

Biological markers of long-term alcohol consumption in alcoholised drivers: impact of the determination of transferrin isoforms by capillary electrophoresis

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INTRODUCTION- Alcohol Abuse

Alcohol use disorders are:

- One of the most frequent addiction problem
- An important issue in clinical toxicology particularly for licence reapplication after driving under the alcohol influence

Adult excessive drinking

The Institute on Alcohol Abuse and Alcoholism (NIAAA) reported this guideline:

- Drinking more than 2 drinks a day is linked to serious health problem including increased risk for motor vehicles crashes, other injuries, high blood pressure, stroke , violence, suicide ,and certain types of cancer

Foster, JAMA, 2003;289: 989-994

- One drink is defined as 250ml can or bottle of beer, 120 ml glass of wine or a 40 ml shot of liquor straight or in a mixed drink.

How to identify patients who are drinking to excess?

- Patients may underestimate and doctors may overlook the condition (subjective measures)
- As excessive alcohol consumption can lead to several biological abnormalities, laboratory tests for excessive alcohol intake have been developed.
- This indicates the strong willingness to have more objectives measures

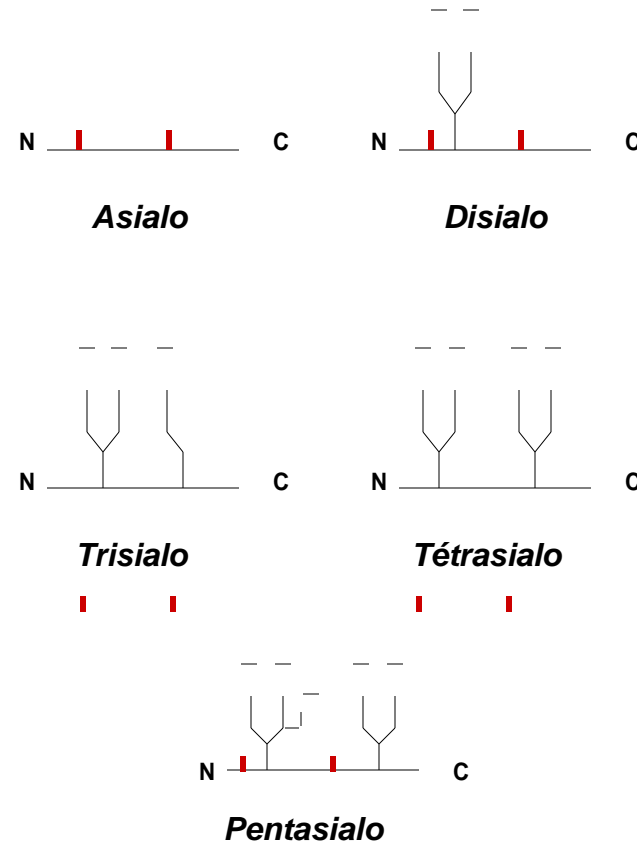
Transferrin

- Changes in the carbohydrate composition of serum glycoprotein such as transferrin (CDT) has been reported to be superior to usual markers like gamma glutamyltransferase (GGT) or mean corpuscular volume.
- Daily consumption of 50 to 80 g per day on 7 consecutive days is necessary for an increase in CDT (Stibler, 1991)
- However concerns about sensitivity and specificity have lead recently to a refinement in the current analytical methods to improve performance.

CDT

Isoforms	ref. val.
Asialo	ND (< 0.5 %)
Monosialo	ND (<0.9%)
Disialo	< 1,7 %
Trisialo	4.5 - 9.0 %
Tétrasialo	64 - 80 %
Pentasio	12 - 18 %
Hexasio	1 - 3 %
Heptasio	< 1.5 %
Octasio	ND

(#) Arndt T., Clinical Chemistry 2001; 47(1) : 13 - 27



Transferrin is an iron transporting protein with different level of sialylation. A-, mono-, disialotranferrin are collectively referred as CDT

Analytical issue

Recent studies compare two analytical methods

- Separation of individual sialic acid-deficient Tf isoform (CDT-CE)
- Traditional method: block separation of CDT desialylated Tf isoforms by anion-exchange chromatography with turbidimetric immunoassay (% CDT-TIA)

- Comparing 111 alcohol dependent patients admitted in a specialized treatment center, Legros and colleagues found that asialo-tf was present in 92% subjects with alcohol dependence and absent in all abstainers (Legros et al, 2002)
- Asialo-tf was present in 92% of 413 medical patients with average alcohol use more than 50 g per day and in 5% of 201 patients with average use <30 gr ethanol per day (Legros, 2003)

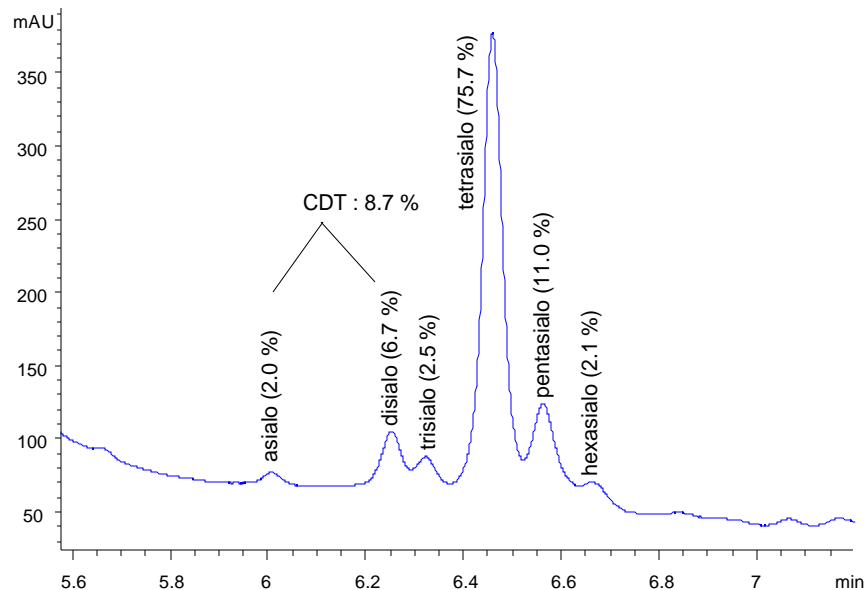
Example

Man, 41 years old)

Consumption: more than one liter of beer per day

Biological tests:

gGT :	60 U/L	(15 - 85 U/L)
AST :	39 U/L	(30 - 65 U/L)
ALT :	26 U/L	(15 - 37 U/L)
CDT (%TIA) :	2.1 %	(< 3.2%)



Tf Isoform (CE-UV)

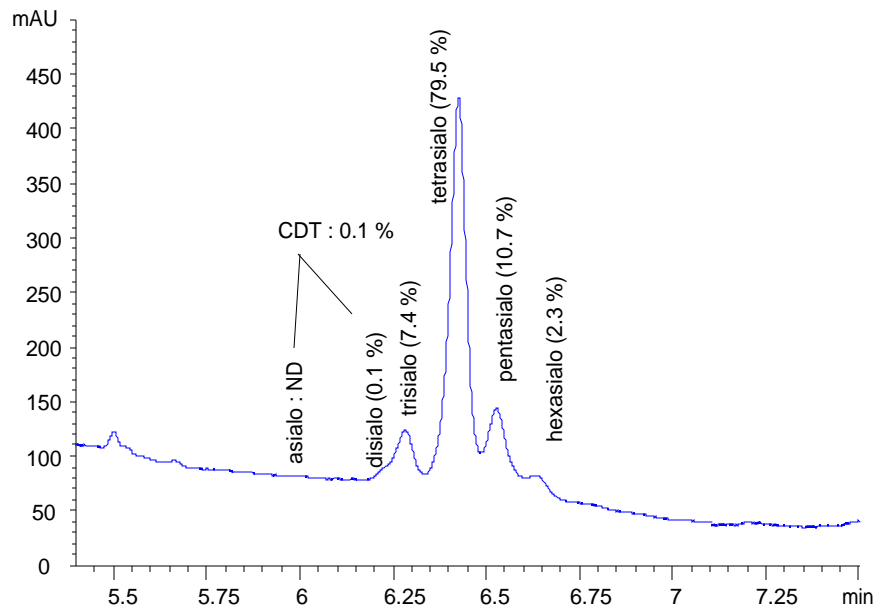
Asialo	2.0 %	
Disialo	6.7 %	CDT : 8.7%
Trisialo	2.5 %	
Tétrasialo	75.7 %	
Pentasialo	11.0 %	
Hexasialo	2.1 %	

Example 2

Case Man, 52

Follow-up of alcohol dependence: abstinence for more than 3 months

gGT : 59 U/L (15 - 85 U/L)
 (decrease from 400 to 50 U/L)
 CDT (%TIA) : 3.6 % (< 3.2%)



Tf-Isoforms (CE-UV)

Asialo	ND	
Disialo	0.1 %	CDT : 0.1%
Trisialo	7.4 %	
Tétrasialo	79.5 %	
Pentasialo	10.7 %	
Hexasialo	2.3 %	

Materials and methods

- We studied 152 consecutive Swiss drivers referred to the institute of legal medicine because of driving while under the alcohol influence. These subjects were consecutively recruited from January to June 2003.
- Alcohol intake was monitored by structured interviews, self-reported drinking habits and the Audit questionnaire as well as information provided by their family and general practitioner. Clinical diagnosis of alcohol dependence or abuse was assessed by ICD-10 criteria

CDT measures

- CE was conducted with a Analis reagent set (Ceofix CDT kit, beckam coulter) on a Hewlett-Packard CE instrument.
- CDT was also detected by anion-exchangechromatography-immunoturbidimetry using the Axis-Shielf %CDT reagent set (Biorad)

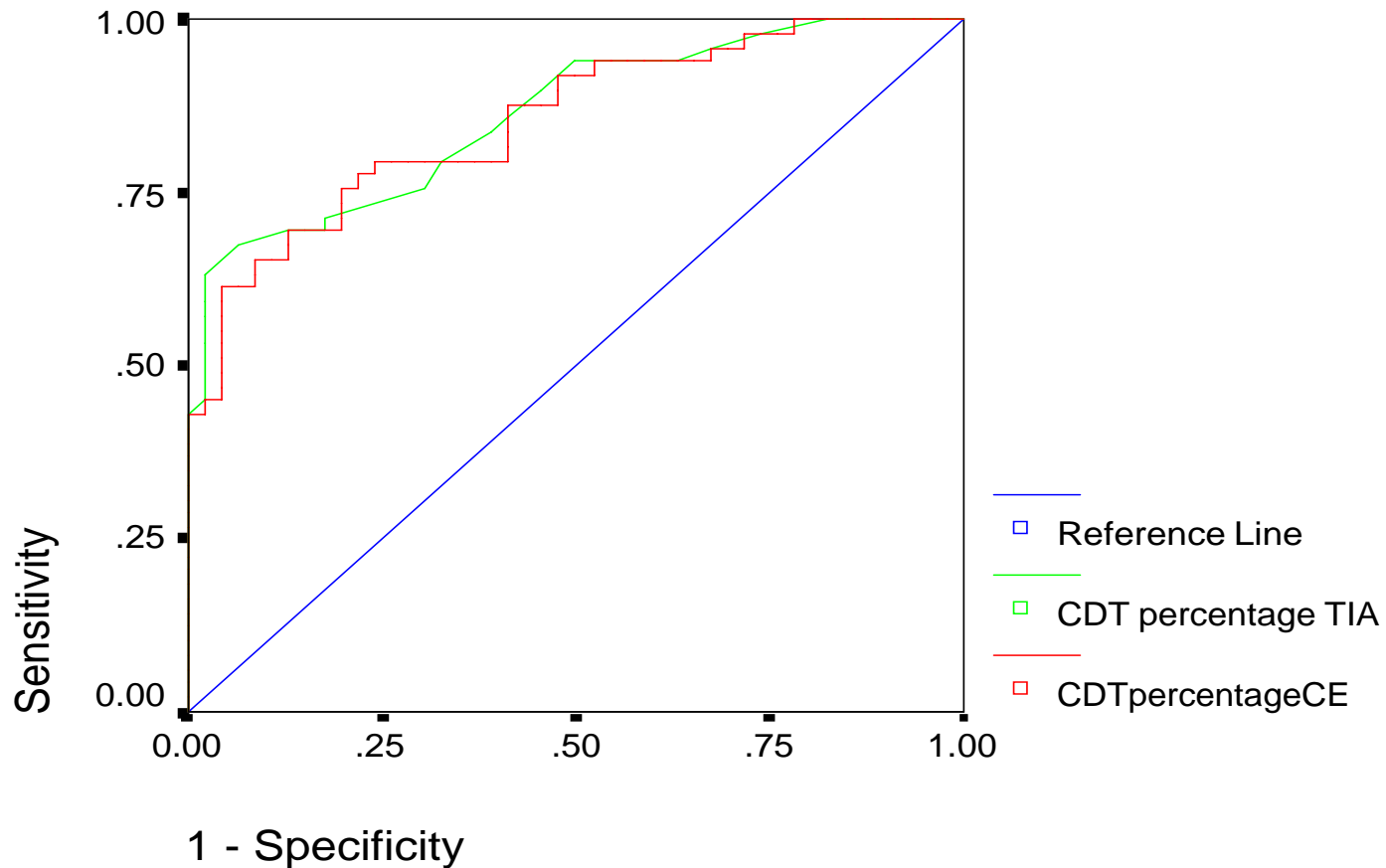
Patients

- N = 152 (134 men and 18 women),
Audit >7: 46%, Audit ≤7: 54%.
- Higher Audit scores seem associated with greater severity of alcohol misuse and scores >12 are a characteristic of alcohol dependence (Allen, 1997)
- Diagnosis:
Actual dependence (CIM-10) 39%
Past dependence 29%

Alcohol consumption

- >2 drinks per day : 32%
- 2 drinks per day: 39%
- abstinent(>1 mois): 29%

Comparison between abstinent subjects (more than one month) versus excessive drinkers (>2 drinks)

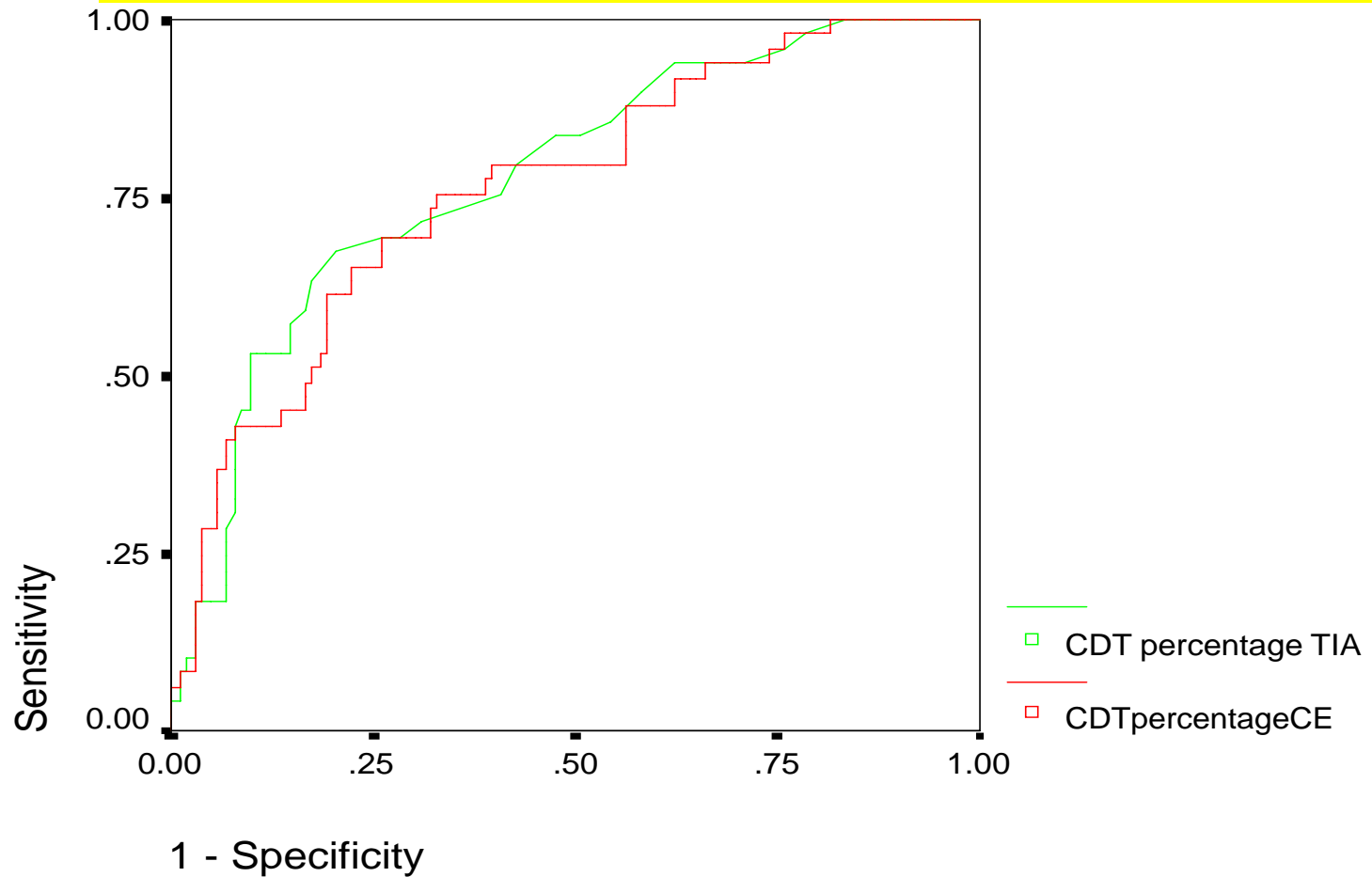


CDT-CE: AUROC 0.85 CDT-TIA: AUROC =0.86

Problem

- Highly contrasted group leads to overestimation of test performance
- Markers to assess whether alcohol is above or below some “safe limit” ?(Whitfield 2002)

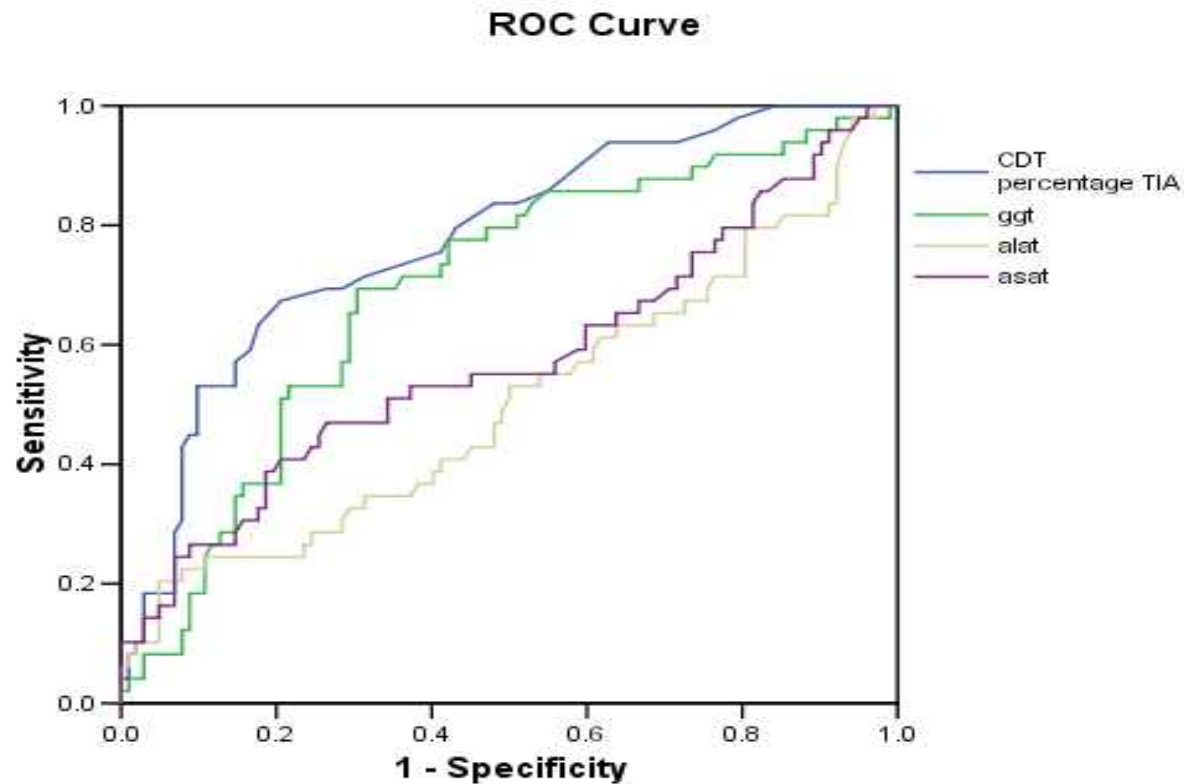
More than two drinks per day or not



CDT-CE: AUROC:0.76

CDT%TIA: AUROC 0.77

Comparison between excessive drinkers (>2 drinks) or moderate drinkers (two or less drinks)



Comparison for excessive versus moderate drinking

Marker	cutoff	sensitivity	specificity
Asialo-Tf	0%	0.49	0.89
Asialo+disialo-Tf	1,2	0.49	0.82
	1,7	0.43	0.92
%CDT	2,6%	0.86	0.45
	3,2%	0.71	0.69

Comparison between abstinent subjects (more than one month) versus excessive drinkers (>2 drinks)

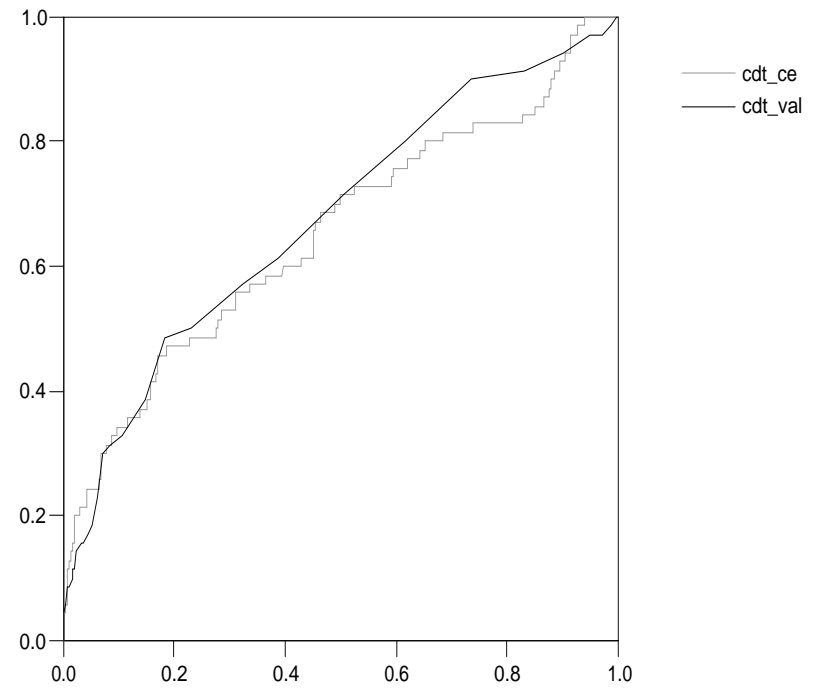
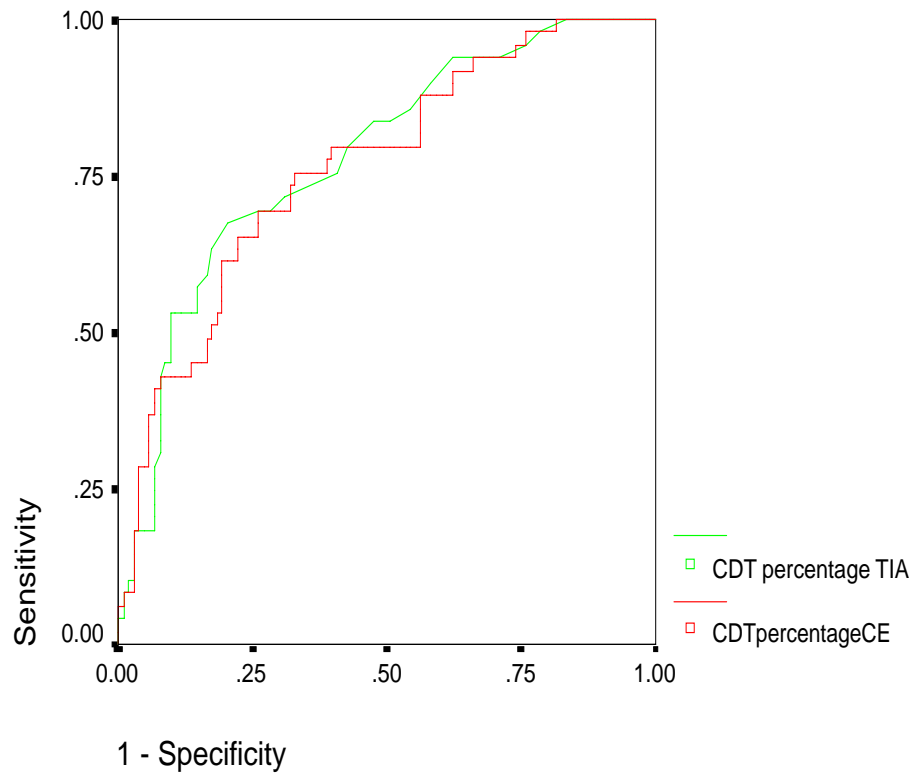
Marker	cutoff	sensitivity	specificity
Asialo-Tf	0%	0.43	0.98
Asialo+disialo-Tf	1,2	0.45	0.98
	1,7	0.42	100
%CDT	2,6%	0.85	0.59
	3,2%	0.69	0.83

- Spectrum bias for CDT CE?
- Definition: diagnostic test performance varies across patients subgroups
- How does it work for a different population?

Comparison of CDT-CE and CDT-TIA
between 581 subjects aged of 19
(mandatory one-day army recruitment)
and our 152 subjects

152 patients:AUROC:0.76

581 patients:AUROC:0.65



Conclusions I

- GGT, AST and ALT are poor markers in our study in accordance with previous studies
- Concerning the debate about trisialo (Arndt, 2002), ROC curves confirm the trisialo-TF is not associated with excessive alcohol consumption (AUROC: 0.52)

Conclusions II

- Asialo-Tf isoform seems to be a very good predictor of non abstinence. In a group of chronic abusers who stopped drinking for more than one month before testing, we found only one positive result ($>0\%$) out of 46. Legros (2002) found 0% in teetotalers. Therefore, this test is very useful in the follow-up of abstinence in the clinical field.
- We found that asialo-Tf was present in 43% of people recognizing more than two drinks per day

Conclusions III

- CDT-CE (asialo+disialo-Tf) was not as good as we thought in comparison with CDT-TIA. However the method is more convenient for the scientist, particularly in the forensic field. Contrary to CDT-TIA which give « black bocks » results, CDT-CE has a good discrimination for peaks of each isoform.
- Moreover, this technique can diagnose congenital disorder of glycosylation that we found in 3 cases out of 152, which is capital to rule out false positive results in forensic medicine .

Conclusion IV

- Finally CDT-CE and CDT-TIA have a low performance in young population. The timing, the pattern of drinking as well as the numbers of years of alcohol abuse may play an important role in the performance of CDT .