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Letter to Editor

Metabolomic Fingerprints in Large Population Cohorts: Impact of Preanalytical Heterogeneity

To the Editor:

Metabolomics studies have provided new insights into molecular disease mechanisms and individual response to treatment. Large scale metabolomics studies can greatly contribute to building a solid data and knowledge basis for future disease prevention strategies as well as better diagnostic and therapeutic approaches (1). A prerequisite, however, is that data from a sufficient number of biosamples are available. This goal can only be achieved by gathering many samples from different cohorts and requiring that the quality of these samples is appropriate to generate reliable and reproducible results.

The impact of the preanalytical procedures on the stability of the human metabolome has been previously described. In particular, systematic simulation of different preanalytical procedures performed on urine and blood serum and plasma have highlighted how the concentration of some key metabolites is altered via 2 main mechanisms: enzymatic activity, mainly, but not exclusively, attributable to the presence of cells; and redox reactions occurring among metabolites and between metabolites and dioxygen (2, 3). The results have led to the development of international specifications such as the 2016 European Committee for Standardization (CEN)/TS 16 945 Specifications for molecular in vitro diagnostic examinations—Specifications for preexamination processes for metabolomics in urine, venous blood serum, and plasma.

We performed a comprehensive nuclear magnetic resonance (NMR)-based metabolomics study of human blood serum and plasma (EDTA-plasma) from, respectively, 5 and 8 leading European population cohorts from the BBMRI-LPC consortium. We addressed the extent to which samples of different cohorts were suitable to be used together for metabolomics studies and whether data integration of studies performed on such samples was feasible and reliable. The analysis was performed via ^{1}H NMR, a highly reproducible tool for untargeted fingerprinting and profiling (4), where all metabolites above the 1 μM detection limit were measured simultaneously. Each participating biobank provided serum and plasma samples from 30 healthy volunteers with equal share of males and females.

Multivariate statistics revealed a clear discrimination of the samples based on the biobank of origin. The accuracy for classification (96% for plasma and 98% for serum, Fig. 1) was assessed by means

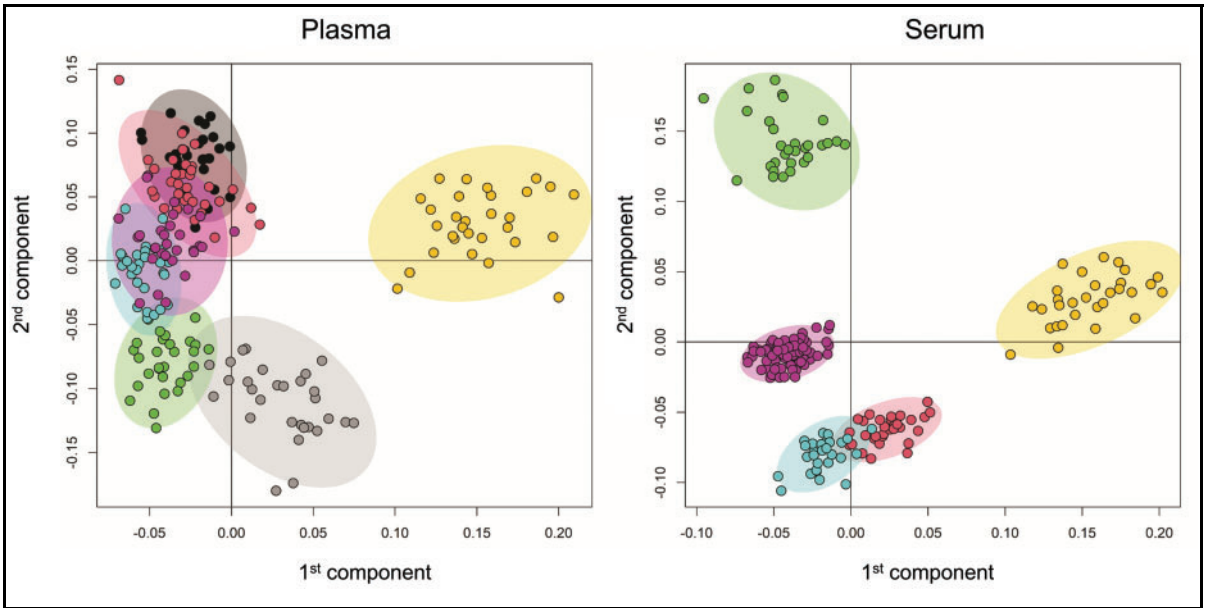

Fig. 1. Supervised discrimination obtained via orthogonal projections to latent structures-discriminant analysis used to increase the separation of the analyzed groups. In the score plots, each dot represents a different sample; samples from different biobanks are represented with different colors.

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of a Monte Carlo cross-validation scheme; each dataset was randomly divided into a training set (90% of the data) and a test set (10% of the data). The training set was used to build the model, whereas the test set was used to validate its discriminant and predictive power; this operation was repeated 500 times. The differences were mainly attributable to a small but relevant set of metabolites that showed different mean concentration values in samples from different biobanks. From an ex post analysis of the standard operating procedures adopted by each biobank, we could interpret the observed trends in terms of differences in preanalytical procedures. A major effect was attributable to the delayed separation of plasma and serum from the blood cellular components; erythrocytes, when removed from the circulation, exhibit severe disturbance of the glycolytic flow (5), which manifests itself mainly in glucose consumption and lactate accumulation. In fact, unusual concentrations of these 2 metabolites, which are key biomarkers of a series of metabolic dysfunctions, were observed for the biobanks allowing for 72 h delayed sample preparation (i.e., centrifugation). Another critical step concerned the delay between serum/plasma separation and sample freezing. This phase is not adequately regulated by the standard operating procedures of the various biobanks, which translates, for example, into variable concentrations of citrate within samples from the same biobanks as well as from the different biobanks.

The situation of Fig. 1 is often encountered in metabolomics studies based on multicenter cohorts whenever samples are not collected under strictly controlled conditions, and can be aggravated by the use of different additives (such as gel separators) that might interfere with components of the sample metabolome. The inaccurate quantification of small molecule biomarkers might severely affect the outcome of metabolomics studies, introducing artificial noise, and thus weakening the profiling performance of the analytical method. In summary, 2 main conclusions can be derived from the present contribution. First, samples from existing cohorts should be used with care and possibly after reviewing the operating procedures adopted for sample collection, processing, and storage. Second, the biobanks interested in creating novel collections to be used for metabolomics must adopt procedures that comply with the existing CEN/ISO standards.

Nonstandard Abbreviations: CEN, European Committee for Standardization; NMR, Nuclear Magnetic Resonance; BBMRI-LPC, Biobanking and Biomolecular Research Infrastructure-Large Prospective Cohorts; ISO, International Organization for Standardization

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P. Turano, K. Zatloukal, C. Luchinat, and P.M. Abuja conceived this study; V. Ghini performed the metabolomic analyses; P. Turano and V. Ghini interpreted the NMR results. O. Polasek, L. Kozera, P. Laiho, G. Anton, M. Zins, J. Klovins, and A. Metspalu provided biobank samples and anonymized patient data. P. Turano, V. Ghini, and C. Luchinat drafted the initial manuscript. K. Zatloukal, P.M. Abuja, C. Gieger, and H-E. Wichmann contributed to the manuscript writing, which all authors subsequently revised. All authors approved the final version of the work.

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