Laboratory medicine in the era of precision medicine – dream or reality?

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Precision medicine or personalized medicine?

Precision medicine (**PM**) is a concept that approaches the customization of healthcare by tailoring medical decisions, treatments or products to the individual. Personalized medicine is an older mien with a similar sense to PM (they are frequently used interchangeably) which was proposed to be replaced by PM because it could be misinterpreted to imply that treatment would be developed uniquely for each individual (the concept does not mean production of unique medication for a subject). The aim in PM is to identify which approaches will be effective for which subjects, based on genetic, life style, environmental factors, etc. It means the ability to classify individuals into subgroups that differ in their predisposition to a particular condition they may develop, or in their response to a particular treatment (1). Therapeutic or preventive actions can then be focused on those who will benefit most, sparing costs and side effects for those subjects who will not.

In 2015 president Obama announced a government funded initiative that aimed to accelerate progress of PM, with a near-term focus on better cancer prevention and treatment and a longer-term aim applicable to the whole range of health and disease (2). A longitudinal cohort of one million people was planned for embarking on who would voluntarily participate with extensive characterization of biological specimens (metabolic biomarkers, cells, DNA, RNA, etc.), physiological and behavioral/ environmental data link to their electronic health records to build a comprehensive scientific base. Growth of digital health as a source of continuous and rich personal data (due to ubiquitous adoption of electronic medical records), dramatic decline in cost and increase in throughput of DNA sequencing in the last 15 years, after human genome was first sequenced, would facilitate building of a new "pool of gene data" as well as genome-phenom interrogation to generate significant knowledge for the network of scientists. However, the ambitious project is suspended due to lack of financial support nowadays.

Scope of Laboratory Medicine in the era of Precision Medicine – where are we today?

PM ecosystem ideally links patients, research and/or clinical laboratories and healthcare providers. Laboratory medicine (LM) is an indis-

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pensable discipline generating 70% of information used in healthcare processes which will play an essential role to support the implementation of PM in clinical settings.

In diagnostic testing precision there are different levels of efficacy: we have good current evidence for diagnostic accuracy and technical efficacy of medical laboratory methods (3), but we need more research for evaluation of patient outcome, therapeutic and societal efficacy. For technical efficacy we have excellent achievements; a "temple of laboratory standardization" is based on reference methods and materials, accredited reference laboratories, traceable reference intervals and decision limits, appropriately organized quality control and proficiency test programs, error measurement and targets for uncertainty (4, 5). The application of the analytical performance specification can be modulated depending on its use. Decision limit is established according to clinical performance aim (it depends on the purpose of the test: screening, diagnosis, detection of recurrence, etc.).

Even if most biomarkers in use are surrogate markers, they are "windows" into pathophysiology of particular diseases, decision support for diagnosis, prognosis and risk stratification. "Gold standard" biomarkers like creatinine clearance/eGFR and Cystatin C in kidney disease, cardiac troponins (cTn) in myocardial damage, natriuretic peptides (NT-proBNP, BNP) in hemodynamic stress and heart failure, HbA1c for diagnosis and monitorization in diabetes, are widely used in spite of their "imperfection" in some subpopulations (eGFR overdiagnoses renal disease in elderly people, cTn needs lower limit of decision for myocardial injury in women, natriuretic peptides overdiagnose heart failure in people with chronic kidney disease, inadequacy of the use of HbA1c as the only indicator of glycemic control in diabetic subjects with cardiovascular disease). Diagnostic thinking is focused nowadays on assessing test results produced by medical labora-

tories against population-based reference ranges, expected in 95-99% of presumed healthy individuals (previously called "normal" or "physiological" ranges) (6). In 2008, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC-LM) revised the recommendations for establishing reference ranges and current guidelines were published by the Clinical and Laboratory Standards Institute (CLSI C28-A3) (7). Producing their own reference ranges is not an easy task for laboratories and nowadays the need to revise the concept is emerging – the theory of reference ranges is back in fashion. This is the main reason which caused alternative methods to be found and indirect methodologies to be encouraged by IFCC Committee on Reference Intervals and Decision Limits (8). Starting 2013, the Romanian Journal of Laboratory Medicine has published several articles proposing Hoffmann technique (9, 10) for determinations of reference intervals for hemoglobin, serum total magnesium and calcium in Romanian children (11, 12), and decision limits for serum uric acid associated with cardiovascular morbidity and metabolic syndrome in this issue (13).

On the other hand, by using the reference ranges concept for interpretation of test results there is a grey area where superposition of values from healthy and ill subject exists. For instance, PSA, which is still worldwide accepted biomarker for prostate cancer screening, at a threshold of 4 ng/ml (recommended for most ELIA techniques) has excellent negative predictive value (>98%) to rule-out healthy individuals, but overdiagnoses prostate cancer (less than 80% of subjects are rule-in after biopsy) in the range of 4-10 ng/ml due to large overlapping with benign pathologies; in symptomatic men with PSA in this range and negative digital rectal examination, cut-offs for biopsy could be raised in different subpopulations in order to avoid men to undergo unnecessary biopsy (14). Due to poor specificity of total PSA, a PSA in the range of "grey zone" in

asymptomatic men aged 50 and over triggers reflex testing of free PSA (fPSA) or complex PSA (cPSA), which could reduce the number of unnecessary biopsies. It is hoped that research into emerging markers will eventually validate more accurate investigations such as PSA glycoforms, *TMPRSS2:ERG* fusion gene, *PCA3* gene, miR-NAs, circulating tumor cells, androgen receptor variants, and *PTEN* gene (15).

A second example for the evolution of clinical decision limit concept would be assessment of the patient with suspected myocardial infarction (MI) based on serum concentrations of cTn. cTn are the biomarkers which define MI, recommended to both rule-out and rule-in myocardial injury patients. Evolution history of definitions of MI has an epidemiologic era than a clinical approach using evidence of elevated serum cTn at least one value above the 99th percentile upper reference limit (URL). Due to the fact that in chronic myocardial injury cTn frequently exceed 99th percentile URL, the fourth universal definition of acute MI (AMI) recently published (16) is based on kinetics of cTn: delta troponin is the rate of changes (%) between two cTn results taken in the same patient within a 3-4 hour time period; a delta troponin > + / - 30%, with one value for cTn>14 ng/L (>9 ng/L for women) in 2-3 hours, is consistent with an AMI in the right clinical setting. Detection of a rise and/or fall of cTn concentrations is an essential early component along with symptomatology and ECG changes. On the other hand, cTn is a quantitative marker of cardiomyocyte injury, but not a screening tool.

Working with the reference ranges will remain the way for interpretation of laboratory results many years from now. However, future works should be directed to the real contribution of the reference ranges and limits to better define their position in medical decision-making. It must not be forgotten that there are primarily evidence-based clinical decision limits (like symptoms, mortality, rehospitalization), but also that frontiers between health and pathology are grey zones influenced by many biological and environmental factors. This kind of approach is absolutely necessary to ensure optimal use of laboratory tests.

Multimarker approach in precision medicine

Using advanced laboratory technology and a variety of "-omics" LM is prone to discover and evaluate analytical performance and clinical value of novel biomarkers. Multiplex technologies are versatile and flexible to enable simultaneous screening of extended panels of biomarkers with reasonable costs, reduced time and labor over traditional assays, integrating laboratory functions on a chip or on a suspension array technology.

A modest proportion of discovered biomarkers are implemented in routine medical practice, mainly due to poor specificity in some settings and poor sensitivity in others. Filling the gap between biomarker discovery and meaningful clinical use (change of the patient outcome) is slow and challenging.

In many pathologies (especially in cerebral-cardiometabolic, oncologic and infectious diseases) targeted multiple biomarker panels is considered a superior approach of precision medicine for early diagnosis, risk stratification, decision support, prognostication, monitorization and/or optimization of therapies, compared with single biomarkers.

Complete blood count (CBC) derived parameters such as Mean Corpuscular Volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) are used routinely in clinical practice in the diagnosis of different type of anemias or hemoglobinopathy. Recently other/new CBC derivative parameters like platelet-to-lymphocyte

ratio (PL/LY), neutrophil-to-lymphocyte ratio (PMN/LY) or red blood cell distribution width to lymphocyte count ratio (RDW/LY) have been proposed to be used for other purposes/pathologies and were verified also in the Romanian subpopulation as predictors for coronary chronic total occlusion in patients presenting with ST-segment elevation MI - PL/LY (17) to support proper clinical decision making in patients with high risk non-muscle invasive bladder cancer – PMN/LY (18), or aiming at maximizing selection of those with the likelihood of celiac disease who shall benefit from confirmatory serologic tests (serum IgA anti-tissue transglutaminase 2 and anti-endomysial antibodies and duodenal biopsy) - RDW/LY (19).

Patient stratification in cardio-cerebrovascular and metabolic disorders is on big demand nowadays; of the most challenging in biomarker development are prognostic markers associated with left ventricular dysfunction or remodeling (GDF-15, metalloproteinases MMP, TIMP and Gal-3 are promising markers). One of the recent challenging multiple biomarker screening (255 plasma proteins) was performed from the ARISTOTLE trial in order to identify novel prognostic markers for major bleeding in patients with atrial fibrillation on oral anticoagulation (20).

In our population we measured a panel of five peripheral biomarkers for neuronal damage, inflammation, and fibrinolysis and we found that D-Dimers and sTNFR-1 have increased discrimination ability to classify poor functional outcome at 3 months after ischemic stroke (21).

Importance of molecular/genetic diagnostic in precision medicine – *from bench to bedside*

Improving the effectiveness of health care is assured by a combination of revolutionary technologies and evolutionary practice which accelerates transformation of future medicine.

Precision medicine is no longer just a promise for the future. Every day, it is moving more and more into clinical practice. Molecular diagnostic is the fastest growing field within laboratory testing; the term "molecular" covers an array of techniques including proteins, DNA, RNA, miRNA, mRNA, the tendency being to make genomics-based diagnostic a routine. This is transforming health care by offering new tests for patient stratification, typically relying on the ability to identify individuals with specific genetic elements that define a disease or therapeutic efficiency. These results may be used not only to select proper treatment modalities, but also to identify subjects who may benefit from the preventive approach.

The ability to provide precision medicine services to patients in routine clinical practice depends on the availability of quick and accurate molecular tests; molecular testing continues to become faster and cheaper (feasible even for point-of-care variants). Nowadays, a long list of molecular diagnostic tests is used in laboratories for unexplained disorders; many of them are analytically validated, some of them are still expected to prove clinical utility and/or public health validity.

A. Identification of genetic variants as the basis of disease

Detection of single nucleotide polymorphism (SNP) 1691G>A of *F5* gene (factor V Leiden, FVL – the most common hereditary hypercoagulable disorder among Eurasians), 20210G>A of *F2(*prothrombin) gene and MTHFR (methylenetetrahydrofolate reductase) C677T and A1298C polymorphisms in patients with deep venous thrombosis; in young Romanian women homozygosity for FVL and for MTHFR A1298C polymorphisms increase the risk for venous thrombosis (22)

Mutations in any of the three genes *LDLR (*low density lipoprotein receptor), *APOB (apolipoprotein B)* and *PCSK9 (*Proprotein convertase subtilisin/kexin type 9) are known to cause autosomal dominant familial hypercholesterolemia, FH (the most prevalent genetic disorder in the general population and a major risk factor for atherosclerosis); cascade screening in descendings from patients with premature acute myocardial infarction permit prophylactic approach. However, FH is underevaluated and probably underestimated, mainly due to the relatively low proportion (~ 40%) of monogenic mutations found in subjects with clinical diagnosis of FH (according to DUTCH LIPID CLINIC NETWORK DIAGNOSTIC, MEDPED or Simon Broome criteria). For the rest of the subjects, classified according to clinical scores, the etiology of hypercholesterolemic phenotype is very probably polygenic – multiple SNPs-Scores could help. The ability to select those subjects with a positive clinical diagnosis who have a monogenic or a polygenic etiology is a model of the application of genomic data using cholesterol-lowering drugs with different efficiency and costs.

Homozygosity for ApoE2/E2 (apolipoprotein E) is correlated with one of the most severe mixt dyslipidemia (type III dyslipidemia with persistent intermediary density lipoproteins IDL) and homozygosity for ApoE4/E4 - with Alzheimer disease.

Either a homozygous DR4-DQ8 genotype or a heterozygous DR4-DQ8 and HLA DR3 risk genotype are used to stratify risk of developing type 1 diabetes.

Identification of HLA-DQ2 and -DQ8 heterodimer are performed to exclude the possibility of having celiac disease in subjects with serology and/or inconclusive biopsy, or to eliminate repeated serology testing in family members.

Mutations -13910C>T and/or -22018G>A of *MCM6* (minichromosome Maintenance Complex Component 6) gene are found in patients with lactose intolerance.

HLA-B27 alleles are checked to rule-out ankylosing spondylitis and other autoimmune arthritis types.

Molecular diagnostics are essential to diagnose and to monitor patients with some infectious diseases, especially in immunocompromised subjects (influenza virus-specific strains as H1N1, HIV, HCV, CMV, Chlamydia, etc.), or genotypes with oncogenic potential (HPV16, -18, HTLV-I, HHV8, i.e.).

B. Drug-gene testing for more precise medications - pharmacogenomics

Pharmacogenomics is the study of how genes determine a person's reactivity to particular drugs. Efforts have been made to apply pharmacogenomics testing to improve patient care in many specialties. The main purpose is identification of people who are likely to benefit from, and people who may require careful monitoring in order to establish a tailored dose of medication, identification of people who are likely to be at increased risk for serious side effects and should not receive the treatment; no longer "one size fits all"! Here are some examples:

Efficiency of treatment with HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A reductase) inhibitors in hypercholesterolemic patients, but also side/toxic effects (like myopathy, hepatotoxicity) are influenced by polymorphism in cytochrome enzyme *CYP3A4* and *CYP2C9*2* genes (according to the type of statin used); the most important interactions appear in combination with CYP3A4 activators (carbamazepine, barbiturates, rifampicin) or inhibitors (macrolides, azole antifungals, amiodarone, foods such as grapefruit juice) which require dose adjustment. The *SLCO1B1* (solute carrier organic anion transporter family member 1B1, also known as *OATP1B*) gene encodes the organic anion he-

patocellular transporter OATP1B of a variety of endogenous compounds and drugs. A *SLCO1B1* variant has been associated with an increased risk of myopathy during simvastatin therapy due to altered pharmacokinetics of this drug.

Subjects carrying *A* allele of the vitamin K epoxide reductase complex subunit 1 *VKORC1* and/or *CYP2C9*2* and/or*3* gene polymorphisms are called "poor metabolizers" of K vitamin inhibitors; variant enzymes resulting is less active than normal enzymes, decreasing the metabolism of coumarin derivatives which results in an associated bleeding risk and need for a lower dose of acenocoumarol.

20-65% of individuals in different populations have loss-of-function *CYP2C19* gene polymorphism leading to a poor metabolization of clopidogrel and determine insufficient *in vivo* activation of pro-drugs P2Y12 inhibitors and poor inhibition of platelet reactivity despite treatment. In a Romanian group with acute coronary syndromes and non-cardiogenic ischemic stroke we found 19% of clinically non-responders to this drug (in line with Western European populations); this occurred especially in individuals who were carriers of *CYP2C19*2* and/or *CYP2C19*3* allele, even if only 10% of them were homozygotes and 29% heterozygotes (23). Other patients possess increase of function polymorphism in the enzyme *CYP2C19*17* gene that makes them rapid metabolizers, but the prevalence is much lower than of poor metabolizers.

Most of the beta-blockers used for treatment of heart failure, angina, and hypertension are metabolized in the liver by CYP2D6 ("debrisoquin –oxidase") enzyme with more than 100 isoforms; normal function alleles are *CYP2D6*1, *2, *33,*35*. In about 10% of the population, no function (the most common include *CYP2D6*3,*4,*5* and *6)* or decreased function alleles (the most common are *CYP2D6*9, *10, *17, *29* and*41)* were found. In addition,

drugs like propafenone, paroxetine, quinidine, and fluoxetine inhibit CYP2D6 activity; "CYP2D6 poor metabolizers" have 5-10 times higher prevalence of side effects after metoprolol administration.

A possible molecular mechanism of multidrug resistance epilepsy involves the silent polymorphisms of drug efflux transporter of antiepileptic drugs *ABCB1* gene (ATP-binding cassette subfamily B member 1), also known as *MDR1* (multidrug resistance 1) gene. In a recently published Romanian study, *ABCB1* -129T>C showed a significant association with the sub-therapeutic plasmatic concentration of antiepileptic drugs, but the frequency of patients who did not reach therapeutic concentrations was reduced comparatively with the frequency of non-responsive patients - other mechanism of resistance being possibly involved (24).

Hypersensitivity reaction to abacavir is strongly associated with the presence of the *HLA-B*5701* allele; carriers of this allele should avoid the drug entirely to eliminate a serious adverse event.

C. Genomic tests for more precise management of cancer

Traditional tumor biomarkers like PSA, CEA, AFP, CA 15-3, CA 19-9, CA 27-29, CA-125 have defective sensibility and specificity especially because most of them are performed in peripheral blood, but they are still routinely used in cancer treatment decisions mainly due to affordable cost and to the fact that sophisticated markers are not easily available (25). Of the nucleic-acid based tumor biomarkers, the US Food and Drug Administrations (FDA) currently acknowledge less than 25 markers for diagnostic/prognostic and predictive purpose (Table 1) (26). Due to the complexity of the topic it needs a deeper approach in another issue.

Analytical validation is only the first part in the process of *in vitro* diagnostic (IVD) tumor

Table 1. Diagnostic, prognostic, and predictive nucleic-acid based tumor biomarkers FDA approved (26)

Cancer type	Predictive markers	Diagnostic markers	Prognostic markers
Breast	HER2, ER/PR* HER2 in CTC **	MG, CK19	HER2, TOP2A 58-, 70-gene RNA expression profile
Prostate		PCA3	tPSA
Gastro-intestinal stromal tumor	KIT mutation*		
Colorectal	KRAS mutations*	BMP3 and NDRG4, KRAS, β-actin	
Esophago-gastric adenocarcinoma	HER2*		
Acute Myeloid Leukemia			EGR1
Acute Promyelocytic Leukemia	PML-RARα*		
Chronic Myeloid Leukemia	BCR-ABL *		
B-cell Chronic Lymphocytic Leukemia		12p11.1-q11	TP53, ATM, 13q14.3 deletion (D13S319), trisomy 12 (D12Z3)
NSCLC	EGFR (HER1)* ALK*		
Lung adenocarcinoma	BRAF*, HER2*, EGFR(HER1)*, KRAS*, ALK*		
Skin melanoma	BRAF V600*		
Ovary		BRCA1 and 2	
Bladder		Aneuploidy of chr 3, 7, 17, loss of 9p21	

ALK, anaplastic lymphoma kinase; ATM, serine/threonine kinase; BRCA1/2 tumor suppressor genes; EGFR, epidermal growth factor receptor; ER, Estrogen receptor; ERK, extracellular-signal-regulated kinases; HER2: Epidermal growth factor receptor 2; NSCLC, non-small cell lung cancer; PML, Promyelocytic leukemia gene; PR, progesterone receptor; RARα, retinoic acid receptor-alpha; CTC-circulating tumor cells MG, mammaglobin; PSA, prostate specific antigen; TOP2A, topoisomerase II alpha; chr. chromosome

* In clinical use

** Under clinical evaluation

biomarkers development. Clinical utility validation and clinical implementation are even more challenging in making markers suitable for clinical diagnostic/prognostic and treatment decision (27).

D. The importance of liquid biopsies for precision medicine

Conventional prenatal diagnostic tests for chromosomal abnormalities currently involve an amniocentesis procedure or chorionic villus sam-

pling with risk of inducing miscarriage. On the other hand, rare fetal cells originating from the placenta can be found in a mother's bloodstream (about one in a million). Using modern micro-fluidic technology, it is now possible to isolate these cells from the mother's white blood cells or even cell-free fetal DNA/RNA which has recently revolutionized the field of aneuploidy and microdeletion screening in pregnancy by enabling non-invasive prenatal testing.

Traditional methods, such as flow cytometry, detect extremely rare subpopulations (i.e. circulating tumor cells CTC) which allows early detection of cancer and treatment effectiveness.

Circulating extracellular RNAs with origins across all cell types have the potential to serve as biomarkers for a wide range of medical conditions. Identifying the specific tissue where the RNA originates along with its molecular structure and its abundance in the circulation has enormous potential for detecting disease processes and discovering new abnormalities. Exciting new technologies and thousands of listing examples of various miRNAs that have been identified as potential biomarkers, outbreak current scientific publications. In previous issues of the Romanian Journal of Laboratory Medicine some miRNA-30c and miRNA-190 were presented with prognostic abilities in patients with colon and lung cancer, but also a series of miRNAs that can serve as biomarkers for the evaluation of the critically ill polytrauma patient respectively (28-30). However, there is still a long way to go for the analytical and clinical validation of these classes of biomarkers.

What are the challenges the precision medicine initiative is facing?

Precision Medicine initiative needs modern technologies; some of them are in the early stages of development and some others are still too expensive to become part of routine healthcare (e.g. DNA sequencing); however, guidance for analytical validation of Next Generation Sequencing (NGS) – based on IVDs intended to aid in the diagnosis of suspected germline diseases, have already been released by FDA (31).

Reimbursement from insurance companies is also likely to become an issue. Healthcare providers will need to know more about genetic and molecular tests, collaboration among different experts will be necessary for better understanding of how that information is relevant to prevent or treat disease, to be used in clinical practice.

Databases to efficiently store and analyze a large amount of information, standardization of data collection are other challenging tasks.

The PM initiative also raises legal and ethical components – patients' privacy and protection of confidentiality of health information will be critical aspects.

Digitalisation of society and of health care system will cause the end of Laboratory Medicine as we know it nowadays. Laboratory medicine will play a key role in the development of precision medicine, not only from a technical point of view, but also by adopting new responsibilities in the clinical translation of complex information generated in a rapidly changing healthcare environment.

Authors' contribution

MD (Conceptualization; Investigation; Methodology; Project administration; Resources; Supervision; Writing – original draft; Writing – review & editing)

ORO (Formal analysis; Methodology; Visualization; Writing – review & editing)

Conflict of interest

None to declare.

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