Plasma proteomic analysis of patients with Coronary Artery Disease with different levels of apolipoprotein CIII

Carmela Chiariello[a], Annalisa Castagna[a], Marcello Manfredi[b], Elia Ranzato[c], Simona Martinotti[c], Emilio Marengo[c], Daniela Cecconi[d], Oliviero Olivieri[a].

[a]Department of Medicine, Section of Internal Medicine B, University of Verona, Italy.
[b]ISALIT S.r.l., Novara, Italy.
[c]Department of Sciences and Technological Innovation, University of Piemonte Orientale, Alessandria, Italy.
[d]Proteomics and Mass Spectrometry Laboratory, Department of Biotechnology, University of Verona, Verona, Italy.

***BACKGROUND*:** Coronary artery disease (CAD) is a multifactorial condition, involving also genetic factors. Among candidate genes associated with CAD risk there are lipoprotein lipase (*LPL*) gene and *APOC3* gene. *APOC3* encodes for the apolipoprotein CIII (ApoCIII) which acts as an inhibitor of LPL. Three different ApoCIII glycoforms (with different LPL inhibitory activity) - characterized by none, one or two sialic acids - have been described. Changes in the relative abundance of these glycoforms have been observed in a variety of pathologies.
The aim of this study was to analyze and quantify the apoCIII glycoforms and to assess their relationship with LPL activity and with the levels of the LPL activator apoA-V.

***METHODS*:** ApoCIII glycoforms in four groups of patients (from “Verona Heart Study” biobank,) classified according to the total plasma concentration of ApoCIII and different TG levels, were analyzed by a classical (isoelectric focusing/western blotting) and by a shotgun MS approach. LPL activity (Fluorescent assay) and ApoA-V concentration (ELISA assay) were determined, and their correlations with lipid metabolism parameters were analyzed. We also validated by 2D-WB some proteins previously identified by 2-DE analysis.

RESULTS & DISCUSSION:

Tabel 1. Characteristics of the subjects

groups	Number of samples	ApoCIII levels	Fatty acids profile
1	7	low (7.25 ± 1.45 mg/dL)	A (poly- unsaturated > 40%)
2	5	low (7.25 ± 1.45 mg/dL)	B (poly- unsaturated < 40%)
3	7	high (17.31 ± 3.98 mg/dL)	A (poly- unsaturated > 40%)
4	7	high (17.31 ± 3.98 mg/dL)	B (poly- unsaturated < 40%)

Note: The level of apolipoprotein C-III was determined using an automated turbidimetric immunoassay; are considered low values <9.2 mg / dL and higher than ≥ 12.6 mg / dL. The polyunsaturated (PUFA) profile has been defined by gas chromatography.

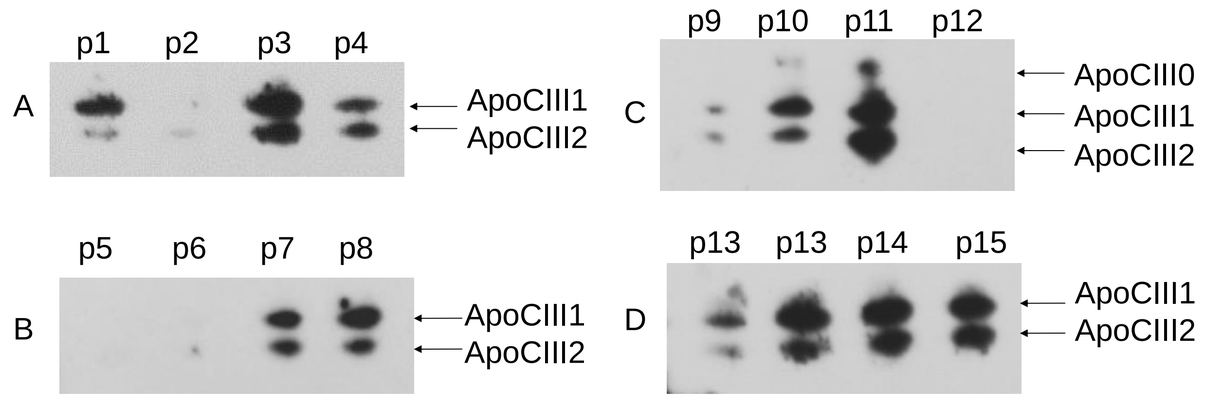

Figure1. ApoCIII detection after IEF and diffusion blotting. (A) GROUP 1 low ApoCIII ;(B) GROUP 2 low ApoCIII; (C) GROUP 3 high ApoCIII;(D) GROUP 4 high ApoCIII

The distribution of the three ApoCIII glycoforms in the selected groups of patients are related to the TG levels, particularly the mono-sialylated isoform (ApoCIII-1) prevails in patients with the highest TG levels.

Table2. ApoAV and LPL correlations with apolipoproteinCIII and triglycerides

Variable		Concentration	Triglycerides	ApoCIII
LPL (µmol/ml)*	3,10 (2,72 -3,53)	r= 0,034 p= 0,805	r= 0,188 p= 0,165	r= 0,153 p= 0,259
Apo A-V ng/ml*	496,90 (369,70-667,87)	r= -0,245 p= 0,143	-	-

r = correlation coefficient, p = p-value

Table 3.Mean values, with standard deviation, range and normal values, relative to the concentration of apolipoprotein and LPL activity

Variable		Range (min-max)	Normality value
Apo C-III (mg/dl)	11,81 ± 3,84	3,17 - 20,44	< 10.5
LPL (µmol/ml)*	3,10 (2,72 -3,53)	0,78 - 7,21	-
Apo A-V ng/ml*	496,90 (369,70 -667,87)	66 - 2395,95	-

The mean concentration of ApoAV measured in our study group (496.90 ng / ml, CI 369.70 to 667.87), falls within the normal range, approaching the upper limit. Our analysis does not show significant correlations between the LPL activity, measured as Vmax, and plasma levels of these apolipoproteins.

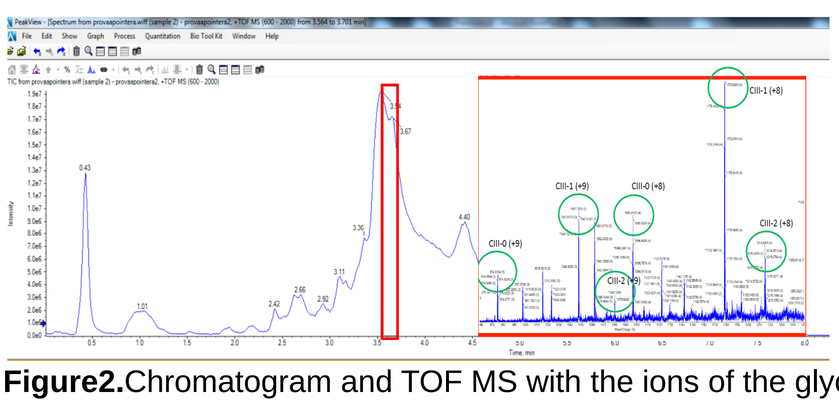

Figure2. Chromatogram and TOF MS with the ions of the glycoforms

Sum of the three most abundant ions for +8 and +9 charge state of each glycoforms for relative quantification

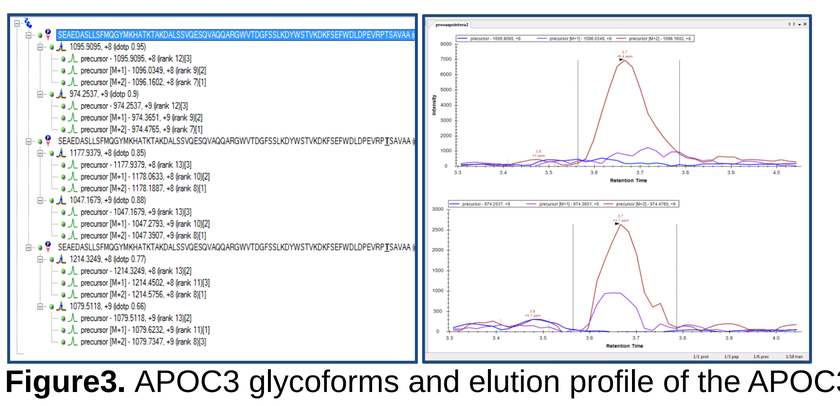

Figure3. APOC3 glycoforms and elution profile of the APOC3_0 isoform

Good agreement between IEF analysis and MS approach in terms of abundance % of isoforms. The MS analysis on a new set (n=60) of CAD patients is actually ongoing!

CONCLUSION: These data could provide important insights regarding the interaction among apoCIII glycoforms, ApoA-V level and LPL activity in predicting cardiovascular risk in predisposed individuals

REFERENCES:

Olivieri O. et al. 2010 Apolipoprotein C-III predicts cardiovascular mortality in severe coronary artery disease and is associated with an enhanced plasma thrombin generation. Journal of thrombosis and haemostasis 2010; 8(3): 463–47.
Yoshinao Wada et al.2012 Mass spectrometry of apolipoprotein C-III, a simple analytical method for mucin-type O-glycosylation and its application to an autosomal recessive cutis laxa type-2 (ARCL2) patient
Wenying Jian et al., 2013 Relative Quantitation of Glycoisoforms of Intact Apolipoprotein C3 in Human Plasma by Liquid Chromatography–High-Resolution Mass Spectrometry