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# Prediction of classical clinical chemistry parameters using a direct infusion mass spectrometry

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ABSTRACT

Recent decades have been marked by advances in omics sciences based on high-throughput technologies, which have enabled the measurement of enormous numbers of molecules in biosamples. This study investigated the capacity of blood plasma metabolome to be used for diagnostics. Blood plasma samples ($n = 120$) were methanol-treated to remove proteins. The remaining metabolite fractions were directly analyzed by mass spectrometry. Various diagnostic substances with different molecular weights, chemical classes, and plasma abundances were directly estimated from mass spectra or predicted from mass spectra by neural networking. Predicted levels of these substances were correlated ($r = 0.51–0.78$) with their actual values. Blood plasma metabolomics may be considered as a diagnostic tool capable of substituting for several clinical laboratory tests.



## 1. Introduction

The development of global high-throughput technologies in "omics" sciences has enabled the measurement of enormous numbers of molecules in biosamples, ranging from DNA sequences (genomics) to proteins (proteomics) and other small biomolecules (metabolomics). In response, it has been expected that the scientific community would develop novel omics-based tests to detect disease more reliably [1,2]. However, the transformation of these new technologies into clinical laboratory tests has been difficult. For example, the verification of omics-based tests requires the application of complex, multistage procedures to collect data. Moreover, the interpretation of omics data differs from that of conventional test results. Thus far, agencies intended to approve omics-based tests (e.g., the Food and Drug Administration) have not approved most of them [3].

With respect to medicine, the metabolome can be considered as the most perspective of the "omes". For example, living systems can be divided into the main omic building blocks of the genome, transcriptome, proteome, and metabolome. The functioning of living systems is realized through the complex interactions of these components, with the flow of information from the static data encoded in genomic macromolecules to the dynamically changeable small molecules, the metabolites. Metabolites are substrates, intermediates, or products of biochemical reactions, building blocks of macromolecules, and sources of energy. The comprehensive set of these small molecules represents the "metabolome" and defines the molecular phenotype of living systems.

Furthermore, there is a limited probability that a given event in the genome, transcriptome, or proteome will actually appear in the phenotype. Generally, the likelihood that genome-level events will have consequences is associated with a certain degree of probability, because only a portion of genes is expressed [4]. Moreover, the transcript level is not correlated with protein expression (average correlation of 0.27 [5]), and the concentrations of proteins, excluding known biomarkers, are not directly correlated with disease. Metabolites could directly report the clinically relevant molecular phenotype. For this reason, the metabolome has a unique position among other omes in terms of its potential clinical application.

The goal of this project was to explore a way to overcome some of the aforementioned problems facing the development of metabolomics-based clinical tests. Among metabolomics technologies, direct infusion mass spectrometry (DIMS) appears to be the quite suitable approach for developing a prototype for clinical analysis. DIMS involves the direct infusion of an analyzed biological material to the ionization source of the mass spectrometer, without any preliminary separation (see, e.g., Fig. 1A) [6–10]. The major advantage of this method is its high reproducibility, which

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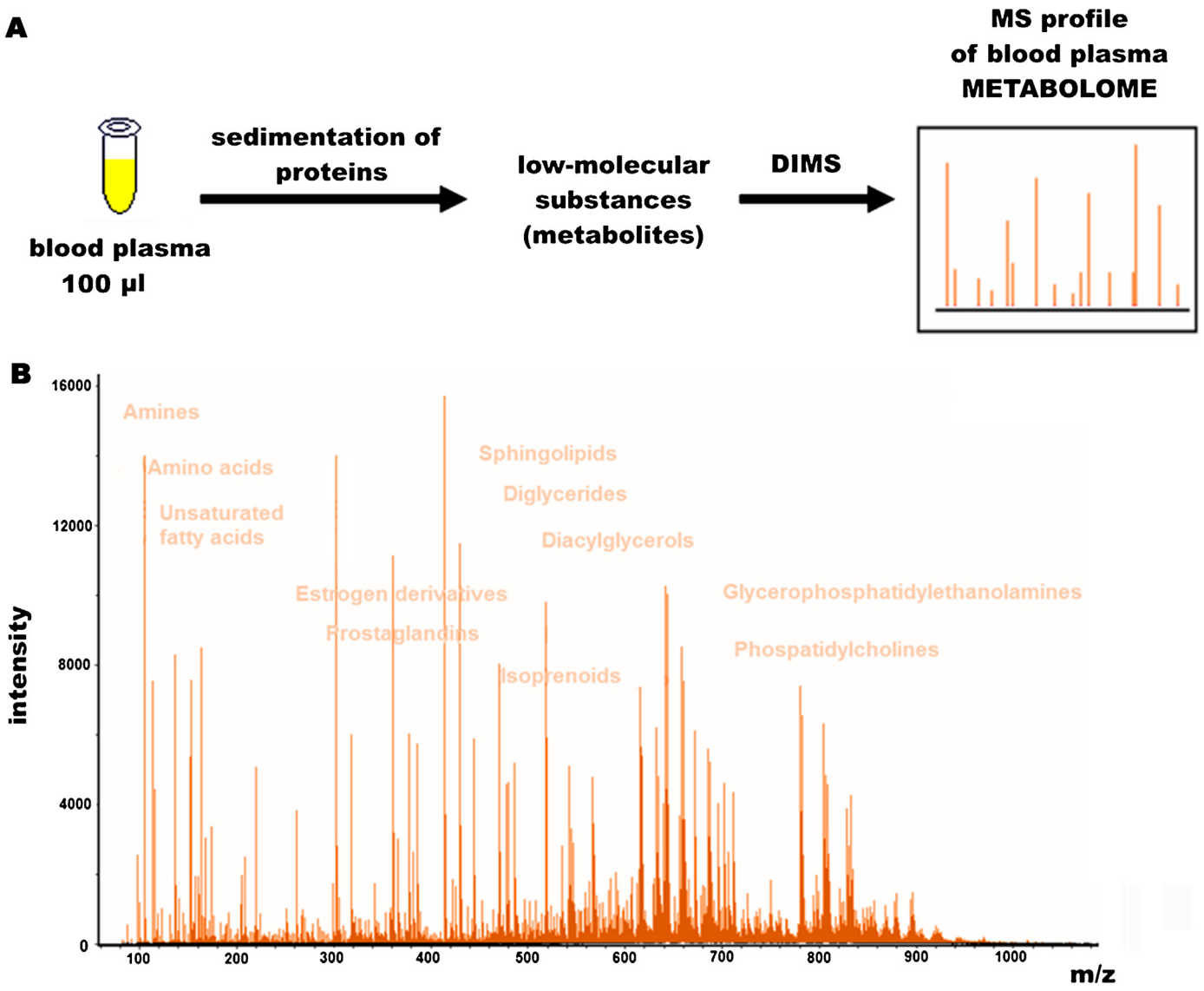


**Fig. 1.** Metabolome profiling of blood plasma by direct infusion mass spectrometry (DIMS). (A) Workflow for obtaining MS metabolome profile of blood plasma by DIMS. (B) Typical mass spectrum of human plasma metabolites. The mass spectrum was obtained after the direct injection of a deproteinized blood plasma sample into an electrospray ion source of a hybrid quadrupole time-of-flight mass spectrometer.

allows the subsequent effective data processing of metabolite mass spectra [11].

We describe a DIMS approach for implementing an omics-based test. This approach is characterized by a single-stage data-retrieval procedure with following data processing by neural network, which may serve as a prototype for clinical analyses. To simplify data interpretation, for evaluation of the metabolic profile we chose the prediction of the concentrations of diagnostic substances widely used in clinical practice.

If the expectations of omics technologies are justified, then the proposed omics-based test should serve, at least, as an effective substitute for currently utilized diagnostic tests and, at best, as a new concept for effective laboratory diagnostics.

## 2. Materials and methods

### 2.1. Patient cohort

Cohort of study participants was recruited at the Polyclinic Department of the Endocrinological Scientific Centre RAMS (Moscow, Russia). The study was approved by the relevant ethical review committee of the Centre #23-01, approval number #14 (statement # 01-02/64). Subjects ($n = 120$) who were admitted to the department and had risk factors of diabetes mellitus were selected to participate in this study. All participants provided their written informed consent to provide blood samples for research purposes. Blood plasma concentrations of diagnostic substances (glucose, uric acid, total cholesterol, insulin, C-reactive protein, triglycerides, LDL, and HDL) were measured with the Architect c4000 clinical chemistry analyzer (Abbott Diagnostics, Abbott Park, IL, USA). Table 1 reports the clinical characteristics of the subjects involved in this study.

**Table 1**
Clinical characteristics of subjects involved in the study.

| Characteristics | Value |
|---|---|
| *N* (male/female) | 32/18 |
| Age (median/range) | 59/34–82 |
| BMI (average/range) | 28.9/22.–49.8 |