Application Note

Study on NMR based Lipoprotein Subclass Analysis

Lipoproteins are supermolecular assemblies that transport water insoluble lipids in blood. Lipoproteins also carry apolipoproteins, which determine structure and function. Apolipoproteins, a monolayer of amphiphilic phospholipids and cholesterol embedded therein, constitute the surface of the lipoproteins that 'hide' the lipids from the aqueous surrounding. The inner core mainly consists of triglycerides and esterified cholesterol. Lipoproteins are commonly classified into five main groups; the chylomicrons, the Very Low-, Intermediate-, Low- and High Density Lipoproteins (VLDL, IDL, LDL and HDL respectively). In the context of assessment of cardiovascular risk, the lipoprotein class-specific concentrations of cholesterol and triglycerides are of interest beyond their total concentrations within the plasma. There is indication that additional sub-classification of lipoproteins may improve cardiovascular risk prediction. For example, the role of small LDL particles is heavily discussed. There, at constant total LDL cholesterol an increased number of small LDL particles on cost of the large LDL particles is assumed to increase risk while the opposite constellation seems less risky.

Lipoprotein Subclass Analysis based on NMR

One method of lipoprotein subclass analysis uses ultracentrifugation, however, it is extremely time-consuming, so alternative approaches are sought.

Bruker is involved in a project to establish lipoprotein subclass analysis based on ^{1}H-Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy on blood plasma or serum samples. The objective is to provide a method that:

- Determines cholesterol, phospholipids, triglycerides and apolipoproteins A1, A2, B for plasma as well as for main and selected subclasses of lipoproteins.
- Works at high throughput (about 100 samples per day) at low cost per sample, with full automation and straightforward sample preparation.
- Provides adequate accuracy at highest reproducibility.

The method of choice is based on the analysis of signals in the ^{1}H-NMR spectrum which are related to the lipoproteins. Differences in lipoprotein composition, size and density translate into respective signal line shape differences, which can be used to extract information on lipoprotein main- and subclasses (see figure 1).

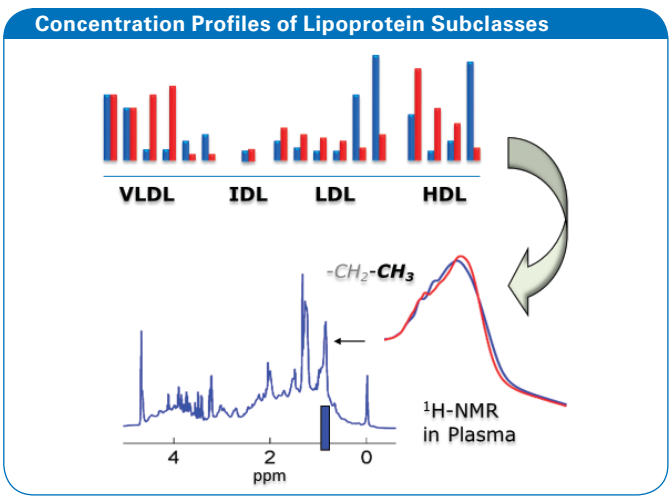

Figure 1: Illustration of the relation between the ^{1}H-NMR line shape of the CH3-group signal of the lipoproteins at 0.8ppm and differences in the concentration profiles of lipoprotein subclasses

First, a so-called regression model is developed using a training data set which consists of (1) lipoprotein analytes from total plasma and ultra-centrifugation-based main and subclasses, as well as (2) ^{1}H-NMR spectra of the same sample set. Once the regression model is established a prediction algorithm calculates the lipoprotein analytes directly from the 1H-NMR spectra of new plasma or serum samples, without further need for ultra-centrifugation (compare fig.1).

Using this ^{1}H-NMR approach, information could be extracted about plasma, the main VLDL, IDL, LDL and HDL classes, as well as six subclasses VLDL-1 … VLDL-6 (sorted according to increasing density and decreasing size, respectively) and LDL-1 … LDL-6 and four subclasses HDL-1 … HDL-4. Information consists of concentrations of lipids, i.e. cholesterol, free cholesterol, phospholipids and triglycerides and apolipoproteins Apo-A1, ApoA2 and Apo-B. In table 1 an overview of all parameters estimated within the ^{1}H-NMR lipoprotein subclassification study is presented.

It is important to note that due to the nature of the regression approach, the analytes listed in table 1 are partially strongly correlated and cannot be assumed to be independent analytes. The relation between the analytes depends on just a few latent factors that are determined by both lipid metabolism and the limits of the training data set from ultra-centrifugation used for training the regression model.

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Principle of NMR Analysis

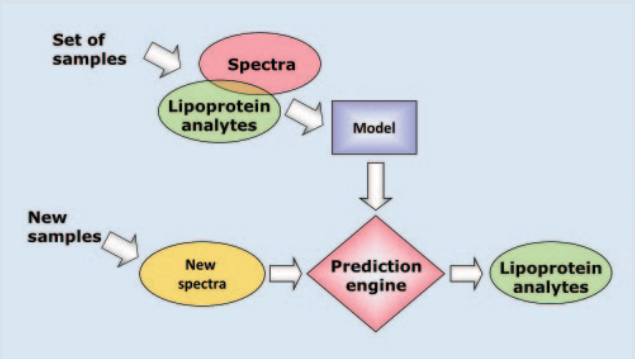

Figure 2: The NMR method principle. In the first phase training of a regression model is needed which can later be applied by a prediction engine to calculate lipoprotein parameters directly from the NMR spectra.

Table 1: Overview of parameters obtained by the ^{1}H-NMR lipoprotein subclassification method

	Triglyceride	Cholesterol	Free Cholesterol	Phospho-lipids	Apo-A1	Apo-A2	Apo-B
PLASMA	√	√	√	-	√	√	√
VLDL, VLDL-1 … -6	√	√	√	√	-	-	√
IDL	√	√	√	√	-	-	√
LDL, LDL-1 … -6	√	√	√	√	-	-	√
HDL, HDL-1 … -4	√	√	√	√	√	√	-

NMR predictions in comparison to the ultracentrifugation

Table 2: Absolute and relative prediction errors (standard deviation) in mg/ dL and (%)

	Triglyceride	Cholesterol	Free Cholesterol	Phospho-lipids	Apo-A1	Apo-A2	Apo-B
PLASMA	5.3 (5%)	6.1 (3%)	4.3 (8%)	n.d.	10.2 (6%)	3.5 (10%)	4.1 (7%)
VLDL	5.0 (9%)	1.9 (11%)	1.0 (13%)	1.4 (7%)	-	-	0.5 (10%)
IDL	3.4 (35%)	2.1 (23%)	1.2 (41%)	2.3 (32%)	-	-	0.8 (29%)
LDL	2.1 (12%)	7.1 (6%)	2.7 (8%)	3.9 (5%)	-	-	3.0 (6%)
HDL	1.8 (16%)	4.7 (8%)	1.4 (11%)	4.7 (5%)	7.4 (5%)	2.4 (7%)	-
VLDL-1	5.0 (18%)	1.1 (30%)	0.5 (25%)	1.1 (18%)	-	-	n.d.
VLDL-2	1.7 (18%)	0.6 (35%)	0.3 (42%)	0.5 (16%)	-	-	n.d.
VLDL-3	1.7 (21%)	0.7 (35%)	0.4 (43%)	0.7 (21%)	-	-	n.d.
VLDL-4	1.6 (22%)	0.9 (30%)	0.5 (34%)	0.9 (20%)	-	-	n.d.
VLDL-5	0.6 (33%)	0.6 (61%)	0.3 (108%)	0.6 (40%)	-	-	n.d.
VLDL-6	0.6 (32%)	0.1 (89%)	0.1 (10%)	0.1 (27%)	-	-	n.d.
LDL-1	1.1 (24%)	6.1 (26%)	1.7 (25%)	3.6 (24%)	-	-	2.1 (25%)
LDL-2	0.6 (24%)	4.2 (25%)	1.2 (25%)	2.4 (24%)	-	-	1.5 (23%)
LDL-3	0.6 (25%)	4.2 (21%)	1.1 (19%)	2.3 (19%)	-	-	1.4 (19%)
LDL-4	0.5 (27%)	4.6 (25%)	1.1 (22%)	2.6 (22%)	-	-	1.7 (22%)
LDL-5	0.5 (26%)	4.2 (28%)	1.1 (25%)	2.4 (25%)	-	-	1.8 (27%)
LDL-6	0.9 (33%)	4.6 (30%)	1.1 (26%)	2.8 (27%)	-	-	2.6 (30%)
HDL-1	0.9 (27%)	3.5 (21%)	0.9 (21%)	3.7 (16%)	4.6 (21%)	0.7 (25%)	-
HDL-2	0.5 (25%)	1.2 (13%)	0.3 (16%)	1.8 (11%)	2.4 (14%)	0.6 (16%)	-
HDL-3	0.5 (20%)	1.0 (8%)	0.3 (14%)	1.9 (9%)	1.9 (7%)	0.9 (13%)	-
HDL-4	0.6 (15%)	2.5 (12%)	0.6 (15%)	3.2 (9%)	4.9 (7%)	2.2 (11%)	-

Table 3: Correlation between NMR and ultracentrifugation (0 = no correlation, 1 = maximum correlation)

	Triglyceride	Cholesterol	Free Cholesterol	Phospho-lipids	Apo-A1	Apo-A2	Apo-B
PLASMA	0.99	0.98	0.87	n.d.	0.82	0.63	0.96
VLDL	0.99	0.99	0.95	0.99	-	-	0.97
IDL	0.84	0.92	0.71	0.78	-	-	0.80
LDL	0.84	0.97	0.85	0.98	-	-	0.97
HDL	0.87	0.95	0.93	0.98	0.96	0.90	-
VLDL-1	0.98	0.96	0.94	0.96	-	-	n.d.
VLDL-2	0.96	0.91	0.94	0.97	-	-	n.d.
VLDL-3	0.95	0.95	0.93	0.96	-	-	n.d.
VLDL-4	0.89	0.93	0.85	0.91	-	-	n.d.
VLDL-5	0.56	0.46	0.61	0.60	-	-	n.d.
VLDL-6	0.56	0.19	0.05	0.43	-	-	n.d.
LDL-1	0.74	0.77	0.74	0.76	-	-	0.72
LDL-2	0.67	0.83	0.77	0.82	-	-	0.81
LDL-3	0.54	0.86	0.80	0.86	-	-	0.84
LDL-4	0.70	0.80	0.83	0.83	-	-	0.80
LDL-5	0.87	0.81	0.76	0.82	-	-	0.81
LDL-6	0.72	0.71	0.65	0.68	-	-	0.70
HDL-1	0.86	0.93	0.92	0.97	0.96	0.93	-
HDL-2	0.84	0.93	0.92	0.95	0.94	0.89	-
HDL-3	0.84	0.94	0.91	0.95	0.94	0.86	-
HDL-4	0.88	0.80	0.70	0.79	0.85	0.81	-

Reproducibility of the NMR based lipoprotein subclass analysis

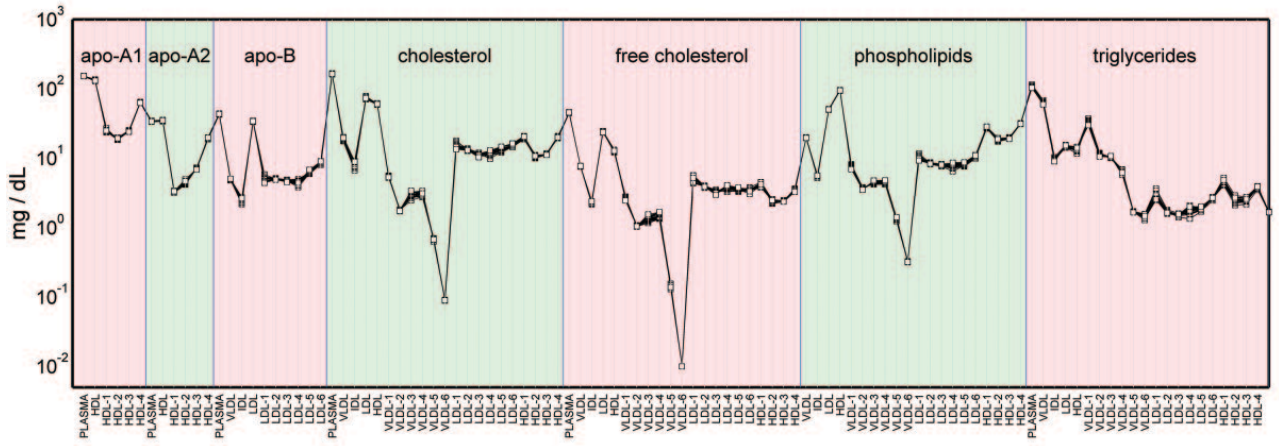

Figure 3: The maximum standard deviation observed in all 105 parameters was found for the parameter LDL-1 triglyceride (10.5%). The minimum standard deviation observed was found for the parameter plasma total cholesterol (0.4%).

Comparison of lipoprotein subclass analysis

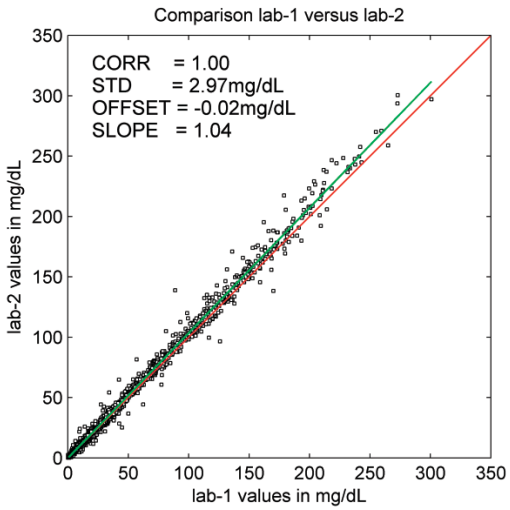

Figure 4: Cross-comparison of the NMR-based lipoprotein subclass analysis from data acquired in two different laboratories. 50 different plasma samples were split into two aliquots each and shipped to the two laboratories for NMR measurement. 105 different analytes were determined for each sample from the measurement by each lab. All 50 x 105 = 5250 values are compared in the figure. The correlation between the values of the two labs is at CORR = 0.997, the in-between lab standard error is at STD = 2.6 mg / dL on average. SLOPE and OFFSET were found at 1.04 and 0.02 mg/dL, respectively.

References

The following organizations are involved in the development of the NMR based lipoprotein analysis:

baltic analytics

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Nestlé Good Food, Good Life

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