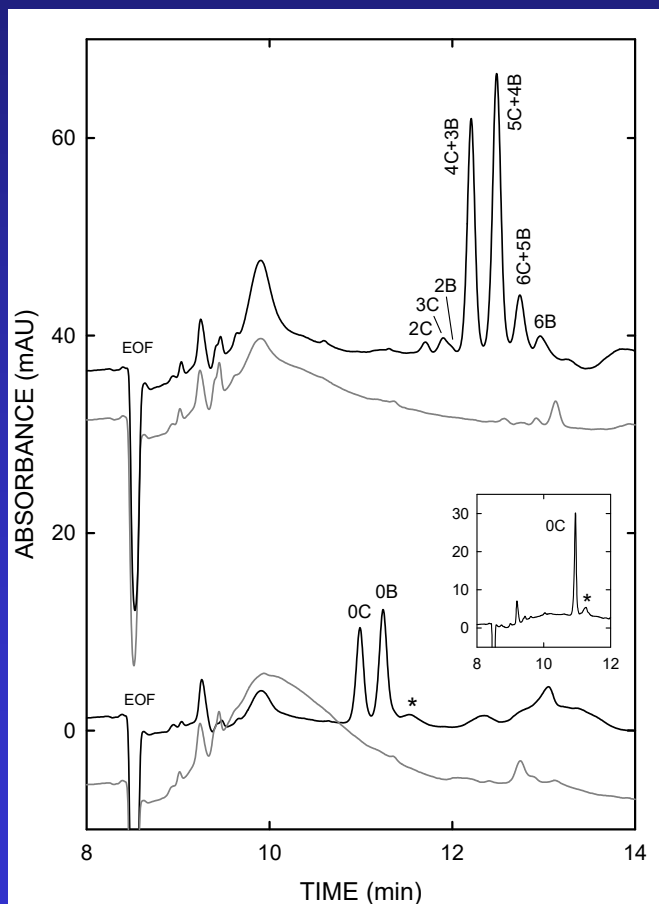


J. Sep. Sci. 37 (2014) 1663; J. Sep. Sci. 41 (2018) 303.

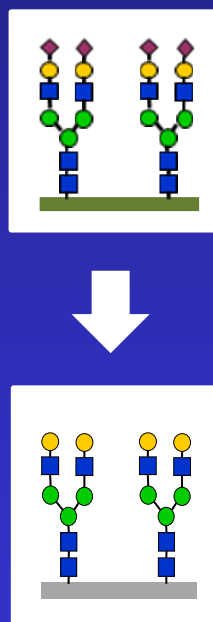
# Transferrin patterns after desialylation

- Removal of sialic acid with neuraminidase
- Analysis of genetic variants by CZE

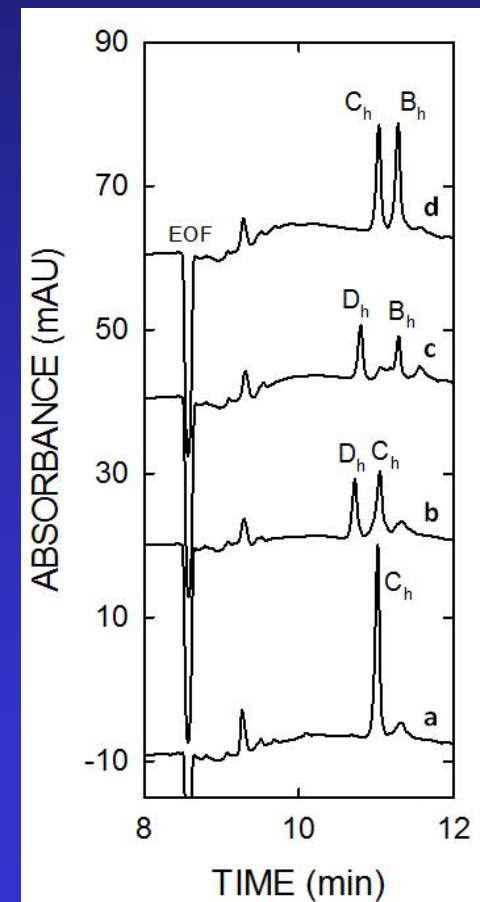
## Effect of enzym. treatment



## Tetrasialo-Tf



## Genetic variants



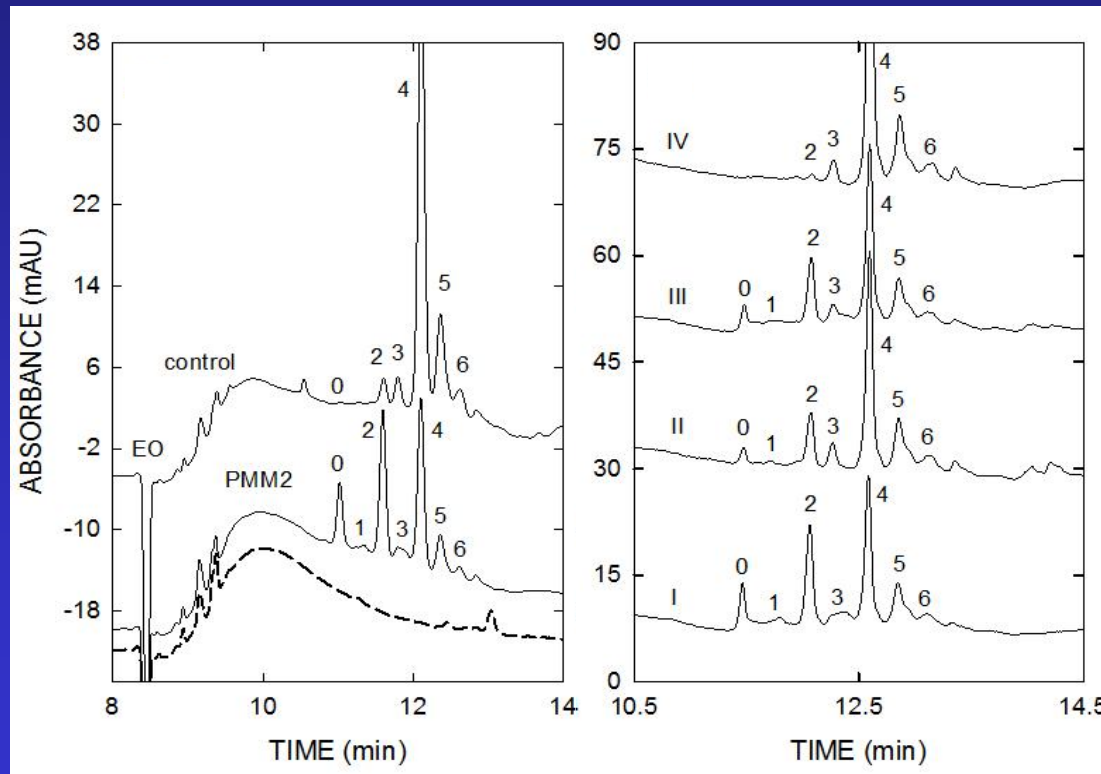


# Congenital disorders of glycosylation

- Type I: defects in the assembly and transfer of the oligosaccharide chain resulting in **lack of complete N-glycans (hypoglycosylation)**
- Type II: defects in the trimming and processing of the protein-bound glycans resulting in **immature, truncated glycans (undersialylation)**

**Type I disorder:**

**CDG-Ia (PMM2; phosphomannomutase)**



IV: Normal subject

III: Mixture

II: Alcohol abuser

I: CDG type Ia

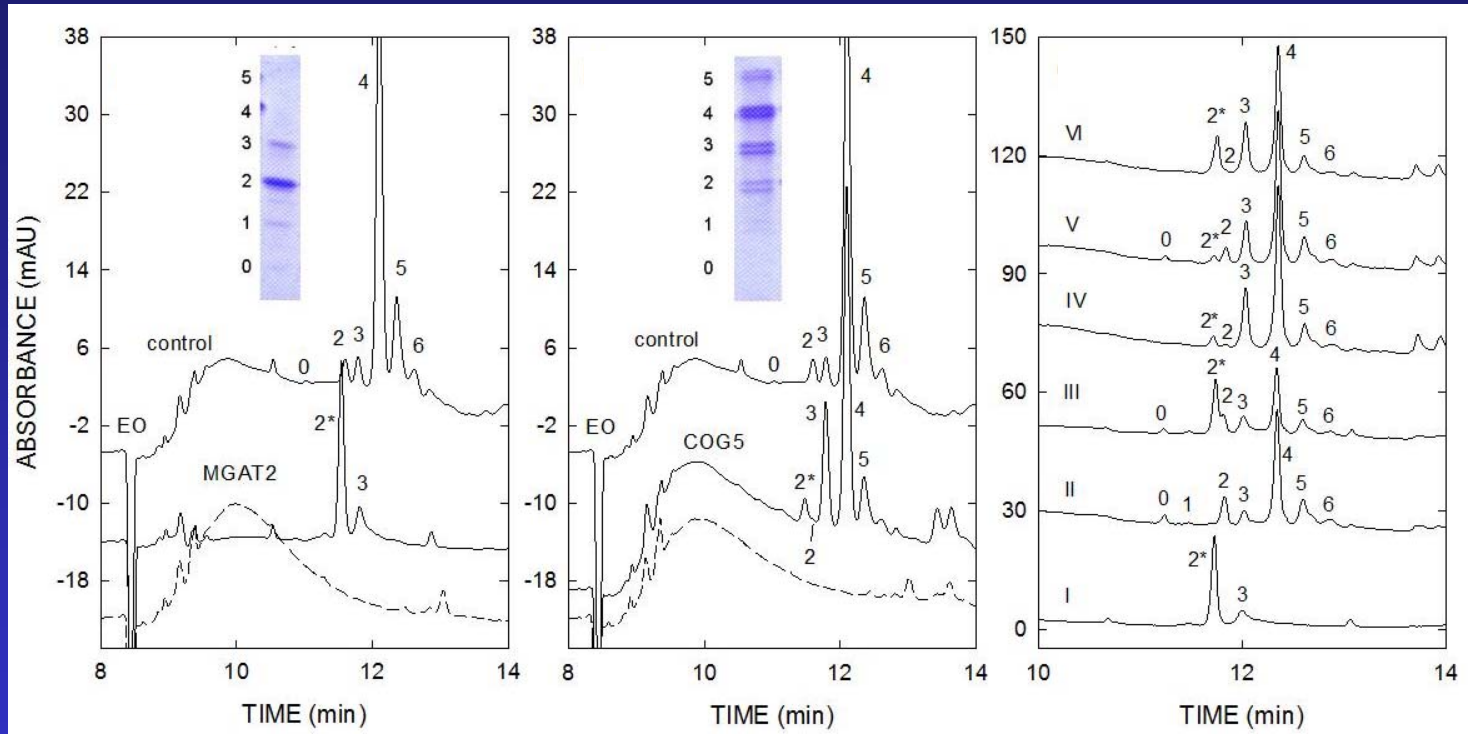
J. Sep. Sci. 41 (2018) 2808.

**Type I CDG: Same glycoforms as healthy person and alcohol abuser**

# Congenital disorders type II

CDG-IIa (MGAT2,  
β1-2 GlcNAc-transferase)

CDG-IIi (COG5,  
conserved oligomeric Golgi complex)



MGAT2 + COG5 (1:2)

COG5 + alc. abuser

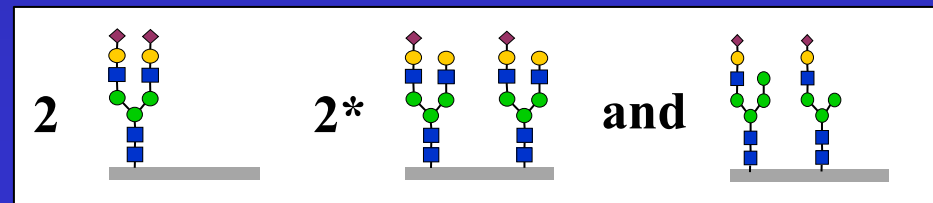
COG5

MGAT2 + alc. abuser

Alcohol abuser

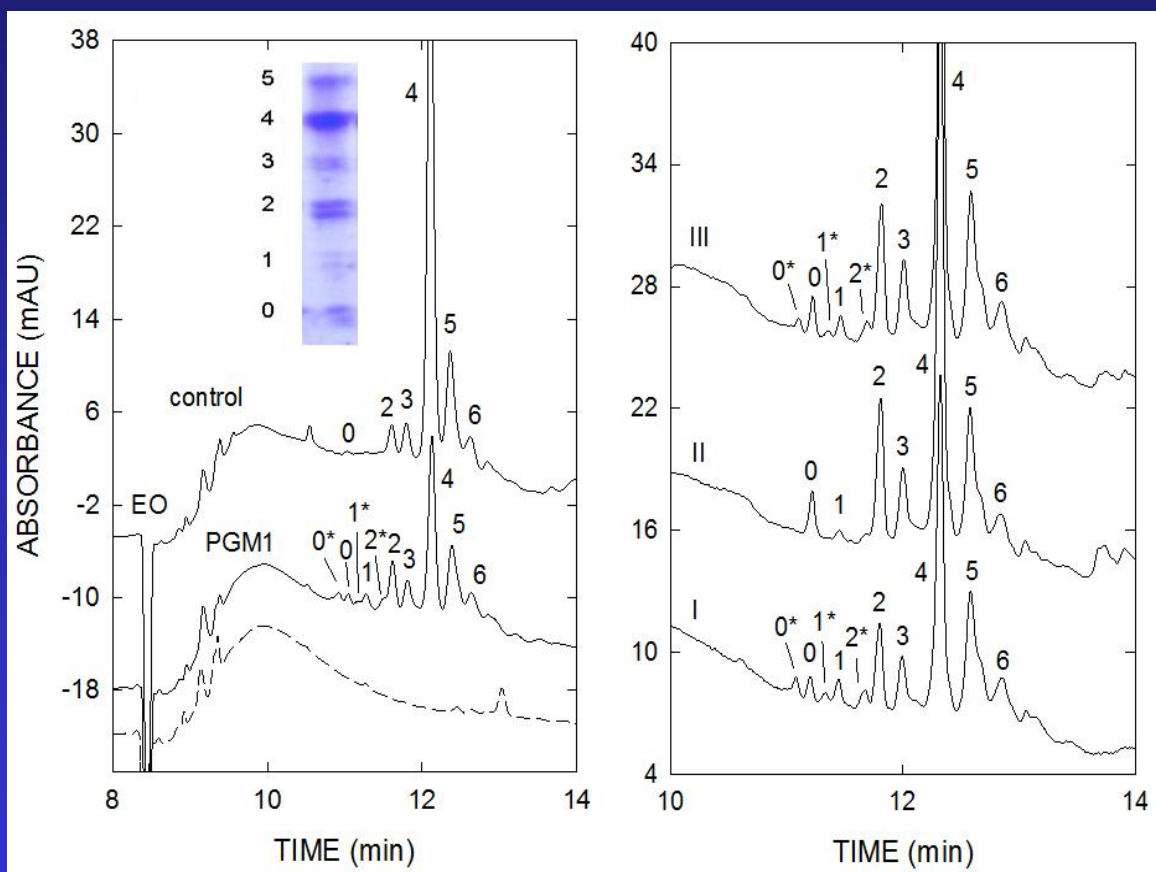
MGAT2

CDG type II patterns have  
undersialylated disialo-Tf glycoforms:



# Mixed type I/II congenital disorders

- Common, hypoglycosylated and undersialylated glycoforms
- PGM1 CDG (phosphoglucomutase)



PGM1  
+ alcohol  
abuser

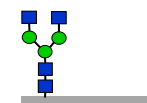
Alcohol  
abuser

PGM1

under-  
sialylated      hypoglyco-  
sialated      sialated

glycoforms:

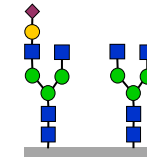
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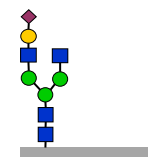
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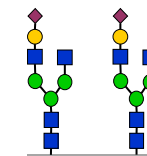
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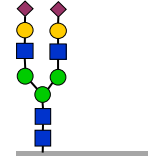
1



2\*

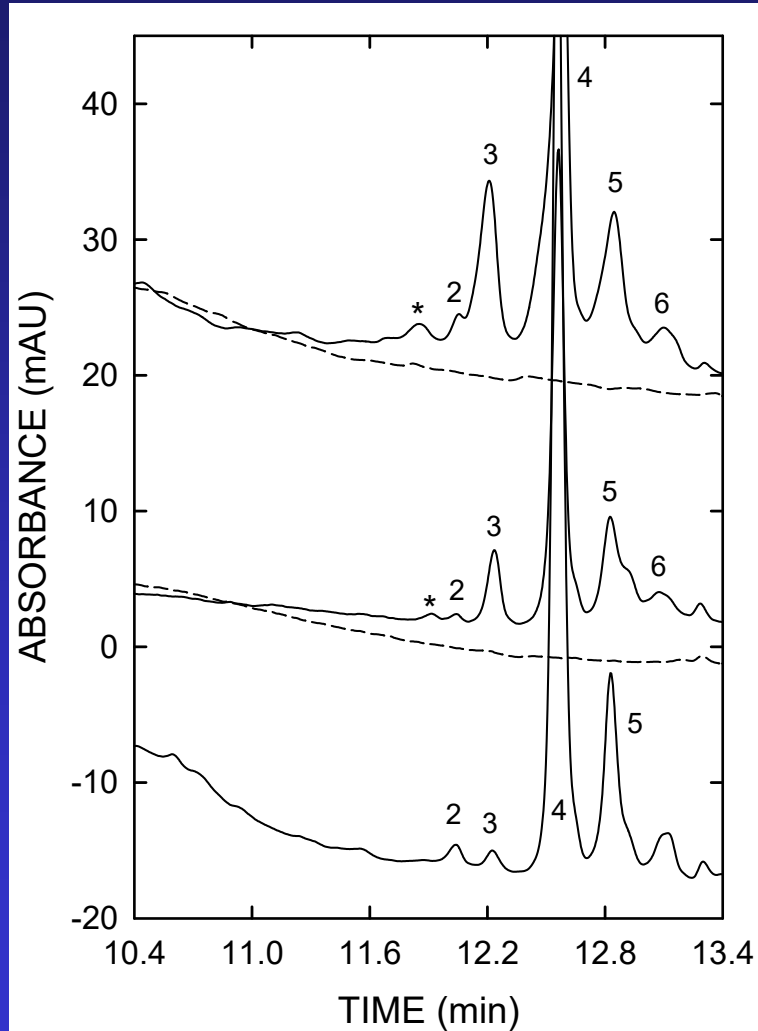


2



# Patient sera with high trisialo-Tf

Normal levels: trisialo-Tf 3-7 %; disialo-Tf < 1.70 %



**High and broad  
trisialo-Tf: 18.0 %  
Disialo-Tf: 0.61 %**

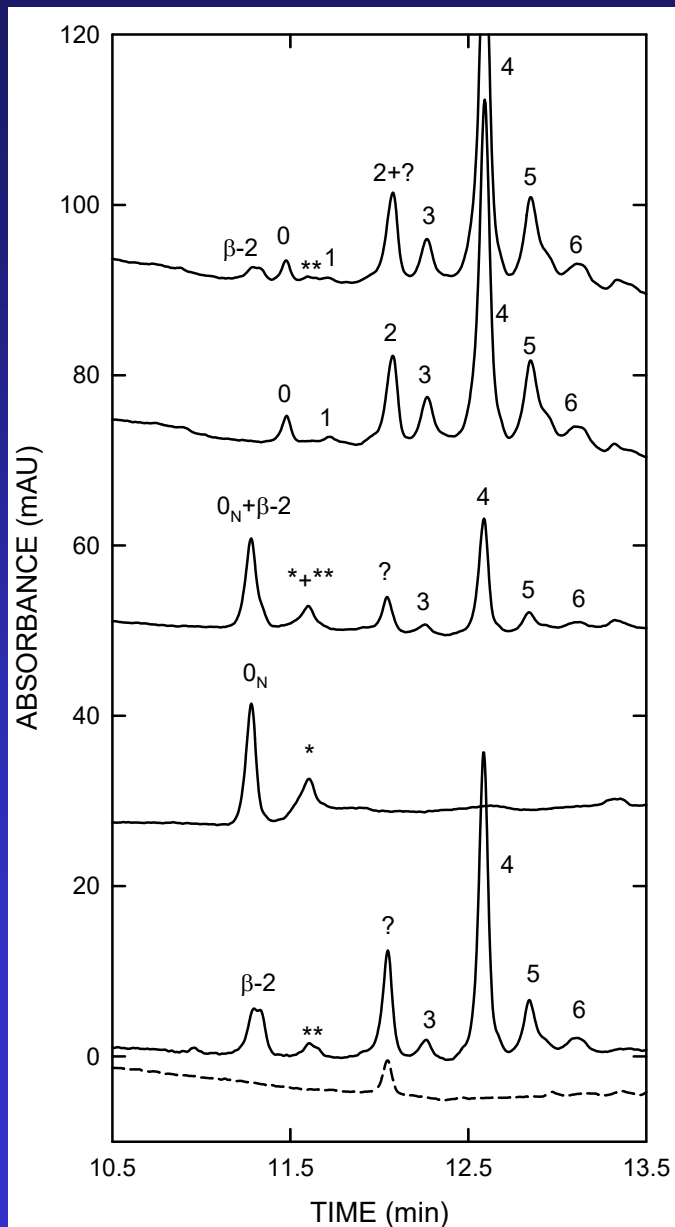
**Elevated trisialo-  
Tf: 11.2 %  
Disialo-Tf: 0.71 %**

**Low trisialo-Tf:  
1.35 %  
Disialo-Tf: 1.52 %**

**Peak marked  
with \*:**

**additional Tf peak  
in sera with  
elevated trisialo-Tf  
which comigrates  
with  
undersialylated  
disialo-Tf.**

# Transferrin in cerebrospinal fluid (CSF)



- $\beta$ -2-Tf in nasal fluid marks CSF leakage
- $\beta$ -2-Tf in CSF as test for neurodegenerative diseases

1:2 mixture of CSF  
and alcohol abuser

Alcohol abuser

1:1 mixture of CSF  
and neuraminidase  
serum

Neuraminidase  
treated  
serum

150-fold  
concentrated CSF  
(----- after Tf  
immunosubtraction)

➤  $\beta$ -2-Tf of CSF  
comprises a double  
peak

➤ First peak of  $\beta$ -2-Tf  
comigrates with  
asialo-Tf of  
neuraminidase  
treated serum

➤ CSF contains  
monosialo-Tf  
which comigrates  
with that of  
neuraminidase  
treated serum

# Clinical Diagnostics of Transferrin Glycoforms by High-resolution CE

- Analysis as  $\text{Fe}^{3+}$  saturated glycoforms at alkaline pH
- Carbohydrate-deficient transferrin (CDT), marker for chronic alcohol abuse
  - Genetic variants, congenital disorders of glycosylation (CDG)
  - Transferrin in cerebrospinal fluid (CSF)

## Pros and cons of Tf analysis by CE

### Pros:

- Sample preparation
- Small amount of sample
- Automation
- Precision (RSD < 5 %)
- High resolution / high throughput
- Abnormal Tf patterns

### Cons:

- Unselective detection (200 nm)
- Occasional interferences
- Insufficient Tf
- Insufficient resolution for selected patient sera

# Acknowledgements

**C. Lanz, J. Caslavska  
M. Kuhn, U. Marti, V. Deiss,  
J. Joneli, U. Wanzenried, J. Schiess, M. Hurni,  
F. Tagliaro, F. Bortolotti, F. de l'Escaille,  
J.-B. Falmagne, M. Tobler, P. Burda, C. Schild**

**Swiss National Science Foundation  
Liver Foundation, Bern  
Analis, Suarlée, Belgium**