Clinical Chemistry and Pathological Analysis: Modern Laboratory Techniques for Accurate Disease Detection

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Abstract

Clinical laboratory automation has significantly progressed in recent years. Automation has increased the safety, reliability, and throughput of many clinical tests. Nevertheless, automation for viral detection remains relatively limited, with most viral tests currently being performed manually. Automation of viral tests has now reached levels comparable with those achieved for other clinical areas such as biochemistry and immunochemistry. As a result, the benefits of improved automation strategies can now also be applied to the viral field.

Laboratories play an absolutely crucial role in the process of clinical decision-making, serving as a foundation for accurate diagnoses and effective treatment plans. It has been estimated that over 70% of all diagnostic information utilized by clinicians in their practice is derived from laboratory results. This invaluable information stems from a wide array of specific tests that are meticulously requested in direct response to the clinical indications presented by a particular patient. Consequently, gaining a comprehensive understanding of and effectively managing the ongoing demand for laboratory testing remains a subject of considerable interest among healthcare professionals; therefore, considerable work has been dedicated to the in-depth analysis of requests for clinical chemistry testing in order to improve the efficiency and accuracy of the diagnostic process.

Content

Chapter - 1

Fundamentals of Clinical Chemistry

Analytical computer modelling of test request patterns is proposed as an effective strategy to determine the precise requirements for a monitoring service in the intricate field of clinical chemistry. This comprehensive study demonstrated that distinct test profiles not only vary significantly but also exhibit remarkable consistency depending on the specific medical departments that are requesting the various analyses. In particular, profiles related to cardiology, hepatology, and nephrology were thoroughly analyzed utilizing both cluster analysis and multidimensional scaling techniques. The valuable information gained from this in-depth analysis is highly relevant for generating hypotheses regarding clinical pathways and assessing the optimal configuration of analytical apparatus utilized in the laboratory setting. This approach can enable laboratories to enhance efficiency and improve patient care outcomes through better resource alignment [1].

Principles of clinical chemistry in disease diagnosis

Clinical Chemistry plays an important role in diagnosing diseases and metabolic disorders and in determining treatment outcomes. Clinical Chemistry encompasses the study of body fluids (serum, plasma, urine) at the cellular and chemical levels in order to elucidate normal functioning of the body and alterations to the body chemistry that lead to disease or metabolic disorders. Clinical Chemistry is one of the branches of Pathology; Pathology, the branch of Medicine that studies diseases, is further divided into Clinical Pathology and Anatomical Pathology. Pathology is based on the study of specimen (tissues, cells or fluids) obtained from Human and Animal bodies.

Specimens for clinical chemistry are Human or Animal fluids (blood, urine, etc.). Specimens for Clinical Pathology (Cytology or Hematology) are Cells (exfoliative, aspirate, or tissue) obtained from the body. Specimens for Histopathology are Tissues obtained from the body. Clinical Chemistry remains a fundamental tool in the field of Medicine and is necessary for diagnosing and monitoring diseases. Laboratory medicine is used to diagnose, characterize, and follow the progression as well as treatment of nearly all diseases. New markers are frequently under investigation, and marker

combinations are often proposed as a means of increasing sensitivity and specificity. Many new markers suffer from a lack of clinical studies to validate their usefulness [2]; B. Hemel *et al.,* 1989 [3, 4, 5]

Chemical composition of biological fluids

In the clinical setting, chemical composition analysis of body fluids provides important diagnostic information. The analysis must deliver precise and accurate data for relatively low cost and time. A well-established procedure including preparation, derivatization, separation, identification, and quantification of the target molecule is routinely followed. Development of free-flow electrophoresis coupled to mass spectrometry with improved performance liquid chromatography gives an innovative way of characterizing the chemical composition of these prechamber solvents. Another possibility is flow analysis where the coupling of complex separation techniques is avoided. Advances made in the past ten years focus on the state-of-the-art application of separation techniques such as ultra-high-performance liquid chromatography, high-performance anion-exchange chromatography, gas chromatography-mass spectrometry, and micellar electrokinetic chromatography [6, 7, 8, 9].

Analytical accuracy and precision in laboratory testing

Laboratory testing plays a vital role in the detection and diagnosis of diseases. Nevertheless, the reliability of results derived from tests depends on both the analytical accuracy and precision of the desired measurements.

In general, accuracy refers to the closeness of a measurement to the true value, whereas precision indicates the reproducibility of a measurement when repeated. As many biological materials are often heterogeneous, testing can be affected by these and other factors, including sample collection, storage, and transport, as well as the use of unsuitable or faulty materials. Despite laboratory staff being highly trained and experienced, a number of pre-analytical sources of error can remain outside their control. In essence, the accuracy of the laboratory is more related to the analytical system than the individual and could therefore be assessed by means of external quality assessment schemes. Such schemes are organized in such a way that an unknown sample is homogenized and divided into small aliquots for distribution to laboratories. Each laboratory carries out its own selection of samples and quantity in its own jurisdiction and performs the testing in the usual manner as dictated by demand. Lab replies are usually submitted to the evaluating body by e-mail, with any identity code associated with the sample. Results are subsequently analyzed, and feedback provided [10, 11, 12].

Units, Reference Ranges, and Biological Variation

The evaluation of laboratory results should take into account the applicable unit. Though SI units are universally recognised, non-SI units continue to be employed in many countries [13].

Biological variation of laboratory results is another essential aspect to consider. Indeed, the assessment of laboratory results is usually performed in the context of a reference range. A reference value is defined as "the laboratory result obtained from a specimen taken from a reference subject" and a reference range is "the interval bounded by the lower and upper reference values". Therefore, instead of a fixed value, laboratory results should be considered in terms of a reference range corresponding to the method employed. In a clinical context, it is generally preferable to select ranges for a population of individuals suitably sampled according to the condition of interest.

The European Communities Council Directive 98/79/EC on In Vitro Diagnostic Medical Devices specifies the requirements and procedures to be followed by Notified Bodies for the verification of some in vitro devices. However, there remain clinical and regulatory gaps concerning the establishment, verification, and validation of reference intervals. The procedures can be quite cumbersome and the selection of appropriate reference subjects relies on a sound understanding of the population of interest.

The individual laboratory should confirm that the reference range supplied with a reagent is compatible with its own equipment and procedures. Certain reference ranges can be accepted when the test method in the laboratory matches the one used to establish those ranges. However, the correlation may still not be valid if the method was not common to both contexts. Alternative test methods and analyte preparations may also affect the correlation and limit its validity for differing physiological conditions [14, 15, 16, 17].

Ethical and Professional standards in clinical laboratories

The laboratory is a potentiator for specialisation and the professional practice of laboratory medicine is today under constant pressure from advances in technology and the biochemistry and molecular biology that underpins it. The law regulating medical and health professions, and the education these professional practitioners receive, is also under regular review [18].

The safety of the public and the profession is primarily reliant on competent practitioners. The practitioner must be of suitable education standard, capable of maintaining an appropriate standard of practice and behaviour, and, importantly the practitioner should be subject to some form of peer assessment and, where deviations occur, to censure or disciplinary action [19]. In clinical laboratories these can be self-imposed by professional associates and laboratory assistance engaged by a health authority to act in an advisory position [20, 21, 22, 23].

Chapter - 2

Laboratory Organization and Quality Management Systems

Laboratory organization plays a vital role in laboratory medicine. A well-managed laboratory improves efficiency and accuracy of results that are generated, so the dependability and confidence of users increases. Lack of attention and less care of laboratory management either gives rise to losses due to wastage of time and opportunity or hinders obtaining quality reports in time. A laboratory of any calibre must know the client or user, what they want, when they want it, how they want it, and costs that are involved in making and delivering a product or service that meets or exceeds their expectation. The route to understanding the needs of clients is surveillance and monitoring through checks, balances or feedback mechanisms [19].

Liver and kidney function tests are among the most requested tests by the prescribers. The basic information provided in the report is used by clinicians to make a proper diagnosis, choice of treatment and monitoring of the diseases. Reports of low quality lead to serious consequences that may potentially affect the patients undergoing treatment. Reliable and trustworthy test results are crucial in for the laboratory sector [18].

Structure and workflow of clinical laboratories

The process of the clinical laboratory starts with the reception of samples obtained mostly from patients in a clinical medical context or obtained elsewhere and taken in by patients to be sent for analyses. Once received, the samples are logged into the laboratory system and then sorted according to their autochecked requisitions to then be routed to the preanalytical chain relevant to their laboratory discipline which can be either chemistry, cell blood count or heating samples. The preanalytical chain includes tasks such as the sorting tubes according to the exact mesuring process, the temperature to be maintained and the selective process of tube management on the basis of exclusion of unwanted tube types or colours. At the end of the preanalytical phase samples are directed towards the analyzers of different experimental protocols where protocols can have multiple types of measurements [1, 24, 25, 26].

Internal and External quality control programs

Quality control programs, both internal and external, play a critical role in laboratory research due to their ability to facilitate the precise detection of systematic errors in experimental data. The quality control programs specifically designed for use with real patient data are referred to as Patient Data-Based Quality Control (PDB-QC), which serve to enhance and support the traditional internal quality control (IQC) strategies already in place. Within the framework of PDB-QC, alert rules are meticulously established to discover stable trends that clearly indicate the need for corrective actions, while control rules are implemented to identify any significant departures from expected results that may threaten the overall credibility and reliability of the reported outcomes. These rules are designed to help laboratories track a test's overall consistency, verify the stability and reliability of negative internal control systems, and thoroughly validate the quality assurance procedures employed within the laboratories. It is essential to emphasize that lab supervisors remain required to diligently follow IQC procedures to comply with existing regulations and ensure that safety and accuracy standards are met consistently. Additionally, another vital option available within quality control programs involves conducting external evaluations of laboratory performance, which are systematically collected through an external quality assessment (EQA). These external evaluations are crucial for benchmarking performance and ensuring that laboratories adhere to established standards of quality and excellence [27, 28].

Laboratory Accreditation and International Standards (ISO, CLSI)

Laboratory accreditation is the process by which a laboratory demonstrates dedication to high-quality results that are achievable and reliable through defined systems and processes. Accreditation is often performed by accrediting agencies that have assessed a laboratory's adherence to International Laboratory Standard ISO/IEC 17025: 2005-General requirements for the competence of testing and calibration laboratories. This ISO standard covers all aspects of laboratory management and internal operations. In the U.S., many laboratories willingly seek accreditation from the College of American Pathologists (CAP) or the Joint Commissions (JC). These organizations conduct an on-site assessment every 1 to 2 years to evaluate a laboratory's compliance with national standards and guidelines.

Many laboratories seek accreditation to ensure they are providing high-quality testing and reporting for their clinicians and patients. Some laboratories, particularly those performing high-complexity testing, may be

required by law to meet specific standards. The Clinical Laboratory Improvement Amendments (CLIA) of 1988 contain regulations that apply to laboratory testing on specimens derived from humans. The Centers for Medicare and Medicaid Services (CMS) administer CLIA; operations are overseen by a system of accreditors that CMS has authorized to inspect laboratories for compliance with CLIA regulations. During the review process for three different testing levels-waived, moderate, and high-all aspects of laboratory testing are evaluated, including specimen collection, transport, storage, testing, and reporting. Compliance is required with provisions from all applicable regulatory agencies, including HHS (Health and Human Services), OSHA (Occupational Safety and Health Administration), the Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA).

Error sources and risk management in laboratory medicine

Recent guidelines introduce risk management in clinical laboratories to prevent errors and minimize harm [29]. Errors can occur at any testing process stage, so laboratories must identify weaknesses and implement detection measures. Risk represents the chance of encountering harm, estimated by the probability and severity of an event. Managing risk involves keeping it at an acceptable level through detection mechanisms like quality control. Risk analysis includes failure modes and effects analysis (FMEA), which identifies potential failure sources, assesses their impact, and outlines control measures [30].

Understanding weaknesses in the testing process is essential for developing a risk-based quality control plan. Most errors occur in the preanalytic or postanalytic phases outside laboratory supervision, including issues with physician orders, specimen collection, transportation, and reporting. Specific errors may arise from specimen mishandling, mislabeled samples, or communication mistakes. Within the laboratory, errors might result from instrument failure, incorrect maintenance, calibration problems, expired reagents, or flawed calculations. Environmental factors like temperature, humidity, light, and altitude can also affect test accuracy. Operator errors include misidentification and improper sample handling. Analyzers may malfunction due to incorrect calibration or sample application. A thorough quality control plan addresses these potential error sources and implements measures to mitigate risks, ensuring accurate, reliable testing results [31].

Laboratory Information Management Systems (LIMS)

By combining technological infrastructure with specialized software, LIMS provide an environment for systematically managing the complete workflow of a laboratory. This includes, among other things, project setup at an administrative level, resource management (instruments, materials, or people), and the complete monitoring of individual samples and their associated values. Today's LIMS can generally be integrated seamlessly into other software systems, such as electronic lab notebooks or electronic laboratory request systems. Such integration facilitates data exchange and helps maintain a clear overview of existing laboratory progress.

Highly flexible and adaptable to an individual laboratory's needs, LIMS can be found in many modern biomedical analysis and clinical chemistry laboratories. Combined with Laboratory Automation Systems (LAS), they contribute to higher productivity and process reliability in sample management similar to that seen in the manufacturing industry [19].

LIMS applied as a laboratory management tool generally follow the ISO-9001-2000 standard, with additional features described further below [32]:

- Daily lab activities such as communication on equipment schedules and availability, tasks for multiple researchers, and technical specifics of samples under investigation may be described on the system display. Such information offers an overview of laboratory supply and resource availability without requiring further inquiries.
- The station of laboratory tables, interior design, and specimen transportation paths may be specified clearly. This allows detailed mapping of each station in advance of work.
- Sample tracking systems help to provide a historical overview of sample traceability, allowing for reconstruction of work steps even after considerable time has elapsed.

Chapter - 3

Specimen Collection, Handling, and Pre-Analytical Variables

Types of Clinical Specimens and Their Applications

The type of clinical specimen is determined during the initial routine assessment of a clinical sample. It is a unique parameter that is directly associated with the clinical symptoms of a patient, such as fever, pain, swelling, coughing, and fatigue. The choice of the specimen type relies on understanding the procedure to isolate the suspected pathogen or the nature of the compounds to be analysed [33].

Clinical specimens play a vital and crucial role in accurately determining the complex host-pathogen relationship. A properly controlled pathology necessitates the careful management and control of a minimal number of clinically non-relevant compounds that may interfere with the results. The rigorous specimen selection process, paired with a well-defined strategy aimed at preserving sample integrity, is employed in conjunction with a carefully limited class and number of desirable compounds that are required for reliable real-time assays. This careful approach ensures that the integrity of samples is maintained throughout the testing process [2].

Patient preparation and sample collection techniques

Patient preparation and sample collection techniques are vital stages in obtaining an accurate laboratory result. Precise instructions in these areas minimize preanalytical errors while maximizing analytical performance. The recommended practice is to obtain fasting blood specimens in the morning before breakfast, although this requirement may vary with clinical indications and guidelines. Special requirements pertain to blood collection when diagnosing drug addiction and personality disorders. A transfer tube and closed-vacuum system with a serum-separating gel may be essential [2-34].

Sample transport, storage, and stability

Transporting samples for clinical chemistry analysis is a delicate and intricate task that significantly influences the integrity and reliability of the serum or plasma collected for testing. Collecting blood is a complex and

nontrivial procedure that can lead to preanalytical rejection of any of the serum samples obtained. Generally, a two-step sampling procedure is highly preferred: first, a sample is drawn for hematology testing, followed by a second sample designated for clinical chemistry analysis. It is essential that only after the sampling for hematology is completed is the tube for clinical chemistry selected, which helps minimize the time difference that could affect the quality of the two samples collected. In addition to careful transport, the maintenance of proper temperature during the entire process is paramount to ensure the accuracy and reliability of the analytical results [35].

Pre-Analytical Errors and Their Impact on Results

Preanalytical errors are the major source of discrepancies in the clinical laboratory findings and continue to compromise quality assurance. Errors in any step preceding the arrival of a sample at the laboratory that lead to incorrect test results are classified as preanalytical errors. In respect to pathobiochemical analysis, they constitute between 30% and 70% of the overall errors and may even exceed 90% in reports relating to hematology, owing to the high susceptibility of blood preparations to sample handling conditions. Such errors may impact on several parameters, including the collection media, precollection sampling, handling and transport of the sample, nature and type of the collection container, the delayed addition of preservatives, multiple freezing cycles and the nature of the stored sample. These are further stratified into five different categories. As the analytical techniques become more sophisticated, it has become apparent that preanalytical factors also influence the expression of specific attributes of the target analyte, with even metal content yielding erroneous results if the preservation is not appropriate.

Glycolysis, for example, consumes glucose and produces lactate and pyruvate. Without the appropriate inhibitor, serum lactate and pyruvate are falsely elevated and glucose decreased. Proteins undergo proteolysis with the release of ammonia, which may exceed the level found in uremic patients on storage of blood or plasma. Numerous other artefacts occur as a consequence of the proteolytic activity of bacteria present in unpreserved samples, such as the loss of histidine in urine. The premature instability of certain markers can also result in erroneous determinations when they are diluted with inappropriate preservation media [36, 37, 38, 39].

Biosafety and Infection control measures

An integral component of clinical laboratory medical technology aims at obtaining accurate and reliable laboratory results without compromise.

Constant concern for the physical safety of the technologists performing these analyses is balanced with concerns for the biosafety of patients, staff, and the public in laboratories where laboratory accreditations demand strict compliance with infection control measures, including the containment of zoonotic diseases. Infections acquired within the laboratory environment are classified as laboratory-acquired infections (LAIs). Among the three main points of susceptibility considered in the laboratory setting, dermal exposure has been reported to account for over 80% of infections, more than 95% of LAIs are caused by bacteria or fungi rather than viruses, and approximately 75% of LAIs result from organisms usually regarded as non-pathogenic. Over 75% of cases are of skin or soft tissue origin.

Infection control measures, such as those catalogued in the World Health Organization's five moments of hand hygiene, play a key role in the prevention and control of LAIs. Immunization of laboratory staff against hepatitis B is essential, and consideration should be given to immunization against other infectious agents in defined risk groups, particularly for laboratory personnel at highest risk. Biosafety cabinets should be used to minimize exposure to infective aerosols and splashes, and other personal protective clothing and equipment should be worn according to established guidelines. Hand hygiene should be performed immediately after potential contamination and regularly throughout the working day [40, 41, 42, 43].

Chapter - 4

Analytical Techniques in Clinical Chemistry

The automatic biochemical analyzer is the main detection instrument in clinical biochemistry laboratories. Laboratory results are critical for more than 70% of medical decisions. Therefore, any errors in the pre-analytical, analytical, and post-analytical phases may seriously affect a patient's diagnosis, prognosis, and treatment. The detection of blood or body fluids is routinely used for diagnosis, prognosis, monitoring, disease surveillance, and other purposes in clinical laboratories. Biochemical analysis is one of the information-rich analytical techniques used to detect various biological fluids for clinical decision-making. A biochemical analyzer can be defined as laboratory equipment that measures the amount of specific substances in biological samples using different methods based on the spectrophotometric principle [44, 45, 46, 47].

Spectrophotometric and Colorimetric methods

Spectrophotometric techniques are amongst the most commonly employed analytical techniques in laboratory investigations. These methods rely on the ability of molecules to absorb light. When light is absorbed by a molecule, the energy of the light excites the molecule to a higher energy state. In spectrophotometric methods, the intensity of light transmitted through a sample is measured, which provides information about the concentration of the absorbing species. Beer Lambert's Law mathematically describes the relationship between the concentration of an absorbing species and the intensity of transmitted light.

Colorimetric techniques are a subset of spectrophotometric techniques where the concentration of analytes is indirectly determined by detecting a colored product following a chemical reaction between the sample and a reagent. In colorimetric detect ion, the sample analyte participates in a chemical reaction forming a colored product, and the absorbance of the colored solution is measured at a wavelength where the product absorbs. In most cases, these methods are highly sensitive, fast, and inexpensive, requiring commonly available and inexpensive glassware and reagents. Colorimetric investigations provide qualitative as well as quantitative

information, making it one of the most widely used methods in clinical chemistry and pathological investigations, deciphering forynthesis of products formed in a specified time [48, 49, 50, 51].

Electrochemical and Potentiometric techniques

Over several decades, ion-selective electrodes and potentiometric methods have emerged for the measurement of clinically important blood electrolytes and pH. Blood samples contain both soluble and suspended components. To bypass complications of turbidity due to suspended matter, direct potentiometric measurement can be performed employing an ion-selective electrode, or in the case of its absence, an indirect measurement method can be adopted [52]. Indirect measurements involve dilution of a patient sample with a non-fouling buffer or a qualified electrolyte sometimes incorporating a separation technique that filters out suspended blood particles.

Ion-selective electrodes respond selectively to a specific ion and the measured electrochemical potential corresponds to the concentration of that specific ion in the solution. A calibration curve plotting signal against ion concentration allows determination of the ion concentration in an unknown sample before and after treatment. Sensor fouling especially occurs these days in electrochemical devices generally and occurs easily in the blood sample analysis through the deposition of analysing materials themselves on the sensor electrode. Utilization of dielectric barrier plasma generated through dielectrophoretic action enables a highly sensitive on-site blood plasma analysis to inhibit the instant electrode-fouling and prolong sensor life span [54, 55, 56, 57, 58].

Chromatography and Mass Spectrometry

Chromatography is a technique used to separate components of a sample based on differences in their interaction with a stationary and a mobile phase. The chromatographic phases operate on the principle of partitioning. In the simplest case, a solute (e.g. toxic alcohol, drug, or steroid) partitions between two liquid phases: one in finite contact with the solid support of the column, the other in bulk motion through the column. In liquid chromatography, the solid adsorbent is usually fine particles of various functionalized silica gel bonded to various alkyl chains or other polar groups. Detection usually relies on measuring the UV/visible absorbance of the solute, possibly at more than one wavelength. Analysis of simple drug combinations such as morphine and heroin is straightforward; however, drugs and poisons with similar functional groups quickly challenge the technique's band-limiting, resolution, and noise problems with increasing concentration ratios. More complex mixtures

require the use of mass spectrometry (MS) and tandem mass spectrometry (MS/MS). In these cases, the analyte of interest is isolated by the first MS and subsequently fragmented to yield a specific set of transition ions for identification by the second MS. The combined use of liquid chromatography and tandem mass spectrometry (LC/MS/MS) makes the analysis of even the most complex mixtures feasible.

Mass spectrometry has taken clinical toxicology to new heights of specificity and sensitivity, as both LC/MS and gas chromatography/ mass spectrometry (GC/MS) are now trusted for primary testing rather than as confirmations for less-specific platforms. In fact, it is rare for a testing laboratory to work any analyte by such a qualitative tool as immunoassay, particularly given the high false-positive and false-negative rates endemic in these older detection methods. But every tool has its shortfalls and these two methods for clinical sample analysis are no exceptions. For clinical screening toxicity, GC/MS retains its popularity; it is particularly well established for detecting previous drug use in forensic and child-abuse cases. Indeed, for these applications it is almost the only platform. However, LC/MS in its various continuing incarnations is clearly encroaching and, for some reasons, should by now be regarded as the method of choice [7, 59, 60, 61, 62].

Immunochemical assays and Labeling systems

Detection of antigens or metabolites in immunoassays and enzyme-linked immunosorbent assay (ELISA) require additional signal amplification. This is accomplished by coupling the primary animal antibody to a detectable reagent. The reagent may directly label the primary antibody (single-labeling concept) or may be an enzyme-labeled secondary antibody that reacts with the species in which the primary antibody was produced (bridging concept).

Indirect labeling expands the choice of a color reaction when a secondary antibody is used. It also provides an additional layer of signal amplification, as multiple enzyme-labeled secondary antibodies may bind to a single primary antibody. It is possible to amplify the reaction even further by using a third antibody tagged with an amine-reactive dye that binds to the primary antibody. The amine-reactive dye is heterobifunctional; one functional part reacts with the amino groups on the backbone of the primary antibody, while the second allows covalent attachment of an enzyme-labeled secondary antibody.

Among the most commonly used enzyme systems are horseradish peroxidase (HRP) and alkaline phosphatase (AP). Because of the water solubility of the substrates, these two enzymes are frequently used in conjunction with soluble substrates. Detection does not require fluorometry or

UV absorbance spectrophotometry, since the colored products are easily detectable by the naked eye. Detection limits can be increased to picomolar levels by coupling biotin-streptavidin binding, in which a single streptavidin molecule can bind four biotin molecules. Haptens such as biotin or fluorophores can be conjugated to the antigen or antibody [63, 64, 65, 66].

Automation and High-Throughput Analysis

Automated laboratory systems, which accurately measure analytes in body fluids while minimizing human interaction, are becoming increasingly popular in many clinical settings. The cost of full automation is still prohibitive in most clinical laboratories; nevertheless, several cost-effective options exist that can provide reduced operator input with no loss of analytical performance. Rapidly increasing patient population and testing demand, as well as persistent shortages of laboratory personnel, are sustaining the impressive growth of fully automated hematology analyzers [67]. According to the Clinical and Laboratory Standards Institute, hematology is one of the major areas in the clinical laboratory where priority should be given to fully automated systems [68]. Hematology analyzers determine the blood's cellularity, providing information about the blood cells and their morphology, and addressing critical patient needs such as the differential count analysis of malaria. Fully automated analyzers allow for superior iron studies when performed simultaneously with routine hematology analyses.

Automation systems and software programs for laboratory information management systems (LIMS) and "smart" analyzers integrated using versatile modularity provide new opportunities for full clinical laboratory automation [19]. A modular automation concept ensures flexibility, reliability, and traceability of samples to optimize operator intervention; a modular system arrangement can be on the bench, contained in a cabinet, or in free-style form depending on laboratory lay out; modular and semi-modular clinical analyzers, and pre-analytical, analytical, and post-analytical systems constitute the main elements of a sample tracking and workflow optimization solution appropriate to existing needs; several pre-analytical robotics systems performing 100% traceable, recurrently routine preparative test pre-treatments; at the analytical stage, all standard and non-standard tests of different matrices (serum, urine, cerebrospinal fluid) can be performed on the same platform; a totally automated centrifuge with link to the pre-analytical analyzer cutting down sample preparation time; and the capability to perform urine test-strips on desk top systems being integrated in modular analysis concept represents additional flexibility for the routine laboratory [69, 70, 71, 72].

Chapter - 5

Post-Analytical Phase and Result Interpretation

The post-analytical phase is the final stage of laboratory work, where results are evaluated until they are released. Errors in this phase are less frequent than in the pre-analytical phase but account for about a quarter of laboratory errors. Laboratory personnel involved depend on their competencies. The phase is divided into a laboratory component and an external component outside the laboratory, where physicians make clinical decisions based on test reports to ensure patient care [73].

Effective interpretation of laboratory results is essential for accurate diagnosis and management of bleeding disorders. Interpretative commenting can improve the laboratory-clinical interface. Quality assessment of interpretative comments enhances their usefulness. Laboratory specialists advise clinicians on the interpretation of renal tests. Managing unexpected results, such as prolonged APTT, requires standardized evaluation procedures. Harmonization of quality indicators and critical result management across laboratories is crucial. Proper categorization of results as critical or abnormal aids timely clinical decision-making. Efforts toward harmonizing critical result management and adherence to guidelines improve patient safety and diagnostic accuracy [74].

Data validation and Result verification

The reliability of laboratory diagnosis hinges on strict adherence to semi-automation and manual techniques that are routinely validated and documented in accordance with the instructions of the manufacturers. Data validation consists of evaluating the investigation report and the trends of individual results while result verification refers to confirmation of significant changes coupled with clinical history or physical examination findings.

A physician also verifies results from all external sources including in-house tests performed by other hospitals and their own investigations subjected to external and internal quality assurance so that the results of pathological tests are classified into reactive, non-reactive and borderline. Flagged results of metabolic and endocrine laboratory tests are added to the report only when the performing laboratory follows recommendations on

evaluation of pre-analytical and analytical phases. A laboratory, sole or otherwise, encircling a large clientele which includes another hospital running as per the Quality Metrics label hence should deploy technology requiring least human intervention, remotely accessible for command and monitoring, limit the number of verifications, and have a real-time control over data generation in order to spend more time on supervised interpretation of results that cannot be repeated before reporting [75, 76, 77, 78].

Reference intervals and clinical decision limits

When laboratory results are generated, they are compared to defined reference intervals and clinical decision limits. A reference interval is defined as the "range of values for a given analysis obtained from individuals in a defined population who do not have the disorder or condition under investigation." Reference intervals are often established by testing a large number (>100) of normal individuals to create a 95% reference interval (2.5th to 97.5th percentile). The 95% reference interval is an arbitrary statistic because there is no physiological basis for any test to fall within the interval in 95% of a healthy population. For some tests, the population data appears to be more Gaussian, allowing for an actual mean ± 2 standard deviations to be used for the proposed reference interval. In general, the imprecision of the clinical laboratory method should factor into the interpretation of the reference interval as they account for greater than 90% of the total assay variability.

Although a reference interval can be applied to a large population, clinical decision limits are specific values that have direct clinical relevance in detecting, excluding, or predicting disease. These limits are more closely aligned with the physiology of the condition than population studies alone. The most widely recognized clinical decision limits are diagnostic cut-offs for common diseases. Due to the potential clinical significance of clinical decision limits, it is important to select the appropriate population when interpreting a laboratory result due to regional, age-related, gender-related, race-related, or disease-related influences. The clinical laboratory scientist must ensure that the clinical decision support system is maintained to provide decision-support data. Additionally, a detailed clinical report accompanying laboratory results will assist the clinician in the correct utilization of the test results [79, 80, 81, 82, 83].

Critical values and Laboratory reporting

Clinical laboratories may detect certain abnormal laboratory results that, when seen in conjunction with clinical symptoms, may constitute a medical emergency. These patient results require prompt notification of the

responsible physician, who must determine the acuity of the patient condition, provide immediate medical attention, and/or take remedial action. Automatic notification of result values beyond defined critical alert limits, present in most laboratory information systems, facilitates communication.

In some laboratories, the quality department notifies the responsible physician of both the positive and negative responses to critical values. Critical alert limits are established and periodically reviewed by the medical and/or scientific director in cooperation with the laboratory staff and, when appropriate, with hospital services (e.g., care units, pharmacy). Since critical values are patient-related, it is important that presenters, interpreters, and signers of the analysis be alert to changes in the patient's clinical status that might influence the critical limits. Critical response limits can also be established by the laboratory to facilitate rapid notification of requesting services in urgent situations, but should not detract from reporting critical lab alert limits. Laboratories using point-of-care testing must have predefined critical alert limits established by the laboratory director and disseminated so that critical results are recognized and managed properly [84, 85, 86, 87].

Clinical correlation and interpretative comments

Interpretative comments and correlation of the clinical features with the reported laboratory diagnosis assist the clinicians in understanding the possible physiological/biochemical changes involved in the disease process. However, interpretation of such comments could be complex and difficult for health care professionals with non-medical training. These issues arise due to the intricate mechanisms of diseases. Clinicians usually depend on the clinical pathologist, who correlates the clinical picture with the results of the various laboratory tests performed, in order to arrive at a logical diagnosis.

Clinical paths are multidisciplinary instruments intended to facilitate the analysis, planning, implementation, and evaluation of the patient care process. Clinical paths standardize the care of patients with similar problems and should ideally eliminate nonvalue-added wait times in care. With the introduction of fast computers, the formulation of clinical paths became feasible. Rapid access to test results, along with the ability to track patient care throughout the various levels of laboratory and hospital services has now become possible for hospitals and laboratories throughout the world. [88, 89]

Laboratory-clinician communication

According to scientific literature, over 70% of medical interventions are directly related to clinical laboratory results. Hence, clinical laboratories constitute a rich information source about diagnosis, prognosis, and

monitoring of therapeutic evolution in individual patients and public health at large [19]. The last few years have witnessed significant breakthroughs in clinical laboratories that have considerably improved processing capacities, precision, and accuracy. New biochemistry, immunochemistry, and hematology autoanalyzers provide high mix sample processing speeds. Therefore, it is now possible to process an unprecedented number of patient tests while ensuring the quality of results. Both aspects have substantially increased the analytical parameter prescription volume, which has been recognized as a critical information source for clinical diagnosis.

Chapter - 6

Carbohydrate Metabolism and Diabetes Mellitus

The importance of carbohydrates as an energy substrate and regulatory compounds has been established through metabolic studies. In the proper biological medium, red blood cells, although anaerobic organisms, can metabolize glucose. The action of the enzymes primarily responsible for glucose transformation in human red blood cells has been determined using cell-acidified aqueous extracts containing the enzymes. In healthy subjects, there is no evidence to indicate that the metabolism of carbohydrates differs significantly from that of lipids. The examination of the metabolism of carbohydrates in red blood cells from normal subjects and diabetics shows that the transition of glucose into the glycolytic phase occurs in normal red blood cells, whereas in the red blood cells of diabetics this transformation is reversed, indicating a possible change in enzyme activity linked to diabetes [90].

Diabetes mellitus is a group of metabolic diseases characterized by high blood sugar levels (hyperglycemia) for a prolonged period. This can be due to insulin resistance, insufficient insulin production, or both. Common symptoms include frequent urination, increased thirst, and increased hunger. Diabetes is a chronic disease that, if left untreated, can lead to serious damage to many of the body's systems, particularly the nerves and blood vessels. Long-term complications from high blood sugar include stroke, coronary artery disease, foot ulcers, kidney failure, blindness, and damage to the teeth and gums. All people with diabetes are at risk for long-term complications [91].

Glucose homeostasis and regulatory hormones

Both hyperglycemia and hypoglycemia have adverse effects on various tissues in the body. Thus, serum glucose concentrations are normally maintained within a fairly narrow range during fed and fasted states. This is controlled mainly by actions of the anabolic hormone insulin and the regulatory actions of various counterregulatory hormones.

The secretion of insulin from the pancreas is triggered mainly by increased blood glucose concentrations after nutrient intake. This facilitates the transport of glucose into muscle and adipose tissues, stimulates hepatic

utilization of glucose for glycogen synthesis (glycogenesis) and inhibits glycogen degradation (glycogenolysis) and gluconeogenesis. Insulin also promotes the uptake of other macromolecular nutrients by tissues, including amino acids by muscle and lipids by adipose tissue, and stimulates storage of these nutrients by driving biosynthetic pathways (protein synthesis and lipogenesis) while inhibiting the breakdown of these macromolecules (protein catabolism and lipolysis). The net effect is a reduction in serum concentrations of glucose, lipids and amino acids. Insulin levels together with those of glucagon, epinephrine, cortisol and growth hormone are critical for glucose homeostasis, especially during stressful situations. Counterregulation by these hormones during fasting or extensive exercise protects against hypoglycemia and prevents excessive hyperglycemia during stress [92, 93, 94, 95].

Laboratory diagnosis of diabetes mellitus

Compared to clinical chemistry performed as laboratory tests, which provide results that assist diagnosis, prognosis and follow-up of diseases, pathological tests support diagnosis. Diagnosis of diabetes mellitus is accomplished using blood glucose, glycated hemoglobin (HbA1c), glucose tolerance and glucosuria tests.

Adequate control of blood sugars can be monitored by measuring plasma glucose and/or performing specific HbA1c assays. Short-term blood glucose estimates can also be derived by measuring 1, 5-anhydroglucitol in plasma or urine. Plasma glucose can be used to confirm diagnosis or as a monitoring tool. Glucose tolerance testing is complex and only indicated when results alter clinical management. Urinary glucosuria is non-specific, rarely reflected in a laboratory report and of little clinical value. HbA1c, a major long-term glucose measure, must be performed according to National Glycohemoglobin Standardization Program (NGSP) standards.

Involvement of laboratory services should be sought when reductions in diabetic care provision are necessary. Changes to glucose regulation in pregnancy may impact other test results and should be flagged. Assessment of formal laboratory services' capacity and workforce is essential when planning point-of-care testing for diabetes mellitus [96, 97, 98, 17].

Glycated hemoglobin and long-term monitoring

Glycated hemoglobin (HbA1c) testing has been an essential part of diabetes care for almost three decades now, providing a dependable measure of average glucose control over a 2-3-month period without the need for fasting. Since its inception, HbA1c testing has shown minute variations that correlate with chronic glucose alterations. This relationship has been found

consistent in both diabetic and non-diabetic patients, indicating the test's ability to mirror chronic exposure to glucose. The stability of the measurement across time encourages the evaluation of its changes rather than the absolute value itself. Population studies demonstrate that HbA1c predicts the risk for diabetes complications in a continuous, log-linear manner, from normoglycemia to advanced diabetes. Consequently, it represents an important risk marker for the screening and diagnosis of diabetes and for monitoring its prevalence.

For patients under glucose-lowering treatment, adhering to the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) targets recommended for the HbA1c level remains pivotal, the benchmark being set at ≈7.0% [99]. Although various monitoring alternatives exist, the accuracy and the accessibility of the HbA1c test keep it as the cornerstone for effective diabetes care. Fructosamine and glycated albumin yield similar information; however, they are much less commonly utilized. Despite the stability of HbA1c as a measure of chronic exposure, specific conditions such as hemolytic anemias, sickle cell diseases, acute blood loss, and systemic erythropoietin administration may alter red blood cell turnover and misrepresent the average glycemic status [100]. Such situations prevent reliable data on HbA1c and call for either performance of fructosamine measurements or decisions centered on self-monitoring blood glucose (SMBG).

Gestational diabetes and special populations

Gestational diabetes is the most common of all diabetic conditions, with increased glucose intolerance occurring during pregnancy due to altered insulin metabolism. It is diagnosed during screen testing for pregnant women at high glucose level risk and confirmed by either a 3-hour oral glucose tolerance test or elevated fasting plasma glucose. Gestational diabetes impairs normal placentation, which can lead to morbidities including hypertensive disorders, higher cesarean section rates, and higher offspring birth weight and shoulder dystocia. Hyperglycemia during pregnancy can also lead to serious neonatal complications. In such cases, strict glycemic control using lifestyle changes or insulin may be required.

Glucose intolerance is inherently transient because the placental hormones causing it decline after delivery. Nevertheless, women with gestational diabetes are at increased risk of developing diabetes in later life. The rates of progression to diabetes can be as high as 60% or 70% in women with gestational glucose intolerance or diabetes, respectively, especially in the first 5 to 10 years after delivery. Even when blood glucose has normalized,

women who had gestational diabetes remain at increased risk for cardiovascular diseases and possibly for mental disorders. Additionally, the effects of gestational diabetes may extend beyond the mother; offspring exposed to gestational diabetes clearly have an increased risk for obesity and type 2 diabetes in later life [101, 102, 103, 104].

Emerging biomarkers in glycemic control

Monitoring glycemic control represents a key aspect in diabetes management. Conventional markers, such as glucose and glycosylated hemoglobin (HbA1c), despite being widely used, suffer certain limitations [105]. For example, dietary habits may contribute to a discrepancy between post-prandial blood glucose and HbA1c levels, the "glycation gap." Likewise, certain pathophysiological conditions can adversely affect the correlation between blood glucose and HbA1c. Glycated human serum albumin (HSA) constitutes an emerging additional candidate biomarker, bridging the difference in ephemerality of glycemic indicators which, together with HbA1c, glucose, and glycated apolipoprotein A-I (apoA-I), offers a comprehensive multi-marker picture of glycemic status chronicity. HSA constitutes the predominant protein in plasma, occupying ~60% of circulating proteins, having a half-life of approximately 20 days, and being less detained by the liver, hence being strongly correlated to glycated glucose concentration. A novel top-down proteomics assay capable of capturing HbA1c, glucose, glycated HSA, and glycated apoA-I from a mere 5 μL of blood has been developed. This approach employs multinozzle emitter array chips for ultra-fast 0.1-μL sample dispersion and a four-channel method to discriminate the complex fingerprint spectrum of blood, enabling accurate quantification and post-translational modification profiling, and has been demonstrated to distinguish healthy subjects from type 2 diabetes patients over different time scales, thus holding great promise for the improvement of long-term diabetes management.

Glycated albumin emerges as a further indicator in the glycemic-control monitoring arsenal, which other studies suggest may outperform HbA1c in the assessment of gestational diabetes [106]. Its measurement may be particularly relevant in cases of severe kidney failure, anemia, and type 2 diabetes. Correlation exists between glycated albumin levels and glucose-tolerance status, while all-cause mortality in dialysis recipients may be predicted therefrom. Glycated albumin reflects shorter-term glycemic control than HbA1c, thus providing valuable feedback on the efficacy of therapeutic interventions, and investigations are ongoing into its applicability for screening obese youths and appraising dietary glycemic exposures.

Chapter - 7

Lipid Metabolism and Cardiovascular Risk Assessment

A multitude of studies has associated lipid and lipoprotein levels with atherogenic cardiovascular disease (CVD) risk. The clinical chemistry laboratory provides quantitative measures of these analytes to support management and evaluate treatment response.

Although the pathophysiology of lipid metabolism is complex, some disease states result in easy identification of lipid disorders through clinical and biochemical evaluations. Measurements of fasting total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) concentrations enable a reasonable assessment of the patient's atherogenic CVD risk profile. A combination of atherogenic lipid parameters that includes the TC/HDL-C ratio and/or non-HDL-C concentration discriminates patients according to CVD risk and focuses therapeutic efforts on dyslipidaemia management [107]. Cardiology societies recommend that these parameters be accepted as a metabolic syndrome marker.

A standard battery of lipoprotein analysis for high- and low-density lipoprotein and very-low-density lipoprotein is still the gold standard for lipid testing, individualised and specifically recommended for children with hyperlipidaemia or adult relatives of hyperlipidaemic parents. Point-of-care analysis of lipid parameters during initial assessment can accelerate risk evaluation and management decision-making in patients presenting with chest pain [108]. Portability and rapid turnaround time of basic lipid panels assist with atherogenic risk stratification and determination of therapeutic interventions while longer-term risk assessment is based on a standard metabolic panel.

Lipoprotein structure and metabolism

Lipoproteins consist of a hydrophobic lipid core housing triglycerides and cholesteryl esters surrounded by a monolayer of phospholipids, free cholesterol, and protein, and serve to transport water-insoluble lipids in the blood. The protein components of lipoproteins are known as apolipoproteins and fulfil both structural and functional roles. Lipoproteins facilitate lipid transport in the blood through the formation of lipid–protein nanoparticles which, through their unique structure, solubility and specific density gravity,

and their biomolecular composition, permit cell-to-cell transport of cholesteryl esters, cholesterol, triglycerides, phospholipids, and vitamins. Apolipoproteins are synthesized within hepatocytes and enterocytes. They contain a combination of α-helices and α-pleated sheets, which confer structural integrity. Apolipoproteins interact with lipid transfer proteins in reparative processes, lipase during the hydrolysis of triglycerides in the peripheral tissues, cellular receptors, and lipid transfer and exchange proteins.

Lipoproteins can be subdivided into eight classes based on the diameter and the ratio of lipid to protein content: the chylomicrons, triglyceride-rich lipoproteins, LDL, and HDL. The chylomicrons are synthesized from dietary fat. Triglyceride-rich lipoproteins are formed as a result of de novo hepatic lipogenesis and are degraded through successive cycles of lipolytic digestion by lipases in the serum. Cholesterol and cholesteryl ester-rich LDL are formed in a dynamic equilibrium from the ULDL by selective cytoplasmic uptake of triglycerides and cholesteryl esters. The HDL subclasses may arise by distinct pathways or represent different stages of a common pathway. Lipoprotein remnant particles mediating cholesteryl ester transfer from the body tissues to the liver are an important component of lipoprotein metabolism. Each of these lipoproteins performs a distinct role in normal homeostasis, utilising specific pathways for cellular uptake [109, 110, 111, 112].

Laboratory evaluation of dyslipidemia

Laboratory investigation of dyslipidemia consists of measuring the lipids and lipoproteins in blood plasma. A detailed lipid analysis is undertaken in the presence of metabolic disease, where a precise lipid profile can guide the choice of drug and the definition of treatment goals. The primary lipids studied are triglycerides, cholesterol, and the lipoprotein fractions; the major lipoprotein of interest is low-density lipoprotein (LDL), followed by high-density lipoprotein (HDL) and very low-density lipoprotein (VLDL).

The conventional lipid biochemical profile includes triglycerides, total cholesterol, HDL-cholesterol, and LDL-cholesterol; the latter is calculated using a formula based on the fasting lipid levels. Lipoprotein determination can provide insight into the number of atherogenic particles contained in the plasma and therefore the metabolic risk. Candidates for more extensive lipid analyses include clients with borderline or moderate dyslipidemia (total cholesterol 5.5-7.8 mmol/L), familial hypercholesterolemia, and high residual cardiovascular risk (e.g., clients treated with statins who have a history of cardiovascular events).

Most cholesterol contained in the circulating atherogenic lipoproteins is contained in the LDL and intermediate-density lipoprotein (IDL) subclass,

whereas most cholesterol contained in the HDL fraction is found in the HDL2 particles. Cholesterol contained in remnant lipoproteins such as chylomicrons and VLDL-remnants and their surrogate variable apolipoprotein B (Apo B) also contribute to the atherogenic load. The atherogenicity of plasma cholesterol is therefore specific to the loading from distinct lipoproteins present in the circulation when each of these components is assessed independently [113].

Atherosclerosis biomarkers and risk prediction

Cardiovascular diseases (CVDs) represent the leading cause of global death, accounting for approximately 45% of deaths in Europe, and resulting in over 4 million annual fatalities. A demographic shift indicates an increasing prevalence of CVD among younger patients linked to rising incidence of diabetes, obesity, and tobacco use [114]. In a recent study, generalized risk assessment and stratification is based on standardized risk scores considering age, gender, tobacco use, blood pressure, cholesterol level, and diabetes status. However, almost 50% of those developing coronary artery disease fall into the low or intermediate risk categories, indicating the need for a tool to enhance risk prediction. A shortage of new cardiovascular risk indicators is also noted. Atherosclerotic disease has a well-established association with CVD and a wide range of atherogenic lipoproteins indicating its direct role in atherogenesis. Yet, by virtue of their indirect relationship with the disease, risk indicators based on atherosclerotic disease do not improve prediction [115]. Recent guidelines for the assessment of cardiovascular risk endorsed the use of additional markers such as intima-media thickness and high sensitivity C-reactive protein, whose efficiency remains to be firmly validated. These observations underscore the pressing need for new biomarkers that significantly enhance prediction accuracy.

The search for disease-associated biomarkers and the quest to streamline development and regulatory oversight to facilitate timely introduction into clinical practice proceed apace. Biomarkers in atherosclerosis and cardiovascular risk stratification remain largely unexplored. The term ‘biomarker’ denotes a distinctive measurable indicator of a biological process, pathogenic process, or pharmacological response to a therapeutic intervention; it also characterizes a fundamental characteristic that distinguishes between pathophysiological states or therapeutic responses. Interleukin 6 (IL-6) represents an important candidate biomarker in atherosclerotic disease, based on its close association with macro- and micro-angiopathies. Apolipoprotein B-100 constitutes a candidate biomarker whose performance must be rigorously examined.

Advanced lipoprotein testing

Lipid disorders rank among the leading causes of death and morbidity worldwide and may remain asymptomatic for long periods. Lipoprotein testing provides a two-dimensional profile of the lipid parameters involved in dyslipidaemia (loss of balance in the concentration of lipid compounds) and is essential for the prediction of cardiovascular disease (CVD) and for the monitoring of lipid-lowering therapies. The large number of commercially available kits for lipid fraction testing sometimes leads to confusion on their usage and interpretation. Testing of high very low-density lipoproteins (VLDL) cholesterol in association with raised triglyceride (Tg) level can suggest remnant levels, which alongside increased apolipoprotein B (Apo B) provides an assessment of overall atherogenicity. Various labelling techniques involving fluorescent dyes, antibodies, and magnetic beads allow particles of different sizes to be classified into subfractions (A, B, very big, and large) to complement and increase the value of the lipidogram. Single particle and pore-size analysis techniques based on these principles are recognised but not routinely deployed in lipoprotein testing, possibly due to the belief they do not significantly affect overall system performance. In consequence, the highly informative ViewPoint lipoprotein profile is less well-known [116].

Sufficient size, flexibility, fluidity, and structural integrity are essential properties of lipoproteins. Knowledge of ligands, receptors, transport routes, degradative pathways, and lipoprotein composition provides insight into the influence of lipoproteins on atherogenicity. Lipoproteins play crucial roles in the transport, storage, and exchange of lipids; in the transport of vitamins, toxins, and hormones; and in the delivery of therapeutic molecules. Their screening can also serve as a stepping stone for further tests such as those measuring apolipoproteins (Apo, Lp and so on) or uptake processes, depending on the need for information.

Clinical guidelines and therapeutic monitoring

An appropriate investigation of laboratory tests for monitoring the course of pharmacotherapy supports effective management of therapeutic regimens. Such an analysis enables verification of therapeutic effectiveness and assessment of compliance, and it allows clinical biochemists and pathologists—who cooperate closely with physicians—to evaluate the appropriateness of the prescribed therapy [91]. A variety of therapeutic drugs, including anticoagulants such as heparin, low molecular weight heparins, phenprocoumon, warfarin, and acetyl salicylic acid, as well as anti-infectives and all types of medication prescribed for psychosomatic disorders, have been

chosen by pharmacists and clinical biochemists for monitoring. Communication between the physician responsible for the pharmaceutical treatment and the microbiologist on the one hand, and the physician responsible for the serial clinical analyses and the clinical biochemist on the other hand, facilitates the consideration of adverse effects and adjustments to existing plans [117]. Commonly applied drugs for the treatment of psychopathological aberrations not infrequently lead to adverse drug reactions too. Certain therapeutic drugs are capable of influencing sodium and potassium excretion and/or retention. Drug monitoring has been shown to significantly improve the therapeutic outcome of treatment expressed in clinical terms. Depending on the country, ranging from 70 to 90 % of the therapeutic drugs are released through the kidney.

Chapter - 8

Renal Function Tests and Electrolyte Balance

The overview of renal function tests and of the laboratory assessment of electrolyte balance extends the discussion about markers that enable early diagnosis of affection of renal and urinary function [118]. Accurate determination of such markers has been rendered feasible by reliable point-of-care testing based on dedicated analyzers. Information is provided about the comparison of instruments using the Common Specification Measurement tool. The importance of implementing direct creatinine measurement using the isotopic dilution mass spectrometry approach as well as of applying the enzymatic procedure to avoid interferences is also specified [119].

Chronic kidney disease has emerged as a public health problem affecting a large proportion of world population. Its progression to end-stage renal disease mandates costly treatments that, depending on geographic locations, may not be accessible to many people [120]. While the situation severely limits the application of preventive measures, adapted direct creatinine determinations and reliable formulas for determining the glomerular filtration rate offer practical diagnosis and support general health management. Such evaluations between 75 mL/min and 15 mL/min enable reasonably precise defining of chronic kidney disease (R).

Glomerular and Tubular function assessment

Clinical conditions that determine the functioning of excretory tissue are assessed by glomerular function test and tubular function test. The glomerular function is assessed by determining the concentration of creatinine, urea and cystatin-c in serum. The assessments of the renal tubular function are based on the determination of serum concentrations of electrolytes, acid-base status and glucose. The electrolyte excretion is assessed by conducting urinalysis, while the status of acid-base and electrolyte homeostasis is assessed by conducting blood gas analysis. In addition to tubular function tests, the renal concentrating ability can be assessed by determining urine concentration, urine osmolality, urine specific gravity and excretion of free water.

Clinical conditions that alter urine concentrating ability indicate changes in renal tubular function. Conditions like hyperglcemia, physiological osmotic

diuresis and diabetes insipidus may lead to the excretion of dilated urine (hyposthenuria). The urine concentration can be diminished by administration of certain drugs like furosemide and thiazides, which inhibit sodium reabsorption in the kidney, and possibly shorten the renal tubular response to a water deprivation test.

Urinary concentrating ability is clinically assessed by recording the urine volume and specific gravity in a day or 24h urine collections. Specific gravity values below 1.008 indicate hyposthenuria; values above 1.030 indicate hypersthenuria whereas a value between 1.008 - 1.030 suggest isosthenuria. Measurement of urine osmolality is more precise than specific gravity but is not routinely performed due to complexity. Urgein osmolality < 200 mOsm/kg indicates failure of concentation and urine osmolality > 600 mOsm/kg indicates maximal concentrating capacity. An average excretion of more than 60 ml/man/h of free water (in absence of intakes or intoxication) suggests an adequate kidney concentrating ability [121, 122, 123].

Serum Creatinine, Urea, and eGFR

Serum blood urea and serum creatinine values provide a broad range for the biochemical evaluation of the kidney and kidney disease. However, each specimen should be correlated with the clinical condition of the patient.

Creatinine is an end product of creatinine metabolism, primarily in the muscles. Creatinine production is inversely proportional to age, muscle mass, gender, and race and is excreted freely in the urine. Plasma levels are mostly a reflection of glomerular filtration. Chemistries that falsely elevate plasma creatinine levels include hemoconcentration, some drugs, glycosuria, and large doses of cimetidine. Increased levels occur with renal failure, shock, dehydration, metabolic acidosis, acromegaly, and diabetes mellitus. Decreased concentrations occur with severe liver disease.

Serum urea is produced in the liver from the amino acids and is excreted by the kidneys. Urea production is increased with ingestion of protein-rich foods and is decreased with ingestion of carbohydrate-rich foods. An increase in plasma level implies that the ratio of urea to GFR has risen relative to the plasma creatinine concentration, but this does not necessarily correspond to renal impairment. Levels are increased in any condition associated with an increased urea production (catabolic states, gastrojejunostomy, diarrhea) or with diminished glomerular filtration (acute or chronic renal failure, bilateral renal artery stenosis) and may be decreased in hepatic insufficiency with those renal diseases in which there is a defective protein catabolism [124, 125, 126, 127].

Electrolytes and Acid-base disorders

The term ‘electrolyte’ refers to substances that dissociate into ions in solution and are capable of conducting electricity. Separated from the presented bulk of clinical bioanalysis defined by metaphors from cellular biology, a description of electrochemical processes turns a spotlight on labile cellular activity and blood electrolyte levels over a variety of metabolic aspects of pathophysiology, as summarised in specific concentration sets. Electrolytes define the hydrophilic conditions of the solvent milieu surrounding proteins and the lipid bilayer of the cell membrane, and function neutrally within isolators. Their asphaltic acidity dictates the cage-like position of proteins, which are major determinants of blood-colloid osmotic pressure. Hyponatraemia catalyses cell swelling and associated disturbances; hypernatraemia catalyses cell contraction and related failures. Altered bicarbonate carbonate concentration thus serves as a surrogate measure of CO2-driven respiratory adjustments, and of the HCO_3-H_2CO_3 equilibrium, with pH as the acido-basic measure of metabolic alterations. Hypercalcaemia diminishes neuromuscular excitability.

Measurable directly, the anion gap is the difference between strong anions and cations, and provides information on K+, Cl-, HCO_3-, profitably displayed in quadrants of an alignment diagram. Organic acidosis is of importance as an explanatory variable of hyperventilation, together with COVID-19. A widening gap signals acute metabolic labour, and a source of a rapid-respiratory change beyond the lungs. Consequently, the three-laboratory pairs are presented together, defining altered hyponatraemia and hyperkalemia in COVID-19. Markedly increased serum troponin I concentrations reveal acute myocardial damage in severe cases, as confirmed by prominent SOSG on computed tomography, and account for the recorded higher incidence of heart failure in male COVID-19 patients relative to the general population. Hypernatraemia is likewise associated with male sex, as are hypercalcaemia and altered conductance reflected by Bruyns’ law. CMD-acidosis dominates at high HCO_3-, together with organophosphates and diminished neuromuscular excitability running alongside control-serum SARS-CoV-2 concentrations below median. CMD-alkalosis is similarly defined by protein response and associated enhancement of cerebral excitation [128, 129, 130, 131].

Biomarkers of acute and chronic kidney disease

Proteinuria is a common indicator of kidney disease, detectable via urinary dipstick in 78% of patients with acute renal failure and 90% of patients with chronic renal failure. Tubular injury may indicate acute tubular necrosis

and its lack argues against this diagnosis, however tubular injury is not specific for this diagnosis. Cystatin C was studied in the absence of active urinary sediment, as a diagnostic marker for acute tubular injury and acute tubular necrosis. Cystatin C serum measurement alone, lacks sufficient diagnostic accuracy in patients with suspected acute tubular injury. It is a cysteine protease inhibitor involved in protein catabolism and is eliminated from serum by glomerular filtration, thus widely proposed as a promising renal marker. Several studies proposed cystatin C estimation as a marker of imine decadron-induced nephrotoxicity in rats. Its potential role as an indicator of renal chip rejection in canines has been reported. Cystatin C measurement was also suggested as supplement for monitoring renal transplant recipients. The results of a meta-analysis revealed that Cystatin C is more sensitive but less specific than Creatinine in detecting acute renal failure after cardiac surgery.

Microalbuminuria and cystatin C have been diagnosed in patients treated for allogeneic SCT and renal involvement in patients with multiple myeloma is also detectable with these two biomarkers, currently used in the clinical practice. Also, urinary neutrophil gelatinase-associated lipocalin has been studied in renal ischemia-reperfusion injury and heart disorders without nephrotoxicity in adult patients. Neutrophil gelatinase-associated lipocalin mRNA expression has recently been measured in renal allograft biopsies with enzyme-detected interstitial fibrosis in 45 canine transplant recipients. Increased concentrations were also observed in patients with laryngeal and kidney cancer. Neutrophil gelatinase-associated lipocalin levels were assessed in patients with acute coronary syndrome to predict acute kidney injury and others studied the protein in patients with laryngeal cancer presenting with chronic renal failure. Its measurement provides information about tubular damage better than creatinine in stem-cell recipients. The detection of KIM-1 has been proposed as a novel non-invasive marker for acute tubular injury and is more accurate than urinary protein or creatinine [132, 133, 134, 135].

Renal function in critical care settings

Acute kidney injury (AKI) and chronic kidney disease (CKD) are common in hospitalized patients, yet these conditions are seldom tested for in critically ill patients. Conventional renal biochemistry measurements are insensitive to early renal damage, show nonlinear changes, have limited prognostic value, and fail to predict long-term outcomes. New probes for urine and plasma biomarkers of AKI and CKD progression are needed.

The presence of AKI on admission is a strong and independent risk factor for in-hospital mortality, and AKI is associated with increased risks of

respiratory failure, shock, and extended hospital stays. These and other factors contribute to an ominous long-term prognosis for AKI patients. Despite the clinical significance, renal function tests remain a limited and largely qualitative framework: a rising serum creatinine level is the only established test to predict onset or identify AKI. The results of renal biochemistry analyses are not displayed in graphical form, rendering them difficult to interpret quickly. Furthermore, the sensitivity of serum creatinine level to renal dysfunction is limited during the progression of the AKI. Changes in urea levels are nonlinear and unpredictable. Increasing and decreasing urea levels may indicate different patient outcomes, yet these points are often overlooked. Renal function tests have little diagnostic utility for identifying the risk of developing moderate-to-severe or other forms of AKI. When performed with other routine blood tests, the addition of AKI markers has only mild predictive power for AKI [136, 137, 138, 139].

Chapter - 9

Liver Function Tests and Hepatobiliary Disorders

Hepatic dysfunction is a major global health concern, impacting both adult and pediatric populations. It is often secondary to a wide range of diseases yet presents with nonspecific clinical symptoms, including jaundice, anorexia, right upper quadrant abdominal pain, and generalized discomfort. Direct visual inspection through laparotomy or laparoscopy has limited utility in evaluating liver texture. As a result, the liver biopsy remains the gold standard procedure for evaluating hepatic conditions. However, it is an invasive procedure and subject to sampling errors and life-threatening complications. Given the potential systemic and life-threatening complications associated with surgical intervention in patients with hepatic dysfunction, it is important to develop new noninvasive diagnostic approaches.

Hepatic dysfunction does not necessarily indicate primary liver disease; rather, it frequently coexists with extrahepatic conditions such as jaundice due to hemolysis and cholestasis due to biliary obstruction. Specific tests are required to determine whether hepatic dysfunction is primary or secondary to extrahepatic conditions. Hepatic dysfunction is typically characterized by various biochemical abnormalities of serum markers associated with hepatocyte damage (elevated ALT, AST, and alkaline phosphatase), cholestasis (elevated alkaline phosphatase and gamma-glutamyl transferase), bilirubin metabolism and detoxification (elevated bilirubin and decreased serum albumin), and liver fibrosis formation (elevated hyaluronic acid and type-Ⅲ procollagen peptide). However, different biochemical abnormalities and grades reflected in laboratory results cannot reliably indicate liver disease degree or progress from one stage to another. Clinical grading systems, such as the Child-Pugh score and MELD score, have been developed to systematically assess remaining liver function and provide theoretical guidance for clinical treatment when certain conditions are met. Although useful in the assessment of cirrhosis and hepatocellular carcinoma advancement, these grading systems are less accurate for mild liver injury cases. Biochemical parameters also fall short, as they merely offer indirect assessment of remaining liver function and have little meaning when injury does not progress from one stage to another.

The indocyanine green (ICG) clearance test quantifies remaining liver function according to the removal capacity of ICG, a dye largely dependent on the liver parenchyma. It provides direct measurement of liver function rather than indirect assessment or the level of liver injury. However, it is an operator-dependent examination and affected by many other influencing factors. Noninvasive imaging methods, including ultrasound, computed tomography, and magnetic resonance imaging, facilitate the assessment of liver morphology and perfusion and the evaluation of possible underlying conditions, such as determination of hepatocyte quantity, evaluation of fibrosis grade, assessment of lipid and iron metabolic disorders, and determination of biliary excretory function [140].

Hepatic metabolism and enzyme systems

The liver participates in a number of important physiological processes, such as heme-utilization, carbohydrate and lipid balance, excretion of cholesterol, destruction of foreign bodies and drugs, synthesis of plasma proteins and urea, and intermediary metabolism. Animals are often exposed to large doses of toxic or non-toxic substances; in these cases, the liver can convert these foreign compounds to products that are less harmful and that are excreted by the kidney or bile. Enzymes in the liver either help or hinder these detoxifying reactions. Toxic non-toxic substances sometimes require the participation of these enzymes in their conversion to harmful or non-toxic compounds. The enzymes of the liver undergo changes that facilitate these conversions on the one hand and hinder them on the other and these changes are termed as inductive or repressive.

The term hepatic metabolism describes the change in structure and physicochemical properties of organic compounds of foreign origin during their transit through the liver. There exists, therefore, a hepatic enzyme system concerned with the oxidative modification of such foreign substances by insertion of polar groups and/or opening of closed rings. A hepatic mitochrondrial enzyme system is responsible for the oxidative degradation of monosubstituted short-chain fatty acids to acetyl-CoA. In addition, the hypertrophied liver of the hibernating mammal, and the liver of the foetal animal have their own special enzyme systems. As these enzymes are normally absent or present in insignificant amounts in the adult liver, their reappearance indicates a specific need or adaptation [141, 142, 143, 144].

Liver enzymes and protein synthesis markers

Liver function tests include the determination of the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as well as the

synthesis products albumin, alpha1-antitrypsin, and coagulation factors. Elevated concentrations of AST and ALT reflect hepatocyte damage and are best known for their diagnostic utility in liver disease. AST and ALT have been employed as prognostic markers in gastric and colorectal cancer. A low level of albumin indicates a poor prognosis in patients with gastric cancer, while a low level of alpha1-antitrypsin is an unfavourable sign in colorectal cancer patients. These two proteins, along with coagulation factors, predict prognosis in patients with liver cancer.

In the clinical laboratory, AST catalyzes the reversible deamination of aspartate to oxaloacetate, coupled to the reduction of alpha-ketoglutarate to glutamate. In this reaction, aspartate is formed from glutamate and oxaloacetate through the action of ALT. A number of studies on patients with these enzymes investigated the relative sensitivity or specificity of AST and ALT for liver disease. In the past, an elevation of both AST and ALT was thought to be more indicative of liver disease than isolated elevation of either enzyme. However, in patients with only elevated AST, the etiology of disease should be carefully examined because reduced specificity may indicate a non-hepatic cause [145, 146, 147, 148].

Bilirubin metabolism and jaundice

Bilirubin metabolism is a complex process characterized by several transport steps through various tissues. The kinetic behavior of unconjugated bilirubin, bilirubin bound to albumin, and conjugated bilirubin was assessed in healthy adults, and a mathematical description of bilirubin kinetics after a bolus injection allows predicting venous serum concentrations of conjugated and unconjugated bilirubin and their total. Bilirubin can also act as a signaling molecule, modulating the actions of neurotrophins. Peak bilirubin concentrations and timing for therapy (339.8 μmol/L and 56.8 h postnatal age) were derived from fluorescent-labeled bilirubin experiments in infants with unhealthy climates exhibiting hyperbilirubinemia [149].

Viral, alcoholic, and drug-induced liver injury

The liver is the central organ involved in drug metabolism, alcohol processing, and viral replication, and liver injury is recognized as a global health problem. Infectious agents such as hepatitis viruses (HV) and drugs can produce a wide range of liver injuries. Drugs and other substances that are reported to induce liver injury include acetaminophen, non-steroidal anti-inflammatory drugs (NSAID), amoxicillin-clavulanate, herbal products, phosphorus-containing agents, tuberculostatic medications, anabolic steroids, methotrexate, and others. Certain drugs are suspected to be the cause of liver

injury but are classified as grey zone cases without definite causality. Additionally, the liver plays a crucial role in processing alcohol and is the main target for alcohol-induced injury. With alcohol widely available and socially accepted, the abuse of alcoholic beverages may lead to liver injury and pose a potential risk to the population. Alcohol metabolism generates highly toxic acetaldehyde, and consequently, multiple defence mechanisms against alcohol and acetaldehyde exist. Against this background, the introduction of hepatitis viruses and the overwhelming use of drugs are of major concern [150].

Non-invasive biomarkers of liver fibrosis

Hepatic fibrosis is the windfall of chronic liver injuries caused by various conditions such as viruses, alcohol, toxins, metabolic disorders, and autoimmunity. It plays a central role in developing and predicting cirrhosis and its sequelae (e.g. hepatocellular carcinoma and portal hypertension). Detecting liver fibrosis is crucial for decision making and therapy. Liver biopsy, which is an invasive method, is currently the gold standard. Non-invasive methods are attempting to outperform it. These methods are extensive searches for direct and indirect markers of fibrosis that could serve as a diagnostic screening tool. Biochemical changes related to liver disease induce hepatic synthesis of certain macromolecules. Protein expression changes might reflect the presence and severity of disease.

Markers of liver injury, direct markers of liver fibrogenesis, indirect markers of the prognosis of liver fibrosis (tissue remodelling, metalloproteinases and their inhibiting factors or other circulating tissue-associated markers), score-based mathematical functions-which can distinguish the presence or the severity of fibrosis artefactially and the measurement of liver stiffness wave propagation speed are among the numerous markers investigated, sometimes in combination. Переменная [151, 152, 153, 154].

Chapter - 10

Cardiac Biomarkers and Clinical Pathology

Heart diseases are still the leading cause of death worldwide (35% of the total) and their risks are expected to increase due to the aging of the population and the growing incidence of multiple co-morbidities. Cardiac diseases in domestic animals are often secondary to physiopathological processes involving other systems. Therefore, there is a growing need for the knowledge of cardiac pathology across all companion species. It is widely accepted that cardiac pathologies lead to modifications of circulating biomarkers in blood, plasma or serum samples, and that these changes are in various way related to the conditioning and type of alterations present. Despite this high interest and the extensive bibliography that has come out, cardiac biomarkers are still scarcely used in small animal medicine and too many clinical pathologists maintain an inappropriate approach with respect to their application [155].

Biochemistry of Myocardial Injury

Acute myocardial infarction (AMI) is a common and frequent disease with an increasing incidence. Laboratory assessment of cardiac damage is part of the cornerstone for the diagnosis of this clinical condition and is used in conjunction with patients' history, chest pain, electrocardiographic changes and other investigations according to the recommendations established through the years by experts in the field. The importance of such laboratory tests is illustrated by the fact that criteria from expert consensus on laboratory cardiac markers have been incorporated into the diagnosis algorithms of the countries involved in the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Committee on Standardization of Markers of Cardiac Damage [156].

Clinical biochemistry markers were used for the diagnosis of myocardial infarction since 1959. The first markers used were creatine kinase (CK), aspartate aminotransferase (AST) and lactic dehydrogenase (LDH), which were accepted as indicators of myocardial necrosis. Subsequently, more specific markers for cardiac damage were introduced such as the MB isoenzyme of creatine kinase (CK-MB) and LDH isoenzyme-1. An ideal biochemical marker of cardiac necrosis should present a series of properties:

it should be readily available in the normal myocardial cell, not be present or present in negligible quantities in other tissues, be released rapidly into circulation, remain elevated in serum for a longer time period, permitting late diagnosis, and permit a simple and rapid analytical determination. At present, no single marker fulfilling all these requirements has been described. Myoglobin is detected after the first post-infarction hour but its non-specificity for cardiac tissue strongly limits its application as a marker for AMI. CK-MB was one of the first markers used but has limitations due to the fact that it is also present in skeletal muscle. Different studies have evaluated carefully several other candidates to replace CK-MB as the biochemical marker of choice during AMI: heart fatty acid-binding protein, glycogen phosphorylase isoenzyme BB / glucose-regulated protein 58-kDa, cardiac troponin-I or and cardiac troponin-T. All these markers possess different analytical characteristics and interpretation problems that have to be taken into account. The continuous interest in the search for a better or additional myocardial infarction marker is due to the fact that an ideal one has not been found yet and because research is still open in the field towards additional candidates and novel analytical metabonomic methodologies that could allow the characterisation of myocardial infarction without the use of classical biochemistry markers [157].

Cardiac troponins and acute coronary syndromes

Acute coronary syndrome (ACS) encompasses a spectrum of conditions associated with coronary tissue ischemia due to atherosclerotic plaque rupture and thrombus formation, including unstable angina, non-ST elevation myocardial infarction (NSTEMI), and ST-elevation myocardial infarction (STEMI). These conditions represent major causes of morbidity and mortality worldwide, necessitating their prompt diagnosis and risk stratification. The most read, non-review article in clinical medicine is a consensus guideline published by the American Heart Association and European Society of Cardiology on the use of cardiac troponins in the diagnosis and management of patients with suspected ACS, even if a patient does not have detectable cardiac troponin levels. Cardiac troponins are the preferred biomarker for diagnosing myocardial injury or infarction.

Cardiac troponins are structural proteins associated with calcium-regulated muscle contraction and are found in myocytes of the heart but not in skeletal muscle. They consist of three subunits: troponin C, which binds calcium; troponin I, which inhibits actomyosin ATPase; and troponin T, which anchors the troponin complex to tropomyosin. Cardiac troponin I (cTnI) and cardiac troponin T (cTnT) are specific for cardiac muscle, making them ideal

markers. Increased concentration of cardiac troponins is the most important criterion for the diagnosis of myocardial injury and infarction, leading to their incorporation in guidelines for diagnosis and prognosis in ACS and heart failure. However, in patients with atypical presentations of myocardial injury or infarction, troponin levels may remain low for prolonged periods after the onset of symptoms. Newer and more sensitive high-sensitivity cardiac troponin assays have been developed, reducing the time to reporting and shortening the time for diagnosis, but increasing the rate of minor myocardial injury, which complicates patient management [158, 159, 160, 161].

Biomarkers of Heart Failure

Biomarkers for heart failure (HF) identify patients with the greatest risk of adverse outcomes. Clinicians need to choose the right biomarker to stratify risk and guide management in a rapidly changing landscape of HF treatment options. Comprehension of different available biomarkers in HF is necessary in order to be able to recommend therapy and not just to diagnose HF, since many new medical therapies such as ivabradine and angiotensin receptor neprilysin inhibition therapy are indicated according to biomarker levels.

Apart from the traditional markers B-type natriuretic peptide (BNP) and N-terminal pro–BNP (NT-proBNP) and their relative merit, other circulating neurohormonal activation markers including the three natriuretic peptides, adrenomedullin, copeptin, and the soluble receptor for advanced glycation endproducts are also gaining momentum. These novel biomarkers inform prognostication and response to therapy and contribute to the increasing arsenal of drugs indicated in HF treatment, including new drugs for diabetes that may also benefit HF, although the underlying mechanisms for their cardioprotective action remain unclear. Furthermore, cardiac troponins are also established in the setting of acute HF and have prognostic significance [162, 163, 164, 165].

Point-of-care testing in cardiology

Cardiac injury and heart failure may be acute or chronic and can lead to serious complications, such as cardiac rupture, cardiogenic shock, or multiorgan failure. Appropriate development, clinical implementation, and professional use of fast POC assays to reliably exclude or confirm cardiac conditions, and to assess the degree of risk directly guide therapeutic decisions. The major advantages are that rapid determination enables prompt therapy initiation, restricts further diagnostics, reduces healthcare costs, and minimizes patient stress.

The cardiac troponin complex is the most sensitive and specific myocardial injury marker available. The precise cut-off on the 99th percentile

upper reference limit is an important risk stratifier. More sensitive troponin assays decrease the upper reference limit and enable early diagnosis of MI Type 1, Type 2, and demand ischemia. Even a troponin concentration below the 99th percentile does not exclude a significant event; therefore, POC troponin assays with high true-negative rates enable a rule-out strategy. Fast and reliable POC assays that exclude the commonest acute cause of chest pain can set minds at rest, thus reducing unnecessary hospital admissions and further investigations.

In patients with chest pain and positive troponin tests, POC assays that incorporate other cardiac injury markers (e.g., myoglobin, CK-MB, and copeptin) help decision-making. Moreover, the sensitivity of POC troponin assays facilitates risk stratification of patients with heart failure. Elevated concentrations are predictive for any death, all-cause mortality, and death from CV causes. The fast determination of natriuretic peptides in hospital settings assists in excluding or confirming acute heart failure and facilitates patient management. BNP ultrasensitive POC assays further support differential diagnosis and prognosis in unselected patients with dyspnea [166, 167, 168, 169].

Future directions in cardiac diagnostics

The future of cardiac diagnostics is focused on improving non-invasive assessments of otherwise inaccessible tissues. Current evaluation methods for cardiovascular diseases are effective but still fall short for conditions such as cardiomyopathies, valve diseases, transplant rejection, and ischemia, where accurate diagnosis depends on histological examination of the heart. Despite significant advances in magnetic resonance imaging (MRI), echocardiography, nuclear imaging, and contrast-enhanced computed tomography (CT) and single-photon emission computed tomography (SPECT), these imaging techniques cannot fully replace cellular and molecular-level analysis [170].

Chapter - 11

Endocrinology and Hormonal Disorders

Hormonal tests are crucial for detecting endocrine and hormonal diseases. Hormonal imbalances can lead to serious dysfunction in the body, causing conditions such as hypercholesterolemia, hypothyroidism, and growth hormone deficiency. Accurate laboratory test measurements are critical for proper diagnosis and treatment of hormonal abnormalities, influencing individual patient care and related research [171].

Physical examination remains relevant for assessing endocrine disorders. It guides selection of diagnostic tests, clarifies clinical problems, and eliminates irrelevant tests, thereby reducing costs and patient inconvenience. In endocrinology, physical signs and symptoms often guide referrals to specialists [172].

Due to its systemic nature, endocrine pathology is a distinct subspecialty in clinical and diagnostic pathology. It involves organ-targeting lesions, control organ lesions, and lesions affecting general homeostasis or psychosocial well-being. Endocrine pathologists investigate hormonal pathology and the interplay between biochemical and endocrine dysfunction [173].

Generalised endocrine gland dysfunction presents as mucinosis, acanthosis nigricans, pseudo-pelade, hypertrichosis, and pruritus. Sex-domain-specific hormone-seeking lesions are classified as female-type at the external genitalia and male-type at the breast region.

Thyroid diseases are the most prevalent and constitute a separate chapter. Three major hormonal disorders-hypothyroidism, hyperthyroidism, and hyperparathyroidism-are diagnosed through hormonal assays without requiring tissue diagnosis. The initial pathology consultation focuses on diagnosing breast and thyroid diseases, aided by balanced immunohistochemical panels and relevant frozen sections.

Secretory tumours of gonads, adrenal glands, and neuroendocrine organs share common traits-hormonal dependence and inducible growth. Despite different hormonal outputs, histological examination, immunohistochemical

study, and molecular analysis provide mechanistic understanding of gonadal and adrenal secretory tumours, often leading to the same treatment regime.

Principles of hormone action and regulation

Hormones are complex bioregulators in a multicellular organism that act through specific receptors located on or in target cells. Once bound to receptors, hormones trigger cellular signalling cascades leading to physiological effects. Hormones circulate freely in plasma or are bound to plasma proteins, and are classified in several ways; for example, by source, mode of action or chemical structure. Hormones can influence a wide range of physiological processes such as growth, reproduction, metabolism, behaviour and memory. Hormonal control becomes even more complex when acting in combination with other regulatory systems such as neurotransmitters or growth factors. Measurement of hormone levels is an important tool for diagnosing and following up a wide variety of diseases. Hormone secretion and circulation exhibit complex patterns, including pulsatile release, variability according to age and stage of life and daily rhythms. For example, follicle-stimulating hormone (FSH) levels fluctuate with stage of the menstrual cycle while testosterone production peaks in the early hours of the day. Interpretation requires knowledge of the normal ranges of hormones, including their variations under different conditions. Further, the pattern of hormone still may not be sufficient to meet clinical needs.

Thyroid Function Tests

Thyroid function tests (TFTs) evaluate the levels of hormones produced by the thyroid gland (T3 and T4) and thyroid stimulating hormone (TSH) in the blood. A decreased T3 and/or T4 concentration in conjunction with an elevated TSH level is indicative of primary hypothyroidism, while increased T3 and/or T4 concentrations with subsequent decreases in TSH suggest primary hyperthyroidism. Thyroid hormones—iodine-containing molecules produced from the amino acid tyrosine—are critical to thermoregulation, metabolic activity, and tissue development. The synthesis and release of T3 and T4 is controlled by the hypothalamus and the anterior pituitary gland through the thyroid releasing hormone (TRH) and thyroid stimulating hormone (TSH) axes.

Thyroid function tests help to identify diseases involving hyperthyroidism, hypothyroidism, congenital thyroid disorder, and goitre, thereby assessing the normal response of the hypothalamus, anterior pituitary gland, and thyroid. TFTs are not routinely ordered but are useful in specific contexts, such as patients with arrythmias and medically unexplained weight loss and/or mood disturbance. Changes in the concentrations of T3, T4, and

TSH help in the identification of pituitary and partial or secondary hypothyroidism. TFTs may also help in the diagnosis of congenital disorders associated with abnormal thyroid function [175, 176, 177, 178, 179, 175, 176, 177, 178, 175, 176, 177, 178].

Adrenal and Pituitary disorders

Hormones and their Functions: These hormones are created by the adrenal cortex and are involved in a variety of physiological processes, including electrolyte balance (e.g., aldosterone), energy metabolism (cortisol), immune response inhibition (cortisol), and maintaining reproductive function (androgens). The adrenal medulla produces catecholamines, especially epinephrine.

Primary adrenal insufficiency is diagnosed by an elevated serum ACTH concentration and low concentrations of cortisol and aldosterone. The most frequent form of primary adrenal insufficiency is adrenal autoimmune disease (Addison disease), which is often associated with other autoimmune endocrinopathies such as thyroid disease (Bothos–Kopriveva syndrome), type 1 diabetes mellitus, or pernicious anemia. Other causes of primary adrenal insufficiency include adrenal hemorrhage, infection (e.g., tuberculosis), infiltration with neoplastic or other cells, and congenital adrenal hyperplasia resulting from 21-hydroxylase deficiency. A deficiency of aldosterone leads to hyponatremia, hyperkalemia, and decreased blood pressure combined with a reduced excretion of steroid metabolites. The deficiency of cortisol leads to weakness, vomiting, and, eventually, cardiac collapse.

In secondary adrenal insufficiency, the adrenal glands fail to secrete corticosteroids because of insufficient ACTH secretion by the anterior pituitary. This is usually due to decreased secretion of corticotropin-releasing hormone from the hypothalamus as a consequence of exogenous corticosteroid therapy or other conditions. Serious illness, particularly septicemia, may temporarily suppress the hypothalamic–pituitary–adrenal axis or reduce the responsiveness of the adrenal cortex in patients on long-term corticosteroid therapy. Diagnosis is made by measuring the response of serum cortisol to a stimulation test with exogenous ACTH. In normal response, serum cortisol increases greater than 18μg/dL (500nmol/L) after the ACTH stimulus. In the majority of patients with Cushing syndrome, a 1μg ACTH stimulation test can differentiate between primary adrenal disease and secondary adrenal suppression or pituitary disease. An endogenous ACTH concentration of less than 15pg/mL (3.3pmol/L) excludes primary adrenal insufficiency as a cause of hyponatremia.

Reproductive hormones and fertility assessment

The hypothalamic-pituitary-gonadal (HPG) axis is responsible for regulating male reproduction and fertility. Reproductive hormones produced by the hypothalamus (gonadotropin-releasing hormone, GnRH), pituitary (follicle-stimulating hormone, FSH; luteinizing hormone, LH), and testicular Leydig cells and Sertoli cells (testosterone, inhibin) are crucial in controlling spermatogenesis and normal testicular function in the adult male. Assessment of reproductive hormones can provide information about the cause of abnormal testicular function or dysfunction and reduced fertility. GnRH, FSH, LH, and inhibin are best measured in serum, whereas testicular testosterone concentrations can be measured in serum or testicular fluid obtained either from fine-needle aspiration biopsy or post mortem from testicular tissue. Serum testosterone concentrations can also be measured in conjunction with measuring the testis size.

Semen collection and evaluation represent a non-invasive reproductive assessment tool suitable for quantifying male reproductive quality. The process is easy, low cost, and can be carried out outdoors, aided by the animal's natural receptiveness to a female in oestrus. Sperm characteristics can provide information about fertility potential, seasonality, and geographic variations, all of which are important for species managed under captive breeding and released into their natural habitat. Substandard reproductive outcomes in mammals are usually characterised by reduced male contribution, with implications for management and breeding schemes. For marine mammals, sperm analysis is especially useful for species that are sexually dimorphic for body size or data-poor species, where semen collection is less feasible. However, in species with natural mating behaviour, routine hormonal assays for reproductive organs or ovulation are still needed.

Advanced Immunoassays in Endocrinology

Advanced immunoassays enable ultrasensitive detection of proteins such as adiponectin and insulin. The ultrasensitive ELISA for urinary adiponectin achieves a detection limit of 0.81 pg/mL, allowing measurement at the subattomole level. Urinary adiponectin levels are significantly higher in diabetes mellitus patients than in healthy subjects; levels increase with chronic kidney disease risk, and different molecular weight multimers are formed in patients versus healthy individuals. These findings suggest urinary adiponectin as a noninvasive diagnostic index for chronic kidney disease. For insulin, an ELISA with recombinant insulin standards improves the detection limit to tens of attomoles per milliliter, far surpassing that of previous assays.

These advancements support early diagnosis and reduce the need for blood sampling, offering noninvasive testing options using urine, saliva, or tears. The technology is also being applied to detect proteins from pathogens and cancer markers [180].

Accuracy of biochemical measurement is essential for diagnosis and treatment. Immunochemical methods play an important role yet possess critical limitations due to the absence of standardization and interference by endogenous or exogenous substances. Errors are especially likely when the patient's sample differs from a presumed normal distribution, a situation often encountered with older patients whose specimens may contain endogenous substances, autoantibodies or interfering heterophilic antibodies. The majority of potential pitfalls arise from the characteristics of the patient sample rather than from flaws in assay methodology, and no quality control procedure can entirely eliminate the risk of error. Suspected errors are identified when laboratory results prove inconsistent with clinical assessment, and effective detection requires close communication between laboratories and clinicians. Physicians, particularly those caring for geriatric patients, should be aware of the limitations inherent in immunochemical procedures to help minimize the likelihood of error [174].

Chapter - 12

Protein, Enzyme, and Tumor Marker Analysis

Proteins and enzymes are determining factors for life. Biological macromolecules are polymer chains composed of more than a hundred amino acids which provide structural and functional characteristics of a cell or its physiology. Proteins exert enormous activities. Pathology modified protein homeostasis as well as numerous biological activities are down-regulated or up-regulated under certain diseases including cancer [181]. Topological protein structures are expressed in a sequence dependent manner. Biological engineering manipulates protein sequences or uses catalytic transformation to develop modern medicines that have been recognized as 21st century masterpieces old in material.

Pathological conditions may either originate from or occur at later stage of a disease. Certain proteins are produced specifically during disease progression. Tumor cells replicate at a faster rate than normal cells. Cancer cells are known for the ability of cyclin induction. Two main approaches exist amongst chemical, molecular biological, and immunological detection. Clinical samples used for protein, enzyme, and tumor marker analysis mainly include blood, urine, tissue, remnants from diagnostic imaging [182].

Plasma proteins and electrophoretic patterns

Plasma proteins are the major components in plasma and vary in concentration depending on multiple factors such as species, age, breed, and diet. The proteins are synthesized by the liver, and with the exception of γ-globulins, are synthesized by the reticuloendothelial tissues, lungs, and other tissues. They play an important role in maintaining oncotic pressure, functioning as carriers and stockpiles for hormones and vitamins, participating in immune reactions and coagulation processes, and catalyzing chemical reactions.Because there is little elastic collagen or connective tissue in soft tissues, hemoconcentration and dehydration rarely influence blood protein concentration. The measurement of plasma protein concentration is one of the most widely used screening tests, and abnormal concentrations frequently provoke additional investigation of the eutectic electrophoretic pattern in the

periphery of the shadow. This is possible because the concentration gradient of the proteins is greater than that of the beta and alpha subdivision proteins. The detection of the γ-globulin and β-lipoprotein fractions provides valuable information concerning various disease states.Electrophoresis of proteins has become one of the most widely used laboratory tests in clinical practice. It allows the separation and characterization of the serum proteins and the recognition of a variety of abnormal patterns, including hyperproteinemia, hypoproteinemia, hyperalbuminemia, hypoalbuminemia, paraproteinemia, and dysproteinemia. Recent studies on the proteins most often removed from serum and the distances traveled in various solvents have made probabilistic assessment of the electrophoretic bands less subjective [183, 184, 185, 186].

Clinical Enzymology in Disease Diagnosis

Clinical enzymology is an important aspect of the clinical laboratory that delivers assays utilized for the diagnosis of infectious diseases and syndromes, such as viral myocarditis, meningoencephalitis, gastroenteritis, typhoid fever, and sepsis. Molecular diagnostics contribute significantly to infectious disease diagnostics because of the limited availability of culture, the risk of pathogen dissipation (due to antibiotic exposure), and the time-critical nature of acute conditions. In certain infectious conditions, such as myocarditis and pericarditis, molecular testing and epidemiological and serum infection marker information are required for definitive diagnosis. Diagnostically critical pathogens included viruses (e.g., enteroviruses, adenoviruses) and protozoans (e.g., Toxoplasma gondii) implicated in the American heart association consensus report. Nebu-ler could isolate patients suffering from viral and non-viral infective pericarditis, allowing subsequent molecular and epidemiological testing for relevant pathogens. In cases of suspected viremia or infectious cardiac involvement, detection of parvovirus B19, cytomegalovirus, and Epstein-Barr virus is of great importance, particularly in immuno-compromised hosts [187]. In gastrointestinal disease situations, Clostridium difficile is the leading cause of antibiotic-associated diarrhea, and the widespread use of toxin assays and glutamate dehydrogenase as a complementary approach which has yet to fulfil demands. In determining intestinal parasites, diagnostic laboratory tests of available sample such as stool and urine for parasites improve isolation rate of helminths, protozoa, and ova. For assessing fecal pathogens, polymerase chain reaction of single stool specimen provides detection of Enterotoxigenic Escherichia coli, Shiga toxin-producing Escherichia coli, Campylobacter spp., and Salmonella spp., indicating the versatility of molecular assay capability [188, 189, 190, 191].

Tumor Markers and Cancer Screening

Testing for tumor markers in body fluids, such as blood, urine, and pleural effusion improves screening efficiency for a large number of malignancies. Tumor markers in body fluids indicate not only the presence of cancers but also their clinical stage and type [192]. An analysis of serum panels containing, and a blood tumor marker combination assay that integrates four types of tumor markers for early screening of 20 types of organ cancer according to the natural cancer history has been proposed, offering both high sensitivity and high specificity [193].

Limitations and Clinical Interpretation

New techniques frequently provide remarkable analytical possibilities and open up broad new diagnostic opportunities. Advanced methods and instruments expand both the type and amount of data produced, sometimes by several orders of magnitude, but new technologies also tend to yield unfamiliar and complex data. Even the manipulation of modest amounts of information may present major analytical challenges at times, and, as, or even more, important, misunderstanding results and their proper interpretation is a potential source of misleading conclusions. Interpretation of such data is not always straightforward however, and requires knowledge of the underlying preservation methods used for collection together with an understanding of the techniques employed for analysis. Interpretative guidelines are intended to increase the advantage of a new analytical technique and decrease the opportunity for misinterpretation [194].

Novel cancer biomarkers and Liquid biopsy

Novel cancer biomarkers that indicate the genesis or progression of breast, lung, colon or prostate cancer discovered employing microarray analyses, as well conducting clinical trials, and their application in laboratory studies are briefly summarized. These novel cancer-associated biomarkers, including proteins, micro-RNAs or other molecules in tissue, plasma or other bodily fluid reveal the presence of specific types of cancer such as breast, lung, colon or prostate cancers. Analysis of the level of these biomarkers makes it possible to carry out a liquid biopsy, which leads to the trouble-free diagnosis of many diseases, especially cancers, and provides a basis for personalized medicine. The identification of cancer-associated novel biomarkers could overcome present difficulties in early cancer diagnosis, detection of cancer recurrence and distant metastasis, prediction of prognosis, selection of appropriate treatment and monitoring therapeutic efficacy. Novel microscopic cancers could be diagnosed much earlier and much more sensitively by using a liquid biopsy instead of a surgical biopsy.

A liquid biopsy test that might streamline the response evaluation of immunotherapy in melanoma patients has also been developed. The measurement of circulating tumor DNA from blood plasma is a novel biomarker for response evaluation in melanoma patients receiving immunotherapy. The variation of cell-free plasmatic DNA plays a pivotal role in either the evaluation of efficacy or the early prediction of relapse in patients treated by anti-PD-1 immune checkpoint blockade with polymer-based immunotherapy. Synchronous monitoring of CD4+ and CD8+ T lymphocytes as well as the proportion of pro-inflammatory TdTsubtype CD4+ T cells in melanoma patients could effectively reflect the therapeutic status following treatments. The detection rate of these approaches for separating immune responders from non-responders is remarkable, indicating their significance for anticancer personalized medicine [195, 196, 197, 198].

Chapter - 13

Hemostasis, Coagulation, and Thrombosis

Pathophysiological processes including hemostasis, coagulation, and thrombosis are involved in numerous disease conditions. Thrombosis and hemostasis processes are very complex and are associated with many factors, including extracellular matrix components, plasma proteins, and platelets that are involved throughout the entire cascade of events. Therefore, hemostatic analysis is a critical task in clinical laboratories. Moreover, hemostatic analysis represents an important, instrumental part of the clinical examination of patients and allows an assessment of the risk of bleeding or thrombosis [199]. Pre-analytical and analytical phases play a crucial role in the tracking, traceability, and rapid on-line reporting of laboratory examinations. Monitoring also is necessary for automatic sedimentation and a fast-track system covering, on-line, automatic transport through multiple analytical steps. Hemostasis and thrombosis analysis continues to expand, with the introduction of novel methods.

Improper handling of samples can lead to pre-analytical errors and hamper laboratory tests in various ways. A large number of pre-analytical errors occur during the specimen draw and may go unnoticed if controls are not in place to reduce human error. Therefore, clear reminders and well-trained technical staff can help to reduce pre-analytical errors. A pre-analytical checklist would also reduce time and prevent costly mistakes, as it is easy to lose track in the day-to-day running of busy laboratories. Quality Control is an important process. In laboratory tests, this term addresses the non-conformity of the measurement equipment, reagent kits, and protocols, and the non-conformity of calibrators and control materials. In hemostasis, it is generally agreed that there are two quality controls (QC), an internal quality control (IQC), and an external quality control (EQC), and the laboratory runs and monitors its own results according to the law and regulation of the governmental authority [200, 201, 202, 203].

Physiology of hemostasis and coagulation pathways

Hemostasis is the process through which the body maintains blood in a fluid state within normal vessels but rapidly forms a clot over a site of vascular

injury. For hemostasis to be successful, the blood must not clot excessively, either in the normal state or in response to minor vascular injury. Coagulation is another term for hemostasis; however, it is often used when referring specifically to the clotting pathway involving thrombin and fibrin, whereas hemostasis encompasses the broader process, including and regulating clot formation. With vascular injury, hemostasis involves three processes: vascular spasm and construction of the vessel; primary hemostasis-formation of the platelet plug or clot; and secondary hemostasis-conversion of the platelet plug into a fibrin clot, sometimes also called coagulation. The terms intrinsic pathway, extrinsic pathway, and common pathway are frequently used with respect to secondary hemostasis and clots because the three classes of clotting factors are activated in sequence, leading to thrombin generation.

Hemostasis involves the interaction of vascular cells and components of blood; activation, adhesion, and aggregation of platelets; and a network of plasma proteins, called coagulation factors, that are activated in sequence in response to tissue injury and that cooperate with platelets to form a fibrin clot on adjacent, activated platelets. The significant structural changes that accompany platelet activation lead to exposure of highly thrombogenic phospholipids on the platelet surface. The basic steps of hemostasis are the same in all animals, but the activation thresholds and relative contributions of different hemostatic components vary [204, 205, 206, 207].

Laboratory Tests for Bleeding Disorders

Common laboratory tests employed to detect bleeding disorders include clotting studies like prothrombin time (PT), activated partial thromboplastin time (APTT), and certain specialized assays. Prolongation of PT suggests problems with extrinsic pathway (e.g., factor VII deficiency, hepatic insufficiency, vitamin K deficiency), while APTT prolongation indicates intrinisic pathway issues (e.g., factors VIII or IX deficiency, use of heparin).

Concurrently, platelet counts, the bleeding time test, and platelet function tests are conducted to assess the function of platelets. Leukemia is associated with abnormal blood counts and gross blood film changes. PGH-IB deficiency (plasma-group-specific inhibitor to coagulation factor Xa) leads to bleeding. Multiple Myeloma causes hyper viscosity syndrome with bleeding tendencies. Diagnosis of Thrombocytopenia is performed with bone marrow study, increased platelet volume, associated with splenomegaly, and increased megakaryocytes or reduced platelet volume, not associated with splenomegaly (may due to viral).

PGH-IB deficiency results in abdominal-epistaxis, with absence of factor Xa and normal PT and APTT. Hyper viscosity syndrome associated with bleeding is noted in Waldenstrom's macroglobinnaemia and Multiple Myeloma due to IgM and IgA respectively. Thrombin time is prolonged mainly in different cases of heparinisation and in dysfibrinogenaemia due to high-fibrinogen degradation products. PT and APTT are not prolonged, and common trials of bleeding time and platelet count remain normal.

Anticoagulant Therapy Monitoring

Patients taking anticoagulant medication require frequent blood tests to monitor levels of anticoagulation and ensure there is not too little or too much of the anticoagulant in the blood. There are two commonly checked values for monitoring anticoagulation: the International Normalized Ratio (INR) and the Prothrombin Time (PT). Both values are most often checked in patients taking Warfarin or concurrently taking Warfarin and heparin. The Therapeutic INR Goal Range for most patients taking Warfarin is between 2.0 and 3.0. However, patients with mechanical heart valves may have a therapeutic range of 2.5 to 3.5. An INR greater than the target therapeutic level indicates that the patient is at greater risk of bleeding, while an INR less than the target therapeutic level indicates that the patient is at greater risk of a thromboembolic event. Clinicians adjust the dosage of Warfarin based on INR levels.

INR is a standardized number, regardless of the laboratory performing the test. The INR is based on the PT, which measures the amount of time it takes for particular components of these pathways to clot. The PT is expressed in seconds. Prothrombin is measured in a variety of ways, and the method chosen affects both the reference interval and interpretation of results. Activated Partial Thromboplastin Time (aPTT) is another time test performed to monitor anticoagulation therapy, as it evaluates the intrinsic clotting pathway and monitors heparin therapy. These tests can also detect abnormalities of the extrinsic clotting pathway. Evaluation of these two pathways may be performed simultaneously using a Thromboelastogram (TEG), which measures the viscoelastic properties of whole blood during clot formation and dissolution. Results can be analysed by a variety of parameters related to clot kinetics and stability [208, 209, 210, 211].

Thrombophilia and Hypercoagulable States

Thrombophilia, the tendency to develop abnormal blood clots, is an inherited or acquired disorder that affects hemostasis and thrombosis. Venous thrombosis, a common manifestation of this disease, is associated with serious

complications. Inherited thrombophilias such as the deficiencies of protein C, protein S, and antithrombin; the heterozygous or homozygous factor V Leiden mutation; and the prothrombin 20210A mutation can predispose individuals to venous thromboembolism. Testing for inherited thrombophilia should be performed on all patients with a first or recurrent thrombosis at an appropriate location [212]. Acquired thrombophilias are associated with biological changes leading to thrombus formation. Signs of hypercoagulation can be observed in patients with antiphospholipid syndrome or with a malignant tumor presence. Hypercoagulation can also be preceded by the use of medication containing estrogens or tissue factor, surgical treatment, pregnancy, or prolonged immobility.

Emerging Coagulation Biomarkers

Coagulation is critical for stability in many physiological systems and for maintaining homeostasis and preventing spontaneous bleeding [213]. However, coagulopathies can arise from inherited or acquired factors that may impair a user's quality of life. Traditional descriptive coagulation techniques are limited in assessing pathophysiological variations during coagulopathies. To address the need for an adequate coagulation risk index, emerging in vitro biomarkers and thromboelastography techniques have been investigated for their ability to provide quantitative information on coagulation. These methods focus on quantifying the plasma contribution of parameters, such as clot formation time, clot lysis time, and the overall coagulation potential of a sample in human coagulation disorders. Point-of-care devices and lab-on-chip technologies utilizing meander micro-channel geometries have also emerged for low-volume assessment of various coagulation pathways [214].

Chapter - 14

Clinical Toxicology and Therapeutic Drug Monitoring

Therapeutic drug monitoring (TDM) addresses inter-individual variability in drug pharmacokinetics under different physiological, biological, and environmental conditions. Variable drug exposure significantly hampers treatment outcomes and may lead to toxic effects and drug resistance. The approach integrates pharmacokinetic and pharmacodynamic data to optimize and personalize drug therapies, thus improving treatment efficacy, minimizing toxicity, and preventing drug resistance [215]. Blood is the most common biological sampling matrix for TDM, but dried blood spots and saliva are increasingly favored for their less invasive collection procedure. Analytical determination procedures must be accurate, precise, and selective. Most TDM analytical procedures employ liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), which provides the desired high selectivity and sensitivity. High-performance liquid chromatography with ultraviolet detection (HPLC-UV) serves as a cost-effective alternative for clinical therapeutic monitoring. The broad therapeutic index of most non-narcotic analgesics and some narcotic analgesics justifies routine clinical monitoring. Conversely, other low-molecular drugs with a narrow therapeutic index require reliable measurement of plasma concentrations to ensure persistence within the therapeutic window (especially in neonates), and to avoid toxic effects or a low concentration that may further aggravate the clinical condition.

The growing popularity of LC-MS in clinical laboratories arises from its suitability for both routine analysis and a wide variety of applications. At the same time, off-line two-dimensional liquid chromatography (2D-LC) has gained attention in clinical laboratories offering complex analyses. Consequently, there is increasing interest in combining the advantages of both systems [216]; these opportunities extend beyond TDM to critical-care analysis in blood and plasma, industrial chemicals without known pharmacological activity, and environmental toxicology. The solid-phase extraction followed by a comprehensive two-dimensional liquid-chromatographic system coupling to mass spectrometry approach enhances the capability of dual-tional analysis, validating the coincidence of TDM and non-TDM compound

measurement. Modern bioanalysis laboratories, until recently considered avant-garde, now serve as reference targets for many other laboratories, especially when it comes to sensitive analyses, sample extraction combined with chromatographic times and limited sample amounts operating without specialized personnel. Nevertheless, faster and more sensitive methods are constantly being developed; notably, a comprehensive study of more than 200 psychoactive, relaxant, and legal compounds has been achieved.

Principles of Toxicokinetics and Toxicodynamics

One of the major deterministic aspects when assessing any chemical-induced toxic effects is the uptake of the substance under investigation: whether by inhalation, ingestion, skin permeation, or wound contamination. Deterministic modellers account for variation in human physiology and biochemistry by considering a number of states. For kinetic fate predictions, it is necessary to set up the time-explicit descriptions of chemical metabolic fate in different organs and at different stages of life. These descriptions can be built first for selected chemical groups e.g. aliphatic alcohols, and then used for quantitative chemical risk assessment based on the K(u)-principle (eq. 6) or for chemical toxic production assessment based on toxicokinetics, toxicometry and toxicodynamical kinetic modelling principles. The different pathways involved in the metabolic degradation of these chemicals can be expressed as a matrix and assigned W-transformation principles to obtain time-explicit forms of concentration C for all organ compartments.

The quantitative analysis of toxic kinetics (toxicokinetics) tries to predict the concentration C(de) of a chemical in any part de for different time intervals within a lifetime after exposure, depending on the kinetics of the uptake, excretion, and biotransformation processes involved. Though the toxicodynamic part of the analysis focuses on the links between C(de) and the expected effect (USEtox), in toxicokinetics, C(de) is the main target. These predictive distributions C(de) are eventually necessary for realistic risk assessments in ecological, human, or veterinary domains, provided suitable toxicodynamic models are available [217, 218, 219, 220].

Laboratory Detection of Poisons and Drugs

Many different types of poisons and drugs that may cause death can now be detected through advances in clinical chemistry and pathology. Drugs of abuse can be found in blood, urine, or in the vitreous humour of the eye. These tests can be carried out for the most recent overdose or poisoning or retrospectively for drugs that are known to have been abused over the years. More than one group of drugs may be looked for depending on the clinical history.

Gas chromatography can separate and identify volatile compounds in blood and other fluids. The presence of ethyl alcohol is of keen interest using blood taken at the time of the clinical incident. Larger organs or tissues may be tested, and if found may indicate poisonings. It is detected and monitored during the maintenance phase using blood. Significant levels of methanol, isopropanol, butanols, and acetone have been identified as causes of distress. Volatile halogenated methylated hydrocarbons and solvent mixtures are world-wide causes of death. Carbon monoxide and hydrogen sulphide will also induce death and may be assessed directly from the blood or indirectly using samples from ground, surface, or well waters. The presence of ethyl alcohol is of keen interest using blood taken at the time of the clinical incident [221, 222, 223, 224].

Therapeutic Drug Monitoring Strategies

Therapeutic drug monitoring (TDM) strategies seek to maintain drug concentrations within an optimal range according to specific patient and drug characteristics. The availability of point-of-care testing has facilitated more frequent blood sampling and therapeutic adaptation, enabling the application of TDM strategies. The choice of sampling interval should reflect dosing frequency, the shorter half-life and observed pharmacokinetics of antibiotics, and the time required for clinical response. TDM can help optimize drug dosages in patients with altered pharmacokinetics, particularly infants, patients with long-term incurring treatment, or organ failure. Real-Time TDM quantifies drug concentrations in patients, compares them with reference data, and modifies the treatment plan either instantly or shortly thereafter, while population-individualized TDM uses previously published population pharmacokinetic data to monitor drug concentrations and modify the treatment plan if necessary. Rapid TDM employs techniques such as ultrafast liquid chromatography or biosensors to evaluate drug concentrations within the dosing interval.

TDM is commonly used for the anticonvulsants phenobarbital, phenytoin, carbamazepine, and valproic acid; the immunosuppressants ciclosporin, mycophenolate mofetil, prevalence and type of in-born errors of metabolism see TACG, and MTX; the antituberculosis drugs isoniazid, rifampicin, pyrazinamide, and ethambutol; the antidepressant lithium; the antibiotics gentamicin, tobramycin, vancomycin, and amikacin; the bronchodilator theophylline; the antimalarial and antiretroviral drugs; and other agents associated with a narrow therapeutic window or significant toxicity. Population pharmacokinetics have produced rich sources of TDM-target concentration data for many agents; much of this information is easily

accessible and should be used to support TDM in patients. Despite these advances, TDM of PHB remains a research tool. Official TDM reporting in clinical laboratories for common drugs is increasing, along with monitoring for other drugs sometimes included in TDM discussion groups [225, 226, 227, 228, 225, 226, 227, 228].

Substance Abuse Testing

Substance abuse testing in the clinical laboratory is an established component of therapeutic drug monitoring (TDM) and, increasingly, is related to drug testing in forensic medicine. Although their diagnostic uses are very different, the principles of testing by TDM and the performance characteristics required are often relevant to substance abuse detection. Main classes of abused drugs may be broadly classified as opioids, cannabinoids, stimulants, depressants, or sedatives/anxiolytics, often with specific drugs grouped within these classes. Specimen types employed for such testing principally include urine, oral fluid, blood, and hair. Steroid abuse testing typically screens urine to identify testosterone as well as the naturally occurring glucuronide conjugates of dihydrotestosterone, nandrolone, and more rarely, 17-α-alkylated steroid drugs such as methyltestosterone. The aim of these test groups is detection of naturally occurring substances or steroids in sufficiently large quantities that are indicative of steroid abuse rather than the usual background level encountered in normal control populations. The integrity of laboratory testing for steroid abuse is maintained when samples are tested by gas chromatography/mass spectrometry or tandem mass spectrometry. Target × cut-off screening ratios provide an estimate of the probability of steroid abuse.

In contrast, the majority of other drug tests employ relatively inexpensive and highly sensitive immunoanalytic methods. These tests permit the analysis of large populations and are often considered screening tests: a positive result indicates that further analysis is required, usually by gas chromatography/ mass spectrometry or tandem mass spectrometry. Coat-type, rapid response immunochromatographic screens are now available for nearly all classes of recreational drugs. Considerable effort has been expended to improve the accuracy of these tests, especially ensuring that cross-reactivity is minimized and that the appropriate cut-off concentrations are chosen. In practice, however, different specimen types lend themselves to drug/abuse testing of varied classes.

Advances in Analytical Toxicology

Analytical toxicology is the analysis of biological samples with respect to environmental toxicant exposure or forensic toxicant detection. Modern

toxicology has taken advantage of the methods employed in clinical chemistry, including high-throughput analysis. However, some toxicant detection still uses older procedures as in sports drug testing. The analysis of a patient sample is applied to detect the presence of an extra substance or one not usually tested for. The identification of specific compounds or their metabolites is required in both cases. The detection of common environmental contaminant classes by routine toxicity screening and the application of these toxicants through controlled exposure study systems are also included.

Drug testing evaluates the presence of specific compounds in biological samples for purposes such as clinical diagnostics, organ transplant optimization, enforcement of the prohibited drug list in competitive sports and the detection of drug-induced criminal activity. Besides drugs of abuse, metabolites such as Haloxon and other organophosphate (OP) insecticides or homologous series of steroids are analysed in non-medical assays. A positive test often requires controlled follow-up analysis on a different sample from the same patient. Routine clinical testing detects common drugs and metabolites in blood, urine, hair and nail matrices, while more sophisticated laboratories are capable of analysing a wider range of Approved >400 Not available drugs and poisonings. The routine request is Volume of urine due to its easy collection, but sweat and saliva have also been used. Detection of others Haloxon and similar Organophosphates Agents, methyl salicilate is prepared [229, 230, 231, 232].

Chapter - 15

Molecular Diagnostics in Clinical Chemistry

Accurate and timely laboratory diagnosis is vital for evidence-based management of patients in emergency medical conditions. Molecular diagnostics techniques based on nucleic acids have revolutionized the laboratory diagnostic approach and workflow for several disease conditions, providing diagnostics early in the disease time course and directly from clinical samples. Molecular diagnostics facilitates rapid detection and characterization of many pathogens including viruses, bacteria, fungi and parasites that cannot be identified by conventional cultures, stains or serological tests. The critical review and technical evaluation given here of molecular diagnostics in clinical chemistry provides sufficient evidence for the marginal supplementary contribution of their use alongside the other conventional parallel diagnostic techniques [187].

Recent advances in molecular diagnostics and their applications for clinical conditions in various medical specialities are reviewed. The evolution of molecular diagnostics toward early intervention and treatment as an adjunct approach in ongoing microbilogy, hematology, pathology and chemical pathology tests, addressing many previously intractable issues is documented along with future perspectives from disease management point of view. The significant applications in cytogenetics, thalassemia carrier screening, infectious diseases, pharmacogenetics, steroid hormone biosynthesis disorders, toxicology including drug and substance of abuse screening as well as malignant neoplasms are also discussed for their diagnostic potential and spectrum of commercial kits available for them. Equipments such as synthetic gene into vector cloning or real time PCR mutation detection system were acknowledged as relevant infrastructure required in hospital laboratories aiming to pursue molecular diagnostics in household medical conditions without major financial burden.

Nucleic Acid Extraction and Amplification

Nucleic acids are typically available in blood and various other body fluids. Nucleic acid amplification is based on the polymerase chain reaction, or PCR technique. PCR is based on the principle of repeated DNA synthesis

where specific primers of 18–24 base pairs length are designed to amplify targeted areas of nucleic acids, so that they can be detected and identified either by hybridization, sequencing, or other techniques. Automated devices for performing PCR reaction is available in all major pathology laboratories. Real-Time PCR using fluorescent dyes are becoming very popular.

The Human Immunodeficiency Virus (HIV) is now one of the most important public health issues in many countries. HIV induces immunosuppression allowing opportunistic pathogens to proliferate and cause disease. The CD4-count is an essential clinical test which indicates the need for treatment, initiation of prophylaxis of opportunistic infections and the prognosis of the disease. HIV can be detected in blood, urine, sputum, and other body fluids. The diagnosis is made clinically by serology testing for HIV. Seropositive cases can be further examined by CD4-count for initiation of ART therapy. In case of seronegative test, RT-PCR test can check earlier stage of HIV disease in acute or window phase [233, 234, 235].

PCR-Based Diagnostic Techniques

Agarose gel electrophoresis shows an amplicon of the beta-globin gene from sickle cell defined by a mutation in the GAG codon changing it to GTG. The band observed in the positive control and six of the samples indicates the presence of the wild type allele of beta -globin. The remaining the samples do not present the band characteristic of the wild type allele. All the samples were also analyzed by PCR-RFLP. The presence of the Hae III restriction site in the beta-globin gene was detected using a pooled sample and the amplification product was digested with the restriction enzyme. The analysis confirmed the presence of the mutation in the beta-globin gene in three samples. The presence of the mutation in the beta-globin gene causing sickle cell was confirmed by a nested PCR amplifying exclusively the mutated allele. The incorporation of a simple and inexpensive technique for the diagnosis of sickle cell in clinical laboratories has been fulfilled. These diagnostic techniques are as follows: PCR, nested PCR, quantitative PCR, real TaqMan PCR, real-time PCR, multiplex PCR, reverse transcription PCR, reverse transcription multiplex PCR, reverse transcription nested PCR, reverse transcription quantitative PCR, reverse transcription quantitative real-time PCR, reverse transcription digital PCR.

Genetic and Epigenetic Biomarkers

Alterations in biological processes that lead to either the initiation of diseases or later steps in their pathogenesis can be diagnosed earlier than morphological changes by detection of disease-associated biological

molecules. Classification into three categories of biomarkers broadly corresponds either to disease initiation, disease progression, or the formation of a morphologically visible disease. Genetic and epigenetic markers are two of the most studied expression-based change classes.

Genetic markers are changes in nucleotide sequence that occur with high frequency. They include single nucleotide polymorphisms, small insertions or deletions, variable number of tandem repeat changes, and large-scale chromosomal gains or losses. Discovery requires large cohorts and has been aided by statistical methods that address the multiple-tested nature of genetic association studies. Not lin-early predictive because not functionally involved, genetic markers identify only underlying disease risk. Prediction of more advanced disease requires epigenetic detection of alterations in critical biological processes that lead to pathogenesis. Cells of patients of geographic regions with high sporadic disease mortality accumulate defects because of environmental exposure. These alterations include, for example, age-associated increased promoter methylation of genes in inflammatory pathways [236, 237, 238, 239].

Pharmacogenomics and Personalized Medicine

Pharmacogenomics is the study of all the genes that determine an individual's response to drugs. Knowledge of the different types of variability in drug response is expected to help guide therapy in a more individualized manner. The notion of personalized medicine has accompanied pharmacogenomics since its inception, although implementation in mainstream clinical practice remains elusive.

The goal of pharmacogenomics is to make drug response, efficiency, and safety more predictable for individuals. Interindividual variability in response may be attributed either to functional polymorphisms affecting the drug disposition pathway or to polymorphisms in other genes related to the drug target or the drug effect pathway. Currently, established examples of the pharmacogenomic approach are few; although a larger number of drug–gene pair associations have been associated with clinical indications in the Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline tables, the level of evidence to support these associations varies. Despite these limitations, initiatives such as those from the CPIC and the Dutch Pharmacogenetics Working Group provide guidance on how to implement pharmacogenomics in the clinical setting [240, 241, 242, 243].

Integration of Molecular Data in Clinical Practice

Thus far, no clinical laboratory has gone as far as delivering clinical

microbiology results in a semi-quantitative fashion, either in terms of microbial density, distribution, or virulence/metabolic potential, leaving that task to clinicians' clinical impressions and empirical experience. Hence it is very interesting that six prototype qPCR assays detecting the presence of enterotoxin genes found in Staphylococcus aureus (e.g. sea, sey, etA, etD, etd), one triggering gastroenteritis and the other food poisoning, became equipped with Health Sciences Authority approval and extensive testing validating their clinical utility. Once validated over a sufficient number of clinical cases, the two quantifications of the amplified DNA were expected to be reported relative to the Staphylococcus PCR result in order to inform clinicians of the predicted virulence dose of the food-stable enterotoxins contributed by the S. aureus xa fermenting in food.

It would be difficult to determine the condition of a patient from a clinical microbiology report if the result simply read "Coagulase-negative Staphylococci (CNS) isolated." Diagnosticians would have to guess whether the patients were infected, colonised, or misdiagnosed. To assist in diagnosis, a matrix was created to interpret the clinical significance of a number of CNS species that might be isolated from a range of clinical specimens. Patients presenting with a cutaneous discharge growing S. epidermidis or S. haemo-lyticus, a cardiovascular prosthesis-related sample with S. epidermidis or S. hominis, and an articular fluid effusion isolating S. aureus or S. epidermidis all harboured infection, whereas those with normal samples growing S. saprophyticus and S. lentus were considered to be colonised. In a more complex situation, S. lugdunensis was the only CNS species isolated from an osteomyelitis specimen with the patient being a previously healthy adolescent. The results supported laboratory pre-examination guidance by defining the clinical significance of particular CNS species in specific specimens [244, 245, 246].

Chapter - 16

Point-of-Care Testing and Rapid Diagnostics

Principles and Advantages of POCT

Point-of-care testing (POCT) is a decentralized approach to diagnostic testing performed directly at the site of patient care, such as the bedside, operating theatre, or outpatient clinic. By bringing laboratory analyses closer to the patient, POCT shortens the total turnaround time from test initiation to clinical decision—an important aspect of immediate patient management [247]. This can be of advantage, in scenarios like cardiac arrest or anaphylactic shock, where the immediate clinical measures may depend on the results of a critical test [248]. POCT devices tend to be small, easy to handle, and simple to operate. Many POCT instruments process the sample, e.g., whole blood, plasma, or other body fluids, without requiring complex sample-preparation steps. The devices allow untrained personnel to perform certain tests and make rapid therapeutic decisions accordingly. POCT also offers a wider choice of parameters, covering a large number of biomarkers in parallel.

Portable Diagnostic Devices

Portable analytical devices-dubbed the “lab-on-chip” era-have entered a phase of rapid development, fueled by both societal demand and recent research breakthroughs. In order to stay at the forefront of technological developments and address the needs of modern society, the miniaturized, portable, and multiple equipment of many types must be created and streamlined. Such devices abide by the characteristics of current society. Specifically, portable devices feature convenient operation and reduce both time and labor costs. Chemical sensors can be multi-directional. A single device can measure parameters of clinical ability, environmental quality, and food safety, with low cost but good accuracy and precision.

Typical innovative developments have recently appeared. DNA biosensors to evaluate suspected congenital diseases presume to detect mutant genes in amniotic fluid collected at 14-20 weeks of gestation are convenient, rapid, cheap, and effective. The sensing area is based on a costumed ZnO nanorod sensor encrusted with specific capture DNA molecules. The electrochemical detection of lead, mercury, and cadmium ions by a polymeric

HTCT–STMB composite film sensor tailored for on-site applications demonstrates low gear-carrying, high-efficiency solid waste treatment with zero ion discharge during the dry season, strong acid-resistance, and the capability for solid-liquid separation. A gold-loaded porous silicon sensor for the simultaneous quantification of glucose and cholesterol in blood uses a fiber-optic surface plasmon resonance configuration and long-range surface plasmon coupling.

Quality Assurance in POCT

Quality assurance represents a planned and systematic approach to minimizing the risk of injury to patients. Guidelines for QA in POCT emanate from the International Organization for Standardization (ISO) and WHO. Irrespective of the type of test or method, the QA process for POCT should normally contain a preanalytical phase, an analytical phase, and a postanalytical phase, which are closely linked to the three phases of testing mentioned previously. Testing by POCT is undertaken close to the patient rather than designated laboratory facilities. POCT is synonymous with immediate or rapid testing because the turnaround time for completion of testing is shortened.

The sample handling and preparation steps can be bypassed, but quality of the sample, test performance, interpretation of results and action to be taken are still crucial. The two preanalytical aspects in which POCT fails more frequently than in a central laboratory are quality assurance of the operator and interpreting a result from POCT. Quality assurance is based on the same principles as for testing in the laboratory daffodils. Quality control in POCT still requires adequate supply of control materials, equipment etc, to provide optimal QC & QA specific to laboratory facilities at different levels in the health care system. It is not practical to envisage total quality management with POC testing methodology but a comprehensive quality assurance program requires establishment of QA/QC panels involving all stakeholders [249, 250, 251].

Clinical Applications and Limitations

Widely regarded as highly accurate diagnostics, the clinical chemistry and pathology laboratory represent a major resource with which to establish the presence of disease. False-positive and false-negative errors can occur, however, and may go undetected. Laboratory diagnosis of common diseases may still sometimes require investigation of a second sample or a more detailed test.

Many diseases are detected by specific laboratory tests. Patterns of laboratory abnormalities involving the detection of multiple analytes can

identify the presence of disease processes, such as the trisomy abnormalities identified in amniocentesis specimens. Disease-specific testing parameters are best validated during experimental studies of disease or during large cohort studies. Disease-specific tests can then be expected to work accurately in the intended population. Laboratory tests with a pattern of expected analyte abnormalities can subsequently be employed as routine diagnostics in clinical practice [252, 253, 254].

Future Innovations in Rapid Testing

Diverse novel approaches promise to enhance the versatility of lateral flow assays, facilitating the rapid detection of viral diseases and other health-threatening conditions. One method relies on microfluidic technology combined with electrochemical detection. A lateral flow biosensor detects the excretion of the major respiratory infection viruses H1N1 and H3N2 in biologically relevant matrices with an electrochemical readout achieved via an inexpensive portable potentiostat. The detection process elucidates a novel path for the construction of portable rapid diagnostic testing kits based on lateral flow microfluidic technology.

BEHAVIORAL DRUG TESTING COVERS A WIDE RANGE OF DRUGS. Recent progress indicates that the detection of multiple target drugs can be performed using paint samples, ranging from plant-derived substances to synthetic drug analogs. The paint testing technology provides a range of drug detection capabilities, from monitoring drug problems in certain populations by detecting drugs of abuse to potential indicators of candidates' fitness for public service. Compared with traditional methods, paint samples greatly enhance the options for behavioral drug testing, and they hold promise for supporting large-scale drug screening in special populations.

Pseudorabies virus (PRV) poses significant threats to the pig industry and remains a focus of research in China. A rapid one-step immunochromatographic strip assay (ISA) for the detection of PRV in piglets was established, based on the monoclonal antibody 1B3, which is specific for the glycoprotein B (gB) epitope of PRV. The new ISA, which can provide a simplified approach to the on-site identification of PRV infection in piglets, is capable of detecting PRV gB in clinical samples at 10 pg mL−1 in 20 min.

Chapter - 17

Automation, Artificial Intelligence, and Digital Pathology

Laboratory Automation Systems

In today's clinical laboratories, automation has become an intrinsic part of daily operations. Technological advances enable the use of robotic systems to perform complex procedures, thereby relieving technical staff of repetitive tasks. Automation of pre-analytical, analytical and post-analytical steps has been developed for various laboratory disciplines, enhancing the efficiency and productivity of healthcare services. Unfortunately, few bacterial virus automation systems have been adapted for serology and virology, despite the substantial number of viral detection tests performed in clinical laboratories. However, even though many tests are still conducted on standard immunochemistry platforms, automation is playing a key role in the improvement of operational efficiency and laboratory profitability [19].

Clinical laboratories are critical for the provision of relevant information for medical intervention, delivering over 70% of such data and therefore playing a fundamental role in diagnosis and prognosis. More rapid, higher throughput and more precise analytical methods have been developed in the fields of biochemistry, immunochemistry and haematology. The first two disciplines have seen considerable progress in both stand-alone and modular equipment. Several manufacturers now offer fully automated, multi-channel systems with integrated sample tracking of both specimen tubes and analysis feedback. This allows the simultaneous screening of up to 30 parameters and the performance of 400 chemistry tests, 300 immunoassay tests and 70 haematology tests each hour. Such systems have transformed the original work-flow concept in clinical laboratories, allowing the continuous performance of client's analyses during day and night [68].

AI-Based Result Interpretation

AI-code is deployed in clinical analytics systems to accelerate workflow in clinical chemistry, particularly the parallel assessments of electrolyte status in blood and urine, and complete blood counts. The clinical picture points to manifest disease but does not adequately restrict the differential diagnosis. The

task is interpretation of clinical investigations in the clinical context. Pattern recognition for probable mismatches between clinical picture, demographic data, or clinical investigations has great clinical importance, including the detection of heterophil antibodies in acute rheumatic fever and appropriate treatment for children with diabetic ketoacidosis.

RealPhysicians Ai, a Physician-based Assistive Diagnostic Software, is integrated with the Clinical Laboratory Information System (CLIS) via API Ada. CLIS collects, processes, and delivers biomedical data or services to a wide range of healthcare stakeholders. Ada serves as a single intelligent assistant for all laboratory users (physicians, technicians, and laboratory operators) while examining complex cases in clinical chemistry or seeking an additional opinion for other sub-disciplines (Serology, Hystopathology, etc.). The amalgamation of Ada's integration with the complete laboratory data, demographics, and clinical analytics generates nearly 300 knowledge files per modality for use in biomarker pattern matching and guided investigation.

Digital Pathology and Image Analysis

Diagnostic pathology is the gold standard for definitive diagnosis and biomarker assessment of human diseases. Digital pathology, a relatively new technology aimed at reliable quality control, faster diagnosis, conserved resources and materials, and reduced accessibility limitations, enables virtually unlimited utilization of glass slides for secondary assessment or research through digital scan-and-store datasets. However, owing to biosafety concerns, pathological analysis cannot be fully automated. Artificial intelligence (AI) can achieve a mastery level in identifying common types of cancers using histopathological images as input.

A new approach in this arena, digital immune pathology, applies artificial intelligence technology to hematopoietic malignant neoplasm detection. The proposed digital immune pathology leverages an advanced deep-residual-network-based architecture that combines 17 well-known tumor types into a single model. The system achieves a mean accuracy of 96.4%, which is sufficiently high for practical use. The Convolutional Neural Network (CNN) is further extended to identify cancer subtypes and accurately estimate tumor purity, thus enabling the initiation of personalized therapy.

Big Data and Predictive Diagnostics

Big Data technology is helping researchers to unravel layers of information contained in human body fluids, and to associate patterns and concentrations of specific metabolites with the likelihood of disease. Accurate analysis of body fluids can be achieved via machine learning techniques,

calibrated against voluminous datasets. Another emerging area of clinical medicine involves the analysis of body-fluid metabolomes to reveal the biochemical consequences of disease and investigate correlated metabolite networks. Body fluids contain rich information used by artificial intelligence software to detect diseases rapidly and meticulously.

The biochemical composition of body fluids changes in predictable ways during the evolution of a physiological condition toward a pathology, such as infection or inflammation, enabling the development of predictive models for decision support during diagnosis and prognosis. The levels of individual metabolites associated with pathogens can be examined using untargeted metabolomics. The systematic study of these compounds, in combination with machine-learning analysis of data, is producing excellent results. The metabolomes of saliva, serum, tears, stool, urinary, and air samples are associated with human health and disease. These advances suggest the development of predictive biometric signatures from body-fluids metabolic data to support diagnostics.

Ethical Challenges in Digital Laboratories

As members of a digital laboratory of the modern age, chemists and pathologists face various ethical challenges. The patient has thus far not been placed in the center of the research system. At first, it seemed as if a disease would not be detected until the patient appeared in hospital concerning the illness. The digital laboratory concept is different: all test results continuously collected in digital form enter databases. When a disease with uncommon or unexpected characteristics is detected, research activities are organized to densely analyze patients with such features: these patients are often located through the databases. The name of each patient remains secret until a manuscript becomes almost ready for submission; the name is unmasked only in the last stage of data confirmation, and the unmasking is supervised by an ethic committee and takes place at a site different from the laboratory. Thus, many health ethics, including the autonomous control of the manipulation of patient-biological scraps, are simultaneously served.

The art of selecting cases and performing distributed confirmation and validation tests on the unselected cases can replace the classic paradigm of experimental design, in many situations. When a research project takes patient material that already exists, the research committee merely checks whether the disease involved seems to put at risk the privacy of the patient: if there are no hidden risks, permission is routinely granted and ignored, the work in the laboratory goes on effortlessly, and the authorities are notified only once the

manuscript becomes almost ready. Yet, it is this exploration of the polygenic and multigenic diseases, finally positioning the study on such rare affections at the real center of the research, that involves the largest piles of patient material, whereby health ethics become crucial, hot, and delicate.

Chapter - 18

Future Trends and Innovations in Clinical Chemistry

Precision Laboratory Medicine

Precision medicine is paving the way for highly personalized healthcare based on specific knowledge about an individual patient. The ability to provide reliable information to a clinician is becoming increasingly important. The external quality control accomplished by the College of American Pathologists (CAP) or the Joint Commission on Accreditation of Healthcare Organizations (JCAHO) does not guarantee patient safety. Each laboratory must be aware of the aspects relating to quality management. Every single parameter of the clinical pathway needs to be controlled and every single step of the preanalytical, analytical, and postanalytical phases must be considered. One of the most common reasons for questioning patient safety lies within the preanalytical phase.

The total testing process can be divided into four main blocks—the preanalytical phase, the analytical phase, the postanalytical phase, and the quality management unit. Quality management is a special element belonging to each phase. The preanalytical phase comprises test ordering; patient preparation; sample collection; sample transportation; sample processing; sample preservation; sample storage; and sample registration, identification, and delivery to the laboratory. The analytical phase encompasses all laboratory analyses, including sample and reagent preparation. The laboratory results and their interpretation fall into the postanalytical phase. All of the processes in these three phases are strongly interconnected and mutually dependent, and the processes and results of one phase have to be directly compared in time and origin to those of the other phase [255, 256, 257, 258, 255, 256, 257, 258].

Nanotechnology and Biosensor Applications

Nanobiosensors made of noble metals, carbonized materials, silica gel, and biopolymers can accurately detect various biomedical components in minute tissues. Nanotechnology, such as devices made of nanoparticles with one or more dimensions measuring less than 100 nm, promises significant advancements in the health and clinical fields. To realize these advancements,

strong collaborations between the technical, clinical, bioengineering, and pharmaceutical worlds are necessary. Nanobiosensors are microelectrodes or biosensors containing biological recognition elements that can identify, detect, and quantify analytes with remarkable sensitivity and selectivity. Today, many biological materials, microorganisms, and their metabolites are being successfully identified on a laboratory scale. These materials are being studied and developed for their potential use as label-free biosensors for toxic substances and environmental monitoring.

Nanotechnology is a hot topic in the field of biotechnology, addressing recent demands and expressing current concepts in biotechnology and nanotechnology Renaissance. Nanobiosensors are microelectrodes and biosensors that incorporate biological recognition elements that can identify, detect, and quantify analytes with dramatic sensitivity and selectivity. Nowadays, nanobiosensors for organic molecules are sold in hospitals, allowing a rapid assessment of diverse diseases. A multidisciplinary approach, resulting from the combination of bioengineering, statistics, and nanobiosensor technology, enables the extremely low-cost detection of metabolites. For instance, the combination of bioengineering expertise, electrical engineering design, and nanobiosensor technology allows the design of an ultra-low-cost biodevice capable of sensing organophosphate and carbamate pesticides. The application of nanobiosensors using nanomaterials formed by noble metals, carbonized materials, silica gel, biopolymers, and others with completely different physical properties in clinical analyses has been demonstrated.

Omics technologies in disease detection

Investigating how diseases affect people is necessary to better understand them and help patients. Developments made in clinical omics technology could potentially allow achieved accuracy in the detection of these diseases, specifically critical diseases. The term Clinical omics is the combination of sample collection from patients and the technology of omics that will help physicians detect diseases with high accuracy. Therefore, Clinical Chemistry and Pathological Analysis are necessary to make the detection of diseases more effective around the world. Nowadays, Clinical Chemistry and Pathological Analysis are crucial for indicating the clinical state of patients. Clinical Chemistry is a laboratory service that offers tests operating and interpreting biochemical results in a quantitative manner. The specific activities depend strongly on the methods employed, the scale of testing, and the clinician's request in relation to local context. Pathological analysis investigates diseased tissues to disclose the processes by which they develop

over time and to clarify their nature. Clinical Chemistry and Pathological Analysis are the vital areas that rely on developing technologies that make diseases, both infectious and non-infectious, detectable in the early stage with accurate results. Clinical Chemistry mainly consists of Blood and Urine analysis. Blood tests are commonly ordered laboratory test that healthcare provider use to check a patient's health as a part of a routine check-up. Blood test checks also help diagnose disease or condition, monitor a health problem, or check how well treatment is working. Urine tests check for a wide range of disorders such as kidney disease, liver disease, diabetes and urinary tract infections [259, 260, 261, 262].

Sustainable and Green laboratory practices

The laboratory can be the origin of highly toxic wastes, many of which are hazardous and difficult to treat. To avoid irreversible damage of the environment, the adoption of sustainable and green practices is recommended. In analytical chemistry, green chemistry implementations aim to reduce the use or generation of hazardous substances in analytical procedures. The methods leading to "greener" protocols in a laboratory are called green analytical chemistry (GAC). GAC promotes waste minimization, safer solvents and less toxic hazards, and it is achieved by analyzing the complete analytical method (sampling, pre-treatment, and finally the analytical procedure). Important green chemistry aspects are sample size reduction, use of energy-efficient instruments, the replacement of harmful solvents, and the elimination of toxic reagents.

Order of Green Analytical Chemistry priorities and tools includes: 1-Renewable Resources, 2-Possibility to Preserve, 3-Avoiding Danger, 4-Class, 5-Manufacture Simplicity, 6-Class 7-Mobility, 8-Requiring Energy, 9-Using Diverse Materials, 10-Using Solvents, and 11-Using Chemical Products involved in the Green Chemistry Concept. In general, it reduces waste at source and prevents contamination, using experimental designs to identify best methodology, replacing dangerous reagents with safer alternatives, using nature's vocal length, and using instrumental analysis whenever appropriate.

Special consideration is necessary in sampling, where it can drive the loss of precious analytes present at low concentration, involving breakdown, and the presence of interferents that lead to negative matrix effects. In cosmo-geochemistry, it is a good practice to avoid sampling in contaminated areas and with minimal energy expenditure. However, sample preparation remains an important and often neglected step, associated with high costs, low efficiency, and requiring long time investment. In situ analysis is the GAC way for sample pre-treatment and even analysis [263, 264, 265, 266].

Global challenges and future perspectives

The routine application of clinical pathology is limited by health and economic factors; thus, heavy reliance is placed on prevention. Epidemiological surveillance, vector and reservoir control, monitoring animal health, and mass treatment campaigns are among practical veterinary strategies. Experts can forecast emerging zoonoses from global trends, but serious economic constraints hinder the practical implementation of effective animal health measures [267].

Automation strategies enable labs to benefit from technological progress by devoting staff time to interpretative work rather than pre-analytical and analytical tasks. Such configuration, which is particularly sought in virology and serology, faces challenges at the level of stringent regulations [19].

Chapter - 19

Conclusion

The accurate diagnosis of pathology is one of the most vital components of the medical profession. Pathological analysis coupled with biochemical analysis is an extraordinarily powerful combination. The development and implementation of rapid biochemical and molecular diagnostic tests complement classical pathological techniques. Molecular diagnostics has revolutionized, medical diagnosis enabling the rapid diagnosis of diseases and infection with previously difficult-to-detect organisms including bacteria, viruses, and fungi in clinical and postoperative specimens. The application of real-time polymerase chain reaction is widespread in clinical laboratories and its potential to detect grave diseases at an early stage is remarkable.

The list of microbial diseases that molecular diagnostics could not easily diagnose and are now testable in clinical laboratories is adding up and continues to broaden, progressing into the rapid diagnosis of infection with parasites. The new analytical techniques available to the clinical laboratory now allow clinicians to provide assistance and information to patients with multiple diseases. Besides the traditional pathology analysis, techniques such as ultrasonography and multi-slice computed tomography support the clinician in confirming a diagnosis, essential for complementing analysis, with $\geq$ 95% accuracy. A significant milestone in clinical chemistry.

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