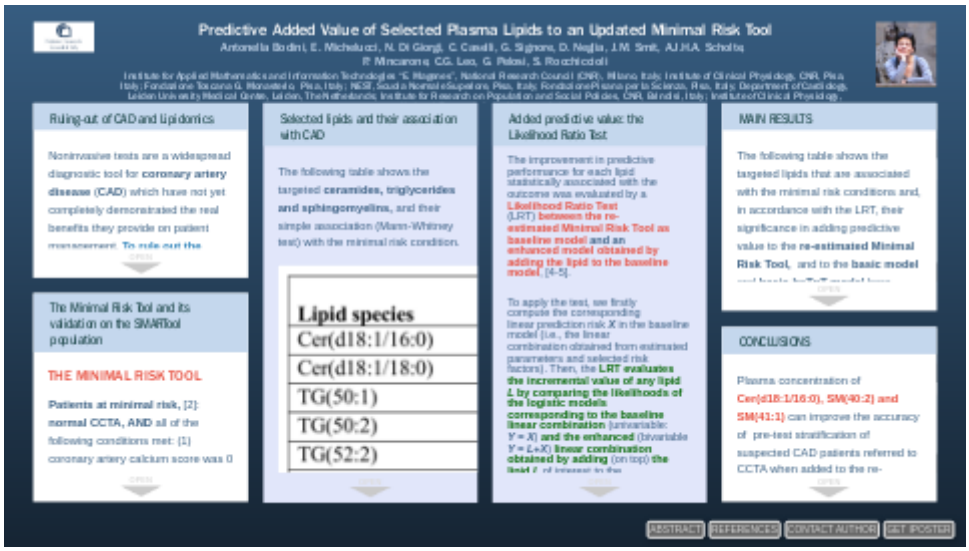
Predictive Added Value of Selected Plasma Lipids to an Updated Minimal Risk Tool



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PRESENTED AT:

RULING-OUT OF CAD AND LIPIDOMICS

Noninvasive tests are a widespread diagnostic tool for **coronary artery disease** (**CAD**) which have not yet completely demonstrated the real benefits they provide on patient management. **To rule out the disease in patients at low risk is of foremost relevance to reduce more complex investigations**. Usually, ruling out is based on a low pre-test probability of *obstructive* CAD, however, the lack of accuracy of this decision rule is well demonstrated. According to a recent systematic review [1], the **Minimal Risk Tool** (MRT) **is the only model directly identifying patients unlikely to benefit from noninvasive testing**,[2]. The MRT is based on traditional risk factors. However, atherosclerosis is the most common underlying cause of CAD, and nowadays **lipidomics is able to improve our understanding of atherosclerosis at very thorough level of detail**. Lipidomics **can quantify hundreds of different molecular lipid species and therefore can provide as many biomarkers of CAD**. **It is then essential that a biomarker will provide a statistically significant predictive added value with respect to the current knowledge, beyond simple association with disease**. In this work, we first validated the MRT on the **SMARTool population**, then the re-estimated model was used to rigourously demonstrate the improvement provided by a few targeted lipids in the prediction of subjects at minimal risk and then the relevance of lipids for ruling out CAD.

THE MINIMAL RISK TOOL AND ITS VALIDATION ON THE SMARTOOL POPULATION

THE MINIMAL RISK TOOL

Patients at minimal risk, [2]: **normal CCTA, AND** all of the following conditions met: (1) coronary artery calcium score was 0 or was not obtained; (2) no evidence of atherosclerosis; (3) overall study quality was diagnostic (i.e., sufficient data quality for interpretation); (4) left ventricular function was normal or not reported; (5) no wall motion abnormalities were present or not reported; and (6) no relevant cardiovascular incidental findings that could account for the patients' symptoms (i.e., aortic dissection or pulmonary embolism) were noted. **All patients with normal CCTA results were included in the minimal-risk cohort in the absence of any of the following adjudicated clinical events during the median 25-month follow-up period**: all-cause death, nonfatal MI, unstable angina hospitalization, or revascularization during the entire follow-up period.

Model development: logistic regression.

Included predictors: sex, age, ethnicity, hypertension, dyslipidemia, smoking, family history of CAD, diabetes, symptoms of physical or mental stress, HDL-C.

VALIDATION OF THE MRT

SMARTool population: 256 subjects from the clinical trial of **SMARTool project (http://www.smartool.eu/)** (H2020-689068-SMARTool project clinical trial - Clinicaltrial.gov Identifiers NCT04448691) with suspected coronary artery disease were recruited in 7 clinical centers from 5 European countries (Finland, Italy, Poland, Spain and Switzerland). **51 subjects (20%) were at minimal risk.**

VALIDATION PROCEDURE

We considered a procedure in 3 steps, [3]:

1. **Calibration-in-the-large**: no significant difference between the mean observed outcome and the MRT predicted probability (19.9% vs 21.1%, p=0.62)

2. **Miscalibration:** overall effect of the MRT predictors significantly reduced (p=0.003);
   effect of sex significantly smaller (p=0.001)

3. **Re-estimation:** only age, sex, smoking, diabetes and hypertension were retained by the backward selection procedure. HDL-C in particular was excluded.

SELECTED LIPIDS AND THEIR ASSOCIATION WITH CAD

The following table shows the targeted **ceramides, triglycerides and sphingomyelins,** and their simple association (Mann-Whitney test) with the minimal risk condition.

Lipid species	**Normal** (μmol/L)[1] **($n = 51$)**	**CAD** (μmol/L)[1] **($n = 205$)**	***p*-value**[2]
Cer(d18:1/16:0)	0.52 (0.31-0.75)[3,4] [51]	0.43 (0.29-0.62)[3,4] [205]	0.067
Cer(d18:1/18:0)	0.09 (0.06-0.11)[3,4] [46]	0.09 (0.06-0.14)[3] [181]	0.61
TG(50:1)	185.5 (108.5-235.5)[3] [51]	198.2 (144.4-269.9) [205]	0.12
TG(50:2)	66.1 (42.8-109.9)[3] [51]	81.7 (55.9-116.5)[3] [205]	0.09
TG(52:2)	182.6 (158.5-219.1)[4] [51]	205.8 (175.5-853.7)[3] [205]	0.008
TG(52:3)	74.1 (62.0-99.4)[3] [51]	92.8 (73.0-112.4)[3] [205]	0.004
TG(54:2)	50.8 (31.5 – 63.4)[3] [51]	56.2 (40.3-85.32)[3,4] [205]	0.027
TG(54:3)	72.1 (51.8-86.6)[3] [51]	82.2 (59.7-99.0)[4] [205]	0.016
SM(34:1)	139.7 (131.6-151.4)[3] [46]	134.3 (121.8-143.7)[5,6] [180]	0.013
SM(36:2)	24.4 (19.9-27.7)[3] [51]	20.9 (17.6-24.7)[3,4] [205]	0.002
SM(38:2)	14.0 (12.1-16.2)[3] [51]	12.4 (10.3-14.7)[3,4] [205]	0.001
SM(38:1)	49.0 (42.6-54.99)[3,4] [51]	44.6 (37.6-54.0)[3,4] [205]	0.078
SM(40:3)	7.3 (6.4-9.2)[3] [50][9]	6.6 (5.3-9.0)[3] [191]	0.046
SM(40:2)	69.4 (61.8-81.2)[3] [51]	61.2 (52.2-73.2)[3] [205]	0.002
SM(40:1)	114.9 (94.2-124.6)[3] [48]	107.1 (92.5-122.5)[5] [195]	0.19
SM(41:2)	49.9 (40.7-60.3) [51]	41.8 (33.8-52.3)[3] [205]	0.003
SM(41:1)	58.9 (49.3-72.2)[3] [51]	52.0 (42.4-68.4)[3] [205]	0.035
SM(42:4)	7.6 (6.4-10-1) [51]	6.8 (5.6-9.2)[3] [205]	0.030
SM(42:3)	99.9 (89.7-115.2) [50][8]	94.7 (77.9-109.1)[3] [204][8]	0.024
SM(42:1)	87.7 (67.0-100.0)[3] [51]	79.0 (65.3-97.5)[3] [203][9]	0.15

[1] Median and interquartile range expressed as an interval, $\mu_{0.5}$, (IQR), and [total number].
[2] The test is the two-sided Mann-Whitney test. $p \ll 0.001$ means order of magnitude less than -4.
[3] Presence of data points that are more extreme than upper inner fences (+1.5 × IQR).
[4] Presence of data points that are more extreme than upper outer fences (+3 × IQR).
[5] Presence of data points that are more extreme than lower inner fences (- 1.5 × IQR).
[6] Presence of data points that are more extreme than lower outer fences (- 3 × IQR).
[7] The missing data has been considered as dyslipidemia.
[8] The missing value has been imputed with the reported median.
[9] The missing value has been imputed with the median of Normal women, 7.79 μmol/L.

ONLY LIPIDS WITH SIGNIFICANT ASSOCIATION ARE NOW TESTED FOR ADDED PREDICTIVE VALUE

ADDED PREDICTIVE VALUE: THE LIKELIHOOD RATIO TEST

The improvement in predictive performance for each lipid statistically associated with the outcome was evaluated by a **Likelihood Ratio Test** (LRT) **between the re-estimated Minimal Risk Tool as baseline model and an enhanced model obtained by adding the lipid to the baseline model**, [4-5].

To apply the test, we firstly compute the corresponding linear prediction risk X in the baseline model (i.e., the linear combination obtained from estimated parameters and selected risk factors). Then, the **LRT evaluates the incremental value of any lipid L by comparing the likelihoods of the logistic models corresponding to the baseline linear combination** (univariable: $Y = X$) **and the enhanced** (bivariable $Y = L+X$) **linear combination obtained by adding** (on top) **the lipid L** of interest to the baseline linear prediction risk X.

This procedure differs from the usual logistic regression with variable selection in that predictors and assigned weights of the baseline model are kept fixed when the enhanced model is estimated. This allows for the assessment of the targeted lipid only.

SENSITIVITY ANALYSIS

Two other baseline models have been developed on the SMARTool cohort and then used as baseline models as well, for the purposes of sensitivity analysis.

- **BASIC MODEL**: including age, sex and typicality of chest pain.
- **BASIC-hsTnT MODEL**: including age, sex, typicality of chest pain and **high-sensitive cardiac troponin T**.

These models are inspired by literature on pre-test probability models of obstructive CAD, [1], but **they are completely new models, due to the minimal risk endpoint here considered in place of the usual obstructive CAD.**

MAIN RESULTS

The following table shows the targeted lipids that are associated with the minimal risk conditions and, in accordance with the LRT, their significance in adding predictive value to the **re-estimated Minimal Risk Tool,** and to the **basic model** and **basic-hsTnT model** here considered for the purposes of sensitivity analysis. The yellow color highlights the significant results ($p < 0.05$).

Lipid species	LRT-*p* value		
	reMRT	Basic model	Basic-hsTnT model
Cer(d18:1/16:0)	0.01	0.01	0.02
TG(52:2)	0.41	0.36	0.89
TG(52:3)	0.49	0.54	0.83
TG(54:2)	0.76	0.25	0.92
TG(54:3)	0.50	0.33	0.57
SM(34:1)	0.12	0.003	0.01
SM(36:2)	0.30	0.34	0.17
SM(38:2)	0.16	0.09	0.03
SM(40:3)	0.88	0.15	0.12
SM(40:2)	0.04	0.006	0.001
SM(41:2)	0.18	0.03	0.01
SM(41:1)	0.052	0.054	0.005
SM(42:4)	0.77	0.17	0.03
SM(42:3)	0.76	0.24	0.10

CONCLUSIONS

Plasma concentration of **Cer(d18:1/16:0), SM(40:2) and SM(41:1)** can improve the accuracy of pre-test stratification of suspected CAD patients referred to CCTA when added to the re-estimated Minimal Risk Tool model. **The correct identification of these subjects, meets the clinical need of a more efficient use of diagnostic imaging with a reduction of unnecessary radiation exposure for subjects and operators**. The sensitivity analysis as well supports the added predictive value of plasma lipidomics derived biomarkers by identifying **SM(34:1), SM(38:2), SM(41:2) and SM(42:4)** as further possible useful predictors.

These results support the future exploitation of plasma lipidomics derived biomarkers in clinical practice, not only to improve the prediction of obstructive CAD, vulnerable plaques or long term adverse cardiovascular outcomes but also **to help ruling out coronary atherosclerosis in patients referred to CCTA as first line test for suspected CAD**.

ABSTRACT

Advancements in analytical technologies and increasing use of Machine Learning make available a wide set of new possible biomarkers from lipidomics as possible predictors of cardiometabolic disease risk. However, from a cost-effectiveness point of view, decisions on which tests to order should consider the improvement over the existing knowledge brought by these new biomarkers in predicting the outcome of interest. **By state-of-the-art statistical methods we evaluated whether and which of a set of lipids, derived from targeted plasma lipidomics profile of stable CAD patients** (H2020-689068-SMARTool project clinical trial), **can significantly contribute to improve the performances of the Minimal Risk Tool** (MRT), a pre-test model developed in a secondary analysis of the PROMISE trial to identify patients with chest pain but normal coronary arteries and no clinical events during follow-up.

The association between lipids and minimal risk endpoint was checked by the Wilcoxon nonparametric test. The MRT has been validated by regression methods considering calibration in-the-large and miscalibration of the predictors effects, and then re-estimated. The updated model (reMRT) was used as a baseline model in a Likelihood Ratio Test approach to assess the added predictive value of each lipid from thirteen among ceramides, triglycerides and sphyngomielines. A sensitivity analysis was carried out by considering two alternative models developed on the cohort as baseline models.

Patient-specific plasma lipidomics is a promising source of diagnostic biomarkers, exploitable not only to assess the risk of obstructive CAD but also to rule-out subjects without coronary atherosclerosis, then meeting the clinical need of a more efficient use of diagnostic testing.

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