April 2016 ISSN 1650-3414 Volume 27 Number 2

eJIFCC

Communications and Publications Division (CPD) of the IFCC

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The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

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Foreword of the editor

Editor in Chief: Gábor L. Kovács, MD, PhD, DSc

Janos Kappelmayer was born in Debrecen, Hungary in 1960. In 1985 he received his medical degree from the University of Debrecen with "summa cum laude". After his residency program in clinical pathology, he obtained his board certification in 1989. He received a second board certification in laboratory hematology and immunology in 2003. He defended the PhD thesis in 1994 and D.Sc. degree in 2008. His scientific interest is in laboratory diagnostics, hematology, and thrombosis research. Since 2004, he is the director of the Institute of Laboratory Medicine at the University of Debrecen. He spent two postdoctoral years at The Sol Sherry Thrombosis Research Center, Temple University School of Medicine, Philadelphia (1990-92) and one year at the Cardiovascular Biology Program, Oklahoma Medical Research Foundation as a Greenberg Scholar (2001). Dr. Kappelmayer has several ongoing international research collaborations, e.g. with the Department of Medicine and Biochemistry at the Oklahoma Health Sciences Center and the Department of Medical Biology at the University of Tromsö, Norway. He was the tutor of five graduated PhD students in the areas of flow cytometry, leukemia diagnostics and thrombosis research. He was invited speaker at several international meetings, in the area of Thrombosis research: Venice 2004, Oslo 2006; Flow cytometry: Odense 1999, Antalya 2010; Laboratory medicine: Belgrade 2003, Berlin 2011, Istanbul 2014. He published 159 original papers, 102 of them in highly ranked international journals. His cumulative impact factor is 325, with over 2000 independent citations in the literature. In 2009-2011 he was the president, since 2015, he is the new president elect of the Hungarian Society of Laboratory Medicine and a board member of the European Society of Clinical Cell Analysis (ESCCA). Due to his excellent research, teaching, clinical service and management activities Dr. Kappelmayer enjoys the full trust of Hungarian laboratorians – including that of the editor-in-chief of this journal – to guest edit the special issue devoted to the 70 year old Hungarian Society of Laboratory Medicine.

In this issue: Celebrating the 70th Anniversary of the Hungarian Society of Laboratory Medicine

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The Hungarian Society of Laboratory Medicine – serving patients for 70 years

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EDITORIAL

The history of laboratory diagnostics is the true tale of the spectacular medical and technical developments of the past decades. Probably no other medical disciplines have undergone this steep development in the same period of time. A typical hospital laboratory in the 1930's reported annually around 200 'chemical assays' and the same amount of histological examinations, about 1000 bacteriological tests and 2-3000 Wassermann reactions. These data obviously show that before the Second World War, this simple formula could be applied: laboratory diagnostics = bacteriology + some rare chemical tests. These figures were considerably rewritten before the end of the 1940's by the widespread introduction of spectrophotometers, mostly based on discoveries by Arnold Beckman, and the subsequent application of standard and reproducible procedures for measuring chemical analytes like creatinine, bilirubin and total protein. Another boost for laboratory testing was obtained when, in the 1950's, Wallace Coulter developed his first simple cell counter that later was developed into a hematology analyzer. The recent forms of this equipment can provide 25-30 biologically / clinically useful numerical results in a very reliable manner and may process over 1000 samples per

day. Hardly anybody could foresee this type of development.

It should be mentioned that dedicated colleagues from Hungary, considerably contributed to the development of laboratory medicine. There were several scientists and practicing physicians in the early 20th century whose work established valuable clinical laboratory methods that are in general use even today. Some of them are listed below.

Sándor Korányi (1855-1944), an outstanding clinician, introduced the technique of freezing point determination for the evaluation of urine osmolality, the first exact test for renal function that later provided the basis for osmometry. He and his colleagues also introduced a titration method for the measurement of gastric HCl. Kálmán Pándy (1868-1945) was an eminent neurologist who performed cerebrospinal fluid (CSF) analysis in his laboratory by his own hands. Once a bottle of phenol accidentally turned over and got mixed with a drop of CSF sample, Pándy noticed the development of an opalescent cloud. Based on this observation, he worked on optimizing the method for measuring the protein content of CSF and, finally, he established the 1:15 phenol dilution as a suitable test. Many senior colleagues may be also familiar with a glucose assay, the so called Somogyi-Nelson method. Mihály Somogyi (1883-1971) was one of the inventors reported the original test, which was later modified by Nelson. Somogyi also made seminal discoveries by describing the 'Somogyi-effect' a paradoxical situation of insulin-induced post-hypoglycemic hyperglycemia. Probably the best known Hungarian laboratorian is Lóránd Jendrassik (1896-1970). His name is linked to the discovery of the chemical reaction for serum bilirubin measurement; the so called 'Jendrassik-Gróf' method, that served for decades as the state-of-the-art bilirubin methodology worldwide. Even today, a modified form of, the Jendrassik method utilizing diazonium salt is used for the detection of bilirubin.

In 1946, the medical and health science workers established the joint Society for Pathologists (Kórbonctan in Hungarian) and Laboratory Specialists (Laboratóriumi Diagnosztika) and thus the Society was named KOLAB. This Society also included a Division for Experimental Medicine. During the next decades, the three areas diverged and the Hungarian Society of Laboratory Medicine evolved, which today counts over 450 members. The Society holds biennial meetings at variable locations. The 70-year old Society will hold its jubilee meeting on 25-27 August, 2016 in Szeged.

The contributors of this 70-year anniversary issue are all well-known to the field of Hungarian Laboratory Medicine. Géza Bödör is a professor at the University of Colorado and Section Chief of the Chemistry and Molecular Laboratories at the VA Medical Center in Denver, Colorado, USA. Gábor L. Kovács was the director and professor of two large laboratories for 25 years, at the Markusovszky Teaching Hospital and subsequently at the Department of Laboratory Medicine at the University of Pécs, and is presently the director of the Szentágothai Research Center in Pécs. Professor Barna Vásárhelyi is the director and professor of the youngest Laboratory Medicine Department at the Semmelweis University in Budapest. Katalin Kristóf is the chief of the Diagnostic Microbiology Division at the same Department. Zsuzsanna Bereczky is the head of the Division of Medical Laboratory Sciences at the University of Debrecen. Éva Ajzner is the acting president of the Hungarian Society of Laboratory Medicine and the head of the Laboratory Department of the Jósa András Teaching Hospital in Nyíregyháza. I, János Kappelmayer, am the director of the Department of Laboratory Medicine at the University of Debrecen. This volume embraces various aspects of Laboratory Medicine and will hopefully demonstrate the multivalency of our discipline.

In this issue: Celebrating the 70th Anniversary of the Hungarian Society of Laboratory Medicine

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Biochemical markers of myocardial damage

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Key words:
coronary artery disease, myocardial infarction, biochemical markers, cardiac markers, cardiac troponins

ABSTRACT

Heart diseases, especially coronary artery diseases (CAD), are the leading causes of morbidity and mortality in developed countries. Effective therapy is available to ensure patient survival and to prevent long term sequelae after an acute ischemic event caused by CAD, but appropriate therapy requires rapid and accurate diagnosis. Research into the pathology of CAD have demonstrated the usefulness of measuring concentrations of chemicals released from the injured cardiac muscle can aid the diagnosis of diseases caused by myocardial ischemia. Since the mid-1950s successively better biochemical markers have been described in research publications and applied for the clinical diagnosis of acute ischemic myocardial injury. Aspartate aminotransferase of the 1950s was replaced by other cytosolic enzymes such as lactate dehydrogenase, creatine kinase and their isoenzymes that exhibited better cardiac specificity. With the availability of immunoassays, other muscle proteins, that had no enzymatic activity, were also added to the diagnostic arsenal but their limited tissue specificity and sensitivity lead to suboptimal diagnostic performance. After the discovery that cardiac troponins I and T have the desired specificity, they have replaced the cytosolic enzymes in the role

of diagnosing myocardial ischemia and infarction. The use of the troponins provided new knowledge that led to revision and redefinition of ischemic myocardial injury as well as the introduction of biochemicals for estimation of the probability of future ischemic myocardial events. These markers, known as cardiac risk markers, evolved from the diagnostic markers such as CK-MB or troponins, but markers of inflammation also belong to these groups of diagnostic chemicals. This review article presents a brief summary of the most significant developments in the field of biochemical markers of cardiac injury and summarizes the most recent significant recommendations regarding the use of the cardiac markers in clinical practice.

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EPIDEMIOLOGY OF CORONARY ARTERY DISEASES

Heart disease, along with malignancies, are the top two causes of death in developed countries. In the United States approximately 25 % of all deaths occur because of cardiac diseases. This is equivalent to 610,000 deaths each year from heart disease [1]. Sixty-one percent of the deaths, or 370,000 events, are due to coronary heart disease (CHD) [1] that is caused by cholesterol plaque buildup with consequent narrowing of the coronary arteries. This is known as coronary artery disease (CAD). When this plaque ruptures it activates the coagulation cascade locally and the developing thrombus restricts or completely stops blood flow to the cardiac muscle downstream from the occlusion, causing the

Non-standard abbreviations (in alphabetical order)

AACC: American Association for Clinical Chemistry,
ACC: American College of Cardiology,
ACS: acute coronary syndromes,
AHA: American Heart Association,
AMI: acute myocardial infarction,
AST: aspartate transaminase,
CAD: coronary artery disease,
CABG: coronary artery bypass graft,
CHD: coronary heart disease,
CK: creatine kinase,
CK-MB: creatine kinase MB isoenzyme,
CRP: C-reactive protein,
cTnI: cardiac troponin I,
cTns: cardiac troponins,
cTnT: cardiac troponin T,
CV: coefficient of variation,
ECG, EKG: electrocardiogram,
ESC: European Society of Cardiology,
HAMA: human anti-mouse antibody,
hs: high-sensitivity,
IFCC: International Federation of Clinical Chemistry and Laboratory Medicine,
IMA: ischemia modified albumin,
LD, LDH: lactate dehydrogenase,
LD-1: lactate dehydrogenase isoenzyme 1,
LoD: limit of detection,
MI: myocardial infarction,
MoAb: monoclonal antibody,
NACB: National Academy of Clinical Biochemistry,
NQWMI: non-Q wave MI,
NSTEMI: non-ST elevation MI,
PCI: percutaneous coronary intervention,
QWMI: Q-wave MI,
SGOT serum glutamic oxaloacetic transaminase,
STEMI: ST-elevation MI,
TF-CB: Task Force on Clinical Applications of Cardiac Bio-Markers,
TnI: troponin I,
TnT: troponin T,
UA: unstable angina,
URL: upper reference limit,
WHO: World Health Organization

clinical conditions known as angina and heart attack (myocardial infarction). According to the 2013 US national statistics, the 370,000 deaths per year are the consequence of approximately 735,000 heart attacks of which about one third (1/3) are recurrent events [1]. Based on population statistics it can be calculated that approximately 310 heart attacks per hundred-thousand people older than 18 years of age and approximately 150 deaths per hundred-thousand adults can be expected each year in developed nations. The number of people with heart disease and the death rate from CHD varies between sexes, racial groups and geographic region within a country and it increases with increasing age even within the same country, but CHD and heart attack are large public health issues and consume large amounts of health care dollars. As the severity of consequences of a heart attack increases with each minute of delay in diagnosis and treatment, early diagnosis is mandatory to minimize long term sequelae of an acute coronary event.

CORONARY HEART DISEASE AND ACUTE HEART ATTACK

For many people the first sign of having CHD is chest pain, caused by heart attack, but others may learn about their CHD from their doctor after receiving results of laboratory tests as part of their annual check up. Diet, smoking, presence of diabetes, hypertension or hyperlipidemia, among other things, can help estimate one's chance of having CHD or CAD, therefore measurement of blood pressure, cholesterol, blood sugar, body weight and body mass index as well as obtaining relevant medical history to collect information about one's diet, exercise and smoking habits can be the first steps of working up a patient for possible presence of CAD. If it appears that one is at high risk for CAD, additional tests can be performed using a variety of diagnostic procedures consisting of electrocardiogram (ECG or EKG), echocardiogram, exercise test, cardiac catheterization and coronary angiogram. During an acute chest pain event the concentration of biochemicals can be measured in one's blood to asses if the person's CAD has progressed into a heart attack or if the chest pain is due to some other disease entity than coronary artery closure. The laboratory markers used in the diagnosis and differential diagnosis of acute chest pain are collectively called cardiac markers, myocardial injury markers or biochemical markers of myocardial injury. This article will present a brief overview of the most significant cardiac markers and it will discuss the use of those markers for the diagnosis of cardiac diseases but it will not talk in details about the non-laboratory diagnostic modalities.

THE EARLY CARDIAC MARKERS

The first blood test to aid in the diagnosis of a heart attack was described in 1954 by Ladue, Wroblewski and Karmen in Science [2]. They found that the activity of the enzyme, serum glutamic oxaloacetic transaminase (SGOT), later known as aspartate transaminase (AST), increased and remained elevated for several days following a heart attack. Because AST activity could be increased in other conditions than a heart attack search was started for other, better laboratory diagnostic markers of myocardial injury. This search lead to the discovery of several other cytoplasmic enzymes that could also be used for the detection of a heart attack. Of the many candidates that were described in the literature lactate dehydrogenase (LDH or LD) and creatine kinase (CK) gained wide clinical acceptance. However, because these enzymes were also present in other tissues than the myocardium, their diagnostic specificity was limited especially in the presence of concurrent liver or skeletal muscle diseases or even strenuous exercise. Their diagnostic sensitivity also suffered from the presence of a sizeable baseline

enzyme concentration in the circulation without any cardiac pathology, sometimes masking a small infarction because of the high "background". To overcome the sensitivity and specificity limitations of LD and CK measurement, their more cardiac specific isoenzymes were introduced to clinical practice. In the mid-1960's it was discovered that the LD-1 isoenzyme of LD had better cardiac specificity than total LD activity and the LD-1 to LD-2 ratio was proposed to be a better diagnostic tool for the diagnosis of myocardial infarction (MI) than total enzyme activity measurement [3]. Unfortunately, LD-1 elevation could also be caused by minor in vivo or in vitro hemolysis [4]. LD-1 isoenzyme measurement was further limited because practical, relatively rapid analytical techniques could not be developed. Most laboratories were required to use gel electrophoresis for LD isoenzyme measurement although an immunoinhibition assay was later introduced to the medical market [5]. In spite of the many limitations of LD and LD-1 measurement it remained in clinical use until the 'troponin era' because LD-1 concentrations remained elevated for up to a week after an acute myocardial infarction (AMI) thus allowing diagnosis of a heart attack in patients who presented to the hospital after the other markers' concentrations returned to normal.

The measurement of the MB isoenzyme of creatine kinase had a better record. The CK enzyme is a dimer of two polypeptide chain, encoded by two genes and translated separately. The CK-M and CK-B monomers form the dimer of CK-MB, and because heart muscle expresses the B gene at a higher rate than other skeletal muscle the CK-MB isoenzyme exhibits better cardiac specificity than total CK. This specificity could be further enhanced by calculating the so called CK-MB ratio from separate measurements of CK-MB and total CK. CK-MB concentration measurement or CK-MB ratio reporting demonstrated sufficient specificity for clinical practice and after the CK-MB specific antibody was developed in the Ladenson lab [6] the possibility of practical, automated CK-MB measurement became reality. The availability of CK-MB immunoassays on automated chemistry analyzers and the significantly improved diagnostic specificity of CK-MB above all the other cardiac enzymes made CK-MB measurement the "gold standard" of AMI diagnosis until the troponins replaced it in this role.

DIAGNOSIS OF AMI ACCORDING TO THE WHO CRITERIA

Although no official definition of heart attack was ever developed, consensus existed for the purpose of establishing the diagnosis of MI. This consensus became to be known as the "WHO criteria" and it had been used since the early 1960's in research publications and later in clinical practice for AMI diagnosis. The "WHO criteria" were also referred to as the "WHO two out of three rule" and it was based on the presence of characteristic chest pain, elevation of cardiac enzymes such as CK, CK-MB or LD, and new abnormalities on electrocardiogram (ECG), such as a newly developed Q-wave or ST-segment elevation. The simultaneous presence of at least two of these three criteria was sufficient for the diagnosis of an acute MI. If a minimum of two criteria was not present the chest pain would be called angina. AMI, caused by the acute occlusion of a coronary artery, was believed to have started at the time the chest pain started and diagnostic cardiac enzyme elevations were timed starting from the onset of chest pain. Because old ECG abnormalities could hide a new ischemic event, biochemical diagnosis was essential to establish the diagnosis of AMI and to start therapy, therefore the ideal cardiac marker was expected to become positive, or exceed a predetermined cut-off concentration, shortly after the onset of chest pain. In addition to cardiac specificity, cardiac markers were also graded on

the fact of how rapidly they would diagnose an AMI. For example, CK-MB concentration could take 4-6 hours to become positive after the onset of chest pain.

Because the early markers didn't have absolute cardiac specificity, characteristic rise and fall pattern of cardiac enzyme concentration was required to rule out false positive diagnosis of AMI. This necessitated multiple sample collections within the first 12-24 hours of hospitalization before the definitive diagnosis could be pronounced. As the concentration of the gold standard CK-MB could become negative approximately three days after an AMI, the need for the much less tissue specific LD and LD-1 measurement remained necessary.

As experience accumulated with these cardiac markers it was recognized that patients with small muscle mass may never reach diagnostic concentrations of CK-MB, and that patients with regenerative skeletal muscle disease such as Duchenne muscular dystrophy or acute skeletal muscle trauma due to recent surgery or moving vehicle accident, could have false negative CK-MB results due to the very high concentrations of the MM isoform of CK released from skeletal muscle [7], [8]. It was also recognized that the elevated CK-MB concentration in patients with regenerating muscle diseases was due to enhanced CK-B gene expression by skeletal muscle [9].

These limitations, while they did not stop the reign of CK-MB, accelerated the search for the "ideal" cardiac marker that would demonstrate absolute cardiac specificity, would not be present in blood without cardiac muscle damage and its concentration would rise rapidly after a heart attack, and it would be inexpensive to test for and the analysis could be performed on automated instruments. The search for this ideal cardiac marker turned from cytosolic enzymes to structural proteins of the myocytes. The first practical result of this search was the discovery of myoglobin as a cardiac marker. Although myoglobin did not provide any cardiac specificity it satisfied another requirement of an ideal cardiac marker [10]: It could become positive within one to two hours of the onset of chest pain so it became the best early biochemical marker of myocardial damage and later it gained a significant role as a "rule out" marker for non-cardiac chest pain patients.

THE EMERGENCE OF CARDIAC TROPONINS AS CARDIAC MARKERS

Several structural muscle proteins were evaluated as possible biochemical markers of myocardial damage, including myosin heavy and light chains, fatty acid binding proteins, natriuretic peptides, tropomyosin and members of the troponin complex. The search was directed by the idea of finding one protein that had different genes and amino acid sequences in skeletal and cardiac muscle, and developing monoclonal antibodies against the cardiac isoform to be used in an immunoassay for quantitative measurement of the marker's concentration in blood following a cardiac event. The target protein was supposed to be a small molecule as it was known by this time that smaller proteins exited damaged cells faster than larger ones after an ischemic event. Many markers showed promise initially but large-scale clinical trials that exposed serious flaws for all of them prevented their acceptance for clinical use except for cardiac troponin I and T.

The troponin complex is part of the regulatory apparatus of the myocyte. It consists of three components: the calcium binding troponin C, the inhibitory troponin I and the tropomyosin binding troponin T. The troponin complex is involved in the Ca-mediated muscle contraction that is exerted via conformational changes of the individual components [11]. Only troponin

I (TnI) and troponin T (TnT) have cardiac specific genes in addition to other genes that encode for slow-twitch, fast-twitch and smooth muscle isoforms. The laboratory of Hugo Katus in Germany focused on developing an immunoassay for the measurement of cardiac troponin T (cTnT) [12] and our research group in Jack Ladenson's laboratory at Washington University concentrated on cardiac troponin I (cTnI) [13]. Phase one clinical studies demonstrated that both cTnI and cTnT will be elevated after a heart attack in patients [13], [14]. The early studies had also proven that the cardiac troponin assays show similar early sensitivities for myocardial damage to that of CK-MB but the cardiac troponins would remain elevated (positive) for longer than CK-MB thus eliminating the need for LD measurement in late-arriving patients.

Both markers demonstrated excellent cardiac specificity allowing for biochemical detection of myocardial infarction in clinical situations where CK-MB could not perform due to concurrent skeletal muscle damage such as after accidental chest contusion or in postoperative situations [15], [16]. While evidence accumulated that cardiac troponins may become the new gold standard for the diagnosis of myocardial damage, doubt also emerged regarding tissue specificity of cTnT [17]. Patients with regenerative skeletal muscle diseases but without recognizable cardiac damage would have elevated cTnT concentration without similar increase in cTnI values. Using immunohistochemistry staining of newborn, and healthy and diseased adult human skeletal and cardiac muscle tissue, we have demonstrated that the difference between cTnI and cTnT expression is real and it is related to re-expression of the cardiac TnT gene by skeletal muscle during muscle regeneration [18, 19]. Our findings were confirmed by RNA expression studies in 1999 [20]. Because the human cTnT mRNA undergoes differential splicing that produces different forms of cTnT protein molecules in heart or skeletal muscle, the cTnT immunoassay was later redesigned and eliminated the unwanted cross reactivity with cTnT of skeletal muscle origin [21]. The current generations of cTnT assays are no longer hindered by compromised cardiac specificity.

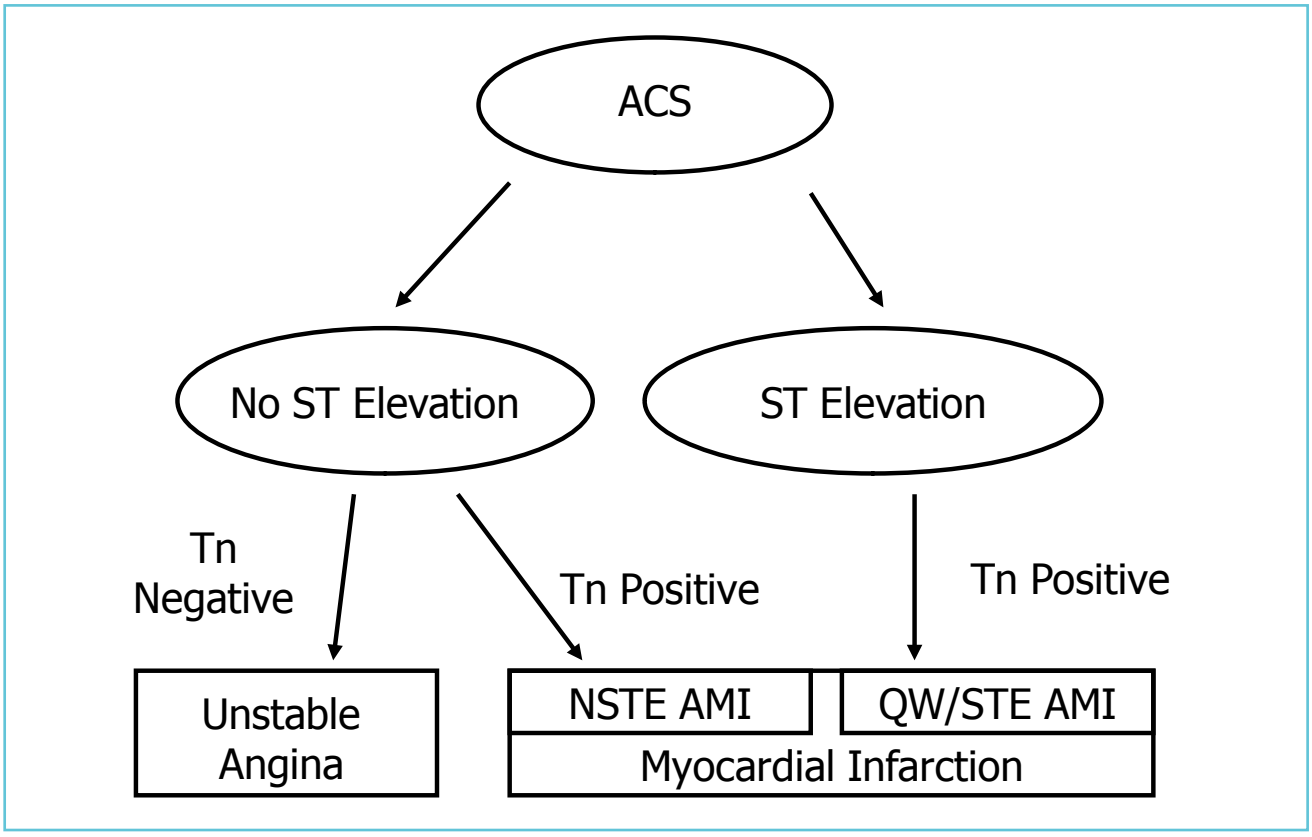
As cTnI and cTnT immunoassays started appearing in clinical practice additional questions were raised regarding the performance of the troponin methods. Both cTnI and cTnT were detected in patients who did not satisfy the definition of MI according to the WHO criteria. These non-MI chest pain patients had elevated cardiac troponins and were diagnosed with unstable angina (UA) or non-ischemic cardiac diseases, but they had increased odds of later cardiac events [22], defined as a later AMI, re-infarction or death, depending on the clinical trial. The increased odds for later events were demonstrated by short term (14-30 days) and long term (up to a year) follow up clinical trials. The patients who had elevated admission cTns without an AMI could have 10-fold increase in the odds of developing later cardiac complications versus those who didn't have measurable troponin elevation. It was also discovered that non-MI patients with elevated cTn would benefit from invasive therapy but those without cTnI elevation would do better with conservative therapy [23], [24], [25, 26]. False positive results due to skeletal troponin interference could be ruled out because troponins were proven to have 100% tissue specificity, therefore new models were needed to explain these findings.

THE NEW ERA OF DIAGNOSIS OF ISCHEMIC CARDIAC INJURY – THE ACUTE CORONARY SYNDROMES

The recognition of increased risk of later myocardial damage after increased troponin blood concentrations in non-MI patients were incorporated into two different practice recommendations

that were published within a year of each other. The National Academy of Clinical Biochemistry (NACB), International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), American Heart Association (AHA) and American College of Cardiology (ACC) published their recommendations in 1999 [27], and the European Society of Cardiology (ESC), ACC and AHA consensus document was released in 2000 [28]. Both documents recommended the revision of diagnosis of AMI and offered guidance for testing of cardiac markers. One of the most important changes in the diagnosis of myocardial ischemic injury, as put forward by these documents, recommended the replacement of cardiac enzymes with cardiac troponins in the WHO's "two out of three" criteria. The second major change was incorporating stable angina, unstable angina (UA), non-Q wave MI (NQWMI), ST-elevation MI (STEMI) and Q-wave MI (QWMI) on a continuum. These diagnoses were now representatives of the same ischemic process, collectively called the Acute Coronary Syndromes (ACS). The NACB document recommended the use of two diagnostic cut-off marker concentrations, one (lower concentration) for the diagnosis of ACS, and the second (higher) concentration as the diagnostic cut-off for AMI. The joint ECS/ACC/AHA document clearly stated that CK-MB was no longer required for the diagnosis of AMI because of the availability of cTns, and any elevation of cardiac troponin I or T would establish the diagnosis of an ischemic cardiac injury [28]. ECG was required to sub-classify cTn positive patients depending on the presence or absence of ECG abnormalities. Later interpretations of the ECS/ACC/AHA document recommended the elimination of the need for ECG abnormalities

Figure 1 **Schematic representation of diagnosis of ischemic myocardial injury, using cardiac troponin measurement and ECG findings**



for the diagnosis of some forms of AMI in cTn positive patients by defining NSTEMI and NQWMI diagnoses that were characterized by chest pain and cTn positivity only. To diagnose STEMI and Q-wave myocardial infarction required cTn positivity in the presence of new ECG findings (Figure 1.) Elevated cTn concentration was defined by the ECS/AHA/ACC consensus document as a concentration that exceeded the 99th percentile of the assay-specific reference limit of a healthy population on at least one occasion within the first 24 hours after the onset of symptoms [28].

Application of the new classification in clinical practice soon revealed the increased financial and social consequences of the new diagnostic algorithm [29] that was the end result of the increased number of patients diagnosed with MI due to the increased sensitivity of cTns for minor myocardial damage. Because the new guidelines did not completely invalidate the WHO "two out of three" diagnostic criteria, the time of myocardial damage was still considered to be defined by the onset of characteristic chest pain. However, because cardiac marker elevation could be present without the traditional diagnosis of an MI, i.e., without the presence of chest pain and ECG abnormalities, this discrepancy had to be reconciled and resulted in research in three separate directions: The search for ischemia versus infarction markers, the development of "cardiac risk assessment" markers, and the eventual redefinition of MI.

Proponents of the 'ischemia versus infarction theory' postulated that there could be myocardial ischemia without the development of a traditional infarction causing only reversible myocardial damage, but if ischemia existed for an extended period of time it could lead to irreversible damage. The logical conclusion of this theory was to try to find biochemical markers that would signal the presence of ischemia before the permanent damage occurred and use cTns or CK-MB for the diagnosis of MI. The best representative of the ischemia markers was the ischemia modified albumin (IMA), measured by the albumin-cobalt binding assay [30-32]. After many clinical trials IMA did not fulfill the expectations for its diagnostic role as an ischemia marker and it lost its significance after the redefinition of myocardial infarction.

Cardiac risk assessment by biochemical markers is based on the observation that certain biochemical markers can be detected at various concentrations in an asymptomatic (healthy) population or may be found during admission for suspected MI even when the diagnosis of AMI can be ruled out. However, on follow up, patients with detectable cardiac marker concentration may do worse after hospital discharge than patients without elevated markers. It was also recognized that higher concentrations of these biochemical markers confer increasing risk for short and long term cardiac events. The risk markers could be either cardiac troponins I or T [33] and CK-MB [34], but they could be inflammatory markers such as myeloperoxidase [35] or C-reactive protein (CRP) [36] also. In addition to these examples several other markers, associated mostly with inflammation or platelet activation, have been described in the literature and were commercially available. One important characteristic of these risk markers is the fact that they are somewhat independent predictors of cardiac risk, therefore simultaneous measurement of the concentrations of more than one marker can provide a more precise forecast of risk of future cardiac events and patient outcome [37-39]. Because the between- and within-individual variation of the risk markers can be great, their concentration is assessed in the general population then the population values are divided into quartiles or quintiles. The relative risks of an event for each segment of the population is determined from clinical

studies. The individual's own result of the risk marker concentration is compared to the age- and gender-related reference ranges and the risk associated with the quartile (or quintile) where the test result falls is assigned to the individual. It is important to recognize that using risk markers is not to establish a diagnosis but to calculate the relative risk of a cardiac event and to offer appropriate risk-mitigating intervention to the patient in the form of life style changes or medical intervention. The major shortcoming of most of the cardiac risk markers is that they are acute phase reactants therefore they can be present at elevated concentrations during minor illnesses. Interpretation of the results of cardiac risk markers requires careful evaluation of the patient's clinical history.

UNIVERSAL DEFINITIONS OF MYOCARDIAL INFARCTION

The most significant impact of cTn measurement is the realization that myocardial cell damage can occur before clinical signs and symptoms of "traditional" myocardial infarction and that this damage may have long term consequences for the patient. Investigating cTn release characteristics revealed that myocardial cell damage may be due to coronary artery occlusion as hypothesized in the WHO model but myocardial cell damage can also happen for other reasons than a coronary artery occlusion. To establish the correct mechanism of myocardial damage, other diagnostic modalities than cTn measurement may have to used. These additional diagnostic modalities includes ECG but imaging techniques are also valuable. This newly acquired insight into the mechanism of myocardial damage had to be incorporated into a new definition of myocardial infarction.

The expert consensus document, entitled Universal Definition of Myocardial Infarction, published by the Joint International Task Force in 2007 developed the first universally accepted practice guidelines that incorporated the new knowledge gained from clinical and research use of cardiac troponins [40]. This document addressed the diagnosis of myocardial infarction due to acute ischemia as well as for other causes, established the decision limits for diagnosis using biochemical markers and recommended different diagnostic cut-offs based on the clinical circumstances around the time the myocardial cell death occurred. The document defined six different types of myocardial infarction, including spontaneous MI, sudden cardiac death and several types of MI that developed during or immediately after an invasive cardiac procedure. All diagnostic cut-offs were based on the 99th percentile of the particular cTn assay in use but different circumstances required different multiples of the 99th percentile cut-off [40]. The document extensively discussed the use of ECG and imaging for diagnosis and differential diagnosis of myocardial damage [40]. The diagnosis of reinfarction, previously hotly debated as to whether it could be assessed by the use of cTns, was also addressed by this document and the expert panel presented recommendations for the use of cTns for this purpose. They proposed that a rise of cTns by more than 20%, or by more than 3 standard deviations for the assay in use, in two sample within 3-6 hours after decreasing cTn concentration had been observed would be diagnostic of a reinfarction [40].

The 2007 consensus document [40] was followed by a revision in 2012 [41]. The 2012 document retained previous recommendations and added a new MI diagnosis to the previous six (Table 1). As in previous documents it also provided a list of diseases that may present with elevated cardiac markers but are not MI or may not even be cardiac diseases. It also listed common abnormalities that could cause

false negative or false positive ECG changes that could be misinterpreted as ischemic myocardial damage. Table 2 is an abbreviated list of common non-MI diseases that may present with cardiac marker elevation. This list is intended to illustrate the fact that tissue specificity of a biochemical marker will not necessarily define the mechanism of tissue injury and to caution against too simplistic interpretation of an elevated cTn result.

Table 1 Types of MI and most significant criteria for diagnosis as recommended by the third universal definition of myocardial infarction [41]

Type of MI	Cause or circumstance defining Type of MI	Multiples of 99th percentile of cardiac marker required for diagnosis	Notes
Type 1	Spontaneous	>1x	MI related to spontaneous rapture of plaque and subsequent coronary artery occlusion
Type 2	Secondary	>1x	MI is due to ischemic imbalance (oxygen supply-demand mismatch)
Type 3	Sudden cardiac death	>10x or undefined	Antemortem blood may not be available for cardiac marker testing. Clinical history is strongly suggestive of cardiac event. Autopsy may be required for diagnosis.
Type 4a	PCI	>5x	>5 x 99th percentile URL after initial normal marker values or >20% increase above stable or decreasing baseline. ECG or imaging may be required for diagnosis
Type 4b	Stent thrombosis	>1x	Stent thrombosis detected by coronary angiography or autopsy
Type 4c	Restenosis	>1x	≥50% stenosis on coronary angiography
Type 5	CABG	>10x	ECG and imaging evidence is required in addition to cardiac marker elevation

Notes: For all diagnosis by biochemical markers characteristic rise and fall of marker concentration is required.

Positive marker concentration is defined as at least one result above the 99th percentile (or above the relevant multiple) of the upper reference limit (URL) for the assay in use.

Table 2	Elevation of cardiac troponin values due to myocardial injury but not due to MI
	tachy/bradycardia
	aortic dissection
	hypertrophic cardiomyopathy
	congestive heart failure
	shock
	respiratory failure
	cardiac contusion
	cardiac surgery or ablation
	myocarditis, endocarditis, pericarditis
	pulmonary embolism
	rhabdomyolisis
	sepsis, viral illness
	stroke
	amyloidosis, sarcoidosis, hemochromatosis
	strenuous exercise
	renal failure
	burns of large body surface area

Note: Compiled from references [40, 41, 57].

ANALYSIS OF BIOCHEMICAL MARKERS OF CARDIAC INJURY

CK-MB was the first cardiac marker that could be measured with a practical immunoassay. The experience gained with CK-MB established the fact that immunoassays, also called "mass assays", would be superior to activity based assays used earlier for cardiac enzyme measurement. The use of monoclonal antibodies provided the necessary analytical specificity and analytical sensitivity could be enhanced to detect marker release from arbitrarily small tissue damage. Even first generation cTn assays were capable of detecting the death of as little as 1 gram of cardiac muscle through the measurement of marker concentration in the circulation and newer, more sensitive assays could detect the destruction of even a smaller volume of heart tissue.

In spite of impressive analytical performance, cardiac troponin assays were not without problems. We mentioned the nonspecificity that plagued the first generation cTnT immunoassay and that later was corrected [17, 21]. Cardiac troponin I assays, and, to a smaller extent, cardiac

troponin T assays, too, had other problems in the form of large discrepancies between results from different manufacturers or between different generations of the same manufacturer's assay. The concentration difference between two tests producing the lowest and highest result on the same sample could be as much as 10- to 30-fold. These differences could be caused by multiple reasons. There was no agreement between how manufacturers established their calibrator values and there was no standardized calibrator available for use by assay manufacturers. Post translational modification of cTnI in the form of proteolysis at both the N- and C-terminal of the troponin molecule, oxidation, phosphorylation or complexation with troponin C could alter or eliminate epitopes that were recognized by the different monoclonal antibodies (MoAb) in the different assays [42]. The final outcome of the many factors in assay differences translated into approximately ten- to 20-fold differences in the limit of detection (LoD) and greater than ten-fold differences at the 99th percentile concentration [43]. False negative results could be also seen if an epitope was deleted because of proteolysis or if autoantibodies completely blocked attachment of the MoAb in the test kit. These large differences made application of practice guidelines difficult and required the establishment of assay-dependent cut-offs for each assay individually, leading to confusion in clinical practice. The troponin T assays, being produced by a single manufacturer, had fewer problems but they were not free of the similar differences between successive assay generations.

In addition to false negative and false positive cTnI results due to cTn specific autoantibodies against some epitopes, human anti-mouse antibodies (HAMA) could lead to incorrect results in assay formulation using mouse MoAbs and other heterophile antibodies could produce similar interference in other assays using polyclonal antibodies. Recently developed assays are less prone to these interferences because of manufacturers' effort to incorporate blocking agents in their reagents but the unwanted influence of heterophile and autoantibodies must be kept in mind when investigating false negative or false positive cTn results.

cTnI assay standardization was proposed and the international Subcommittee for Cardiac Troponin I Standardization was established by the American Association for Clinical Chemistry (AACC). This committee identified and validated cTnI candidate reference materials [44], [45] to be used as primary standards by test kit manufacturers and the final calibration standard is now available from the US National Institute of Standards and Technology (NIST) for cTnI assay developers or troubleshooting in the clinical laboratory. Unfortunately the availability of reference standard did not fully eliminate cTnI assay differences although it minimized them, therefore establishing LoD and 99th percentile cut-off is still required for each cTnI immunoassay.

HIGH SENSITIVITY TROPONIN ASSAYS (HS-TN)

As the clinical importance of even small amounts cTn in the circulation was recognized, successively newer generation cTn assays were developed with the intention of providing improved analytical sensitivity, but many assays demonstrated only marginal improvement in LoD or better precision at low analyte concentration. Second, third or fourth generation cTn assays were marketed as "sensitive", "high-sensitive", "highly sensitive", "high performance" or "high-sensitivity" without an exact definition of this terminology. Most of the time the operational characteristics, such as LoD or 99th percentile cut-off, of the various assay generations remained within the ng/mL concentration range, essentially unchanged from previous generations. Starting around 2010 true high-sensitivity

troponin assays started appearing for clinical laboratory use. The main defining characteristic of these high-sensitivity assays was an increase in analytical sensitivity of two orders of magnitude as compared to traditional cTn methods [46], creating new challenges for the user. Traditional cTn immunoassays reported marker concentration in ng/mL (nanogram/milliliter) units, numerically equivalent to microgram/L (microgram/liter) measurement units. The new, high-sensitivity cTn assays, therefore, had to express cTn concentrations using multiple zeros in the traditional unit of measure, a potentially very error prone procedure. The other option was to use different measurement units for traditional and high-sensitivity assays, a confusing proposition. Although no solution has been found for this problem, recommendation has been made to use ng/L (nanogram/liter) measurement unit for all cTn assay results [46], regardless if it is traditional or high-sensitivity method. If this recommendation is adopted by both assay manufacturers and clinicians traditional and high-sensitivity results can be easily distinguished. For example, a traditional cTn assay that has a 99th percentile cut-off of 0.05 ng/mL (in traditional units) and a hs-cTn assay that has a 99th percentile cut-off of 5 ng/L would be reported as 50 ng/L and 5 ng/L, respectively. As a practicing laboratory physician I strongly agree with this recommendation and encourage its adoption.

The emergence of hs-cTn assays created new challenges for the laboratory and clinical community. The significantly lower LoD made it possible to measure cTn concentration in up to 95% of healthy control subjects [47] contrary to the approximately 15% with the traditional assays. Later studies indicated that age and gender specific reference ranges may be needed both for hs-cTnT [48] and hs-cTnI [49], but other investigators did not confirm these findings [47]. Whether gender specific reference ranges are truly necessary must be decided by additional clinical trials, but using sex-related hs-cTnI reference range was found to improve detection in women, but not in men, of major cardiac events within one year after an initial presentation for ACS [50]. The benefits of improved early detection of ACS by hs-cTns are offset by reduced specificity for ACS because more patients are detected with myocardial injury not due to ischemia [51].

The low 99th percentile cut-off of hs-cTn assays and their even lower LoD when combined with high precision (low coefficient of variation or CV) at the decision limit provide speedy diagnosis and accurate identification of patients who can be safely discharged from the emergency room [52, 53] and produce better than 99% negative predictive value for subsequent MI or cardiac death at 30 days after initial hospital visit [53]. If patients with symptoms suggestive of ACS can be safely and rapidly discharged from the hospital, it can reduce inpatient admissions, cost of hospitalization and have major benefits for patients also.

Because an inherent characteristic of cardiac marker release in acute ischemic events is the rise and fall of marker concentration, non-ACS, non-MI diagnoses may be ruled out by repeat measurements of cardiac marker concentrations by hs-cTn assays. The improved precision and the very low LoD of hs assays allows for repeat marker testing within a shorter time frame than with traditional troponin assays. Both absolute concentrations and percent change from baseline or admission values (delta values) within the first six hours have been evaluated for this diagnostic algorithm and found to be valuable to ‘rule in’ ACS or to ‘rule out’ significant stenosis, recurrent infarction or death within one year after initial admission in patients with non-ST elevation chest pain [54]. Jeager et al. investigated the diagnostic utility of absolute concentration change of hs-cTnI from baseline

within one hour of admission in patients with suspected AMI [55]. They have reported their one hour delta hs-cTnI protocol could rule out AMI with 100 % sensitivity (negative predictive value = 100%), and it could rule in AMI with 96 % specificity (positive predictive value = 70 %) [55]. Their one hour delta hs-cTnI protocol exhibited better diagnostic accuracy than a combination of ECG and hs-cTnI measurement while providing the improved diagnostic performance faster.

Only a handful of hs-cTn assays are on the market at this time but their number is growing. Laboratory professionals and clinicians may be still digesting results of recent research and may have just started adopting practice recommendations when newer discoveries are published. Professional organizations have attempted to further this process via various publications that distill the essence of the detailed practice guidelines. A recent publication by the IFCC Task Force on Clinical Applications of Cardiac Bio-Markers (TF-CB) is a valuable educational aid to help with adopting hs-cTn assays in every day practice [56].

SUMMARY AND CONCLUSIONS

Sixty years of research into the physiology and pathology of ischemic and non-ischemic cardiac injury has greatly increased our understanding of the events taking place during and after myocardial cell death. Clinical signs and symptoms, ECG, combined with measurement of biochemical markers and imaging studies improved our capacity to detect and respond to an acute coronary event. Successively newer biochemical markers of these injuries have evolved from cytosolic enzymes to tissue specific structural proteins, culminating in our current, best biochemical markers of cardiac troponins I and T. Improved analytical techniques of cardiac troponin measurement produced high-sensitivity cTn assays with detection limits two orders of magnitude better than the first methods and enhanced our diagnostic sensitivity and specificity of cardiac damage. The same cTn assays forced us to reassess our understanding and practices of diagnosing and treating myocardial injury, but did not close the chapter on biochemical diagnosis of these clinical entities.

The fast pace of change in our understanding of myocardial injury and the use of biochemical markers for the diagnosis of these diseases provide challenges for practicing laboratory professionals, emergency medicine physicians and cardiologist to keep up with the new recommendation and practice guidelines that are the result of research in this field. Hopefully this review article presents information on the most significant aspects of the use of biochemical markers for the diagnosis of myocardial injury. The historical approach was elected in the hope of providing additional explanation why certain markers or practices are preferred over others when dealing with the patient who has cardiac damage.

It is impossible to discuss all the biochemical markers of myocardial damage in a single review article. The number of markers are too numerous and many of them were short lived. This article attempted to present the most significant milestones on this field with emphasis on the recent discoveries and issues related to high-sensitivity cTn assays. It is expected that this area of laboratory medicine and cardiology will experience additional growth in the near future as results of new clinical trials get published, leading to further refinements in our understanding of ACS and MI.

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In this issue: Celebrating the 70th Anniversary of the Hungarian Society of Laboratory Medicine

The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Non-invasive assessment of viability in human embryos fertilized in vitro

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Key words:
in vitro fertilization, embryo viability assessment, morphology, biomarker, mass spectrometry, non-invasive diagnostics

Ethical approval and supervision:
The study protocol was approved by the Committee of Human Reproduction, National Science Council of Hungary (5273-2/2012/HER), and later supervised by the Public Health Officer, Hungarian Government Office in Baranya County (BAR/006/58-2/2014).

Acknowledgements:
The presented publication was supported by the NKFI-EPR K/115394/2015 (Early biochemical indicators of embryo viability).

ABSTRACT

Human reproduction is a relatively inefficient process and therefore the number of infertile couples is high. Assisted reproductive technologies (ART) have facilitated the birth of over five million children worldwide. ART, however, superimposes its own relative inefficiency on the preexisting inefficiency of normal reproduction. The efficiency (expressed as pregnancy rate) is generally not more than 30%. Modern reproductive medicine is gradually moving from multiple embryo transfer to the transfer of a single embryo, mainly because of obvious and unwanted side effects of multiple embryo transfer (e.g. „epidemic" multiple pregnancies). This concept, however, requires a fast, professional selection of the most viable embryo during the first few days of ART. Thus the aim of a modern ART is the safe transfer of a healthy, viable, single embryo. Accurate and rapid methods of quantifying embryo viability are needed to reach this goal. Methodological advances have the potential to make an important contribution, and there has been a drive to develop alternative non-invasive methods to better meet clinical needs. Metabolic and genetic profiling of spent embryo culture (SEC) media should offer an exceptional opportunity for the assessment

of embryo viability. The current review focuses on the latest non-invasive diagnostic approaches for pre-implantation viability assessment of in vitro fertilized embryos.

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INTRODUCTION

Infertility has been recognized as a public health issue worldwide (1) leading to an increasing need to the use of assisted reproductive technologies (ART), including in vitro fertilization (IVF). After the first reported case of IVF in 1978 (2) ART enabled millions of people to have their own children in cases when pregnancy did not occur under natural circumstances. ART has advanced significantly and became more and more widespread resulting in ca. 700,000 cycles a year in the USA and Europe together (3,4). Despite of evolving intracytoplasmatic sperm injection (ICSI) technique the rate of the successful embryo implantations is surprisingly low (5,6). A success rate of 25% and 28% has been reported in 2005 (7) and 2008 (8), respectively. Nowadays, this rate went up to 32% (9), which cannot be considered as a significant development. Earlier clinical protocols preferred multiple embryo transfer, but multiple gestations can result in the increased risk of preterm delivery (10-16). Other studies report that multiple gestations also increased the risk of low birth weight cerebral palsy (17). In the US alone, preterm births resulting from multiple pregnancies during IVF cause a 1 billion USD extra cost to the social insurance (18). In order to exclude the discussed risk factors, single embryo transfer becomes the standard of care for all. It is imperative, however, that accurate and economical methods should be developed to ensure that the most viable euploid embryo is selected for transfer. Ideally, such tests would be noninvasive, lessening the risks to the embryo and reducing costs and workload in the embryology laboratory (19). The biggest issue with pre-implantation viability assessment is that due to ethical reasons any assay should be completely non-invasive because no one can predict what kind of interference would be the unwanted result in the later embryonic development.

THE MORPHOLOGICAL APPROACH

The most apparent – and routinely applied - way of the assessment of viability is the morphological evaluation of in vitro fertilized embryos using microscopy. There are several morphological features described which could be used for viability assessment purposes, these are dependent on the time spent after fertilization. Right after fertilization in the 1-cell embryo the size and symmetry of the two pronuclei can be examined. The time of the first cell division is also a good predictor of later implantation potential, as zygotes that divide early tend to develop more frequently to the blastocyst stage. Criteria as cleavage rate and blastomere shape and symmetry, an adequate trophectoderm layer (TE) and an inner cell mass (ICM) is a morphological marker of the later stages (5,20). Not only can the morphology of the fertilized embryo be used for further prediction of implantation potential, but morphological defects of the retrieved oocyte as well. Fertilization and pregnancy rate correlates with the grade of cumulus-oocyte complexes, and embryos originating from dysmorphic oocytes show a larger grade of pregnancy loss (21-23). The cleavage stages of morulae and blastocysts or the symmetry and patterns of cell division are also notable and frequently used aspects, and are often examined during the prediction of embryo viability (23). The biggest issue of morphological assessment is that it is still a highly subjective method (20). The reason is partly due to the fact that the final decision is made by a clinician, and not by an objective test result, and secondly it is does matter how important are the individual

morphological features in the final conclusion (24-26). To overcome the different practice of laboratories worldwide in 2011 an international consensus (Istanbul Consensus) has been reached on embryo viability assessment (27). The selected morphological markers of respective stage embryos, the weighing of individual features and a scoring system has been set up. The limitations due to static time-point observation, is now solved with the use of time-lapse microscopy (28,29). Time-lapse microscopy also enables the observation of dynamics of cytoplasmic movements and cytokinesis, reflecting the functionality of microtubule and actin cytoskeleton, which is critical for proper development. In our laboratory, we aimed to improve the success rate of implantation by adopting and further optimizing the Istanbul consensus. This score has been called as the optimized criteria system (OCS). According to this scoring, 3-Day old embryos were divided into two subgroups: the subgroup with low blastomere number (less than 7) and with high blastomere number (7 or more). Symmetric position of blastomeres indicates the rate of symmetry of holoblastic cleavage along the embryo axis. It was classified as good (full symmetry); fair (light asymmetry); or poor (evident asymmetry). The percent values of fragmentation are based on the ratio of fragmented to total cell numbers. As a further modification to the Istanbul consensus, the assessment of fragmentation was slightly changed. Embryos were considered as good if the fragmentation rate was <15% (instead of the original 10%). This shift from 10 to 15% was the result of our observation that a fairly high proportion of the embryos between 10-15% appeared to be viable. In summary the optimized criteria system (OCS) highlights 3 modified or new parameters: fragmentation (with a more permissive criterion of <15% in the "good" category); symmetry and the blastomere number. In addition, the blastomere size was evaluated according to the original Istanbul consensus. A scoring-map was created to facilitate the evaluation (Table 1) As far as the 5-Day old embryos are concerned, we modified the original Istanbul Consensus for blastocysts by leaving out the hatched stage from the evaluation. The Istanbul Consensus for the 5-day old embryos has a shortcoming, i.e. it does not express the viability of embryos with a single category (good, fair, poor). We tried to overcome this by using a scoring map (Table 2). In conclusion, we constructed a composite score for Day-3, as well as Day-5 old embryos, based on morphological parameters. As it is evident from the results, this composite score is sensitive to evaluate viability (Figure 1)

THE BIOCHEMICAL APPROACH

Another possibility for non-invasive embryo viability assessment is the metabolomic examination of the culture medium surrounding the in vitro fertilized embryo. Metabolomic, (proteomic) profiling of spent embryo culture (SEC) offers an exceptional, non-invasive opportunity for the assessment of embryo viability (30,31). The metabolomic profiling (32,33) of early embryo development might mean the analysis of the total metabolome by following the changes of several selected compounds, metabolomic analysis using unidentified, but significantly differing metabolomic changes, or by the analysis of a limited population of nutrients or end products. The common feature in all three concepts is that they are concentrating on the metabolomic alterations caused by differently developing embryos in the culture medium. Very simple idea is the monitoring of glucose consumption or pyruvate formation, since this would directly indicate the metabolism of the developing embryo and it is an obvious conclusion that a metabolically active embryo would have higher implantation potential. Some authors reported that the identification of these parameters

Table 1 The composite score of the "optimized scoring system" for Day-3 old embryos

ICCS for cleavage stage embryos		
Good	**Fair**	**Poor**
<10% fragmentation	10–25% fragmentation	Severe fragmentation (>25%)
Stage-specific cell size	Stage-specific cell size for majority of cells	Cell size not stage specific
No multinucleation	No evidence of multinucleation	Evidence of multinucleation

OCS for cleavage stage embryos							
Fragmentation		**Blastomere size**		**Number of blastomeres**		**Symmetry**	
1	Good (<15%)	1	Stage specific	1	≥7	1	Symmetric cleavage
2	Fair (15-25%)	2	No stage specific	2	<7	2	Light asymmetry
3	Fair (15-25%)	-	-	-	-	3	Evident asymmetry

Scoring map							
Good		**Fair**			**Poor**		
1111	1121	1112	1112	1131	2132	2212	2221
1211	1221	1132	1212	1222	2222	2231	2232
2111	2121	1231	1232	2112	3131	3132	3211
2211	-	2122	2131	3111	3212	3221	3222
-	-	3112	3121	3122	3231	3232	-

resulted in successful prediction of embryo implantation potential, but other research groups describe contradictory results (34, 35). The amino acid profile of culture media is also used in the prediction of implantation potential, though not exclusively as an independent parameter, rather in combination with morphological features (36). The detection of unidentified metabolomic changes using near infra-red (NIR) or Raman spectroscopy (37, 38) is a very interesting and challenging possibility.

More complicated is the concept when unknown, new biomarker molecules of embryo viability are searched for, assuming that these biomarkers were secreted by the embryo. The difficulty of the concept is that only 4-8 cells are present in the culture medium; thus a very sensitive analytical tool is required. Mass spectrometry (MS)

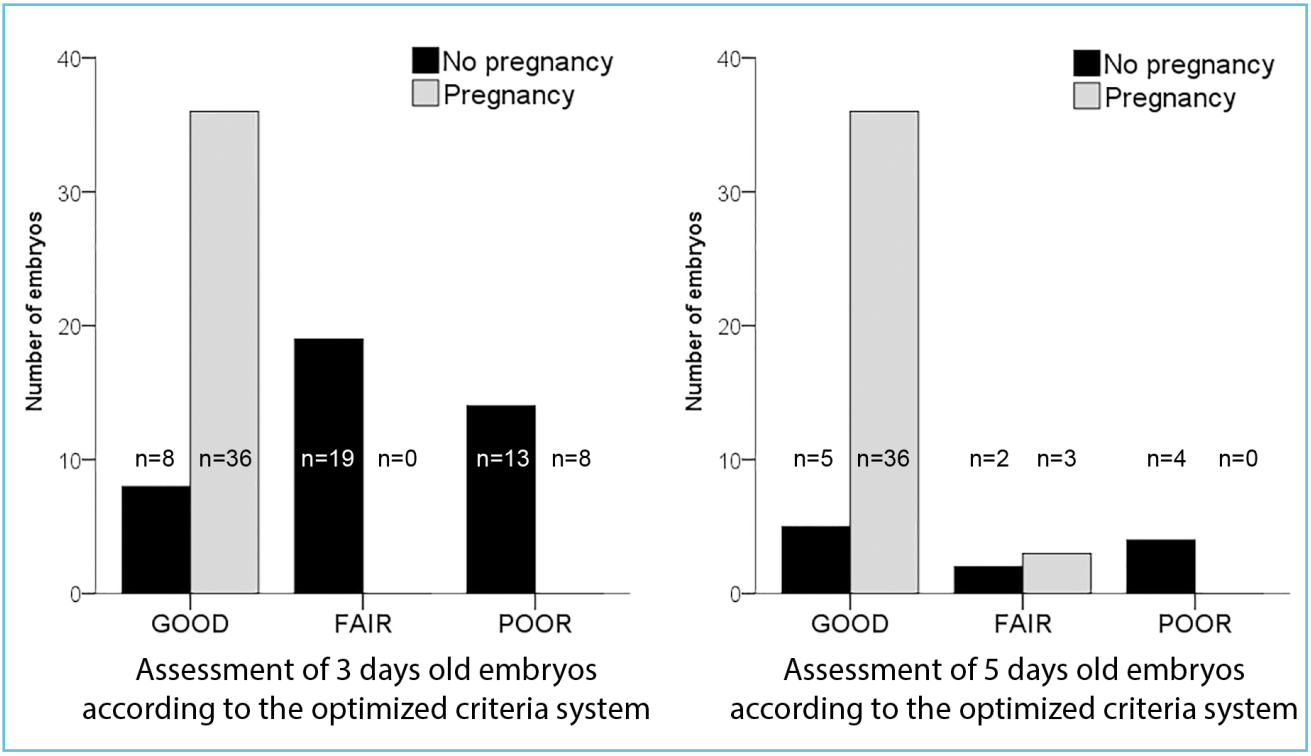
Table 2 The composite score of the "optimized scoring system" for Day-5 old embryos

ICCS for blastocysts (Day-5)	
Stage	
1	Early blastocyst
2	Blastocyst
3	Expanded blastocyst
4	Hatched/hatching
ICM	
Good	Prominent, easily discernible, with many cells that are compacted and tightly adhered together
Fair	Easily discernible, with many cells that are loosely grouped together
Poor	Difficult to discern, with few cells
TE	
Good	Many cells forming a cohesive epithelium
Fair	Few cells forming a loose epithelium
Poor	Very few cells

OCS for blastocyst (Day-5) – scoring map					
Good		**Fair**		**Poor**	
111	112	122	132	133	223
113	121	213	222	231	232
123	131	313	322	233	323
211	212	-	-	331	332
221	311	-	-	333	-
312	321	-	-	-	-

has the potential of specific and sensitive quantification in a wide spectrum of molecular mass ranges and therefore suites well the needs of metabolomic or proteomic fingerprinting and quantification. In parallel to the spreading of mass spectrometry, proteomics is also an emerging field in the understanding of embryo development (39,40). The analysis of the embryonic secretome (41,42) provides information of the total transcriptome of the developing embryos. Mass

Figure 1 The sensitive "optimized scoring system"



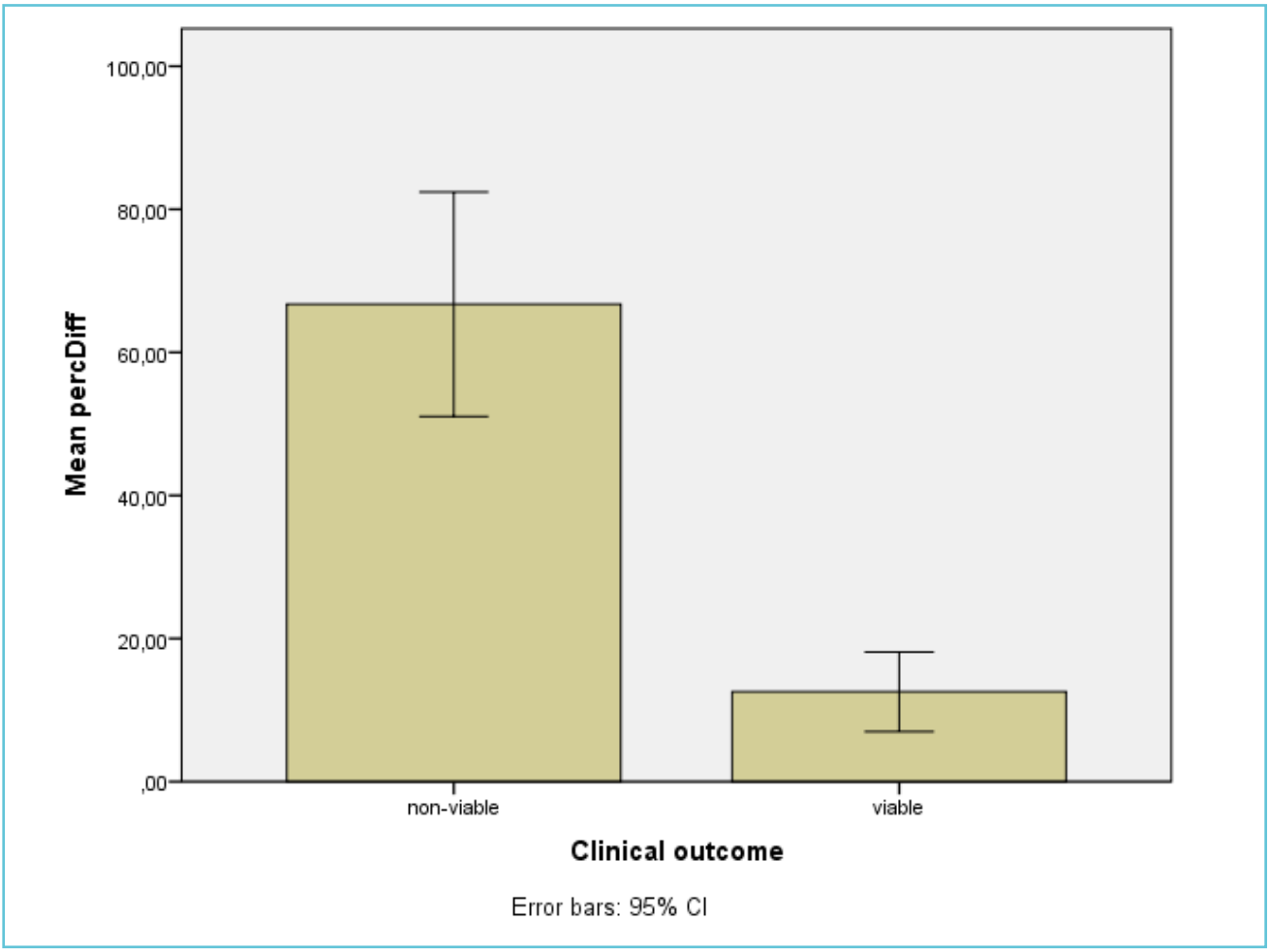
Day-3 old embryos (left panel): Pregnancy occurred only in the group evaluated as good quality embryo. No pregnancies occurred if the embryos were assigned to the fair or poor quality groups. It should also be noted the good quality embryo does not necessarily mean pregnancy (maternal causes of infertility may be present).

Day-5 old embryos (right panel): With the exception of a very few cases, most pregnancies occurred in the good quality group.

spectrometry can be used both in targeted and discovery analysis with accurate quantification of identified biomarkers after molecular identification by bottom-up or top-down proteomics using tandem or multiple MS (43-46).

In a recent publication from our laboratory (47) using liquid chromatography coupled mass spectrometry (LC-MS), a fragment of the human haptoglobin molecule was identified in the culture medium. Rather than analyzing the embryonic secretome, the aim this experiment was to use preexisting molecules present in the cell culture media as biomarkers. Haptoglobin - which was detected in the culture medium - is not a product of the developing embryo; the polypeptide is a contaminant of the human serum albumin standard used to supplement the culture medium (47,48). During the first three days of embryo development the formation of a subunit (alfa-1) of the human haptoglobin molecule was observed. This subunit similar to the total haptoglobin molecule was detectable in the blank control medium samples as well. The differentiation of the viable and non-viable embryos was done using the observation that compared to blank controls the samples of embryos which later did not resulted in pregnancy contained the alpha-1 subunit in a much larger quantity than the samples of embryos which did (Figure 2). 160 samples of 77 Day-3 old embryos were analyzed. Clinical statistical analysis of the results revealed that the specificity of the diagnostic test was 64%, while the sensitivity was 100%. It is more informative that the

Figure 2 The mean difference in the amount of the haptoglobin alpha-1 peptide fragment between viable and non-viable embryos



The mean difference in the amount of the haptoglobin alpha-1 peptide fragment between viable and non-viable embryos.

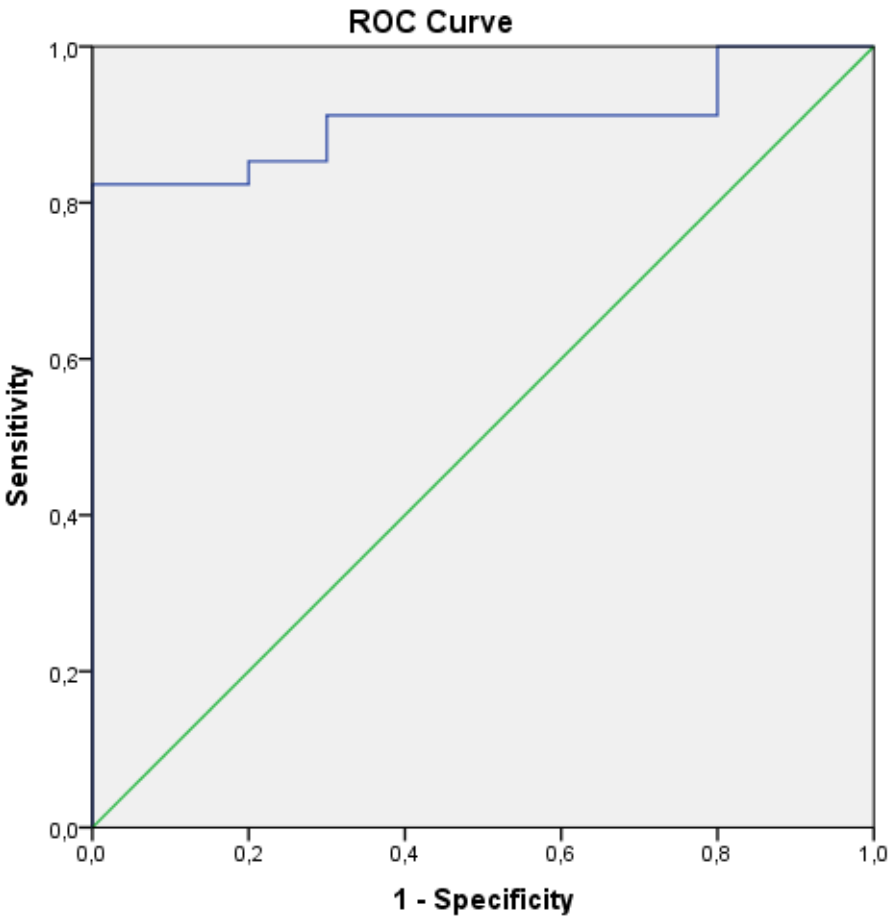
The quantification of the biomarker (n=160) was carried out by mass spectrometric detection following reverse-phase HPLC separation; the analyzed sample volume was 25 µl. In the samples of the non-viable embryos (no pregnancy) the fragment was present in a significantly larger (p<0.001) content than in the sample of the viable (live birth) embryos.

positive predictive value of the assay was 51% and – maybe more importantly – the negative predictive value was 100%.

Receiver operating characteristic (ROC) analysis provides tools to select possibly optimal models and to discard suboptimal ones. ROC analysis is related in a direct and natural way to cost/benefit analysis of diagnostic decision-making. The ROC curve of the morphological versus metabolomic approach in relation to the correct prediction of pregnancy outcome is illustrated in Figure 3. It is obvious that our biochemical investigation method enables a selection of the embryos by sorting out the non-viable ones. The test selected with 100% potential the embryos, which did not lead to successful implantation at all.

One of the areas of collaboration between clinicians, the clinical laboratory and the research laboratory at the University of Pécs is related to the research of infertility. Since the clinical background gives the beauty and the medical

Figure 3 Receiver operating characteristics (ROC) curve of the blinded, retrospective cohort study



Embryo viability prediction was made using the data of the mass spectrometric haptoglobin alpha-1 chain quantification. n=160, the area under the curve (AUC) is 0.906.

A ROC curve (blue line) visualizes a calculation by graphing the sensitivity on the y-axis (vertical) and the false positive rate (1-specificity) on the x-axis (horizontal) for all possible cut-off values of the diagnostic test. The green line is the reference line. The AUC is used as a measure of the performance of a diagnostic test against the ideal and may also be used to compare different tests.

importance of laboratory research, it was of outstanding importance for us to receive the EFLM-Abbott Diagnostics Award for Excellence in Outcomes Research in Laboratory Medicine (Paris, 2015), the award given to the best published paper (47), as judged by an independent panel of experts, which demonstrates improved outcomes arising out of the application or improved utilization of an in-vitro diagnostics test. This short review summarizes some of our recent findings and views on this field.

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In this issue: Celebrating the 70th Anniversary of the Hungarian Society of Laboratory Medicine

The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

The clinical value of soluble urokinase plasminogen activator receptor (suPAR) levels in autoimmune connective tissue disorders

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Key words:
autoimmune disease, biomarker, inflammation, rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, systemic sclerosis, soluble urokinase plasminogen activator receptor

Declaration of conflict of interest:
The authors declared no conflict of interest.

ABSTRACT

The assessment of the general inflammatory condition of patients with autoimmune connective tissue disorders (ACTD) is a major challenge. The use of traditional inflammatory markers including CRP-levels and erythrocyte sedimentation rate (ESR) is limited by several preanalytical factors and their low specificities. Soluble urokinase plasminogen activator receptor (suPAR) is one of the novel candidate markers that is increasingly used in immune mediated disorders. In our studies we compared suPAR levels of patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis (SSc) and ankylosing spondylitis with those of healthy controls.

suPAR provided valuable clinical information on disease activity in RA, SLE and SSc. We identified a subgroup of remitted RA patients, who presented still clinical symptoms of inflammatory activity which correlated to high plasma suPAR (while ESR and CRP were normal). In SLE we established specific suPAR cut-off values that support the discrimination between patients with high and those with moderate SLE activity. In patients with SSc suPAR correlated with objective measures of lung and other complications.

In the majority of ACTDs including SLE, SSc or RA, suPAR is seemingly a good biomarker that would provide valuable clinical information. However, before the introduction of this novel parameter in laboratory repertoire important issues should be elucidated. These include the establishment of appropriate and disease specific cutoff values, clarification of interfering preanalytical values and underlying conditions and declaration of age- and gender-specific reference ranges.

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INTRODUCTION

Inflammation is a characteristic hallmark of relapsed autoimmune connective tissue disorders (ACTD). The treating physician's challenge is to determine the extension of inflammation and to decide whether the patient requires an intervention or therapy should be modified. Inflammatory markers, therefore, are generally used to assess ACTD patients' general condition. CRP-levels and erythrocyte sedimentation rate are among the most frequently ordered lab tests. However, the information provided by these tests is limited by their low sensitivity and the number of interfering preanalytical factors such as diurnal cycle, way of sampling or even physical exercise. Therefore, novel biomarkers that indicate the presence of severe inflammation in ACTD are highly warranted. suPAR is one of the promising candidates that we investigated extensively in patients with different ACTDs.

Urokinase-type plasminogen activator receptor (uPAR) is expressed on various cell types, including immune, smooth muscle and endothelial cells (1,2). When this receptor dissociates from the cell surface, suPAR, the soluble form of uPAR is created. suPAR is detectable with standard ELISA tests in low concentrations in non-diseased people. Its benefits over traditional acute phase proteins are that its levels do not depend on diurnal variation and fasting state (3). suPAR is readily resistant to preanalytical conditions such as freezing and thawing (4). Due to its stability it may be a candidate as an assessable biomarker for inflammation. According to the data available, inflammatory response leads to elevated plasma suPAR levels in many inflammatory diseases (5) which is predictive to a worse prognosis. The clinical value of suPAR was investigated most extensively in systemic inflammatory response syndrome (SIRS) and in patients with septic conditions. Current evidence unanimously indicates that levels of suPAR are increased in SIRS and may be used for risk stratification of patients with SIRS (6,7). Findings indicate that suPAR predicts better adverse outcome following sepsis than traditional markers including CRP levels (8,9,10).

During the last five years our team made an extensive work to assess the clinical utility of suPAR levels in ACTD. In our studies we compared plasma suPAR levels of different and well-characterized patient ACTD subgroups such as those with rheumatoid arthritis (RA) (11), systemic lupus (SLE) (12), systemic sclerosis (SSc) (13), and ankylosing spondylitis (AS) (14) with 29 healthy control subjects. Healthy controls had a negative history of rheumatic symptoms and negative status upon detailed physical and laboratory examination. Written informed consent was obtained from all participants, and our study was reviewed and approved by the Ethics Committee of the institution. The studies were adhered to the tenets of the most recent revision of the Declaration of Helsinki.

For the purpose of suPAR determination we collected EDTA anticoagulated fasting blood samples from patients and controls, separated plasma and stored at −80°C until measurement. Plasma suPAR concentrations were measured with the suPARnostic Flex ELISA assay (ViroGates A/S, Birkerod, Denmark) and were related to ESR, CRP and clinical status.

Table Summary of suPAR, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) values in autoimmune connective tissue disorders (ACTD) including rheumatoid arhtritis (RA), ankylosing spondylitis (AS), systemic lupus (SLE) and systemic sclerosis (SSc)

Demographic characteristics and inflammatory markers	Healthy controls n = 29	RA (n=120)	AS (n=33)	SLE (n= 89)	SSc (n = 83)
Age, years	55 [46-69]	61 (48 – 72)	41* [35–45]	44 [34–59]	51.5 [44-60]
Gender, male/female	10/19	46/74	24/9	10/79	16/67
suPAR, ng/mL	2.80 [2.06-3.42]	4.24 (3.19 – 5.40)	2.97 [2.57–3.80]	4.58* [3.72–6.30]	4.02* [3.19-5.53]
CRP, mg/L	2.70 [BLD-4.15]	4.00 (BLD – 9.83)	10.00* [2.75–25.80]	3.90 [BLD-9.55]	3.50 [1.80-8.40]
ESR, mm/h	10 [7-14]	21 (12 – 36)	17* [10–33]	28* [17–50]	18* [8-28]

*BLD=below the level of detection; * p<0.05 compared to the control*

Based on data published in refs 11–14.

suPAR IN RHEUMATOID ARTHRITIS (11)

Rheumatoid arthritis (RA) is a chronic inflammatory disease leading to the erosion of the cartilage and bone, and invasive growth of synovial pannus tissue. The Disease Activity Score (DAS28) reflecting the severity of RA is based on clinical signs and symptoms along with CRP and ESR (15). RA is regarded as active above a DAS28 score of 2.6, however, patients in remission (DAS28 score ≤2.6) might also be affected by inflammatory activity.

In our RA study we enrolled 120 RA patients at various stages of disease duration and activity and related their clinical parameters and DAS28 score to suPAR levels. The median DAS28 score (calculated at the time of sampling) was 2.8, corresponding to a median low disease activity. All the 120 RA patients received a variety of disease modifying anti-rheumatic drugs (DMARDs); 34 and 60 RA patients received add-on anti-tumor necrosis factor (TNF) therapy and glucocorticoid treatment, respectively.

suPAR, CRP and ESR values were higher in RA patients compared to healthy individuals (see Table). We identified correlation between suPAR and DAS28 in RA patients (p=0.02, r=0.26), suPAR values and ESR values in RA patients (p=0.05, r=0.30) and suPAR values and CRP values in healthy individuals (p=0.02, r=0.32). CRP and ESR values were also analyzed according to DAS28 scores. Irrespectively of anti-TNF and glucocorticoid therapy, CRP and ESR values were higher with a DAS28 score >2.6 than in RA patients in remission (DAS28 score ≤2.6) or in healthy individuals.

The evaluation of RA patients' condition is based on laboratory markers and clinical symptoms.

Appropriate determination of disease activity has a significant impact on therapeutic decision making process. The elevated suPAR, CRP and ESR values are in agreement with earlier results indicating higher plasma suPAR levels in RA. Furthermore, Slot et al's study indicated a positive correlation between suPAR and CRP and ESR in RA (16). We also found a correlation between ESR and DAS28 but not CRP values and suPAR levels when all RA patients were analyzed. A reason for this apparent controversy might be that patients enrolled in our study were more heterogeneous in terms of disease severity, including patients with milder RA. In contrast with the findings in RA, CRP values were correlated to suPAR levels in healthy individuals in our study either.

When RA patients were grouped according to anti-TNF and glucocorticoid therapy, or CRP and ESR values, no differences were detected between the corresponding therapeutic subgroups. However, when we compared RA subgroups according to DAS28 scores, a difference between remitted RA patients (DAS28 ≤2.6) and patients with different stages of active disease (DAS28 >2.6) was detected.

Of note, while CRP and ESR values were comparable with healthy individuals in remitted patients, suPAR values were still elevated (but were lower than in patients with DAS28 >2.6). In addition, the number of affected joints was strongly correlated to elevated plasma suPAR levels, indicating that suPAR levels represent well ongoing inflammatory activity in remission. While CRP and ESR values were similar in all subgroups of RA patients in remission to the levels seen in healthy individuals, suPAR values were elevated indicating the inflammatory activity in patients with 2–3 or four affected joints. Highest suPAR values were observed in patients with the highest number of affected joints. This subgroup represented almost 10% of the whole RA group of our study and over 20% of remitted RA patients, indicating that in remitted RA regular monitoring of plasma suPAR values would support the early detection of inflammatory activity. This is of particular importance as recent data indicate that patients in remission according to DAS28 scores could have slowly progressive structural damage without relevant clinical symptoms and with normal CRP and ESR (17). In such cases only ultrasound investigation of the joints supports the presence of synovitis. However, the use of ultrasound has limited as its availability is restricted, it is time-consuming, and investigator-dependent. Our analysis indicated measuring suPAR with a 4.8 ng/mL cut-off value would support the identification of patients under risk.

These results suggest that suPAR is a sensitive marker of inflammatory activity even in remitted RA patients. We identified a subgroup of RA patients in remission according to DAS28 scores, who present still with clinical symptoms of inflammatory activity (tender and/or swollen joints) which correlate to elevated plasma suPAR levels. Importantly, ESR and CRP values showed no alteration in these patients compared to healthy controls. Hence, suPAR levels might help the follow-up of remitted RA patients with mild clinical signs. Our finding might also have important therapeutic consequences, since this subgroup identified by elevated suPAR levels may benefit from earlier anti-RA treatment.

suPAR IN BECHTEREW'S DISEASE (14)

Ankylosing spondylitis (AS) is an immune-mediated rheumatic disease characterized by chronic inflammation. The autoimmune reaction principally affects the axial and sacroiliac joints in AS eventually leading to spondylitis, extra bone formation and vertebral fusion (ankylosis). In later stages of the disease, systemic autoimmune reactions are hallmarked by the

inflammatory involvement extraskeletal organs (eye, gastrointestinal tract or heart). Therefore, early and reliable detection and monitoring of inflammation and the initiation of targeted therapy are of utmost importance in AS.

In order to determine whether suPAR is a marker of inflammation in AS, we enrolled 33 AS patients. AS patients were classified according to the modified New York criteria (18). The median of Bath ankylosing spondylitis disease activity index (BASDAI) was 5.49, indicating an active disease. Ten of 33 patients received sulfasalazine treatment, while 15 of 33 AS patients received anti- TNF therapy.

In AS CRP and ESR values were higher than normal, while suPAR values were comparable to the control (see Table). When suPAR levels were analyzed according to different subgroups of AS patients, AS patients with an ESR value greater than 20mm/h exhibited higher suPAR levels than those with an ESR value ≤20mm/h and healthy controls. suPAR correlated with CRP and ESR values in AS patients. Of note, while BASDAI scores correlated with CRP and ESR, they did not interact with suPAR.

These observations indicated that suPAR failed to detect the ongoing inflammation in AS. This pilot study does not support the usefulness of suPAR in the assessment of AS.

suPAR AND SYSTEMIC LUPUS ERYTHEMATOSUS (12)

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease that affects almost all vital organs and tissues and is characterized by a wide spectrum of clinical signs and symptoms.

Nowadays, C-reactive protein (CRP) is regarded as the gold-standard for the assessment of systemic inflammation. However, SLE is an important exception, as CRP levels are not necessarily elevated and do not reflect inflammation in SLE (19). In clinical practice, a significantly elevated erythrocyte sedimentation rate (ESR) with a normal CRP is a strong indicator of SLE. ESR is, however, a rather unspecific marker of inflammation.

In 89 SLE patients with various stages of disease duration and activity we aimed to assess plasma suPAR levels and to determine if suPAR could serve as an inflammatory biomarker in SLE. SLE patients were diagnosed and classified according to the updated American College of Rheumatology (ACR97) criteria (20). The median of SLE duration was 8 years, and the median of Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score was 2, corresponding to moderate disease activity (21). Patients with a SLEDAI score of 0 were considered to be in remission, a SLEDAI score between 1 and 8 was regarded as moderate disease activity, and a SLEDAI score above 8 was regarded as high disease activity.

suPAR and ESR values were higher in SLE patients than in controls, while CRP levels were comparable (see Table). We performed further analyses of suPAR levels based on several subgroups created according to SLE complications. Of note, suPAR levels of patients with vasculitis in their history was higher than that of patients with no vasculitis (5.84 [4.12–7.01] vs. 4.21 [3.57–5.47] ng/mL, $p = 0.04$). CRP and ESR values did not differ between subgroups of SLE patients with different disease activity. suPAR levels behaved in a different manner; patients with high disease activity exhibited higher suPAR levels than those with moderate disease activity or in remission.

ROC analysis to discriminate healthy individuals and SLE patients based on suPAR yielded an AUC of 0.85 (ESR performed in a comparable manner with an AUC of 0.87). The cut-off value of suPAR was 3.54 ng/mL (sensitivity%: 82.02, specificity%: 79.31). ROC analysis of suPAR values in SLE

patients according to SLEDAI scores yielded an AUC of 0.68; the cut-off value of suPAR to discriminate between patients with high and with moderate disease activity was 5.70 ng/mL (sensitivity%: 61.54, specificity%: 78.72).

These findings indicate that suPAR levels are elevated in SLE. The use of CRP in SLE is limited its insensitivity to inflammation in this condition. This is due to different factors including decreased responsiveness of monocytes producing CRP-inducing cytokines in SLE, the common presence of CRP gene variations associated with lower CRP levels and an increased risk of SLE and the presence of the autoantibodies against CRP in SLE (12)). In contrast with CRP suPAR levels are increased in SLE patients, particularly in those with vasculitis. One might hypothesize that inflammatory cell adhesion to endothelial cells leading to extravasation into the intima is promoted by higher suPAR levels in SLE. (Indeed, experimental evidence suggests that suPAR might promote cell adhesion by binding to very late antigen-4 (VLA-4) on inflammatory cells as a ligand, promoting their extravasation via the activation of other molecules regulating cellular adhesion and migration (22)).

Importantly, these results indicate that suPAR is a novel marker that may help to discriminate between patients with high disease activity and those with moderate disease activity or in remission.

suPAR IN SYSTEMIC SCLEROSIS (13)

Systemic sclerosis (SSc) is a chronic connective tissue disorder characterized by microvascular injury, fibrosis and autoimmunity that affects the skin and internal organs (23). There are two major subtypes of SSc; dcSSc (dominantly affecting the skin) and lcSSc (involving the lung). To date, there is still no systemic marker that supports the clinical follow-up of organ specific disease activity. Currently, erythrocyte ESR and CRP levels are routinely used to assess SSc disease activity and severity (24).

We measured suPAR in 83 SSc patients who fulfilled the criteria proposed by the American College of Rheumatology (25). While CRP levels were comparable, suPAR and ESR values were higher than normal in SSc patients (see Table). suPAR values were higher in lcSSc than in dcSSc and correlated with the presence of anti-Scl70. Interstitial lung disease assessed by diffusing capacity for carbon monoxide (DLCO) and forced vital capacity (FVC) was more severe in patients with high suPAR; these parameters correlated inversely with suPAR levels. SSc patients with pulmonary fibrosis and pulmonary arterial hypertension also exhibited higher suPAR levels than those without these complications. Microvascular changes including the presence of digital ulcers, Raynaud phenomenon and NC abnormalities and arthritis were also more prevalent with high suPAR values.

These findings support the notion that suPAR may provide additional information to traditional biomarkers that help the objective assessment of complicated SSc.

CONCLUSIONS

These studies indicate that suPAR may be a useful biomarker of inflammation in several types of ACTD characterized by low-grade or transient inflammatory periods. However, the clinical use of suPAR in these conditions requires the clarification of several issues.

What suPAR levels should be used for decision making?

In several conditions the decision should be based on well defined cutoff values; suPAR above the limit may indicate an increased risk of inflammation and/or complications. However, based on the currently available data it is still uncertain which cut-off values are to be used. (The similar

uncertainty exists for sepsis, a much more extensively investigated condition with different cut-off values suggested [6,8].)

This uncertainty is partly due to methodological issues. It is still unclear whether any change in lot numbers / manufacturers would affect the results. In addition, the lack of external quality control programs for this parameter still prevents the routine use of suPAR.

What is the physiological basis of suPAR alteration?

suPAR is cleaved from the cell membranes by the action of proteases including cathepsin-G, PI-PLC, plasmin, chymotripsin, matrix metalloproteases (MMPs) etc. Inflammatory and endocrine milieu clearly influences the activity of these enzymes, and, probably, suPAR levels. The over-activation of the adrenal gland is a common feature in the diseased patients. Of note, adrenal hormones clearly influence MMP activity and, therefore, may contribute to suPAR production (26). Therefore, one may assume that any increase of suPAR levels may be a surrogate marker of increased adrenal activities. The increase in adrenal hormone levels including glucocorticoids may be due to the progression of disease, but is also inherent with therapeutic interventions routinely applied in autoimmune disorders. However, still there are no data to test this possibility.

What clinical factors additional to systemic inflammation influence suPAR levels?

The factors and conditions interacting with suPAR levels are less clarified. Patients with autoimmune disorders often suffer from a polymorbid state and from complications partly associated with their background condition. Some data indeed demonstrated an inverse association between suPAR and renal function and a positive association between suPAR and age (6). It is unknown, however, how other factors such as hepatic failure, or different therapeutic regimes influence suPAR levels.

From these pilot data suPAR is seemingly a good biomarker to obtain an impression whether patients with SLE, SSc or RA are subjected to an increased inflammatory status. However, the introduction of such a novel parameter in everyday practice requires more extensive clinical observations collected during prospective studies. The results will serve to decide whether suPAR is suitable to be used as a clinical biomarker in patients with autoimmune connective tissue disorders.

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In this issue: Celebrating the 70th Anniversary of the Hungarian Society of Laboratory Medicine

The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Deficiencies of the natural anticoagulants – novel clinical laboratory aspects of thrombophilia testing

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Key words:
thrombophilia, antithrombin, protein C, protein S, thrombophilia testing

ABSTRACT

Venous thrombosis is a typical common complex disease as acquired and genetic causes play a role in its development. The different „loss of function“ mutations of the natural anticoagulant system lead to antithrombin (AT), protein C (PC) and protein S (PS) deficiencies. Since thrombophilia testing has high cost and it has several methodological issues (analytical, pre-analytical), which makes the interpretation of results difficult, considerations should be made on the indications of testing, on the parameters that are measured and on the best available method to use. The latest guideline on clinical and laboratory management of thrombophilia kept the relatively old laboratory recommendations unchanged. This is partly because of the existence of unresolved problems with the laboratory tests used for diagnosis. Based on the literature and our previous research here we discuss the unresolved problems, the recently raised questions and issues concerning AT, PC and PS laboratory diagnosis and summarize the recent findings in molecular genetic investigations.

INTRODUCTION

Thrombosis is a common pathology underlying atherothrombotic diseases and venous thromboembolism (VTE), which are highly frequent and the major determinants of morbidity and mortality (1). Primary and secondary prevention is key to reducing death and disability from these diseases. VTE is a typical common complex disease as acquired and genetic causes play a role in its development (2). The different „loss of function" mutations of the natural anticoagulant system lead to antithrombin (AT), protein C (PC) and protein S (PS) deficiencies and the "gain of function" mutations known as Factor V Leiden (FVL) resulting in activated PC (APC) resistance and prothrombin 20210A allele (FII20210A) are responsible for the majority of inherited thrombophilia. Further hereditary factors are non-O blood group, elevated factor VIII, IX and XI, certain types of dysfibrinogenaemia and hyperhomocysteinaemia, however except for blood type all of these may have acquired components in their variability. Antiphospholipid syndrome (APS) is an acquired condition (3). The incidence of thrombosis in individuals having inherited thrombophilia is variable; it depends on the particular genotype, the co-existence of other genetic alterations (polymorphisms) and environmental factors (4). Moreover, several so far unidentified genetic factors may contribute to the risk of VTE, as it is suggested by the different genome-wide –association studies, like MARTHA or FARIVE (5).

RECOMMENDATIONS FOR THROMBOPHILIA TESTING-RECENT STATEMENTS

After the discovery of the above-mentioned hereditary risk factors testing for thrombophilia became more and more popular and the number of laboratory requests showed a rising tendency. Since thrombophilia testing has high cost and it has several methodological issues (analytical and pre-analytical ones; mentioned later in detail) which makes the interpretation of results difficult, considerations should be made on the indication of testing, on the parameters that are measured and on the best available method to use. In recent years, several contradictory papers and recommendations have been released by experts, different committees and working groups on the indications for thrombophilia testing and on the laboratory parameters to be determined (6-16). One can conclude from these that thrombophilia testing should be performed in a very carefully selected population in which the test results have a direct impact on the clinical decision either on primary or secondary thrombosis prophylaxis. Thrombophilia testing is not recommended routinely after a provoked VTE according to most of the guidelines, however the definition of "provoked" itself is not always clear in the different papers. There are situations, or conditions in which thrombophilia testing is advisable according to most of the recommendations. These are idiopathic (unprovoked) VTE, especially below the age of 50 years, thrombosis in unusual sites, recurrent VTE, first VTE with strong positive family history, asymptomatic family members of relatives having severe inherited thrombophilia, pregnancy complications or in women taking contraceptive pills, or under hormonal replacement. Thrombophilia testing, although its association with arterial thrombosis is uncertain mainly due to the lack of large population-based studies, may be considered in young patients especially without any well-defined risk factors of arteriosclerosis. A comprehensive review has been published most recently on the clinical aspects of thrombophilia testing, in which the existing guidelines are summarized (17). The major question is to estimate the risk of recurrence after the first VTE, which influences the duration (and perhaps the aggressiveness) of

anticoagulation. The VTE risk for asymptomatic family members of a proband with thrombophilia is the second important issue, when primary prophylaxis is considered in different risk situations. If thrombophilia testing helps to answer these questions its execution is definitely worthwhile.

THE THROMBOPHILIA PANEL

As no single well-standardized and widely accepted method exists for thrombophilia screening a list of investigations should be performed in a patient suspected for thrombophilia. The latest guideline kept the old (2001) laboratory recommendations unchanged (8). This is partly because of the existence of unresolved problems with the laboratory tests used for diagnosis, especially in the case of AT, PC and PS deficiencies.

Investigations for thrombophilia usually include AT, PC and PS assays, tests for APC resistance and/or FVL and the FII20210A. This panel is completed by the laboratory investigations for APS (18). It is advisable to perform the screening tests of coagulation (i.e. prothrombin time, activated partial thromboplastin time, thrombin time) to detect the presence of different anticoagulant drugs, which may interfere with certain laboratory tests. Thrombin time is also useful to screen for fibrinogen abnormalities, like dysfibrinogenaemia. Some authors also recommend testing for elevated FVIII and for APC resistance not due to FVL. Thrombophilia testing should be completed by measurement of plasma homocysteine and blood typing is also advisable (19). Besides taking the correct parameters to be tested into consideration, appropriate timing of investigation is also important (18).

LABORATORY DIAGNOSIS OF INHERITED AT, PC AND PS DEFICIENCIES

Two reviews are recommended for interested readers, which describe the molecular basis and epidemiology of AT, PC and PS deficiencies and introduction into the laboratory issues (20, 21). In this paper we are going to discuss the unresolved problems, the recently raised questions and issues concerning AT, PC and PS laboratory diagnosis and summarize the recent findings in molecular genetic investigations.

STRUCTURE AND FUNCTION OF ANTITHROMBIN; ANTITHROMBIN DEFICIENCIES

AT is the most important circulating inhibitor of blood coagulation proteases, synthesized by hepatocytes and is a member of the serine protease inhibitor (serpin) superfamily (20). The mature AT molecule is a single-chain 58 kDa glycoprotein with half-life of 2.4 days. The plasma concentration of AT is around 150 mg/L in the circulation. AT contains an N-terminal heparin-binding domain, a carbohydrate rich domain and a COOH-terminal serine protease-binding domain. It has two isoforms that differ only in the extent of glycosylation. The major α isoform, which represents 90-95% of total AT, is N-glycosylated on 4 Asn residues (127, 167, 187 and 224), while the β isoform (5-10%) is not glycosylated at Asn167. This latter isoform has higher affinity to negatively charged glycosaminoglycans, like heparin. The AT encoding gene, *SERPINC1* is located on the chromosome band 1q23-25, has 7 exons and 6 introns. The heparin-binding site of AT is encoded by exon 2 and exon 3. The reactive site, which is located in the carboxy-terminal part of the protein, is encoded by exon 7.

AT primarily inactivates thrombin mediated fibrin clot formation and the generation of thrombin by activated FX (FXa). It is also able

to inhibit activated coagulation factors FXII, FXI and FIX (FXIIa, FXIa and FIXa) in the intrinsic and FVIIa-tissue factor complex in the extrinsic pathway (22).

AT deficiency was first described by Egeberg in 1965 (23) and the first functional AT defect, named as AT Budapest, was reported by Sas et al in 1974 (24). AT deficiency is classified as type I (quantitative) and type II (qualitative) (25). In type II deficiency, the defect may involve the reactive site (II RS), the heparin-binding site (II HBS) or it may exert a pleiotropic effect. Individuals with inherited AT deficiency have a highly increased thrombotic risk and homozygosity in type I deficiency and in most type II deficiencies, with notable exception of type II HBS variants, are incompatible with life (26). The type II HBS deficiency is considered as a lower risk of VTE (27). The mutation profile of *SERPINC1* is highly heterogeneous and the most prevalent mutations are AT Cambridge II (p.Ala416Ser), AT Budapest 3 (ATBp3, p.Leu131Phe) and AT Basel (p.Pro73Leu), which were reported in a number of studies (28-34). AT Cambridge II is frequent in the British population; the mutation has a prevalence around 0.5-2.0% in French, Spanish and German VTE patients. AT Cambridge II, however was not detected in other populations like in Hungary and in Southern China (32, 35). The ATBp3 is a founder mutation in the Hungarian population with prevalence of 86.5% within type II HBS deficiency (32).

METHODOLOGICAL PROBLEMS AND RECENTLY RAISED QUESTIONS IN ANTITHROMBIN DEFICIENCY

A first-line test for the diagnosis of AT deficiency is based on a chromogenic functional assay, in which the inhibition of FIIa or FXa by AT in the presence of heparin is detected by measuring the residual enzyme (FIIa or FXa) activity using their specific chromogenic substrates (20). If the assay is performed in the presence of heparin, which ensures a fast inhibitory effect of AT, the assay is named as heparin-cofactor AT test (hc-anti-FIIa, or hc-anti-FXa AT assay). If heparin is not used in the assay then the so-called progressive AT activity is measured (p-anti FIIa or p-anti-FXa AT assay). Several commercially available reagents can be used for measuring AT activity and the heparin cofactor AT activity assays are dedicated to detect all types of AT deficiency. In case of using hc-anti-FIIa AT activity assay, it is important to choose those with bovine thrombin instead of human thrombin to avoid the influencing effect of heparin cofactor II on AT activity results (36). Human vs. bovine source of enzyme is not a problem in hc-anti-FXa assays, since FXa does not react with heparin cofactor II at all.

According to the latest results of the external quality control surveys both hc-anti-FIIa and hc-anti-FXa assays are used in equal number by the different laboratories. In the latest UK NEQAS program 119 and 184 laboratories used hc-anti-FIIa and hc-anti-FXa assays, respectively and in the ECAT program 154 and 145 laboratories reported results by using hc-anti-FIIa and hc-anti-FXa assays, respectively. Among hc-anti-FIIa assays, Siemens Berichrom AT and Diagnostica Stago Stachrom AT are the most popular ones. Among hc-anti-FXa assays Siemens Innovance AT, Werfen HemosIL (liquid) AT and Chromogenix Coamatic AT tests are performed by most of the laboratories. All these kits have very similar performance within the reference interval (i.e. in the case of non-AT deficient samples). It is of great importance, however, to realize that despite the numerous functional assays available on the market, no single one appears to be able to recognize all defects (37). FXa-based assays in general are less sensitive to detect AT deficiencies caused by certain mutations around the reactive site, like in the case of AT Cambridge II (28,29). On the contrary, it was demonstrated

by some studies that hc-anti-FXa assays had higher sensitivity to type II HBS AT deficiency (37, 38). In our study Siemens Berichrom AT test was inferior to Siemens Innovance AT and Labexpert AT H+P assays. The latter two tests, which are based on hc-anti-FXa methodology, gave practically identical results with all AT deficient patients (n=37) (39). By the investigation of the highest number of patients with the type II HBS ATBp3 (n=102), we confirmed that the hc-anti-FXa assay (Innovance AT) was the method of choice in type II HBS AT deficiency (32). In the study of Puurunen et al. patients with AT Basel (n=88) were also successfully detected by Innovance AT reagent (34). It is interesting that there are big differences in the sensitivity even among hc-anti-FXa assays to type II HBS AT deficiency (37, 40). According to the results of these studies the HemosIL AT and the Coamatic AT reagents were less sensitive, while Innovance AT

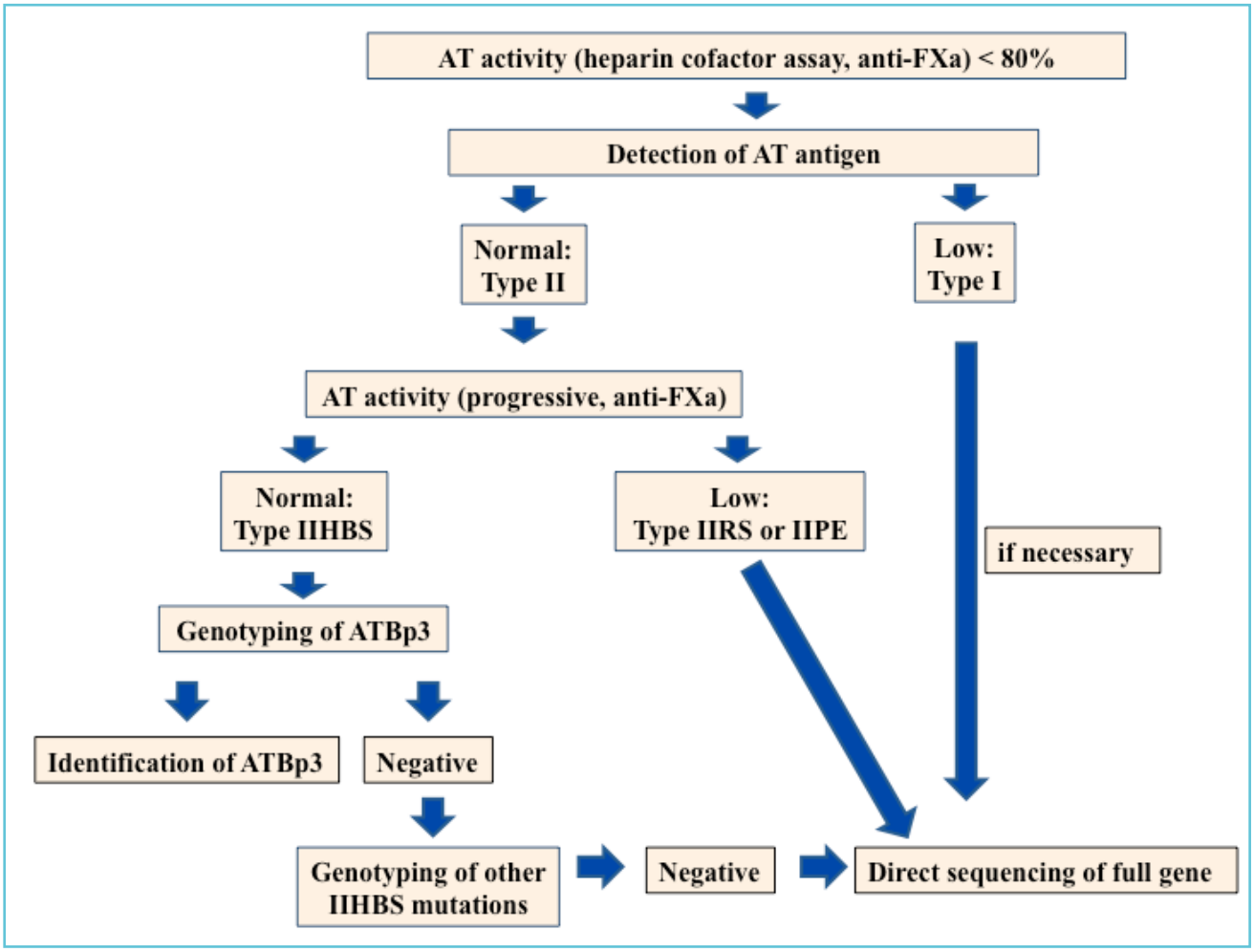
Table 1 Standard characteristics of the different commercial heparin cofactor anti-FXa AT activity assays

	Substrate source	Incubation time	Heparin concentration	Chromogenic substrate	Sample predilution	Dilution buffer	Final dilution of sample
Siemens Innovance® Antithrombin	Human FXa	180-190 s	1500 U/L	Z-D-Leu-Gly-Arg-ANBA-methylamide-acetate	1:4	Tris/HCl pH 8.0	1:20
HemosIL® Liquid Antithrombin	Bovine FXa	100-140 s	3000 U/L	S-2765 (N-α-Z-D-Arg-Gly-Arg-pNA•2HCl)	1:40	0.15 M Sodium Chloride	1:120
Chromogenix Coamatic® Antithrombin	Bovine FXa	100-140 s	5000 U/L	S-2765 (N-α-Cbo-D-Arg-Gly-Arg-pNA·2HCl)	1:121	0.15 M Sodium Chloride	1:484
Hyphen Biophen Antithrombin	Bovine FXa	60 s	(ready to use)	SXa-11 (Suc-Ile-Glu-(γPip)Gly-Arg-pNA, HCl)	1:20	0.15 M Sodium Chloride	1:170
Labexpert Antithrombin H+P	Bovine FXa	60 s	1 USP units/mL	BIOPHEN CS-11 [Suc-Ile-Gly-(γPip)Gly-Arg-pNA, HCl]	1:50	50 mmol/L pH 8.4 Tris-HCl	1:150

showed practically 100% sensitivity. This suggests that not only the source of enzyme (FIIa or FXa) is responsible for the difference in performance of AT functional tests. Table 1 demonstrates the assay characteristics of the most commonly used hc-anti-FXa AT assays. All but one reagent contains bovine FXa as enzyme in the reaction. The different tests differ in plasma dilution, in the added heparin concentration, in the source of chromogenic substrate and also in the incubation time. It can be concluded that clarification of the situation concerning heparin cofactor AT functional assays warrants future research to establish improved recommendation for AT testing.

Progressive AT assay is based on the same principle as heparin cofactor assays, but it is performed in the absence of heparin on less diluted plasma samples usually with prolonged incubation time. It was demonstrated in several clinical samples that comparison of p-anti-FXa and hc-anti-FXa activities is a useful tool in the diagnosis of type II HBS AT deficiency since in this type hc-anti-FXa decreases, while p-anti-FXa remains

Figure 1 **Laboratory diagnostic algorithm in antithrombin deficiency used in the authors' laboratory**



AT, antithrombin; type II HBS, heparin-binding site AT deficiency; type II RS, reactive site AT deficiency; type II PE, AT deficiency with pleiotropic effects; ATBp3, AT Budapest 3 deficiency caused by p.Leu131Phe mutation

normal, or shows only a slight decrease. The ratio of p-anti-FXa and hc-anti-FXa therefore is well above the upper limit of the reference interval in heterozygous type II HBS patients and even higher in homozygotes (41). The reagent developed in our laboratory (Labexpert AT H+P) is able to measure both hc-anti-FXa and p-anti-FXa AT activities and reference interval has been determined according to CLSI guidelines for both. A diagnostic algorithm that is followed in our laboratory is shown in Figure 1.

MOLECULAR GENETIC DIAGNOSIS IN AT DEFICIENCY

Molecular genetic testing is a useful diagnostic tool for confirming inherited AT deficiencies especially for patients with borderline activities (31). Moreover, genetic analysis helps to distinguish among the different AT deficiency subtypes that has of great importance from the point of view of clinical patient management. The mutation detection rate in the case of *SERPINC1* in general is quite high, more than 80%. Current evidence shows that not all *SERPINC1* mutations causing AT deficiency lead to decrease in AT activity in the commercially available functional assays (42). Genetic analysis has been therefore recently suggested to be included in clinical practice when screening for AT deficiency in individuals experiencing unprovoked thrombotic diseases, even if the hc-AT activity is above 80% (43).

In *SERPINC1* 215 causative mutations were found before 2010; since then 61 mutations were reported in HGMD database (Table 2). It is to be noted, however, that several novel variants have not been reported in the HGMD, yet (Table 3) (44-50).

Table 2 Distribution of different mutations within the genes for antithrombin, protein C and protein S

Mutation types	*SERPINC1*	*PROC*	*PROS1*
Missense/nonsense	155 (56%)	231 (74.5%)	171 (64%)
Splicing	17 (6%)	25 (8%)	27 (10%)
Regulatory	0 (0%)	12 (4%)	3 (1%)
Small deletions	52 (19%)	24 (8%)	28 (10%)
Small insertions	23 (8%)	13 (4%)	15 (6%)
Small indels	2 (0.8%)	3 (1%)	4 (1.5%)
Gross deletions	24 (9%)	2 (0.5%)	14 (5%)
Gross insertions/ duplications	1 (0.4%)	0 (0%)	4 (1.5%)
Complex rearragements	2 (0.8%)	0 (0%)	2 (1%)
Total	**276**	**310**	**268**

According to HGMD database (The Human Gene Mutation Database http://www.hgmd.cf.ac.uk/ac/search.php*), accessed on 14th December 2015.*

Table 3 Novel mutations in *SERPINC1* published in the last five years and not indicated in the HGMD database

	Nucleotide exchange	Amino acid exchange	Type of deficiency	References
Missense/ nonsense	c.133 C>T	p.Arg45Trp	I†	Caspers (2012)
	c.134 G>A	p.Arg45Gln [1]	I	Deng (2013)
	c.335 C>G	p.Pro112Arg [2]	I	Maruyama (2013)
	c.342 T>G	p.Ser114Arg [1]	I	Deng (2013)
	c.347 C>T	p.Ser116Phe	I†	Caspers (2012)
	c.452 T>G	p.Ile151Ser	I†	Caspers (2012)
	c.455 A>C	p.His152Pro	I†	Caspers (2012)
	c.458 T>A	p.Phe153Tyr	NA	Zeng (2015)
	c.464 T>G	p.Phe155Cys	I	Ding (2013)
	c.491 G>A	p.Arg164Gln	NA	Zeng (2015)
	c.539 G>A	p.Gly180Glu	I†	Caspers (2012)
	c.569 A>C	p.Tyr190Ser	I†	Caspers (2012)
	c.569 A>G	p.Tyr190Cys	I†	Caspers (2012)
	c.598 G>C	p.Ala200Pro	NA	Zeng (2015)
	c.883 G>A	p.Val295Met	NA	Zeng (2015)
	c.886 G>C	p.Ala296Pro	I†	Caspers (2012)
	c.899 A>G	p.Gln300Arg	I†	Caspers (2012)
	c.934 A>G	p.Thr312Ala	IIRS	Bhakuni (2015)
	c.938 T>C	p.Met313Thr	NA	Zeng (2015)
	c.1114 C>T	p.Leu372Phe	I	Ding (2013)
	c.1121 A>G	p.Asp374Gly	IIRS	Castaldo (2012)
	c.1307 C>G	p.Ala436Gly	I†	Caspers (2012)
	c.178 A>T	p.Lys60X	I†	Caspers (2012)
	c.203 C>G	p.Ser68X	I	Ding (2013)
	c.1016 G>A	p.Tyr339X	I	Ding (2013)
	c.1024 G>T	p.Glu342X	I†	Caspers (2012)
	c.1394 A>C	p.X465Sext28*X	I	Castaldo (2012)

Splicing	c.408 +1 G>A	-	I†	Caspers (2012)
	c.409 -10 G>A	-	I†	Caspers (2012)
	c.624 +1 G>T	-	I†	Caspers (2012)
	c.1219 -1 G>A	-	I	Castaldo (2012)
Small deletions	c.86_87delinsCT	p.Cys29Ser	NA	Zeng (2015)
	c.173del	p.Pro58ArgfsX3	I	Castaldo (2012)
	c.412_417del	p.Phe138-139Lysdel	I†	Caspers (2012)
	c.457_459del	p.Phe154del	I†	Caspers (2012)
	c.462_464del	p.Phe155fs	I†	Caspers (2012)
	c.490del	p.Arg164GlufsX8	I	Nadir (2015)
	c.614del	p.Leu205fs	I†	Caspers (2012)
	c.712_719del	p.Asn240fsX1	I†	Caspers (2012)
	c.1332_1333del	p.Ile444MetfsX19	II	Castaldo (2012)
	c.1390_1393del	p.X465MetfsX13	I	Castaldo (2012)
Small insertions	c.1172dupG	p.Asp392fs	I†	Caspers (2012)
	c.1340_1341insA	p.Pro448SerfsX16	IIHBS	Bhakuni (2015)
Gross deletions	c.243_263del	p.80-86del	I	Castaldo (2012)
	Exon 6-7	-	I	Caspers (2012)
Large in-frame insertion/ deletion	c.625_630del_30ins	p.Glu241_ Leu242del_241_243ins_ Val_Leu_Val_Leu_Val_ Asn_Thr_Arg_Thr_Ser [3]	IIHBS	Martínez-Martínez (2012)
	c.1066_1083del	p.Arg356_Phe361del [4]	I	Zeng (2015)

These data were collected from publications available on NCBI-PubMed (indexed by MEDLINE) database.
Nucleotide and amino acid numbering follows the HGVS nomenclature.
NA, non applicable (i.e. AT functional assay showed normal result)

† These mutations seem to lead to type I deficiency, however they were not confirmed. In vitro expression studies indicated:
[1] decreased AT secretion and heparin affinity
[2] impaired secretion and intracellular degradation
[3] impaired heparin affinity and the mutation transforms the structure of serpin
[4] represented impaired secretion and reduced functional activity

STRUCTURE AND FUNCTION OF PROTEIN *C* AND *S*; PROTEIN *C* AND *S* DEFICIENCIES

Protein C and S are Vitamin-K-dependent plasma glycoproteins with molecular masses of about 62 and 71 kDa, respectively (21). Plasma concentrations and half-lives of PC and PS are 3-5 mg/L and 20-25 mg/L, and 8h and 42h, respectively. The domain structure of PC and PS is highly homologous to other vitamin K-dependent coagulation factors (pre-pro leader sequence, Gla-domain and epidermal growth factor like (EGF) domains). PC is a two-chain protein in its mature form and it contains an activation peptide domain and the serine protease domain, which is responsible for its anticoagulant effect. PS is a single-chain molecule having a thrombin-sensitive region (TSR) and a C-terminal region homologous to the sex-hormone-binding globulin (SHBG-like domain). The gene encoding PC (*PROC*) is positioned on chromosome 2q13-q14 and contains nine exons, eight of which encode the protein and the 1.7-kb messenger RNA (mRNA) and 8 introns (51). The human PS gene, *PROS1*, is located on chromosome 3q11.2, where it spans 80 kb of genomic DNA and contains 14 introns and 15 exons. In addition to the active gene, a transcriptionally inactive pseudo gene (*PROS2*) is located on chromosome 3. It shows 97% homology to the active gene. This makes the molecular genetic investigations of PS deficiency difficult (please see below).

The zymogen PC is activated by the thrombin-thrombomodulin (TM) complex on the surface of endothelial cells, and binding to endothelial protein C receptor (EPCR) further increases its activation rate (4). Activated PC (APC) inactivates membrane bound activated factor V (FVa) and activated factor VIII (FVIIIa). The free form, approximately 40% of total PS, which is not bound to its natural binding protein (C4bBP), acts as a cofactor for APC. It is to be noted that PS also has APC independent anticoagulation effects that are not investigated in routine laboratories (52, 53). Both APC and PS have roles in a variety of physiological processes distinct from hemostasis. APC has a direct cytoprotective nature (54). PS is involved in cell proliferation/survival, apoptosis, regulation of inflammatory cytokine release, atherosclerosis, vasculogenesis and cancer development (55). This issue, although very interesting, is beyond the scope of this review.

In type I PC deficiency, which is more common (75-80%), both the activity and antigen concentration of PC is decreased, which suggests defective protein synthesis and/or secretion. In type II deficiency, normal amount of dysfunctional protein is synthesized, and the functional defect can be due to abnormalities in substrate, calcium-ion or receptor binding (56). Type I PS deficiency is associated with a decrease in the total PS antigen and free PS antigen, and hence a decrease in APC cofactor activity; type II is a qualitative deficiency, characterized by a normal total and free PS antigen level but a decrease in the APC cofactor activity; and in type III deficiency there is a normal total PS antigen but a decrease in free PS antigen and in APC cofactor activity. Several reports have proposed that types I and III deficiencies may be phenotypic variants of the same genetic disease (57).

METHODOLOGICAL PROBLEMS AND RECENTLY RAISED QUESTIONS IN PROTEIN C AND S DEFICIENCY

For routine screening and classification of PC and PS deficiencies two types of assays are available, functional tests and antigen assays (21). In the diagnosis of PC deficiency, first, a functional test should be performed and in case of abnormal results, a PC antigen is measured. The ratio of PC activity to PC antigen is then calculated, which can distinguish type I from type II deficiency (58). PC activity can be measured in

plasma by either a clotting-based assay (mostly based on APTT measurement) or chromogenic (amidolytic)-based assay, and PC antigen is usually measured by ELISA. In the diagnosis of PS deficiency, the clotting-based PS activity assays are designed to measure APC-dependent anticoagulant activity. PT-, APTT- or FXa-based assays are commercially available. No chromogenic functional assay is available. Free PS antigen is considered as the "functional" anticoagulant fraction of PS although it is not a true measure of activity. Free PS antigen is determined using ELISA, or immunoturbidimetry. Total PS assays measure both the free and bound fractions of PS by immunological methods. The principles of these assays are detailed elsewhere (21).

Functional assays of PC and PS have several advantages and disadvantages. Clotting-based assays of PC can detect all aberrations regarding the activation, activity, cofactor-and phospholipid (PL) binding, while chromogenic PC assay can analyze only the core function (i.e. activation and activity) of the protein and can not detect defects in cofactor binding, PL surface binding, and receptor binding (59). Due to this phenomenon some cases with mutations affecting regions, which are responsible for these interactions (PC deficiency type IIb) are not detected by the chromogenic functional assay and remain undiagnosed. Chromogenic assays are subjected to interference from haemolysis, icterus and lipemia. Results of clotting tests of PC and PS are influenced by a lot of factors that have an effect on clotting time (i.e. lupus anticoagulant, anticoagulant drugs including direct FXa or thrombin inhibitors and high FVIII level). Presence of APC resistance (FVL) is a special issue. In patients having FVL mutation the PC and PS activity values measured by the clotting assays (despite diluting the patient's plasma in PC or PS deficient plasma wild type for FVL and using wild type FVa as substrate) are falsely decreased. Since antigenic assays are not influenced by APC resistance and give normal result, FVL patients may be misdiagnosed as type II PC or PS deficiency (60-62). Antigenic assays may be subjects of interferences generally seen in immunological methods (e.g. rheuma factor).

According to the experiences in the international external quality control programs there is no consensus among the laboratories as to which functional assays are better for detecting PC and PS activity. Several assays (both chromogenic and clotting based for PC) are commercially available with different sensitivity, specificity and significant variability. Table 4 demonstrates the most frequently used methods in the routine diagnosis of PC and PS deficiencies as it is indicated in two ECAT surveys. Concerning PC functional assays, chromogenic methods that show lower between-laboratory variability are used by the majority of participants (approximately 75%). In the latest NEQAS survey n=290 vs. n=14 laboratories preferred the chromogenic method. PC antigen is not measured routinely by a large number of laboratories. The ratio of laboratories measuring PS activity vs. free PS antigen is approximately 0.7 in the ECAT and 0.24 (!) in the NEQAS program, that reflects the unresolved methodological problems in PS functional assays. Total PS antigen is measured only by a minority of the laboratories. It is well demonstrated in the table that presence of FVL influences the results of clotting-based PC and PS assays, the lowest values are obtained by Siemens reagents. PS activity in general is markedly lower than free PS antigen. Baron et al analyzed North American Specialized Coagulation Laboratory Association (NASCOLA) PC deficiency testing data from six surveys conducted in 2009 and 2010 (63). They demonstrated that performance of the assays showed considerable variety.

Based on the above-mentioned problems there are controversies in the different recommendations

Table 4 Most commonly used assays for PC/PS determination and their results in the latest surveys of ECAT Foundation

Type of assays *n*		Plasma of a patient with a heterozygous PS deficiency			Abnormal Coagulation Control Plasma			Normal Coagulation Control Plasma			Plasma of a patient with a heterozygous Factor V Leiden mutation		
		assigned value	*CV (%)*	*n*	*assigned value*	*CV (%)*	*n*	*assigned value*	*CV (%)*	*n*	*assigned value*	*CV (%)*	*n*
Protein C	**Chromogenic activity**	**267**	**89**	**5.2**	**267**	**20**	**14.9**	**255**	**98**	**5.0**	**255**	**109**	**5.1**
	Chromogenix Coamatic Protein C	25	89	5.2	25	18	20.9	27	97	6.8	27	109	6.4
	Hyphen Biomed Biophen Protein C	16	89	4.1	16	21	9.0	14	97	4.6	14	110	4.2
	I.L. HemosIL Protein C	71	88	4.5	71	17	9.4	62	95	3.4	62	107	4.1
	Siemens Berichrom Protein C	94	91	5.7	94	21	10.1	96	98	5.5	96	110	5.5
	Diagnostica Stago Stachrom Protein C	59	90	5.3	59	21	7.3	54	100	3.3	54	108	4.2
	Clotting activity	**86**	**90**	**10.0**	**84**	**18**	**30.4**	**77**	**105**	**10.9**	**77**	**98**	**15.9**
	I.L. HemosIL Proclot C	13	94	8.2	13	12	40.7	14	105	9.4	14	92	10.6
	Siemens Prot C Reagent (coagulometric)	20	86	6.0	20	25	16.2	18	98	5.6	18	85	9.2
	Diagnostica Stago Staclot Protein C	42	92	9.1	42	16	12.7	35	112	10.0	35	109	12.4
	Antigen (Enzyme Immuno Assays)	**70**	**81**	**9.8**	**71**	**20**	**13.7**	**67**	**96**	**11.9**	**67**	**99**	**9.8**
	BioMerieux Vidas Protein C	13	76	8.3	13	21	ND	13	89	5.5	13	96	2.9
	Diagnostica Stago Asserachrom PC	28	82	8.9	28	20	13.5	28	98	11.6	29	100	10.5

Protein S	**Activity**	**155**	**34**	**12.7**	**155**	**26**	**19.2**	**148**	**76**	**11.2**	**148**	**61**	**19.8**
	I.L HemosIL ProS	28	37	8.9	28	19	20.2	28	74	8.3	28	62	12.6
	Siemens Protein S Ac	40	35	14.8	40	25	13.1	40	68	10.2	40	45	19.4
	Diagnostica Stago Staclot Protein S	80	33	9.9	80	28	10.6	72	80	6.8	72	68	8.3
	Free PS antigen Latex Immuno Assays	**220**	**37**	**10.8**	**220**	**31**	**7.2**	**211**	**90**	**6.4**	**211**	**80**	**6.7**
	Coamatic Free PS/I.L. Hemosil Free PS	93	35	11.4	93	31	7.8	92	91	6.7	92	81	8.6
	Siemens Innovance Free Prot. S antigen	53	40	5.5	53	30	5.8	47	91	3.7	47	80	4.4
	Diagnostica Stago Liatest Free Protein S	71	37	8.3	71	30	7.3	67	87	6.0	67	80	5.7
	Enzyme Immuno Assays	**38**	**38**	**22.6**	**38**	**29**	**15.7**	**37**	**85**	**12.1**	**36**	**78**	**9.6**
	Diagnostica Stago Asserachrom Free PS	24	35	13.1	24	27	9.1	24	83	6.6	23	76	6.7

The table summarizes the most frequently used (n>10 laboratories provided results) commercially available assays for PC/PS according to the data provided in ECAT survey 2015-3 and 2014-4. Since CV was not calculated if less than 10 laboratories sent results obtained by a certain method, these methods are not described here. Total PS antigen is measured by the minority of the laboratories therefore no data is shown here. ND: not determined

for diagnosis of PC and PS deficiencies. Most of the guidelines recommend the use of chromogenic PC assays because these assays are less subjected to interference and more specific than clotting based assays (8, 58). Furthermore, chromogenic assays seemed to be cost-effective (64). In the contrary, clotting based assays are preferred by others since they ensure the diagnosis even in type IIb PC deficiency (65). Since the prevalence of IIb PC deficiency is not known and may show differences in different populations the ratio of undiagnosed cases remains uncertain by using chromogenic assay alone. For example, the p.Asn44Ile (c.131 C>T) causative mutation in the Gla-domain of PC was detected only by clotting assays, moreover there was a great discrepancy among the PC activity values of the different clotting assays (66, 67).

Recommendations concerning PS deficiency are also heterogeneous, free PS antigen is considered as superior over clotting functional assay, however, type II PS deficiency is misdiagnosed by the exclusive usage of it. The Plasma Coagulation Inhibitor Subcommittee of the International Society of Thrombosis and Hemostasis (ISTH) is now working on exploring the background of the discrepancies in PS activity results obtained by the different reagents. Until the development of a reliable PS functional assay that is free from FVL (and other) interferences the PS activity assay alone is definitely not suitable for the laboratory diagnosis of PS

deficiency and the investigation of free PS antigen and sometimes, molecular genetic tests are also suggested.

A Japanese study group developed a novel assay system for precise simultaneous determination of total PS activity and total PS antigen level, allowing PS specific activity (ratio of total PS activity to total PS antigen level) to be calculated (68). In this assay, first PS in the patient's plasma and C4bBP are dissociated by high dilution and adding liposome A that has high affinity to PS. PS is then activated by APC in the presence of PL and calcium and human FVa as substrate is added. The degradation of FVa is followed by a chromogenic reaction in which bovine FXa, prothrombin and S-2238, the chromogenic substrate of thrombin is added in the presence of PL and the change in absorbance is detected at 405 nm. To measure total PS antigen levels, first purified C4bBP is mixed with free PS in plasma and the concentration of total PS is then measured using a latex agglutination method. This assay showed good performance in detecting type II PS deficiency caused by PS Tokushima, p.Lys155Glu, a frequent mutation in the Japanese population. Interference of factors that make the results of commercial PS clotting assays difficult to interpret, especially FVL, however has not been evaluated with this reagent, yet.

MOLECULAR GENETIC DIAGNOSIS IN PC AND PS DEFICIENCY

Molecular genetic analysis of PC and PS deficiencies is also seemed to be useful like in the case of AT deficiency, but testing of patients with PC levels above 70% and with PS levels above 55% may not be indicated (31). The mutation detection rate by Sanger sequencing in the cases of *PROC* (69%) and *PROS1* (43%) is rather low. It is important to mention, that in the case of PS deficiency the presence of *PROS2* requires careful primer design to avoid amplification of pseudogene fragments. As second line test multiplex ligation-dependent probe amplification (MLPA) is suggested to detect large rearrangements and increase the mutation detection rate.

In *PROC* and *PROS1* 299 and 147 causative mutations were found before 2010; since then 11 mutations within *PROC* and 24 mutations within *PROS1* were reported in HGMD database (Table 2). It should be mentioned that several novel variants have not been reported in the HGMD, yet (e.g. ref. 69-72).

CONCLUSIONS

The majority of recommendations concerning AT/PC/PS deficiencies are based on low-quality evidence or on experts' opinions because they belong to the group of rare diseases (17) and it is impossible to conduct large clinical trials to explore the impact of diagnosis and treatment (prevention) in VTE patients, in asymptomatic affected individuals and in patients with arterial thrombosis. As in all rare diseases the impact of well-documented case reports and high quality research on genotype-phenotype associations and structural-functional studies is highly important in these diseases. More research is warranted in method development concerning well-established risk factors, like AT, PC and PS deficiencies and in clarifying so far unknown functional aspects of them. Due to the heterogeneous background of thrombosis and the different gene-gene, gene-environment interactions, population-based guidelines maybe helpful for thrombophilia testing regarding the patients' selection, the parameters to be tested and the correct methodology.

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The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Interpretation of blood microbiology results – function of the clinical microbiologist

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ARTICLE INFO

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Key words:
blood culture, sepsis, rapid diagnostics, microbiology techniques, laboratory workload

ABSTRACT

The proper use and interpretation of blood microbiology results may be one of the most challenging and one of the most important functions of clinical microbiology laboratories. Effective implementation of this function requires careful consideration of specimen collection and processing, pathogen detection techniques, and prompt and precise reporting of identification and susceptibility results. The responsibility of the treating physician is proper formulation of the analytical request and to provide the laboratory with complete and precise patient information, which are inevitable prerequisites of a proper testing and interpretation. The clinical microbiologist can offer advice concerning the differential diagnosis, sampling techniques and detection methods to facilitate diagnosis. Rapid detection methods are essential, since the sooner a pathogen is detected, the better chance the patient has of getting cured. Besides the gold-standard blood culture technique, microbiologic methods that decrease the time in obtaining a relevant result are more and more utilized today. In the case of certain pathogens, the pathogen can be identified directly from the blood culture bottle after propagation with serological or automated/semi-automated systems

or molecular methods or with MALDI-TOF MS (matrix-assisted laser desorption-ionization time of flight mass spectrometry). Molecular biology methods are also suitable for the rapid detection and identification of pathogens from aseptically collected blood samples. Another important duty of the microbiology laboratory is to notify the treating physician immediately about all relevant information if a positive sample is detected. The clinical microbiologist may provide important guidance regarding the clinical significance of blood isolates, since one-third to one-half of blood culture isolates are contaminants or isolates of unknown clinical significance. To fully exploit the benefits of blood culture and other (non- culture based) diagnoses, the microbiologist and the clinician should interact directly.

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BLOOD CULTURE – PRINCIPLE, INTRODUCTION

Bacteraemia/fungaemia can induce a systemic inflammatory response syndrome and as a clinical continuum can fall into life-threatening severe sepsis or septic shock. Huge number of studies have inferred that clinical outcomes in severe sepsis and septic shock hinge upon the optimized selection, dosing, and delivery of highly potent antimicrobial therapy (1, 2, 3). With this in mind the recovery of the causative agent is one of the most important tasks of the microbiological laboratory. Blood cultures- as a gold standard -, in which a sample of blood is allowed to incubate with a medium that promotes bacterial growth, are used to diagnose bacteraemia or fungaemia, confirmed by isolating one or more microorganisms from the blood culture. Clinicians are supposed to collect blood cultures (BC) from patients with clinical signs and symptoms indicating sepsis, or if the laboratory or imaging results suggest an infection, and the presumed infection is known to result in haematogenous spread, or the patient has a fever of unknown origin. For sample collection they should use national guidelines and recommendations of the local microbiological laboratory (4, 5, 6). Proper formulation of the analytical request by the treating physician is essential in order to provide the laboratory with complete and precise patient information, which are inevitable prerequisites of a proper testing and interpretation. The partner microbiological laboratory should prepare useful guidelines, which contain every important preanalytical rule (timing and sampling of blood culture – sample collection, volume of blood required, blood-to-broth ratio, formulation of the analytical request, and transportation. The microbiologist should aim to provide the clinician with proper results as soon as possible, utilizing every available diagnostic method when evaluating culture results (7, 8, 9). The clinician and the microbiologist should cooperate during the whole test procedure, but especially during the evaluation of the results, to ensure the highest possible standard of patient care. In this short summary, as microbiologists, our goal is to provide answers to the clinicians' most frequent questions.

WHEN SHOULD THE SAMPLE BE COLLECTED FOR BLOOD CULTURE?

In case of periodical bacteraemia and fungaemia blood should be collected at the beginning of the fever episode, during the chills or at the start of the fever curve. In case of continuous bacteraemia or fungaemia (e.g. suspicion of endocarditis) sample collection time is not critical (4).

WHAT AMOUNT OF BLOOD SHOULD BE COLLECTED, AND FROM WHERE?

In adults, if local infection is present or suspected, or the patient presents with fever of unknown origin, an amount of 20-30 ml of blood

collected from 2 venipuncture sights (the total amount of blood should be at least 40 ml) is sufficient. The samples should be collected strictly aseptically. If endocarditis is suspected, at least 3 samples are necessary because of the low bacterial count in the blood. In case of children, an amount decreased proportionally with body mass should be collected (see guidelines). Venipuncture should be performed on intact peripheral veins; except if catheter-associated infection is suspected, which case will be addressed separately. Theoretically, it is possible to distribute a sufficient amount of blood sampled from one sight to four different bottles, but in this case, the microbiology laboratory findings cannot aid in the evaluation of the clinical relevance of certain potential pathogens which colonize the skin (and may contaminate the sample), but can also cause infection in case of certain risk factors. Since the number of pathogens in the blood during bacteraemia/fungaemia is very low (0.1-300/ml depending on the patient's age and the pathogen), the sensitivity of BC is mostly determined by the amount of blood collected. Usually, BC containing samples from two or three venipuncture sights is sufficient to support or rule out sepsis; however, a single sample is insufficient (4, 10, 11).

WHAT IF CATHETER-ASSOCIATED INFECTION IS SUSPECTED?

In this case, blood samples should be taken through the catheter and a peripheral vein at the same time. Two pairs of BC is not necessary (one sample is enough), but if the catheter has multiple lumens, a sample should be taken through each lumen. If the time to positivity (TTP) of the sample taken through the catheter is at least 2 hours shorter than that of the sample from the peripheral vein, and the cultured microbe and its antimicrobial susceptibility is the same, catheter-associated infection can be diagnosed (12). If the catheter is removed because of suspected catheter-associated infection, the catheter end should be sent for culture as well.

WHAT TYPE OF BLOOD CULTURE BOTTLE SHOULD BE USED? IS THE ANAEROBIC BOTTLE OR THE SPECIALIZED FUNGI BOTTLE NECESSARY?

The blood collected from one sampling is usually distributed into two (an aerobic and an anaerobic) commercially available blood culture bottles in the amount specified by the manufacturer. An anaerobic bottle is recommended in patients with neutropenia, in case of complications following abdominal surgery, patients with diabetes and in patients with complicated wound infections. Small amounts of blood samples (1-5 ml) collected from children should be distributed into special childrens' bottles containing a smaller amount of media. Fungi usually grow well in aerobic BC media prepared for the culture of bacteria, but certain studies showed that the TTP is shorter when special fungi bottles are used. In case of patients under antibiotic treatment, bottles containing agents that inactivate antibiotics (activated carbon, resin) are recommended (4, 13).

HOW SHOULD INOCULATED BOTTLES BE STORED?

The inoculated BC bottles – if it is possible – should be sent to the microbiology laboratory immediately, otherwise the bottles should be stored at room temperature. Several studies have demonstrated (and it is included in the references of the commercially available bottles) that a certain time (12-16 h) of storage at room temperature, otherwise called delayed time vial entry, does not impact the BC result significantly (4, 14).

HOW LONG UNTIL A RESULT IS AVAILABLE?

Continuous monitoring systems have revolutionized blood culture practices, because the time to detection of microbial growth is significantly shorter by continuously agitating the bottles and checking them every 10 minutes. Depending on the system, the detection method can be an indirect measurement of the CO_2 produced by the microorganism in the bottles (at the bottom of the bottles there is an integrated CO_2 sensor containing a pH indicator or the level of fluorescence change because of the reduction in pH). If the system signals a positive bottle, the microbiology laboratory should initiate analytical tests immediately, according to laboratory protocols based on international and national guidelines, and all relevant information is documented and communicated to the clinician as soon as possible. The result of a Gram stained mount is available in 30 minutes, the result of presumptive or definite identification in 4-48 h (depending on the type of microbe), and the preliminary or final antimicrobial susceptibility report is available in 16-48 h (depending on the type of microbe).

The usual incubation time of BC is 5-7 days at 35-37°C. Positive bottles usually signal in the first 24-48 hours of incubation. The latest guidelines do not recommend longer incubation time in certain cases as previous recommendations did (21 days for the detection of *Brucella*, *Legionella,* the fastidious HACEK group bacteria that cause endocarditis (*Haemophilus, Aggregatibacter, Cardiobacterium, Eikenella, Kingella),* and in case of fever of unknown origin). The isolation of clinically relevant pathogens after 7 days of incubation is improbable (except for dimorphic/filamentous fungi) (4, 7, 8, 9).

WHY IS THE RESULT OF THE BLOOD CULTURE NEGATIVE (NO POSITIVE SIGNAL DURING INCUBATION), WHEN THE CLINICAL DIAGNOSIS OF BACTERAEMIA/FUNGAEMIA IS CERTAIN?

Blood culture is a microbiological test that is heavily dependent on the clinician's procedures (timing of sample collection, the amount of the collected sample, the number of BC bottles used), and the evaluation of the clinical symptoms (estimating the likelihood of bacteraemia/fungaemia and sepsis, the correct assumption of the probable etiological agent, and the proper evaluation of the results) (4, 7). Based on the literature. the preanalytics and analytics of blood culture testings are performed properly if 8-14% of the total number of blood cultures is positive. The assessment of this parameter is recommended in every medical institute/clinic with the help of the microbiology laboratory. If a significantly different percent is determined, the whole procedure should be revised and corrected (with the cooperation of the clinician and the microbiologist).

Sensitivity is basically determined by the type of sepsis. The BC is positive e.g. in endocarditis in 53-99%, in *S. pneumoniae* pneumonia in 25-30%, in neutropenic fever in 10-20%, in abdominal infection in 30-40%, and in disseminated fungal disease in nearly 50%.

If the symptoms of sepsis still subsist, and the BC from the day before is not positive, another 2-3 sample collections are recommended in the next 24 hours. If infective endocarditis is suspected, and the 3 pairs of BC collected on the first day are negative, another 2 pairs should be collected the next day. If an infection caused by a fastidious microorganism requiring special culture conditions is suspected despite negative BC results, consultation should be performed with the microbiologist before taking a new sample (recommendations for specialized

BC bottles, longer incubation time, alternative microbiological testing methods – e.g. serology, molecular diagnostics.) (8, 9).

WHY IS THE CULTURE RESULT NEGATIVE WHEN THE BLOOD CULTURE SYSTEM YIELDS A POSITIVE SIGNAL?

Non-conformity with preanalytical methods, namely overfilling the bottles may lead to a false positive signal in systems based on CO_2 detection, which is caused by the CO_2 contained by the excess amount of RBCs in the blood sample. A false positive signal may also be detected in BCs of ventilated patients (elevated partial CO_2 pressure), and blood samples containing high amounts of WBCs (haematology patients). The microbiologist can immediately confirm this to the clinician by assessment of BC bottle monitoring and the Gram stained mount.

In some cases, the bacterium in the blood may start to multiply, it may be seen in the mount from the positive BC bottle, but it does not grow in subculture. *Streptococcus pneumoniae,* for example, grows well in the rich BC media, but also produces a large amount of autolysin enzyme, which causes the bacteria to die. However, the antigens of the bacteria can be detected with antigen detection kits. B_6 vitamin-dependent streptococci also propagate in BC media containing pyridoxal, but may not grow on media usually applied for the culture of streptococci. Media containing pyridoxal should be used for subculturing such strains (4, 7, 8).

HOW SHOULD A POSITIVE CULTURE RESULT BE INTERPRETED? DOES EVERY MICROBE CULTURED HAVE CLINICAL RELEVANCE?

The following microorganisms are considered significant: *Staphylococcus aureus, Enterobacteriaceae* spp., *Pseudomonas aeruginosa, Streptococcus pneumoniae, Streptococcus β-haemolytic, Haemophilus* spp, *Neisseria meningitidis, Listeria monocytogenes, Enterococcus spp., Salmonella* spp., *Brucella* spp., *Pasteurella* spp., *Campylobacter* spp., *HACEK* group, anaerobes, *Candida* spp. These microbes are always clinically significant, even if they are cultured from only one of the (properly collected) four-six bottles.

The following microbes are considered significant in only certain cases: *Streptococcus α-haemolyticus (40-60%), Staphylococcus coagulase-negative (20-40%).* If a coagulase-negative S*taphylococcus* or α-haemolytic *Streptococcus* is cultured from only one of the ≥2 bottles from a set of BC, the isolate is probably a contaminant. However, an α-haemolytic *Streptococcus* cannot be considered as a contaminant if there was only one bottle. In this case, repeated sampling is recommended: if ≥2 bottles are positive, the α-haemolytic *Streptococcus* is more probably a significant pathogen. Some studies say that a bacterium is more probably a contaminant if it is cultured after a longer than usual incubation time. However, this observation cannot be used in the assessment of the positive results of an individual patient, because there is significant overlapping in the growth rate of contaminants and real pathogens. Further, parallel microbiologic sampling/testing from the source of the suspected bloodstream infection (e.g. urine, lower respiratory samples, removed catheter, etc.) complements and supports the interpretation of the relevance of the microbes cultured from the blood, and aids in identifying the etiology of the infection.

The following microbes are usually considered contaminants: *Staphylococcus coagulase-negative, Micrococcus* spp*., Corynebacterium* spp*. Propionibacterium* spp*.* and *Bacillus* spp*.* However, there is no general rule that they are contaminants in all cases.

The specificity of BC is determined by the percentage of false positive isolates. The interpretation of contaminants depends to a certain degree on patient characteristics. The spectrum of real pathogens and contaminants can be easily determined in the case of community acquired infection. In nosocomial infections, however, bacteria that are considered contaminants in "healthy" (immunocompetent) people may be real pathogens in immunocompromised patients. Specificity can be improved primarily by strictly abiding to sample collecting guidelines, mainly the methods to ensure asepsis, and to have multiple samples collected in cases of sepsis in which potential pathogens are the same as potential contaminants (e.g. catheter or other indwelling device associated infection, neutropenic fever). The number of positive blood cultures containing a contaminant can be assessed at a certain medical institute. If the rate of these bottles is significantly more than 3%, the situation should be remedied by education and consultation (4, 5, 8, 9, 10).

WHAT DOES A POLYMICROBIAL BLOOD CULTURE RESULT SIGNIFY? WHAT IS ITS CLINICAL SIGNIFICANCE?

In about 15 % of cases multiple microorganisms are grown from blood cultures. The rate of polymicrobial blood cultures ranges from 10% to 30% in immuno-compromised patients and in nosocomial BSI of patients treated at intensive care units. Polymicrobial BSI often indicates catheter-related or intraabdominal infections (15).

SHOULD PATIENTS WITH POSITIVE BLOOD CULTURE RESULTS BE RE-SAMPLED FOR FOLLOW-UP?

The blood may not become sterile even after 2-4 days of adequate treatment; the assessment of the recovery of patients with bacteraemia/fungaemia is the clinician's task. So-called "follow-up" BC is not necessary, except for some special cases. In infective endocarditis, it is recommended to guide treatment (the antimicrobial susceptibility of the pathogen may change after prolonged treatment). In every case of bacteraemia caused by *Staphylococcus aureus,* when isolation of the pathogen in the repeated BC taken after 2-3 days may indicate complicated sepsis caused by *S. aureus* (e.g. secondary metastatic infection), and the need for a change in therapy. Several recommendations contain "follow-up" BC in case of fungaemia to determine the necessary duration of treatment (4, 16).

ARE THERE ANY METHODS THAT SIGNIFICANTLY DECREASE THE TIME TO IDENTIFICATION OF THE PATHOGEN OF SEPSIS?

Timely initiation of adequate therapy significantly affects the patient's life expectancy; therefore microbiologic methods that decrease the time to obtaining a relevant result are more and more utilized today.

In the case of certain pathogens, the pathogen can be identified directly ***from the BC bottle after propagation*** with antigen detection or rapid identification methods (e.g. *Streptococcus pneumoniae, Neisseria meningitidis, Streptococcus agalactiae* – antigen detection by latex agglutination) (4, 7, 8).

Identification and susceptibility testing performed with automated/semi-automated systems can identify Gram-negative sepsis pathogens in 92-99% of cases, while Gram-positives are identified in 43-75% of cases. The advantage of these systems is that the most frequent pathogens in routine microbiology can be identified in 4-16 hours. Susceptibility results show 95% correlation with conventional methods (17, 18).

Most laboratories have access to MALDI-TOF MS (matrix-assisted laser desorption-ionization time of flight mass spectrometry) to identify cultured bacteria and fungi. Since the method is based on the mass spectrometry measurements of conserved microbial ribosomal and other proteins, the result is precise, mostly equivalent to DNA sequencing. Since a test can be performed from very little sample size ($10'^4$ - $10'^6$ CFU/ml), testing of barely visible isolated colonies after short incubation time can often be performed and the species identification result can be communicated to the treating physician.

It should be emphasized that MALDI-TOF MS can be used to identify pathogens directly from the blood culture bottles as well. Different separation and lysis protocols are available to remove proteins of human origin from the media, and concentrate the bacteria in the sample to the appropriate amount, resulting 80-96% correct identification results (compared with conventional culture and identification methods). However, the method is not always applicable (e.g. BC media containing activated carbon, polymicrobial infection) (9, 19).

Commercial and/or validated "home-made" molecular methods are also available. Another method is PNA FISH (fluorescent in-situ hybridization) which identifies microbes from positive BC bottles with 95-99% sensitivity and specificity. It is a quick method, since the whole procedure takes 90 minutes, but its disadvantage is that it is only able to identify a small number of microbial species (though the most frequent ones) (e.g. *S. aureus* and coagulase-negative *Staphylococcus* (without identification to species level), *Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*). The identification of yeast groups is based on intrinsic azole sensitivity: *Candida albicans/Candida parapsilosis, Candida krusei/Candida glabrata, Candida tropicalis*. The disadvantage of the method is that antimicrobial susceptibility can only be performed after conventional culture (2, 5, 9, 20).

Because of the pronounced significance of sepsis, more and more manufacturers produce complex tests based on molecular techniques (PCR), providing identification of the most frequent pathogens from positive BC bottles after a simple or more complicated protocol, with adequate sensitivity and specificity, in 1-3 h (e.g. FilmArray BCID Panel (BioFire): 19 pathogens, Hyplex BloodScreen (BAG):10 pathogens, Prove-it Sepsis (MobiDiag):50 pathogens). Molecular methods are suitable for the detection of certain resistance genes as well e.g. *mecA*, *van*-gene detection (20).

Molecular biology methods are also suitable for the rapid detection and identification of pathogens from aseptically collected ***blood samples*** (plasma, serum or EDTA-treated whole blood). Certain pathogens can be detected directly from blood with species-specific real-time quantitative PCR tests (e.g. *Neisseria meningitidis* DNA detection). Broad-range real-time PCR tests can be performed directly from blood samples: Gram-positive and Gram-negative bacteria and fungi can be detected (the clinically most important species in every group), along with certain resistance genes e.g. *mecA*, *van*-gene detection. Several commercially available multiplex molecular tests (e.g. Septifast Test (Roche), Sepsi Test (Molzym)) are able to detect the most frequent bacteria and fungi/the ones included in their panel, after more or less complicated test protocols in approximately 1-8 hours. The advantages of PCR testing performed directly from blood are rapid detection, it is not influenced by antibiotic treatment administered at the time of sample collection, and quantitative detection is available; its disadvantage is that it

detects bacterial DNA and not viable bacteria. A further disadvantage is that it does not detect the fastidious HACEK group. Antibiotic susceptibility/resistance detection is limited to certain resistance genes, the sample may be contaminated, and background bacterial DNA in the blood may be troublesome. After review of the currently available diagnostic palette, attention should be raised to the fact that although these methods are useful, conventional blood culture testing is still necessary. Several studies show that the two methods agree "only" in 55-85% of cases, depending on the patient population studied. In the future, if these methods become more widespread, their clinical significance should be assessed (it will be interesting to see which method will be the gold standard – how PCR positive but culture negative test results should be interpreted) (2, 9, 20).

CONCLUSION

To fully exploit the benefits of blood culture diagnoses, the microbiologist and the clinician should interact directly and discuss both the differential diagnosis as well as the treatment options. The final interpretations of the results will rest on the assessments made by the clinician and the microbiologist, taking into consideration microbiological and clinical findings.

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In this issue: Celebrating the 70th Anniversary of the Hungarian Society of Laboratory Medicine

The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Clinical laboratories – production factories or specialized diagnostic centers

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ARTICLE INFO

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Key words:
turnaround time, autovalidation, interpretative results

Acknowledgements:
We would like to thank Zsuzsanna Hevessy, Anikó Ujfalusi, Bettina Kárai and Gábor Nagy for their helpful comments & Ildikó Kópis for secreterial work.

ABSTRACT

Since a large proportion of medical decisions are based on laboratory results, clinical laboratories should meet the increasing demand of clinicians and their patients. Huge central laboratories may process over 10 million tests annually; they act as production factories, measuring emergency and routine tests with sufficient speed and accuracy. At the same time, they also serve as specialized diagnostic centers where well-trained experts analyze and interpret special test results. It is essential to improve and constantly monitor this complex laboratory service, by several methods. Sample transport by pneumatic tube system, use of an advanced laboratory information system and point-of-care testing may result in decreased total turnaround time. The optimization of test ordering may result in a faster and more cost-effective laboratory service. Autovalidation can save time for laboratory specialists, when the analysis of more complex results requires their attention. Small teams of experts responsible for special diagnostic work, and their interpretative reporting according to predetermined principles, may help to minimize subjectivity of these special reports. Although laboratory investigations have become so diversely developed in

the past decades, it is essential that the laboratory can provide accurate results relatively quickly, and that laboratory specialists can support the diagnosis and monitoring of patients by adequate interpretation of esoteric laboratory methods.

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INTRODUCTION

Since about 2/3rd of medical decisions are based on laboratory test results (1), it is obvious that clinical laboratories need to be organized in the best possible way to meet this demand. Optimizing, in the economic aspect, usually means fusing smaller units into larger ones to save costs, as well as trying to automate as much as possible. Undoubtedly, this has been an ongoing tendency for decades and has resulted in centralized, mega-laboratories that may process 15-20 million tests per year. There are two key concepts in these large laboratories: integration, where analytical instruments or groups of instruments are linked with pre- and post-analytical devices, and consolidation, where different analytical technologies or strategies are combined in one instrument or in a group of connected instruments. However, there is a logical limit to centralization, since no laboratory expert anticipates that a dozen 'ultra-mega-large' laboratories would be enough for a mid-size european country, or that these laboratories would be the best from the point of view of optimal patient care. Politicians and health economists, on the other hand, often tend to think differently, and, as they are unaware of the details of the laboratory professsion, such conceptions may actually prevail.

The majority of the laboratory tests are basic clinical chemistry, hematology, urinalysis and hemostasis screening tests. In many smaller laboratories this comprises the whole repertoire of the laboratory. There are two expectations from the patients and their caretaking doctors: the results should be accurate and they should be delivered fast. The laboratories are putting a lot of effort in the former by using internal and external controls, investigating interfering factors and linearity values, however laboratories are sometimes not paying enough attention to delivering the results on time. The timely delivery of laboratory results, however, is also very important. It may become unnoticed by the doctor if the laboratory is underestimating an enzyme activity by 10%, but the clinician probably does not accept if the same result is delayed by a few hours.

METHODS TO IMPROVE LABORATORY PERFORMANCE

The measuring clock of clinicians' satisfaction: turnaround time

Thus, each laboratory should monitor this key 'satisfaction factor' entitled turnaround time (TAT) and try to improve it as much as possible (2-5). One way for improvement is to modernize courier services in hospitals. The past years have proved that this is best achieved by automated transportation systems, the most widely used method being the pneumatic tube system. In these long tube systems that may reach a complete length of over 20 kilometers, numerous compressors are utilized that produce the pressure for independent circuits, which transport the capsules containing the laboratory samples. In the advanced systems, automated capsules are used, i.e. the capsule itself does not appear in the laboratory, but after its content is automatically unloaded, it returns to the station of origin by the aid of a radiofrequency tag that is attached to the surface of the capsule. Such systems can also optimize the travelling speed of the capsule as being faster when the capsule is empty and slower when it is carrying a sample

(6 m/s versus 3 m/s) (6). Another way to decrease TAT is to deploy laboratory testing to the actual site of patient care in the form of point of care testing (POCT). All POCT results, however, should be registered in the laboratory information system (LIS) and only results that are appropriately controlled, registered and validated should be used in patient care. (7)

Central laboratories usually have three types of assays based on TAT:

- *Emergency testing.* Here the complete 'from vein to brain' TAT should be below 60 minutes. In some cases, extra-urgent samples may need to be further prioritized, such as in the case of patients with ischaemic stroke waiting for thrombolysis.
- *Routine testing.* The TAT for routine test results today may be quite close to the emergency results, but a more realistic maximal routine TAT value is 3 hours. Nevertheless the median TAT for most the routine assays is around 80-90 minutes.
- Special *testing.* The TAT for these assays may be highly variable ranging from 2-20 working days. It can be assumed that no laboratory test should take more than 20 working days, as it would not be possible to effectively implement those slowly generated results into actual patient care.

The first two types of testing are usually part of the 'production factory' (8) while special testing occurs in specialized centers. A delicate balancing is required to devote sufficient resources from the laboratory to each of these test groups.

Ways to optimize test ordering

While we provide a medical service for the patients, whether we like it or not, with a large part of laboratory testing we implement a factory-type work flow, mostly for bulk tests described above (9). It may be assumed that, indeed, doctors often use too many diagnostic tests, and these tests are requested too frequently. This may be because they have erroneous expectations of the tests, are unaware of tests carried out previously, or are simply trying to be rigorous. Because these tests can be easily requested, it has been estimated that 8-30% of test requests may be superfluous (10). Thus, it is plausible that laboratory performance may also be improved by eliminating overtesting. This is, however, somewhat difficult to carry out optimally, and several techniques have been suggested to manage, or rather, to limit the ordering of test requests. One option is to allocate the whole laboratory budget to the requesters or to use a computerized clinical decision support system (CDSS) in medication as well as laboratory test ordering. Most other possibilities refer to tricks that the laboratory can do to prevent overtesting. These may include discouraging or not automatically fulfilling test requests, or creating explorative and reflective testing, such as beginning with a nonspecific, cost-effective but sensitive test, and then performing more targeted and usually more expensive tests only when the results of the initial screening tests are abnormal. A quite useful method could be to exert influence through setup of request forms, or to reduce the availiability of testing at certain times. A relatively low percentage of superfluous tests can also be eliminated by the laboratory through barring tests on predetermined principles of frequency filtering (11).

How to make the most of the laboratorians' time: autovalidate

One way to achieve meaningful organization is by automated evaluation of laboratory results for straightforward cases using autovalidation. If a laboratory is not using autovalidation in 2016, it is frustrating for the laboratory specialists, who are under constant pressure to devote their skills to checking the correctness of tens

of thousands of numerical values for 'simple cases', which may belong to any of the groups below:

i. each laboratory result is within the age specific reference range;

ii. only minor, clinically insignificant laboratory changes occur or

iii. many laboratory results are pathological, but all are similar to preceding values and are compatible with the diagnosis provided.

Tedious manual validation of simple cases by laboratory specialists carries the risk of serial mistakes, since after a while it is impossible to responsibly evaluate large quantities of data. Additionally, this laborious task takes the expert laboratorian's attention away from quality validation, where their time should be devoted to more complex cases.

In a large laboratory with a wide portfolio, the following simple rule may apply:

- Around 90% of the samples require 10% attention and
- The remaining 10% of the samples require 90% attention.

REPORTING AND INTERPRETATION OF SPECIAL LABORATORY RESULTS

Expert opinion of simple tests

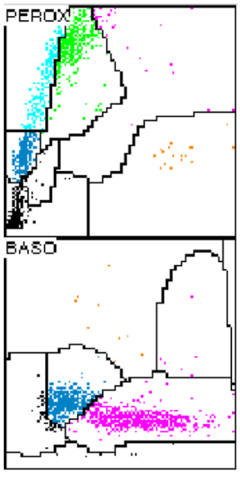
Now, what are those 'more complex' cases that require considerably more time than a handful of 'simple cases'?

If we just consider the basic laboratory portfolio, several complex cases could be mentioned. The automated hematology analyzer reports should be confirmed and validated, since falsely low neutrophil percentage may be reported with erroneously high monocyte numbers in cases with partial or complete myeloperoxidase (MPO) deficiency (12) if differential counts are based on volume and MPO activity (Figure 1A). In the case of unexpectedly high creatine kinase activity, further testing may be required to verify the presence of type I and type II macro-CK isoenzymes, an entity that results in falsely elevated CK-MB values in the immunoinhibition test

Figure 1 **Production factory: cases when more attention is needed in the routine laboratory**

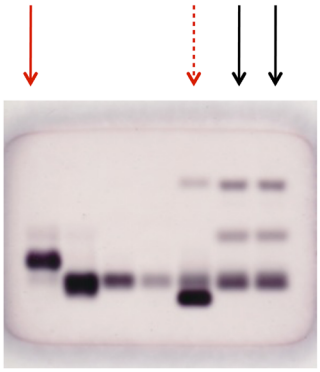
A

Types of white blood cells	Results on hematological analyzer using myeloperoxidase staining	Results on hematological analyzer using Coulter principle	Reference range
Neutrophil	**8.4%**	**62.5%**	43.0 – 76.0%
Lymphocyte	28.5%	29.4%	17.0 – 41.0%
Monocyte	**48.1%**	**5.6%**	3.4 – 9.0%
Eosinophil	1.8%	2.4%	0.0 – 5.0%
Basophil	0.3%	0.1%	0.0 – 1.5%
LUC	**12.9%**	-	
MPXI	-40,7	-	-10.0 - 10.0



B

CK isoenzymes	Patient 1	Patient 2	Reference range
CK-MM	5.7%	20.7%	92 – 100%
CK-MB	3.6%	0.9%	< 6%
CK-BB	0.1%	3.7%	< 2%
Macro CK 1.	**90.6%**	0.0%	
Macro CK 2.	0.0%	**74.7%**	
Atypical isoenzyme	0.0%	0.0%	



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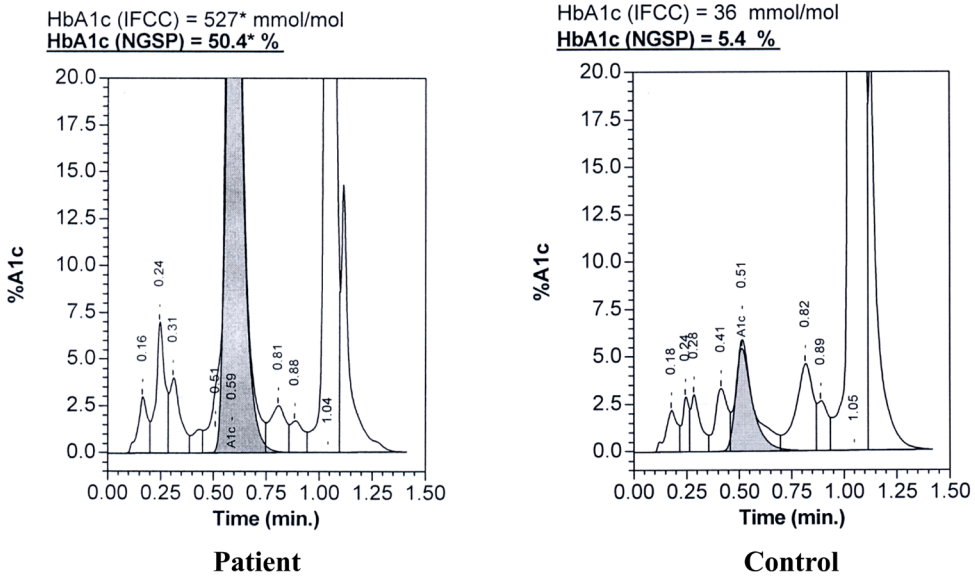

(A) Myeloperoxidase deficiency results in decreased ratio of neutrophiles, and elevated ratio of monocytes and large unstained cells (LUC) when the sample is measured on hematological analyzer using myeloperoxidase staining.

(B) Macro CK results in high CK activity and disturbs the measurement of CK-MB activity using immunoinhibition method. On the CK electropherogram either macro CK 1 (red continuous arrow; patient 1), or macro CK 2 (red dotted arrow; patient 2) are shown compared to control (black arrows).

(C) Extremely elevated hemoglobin A1c concentrations can be measured by HPLC in the presence of some rare hemoglobin variants (e.g. Sherwood forest hemoglobin variant).

(Figure 1B). Similarly, a clinically silent hemoglobin variant, like the rare Hemoglobin Sherwood, can cause an extremely high value in the automated HPLC testing for glycated hemoglobin, where the diagnosis is provided by mutation analysis (Figure 1C). In addition to such cases several other areas exist that require interpretative reports (13) that has been shown to contribute to physician satisfaction (14). Aside from such cases, most of the quality time for general

routine analysis is devoted to microscopic investigations of peripheral blood or cerebrospinal fluid samples.

Expert opinion of special tests

Another area of interpretative reporting is when samples are sent for more esoteric tests, and in many cases no test requests are indicated, rather, a hypothesized diagnosis need to be confirmed or rejected.

These types of investigations mostly, but not exclusively, involve flow cytometric analysis of peripheral blood or bone marrow, cytogenetic analysis for G-banding or FISH, autoantibody pattern description, dynamic endocrine tests and special hemostasis assays for bleeding diathesis or thrombosis. Many of the nucleic acid-based tests can now be easily set up, but in some cases whole-genome sequencing and the interpretation of rare mutations may take many hours, or even days of qualified work from the laboratory specialists to delineate the diagnosis. Many of these techniques also require months or years of experience/training to gain sufficient expertise. Morphological skills are essential to evaluate pathological peripheral blood, bone-marrow or cerebrospinal fluid samples, or to describe autoantibody staining patterns. However, sometimes these skills become additional to other specialized skills, such as the ability to confidently read DNA sequencing curves, operate the software for flow cytometric dot plot analysis, or learn the details of a karyotyping software operation. It is also imperative to sustain the TAT concept in the case of these special tests. This means such a service cannot rely on a single expert, thus a minimum team of two people should handle the reports in each of these subspecialities. The best scenario, however, is a team of about three experts who take turns writing the reports while sticking to predetermined principles of data reporting to minimize subjectivity of these special reports. In our Department, the Divisons that exert the highest time-demand for special diagnostic work are summarized in Table 1. In all of these Divisions, a minimum of 3-5 specialists take turns reporting, and in some areas, two people are required for one type of subspeciality for parallel reporting.

These reports have a generally accepted format and the result sheet should include considerably more data than a general chemistry assay.

A typical request of clinical flow cytometry for the investigation of hematological malignancies should include the followings (15):

- demographic identification of patient;
- identification of the hospital or division sending the sample;

Table 1 Special diagnostic work with the highest time - demand in the Department of Laboratory Medicine at the University of Debrecen

Special divisions with the highest time-demand of diagnostic work	Special diagnostic work (hours/week)	Annual interpretative reports (and its ratio of reports of division)	Turnaround time (working days)
Flow cytometry	70-80	3 000 (67%)	3
Molecular genetics	50-60	2 400 (33%)	12-20
Laboratory immunology	50-60	1 200 (5%)	10-20

- type of specimen (bone marrow aspirate, peripheral blood, other biological fluids);
- timing of observation (first sample or follow-up);
- diagnostic hypothesis of the sender.

When reporting the results of the flow cytometric analysis, the following elements are required (15):

- list of antigens and type of immunofluorescence analysis;
- absolute number of cells in the sample;
- quality of the sample, in terms of viability;
- general description of the gating procedure;
- immunophenotype of blast cells;
- description of cells surrounding blasts;
- diagnostic conclusions.

In special cases, other parameters may be required, like the definition of an antigen panel for the detection of minimal residual disease. In addition, a representative dot plot is also part of the interpretative report. These attributes minimize the subjectivity of the special reports. Nevertheless, there are several flow cytometric analyses that do not require interpretative reporting. This usually depends on the question raised, when reporting of a sheer number is sufficient, like in the case of CD34 positive cell count, or when a qualitative answer is required, like in the flow cytometric heparin-induced thrombocytopenia assay.

Another example is genetic test reports that undoubtedly carry a serious clinical implication for prediction of susceptibility to disease, patient diagnosis, prognosis, counselling, treatment or family planning. Therefore, such laboratory reports should provide a clear, concise, accurate, fully interpretative and authoritative answer to the clinical question (16).

These reports should include a clearly structured format, comprised of the following information:

- administrative;
- patient and sample identification;
- restatement of the clinical question;
- specification of genetic tests used;
- results;
- interpretation of results.

Upon interpreting the results, the expert draws a conclusion that should contain any of the five subsequent possibilities:

- normal finding(s);
- non-specific finding(s) without clinical relevance;
- incidental finding(s) with possible clinical relevance;
- finding(s) of uncertain significance;
- pathognomonic (disease-specific, pathological) finding(s).

When a new diagnosis is made based on these reports, it is appropriate to state specifically that the result has 'potentially important implications for other family members'. Depending on the context, it may be appropriate to explicitly mention the recommendation to test the partner, the possibility of cascade screening tests in relatives, and the possibility of prenatal diagnosis or preimplantation genetic diagnosis. Genetic testing is unique in the respect that when appropriate, the risk for future offspring should be calculated and provided.

Several other areas of laboratory medicine exist where interpretative reports are required. One such area is autoantibody testing. In these studies, two or more methods are frequently used to identify an antibody marker and sometimes the results disagree. When this happens, an interpretation is always required, specifying the

diagnostic accuracy of the tests. Similar to the previously described genetic tests, some autoantibodies that are not requested, and consequently not expected, may be identified by chance. Such cases should only be interpreted when these antibodies have a significant clinical correlation (17). Examples from our laboratory are provided for interpretative flow cytometric, genetic and autoantibody reports in Figure 2.

Since laboratory tests are usually requested by well-trained clinicians who are aware of the diagnostic, prognostic and monitoring value of the results, the over-interpretation of self-explanatory numerical tests can be useless and

Figure 2 Specialized diagnostic centers: interpretation of special laboratory results

A

Sample type	Bone marrow
Panels	Panel of acute leukaemias, de novo T-ALL, AML
CD markers	CD2,CD3,CD4,CD5,CD7,CD8,CD10,CD13,CD14,CD15,CD16,CD19,CD1A, CD30,CD33,CD34,CD38,CD45,CD56,CD64,CD71,CD99,CD117,CD11b,CD123, CD300e,cy3,cyFXIII,cyMPO,cyTdT,HLA-DR,syto16
Interpretation	Nucleated cell count in the bone marrow sample was 110 G/L. Besides 2% mature lymphocytes, 2% erythroid precursors and 4% mature myeloid cells 87% blasts were detected based on CD45 expression and side scatter characteristics. Blast population is not homogenous, blasts belong to two different cell lines, 15% of the bone marrow cells are T lymphoblasts characterized by an immature immunophenotype: CD7+/CD99+/nTdT+/CD34+/CD10+/cyCD3+ and CD45-/CD3-/CD2-/CD5-/CD4-/CD8- while 72% of the bone marrow cells are pathological monocytes with CD64+/CD33++/cyFXIII+/CD4+/cyMPO+/CD15dim and CD14-/CD11b-/HLADR+-/CD13-/CD34-/CD117- immunophenotype. Based on the WHO 2008 classification of acute leukemias the formerly bilinear leukemias are now classified as MPAL, in this case consisting of myeloid/monocytic blasts and T lymphoblasts. **Summary: MPAL, with 72% myelo-monocytic blasts and 15% T lymphoblasts in the bone marrow aspirate.**

B

Sample type: Bone Marrow
Referral Indication: MDS? Neutropenia and low platelets
Karyotype: 46,XY,der(7)t(1;7)(q10;p10)[15]
Interpretation: Analysis of fifteen metaphases from the bone marrow showed an abnormal clone containing a translocation between the long arm of chromosome 1 and the short arm of chromosome 7 with breakpoints at 1q10 and 7p10. This translocation results in the deletion of the whole long arm of chromosome 7 and trisomy of the long arm of chromosome 1. The result is consistent with the referral indication and confirm the diagnosis of MDS. MDS associated der(7)t(1;7)(q10;p10) occurs as a sole abnormality in the majority of cases. According to the literature, this unbalanced translocation defines a unique clinicopathological subgroup of myeloid neoplasms. The der(7)t(1;7)-positive MDS cases show lower blast counts and higher hemoglobin concentrations at diagnosis and slower progression to acute myeloid leukemia than other -7/7q- cases.

C

Test	Result	
Liver specific autoantibodies (immunoblot)		
AMA M2 antibody	negative	
Anti-Sp-100 antibody	positive	!
Anti-LKM1 antibody	negative	
Anti-GP 210 antibody	negative	
Anti-LC1 antibody	negative	
Anti-SLA antibody	negative	
Autoantibodies on rat tissues (rat LKS IIFA)		
Anti-smooth muscleantibody	negative	
Anti-LKM antibody	negative	
Anti-gastric parietal cellantibody	negative	
Anti-mitochondrion antibody	negative	

Test	Result		Reference range	Unit
Anti-cellular antibodies (HEp-2 cell IIFA)				
ANA pattern 1	multiple nuclear dots	!		
ANA titer 1	> 1:5120	!	< 1:160	titer
ANA pattern 2	speckled	!		
ANA titer 2	1:5120	!	< 1:160	titer
Anti-cytoplasmic pattern	negative			
ANCA IIFA				
C-ANCA	negative			
P-ANCA	negative			
Atypical ANCA	negative			
Anti-MPO antibody (ELISA)	<5,0		<5	U/mL
Anti-PR3 antibody (ELISA)	<10,0		<10	U/mL

Interpretation:
Antinuclear antibodies of multiple nuclear dots and speckled patterns are detectable in high titer. MND pattern is caused by anti-Sp-100 antibody which is highly specific for primary biliary cirrhosis. However it may be present in other diseases (such as rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, Sjögren syndrome) and may precede disease symptoms by years. To elucidate the antigen specificity of the speckled ANA component anti-ENA antibody determination is recommended.

(A) An interpretative flow cytometric report of a patient with mixed phenotype acute leukaemia
(B) karyotype determination of a patient with myelodysplastic syndrome
(C) the interpretation of autoantibody pattern of a patient with primary biliary cirrhosis are shown

harmful. However, laboratory investigations have become so diversely developed in the past decades that in the aforementioned cases, as well as in case of many other special tests, it is essential that the laboratory specialist provides a meaningful interpretation to the laboratory findings.

CONCLUSION

A clinical laboratory should be organized in a way so that the clinical pathologist can utilize most of his/her trained skills in evaluating results of specialized diagnostic areas and in interpreting laboratory reports for the physicians. This can be best achieved by introducing automated evaluation in the form of autovalidation in several routine laboratory fields in case of numerous samples that do not require direct medical surveillance. All these measures would facilitate that the laboratorian will become an indispensable part of the medical team.

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The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Adding value in the postanalytical phase

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ARTICLE INFO

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Key words:
added-value, clinical interpretation, critical-risk result, significant-risk result reporting, outcome-driven testing, postanalytical phase, reflex testing, reflective testing, test utilization

Author's disclosures:
The author states that there are no conflicts of interest regarding the publication of this article. The author is the functioning chair of the joint Working Group on Postanalytical Phase (WG-POST) of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) and European Organisation for External Quality Assurance Providers in Laboratory Medicine (EQALM)

ABSTRACT

Apart from maintaining the highest quality of analytical test results, laboratories are now getting more focused on how to achieve the greatest impact of laboratory results on their patient's outcome. Laboratory professionals are now in the learning phase of implementing new practices at different steps of the extra-analytical phases of the testing process where laboratories used to contribute seldom, only sporadically. Recently, the achievable levels of harmonization and responsible contributors at various steps of the testing process have also been proposed. Based on this proposal some tasks of the extra-analytical phase should become primarily the responsibility of laboratories with the involvement of clinicians, like additive testing, individualized interpretative commenting and reporting results with clinical urgency in postanalytical (PA) phase. These tasks can be good targets to start with or to increase patient outcome-oriented extra-analytical activities of laboratories.

The status of the present practice of the PA activities for which laboratories proposed to be primarily responsible in the testing process - laboratory-driven PA tasks - will be reviewed below. In addition, approaches of quality assessment (QA) with quality specifications of these laboratory-driven PA tasks and

the available best practice recommendations in the light of the achievable level of harmonization will be discussed.

Laboratory professionals are encouraged to improve their methodological, theoretical and communicational skills and take the lead and participate in the discussed PA activities that can assist in translating laboratory test results into clinical meaning and thereby lead to better clinical utilization of laboratory test results.

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INTRODUCTION

In the era of changing healthcare environment, fast technological development and increased patient consciousness about their health, clinical laboratories face major challenges to look outside the laboratory and pay more attention to activities which optimize the clinical outcomes of laboratory testing. These new challenges require identification of all the activities and benefits of laboratory medicine that can provide the best utilization of laboratory tests in the interest of the patient. This leads to the renewal of the total testing process concept (TTP) (1) and, besides maintaining the highest quality of analytical test results, laboratories are now getting more focused on how to achieve the greatest impact of laboratory results on their patient's outcome. Recently, the need for harmonization with the likely achievable levels of harmonization has been proposed for all the different phases and steps of the TTP where laboratory profession can have a significant impact (2). In addition, responsible contributors at the various steps of the testing process have also been proposed for activities. Now laboratory profession has started to explore areas where they can successfully participate in extra-analytical phases where laboratories used to contribute seldom, only sporadically to better patient outcome. Based on the proposed levels of harmonization, some of these areas will remain mainly the task of clinicians and laboratory should only provide more assistance, while others should become primarily the responsibility of laboratories with the involvement of clinicians. (2) The activities for which laboratories should be primarily responsible in the PA phase can be good targets to start with or to increase patient outcome-oriented extra-analytical activities of laboratories. Regarding harmonization efforts within the TTP, the present use of these PA activities in laboratories as well as the status of their quality assessment (QA) readiness need to be reviewed first.

UPDATED CONCEPT OF TTP

TTP or brain-to-brain laboratory test loop is a concept which describes the journey of laboratory testing from requesting laboratory tests to the clinical actions taken based on reported results. The TTP therefore includes test requesting, identification (at several stages), collection, transportation, preparation and analysis of samples, interpretation and reporting of analytical results, and finally actions based on the results and their communication (3). The many intermediary steps are further classified in their relation to laboratory analysis as pre-preanalytical, preanalytical, analytical, PA and post-PA phases. (3,4) By definition the PA phase includes those laboratory actions that are induced by a certain laboratory result and taken before the result is communicated to the clinician, e.g. reflex testing, validation of results done by medical technologists or interpretation by laboratory specialists. The post-PA phase means the interpretation of laboratory results by clinicians, which results in clinical decision-making. Although laboratory profession can have significant impact in all the different phases and steps of the TTP (2), extraanalytical phases were due to historical reasons less

in the focus of laboratory attention. Thus, some parts of PA phase, such as analytical and medical validations (with evolving information technology and also the autovalidation) as well as selection of units and correct reference range of the measured analytes became typical routine tasks of laboratories. However, activities related to test interpretation are less practiced and test interpretation remained mainly clinical activity with little, sporadic input from laboratories. In addition, the post-PA phase – the clinical consequences of the laboratory result for the patient- is not in any way under the laboratory's control. (3)

The relatively new concept of „added value" in Laboratory Medicine focuses on the range of opportunities that ensure that the laboratory medicine service achieves optimal clinical relevance for users and that it takes advantage of rapid advances in technology and our understanding of the disease process and treatment opportunities. (5,6) Added value in laboratory medicine is represented by the effectiveness (usefulness/utility) of laboratory tests in influencing the management of patients and related clinical outcomes (7). The use of a diagnostic test, besides having clinical impact, may also involve operational and economic benefits which should be considered by laboratories. (9) The analysis of the outcome of laboratory testing, whether the performance of the test was useful for the patient or for public health, has been integrated as the 10th step in the brain-to-brain laboratory test loop concept (1).

OPPORTUNITIES TO ASSURE THE EFFECTIVENESS OF LABORATORY TESTS IN THE PA PHASE

The main focus of the everyday operation of laboratories used to be to achieve and maintain the highest analytical quality of test results. Recently this task has broadened to encompass activities for optimizing patient outcomes in all steps of the TTP. (6) Achievable harmonization goals for all the different phases and steps of the TTP have recently been proposed with indication of the responsibility for each step. (2) Based on this harmonization proposal, some of the steps where responsibility should be shared between clinicians and laboratory will remain mainly the task of clinicians, and laboratory should only provide more assistance (e.g. test requesting and laboratory result-based clinical actions in the pre-pre- and post-postanalytical phases). Some other steps should become primarily laboratory responsibility with the involvement of clinicians, like additive testing in PA phase, individualized interpretative commenting and also reporting results with clinical urgency. These latter PA activities, where laboratories are designated as being primarily responsible for the task in TTP, can be good targets to start with or to increase extra-analytical activities of laboratories.

PA ACTIVITIES WHERE LABORATORIES ARE PRIMARILY RESPONSIBLE FOR THE TASK IN TTP

Additive testing

Laboratory specialists are expected to assist their clinicians in requesting appropriate tests to help them answer their clinical questions. This includes assisted test requesting techniques in the pre-preanalytical phase as well as additive test requesting techniques applied in the PA phase. Failure to order appropriate tests in diagnostic work can cause harm to the patient either because the clinician misses key information to form the correct diagnosis or because unnecessarily ordered tests can lengthen the patients' investigations. (15) Known interventions to optimize test requesting such as educational strategies, feedback and changing test order forms were found to improve the

efficient use of laboratory tests in primary care very differently, with effect sizes ranging from 1.2% to 60%. (14) However, the impact of inappropriate testing on patient outcomes is rarely reported. (16) There are far more data available on the heterogeneity of test requesting practices where the extent of variation in the requesting patterns cannot be explained by differences in the local prevalence of the disease. (2) Many approaches exist for rationalized test requesting starting from implementing minimum retesting intervals in electronic request systems (11) through harmonized test profiles (12) to implementing artificial intelligence methods to predict the benefit of proposed future laboratory tests. (13) Problem-based test requesting (2,17) and additive testing (18,19) are both approaches when laboratory tests are selected by laboratory specialists in order to respond to a clinical question. During additive testing laboratory investigations are added to existing test results either automatically on the basis of algorithms (reflex testing) or by laboratory professionals who – apart from results – also consider the clinical context of the patient (reflective testing). Typical examples of reflex testing are the addition of free thyroxin when thyroid stimulating hormone is abnormal or free prostate specific antigen in case of an increased level of total PSA. In cases with multiple abnormal test results, addition of appropriate tests –reflective testing- requires professional medical experience combined with the knowledge of patient characteristics and cannot be done by automated protocols. In problem-based test-requesting, the sequence and variety of laboratory tests necessary to answer the laboratory test-based clinical question are selected by the laboratory specialist during investigations. Although reflective testing is considered to be a useful way to improve the process of diagnosing (and treating) patients by different general practitioners or other clinicians and patient populations (18), there is no consensus yet on the point when additive testing should be indicated, for which tests, and for what type of results. (2) So far no quality indicators (QIs) or performance criteria in added testing have been set. (28) There is no strong evidence either on the positive outcome of reflective testing on patient management. (18)

Interpretative commenting

Interpretive comments are narrative interpretations of laboratory results in the context of the clinical situation of the patient. Those comments that are only result-specific and do not generally refer to the patient context do not represent interpretative commenting, e.g. cautionary or explanatory notes on quality or adequacy of the primary sample appended automatically by the laboratory information system such as “sample is haemolysed”. An increasing number of studies has been published reporting that some physicians have either found laboratory assistance useful, or required such laboratory assistance in the interpretation of common laboratory test results. (17,18,20,21) It has also been shown that clinicians found interpretative comments time-saving and improving the accuracy of their diagnoses. (20,22,23) Several studies show that although most of the interpretative comments given by laboratory specialists are appropriate, inconsistencies in comments are observed and some comments may be directly misleading when laboratory specialists are presented with the same case histories. (24,25,26) It is generally advisable that only professionals with clear expertise in the particular laboratory field should be charged with interpreting laboratory results. (24, 25) Recommendations on TTP harmonization suggest that mainly interpretative commenting for complex testing or for laboratory tests should be an integrated and central part of laboratory specialists’ daily activities. (2) However, some studies maintain

that laboratory professionals should even be trained in the interpretation of ordinary laboratory tests because when laboratory specialists were asked to add interpretative comments to non-esoteric laboratory test results, more than half of the interpretations were inappropriate and/or misleading. (24, 27) Therefore, it is absolutely essential that the quality of interpretative commenting should be improved. Improved quality can be achieved by education, availability of best-practice and evidence-based guidelines and by establishing or expanding EQA programs to assess this PA activity. (2) Some EQA schemes already include or focus on interpretative commenting. (2) It is noticeable that the only QI which has been proposed to measure the performance of interpretive commenting, interpretative comments with a positive impact on patient outcome (28), was found not to have been used by a survey looking to provide preliminary results on QIs and related performance criteria in the PA phase. (29) This finding can reflect that it is difficult to collaborate with clinicians in order to evaluate an outcome following the introduction of a specific interpretative comment in the patient's report.

Reporting results that need urgent clinical review for patient safety

Medical laboratories often produce clinically unexpected results that require timely clinical evaluation. The recently proposed risk-based definition of these results differentiates between two risk categories. (30) Critical-risk result (CRR) is defined as results requiring immediate medical attention and action because they indicate a high risk of imminent death or major patient harm. The other risk category, significant-risk results (SRR), labels test results that are less urgent but need to be reported within a shorter timeframe than that for routine results. SRRs are defined as results that are not imminently life-threatening, but signify significant risk to patient well-being and therefore require medical attention and follow-up action within a clinically justified time limit. Examples of common CRRs include very abnormal potassium or glucose concentrations in serum/plasma, whilst examples of SRR might be elevated leukocytes commonly seen in chronic leukemia or early-stage adenocarcinoma in a routine appendectomy specimen.

High-risk results (HRR) as an appropriate umbrella term for critical and significant risk results has also been introduced. (30) Laboratories need to have systems and mechanisms for rapid identification and timely reporting of these HRRs that need urgent clinical review for patient safety. Many studies all over the world, including the one which was organized by the joint working group of EFLM and AACB in European laboratories (31), demonstrated that the reporting of CRRs is very heterogeneous when it comes to procedures on how and what results to report. (32,33) Reporting of CRRs is a field where efforts must be made to improve the quality at many levels. Principally, HRR procedures should be organized in agreement with clinical users considering the local institutional needs and resources. In addition, both HRR practices and alert lists should be designed to serve patient safety. CLSI guidelines on management of CRRs and SRRs have recently been published to provide guidance for laboratories in the field. (34) The QIs proposed for critical values aim to determine the level of successful reporting of CRRs in the laboratory, and turn-around-times (TAT) in CRR notification both for inpatients and outpatients. (28) Reports on preliminary results on QIs and performance criteria in the PA phase showed improvement of laboratories in recent years in successful reporting, and records of time taken to communicate results indicate that procedures are carried out rapidly and effectively. (29)

CONCLUSIONS

The addition of value to laboratory medicine services involves working with users of the service (clinicians). Based on the proposed achievable harmonization goals for all the different phases and steps of the TTP, laboratories should take the lead in several PA activities where laboratories and clinicians should work together for the sake of patient safety. These steps of TTP are good targets to start with or to increase extra-analytical activities of laboratories. All these activities are a new challenge to the laboratory profession since they require communication and cooperation with other professions and most recently they have also become targets of harmonization efforts in laboratory medicine. (2, 7, 10)

Despite lot of communication about extra-analytical activities of laboratories, little is known (mostly sporadic data available only (2,27)) about the practices that laboratories apply in PA phase (neither about those that are proposed to be led by laboratories nor those where clinicians should lead the activities). The forthcoming survey of the Joint Working Group on Postanalytical phase of the European Federation of Clinical Chemistry and Laboratory Medicine. (EFLM) and European Organisation for External Quality Assurance Providers in Laboratory Medicine (EQALM) in 2016 intends to collect the applied PA practices in European laboratories. (35)

Exteral QA programs for the discussed PA activities for which laboratories proposed to be primarily responsible in the testing process and their quality specifications are developing areas of quality assessment. (2) Although performance criteria of the TTP have been set, only very few of the proposed QIs focus on the PA phase. Thus QIs on additive testing and outcome of CRR reporting are not specified at all. In addition, the only QI which has been proposed to measure the performance of interpretive commenting - interpretative comments with a positive impact on the patient outcome (28) - was found not to have been used by a survey looking to provide preliminary results on QIs and related performance criteria in the PA phase. (29) This finding emphasizes that the work at clinical interface is rather challenging. In order to characterize performance criteria and outcome-based QIs in the extra-analytical phases, EFLM established a new task force group, the Task Force group on Performance specifications for the extra-analytical phases (TFG-PSEP). (36) A survey of TFG-PSEP to collect existing QIs in PA phase and ideas of laboratories about quality and performance specifications of extra-analytical phases in European countries has been launched just recently.

Despite the fact that work at clinical interface is rather challenging, laboratory professionals should be encouraged to improve their methodological, theoretical and communicational skills and take the lead and participate in the discussed PA activities that can assist in translating laboratory test results into clinical meaning, improve laboratory test interpretation and thus lead to better clinical utilization of laboratory test results.

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The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

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Book review — "Patient safety"

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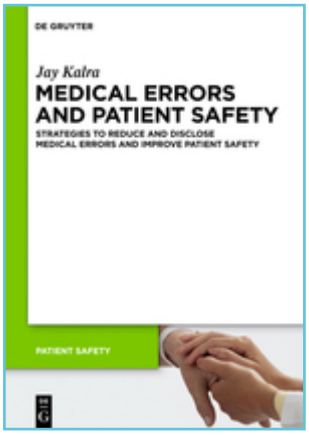
RECENSION ABSTRACT

Mario Plebani and Oswald Sonntag are editors of a book series on patient safety. Since the Institute of Medicine (IOM) report titled "To Err is Human", numerous publications have been published on the topic. As such, since 2011, a new series of publication was conceived with deGruyter as the publisher.

Herewith I like to give an overview of the booklets which have been published so far and those which are in the pipeline.

VOLUME 1: JAY KALRA

Medical Errors and Patient Safety - Strategies to reduce and disclose medical errors and improve patient safety – published: May 2011 - ISBN: 978-3-11-024950-7



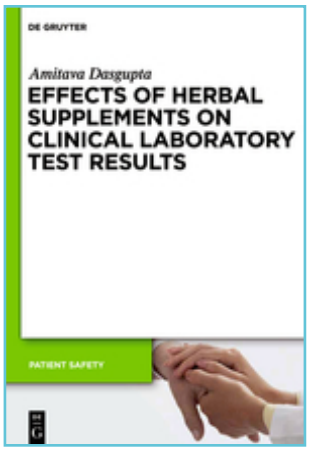
Is the reporting of medical errors changing? This book illustrates, citing true cases from health care and beyond, that most errors come from flaws in the system. It also shows why they don't get reported and how medical error disclosure around the world is shifting away from blaming people, to a „no-fault" model that seeks to improve the whole system of care.

The book intends to provide an introduction to medical errors that result in preventable adverse events. It discusses efforts made to reduce preventable adverse events and medical errors, and moreover highlights their impact on clinical laboratories and other areas, including educational, bioethical, and regulatory issues. Varying error rates of 0.1-9.3% in clinical diagnostic laboratories have been reported in the literature. While it is suggested that fewer errors occur in the laboratory than in other hospital settings, the quantum of laboratory tests used in healthcare entails that even a small error rate may reflect a large number of errors. The interdependence of surgical specialties, emergency rooms, and intensive care units - all of which are prone to higher rates of medical errors - with clinical diagnostic laboratories entails that reducing error rates in laboratories is essential to ensure patient safety in other critical areas of healthcare.

The author maintains that many such errors are preventable provided that appropriate attention is paid to systemic factors involved in laboratory errors. This book identifies possible intelligent systemic approaches that can be adopted to help control and eliminate these errors. It is a valuable tool for physicians, clinical biochemists, research scientists, laboratory technologists and anyone interested in reducing adverse events at all levels of healthcare processes.

VOLUME 2: AMITAVA DASGUPTA

Effects of Herbal Supplements on Clinical Laboratory Test Results - published: May 2011 - ISBN: 978-3-11-024562-2

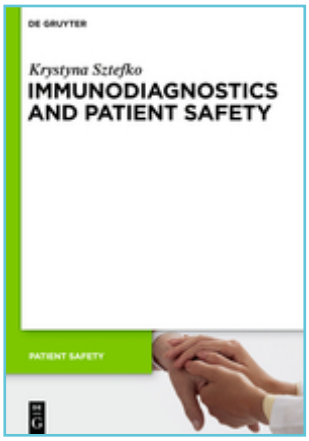


Herbal supplements are available without prescription in many countries throughout the world and accounting for over $30 billion U.S dollar in sale. A majority of U.S population (25-40%) use herbal supplements while alternative medicines are major forms of therapy in third world countries used by as much as 80% of the population. Contrary to the popular belief that herbal remedies are safe and effective, many herbal supplements have known toxicity, and unexpected laboratory test results may be the early indication of such toxicity. In addition, some herbal products such as St. John's wort can interact with many Western drugs causing increased clearance of these drugs and hence treatment failure. This monograph would provide information on how herbal supplements affect laboratory test results as such patient safety. This monograph would provide a comprehensive and concise practical guide for laboratory professionals, physicians and other health care

professionals. The emphasis of this monograph is to provide clinically relevant information rather than discussing in detail mechanisms of such effect, although brief explanations would be provided for such unexpected test results.

VOLUME 3: KRYSTYNA SZTEFKO

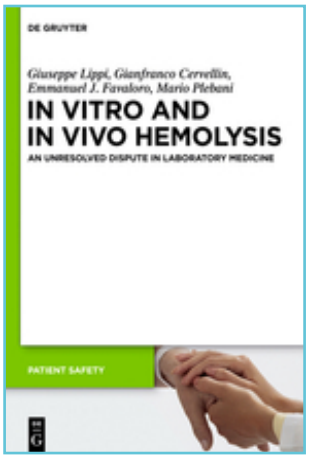
Immunodiagnostics and Patient Safety - published: May 2011 - ISBN: 978-3-11-024948-4.



Today most of immunochemistry methods for the determination of proteins, peptides, drugs, and many small molecules are fully automated, with good precision, excellent sensitivity and short reaction time. However, inaccuracy due to poor standardization and the presence of interfering substances in biological samples is still a serious and life-threatening issue. Proper validation of methods and quality assurance have little effect on frequency of occurrence of false positive or false negative results, which, if unrecognized, may lead to patient's misdiagnosis, unnecessary treatment or even unnecessary surgery. Deep knowledge of basic principles of immunochemical methods (antigen-antibody reaction, standardization, matrix effect, limit of detection, cross-reactivity, etc.), sources of analyte-independent interferences (preanalitycal errors, the presence of binding proteins, the presence of autoantibodies) and analyte-dependent interferences (presence of heterophilic antibodies, high-dose effect) are very important to understand, detect, reduce and/or eliminate the interferences. This book helps to reduce false results and, at the same time, improve patient's care and patient's safety.

VOLUME 4: GIUSEPPE LIPPI, GIANFRANCO CERVELLIN, EMMANUEL J. FAVALORO AND MARIO PLEBANI

In Vitro and In Vivo Hemolysis - An Unresolved Dispute in Laboratory Medicine - published: July 2012 - ISBN: 978-3-11-024614-8

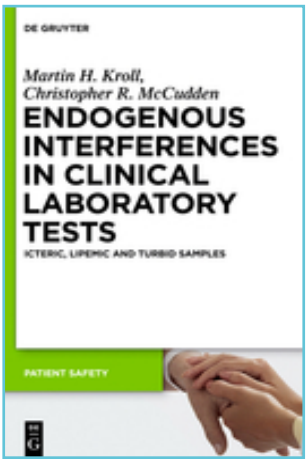


Introduces clinically relevant findings about hemolysis. Indicates possible laboratory errors caused by hemolysis. Covers management of hemolytic specimens.

Defined as red blood cell break down and the release of hemoglobin and intracellular contents into the plasma, hemolysis can seriously impact patient care as well as the laboratory's reputation through its affect on test results. Therefore, the European Preanalytical Scientific Committee, in collaboration with the International Federation of Clinical Chemistry Working Group on Patient Safety, have designed a questionnaire to collect data on prevalence and management of hemolytic specimens referred to the clinical laboratories for clinical chemistry testing. This book will help identify the areas where hemolysis occurs most frequently, which can, in turn, guide further analysis about why it is occurring. Once these elements are known, practices and procedures can be implemented to dramatically reduce hemolysis and avoid erroneous laboratory results affecting patient care and increasing laboratory costs.

VOLUME 5: MARTIN H. KROLL AND CHRISTOPHER R. MCCUDDEN

Endogenous Interferences in Clinical Laboratory Tests - Icteric, Lipemic and Turbid Samples - published: December 2012 - ISBN: 978-3-11-026622-1



Offers the proper approach to evaluating the impact of bilirubinemia and lipemia on clinical laboratory results. Provides the empirical and theoretical foundation for evaluating the effect of bilirubinemia and lipemia on clinical laboratory test results.

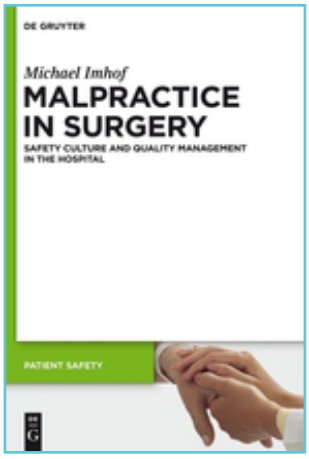
The goal of clinical laboratories is to produce accurate information for clinical decision making in medicine. More than half of the medical decisions made depend on clinical laboratory tests.

Patient safety represents an important and critical problem for laboratories. They need to assure that the information they deliver to physicians is accurate, and therefore safe for clinicians to use. Endogenous compounds can interfere with laboratory tests, decreasing accuracy and threatening patient safety. Elevated bilirubin (bilirubinemia) and elevated lipids (lipemia) are common conditions that cause significant interferences with laboratory results. Clinicians depend on laboratories to detect these endogenous interferences. Laboratories must have the means to detect these endogenous interferences, make decisions about reporting results, and evaluate their impact.

Most clinical pathology books provide only an abbreviated introduction to the subject, or provide a long list of references, without the necessary foundation for evaluating their significance. Package inserts typically provide scant information. This book provides the empirical and theoretical foundation for these interferences, describes the clinical settings where they occur, and explains their evaluation and detection, allowing the laboratory to interpret the available data on interferences and make the appropriate decision to effectively report test results while protecting patient safety.

VOLUME 6: MICHAEL IMHOF

Malpractice in Surgery - Safety Culture and Quality Management in the Hospital - published: December 2012 - ISBN: 978-3-11-027160-7



This book covers the improved management of medical errors and a description how to establish an efficient quality management system in hospitals. Explains medical errors and their negative influence on doctor-patient relationship.

Tentative estimates suggest that one in ten patients suffers from an adverse event in hospital. In Germany, approx. 1.8 million out of approx. 18 million inpatients suffer from adverse events; 50 percent of these cases are estimated to be avoidable. In the US, nearly 100,000 people die from the consequences of mistreatment.

The intensive care units record 1.7 medical errors per patient and day. The most affected disciplines are the operative disciplines, particularly general surgery. Medical errors mainly occur when the indication for surgery is being made, during surgery and post-surgery. Suspicious oncological diagnostic results and post-operative complications are also often ignored.

This book deals with complications and typical medical errors in surgery. It shows solutions and ways of dealing effectively with these errors and how to establish an efficient security management system

VOLUME 7: GIUSTINA DE SILVESTRO, ARIANNA VERONESI AND MARIA VICARIOTO

Transfusion Medicine and Patient Safety – published: December 2012 - ISBN: 978-3-11-028707-3

Blood transfusion is considered a life-saving therapy since ancient times, but, at the same time, a high-risk procedure. Nowadays the common perception is that infection is the greatest risk, even if the blood has never been safer from this point of view. Currently, the residual risk of transfusion must be related mainly to immunological mechanisms underlying to AB0 and minor blood systems, to compatibility of blood transfused and to development of irregular antibodies in transfused patients.

„Transfusion Medicine and Patient Safety" aims to provide the basics of immunohematology to readers and to analyze the *transfusional* process highlighting the most critical points, thus more exposed to errors.

Screening on blood and blood components for infectious diseases along with the surveillance action on emerging viruses results in the drastic reduction of post-transfusion infection, together with the potential to further increase the level of security from infection through the inactivation of blood components. The text also describes the major diagnostic systems and organizational models that modern technology provides us with a correct *immunohematological* diagnosis and an appropriate trasfusional therapy.

VOLUME 8: HANNES ZACHER

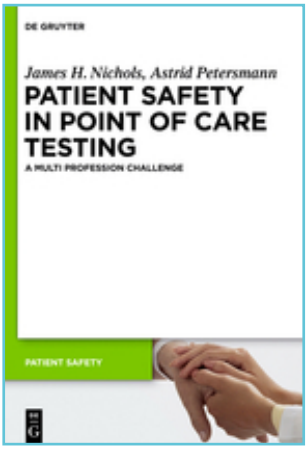
Patient Safety - A Psychological Perspective – published: February 2014- ISBN: 978-3-11-028192-7

This comprehensive book takes a psychological perspective on patient safety. It is based on the most recent theoretical and empirical research evidence from psychology (including clinical, work, and organizational psychology) and adjacent social and behavioral sciences such as human factors. Factors that influence safety-related experiences, behaviors, and outcomes of patients and professionals working in clinical settings such as medical practices and hospitals are reviewed, structured, and critically evaluated. Consistent with the complexity of the topic, the author takes a multi-level approach to patient safety, which includes a review of individual, team, and organizational factors and outcomes. The book describes how these factors, by themselves and in combination, can facilitate or impede patient safety. Individual factors include safety-relevant knowledge, skills, abilities, and personality traits such as conscientiousness and emotional stability. Team factors include group communication, training, and leadership. Finally, organizational factors include the safety culture and climate. Throughout the book, different evidence-based intervention programs are described that can

help practitioners promote patient safety and prevent accidents. The book is a valuable resource for both researchers and practitioners interested in understanding, maintaining, and improving patient safety in a variety of applied settings. It is based on the most up-to-date research evidence from psychology and neighboring disciplines, and it is written in a clear and non-technical language understandable for a wide audience.

VOLUME 9: JAMES H. NICHOLS, ASTRID PETERSMANN AND ANDERS KALLNER

Patient Safety in Point of Care Testing - A Multi Profession Challenge - planned: 2016 - ISBN: 978-3-11-027285-7



Having patient results available immediately at the patients' bed side has led to obvious improvements in patient care. The introduction of automated blood gas analyzers saved many lives. Glucose measurements close to the patient originated in the field of patient self control. Besides these two traditional application of POC tests many more are available on the market today their use stretching from ambulatory to highly complex sites such as intensive care units.

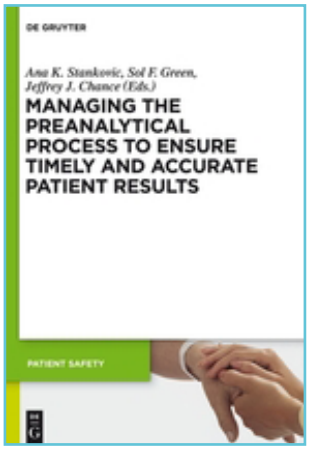
In POCT an impressive potential of improving patient care is combined with increased risks for patient safety. Thoroughly balanced decisions for the use of POCT are a key to patient safety in this field. Depending on the extent of use within an organization, a POCT concept can aid to minimize risks for patient care. As the use of POCT expands experiences gained can serve as guides for future use. POCT is closely related to laboratory medicine but follows completely different rules with a tremendous impact on patient safety. To create awareness for this fact is one aim of this book addressing all involved professions.

The reader will find a comprehensive overview ofŞ

- benefits of POCT and the potential risks for patient safety,
- perspectives of involved professions on POCT,
- POCT strategies for health care providers
- Checklists of important aspects of patient safety in conducting POCT for both individual and institutional use.

VOLUME 10: ANA K. STANKOVIC, SOL F. GREEN AND JEFFREY J. CHANCE

Managing the Preanalytical Process to Ensure Timely and Accurate Patient Results – planned: 2016 - ISBN: 978-3-11-028161-3



The preanalytical phase of laboratory testing—defined as the time from test ordering by the physician until the sample is ready for analysis—encompasses numerous critical steps. The correct specimen must be obtained from the correct patient using correct technique, and specimens must be handled and processed in a manner which ensures specimen integrity. Along the way, there are numerous factors and variables where medical error may be introduced, with the potential to impact patient safety. It is estimated that preanalytical errors may account for up to 70% of total laboratory errors. In addition, delays and rework in the preanalytical phase prolong the time from

order to results. As such, improvements in this area may deliver the greatest gains in the overall quality of laboratory services and patient care, and can help control the overall costs of health-care delivery. This book provides an overview of the preanalytical phase, including test ordering, patient identification, specimen collection, specimen handling and processing. In each step of the process, the variables which influence result accuracy and turnaround time are identified and discussed. Guidelines and best practice recommendations are also included.

Publisher: IFCC Communications and Publications Division (IFCC-CPD)

The eJIFCC is a member of the **Committee on Publication Ethics (COPE).**

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