Superior performances of Capillary Electrophoresis (CE) vs. HPLC to detect asialo-Transferrin, an important, although often neglected, component of human transferrin (Tf) glycoforms

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Introduction

- ✓ Carbohydrate deficient transferrin (CDT) is the acronym for two of the glycoforms of Tf with the lowest degree of glycosylation: asialo-Tf and disialo-Tf.
- ✓ Asialo-Tf is the form of CDT lacking any oligosaccharide chain (Fig. 1).
- ✓ CDT is a highly specific biomarker of chronic alcohol abuse but asialo-Tf is excluded from CDT computation due to its low concentration in serum [1].
- ✓ However, asialo-Tf could provide precious additional information to confirm an elevated CDT result, merely based on disialo-Tf analysis.

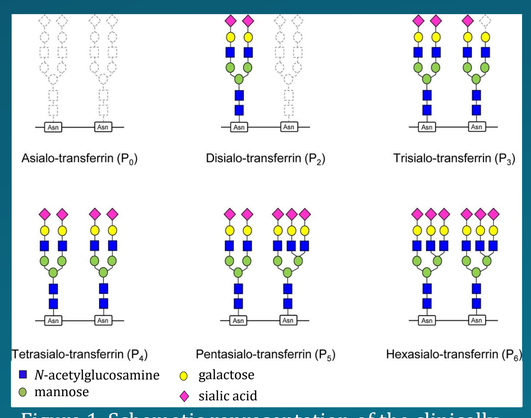


Figure 1. Schematic representation of the clinically relevant Tf isoforms. [2]

Aims of the work:

1. **comparison between CE and HPLC in their ability to detect and measure asialo-Tf in human serum;**
2. **re-evaluation of the potential of asialo-Tf as additional, potentially more specific, biomarker of chronic alcohol abuse.**

Methods

Sample preparation

- ✓ CE: serum samples saturated with a commercial ready-to-use ferric solution.
- ✓ HPLC: serum samples treated for lipoprotein precipitation with $CaCl_2$ and saturated with a commercial ready-to-use ferric solution.

CE vs. HPLC analytical conditions

CE

- **Running buffer: 120 mM H_3BO_3, pH 8.2 + 6 mM DAB (1,4-diaminobutane)**
- **Hydrodynamic injection: 0.5 psi x 25 s**
- **Separation voltage: 30 kV**
- **Capillary: 30 µm i.d. x 60 cm T.L.**
- **Detection: UV absorbance at 200 nm**
- **Cut-off: 1.8 %**

HPLC

- **Chromatography by salt gradient elution**
- **Column: anion-exchange column [65 x 4.6 mm (i.d.)]**
- **Sample injection: 100 µL**
- **Flow rate: 1 mL/min**
- **Detection: absorbance at 460 nm**
- **Cut-off: 1.9 %**

- ✓ **Data were also verified using the commercial assay Minicap CDT (Sebia, Lisses)**

Results and Discussion

- ✓ **221 serum samples were analysed**
- 73 CDT positive in CE, 40 asialo-Tf
- 71 CDT positive in HPLC, 2 asialo-Tf
- 148 CDT negative in CE, 0 asialo-Tf
- 150 CDT negative in HPLC, 0 asialo-Tf

Feature	CE	HPLC
LOQ (disialo-Tf)	0.32 %	0.65 %
Advantages	More rapid Simpler sample preparation	More selective (detection at 460 nm)
Separation efficiency $N = 5.54 \cdot (t / w_{1/2})^2$	51,000 plates/column	2,680 plates/column

Correlation studies

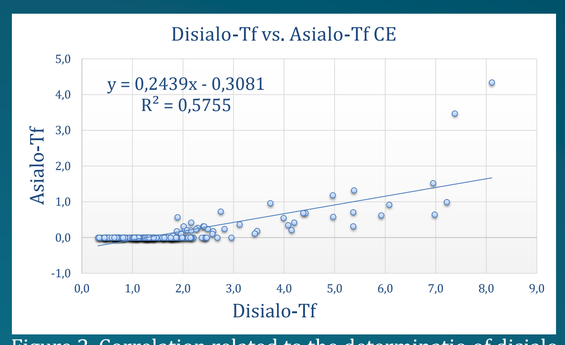


Figure 2. Correlation related to the determinatio of disialo-Tf and asialo-Tf by CE.

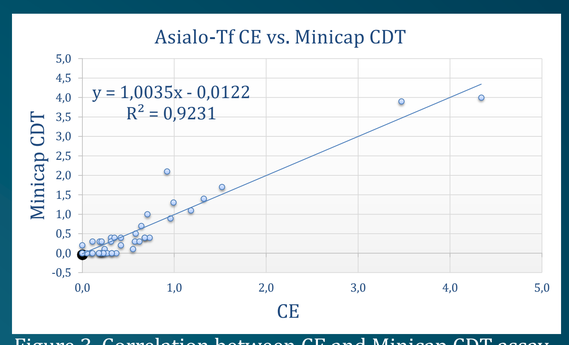


Figure 3. Correlation between CE and Minicap CDT assay (Sebia) in the determination of asialo-Tf.

Sensitivity in detecting asialo-Tf: CE vs. HPLC

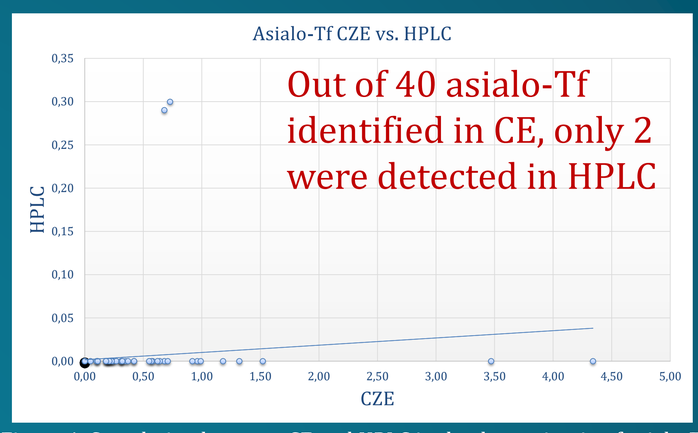
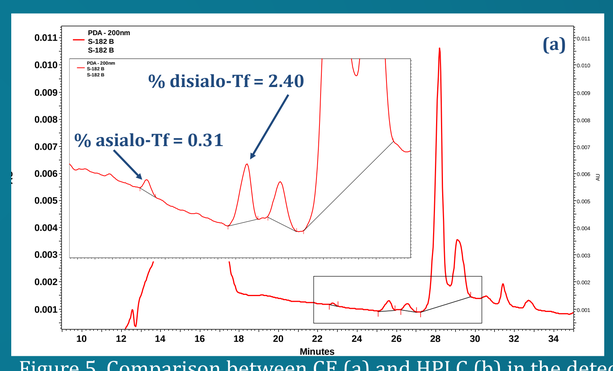


Figure 4. Correlation between CE and HPLC in the determinatio of asialo-Tf.

Relevance of asialo-Tf in CDT determination: case reports



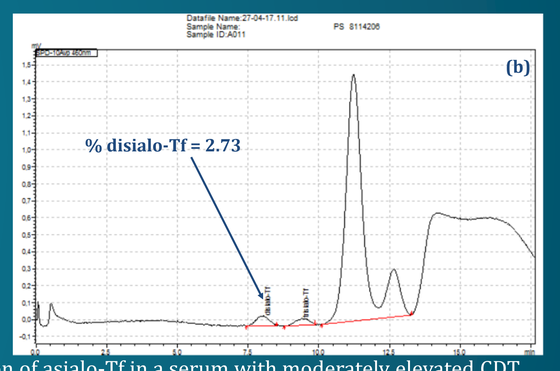


Figure 5. Comparison between CE (a) and HPLC (b) in the detection of asialo-Tf in a serum with moderately elevated CDT value.

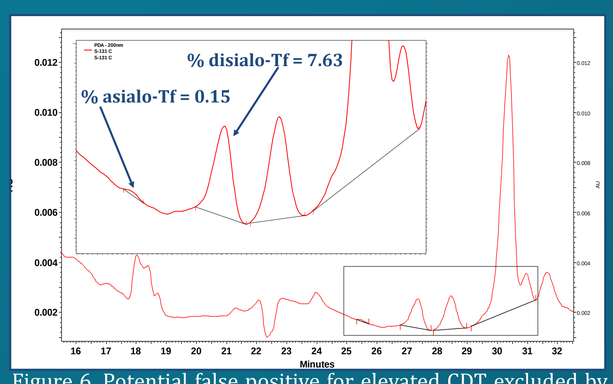


Figure 6. Potential false positive for elevated CDT excluded by the detection (in CE) of a normal asialo-Tf value (child affected by fructosemia).

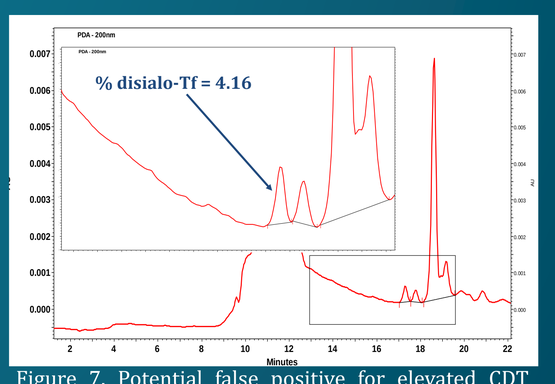


Figure 7. Potential false positive for elevated CDT excluded by the absence (in CE) of asialo-Tf (adult affected by congenital glycosylation disorders).

Conclusions

- ➢ Asialo-Tf could confirm the diagnostic value of elevated CDT results.
- ➢ Re-evaluation of asialo-Tf as additional, possibly more specific, biomarker of alcohol abuse.
- ➢ CE provides information on asialo-Tf in most of the "CDT positive" samples, whereas HPLC is inadequate for this purpose.
- ➢ Future directions:
  - improving the selectivity of CE for asialo-Tf;
  - increasing the knowledge of clinical meaning of an "elevated CDT".

References

[1] Schellenberg, F. et al. (2017), *IFCC approved HPLC reference measurement procedure for the alcohol consumption biomarker carbohydrate-deficient transferrin (CDT): Its validation and use*, Clin Chim Acta - 465:91-100
[2] Kohler, I. et al. (2014), *New insights in carbohydrate-deficient transferrin analysis with capillary electrophoresis-mass spectrometry* - Forensic Sci Int; 243:14–22

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