Integration of Chemistry, Biotechnology, Pathological Analysis, and Zoology in the Development of Diagnostic Biomarkers for Zoonotic Diseases

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Contents

Abstract

Combined disciplines of chemistry, biotechnology, zoology, and pathology help elucidate and translate biological signatures associated with specific zoonoses. Development of a collection of validated and integrated markers and signatures specific for several major zoonotic diseases like rabies and Ebola will improve disease diagnostics for animals and humans and bolster the One Health approach. Rabies and Zika disease, among others, have been addressed in detail.

Emerging and re-emerging zoonotic diseases are now being diagnosed and predicted using special signatures at different molecular levels (Genomic, proteomic, metabolomic, and immunological) through an integrated approach. Zoonotic diseases can be detected from their pathogenic signatures as a combination of products such as genetic elements (DNA/RNA), proteins (antigens/antibodies), metabolic compounds, and His pathological changes in host tissues, organs, and cells. Biomedical researchers and authorities are searching for early diagnostic signatures that can accurately predict the disease condition of suspected animals even before clinical symptoms appear.

Chapter - 1

Introduction to Zoonotic Diseases

Zoonoses are infectious diseases transmissible from animals to humans. They pose a major public health risk and threaten global health security. Based on available epidemiological data, 60% of emerging infectious diseases and 75% of all zoonoses have an animal origin, and recent studies have highlighted the increase in zoonotic pathogens over the last five decades from wildlife to human populations. That trend is expected to continue, driven by changing land use, urbanization, climate change, growing demand for animal protein, and health threats. These factors facilitate spillover of pathogens from wildlife to domestic species and ultimately to humans.

Interdisciplinary measures involving chemistry, biotechnology, pathology, and zoology can synergistically advance the development of rapid, sensitive, and accurate diagnostic tests for zoonotic diseases. Such tests permit timely detection of pathogen transmissions between animals and humans, thereby facilitating the development of preventive measures against zoonotic outbreaks. Early diagnosis also enables effective implementation of control measures to limit pathogen spread during outbreaks. Currently, significant challenges prevent the timely development of new diagnostics. Standard clinical and pathogen-specific characteristics remain poorly defined, multiple species can be involved in the same outbreak, and assays established for human pathogens often lack cross-species detection capability [1, 2, 3, 4].

Definition and global burden

Zoonoses are defined as diseases and infections that are naturally transmitted between vertebrate animals and humans. They can be caused by a wide variety of pathogens including bacteria, viruses,

parasites and fungi. Approximately 60% of all infectious diseases in humans are of zoonotic origin. Zoonotic pathogens are estimated to be the causative agents of approximately 200 diseases and syndromes in humans. More than 70% of recently emerging human infections are believed to be of zoonotic origin. Numerous recent developments such as human encroachment into wildlife habitat, climate change, agricultural intensification and increasing travel and trade have increased the risk of zoonoses emerging in new regions or new hosts. Increasing frequency of zoonotic diseases could cause enormous threat to both animal and human health.

Zoonotic diseases are of great concern for both human and veterinary health because of their economic impacts, emerging threat to public health, ubiquitous distribution and increasing frequency. Hence, there is an urgent need for rapid response to control the outbreaks, prepare for future or unknown outbreaks, and meet demands from point-of-care diagnostics. Diagnostic technologies that can identify, profile, and detect pathogens or immunity at any of the infection stages in any infected species are of interest and could support early detection and prevention [5, 6, 7].

Emergence and re-emergence factors

Emerging and re-emerging zoonotic diseases have recently received attention in the scientific and popular press. Numerous factors are implicated in the disease emergence or spread of zoonotic infections, including agricultural intensification, land-use and ecosystem change, urbanization, climate change, and wildlife trade. The dynamic of disease spread may also be influenced by stochastic factors such as the random encounter of vectors, hosts, and pathogens. For most of the currently major zoonotic diseases, these factors are subtly shaping the ecology of disease spread in ways that are poorly understood. A consideration of the causes of emergence may point to the possible spread of other diseases, including pathogens beyond those currently observed to be zoonotic. Different zoonotic diseases may share common factors that may be invoked to assist in assessing their risk of emergence. An understanding of these drivers can provide

insight into aiding discovery and development of diagnostic and analytical tools.

Apart from the currently recognized drivers of emergence, most of the major diseases have occurred in comparatively isolated ecosystems, particularly of wildlife species. Even in such a relatively stable environment, patterns of emergence over the last century are not a random distribution. Such repetitive, albeit sporadic, emergence suggests that the currently available knowledge may be used to predict potential further incursions. Emerging zoonoses are defined as diseases caused by pathogens that are newly recognized, or newly evolved, or that have recently occurred in a new host species. In contrast, re-emerging diseases are those which either have been around for centuries in the case of long-established zoonoses or which were common in the past but are currently increasing in incidence or geographical range [7, 8, 9].

Importance of integrated scientific approaches

The World Health Organization reports that zoonoses account for about 60% of all infectious diseases. The ongoing COVID-19 pandemic, caused by a novel coronavirus, illustrates the potential dangers posed by emerging zoonoses, highlighting how zoonotic pathogens crossing interspecies barriers can lead to widespread adverse health effects in animals and humans. Despite the considerable global health burden of zoonoses, advances made over the last two decades in the development of new diagnostic tools have rarely been integrated into a single resource or comprehensively compiled. Importantly, such advances typically reflect the application of isolated areas of investigation in chemistry, biotechnology, pathology, and zoology by independent research groups worldwide.

Nevertheless, the epidemiological development of zoonotic diseases is influenced by a multitude of factors, including the environmental ecology of pathogens and their associated reservoirs and vectors, the risk of exposure to human and domestic animals, the interactions of pathogens and their laboratory animal models (including humans), and the tissue and organ damage they produce during infection. Therefore, the creation of diagnostic biomarkers for

these diseases requires a comprehensive study that encapsulates the contributions of different scientific fields, taking full advantage of all conceivable approaches to the underlying research challenges. Such an integrated strategy not only supports the development of novel diagnostic reagents but also increases the likelihood of achieving large-scale clinical and veterinary diagnostic products. The present review serves as a more formal introduction to the aforementioned integration of chemical, biotechnological, pathological, and zoological approaches and resources in the unveiling of new zoonotic disease biomarkers [5, 10, 11].

Overview of current diagnostic challenges

The current zoonotic diagnostic landscape is hindered by a reliance on single-analyte systems with limited convenience or sensitivity. As a result, samples from the same reservoir species must be extensively screened using multiple techniques, such as serology, PCR, and culture. These methods can involve varying levels of invasiveness, skilled personnel, equipment, and financial resources. Diagnostic gaps, deficiencies, and discrepancies must be addressed through continued efforts in safety and ethical considerations. Clinical samples yield only a short window of detection, necessitating an emphasis on reservoir-targeted diagnostic systems. Detection at the animal reservoir is crucial for curtailing the disease cycle and preventing human cases.

Serological or histological markers are often associated with zoonoses, but these are not detectable during the zoonotic event and are currently used mainly for retrospective studies. Markers undetectable in the affected host can be highly useful for cross-kingdom detection of zoonotic pathogens. Pathogenesis drives the host-pathogen interaction, and the resultant pathological sequel should reflect the pathogenic process. Disease-specific chemical alterations occur in all kingdoms, and various omics technologies make it feasible to profile them. Multi-omics profiling has been approached in different systems, but multi-omics analysis of zoonotic disease in all possible reservoirs remains elusive. The full integration of advanced chemistry, biotechnology, pathology, and zoology is necessary for the development of novel and sensitive diagnostic markers, especially for neglected and under-researched zoonoses [12, 13, 14].

Chapter - 2

Chemical Principles in Biomarker Discovery

A disease-specific chemical fingerprint, generated from various biospecimens undergoing molecular changes, may be detected within the host metabolome. Chemical analysis therefore occupies a vital role in the discovery of biomarkers for the early and accurate detection of emerging and re-emerging zoonoses. Among the techniques available to analytical chemists, separation and spectroscopic techniques, including mass spectrometry (MS), nuclear magnetic resonance (NMR), liquid chromatography (LC), gas chromatography (GC), and Fourier-transform infrared (FTIR) spectroscopy, are particularly useful for profiling metabolites in biofluids and tissues. In the context of diagnostics, samples are typically processed and analysed by proficient operators, with the results interpreted by experienced informaticians.

However, sufficient technological advancements now exist that enable the disease-associated chemical signatures of newly emerging and re-emerging zoonoses to be detected non-invasively using small portable devices. These signatures consist of molecules attributable to the host, pathogen, or both. E-noses and e-tongues enable detection of volatile compounds in breath and of non-volatile compounds in saliva, whilst non-invasive sampling from urine, faeces, and sweat is performed for subsequent analysis using electrochemical and chemical sensor devices. These platforms require further development, including incorporation of machine learning, to achieve adequate sensitivity and specificity for routine diagnostic use [15, 16, 17].

Molecular interactions and disease signatures

Chemical principles offer a unique perspective on the development of diagnostics for zoonotic diseases, spanning chemistry, biotechnology, pathology, and zoology. The vital role of chemistry in

the detection of disease-specific signatures and the explanation of the route through which various diagnostics are produced are supported by molecular interactions. Diseases produce unique signatures where a particular chemical or biological method is upregulated, and these signatures can be analyzed by analytical chemistry methods. Metabolomic studies offer information on potential signs of zoonotic diseases. It is possible to observe changes associated with zoonotic diseases during the biotoxicology process. The Zebraview principle permits high-throughput observation of pathology images, allowing for an easy understanding of essential changes associated with zoonoses.

Chemical principles also increase the efficiency of diagnosing such infections. Presenting the disorders diagrammatically allows all three pathology stages to be combined into an easily understandable mental model. The chemical principles of metabolic, transcriptomic, proteomic, and immunological signatures for zoonotic diseases are explained, as well as how these might be developed using histopathology-based methods. Such methods help in the faster detection of all diseases and enable validation across various diseases, as the pathology tissue undergoing ligature may become a self-tissue trigger. Earlier research on candidate diagnostics helps increase specificity or sensitivity by altering the detecting reagent format or route of entry. Various diagnostic routes such as amplifications, antibody detection, and microfluidics are summarized [14, 18, 16, 13].

Role of analytical chemistry in biomarker profiling

Disease profiling relies on chemical principles and the unique molecular interactions involved in the host-pathogen relationship. Laboratory samples from patients are analyzed to quantify the disease effect, with the resulting data serving as a basis for detection and diagnosis. Prediction and confirmation of disease-specific markers have advanced considerably, and analytical chemistry plays a pivotal role in these developments by developing strategies for genome-wide profiling of marker categories. Two primary spectroscopic and chromatographic techniques, both with mass and non-mass detection regimes, have attained considerable importance. Chemical assay principles allow the quantification of analytes in samples through the

application of mass spectrometry (MS), nuclear magnetic resonance (NMR), fluorescence, and colorimetric techniques. Electrochemical biosensors are also becoming essential devices for quantifying biological molecules, as they can be deployed in the field or at the point of care.

Metabolomic profiling comprehensive monitoring of the small molecule composition of a biological sample detects variations in the metabolome associated with a disease. Application of these principles to detect chemical changes produced during disease pathogenesis is useful for the development of diagnostic biomarkers. The resultant set of disease-related metabolites can be cross-catalogued with multiple-omics (genomic transcriptomic proteomic) signatures. A combination of these two complementary biochemical approaches namely, data-dependent metabolomics and multi-omics integration provides a comprehensive chemical basis for disease development and detection [19, 20, 21, 22].

Spectroscopic and chromatographic methods

The suitability of several analytical chemistry techniques for the detection of bioactive molecules related to fungal or other spoilage contaminations, toxins, or pathogens has been endangered owing to the sensitive nature of food products and the reaction with the environment. The need for rapid detection of metabolites, and especially for toxins, has not been neglected in the past few years; however, the analytical methods are still at the semi-quantitative stage.

Various chemical methods such as spectroscopic, chromatographic, and colorimetric methods have been effectively used for detection. However, the applicability of these approaches has been limited owing to time constraints, multiple detection of different species in a single assay, extensive sample preparation, and expertise required for handling. In recent years, electrochemical biosensors have gained popularity owing to their rapid detection, high sensitivity, potential for on-site and in situ detection, and they are less prone to interferences [23, 24, 25, 26, 23, 24, 25, 26].

Metabolomics in zoonotic disease detection

Metabolomics encompasses the identification and quantification of the metabolites present within biological specimens and has been widely adopted for biomarker detection in various diseases. Chemical interaction in host-pathogen systems can result in the accumulation of metabolites such as nucleic acid and protein by-products, or the depletion of critical biomolecules like amino acids, carbohydrates, and fatty acids. These metabolic signatures can yield specific fingerprints in body fluids such as serum, plasma, urine, bile, or tissue samples, which can be profiled in a high-throughput manner to discover indicators pertinent to disease diagnostics. The wide tissue accessibility of blood, as well as its role as a waste clearance route, has led to its profiling by the majority of metabolomics studies and makes it an obvious candidate for detecting zoonotic emerging disease traces.

Metabolomic profiles based on Nuclear Magnetic Resonance (NMR) spectroscopy or Mass Spectrometry (MS) analysis can also be generated through a multiplexing approach and compared with control datasets to profile metabolites that are significantly different between healthy and diseased individuals. At least two independent experimental replicates are suggested to rule out false positives. The peripheral blood of both infected and non-infected animals can be pooled, and a cross-species examination can also be performed to validate the presence of specific metabolites in additional genera.

Chapter - 3

Biotechnological Innovations in Diagnostic Development

Expanding on the importance of an integrated approach that combines the expertise of scientific disciplines such as chemistry, biotechnology, pathology, and zoology, traits and explorations in the field of diagnostics and biosensing, particularly for diseases experienced by wildlife, domestic, and farm animal species and considered notifiable to the World Organization for Animal Health, constitute the elaboration. Recent developments in diagnostic research, where biologically derived markers are applied, are presented. The focus is on novel methodologies that lead to the generation of various one-off-only or cross-species biomarker systems and translational diagnostics that predict the occurrence of disease currently applied or under development in communication with the affected scientific communities.

The techniques and concepts from the various fields are also reflected in the exploration of diagnostic systems for detection of Zika virus infections, determination of acute infection-induced alteration of the host immune response against the course of Chikungunya virus and Zika virus superinfections, expression profiling of potential biomarkers for Nipah virus infection in a cross-species animal model, and the potential of Mallophaga-borne pathogen transmission to mammals [27, 28, 29, 30].

Genetic engineering tools

Recent progress in genetic engineering techniques has enabled the production of disease-associated markers in host organisms. These markers correspond to information molecules released during the onset and progression of disease in host tissues and body fluids. Enabling a

wide range of studies, advanced nucleic acid amplification methods allow the detection of target sequences in minute quantities. These methods can be applied for detection in infected animals, carriers, and vectors, or to confirm the presence of a particular disease in non-target species such as humans who are incidental hosts of the pathogen. However, these methods are still not sensitive enough for use in point-of-care diagnostic kits.

Two approaches can increase sensitivity: the creation of biosensors, and the intersection of nucleic acid-based detection with DNA replication and amplification technologies. Nucleic acid-based biosensors with high sensitivity, specificity, and stability can be developed using ultrasensitive detection systems to determine the burden of nucleic acid targets even in a small sample size, while the detection limit of a simple reaction set can be enhanced by integrating the platform with other nucleic-acid-based amplification methodologies such as the Loop-Mediated Isothermal Amplification (LAMP) reaction, the Rolling Circle Amplification (RCA), or the click-based amplification technique for DNA probes and aptamer-based sensors. These methods are also ideal for microfluidic integration, making them suitable for point-of-care analysis in biosafety levels 1 and 2 laboratories [31, 32, 33, 34].

Molecular amplification technologies

Genetic engineering tools offer never-before-seen opportunities for enhanced sensitivity while employing nucleic-acid-based targets for early detection of pathogens. Although some nucleic-acid-based detection formats cannot detect direct infection and provide only indirect evidence, they are nonetheless highly sensitive and often recognized long before the onset of symptoms. To prevent false-positive results during sample testing, changes in signals must be monitored in a sufficient number of samples and in conjunction with clinical signs. The sensitivity and specificity of nucleic-acid-based detection methods have been greatly enhanced with the advent of the Polymerase Chain Reaction (PCR)-based approach, which has made it easier to detect directly the genetic material of a pathogen in or related to an infected host. The CRISPR-Cas system has recently generated

great interest in diagnostic applications. CRISPR-based systems have shown impressive sensitivity and accuracy in detecting nucleic acids from bacteria and viruses.

One of the main disadvantages of these techniques is that they cannot be performed without laboratory facilities and experienced personnel. To overcome this issue, they are also being integrated into microfluidic devices that permit detection of the target in minimum time. These devices are capable of integrating the entire testing process into a few or even a single body using automatic systems. In such systems, sample processing, amplification, and detection can be performed in a very short period, allowing diagnostic testing within a day [35, 36, 37, 38].

Recombinant protein production

Several diagnostic platforms rely on antigenic molecules that must be produced in large quantities. Recombinant proteins can be generated using expression systems that allow for rapid large-scale production of pure and functionally active target proteins. Production of glycoproteins requires eukaryotic expression systems, such as yeast, insect, or mammalian cells, or Pichia species in particular, which have well-developed expression protocols. These systems are convenient for large-scale production since they permit secretion of proteins at high yields in relatively simple media, without the need for complicated lysis steps. Yeast are recognized as the most suitable alternative to mammalian cell culture systems for large-scale production of eukaryotic glycoproteins. They can also perform certain protein modifications, such as the addition of high-mannose-type and some complex-type N-linked glycans, which may be a requirement for certain vaccines.

Pichia pastoris is a well-established eukaryotic expression system for producing large quantities of recombinant proteins and is capable of performing post-translational modifications. It offers distinct advantages over existing bacterial systems in particular with respect to proper folding, solubility, and activity of the desired product, and over mammalian expression systems with respect to economy and safety. Pichia pastoris possesses many advantages, such as a short growth

cycle, easy handling, transport, and storage; a GRAS status; and the potential to synthesize proteins with advanced post-translational modifications [39, 40, 41].

CRISPR-based diagnostic systems

Emerging CRISPR genetic tools offer new avenues for precise biomarker detection by leveraging signature genetic sequences. These systems, developed for CRISPR genome-editing applications, introduce a novel approach to disease diagnosis, detection, and identification based on specific nucleic acid sequences. The architecture of these detection systems comprises two primary components: A guide RNA (gRNA) that recognizes the signature nucleic acid sequence of interest, and a CRISPR-associated endonuclease (Cas) enzyme that acts as a signal amplifier for the detection platform. The principles of these biosensors remain consistent across various target analytes, spread across unmodified, reverse-transcribed, cDNA, and messenger RNA (mRNA). The specificity of these detection systems relies on proximity probes composed of gRNA/CRISPR-associated (Cas) complex pairs that respond to the signature sequences of diverse class-1 and class-2 viruses. Given the fundamental importance of nucleic acids in driving pathogens and markers' production, the development of nucleic-acid detection systems especially qRT-PCR-reverse-transcription-quantitative polymerase chain-reaction, droplet-digital PCR, loop-mediated isothermal amplification, and CRISPR-Cas-based designs aligned with these response mechanisms ensures significant sensitivity and specificity for biomarker concentration in different species.

Two major obstacles currently hinder the widespread use of these techniques: limited throughput and the requirement for sophisticated detection instruments. Microfluidic Devices developed with integrated functional elements for application in biomolecular detection enable accurate multiplex-assay detection within compact platforms. These systems link multiple detection stages into a single device, thus enabling sample span, washing, and detection processes to be integrated within microfluidic channels facilitating enhanced speed and simplicity. Other Crick-related approaches exploit integrated synthetic-biology principles to devise fully cellular biosensors that function

irrespective of the detection environment. These fully synthetic detection systems combine interaction landscapes from multiple sources with genetic control to route signal-production pathways through cellular transcription and translation machinery, thus allowing complex sensor design, assembly, and optimization to be accomplished with relative ease [42, 43, 44, 45].

Chapter - 4

Pathological Basis of Zoonotic Diseases

Three types of host alterations are associated with zoonotic diseases: tissue-level changes, laboratory-scale cellular injury, and immunological reactions. The established interaction can be exploited for diagnostic marker design. The tissues of infected hosts exhibit characteristic histo-pathological alterations related to various zoonotic diseases. Pathology associated with zoonotic diseases can be a critical aspect in designing diagnostic markers for these diseases. The tissue-change links or genes and proteins upregulated in infected hosts can also induce components of the immune response. Pathological findings in tissues and histo-pathological changes can, therefore, be integrated into the identification of potential diagnostic markers for zoonotic diseases.

Change in the shape of the tissue and its cells in infected hosts are linked to the presence of potential biomarkers in the laid-down proteinous matrix. Pathological alterations in host tissues can be classified into three categories: histopathological changes, specific immune-response histo-architecture, and tissue transcriptome signatures. Histopathology reveals the development of transmitted infections in the hosts such as blisters, necrotic foci, acid-fast lesions, mixed leukocytic infiltrates, mesogeal granulomas, and cysts present in, on or around the organs and tissues of both wild and domestic animals. The histo-pathological hallmarks represent the presence of trypanosomiasis, dotillariasis, fowl cholera, anthrax, pseudopeste, leptospirosis, chikungunya, toxoplasmosis, creutzfeldt-jakob disease, zoonotic schistosomiasis, and vector-borne infectious diseases. Specific patterns of tissue distribution and/or extreme pro-and anti-inflammatory immune responses provide an opportunity for utilizing quantitative histopathology as a basis for the identification of

diagnostic biomarkers. The dynamics and magnitude of the immune response characteristic of particular diseases can also furnish antibodies that serve as diagnostics [46, 47, 48, 49, 46, 47, 48, 49].

Tissue-level alterations in host organisms

The changes occurring at the tissue level in animals infected with zoonotic pathogens can serve as vital links towards the release of disease-associated biomarkers. The location, type, and extent of changes in the host organism during infection are unique for each pathogen, but there are general pathways that can be identified. Understanding these characteristic tissue alterations can aid in identifying the type of cells sustaining the injury, as well as their functions and patterns of response that give rise to the observed alterations. These relationships would then allow for the identification of histopathological markers that could be present in animals tested "positive" for the studied zoonosis, making them useful to complement current diagnostic panels in the respective animal species infected with the pathogens. Finally, examination of the altered processes and cellular functions sheds light on the possible influence on the signature metabolites, proteins, and immune response biomarkers that may be induced/released in the host organism, thus linking pathological findings to the pathways generating the aforementioned biomarkers.

The host organism undergoes a wide array of changes in response to infection with zoonotic pathogens. Affecting different tissues of the body, the alterations are ultimately responsible for the specific symptoms exhibited by the animal infected with the pathogen. By identifying the type of tissue or organ sustaining injury, it is possible to pinpoint the immune cells responsible for the repair process as well as their role in the defence against the pathogen. The nature of tissue alterations associated with different zoonotic diseases may also reveal if apoptotic or necrotic processes are dominant, as well as the implications of these injuries for the concentration of metabolites or proteins released into the blood or other body fluids. Such knowledge may serve as an important basis for the identification of histopathological features that would be expected to correlate with the presence of the pathogens or their products.

Cellular injury patterns and immune reaction

Cytological analysis of infections has revealed that the main alterations observed in the cells comprise injury pattern for the specific infection, for instance; apoptosis, necrosis, cytolysis, multinucleate giant cells, desquamation or keratinization of epithelial cells observed in viral infection. Immune response patterns such as presence of mononuclear granulomatous or neutrophilic or eosinophilic infiltrate can also be consider for design and development of detection tool for respective pathogen. Presence of particular immune response such as development of antibodies in serum and anti-nuclear reaction is also very helpful in detection of disease. Etiological diagnosis of any disease is also made easy by studying the various cytological alterations manifested.

Detected Cellulitis and bulla formation in the skin of the host induced by Pseudomonas aeruginosa in chronic stages and Mullusca d. exposed to herpes virus were diagnosed through presence of fibroblasts and macrophages showing different stages of dermal alteration. Weakening of barrier defence and presence of gastric and interstitial cell hyperplasia due to necrotizing fungi and Entamoeba histolytica indicted the persistence factor for other infection also. presence of Chlamydia sp. and Fungi in genital smear were noted as pathogen detected in the reproductive cytology. Cytological study has enhanced the existing diagnostic techniques for avian abortions caused by fowl ectoparasitic infection with associated bacteria, viruses, etc. Synchronous inflammatory changes were noted as associated factors for Moraxella bovis and M. lacunata and were used to look for the presence of these bacteria and virulence factors through serological, cytological and immunological examination [50, 51, 52, 53].

Histopathological markers of infection

Pathological analyses play an essential role in identifying accurate and disease-specific diagnostic biomarkers. Certain underlying cellular alterations, damage patterns, and the associated immune responses serve as critical indicators of the specific infection present within a host. If the disease is successfully validated, any of these signals may hold significance in the identification of the corresponding infection in

any of the different host species involved in its life cycle, any animal or environmental sample that may be a source of contamination, as well as vectors of the disease. The general histopathological aspects related to common zoonotic and other diseases differ among various species, supporting the identification of genetically, protein, or metabolite-derived biomarkers. These histopathological changes are, therefore, important for defining novel diagnostic biomarkers, especially when no specific marker for a particular disease has yet been discovered.

Histopathological patterns of damage, associated immune responses, or similar effects on various host species can be harnessed for the identification of potential diagnostic biomarkers for the particular disease under consideration. While it may not always be possible to identify a specific target for a given zoonotic disease in the histopathological analysis, the investigation and detection of certain general alterations may still lead to the successful generation of useful diagnostic markers. Moreover, even in cases where host-pathogen interactions have been well characterised, the validation of such markers in naturally occurring infections remains challenging. Histopathological evaluation thus complements all other available components, creating a greater probability of identifying disease-specific diagnostic markers for virtually any zoonotic infection [54, 55, 56, 57, 58].

Linking pathology findings to biomarker generation

Pathological analysis reveals specific alterations associated with various zoonotic diseases in different animal species. These alterations include molecular-level injuries in cells or tissues that are detectable through histopathological examination. The identified disease-specific cellular injury patterns can be linked with the cellular and humoral immune responses of the host. Histopathological markers indicated by these immune responses have been successfully integrated into diagnostic systems for specific diseases. Collectively, these findings establish the connection between histopathological analysis and chemical principles in detecting zoonotic diseases. Thus, the integrated approach demonstrates how pathology provides essential foundations for the further development of disease biomarkers, linking chemical principles, biotechnology, and pathology in biomarker generation.

The link between pathology and chemical principles starts with the concept of disease signatures. In general, zoonotic diseases induce host-specific tissue changes at the molecular level. These changes can be linked to cellular injuries and the immune response of the affected animal species. The histopathological markers reflected by the immune response can then be integrated into diagnostic systems for specific zoonotic diseases using presently available diagnostic measurements. The alterations are not only unique for individual disease agents but may also favor the detection of certain diseases in selected host animals. The analysis, therefore, correlates pathology findings with the involved chemical principles for detecting the associated diseases [59, 60, 61, 62].

Chapter - 5

Zoological Perspectives in Zoonotic Transmission

Zoo animals are widely regarded as potential virus reservoirs, contributing to the emergence of new zoonoses and disease outbreaks in humans, domestic animals, and wildlife. The occurrence of zoonoses is affected by ecological and anthropogenic factors that influence the pathogen reservoir and directly affect the likelihood of virus transmission to humans. Ecological factors affecting wildlife-human virus interaction include distribution of wildlife hosts, viruses, and people; traffic of wildlife; and interaction with domestic animals. Anthropogenic factors include land-use changes that bring wildlife into closer contact with domestic animals and/or people, as well as trade in wildlife. Changes in host behavior as a result of ecological and social pressures have potentially important effects on human-wildlife disease dynamics. Apart from these general factors influencing parasite emergence and re-emergence, the life histories of animal hosts could be a more fundamental determinant of viral diversity and including viral cross-species transmission. A number of such biological, behavioral, and ecological factors related to hunting, fishing, and farming seem to highlight potential hotspots of zoonotic emergence. Surveillance studies play an important role in uncovering the presence of pathogens in wildlife, based on the detection of viral genetic material or the presence of viral antibodies.

While diagnosis of zoonotic pathogens in wildlife still primarily relies on direct detection, a wider application of serological tests would allow a more general description of the viral diversity present in non-sampled species and in less accessible areas of the planet. Ecological studies also add context to the identification of nosocomial and non-nosocomial spillover cases. Validation of all these diagnostic biomarkers is an important step towards utilizing them as potential

early-warning indicators of zoonotic transmission from wildlife to domestic animals and humans [63, 64, 65, 66].

Animal reservoirs and vectors

Reservoir and vector hosts drive zoonotic transmission, typically limited to cross-species interractions involving wildlife. The potential for overlap with livestock species is crucial, enabling domestic variants to serve as conduits for zoonotic spillover and maintenance of infection. Reservoir host ecology informs circulation routes, prevalence, and contact networks while behaviour shapes the risk of exposure to zoonoses. A range of animals have been implicated as reservoirs in cross-species infections, leading to a variety of wildlife sampling techniques. Reservoir hosts can be detected via histopathology, but diagnostic validation in ecological reservoirs and for vectors is rarely integrated. Knowledge pertaining to animal reservoirs, vectors, and associated contamination is essential for effective surveillance development.

are often used interchangeably, but the fine-scale focus on animals that maintain the parasite in a limited ecological setting is important. Reservoirs can remain asymptomatic and support the occurrence of the disease in other susceptible species, yet often encompass wildlife. Vectors can act as incidental hosts, harbouring the agent but failing to facilitate transmission along population cycles. The transmission route may include other species only as dead-end hosts during spillover events. Van Soolingen investigated the influence of wild animal populations on the emergence of highly virulent strains of Mycobacterium bovis in cattle. The correlation between the relative abundance of badgers and the emergence of these strains indicated the reciprocal importance of domestic and wild reservoirs of M. bovis for zoonotic transmission.

In cross-species infection, domestic animals are often the reservoirs that maintain the disease in livestock populations, soybean potentially corralled by feral and wildlife species that occasionally serve as dead-end hosts in outbreak events. In contrast, a wider range of zoonotic pathogens harboured by wildlife is associated with its

breeding and foraging ecology. The spread of these zoonotic diseases follows an animal contact network based on the ecological co-occurrence and abundance of wildlife species, with intersections at environmental reservoirs. Even spillover events involving zoonotic parasites in carnivores follow environmental habitat variety at micro-geographical maps, underlining the importance of localised circulation in natural wildlife habitats for urban centres nearby. Host behaviour can therefore increase the risk of intense exposure to zoonotic diseases, resulting in human-influenced patterns of rodent-borne hantavirus activity [67, 68, 69, 70].

Ecology of disease spread

Animal reservoirs and vectors of zoonotic diseases are known, but not always accurately documented with respect to infection and transmission potential to humans and domestic animals. Species ecology, enabling spillover, is poorly studied, hindering epidemic risk assessment and planning. Infection emergence, maintenance, and spread depend on contact rates and exposure likelihood, linked to ecological niche. Detailed knowledge of host-pathogen relationships is crucial for spillover risk evaluation.

Potential disease transmission by wildlife is increasingly acknowledged, with special attention to mammals sharing habitats with human and domestic-animal populations. Avian pathogens are also examined for wildlife origins. Although many wildlife species (especially birds) are infected, actual transmission risk varies and can rarely be conclusively confirmed. Mammals often support large pathogen loads with severe pathology, especially when commensal in humans and domestic animals, and modelling suggests a zoonotic role. Factors affecting spillover probability remain largely unexplored for these key reservoirs. Little attention is devoted to the transmission risk posed by infection of host species that do not support sustaining infections but are in contact with reservoir species. Evaluating evolving risk posed by a host requires consideration of exposure quantification and spillover ecology. For carnivorous or omnivorous pathogens, risk is greatest when infected species are in direct contact or share habitats with disease reservoirs [71, 70, 72, 68].

Behavioral and physiological factors

Zoology provides important insights into the emergence and transmission dynamics of zoonoses. Certain animals are also known to act as reservoirs (hepatitis E virus in pigs and the NiV in bats) as well as vectors (Borrelia burgdorferi in Ixodes ticks) of zoonotic pathogens or play a critical role in creating a conducive environment for pathogen maintenance or amplification (coral reefs for pathogenic aeromonads). These and many other host-pathogen interactions remain poorly understood. Consequently, these species are often not monitored for novel pathogens or placed in inappropriate ecological niche. Identifying these reservoirs, vectors, intermediary hosts, and amplification hosts; understanding inter-species contact networks in which these species interact with wild and domestic animals; and deciphering the biology that exposes susceptible species to infection can provide vital information when planning broader epidemiological studies.

These studies are crucial for controlling infectious agents of animal origin. Indeed, understanding the ecology of contact or spillover events is critical for characterising the risk involved in transmission among the animal reservoir/vector/inter-object of the pathogen establishment and the susceptible species, including humans. Factors such as life history traits, behaviour, physiology, ecology, etc., can enhance the probability of exposure of susceptible species to infected reservoirs/vectors. Such factors include metabolic fever in insects, heightened behavioural activity during estrus in mammals, attraction of mammals to semiaquatic habitats during the dry season, and hibernation of bats. Wildlife surveillance and diagnostics of pathogens of animal origin should be designed to sample as many of these species as possible to improve the probability of detecting these pathogens when present. Grouping species according to expected exposure to common zoonotic agents is key, as is a nested sampling design that samples from each group [73, 74, 75, 76].

Wildlife surveillance strategies

Wildlife are frequently implicated in the novel emergence or re-emergens of zoonotic infectious diseases. High-risk pathogens

circulating in wildlife populations require rigorous surveillance, particularly in species that serve as reservoirs or vectors. Community ecology serves to identify species that, due to ecological factors, are highly likely to transmit disease to humans or livestock, and must therefore be monitored. Testing these species for potential zoonotic pathogens is essential to pre-empt infections of humans and livestock, but the zoonotic nature of the pathogen must also be taken into consideration. The signature disease phase of the host, or the period of zoonotic transmission, can often be determined from either a detailed understanding of the host-pathogen interaction or analysis of earlier research on the species. Likewise, temporal factors affecting the contact rate between wildlife and livestock or humans must be taken into account when designing the surveillance strategy, with the potential effect of environmental change or disease epizootics considered.

The biomedical community would greatly benefit from a consistent relationship with the zoological community, allowing the integration of diagnostic and ecological information. Diagnostic biomarkers for potentially high-risk pathogens should be developed and made available for use in wildlife surveillance studies that monitor these species for circulating pathogens. These two approaches would allow a complete diagnostic picture of these species to be established, helping to identify changes in host pathology, and therefore risk of zoonotic transmission, associated with past disease events, environmental change, or social change and introducing eco-epidemiological modelling to the world of zoonotic disease [77, 78, 79, 80].

Chapter - 6

Host-Pathogen Interactions

Mechanisms of transmission involve contact between the host and pathogen reservoir, transmission by insect vectors, or aerosol transmission. The initial colonization of the potential host and the complex process of establishment requires penetration of various physical barriers at several tissues. Pathogens apply strategies to invade into host cells or tissues. As invading pathogens start their life cycle, host cells recognize the threat, sending alarm signals and forming an immune response. Thorough understanding of these pathways in each host-pathogen interaction system may contribute to future developments of disease-specific diagnostic biomarkers. Disease-specific signatures released and/or produced in multiple hosts during the facilitation of infection and disease development have the potential to serve as diagnostic biomarkers.

Zoonotic pathogens are primary or secondary parasites for humans, but the ecological hosts that harbour the pathogens and support the life cycle are different. Thus, pathogens have potential to infect different host species at different stages of their life cycle. Understanding genome evolution and expression paths may provide clues related to cross-species pathogenicity as an additional validation approach for any disease-specific diagnostic biomarkers. Overall, life processes of pathogens in different host species should be carefully considered during the development of disease-specific signatures for diagnostic applications [81, 82, 83, 5].

Mechanisms of transmission

Disease-causing agents enter the host primarily through contact with contaminated fluids or live vectors. After entry, the pathogen multiplies and reaches the target area through the bloodstream,

lymphatic vessels, or other body fluids. The establishment of an infection is largely determined by the inoculum used for infection. Pathogens can modify the contact route by producing factors that facilitate invasion and support virulence. Host resistance to infection is provided by early innate and subsequent adaptive immune responses; the latter can lead to tissue damage. Successful infection also requires replication in a new host, which can occur during a nonsymptomatic period. When the pathogen is eliminated, the animal can recover. The initial disease biomarkers are linked to the immunological and metabolic pathways activated during the response, and a change in the level of the key transcription factors will trigger the release of these biomarkers. A robust post-genomic approach supported by multi-omics is essential for a deeper understanding of the host-pathogen interactions and for the identification of reliable biomarkers, which must be validated in tissues.

Host-Pathogen interactions are important in order to put forth the mechanisms of transmission and the establishment of the disease. The viral agents, vectors and potential animal reservoirs and hosts for pathogens are well established in the literature. However, the steps involved in the establishment of the disease, the major host risks and the pet animals that pose a major threat toward zoonosis development remain to be established in order to identify the key biomarkers at molecular level. Transmission occurs mainly through the following routes: direct contact with infected animals, through droplets, aerosols, blood or tissues; exposure to animal waste; accidental or intentional food-borne infection; contact with vectors; inhalation of spores or cysts from the environment; and contact with contaminated materials, livestock products or bodies of infected wild animals [84, 85, 86, 87, 84, 85, 86, 87].

Pathogen invasion and host defense

Various pathways are involved in pathogen transmission and establishment within a host, but they all converge at their ability to successfully invade host tissues and reach the underlying vascular systems. Once in circulation, pathogens gain access to virtually all tissues through active or passive processes, and they interact with host

cells by disturbing the natural cellular cues that minimize contact. In response to these invasions, hosts activate innate and adaptive immune mechanisms to prevent the establishment and spread of new infections. The combination of these mechanisms dictates the extent of damage caused by pathogens and their products and is usually responsible for the clinical signs exhibited by infected hosts.

In addition to providing vital physiological and/or nutritional signals that promote cell proliferation and migration, these pathways often include the expression of various inflammatory mediators, such as cytokines, chemokines, and/or growth factors, which can be classified into groups according to their signaling pathways. The proteins in these categories play key roles in pathogen invasion and/or evasion by affecting the permeability of blood and/or lymphatic vessels and/or attracting leukocytes to the affected sites. Furthermore, the detected proteins and cytokines in tissues infected by any pathogen are then released into circulation and/or local tissue fluids, thus representing possible biomarkers.

Molecular pathways influencing biomarker release

The establishment of infection depends on the capacity of the pathogen to devise mechanisms to evade or modify the host's response, including molecular pathways involved in the production and release of biomarkers. There are two aspects here that are critical for understanding the dynamics of disease development. The first aspect deals with how the pathogens enter the host and how they trigger signals allowing them to invade more tissues. It indicates the potential pathways involved, even through indirect signals, and potential biomarkers associated with the transmission of zoonotic agents. The second aspect relates to how the host responds to the presence of signaling molecules or to cellular injury patterns during infection. The major elements to consider in this picture are the type of host-pathogen interaction, the kinetics of disease development, and the responses activated by the host.

Transmission has been described according to the recognized patterns of association between hosts (human) and pathogens; that is, by the signs (symptoms) that have been associated with zoonotic

infections (in particular, those of zoonotic nature). The initial steps of infection are also one of the most important features, as they provide the first connection between vector, reservoir and human. The capacity of viruses of the Coronaviridae and Filoviridae families to rapidly enter the cells of the human respiratory tract and then follow different infection routes may lead to a greater spread of these pathogens in zoonoses than of other agents.

Any disease represents a complex process, with associated molecular control. The entire cellular and tissue framework of the infected hosts usually responds to the presence of these agents. Pathogenicity and virulence have been defined due to the ability of certain strains to induce specific pathological lesions at the level of specific tissues. Most of the zoonotic agents induce specific lesions; thus, the injury caused by the pathogen should not be neglected, as such injury, injury patterns, inflammatory signals and other similar patterns may also directly lead to the presence of the corresponding biomarker in body compartments.

The interaction of the host with the pathogen should thus trigger the release of various pathogens. Hence, either direct signals from the pathogen and early signs of injury or alterations in infected cells can be examined, as their combination with other infectious or haematological studies can work together to support earlier diagnosis. For all these reasons, the expression of putative biomarkers in different compartments due to specific infections (e.g. those due to viruses of the Coronaviridae or Filoviridae families) should also be considered [88, 89, 90, 91].

Cross-species pathogenicity

For diseases affecting multiple species, particularly at the zoological interface, it is essential to establish whether the pathogen has been reported to infect species within the respective group of interest. Additionally, for zoonoses and diseases affecting domestic and/or wild mammals, it must also be evaluated whether the pathogen has been reported to infect other mammals (especially near relatives) beyond those of the respective group. The knowledge that a pathogen can infect multiple species generally supports the likelihood that it may

have escaped from one of these species to spill back towards the other group, although with some limitations depending on the ecological connectivity of the animal groups being examined.

For zoonoses affecting birds, reassortment - the exchange of segments of genomic material between genome types of similar character (but not necessarily all segment types) within a single cell resulting in a new hybrid genome that differs significantly from the parent viruses - will have greater importance than mere pathogenicity. Reassortment may also be an important consideration in diseases affecting reptiles, amphibians and fish although, because these genomic types typically have no known case of aquiferous zoonosis, detailed ecological-switch disease studies involving these animals have yet to be conducted [92, 93, 94, 95].

Chapter - 7

Biomarker Types and Validation Strategies

Biomarkers consist of host genetic signatures, metabolomic responses, specific pathogen proteins or metabolites, as well as immune system indicators. Successful diagnostics require cross-species consideration of each biomarker type, alongside sensitivity and specificity validation, clinical and analytical reproducibility, and disease-specific response profiles. Meta-analysis of available data can minimize the resource demands of developing individual markers.

Pathogen reservoirs must be considered to confirm diagnostic applicability. Detecting the pathogen and its components usually provides the most reliable diagnosis, though lack of genetic variability may limit marker usefulness. Cross-species amplification can enhance detection sensitivity, especially when coupled with convergent evolution or functional significance. Metabolomic signatures are advantageous as they reveal the host response without requiring prior knowledge of the pathogen or its pathology; however, their diagnostic utility is more difficult to validate and they are especially vulnerable to confounding factors. Protein markers are particularly valuable for developing low-cost, high-speed assays based on nucleic-acid probes, while anti-pathogen response proteins and secreted factors are of fundamental ecological importance, but often lack species-dependent variability. Well-designed studies are essential to fulfil these requirements for all indicators [96, 97, 98, 99].

Genetic biomarkers

Destructive diseases of human beings are caused by various pathogens present in animals or animal products. Currently, the detection of such zoonotic infections mostly depends on the cultured samples taken during clinical investigations. Due to the time-

consuming nature of this method, it is necessary to screen for such infections in a host-preferable manner. Use of resistance genes against such pathogens can act as diagnostic markers. From TY604448 genomic data, a factor XII gene was found which is suggested to play a critical role in interacting with *Raoultella ornithinolytica* in *Micromys minutus*. Bacteriophage of its distinct bacteria, having lytic activity, can be used for control of the disease; gene can be incorporated to JakD-transformed lines for resistance screening. Infection causes enteritis in domestic. Human infection by *M. szulgai* is associated with geophilic mole-rat activity in geographic region, possessing eight novel 18S rRNA and three new 28S rRNA secondary structures, comparative analysis of stomatitis was performed on twenty human cases of whom few cases were detected positive for PCR.

Pathogenicity is related to host-specific biosynthesis of metal-chelating agent, 2,3-dihydroxybenzoate except in *S. marcescens*. MglC interacts with AHL molecules for virulence regulation. From transcriptomic data, one of the genes putatively involved in quorum sensing and critical for swarming motility was selected as a candidate diagnostic marker. Differential mRNA-seq analysis of S. m. ventriculi-grown and S. m. musculi-adapted identified an injury-related protein (MMP9) that may induce a strong inflammatory process in animal models. Concomitant with invasion of skeletal: striated muscle and cardiac tissues, consecutive upregulation of transmission metal ZIP4, and value toll-like receptor (TLR)-2 expression in the earthworm *Eisenia fetida* provides compelling preliminary evidence of attenuated invasion by a hypervirulent clinical *K. pneumoniae* isolate during co-infection [100, 101, 102, 103].

Protein biomarkers

Protein signatures specific to different zoonoses can serve as important guides for confirming infected status in various animals, as well as for appropriate species and geographical identification during times of disease outbreaks. Animals act as amplifying hosts harbouring a large number of pathogens or can be vital for pathogen transmission to humans. Expression of zoonotic pathogens in infecting hosts can be determined through techniques including transcriptomics, proteomics

or tissue-specific down-stream analysis by quantitative polymerase chain reaction (qPCR). In addition to the use of such methodological approaches, an understanding of frequent cellular injury or morphological patterns produced by zoonotic pathogens enables logical selection of protein biomarkers that would be elevated or diminished due to infection. Analysis of specific protein signatures associated with zoonotic infections allow not only diagnostic comparisons between humans and other infected hosts but also detection by amplification of in-situ expression. Pathological-species-braided studies aid in the construction of a catalogue of species-specific protein signatures to the respective zoonotic pathogens.

Antibodies directed against a pathogen or its products represent important additional parameters for use in the epidemiology of zoonoses. In high-agricultural-density countries like India, where many zoonoses are endemic and a large number of seroprevalence surveys have been conducted, serosurvey data can be quantitatively integrated with human population and livestock density density data to confirm the possibility of pathogens being transmitted from animals to humans. In addition, during zoonotic outbreaks, in-depth knowledge of the ecology of the pathogen enables screening of animal species and environmental factors associated with the occurrence of zoonotic pathogens. Such studies can provide further guidance for identifying animals that need to be screened for either active infection or for antibodies against zoonotic pathogens [104, 105, 10, 106].

Metabolic and immunological biomarkers

Genetic changes during disease development may also affect the profiles of circulating metabolites. The protein composition of body fluids, including serum and plasma, is strongly age-dependent, with immunoglobulins accounting for a major part of the protein content in mature organisms. Metabolite or protein profiles of anybody fluid can therefore be considered when searching for diagnostic markers of a particular disease. Disease-specific immunoglobulin production can also be measured, and distinctive changes in the titers of certain anti-pathogen antibodies can assist in diagnosing a particular disease. Antibodies directed against certain metabolic products of bacteria or

viruses may be detected, while humoral immune responses to specific antigens can be evaluated.

Depending on the analytical platform, all classes of compounds may be probed. Identification of characteristic molecular signatures associated with a disease represents one of the first steps toward developing a point-of-care diagnostic solution. A well-defined multidimensional multi-omics signature of a specific pathogen enhances detection accuracy. Moreover, species that lack a cross-species genetic marker in a pathogen can exhibit a different species-species pattern of response, with detection enhanced through the monitoring of disease-indicative immunological markers or meta- bolic changes at the species level [107, 108, 109, 110].

Clinical validation and reproducibility

A disease-associated biomarker can be defined as a biological property detected within a host that correlates with the presence of a pathogen and its pathophysiological effects. Such markers are generated upon pathogen transmission and subsequent changes within the host due to pathogen activity. Genetic biomarkers are sequences of nucleic acid specific to a pathogen, while protein and metabolite biomarkers are produced by the pathogen, hosted by an infected organism, or the combination of both species. The biological fluids (e.g., serum, saliva, urine) of the infected host expose the presence of the consequential pathogens. Animal viruses have been reported to produce specific proteins and metabolites that can serve as metabolic indicators of infections. The validation process varies among the above groups of disease-associated markers, but their respective marker systems must be validated before progressing to clinical diagnostic tests. Reproducibility and subsequent generalizability across different hosts and various environmental conditions, when data are supported by several independent datasets, can be considered mini-testing grounds to support a validated signature.

The major clinical validation steps include determination of sensitivity and specificity. Sensitivity can be defined as the ability of a test to correctly identify patients with the disease, and specificity can be defined as the ability of a test to correctly identify patients without

the disease. Sensitivity and specificity must be determined in accordance with clinical standards and the implied application:

1. For a screening tool, sensitivity is paramount, as a new coronavirus or influenza virus infection detected in asymptomatic individuals can subsequently be triaged with higher specificity tests;
2. Novel zoonotic viruses require tissue samples from the reservoir species; or
3. Most human, animal, and environmental samples are non-infectious and scraping from live animals can be made in accordance with ethical guidelines [111, 112, 113, 114].

Chapter - 8

Chemistry-Based Approaches for Biomarker Quantification

Chemical signatures provide an important component in disease diagnosis. Different classes of chemical biomolecules can be utilized independently or in combination to design diagnostic assays. List of low-molecular-weight metabolites that serve as biomarkers is expanding with the use of different metabolomic platforms. Synthesis of various disease-specific chemicals in affected hosts is a result of biochemical and physiological disturbance. Mass spectrometry is widely used instrument in the detection of biomarkers. Types of sampling procedures, sample preparation and mitigation of matrix effect should receive careful attention. Nuclear magnetic resonance performs compound identification and quantitative measurements of the entire metabolites profile.

Chemical signals constituting the basis of diagnostic assays can be detected at the genetic, protein, metabolite and immune levels. These different classes of diagnostic markers can be placed in a combinatorial manner to increase sensitivity and specificity of detection. Simple and cost-effective colorimetric assays allow rapid on-site quantification of clinically important biomolecules. Fluorescent molecular probes remain important imaging agents in living systems. Development of a wide range of electrochemical biosensors and quantitative measures using point-of-care devices facilitate fast investigation by non-specialized personnel under field conditions. Transfer of testing to the place of sample collection by employing microfluidic devices reduces transfer-related time delays [115, 116, 117, 118].

Mass spectrometry applications

Mass spectrometry has gained traction in quantifying planar or

high-molecular-weight biomarkers, including metabolites generated by pathogens, during the last two decades. The exploitation of mass spectrometer features especially various ionization types or Matrix-Assisted Laser Desorption Ionization (MALDI) in conjunction with mass spectrometry imaging and unbiased separation through High-Performance Liquid Chromatography (HPLC) or gas chromatography strengthens detection capabilities.

In combination with nuclear magnetic resonance, mass spectrometry reads biochemical alterations in body secretions, serum, tissues, or other samples originating from those severely infected. It also plays a prominent role in testing proofs of concept for immunoassays and other detection tools such as micro-fluidic or electrochemical systems. These approaches determine analyte concentrations at various instars of the infection and generate signatures or panels that enable early diagnosis and surveillance in humans, domestic animals, and wildlife, thereby providing an early indication of zoonotic potential and the need for stringent monitoring [119, 120, 121, 122].

NMR spectroscopy for metabolite detection

Mass spectrometry is a renowned technique for the elucidation of metabolites, playing a pivotal role in the development of chemical fingerprinting. It's also highly informative for scanning major classes of metabolites when a standard compound library is available for identification. Alternatively, NMR spectroscopy delivers simultaneous, qualitative, and quantitative information regarding several classes of small molecules. However, sole reliance on a spectral library primarily limits the sensitivity of the approach. Nevertheless, with appropriate optimization and application to specific problems, it can provide powerful insights. Insights of this nature have helped link metabolic alterations in rabies virus and *Leptospira* spp. infections and have also underscored the relevance of NMR-detectable signals in the late-stage metabolic characterization of tuberculosis, schistosomiasis, and COVID-19.

Changes in the metabolic profiles of different mammals due to zoonotic diseases such as rabies and leptospirosis have yielded

informative NMR-detectable signals, while specific metabolite perturbations during the late phase of schistosomiasis have formed chemically defined signatures. The selection of the metabolites under investigation has generally focused on those that have been negatively affected in hosts that are or are implicated in zoonotically transmitting diseases to humans. A similar rationale can also direct the detection of metabolites through a combination of suitable approaches. Other NMR-detectable signals can nonetheless be used to complement such evidence, particularly when no dedicated metabolomic analysis is available for the sample in question [83, 123, 124, 125].

Fluorescent and colorimetric assays

Fluorescent and colorimetric detection formats offer distinct advantages for biomarker quantification. Fluorescent assays enable highly sensitive determination of concentration, with detection limits oftentimes down to the picogram range. They have been integrated into Enzyme-Linked Immunosorbent Assays (ELISAs), through the use of organosilica nanocrystals as immunoassay labels, with a modular architecture suitable for the detection of diverse tumor markers. Additionally, large-format fluorescent immunoassays labelled with quantum dots have provided direct readouts in an intensity format without the need for specialized readout equipment. Furthermore, magnetic fluorescence immunoassays coupled with a portable fluorescence reader have demonstrated subfemtomolar sensitivity.

However, the disadvantages of requiring expensive and complicated instruments for readouts often restrict their adoption in resource-limited settings. On the other hand, colorimetric assays provide good sensitivity at a relatively low cost when compared to fluorescent assays. Colorimetric detection has also been integrated into ELISAs, achieving a detection limit of 3 pg mL−1 through multienzyme signal amplification. Nanoparticle-based colorimetric and electrochemical triple-signal amplification strategies have further enhanced sensitivity in cytokine determination to low femtogram levels. In a promising development, a dual-purposed colorimetric and naked-eye detection of disease-associated proteins has enabled specific monitoring of picogram levels without any sophisticated instrument. A

similar approach has been extended for sensitive detection of two different hybridization probes based on dual-nanoprobe amplification modules containing gold and silver nanoparticles. Such novel detection strategies develop signal transduction mechanisms that circumvent limitations associated with ELISAs employing traditional substrates. These methods therefore serve as alternatives to ELISA and may be adapted in combination with multiplex biomarker detection panels [126, 127, 128, 129].

Electrochemical biosensors

represent an advanced diagnostic platform that combines analyte specificity with the potential for rapid, point-of-care testing. In the field of zoonoses, the prospect of T = A detection for early-warning systems is significant, but the niche nature of emerging diseases often limits the number of infected samples available for multiplex workflows. A discussion of the advantages of electrochemical transducers is therefore relevant, particularly for low-to-middle-income countries with limitations on laboratory infrastructure. Such sensors have been developed for the identification of nucleic-acid targets, proteins, and small molecules, and the biosensing layer is typically confined to a few microns in thickness. This high surface-to-volume ratio, together with the vast range of different materials available for detection, enables high-performance devices to be assembled, while still allowing the use of more affordable screen-printed electrodes when needed.

Nucleic-acid electrochemical biosensors detect a complementary strand to a target sequence and have been developed for a wide number of viruses, bacteria, and parasites. The complementary sequences are often labelled to provide an output signal, and indicators rely predominantly on the labelling method rather than the electrochemical sensing principle. The use of redox-mediator-free Aptamer-based sensors has also been explored but achieving the required sensitivity is challenging, particularly for clinically relevant pathogens that are usually present at low levels in the sample. In addition to biomarkers of infection, nucleic-acid-based sensors can also be applied to provide evidence of vector infection [32, 130, 33, 131].

Chapter - 9

Biotechnology-Driven Biomarker Production and Assays

Advances in biotechnology have equipped researchers with a powerful molecular toolkit for the production and detection of biomarker candidates. These methods are successfully applied in many studies to enable or enhance diagnosis, and their integration into future diagnostic developments promises improved specificity and sensitivity. Condensed surveys follow.

The worldwide spread of zoonotic diseases demands easy-to-use, accurate, and readily available diagnostic tests. The potential diagnostic utility of candidate biomarkers should ideally be subjected to histopathology evaluation at the cross-species level. The relevance of tissue injury in the search for such biomarkers cannot be overstated and should be considered essential. Consideration of common damage-associated molecular patterns is also useful. A multiplexing approach that assesses multiple biomarkers in a single assay rather than a one-at-a-time testing scheme can share costs and increase efficiency. Recent developments in Other Technologies Zoonotic Detection, Rapid Nucleic Acid Detection, Lateral Flow Test, Animal Disease-Associated Diagnostic Development Using Synbio, CRISPR and Related Technology, Continuous Monitoring and Detection Systems, Microfluidic Biomarker Detection, Microfluidics Combined with Search Engines, and Virus Detection tools suggest these areas should be explored to exploit available technologies for developing Zoonosis and Animal Disease-Related Tests.

Recombinant protein biological analyses should be extended to all possible candidate biomarkers to maximize specificity and sensitivity, ideally by using matched pairs and avoiding cross-reaction through

appropriate epitope selection. In addition, the successful amplification of even rarer biomarkers would significantly enhance detection capacity. For nucleic-acid based detection, in-depth consideration of potential *in silico* false lamina between closely related species and the avoidance of using only one oligonucleotide per primer should be established. Moreover, it is important to statistically justify the capacity of these types of integrated testing approaches if the integration is purely from a user exercise of speed [64, 132, 133, 134].

ELISA and immunoassay refinement

Immunoassays are vital for biomarker detection, and the development effort can be streamlined when multiple immunoassays are incorporated into a single ELISA-microarray platform. However, the format's utility is limited by cross-reactivity and inadequate specificity in the determining antibodies of the individually developed ELISAs. Refinements targeting these limiting factors have the potential to enhance multiple-assay detection capacities. For example, trained non-specialists can be successfully guided in setting up more complicated immune-based assays such as IgA IgG T cell bioassay formats and antibody-surrogate assays.

The analytical sensitivity of immunoassays can be improved by including an amplification step that augments the detection signal, with one promising amplification strategy being a novel electrochemical gene-affinity biosensor. In this system, a label-free DNA probe with high specificity recognizes double-stranded DNA (dsDNA) or specific motifs of dsDNA and forms a dsDNA-methyltransferase fusion protein-polymerase complex on the surface of an electrochemical biosensor. The receptor's special bindings to biotin-dsDNA can be exploited to couple streptavidin-gold nanoparticles-conjugated horseradish peroxidase and horseradish peroxidase-labeled DNA signal probes, which afford amplification in electrochemical immunosensor readings [135, 136, 137, 138].

Nucleic-acid based detection platforms

Nucleic Acid Amplification Tests (NAATs), encompassing reverse Transcription Polymerase Chain Reaction (RT-PCR), Loop-

Mediated Isothermal Amplification (LAMP), lateral flow Nucleic Acid Test (LF NAT), and Recombinase Polymerase Amplification (RPA), stand out as the most sensitive strategies developed for the molecular detection of pathogens. NAATs enable the detection of nucleic acid molecules, serving as genetic fingerprints, even in low amounts, through an amplification process, while their inherent specificity can be further widened and reinforced through the use of two or more different primer pairs. Furthermore, the diversity of the NAAT toolbox permits the development of assays with a wide range of skills and characteristics optimized for different purposes: detection in a laboratory or in the field, on-site costless equipment less liable to errors, and reduction of contamination risks.

The routine or large-scale use of classic NAATs is often hampered by the relative high costs of performing RT-PCR assays. Since the introduction of LAMP, several alternatives with lower materials, equipment, and time requirements have emerged. The label-free nature of these methods has allowed their coupling to more easily interpretable and portable detection forms such as colorimetric or lateral-flow detection. NAATs can also be employed for the detection of immune responses, such as in multiplexed detection of antibody responses for subsequent seropanorama evaluations [139, 140, 141, 142].

Microfluidic diagnostic devices

Integration of processes typical of microfluidic devices could be exploited for the integrated use of markers of different types for organisms. A microfluidic processing chamber with a μPDA and a droplet-based biosensing directly linked with a biosample capture has been proposed (Martin *et al*., 2017). The presence of a biosample in a trapping zone impact an optimised mixture maintain and detect (colourimetrically and fluorescent) droplets with molecules linked to the disease by a Time-in-Shade Method (TISM). The sample information is obtained by simultaneous detection, and only a single exposure of biosensor molecules during sensor developing. In another work, they developed a colourimetric - fluorescent biosensor based on a reversibly-colored pH-optode that could simultaneously detect two different ions in two different channels in a microfluidic device. More

complex approaches with the integration of several genes in plants are also possible. A synthesis process based on different types of genetic systems for the simultaneously detection of different biomarkers of M. tuberculosis was already proposed. Such approach could also be implemented for the combination of several chemical processes or different genes working in the same xenobiotic. Another example is the use of bacteria containing the whole mechanism to synthetize nucleic acids and with an activation of the response develop in a microfluidic device [143, 144, 145].

Synthetic biology in biomarker design

Molecular components produced using synthetic biology enable the design of a wide range of diagnostic tools. The underlying principles of synthetic biology involve the manipulation of biological components, such as nucleic acids and proteins, through assembly into pre-constructed molecular devices and complex structures that can execute a desired task. Genetic components are assembled from standard parts by means of Golden Gate or Gibson assembly methods. DNA pools for gene expression are constructed by a hierarchical combination of standard functional parts, enabling multiplexed signal production in intelligent circuits. Researchers have also installed translation initiation sites onto pre-existing libraries of DNA with known activity, allowing predictions of systems responses to translation timinig. At a higher level of organization, multiple DNA sequences can be combined into 3D shapes that reproduce molecular features of natural structures, such as the siRNA processing complex Dicer, RNA catalysts, virus capsids, or bacteriophage-based nano-flare probes.

These advancements have unlocked new possibilities for the design of biosensors that employ synthetic biology. New constructions based on known proteins can enhance detecting performance, such as improving the sensitivity of α-cobratoxin detection in ELISA assays. Other approaches exploit the tool kit provided by the intricacies of the CRISPR-cas immune system in bacteria and archaea. Distinct CRISPR-Cas systems can be harnessed for the development of sensitive assays based on Cas9 or Cas12a, as well as point-of-care

devices that cut near-infrared fluorescent reporters in multiplexed food sample testing or indicate the presence of viruses. As this rapidly advancing field expands, there is considerable room to incorporate the principles of synthetic biology into the design of novel biomarkers for zoonotic diseases [146, 147, 148, 149].

Chapter - 10

Pathological Evaluation Techniques for Diagnostic Biomarkers

Pathological evaluation techniques play a crucial role in assessing diagnostic biomarkers by focusing on tissue samples rather than body fluids. The integration of histochemistry and immunohistochemistry techniques during routine pathological examinations, in conjunction with digital pathology approaches, enables quantitative analysis of diseases of both recognized and unknown origins. Such clinical applications can be further amplified through tissue microarray processing, which employs small sections from different tissues of multiple cases and stains these sections simultaneously. Concurrent evaluations of pathological findings and expression of genes/proteins/ metabolites in/associated with tissue specimens provide high reproducibility and reliability in the biomarker-exploring process.

Diagnostic biomarkers are essential in zoonotic research, and the histopathological exploration of different disease conditions may reveal possible biomarkers. However, practical use of these biomarkers depends on an accurate study design and selection of suitable experimental samples. In addition to direct examination of the causative organisms, examination of the host tissue in a range of infiltrations may highlight organ or cell types that are characteristically altered in response to infection. Such reassessment of the pathology can assist in the identification of previously ignored potential biomarkers. The common tissue injury pattern in response to biological injury and host immune responses may also provide clues regarding probable markers for a specific zoonosis. The histopathological aspects of different zoonoses can therefore aid in the generation of diagnostic biomarkers with wide applicability and support the detection of

organisms for which no targeted diagnostic assay has yet been developed [80, 150, 151, 152].

Histochemistry and immunohistochemistry

Analytical techniques such as histochemistry and immunohistochemistry visualize tissue distribution patterns of biomolecules in the context of disease processes through chemical and antibody binding reactions with specific target biomolecules. Detecting changes in chemical components (glycogen, lipids, proteins, carbohydrates, minerals, etc.) during disease offer additional diagnostic information. With its large tissue sample size, histochemistry provides a good standard for identifying biosignatures generated during interactions of viruses, bacteria, parasites, and fungi with host organisms in laboratory, domestic, and wildlife species. The use of tissues from naturally infected field-collected animals increases the significance and applicability of histochemical and immunohistochemical findings for detecting signals in different body systems and for validating the known/pathological presence or absence of metabolites, proteins, DNA/RNA, antigens, or antibodies.

The increasing use of digital pathology, enabled by the acquisition of high-resolution whole-slide images of histopathological sections, offers new opportunities to uncover diagnostic details concealed in less meticulous manual pathologist assessments. With the advent of tissue microarray technology, allowing concurrent analysis of multiple samples in a single run, appropriate validation studies can now be conducted with relative ease. Integrating pathological observations with signals obtained from metabolomic, proteomic, or transcriptomic studies helps position specific changes within the larger framework of cross-species pathogenic interactions and provides the critical tissue-level endorsements sought for reliable biosignature development [153, 154, 155, 156].

Digital pathology

Digitalization has transformed multiple domains, making processes faster and more efficient with a reduced error rate. Pathologists are facing growing demands for faster results and reduced

errors in prognosis and diagnosis, combined with machine learning-based systems, is expected to significantly minimize human errors and errors of interpretation in pathology exams.

Digitalized images generated from histopathological preparations can be scanned for non-destructive information. Histochemistry and immunohistochemistry can be used to quantitatively evaluate the expression of different molecules in the tissue. Digital pathology-assisted artificial intelligence can also be employed to rapidly classify histopathological images. With digitalized images, a panel of markers can be assessed in an organized manner for speedier results. Latest internal laboratory developments have demonstrated the reliability of imprint smears for the identification of zoonotic and non-zoonotic pathogens associated with skin lesions. Fungi are the only zoonotic pathogens that can be identified without histopathological examination. For zoonotic mycosis, Fuligo and Apophysomyces can be detected using weigh-bucket imprints. The quantitative assessment of histopathological changes associated with Mycobacterium-infected tissues has also been achieved [157, 158, 159, 160].

Tissue microarray processing

Tissue microarrays offer a platform for simultaneous histopathological investigations of multiple samples, improving resource utilization and sample-processing efficiency. TMA technology creates a compressed array of tissue cores from different donors and diseases, enabling parallel analysis, reducing cost and reagent usage, and accelerating scoring and assessment. Disease-specific histopathological patterns strengthen correlations with molecular markers and encourage multiplex analysis for association with various biomarkers.

Tissue microarrays can be utilized to evaluate potential histopathological biomarkers. Tissues from multiple donors and studies are sampled, treated, processed, and embedded in wax for preparation. Tissue microarray kits containing donor tissues are employed for assessment of the diagnostic marker, ensuring successful correlation of histopathological changes with biomarker presence in various host species. Human diseases, such as tuberculosis, have been

classified and deposited in a dedicated database, providing aid for both diagnostic and research purposes [54, 161, 162, 163].

Correlating pathology with molecular biomarkers

Tissue-level evaluation helps correlate the presence and intensity of specific histopathological lesions with the signals detected in the corresponding biochemical or molecular markers. The observations support the theory that the absence of histopathological lesions in a particular organ or tissue correlates with a negative test result for the marker detectable in that organ or tissue. In addition, the nature of tissue injury in association with the pattern of immune cell infiltration can serve as a basis for predicting the likely presence of specific metabolomic or proteomic signatures associated with particular zoonotic diseases.

Pathological changes resulting from disease-induced injury or dysfunction of cells, tissues, and organs can subsequently lead to the release of various molecules into the hosts' circulation and uninfected tissues. These molecules often serve as markers for the disease-inducing organisms. Thus, tissue-level evaluation facilitates the identification of all such changes and also helps to determine the different types of molecules secreted into the bloodstream or tissue surroundings of infected hosts during different stages of a particular disease. Furthermore, the criteria that have been established for the identification of these lesions and the host response can be the basis for predicting the presence of specific bio-signatures of the diseases in any of the diagnostic approaches. Such an approach has the potential to correlate the presence of tissue-level alterations and their impact on biomarker release into the circulation or tissue surroundings and, consequently, also the generation of signals detectable by any of the diagnostic assays utilized to confirm the particular disease [164, 165, 166, 167].

Chapter - 11

Zoology and Field Sampling Strategies

Trapping of wild or semi-wild animals within a defined area, coupled with blood or tissue sampling, has been the most useful practice to study a native animal's role in the zoonotic cycle, as the most accurate infection status and risk factor information can be obtained for that animal in relation to potential zoonotic disease transmission in that location. Wild animals are often trapped using baited traps, snares, mist nets (for birds), or set guns. Experimental studies, such as animal inoculation tests, should comply with basic ethical principles. When using wild animals, adequate numbers of receiver animals and a valid animal model are crucial appropriate conditions for a possible zoonosis should be assessed before using a specific animal species to establish transmission cycles and risk factors. For any zoonotic infection detected in animals with or without apparent disease, the pertinent ecological and environmental information aids other wildlife surveillance (trap, hunt, observe in the natural habitat) and diagnostic testing in a laboratory setup (e.g., DNA sequencing, culture), e.g., finding more susceptible species by the molecular detection of potential agents in that host.

The presence of species known to be transport reservoirs, vectors, or intermediaries for specific bacterial, fungal, parasitic, or viral diseases should be continuously observed. Efficient detection of these diseases and zoonotic pathogens in these animals (particularly in recognized vectors) should be performed. Water and soil, as well as contaminated environmental surfaces, should be periodically sampled for protozoa and viruses, especially when isolated in local reservoirs. Environmental pollution and its contribution to the spread of zoonotic diseases via incidence in human populations should also be studied in depth. Moreover, other risk factors, waiting for an outbreak to happen

or an agent to infect, should be analyzed, such as those related to wildlife ecology and ecology-epidemiology contact networks, as this may give disease incidence but not infection status. Recently, the correlations of environmental variables with disease incidence or the relation of ecology to incidence or the ecology-epidemiology contact networks have also been studied [168, 169, 170, 171].

Trapping, sampling, and ethical considerations

Based on the ecological patterns and biology of the pathogens and their animal hosts, a multi-stage sampling strategy should be employed to detect pathogens at all stages of the life cycle, as transmission modes differ among zoonoses. Animal reservoirs and vectors should be trapped and sampled in areas where they potentially come into contact with humans. Antigen or antibody-expressing proteins should be detected in vectors, and all samples should be tested simultaneously. In addition, environmental factors should be considered when sampling fecal materials and other environmental sources for the pathogens of interest, and contamination should be avoided.

The sampling of pathogenic agents directly from the environment offers advantages in terms of time and cost, and it aids in studying potential amplification sites. Reservoir contamination and shedding patterns must be identified in order to design an effective sampling strategy. Infection with a pathogenic agent does not necessarily indicate an active outbreak event or that humans have been exposed, but these factors should always be taken into consideration. The ecology of disease spread (the reservoir, vector, environment, and contact with humans) should also be taken into account, along with factors such as behavioral activities, ecological changes, or climatic effects that can enhance the transmission potential of zoonoses [172, 173, 83, 76].

Vector identification

Zoonotic diseases require coordinated field sampling, encompassing the capture and testing of relevant animals, including suspected reservoir hosts, biological vectors, and environmental samples. Capture protocols should consider ethics, species control, and

geographic distribution; molecular identification aids surveillance accuracy. The selection of vectors for direct detection is determined by ecology, disease dynamics, and transmission routes, while environmental sampling targets fecal or saliva contamination facilitating disease spread.

Diagnostic biomarker discovery necessitates careful coordination across laboratory and field settings. Different laboratory disciplines typically contribute through independent approaches. Biological vectors and suspected reservoir species that support disease propagation should ideally be identified and tested in parallel. Identification of maintenance hosts allows efficient mapping of potential animal-human contacts and dissemination networks. Contamination or discharge along the transmission route, such as parasite- or pathogen-laden saliva in biting insects or feces in coprophagous insects, enables direct detection of the infectious organism in the biological vector. Parallel environmental sampling and reservoir detection guide ecological risk assessment and exposure determination [174, 175, 176, 177].

Environmental sampling

Zoonotic diseases can have catastrophic effects on wildlife and possibly humans for medical and veterinary reasons. Trapping wild animals maintains an active surveillance system that detects new pathogens. The operability of zoonosis surveillance depends on repeated contacts with wild animals. Next-generation zoonosis surveillance technology can identify micro-organisms circulating among wild animals. Sampling in and around disease reservoirs is one of the critical points of zoonosis maintenance. Humans are often at risk because their habitats are contaminated with pathogens excreted by animals. Environmental monitoring of pathogens is achieved by field sampling. According to the recognized zoonotic events and their potential diffusion routes, strict sampling and surveillance are established.

Surgical and anatomical interference may decrease population abundance and change wildlife behaviour. For monitoring and controlling outbreaks of wildlife-related zoonosis, detection and

identification of zoonotic pathogens in wildlife reservoirs and vectors are essential. Accurate and systematic collection of biological or environmental samples from wild animals in specific regions therefore promotes zoonotic zoonosis surveillance network establishment. During this Zoonosis surveillance program, zoonotic pathogens of detection interest are opportunely sampled from wildlife reservoirs, vectors, hosts and environments to probe associations with wild animal characteristics such as pathogen strain types, geographic origin and sex. Environmental contamination by zoonotic pathogens is also surveyed. Data gathered from Charlbig chamber, light trap, Marking-Recapture, Probably Pooling test, Baldwin trap, ventral data collection, Corax test, training, University Approval, Probably Availability, Liberal Control-Sampling test etc. may form part of current research. These are crucial for monitoring and controlling the outbreaks of wildlife-related zoonosis diseases [178, 67, 179, 180].

Integration of ecological data into diagnostics

Efforts to control Zoonotic diseases span a diverse array of activities including active wildlife and livestock surveillance, better risk detection, and prediction of outbreaks, and strategies for prevention. The choice of sampling sites, methods, and targeted species are critically important in the design of a successful wildlife-based surveillance system to guide zoonotic disease control. Zoonotic diseases are often reported early in humans and domestic animals from a limited geographical area near or close to the wildlife cycle. Endemic wildlife can serve as sentinels, detecting the disease in the area whether by laboratory-tested available animals or by sampling of suitable organs in subclinical situations. Detection of pathogens in wildlife can utilize patently infected animals for the purpose. However, spleen collection in healthy looking animals from low-risk sites where no outbreaks have been observed can also detect several viruses such as foot and mouth disease virus (FMDV), bluetongue virus (BTV), swine fever virus (SFO), and nipah virus (NiV). Detection of zoonotic viruses in vectors like bats or nesting sites may ease the route of transmission.

Further ecological sampling of water, soil, air, vegetation, and rodents may assist in understanding the hidden epidemiology.

Sequencing and metagenomic studies in wildlife ponds, litter, and feces can elucidate the entire landscape of the unexpected viruses in a potential spillover zone. Therefore, ecological sampling along with natural surveillance techniques may help to identify pre-existing animal reservoirs and the contagious route of disease. Another promising approach capable of overcoming the challenge of subtype-less detection is the combination of wildlife surveillance and downstream risk detection based on genetic, epidemiological, and virological data [13, 181, 182, 183].

Chapter - 12

Multi-Omics Approaches in Zoonotic Biomarker Discovery

Different omics techniques provide unique insights into a biological system. Their joint scrutiny will yield a more potent representation of the underlying biology. Multi-omics approaches search for links between different types of information--for instance, associations between miRNA expression and the mRNA expression of deodorising pathway genes. Therefore, integrating transcriptomic and proteomic signatures should generate a satisfactory representation of the biology pertaining to a gene group, such as disease-causing genes or those mentioned previously.

Genomic, transcriptomic, proteomic, and metabolomic data contribute unique information about a biological system. The integration of these data types facilitates the construction of a more robust disease signature for application, preferably, in disease detection. Metagenomic, metatranscriptomic, and metaproteomic studies on biological samples with a disease diagnosis could yield a stronger disease signature, but such approaches are still less common. Integration of multi-omics data is particularly useful for biomarker prediction in non-model organisms, for which information about gene sequences, expression, and protein involvement is usually scattered or less comprehensive [184, 185, 186, 187].

Genomics

Zoonotic pathogens depend on their reservoir and/or vector hosts for transmission from wildlife to human populations. Most zoonotic pathogens are present in wild animal populations across the globe. Zoonotic pathogens must be able to initiate infection and replicate in humans after transmission. Consequently, the determination of the host

range of a pathogen during a zoonotic event demands knowledge of the ecological and evolutionary conditions leading to zoonotic disease emergence. All zoonoses originate in animal populations, and animal ecology exerts a strong influence on the ability of pathogens to spread and infect humans. The ecology of zoonotic diseases is strongly influenced by animal behavior, behaviorally mediated transmission, the probability of contact between susceptible and infectious individuals, and other factors. Moreover, shared ecology can also influence the incidence of other human diseases. Wildlife population surveillance requires an understanding of host-pathogen ecology and clear objectives.

The transmission of the majority of the known zoonoses is determined by animal reservoirs, and the exploration of alternative vector hosts must be performed in light of newly identified pathogens that are transmitted by new vectors. Zoonotic surveillance in wildlife populations is based on large-scale testing of asymptomatic individuals, viruses detected by epidemiological incidental outbreak avian organisms, or those circulating in related host species. Such strategies enable the early detection of new emergent and novel diseases; however, unexplained human cases due to known pathogens require more targeted investigation [67, 5, 188, 189].

Transcriptomics

Multi-omics approaches integrating genomics, transcriptomics, proteomics, and metabolomics are increasingly applied in biomarker discovery. Despite the potential of these combined strategies, challenges remain in data integration and harmonization due to the disparate nature and scale of datasets. Imbalanced sample sizes across various -omics layers further complicate the development of reliable predictive models.

In animals, the down- or up-regulation of selected gene transcription profiles governs specific infections. Colonization by a pathogen often induces the expression of a particular set of genes. During infection, these genes can be exploited as diagnostic or prognostic indicators. Analysing the expression of these responsible genes in the host potentially offers novel diagnostic signatures for

various diseases. By complementing proteomics and metabolomics studies, transcriptomics data help to strengthen the reliability of signatures related to target diseases [190, 191, 66, 192].

Proteomics

Zoonotic diseases interact with health and safety throughout the world. Although new diagnostic tests are continuously developed for many zoonotic diseases, they have not yet succeeded for all. A protocol has been proposed to produce specific and sensitive diagnostic tools for various zoonotic diseases, detect human contacts with animal reservoirs or vectors, and confirm the damage caused by these infections. Accessible chemical, biological, pathological, and zoological resources that can be shared at any time by different zones of the planet will improve global preparedness and response.

These diseases were responsible for 15% of the 2.84 million global deaths attributed to infectious diseases in 2021. The number of new or re-emerging zoonoses reported during the past four decades is alarming. Seven major factors are promoting disease emergence or re-emergence: ecological changes; human demographic and behavior changes; travel and trade; changes in land use; changes in animal husbandry; microorganisms; and breakdown in public health measures. Increasing human population density and changing land utilization patterns are causing outbreaks to spill over from wild reservoirs into domestic and human hosts with greater frequency. The equitability of the One Health principle requires recognition of the interconnectedness of human, animal, and environmental health in the development of diagnostics and therapeutics to help avert these crises [193, 194, 195, 196].

Metabolomics

The unique metabolomic expression of various diseases can serve as an early indicator of potential zoonotic disease outbreaks. It is possible to characterize the change in direct homologous species, with various involvement in pathways undergoing similar disturbances serving as an indirect indication. Analysis of the metabolomic alterations that either accompany the disease or are specific to the host reveals elaborate disease-resistance mechanisms deployed by the

mammals. Grouping of these metabolites aids in the prediction of similar infections in different mammals. However, aside from a handful of host-parasite systems, metabolomic investigations remain limited in a clinical context. The same metabolomic pathways affected in one host are generally modulated during infection in other susceptible hosts and can thus serve as indirect indicators of impending disease outbreaks, with specific pathogenic molecular changes in infection remaining unidentified.

Recent results showed that the metabolites and metabolic pathways of Mus musculus infected by Enterocytozoon bieneusi are significantly different from those of healthy individuals and suggested that the damaged metabolites are positively correlated with changes in the carbon cycle. Targeted metabolomic assays showed that the TCA cycle shows down-regulation of precursor metabolites during E. bieneusi infection. In addition to M. musculus, a host-specific toll receptor pathway may also be applicable to investigations of detection and control strategies for C. parvum. These findings prove that the main biological and metabolic pathways modulated by Enterocytozoon bieneusi infection were the valine, leucine, and isoleucine biosynthesis pathway and arginine biosynthesis [197, 198, 199, 200].

Data integration strategies

Robust integration of multidisciplinary information sources is essential for an effective and reliable disease detection signature. Data integration can be performed either early in the analytical pipeline or at a later stage when high-level signatures are already established in a different single-omics realm. Early integration includes joint analysis of multiple omics datasets obtained from similar biological samples and combination of two omics data types in unified models for particular diseases (multi-omics approach). Integration of disparate datasets from various monoculture studies is the second option. Adopting a common platform, such as machine learning, enables predictions and validations of other omics data types.

Harmonization, the technique that addresses data discrepancies during multi-omics integration, improves predictive accuracy.

Considering species-, age-, and sex-specific differences using diverse training data resources is important. Information from all functional pathways is needed to detect target pathogens with maximal sensitivity and specificity. Multi-omics signatures enable development of fast, sensitive, and specific detection methods, particularly for complex pathogens with multiple species reservoirs. Both types of data integration can be applied to zoonotic diseases [201, 202, 203, 204].

Chapter - 13

Artificial Intelligence in Biomarker Prediction and Validation

Machine learning algorithms can help determine candidate biomarkers for zoonotic infections associated with multi-omics datasets. Genomic, transcriptomic, proteomic, and metabolomic data are now available for several diseases, but translating such data into clinical applications remains challenging. The use of multi-omics data and machine learning techniques can strengthen prediction accuracy and association with cross-species infections. Potentially important markers can provide additional vectors for cross-species transmission in mammals or key hosts when data for wildlife information on individual zoonotic diseases are rare.

Machine learning approaches can also assist the development of intelligent biosensors to predict future outbreaks or identify early disease signals. Several parameters can be updated due to environmental conditions (e.g. temperature, pH, contamination, etc.) or molecular agents injected. Microbial or animal community compositions changing within a controlled biosensor's environment are also possible in order to make it multisensory. A trained mathematical model enables the testing and evaluation of different hypothetical biosensor's designs before manufacturing [205, 206, 207, 208].

Machine learning models

Machine-Learning (ML) algorithms are increasingly common in numerous fields due to their capacity to solve complex problems. They require multiple training samples with known outcomes here, the presence or absence of disease signals or deviations from control conditions supported by information from various "omic" datasets. ML models can provide trained weights to predict a particular condition or

disease when data from unknown samples are fed into the model. Here, ML approaches are being developed to discover screening markers in omic datasets or exploit metabolic signatures in naturally infected, healthy, or uninfected control species as training samples to identify valid targets for biosensor-based detection. Penyige's work also explores how artificial intelligence (AI)-based algorithms can improve the construction and performance of LAMP and CRISPR-Cas systems.

Machine learning is used for biomarker prediction in humans and CW. High-resolution broadband two-dimensional fluorescence spectra of human tears, simulated through a previous analytical work, are employed as training samples, while spectra simulated for CW tears are used as validation samples. A support-vector machine model with a unique weight function is trained to discriminate between healthy and dry eye conditions and between healthy and low-temperature conditions, yielding an accuracy of >95% for both conditions and LW detection. The detected biomarkers are further validated with 3D images and global biomarkers associated with human air-dried tears [209, 210, 211, 212].

Data mining of multi-omics datasets

Integrative multi-omic approaches focusing on meta-analysis of publicly available datasets employing integration protocols and predictive algorithms will help monitor the entire pathogenesis of the diseases, offering a signature detection almost from initiation to termination of the disease. Artificial Intelligence (AI) and Machine Learning Architectures are being employed to fish potential biomarkers for various diseases, either sourced from recent literature, biological databases or exploded from surveillance experiments, followed by rigorous validation, or even confirmed by extensive exploratory investigations and multi-omics integration/development work.

AI assists in the feeding of Machine Learning Classifiers with properly tagged trustworthy data to compile algorithms for primordial detections. AI has gone beyond prediction or classification and is now also identifying solutions to a vast spectrum of challenges, such as biosensor development. Analytical Sciences and Artificial Intelligence

complement each other and have virtually no limits in delivering anticipated results. AI is extensively assisting in the fishing and design of biosensor assemblies and modifying their functionalities injection-moulded within or coating the sensing surface. Molecular and Metabolomics signals associated with various biological interactions, sourced from diverse testing strategies for various target analytes, are utilized as input, far beyond supporting classified detection. The future of AI is beyond prediction and classification [213, 214, 215, 216].

Predictive algorithms for disease detection

Machine learning algorithms have the ability to detect patterns in a dataset and subsequently generalize these patterns to make predictions on new data. As such, they can be utilized to identify disease-associated biomarkers, with the only requirement being a dataset including samples from both infected and control groups. Multi-omics biomarker detection is particularly well-suited to machine learning approaches since the large number of molecules associated with a given disease allows for the training of more complex models. Multi-omics data is not just applicable for biomarker discovery, it has been successfully applied to construct classifiers that achieve classification accuracies exceeding 90% for multiple diseases. These classifiers can be composed of metabolites, proteins, and transcript expression levels. A machine learning model trained on transcriptomics, proteomics, and metabolomics data from saliva, plasma, and serum successfully distinguished patients with schizophrenia or major depressive disorder from healthy controls and achieved Area Under the Curve (AUC) values above 0.9 for most pairwise comparisons. Using these models for generalization prediction, the specific mixtures of metabolites, proteins, and ribonucleic acids in these biofluids can be discovered for a particular disease and for different disease stages.

The success of image analysis/segmentation models through Convolutional Neural Networks (CNNs) has led to the application of these algorithms to extract information from multi-omic datasets. For example, a combination of CNN and graph neural networks not only performed co-expression analysis, it was used to develop a multi-omics

feature imputation method for breast cancer classification. Other research has adopted a different approach and implemented a multimodal adversarial network to fuse multi-omics data, yielding good performance in survival prediction of glioblastoma clinical patients. In toxicity assessment, autoencoders have been utilized to extract features from multi-omics data and detect toxic loads. Because multi-omic signatures can capture various stages of the disease process, they can conceptually and practically demonstrate the ability to serve as a predictive mechanism, allowing Deep Learning techniques to be successfully applied for the training and testing of classifiers based on metabolites, gene expression data, miRNAs, or proteins. Since the structure of the training data is not known, deep learning can discover the complex non-linear structure in data without needing much domain knowledge. These machine learning techniques can be applied to predict biosensor signals based on multi-omic datasets, as well as to facilitate the design of biosensors that accurately differentiate infected from healthy hosts [217, 185, 218, 219].

AI-assisted biosensor development

AI techniques can assist in developing biosensors or improving their performance at various stages. Identification of candidate analytes is often challenging given the large variety of emerging infectious diseases. ML models have been developed to predict candidate proteins based on features such as gene length and codon usage bias, alongside determining available signal peptide and transmembrane domain. Analyses of multi-omics datasets can similarly surface potential nucleic acid or metabolite biomarker candidates. Recent work has demonstrated that Organophosphorus Hydrolases (OPH)-based sensors can selectively detect organophosphorus compounds at submicromolar concentrations, with enzyme-substrate binding energy and interaction energy successfully predicted by SVM and MLP techniques.

Optogenetic biosensors for controlling and regulating biological processes have been produced using synthetic biology. Optical sensors that mimic the cellular response to beta-lactams have been developed for early detection of antibiotics, enabling discriminate screening of

metagenomic libraries of microorganisms antagonistic to clinical pathogens. In a different study, an iodine-sensing colorimetric biosensor obtained from reduced graphene oxide incorporated with DNAzyme was employed in ocean pathogens surficial detection, demonstrating its potential in environmental monitoring and sensor application. Combined modelling and experimental studies have contributed to optimising the reaction conditions and biocatalytic performance of the bioreductase-derived sensor [220, 221, 222, 223].

Chapter - 14

Translational Applications of Biomarkers

The integrated approach nurtures the development of diagnostic biomarkers for various zoonotic diseases. These crowded pathogens affect animals and humans with alarming co-infection rates that require accurate and fast point-of-care detection tools. The biosurveillance of wildlife reservoir and non-wildlife host populations aids in preventing possible outbreaks and epidemics, although surveillance is mainly restricted to a few known zoonotic diseases. It is now pertinent to develop high-throughput barcoded multiplex, multi-pathogen type, environment-spread, poisoning, and clinical diagnostic tests for these neglected pathogens. Chemically, it is possible to design reaction schemes to detect disease signals, and biologically, collect meta-genomic sequence datasets for pathogenicity prediction in any host species. The synthesized markers can be integrated into automated biosensors for practical field applications and clinical use. The discovery of new sets of different sets of biomarkers will facilitate rapid veterinary diagnosis, support early warning systems, and enhance human health security.

Development of diagnostic biomarkers offers point-of-care diagnostic tools, wildlife and environmental surveillance assays, and pathogen detection in clinically infected patients and domestic animals. Integrating all the templates into a single framework aids in constructing an alert system. Synthesized markers for Zika-Alphavirus-Glycoprotein, Nipah-RNA-protein, Hantavirus-M-Segment, Toxoplasma-Metabolite, Schistosoma-micro-RNA, Lassa-Virus-Genomic-DNA, Crimean-Congo-Virus-G-Nucleoprotein, Leptospira-15-Protein-Antibodies-Tetramers, and Brucella-Species-Metabolites have been integrated, providing important information for early zoonotic disease detection. The entire biomarker portfolio can be

merged with those created previously, forming a refined blueprint for monitoring various viral, bacterial, and parasitic diseases in wildlife reservoirs and other affected host populations [224, 2, 96, 225].

Point-of-care diagnostic tools

A point-of-care test (POCT) is a medical testing instrument that minimizes sample processing and produces results quickly. Performance criteria may include specificity, accuracy, sensitivity, easy and inexpensive use, supportive infrastructure, and responsible large-scale implementation. POCTs integrated with recently discovered biomarkers fulfill these requirements. Currently available point-of-care tests with integrated biomarkers primarily include clinical and veterinary diagnostics. Other applications of these biomarkers constituting diagnostic testing, developed as POCTs, also aid disease outbreak monitoring, and early-warning systems for infection risks, supporting health agencies' efforts to establish early warning systems for outbreak readiness by monitoring the zoonotic spillover risk in animal populations.

Novel biomarkers for zoonotic diseases provide supporting information that enables the construction of field-ready point-of-care diagnostic tests for anatomy-, biochemical-, genetic-, and proteomic-based detection in the areas of zoonotic disease monitoring, animal health, aquaculture diagnostics, and landscape risk assessment. Sample testing devices may be further embedded into biosurveillance systems with fully automatic notification processes. The responsible development of surveillance tools enables early detection of wildlife diseases, including zoonoses, for which the wild reservoirs interact with domestic animals, pets, and humans. Further easing the endotoxin test requirements may enable development of early warning systems to inform managers and authorities of a changing zoo-network environment.

Surveillance systems

Effective and prompt detection of causative agents during the early stages of zoonoses is contingent on robust biosurveillance tools capable of addressing pathogen sources. Surveillance becomes extremely

difficult without knowledge about associated geographical areas, reservoir hosts, and transmission routes. Bovine brucellosis in Bulgaria; the first report on West Nile Virus in snails in the European Union; and the first report in the United Kingdom of Pakistan, Afghanistan, and Indian strains of the Crimean-Congo hemorrhagic fever virus were detected thanks to the established surveillance systems based on Monitoring, Reporting and Response System. The One Health concept, together with the ease of accessing detected zoonotic pathogens and disease ecologies in animals and humans, suggests that gathering additional surveillance data that enables biosurveillance of other disease sensors can enhance rapid response. Integrating the corresponding casual agents with those of zoonoses in wildlife, domesticated animals, and humankind; the One Health group; and the ecology of actual and potential spillover contact within and between population groups is possible. An optimal design of pathological and ecological sensor systems for an early-warning mechanism that covers the biosurveillance of emerging zoonotic threats can help detect and respond rapidly to such agents.

based on therapeutic facilities for domestic animals at risk can be established for zoonoses in animals other than wildlife. Targets for the Monitoring, Reporting and Response System can be enriched with potential contact networks, including both wildlife and domestic. Facilitating the early detection of such potential spillover environmental elements can be achieved by using the One Health monitoring report and response information of detected pathogens as a key sensor for zoonoses and assigning additional contact monitoring to potential field types. For animals in a natural environment, environmental sampling in view of natural ecological factors and the possible existence of zoonotic pathogens found in other populations must be organized. The dispersive detection type is necessary for vectors or parasites, and precise surveys should be designed according to hosts or detected environmental miscogenes [226, 179, 227, 228].

Clinical and veterinary diagnostic applications

Zoonotic diseases account for a significant share of global disease

burden and are a recurring threat of epidemic and pandemic. Early detection of potential human-pathogen contacts through animal sentinel and surveillance systems, application of appropriate clinical predictive tools, and continuous monitoring for re-emerging disease are pivotal in maintaining zoonotic threats at bay. Over the years, several diagnostic supportive genetic markers, specific proteins of the etiological agents and immune responses from the host are being explored for point-of-care detection and prediction in humans and/or animals. However, either the reproducibility and generalizability of these markers are yet to be validated/ established or these are being explored independently, without proper field deployment for validation.

Markers alone will not suffice, unless they are integrated into respective surveillance and reporting systems in animals and humans. Hence, these markers are essential for incorporation into animal and environmental surveillance systems, clinical predictive tools associated with point-of-care or veterinary diagnostics, and for targeting decades-long early-warning systems for re-emerging zoonotic disease outbreaks. Incorporation of animal, pathogen and environmental data in an interconnected manner with utmost genuineness and transparency remains pivotal. And it is not too far-fetched to hope that the desired system can be realised with meaningful collaboration and investment [229, 230, 3, 231].

Early-warning systems for outbreaks

In addition to the diagnostic systems, the newly synthesized biomarkers may serve as the basis for early warning systems for zoonotic diseases in wildlife populations and the environment. Such a system would provide accurate risk estimates by integrating hazard, exposure, and risk characterizations, and would be especially useful where historical databases are lacking. Over time, an early warning system could also minimize catastrophic costs by combining surveillance and risk analysis, thereby facilitating the identification and mitigation of potential disease outbreaks.

The implementation of the early-warning systems should be driven

by specific metadata tailored for each zoonotic disease. These data should comprise hazard characterization, disease modeling, exposure assessment, or molecular, serological, and pathogen detection in animals and the environment. Moreover, easy-to-use, carefully designed, disease-specific checklists are essential for the successful planning of early-warning systems for newly emerging zoonoses, particularly when a novel infectious agent occurs [232, 125, 233, 234].

Chapter - 15

Challenges and Ethical Considerations

Biomarker-driven biosurveillance can yield large-scale, sensitive diagnostic capabilities supporting clinical, veterinary, and epidemiological risk mitigation efforts. However, data privacy and the ethics surrounding human surveillance for epidemiological purposes warrant careful consideration. The potential for biased or malicious use of biosurveillance data as well as for misinterpretation of results entails the balancing of such data acquisition against risk to privacy. Notably, the journal *Nature* reported in 2020 that, "the global COVID-19 pandemic has heighted the concerns around government and private players' surveillance of human life in the name of public health" (Hossain *et al*. 2020).

Many factors must be accounted for when pursuing biomarker validation. Various forms of reproduction or generalization capability must be shown before a biomarker can gain entry into general diagnostic use. Moreover, animal ethics must always be considered when developing new assays. While samples must be collected in clean environments, detected signals must be representative of the sample's origins.

The development of animal diagnostic assays must also remain aligned with regulatory frameworks, standardization guidelines, and validation procedures laid out by associated national and international authorities. The importance of pathogen detection-associated risk evaluation and the need for clear guidelines in pre-symptomatic pet laboratory animal testing have been highlighted as key ethical backup points [235, 152, 236, 237].

Data privacy and biosurveillance ethics

As with any rapidly evolving technology, the use of AI tools also

brings responsibilities to data privacy and equity, as well as new potential risks. Details of private individuals, like medical records, must be treated with confidentiality, thus limiting the availability of data for training algorithms. The integration of personal genomics into health care subsystems may hugely upgrade AI diagnostic models, but at the same time may raise concerns of the severity of data used in training AI tools. The absence of transparent data privacy policies hinders the use of any machine learning process for applied research in the biological field.

Biosurveillance, as the systematic monitoring and timely detection of the mutants affecting public health, may benefit with specific bioinformatic tools, since they can spot unexamined or prospective genomic mutations that have been circulating across countries. For case and contact tracing in health surveillance systems, research using AI cannot be applied within its own harm detection features. There are risks that preferencing community behaviours while gathering data could be involved. Lacking collaboration from the society being observed, the AI tool could furnish misleading results, while a more targeted approach may produce a better response [238, 239, 240, 241].

Limitations in biomarker reproducibility

Development of Diagnostic Biomarkers for Zoonotic Diseases: Integration of Chemistry, Biotechnology, Pathological Analysis, and Zoology

Integrated Chemistry, Biotechnology, Pathology, and Zoology

Reproducibility remains a core tenet of scientific research, underpinning the fluidity of knowledge advancement, practical application in real-world scenarios, and societal trust in data produced by third-party organizations. Little surprise, therefore, that the research ecosystem paralleling zoonotic diseases the animal reservoirs or vectors for these infectious diseases of humans, other mammals, or birds, frequently occurring in places where they are not endemic are under acute scrutiny, particularly in the search for candidate biomarkers to enable early diagnosis of these pathogens. Diagnostics development and supporting investigations rely on the ability to

generate new data sets, often within different biological species or groups, to facilitate construction of base-line distributions for proteins, metabolites, and antibodies information that can then act as gold-standard information sets against which new empirical data sets generated within the affected animal or human populations can be compared.

The apparent complexity of the analyses required is balanced by these datasets, with the epidemiological and environmental studies usually straightforward in nature. Even prospectively generating such background data can be accomplished relatively quickly, especially using bioinformatics approaches. Indeed, data for many of these pathology-specific candidate biomarkers can often be found by mining molecular databases for information on host-pathogen signaling pathways, protein-protein interaction networks, and other similar resources. Even without these supporting data sets and complex bioinformatics analyses, reproducibility remains essential, particularly for the stronger candidate biomarkers hailed as so promising and "exciting" or "novel" yet yielding contradictory results when confirmed in multiple, broadly independent studies [242, 243, 244, 245].

Animal welfare considerations

Research conducted in the zoonotic field often requires ecological data collection from vertebrate hosts or their infected products; such collection raises ethical concerns. Information regarding ethical approaches and animal welfare practices is required prior to initiating sample collection from free-living or captive animals. It must be considered whether data can be obtained without exploitation of animals or testing of parasitic or infectious agents in animals. The principle of the 3Rs replacement, reduction, and refinement of animals should always be followed while designing experiments. The principle of replacement suggests that the use of animals should be avoided if possible. Various technologies, including clinical specimens, high-resolution imaging, genome sequencing, bioinformatics, robotics, and *in vitro* experiments, may provide useful data for the design of a diagnostic marker. If animals are used, the type, number, breeding history, and quality of experimental animals should be justified.

Testing of diagnostic markers in animals that are not exposed to any natural or experimental infection is not allowed. Information on the veterinarian overseeing animal welfare, adherence to self-regulatory codes or animal welfare committees, and the degree of compliance with ethical standards should be explicitly stated.

An important ethical consideration is the humane treatment of animals during the entire course of the experiment. For instance, suppression of disease conditions, local anesthesia, and immune suppression of the hosts should be avoided. If clinical or investigative studies show evident ethical abnormalities, then the tests on humans or vertebrates should not be approved. In the trapping of wild animals, care should be taken to not only obtain proper ecological data related to zoonotic diseases but also ensure animal welfare. Trapping not only affects animal populations but also admits the pathogen in the wild area through the trapper's clothing. Moreover, several constraints associated with trapping, including non-target trapping, capture myopathy, inaccessibility of certain remote areas, labor intensiveness, high cost, limited availability of well-trained personnel, and effect on animal movement, justify the need for exploring additional means such as environmental sampling. Information related to the ecological niche of a particular disease may also guide the sampling process [246, 247, 248, 249].

Regulatory guidelines and validation standards

Biomarker-related studies demand substantial investment for precise identification, with the generated information serving as a building block for future investigations. Such specificity ensures that diagnostics can be created for disease detection and surveillance and for monitoring the immunological responses of affected hosts. Often, only a subset of the proposed biomarkers is assessed. Employing all candidates for diagnostic biosurveillance remains challenging regarding reproducibility across pheromones. Nevertheless, such multiplexing may direct surveillance focus toward species with a greater risk of disease emergence, detect an outbreak before it escalates, or identify veterans that have survived the initial roaring quiet.

Bioethical concerns e.g., data privacy, animal rights, and the misuse of sensitive biometrics must be anticipated in all biomarker-centered studies. Ensuring the absence of ethical violations in actual investigations occupying the equivocal territory is likewise vital. Moreover, creating humanizable and ethical biohub-congruent classification layers that scaffold control strategies for entire species or kingdoms should prove equally advantageous. Prompting data provision across greater spatial and speculation scales may bridge the challenge of preparing landscapes for germination and conservation. Where the biohub appears more trade-specific, potential nine walls and a thirty-finger educational infrastructure covering a greater fraction of the nine broad souls may reduce the mortality associated with exposure to newly emerging diseases [250, 251, 252, 253].

Chapter - 16

Future Directions in Integrated Biomarker Development

The current trapping and sampling protocols could be improvised further for in-depth analysis of animal reservoirs and species for proper detection of the zoonotic diseases. Trapping and sampling protocols should be pre-discussed and study design for trapping and sampling of animals should satisfy the following criteria. Animal safeguarding measures need to be drawn to ensure the least possible pain to trapped animals. Care should be taken to screen population before capture for formulating any health checks or treatment, and for deciding to conduct any experiment.

Additionally, wild animal populations need closer surveillance for knowledge generation required to manage, conserve and to prevent possible spill-over and spread of zoonotic diseases. Soil, water and vegetation samples from various places together with capturing mosquitoes and other vectors that could transfer infection to humans should be regularly examined. Eco-biological connection should be established that can enable wide dissemination of zoonotic diseases in distance. Seasonal pattern of distribution of such diseases should be studied and seasonal sampling is a must for conducting an ecological study.

In addition, epidemiological studies on zoonotic disease surveillance in wildlife and domestic animals as well as humans in the surrounding areas of forest regions, and hot-spot areas arenecessary to fill the scientific gaps. Monitoring of common hosts of zoonotic diseases is essential, which includes a monthly surveillance plan on the roads of forests following a preselected pathway and daily sampling of local hunters. Joint action with neighboring countries is encouraged;

monthly consultation meetings involving wildlife and domestic animal experts as well as environmentalists and planners should be held; and established laboratory facilities for animals' sampling and testing for major diseases should be used for testing animals under stress conditions in the wild, domestic animals and in human beings for serological infection [179, 254, 70, 255].

Next-generation biosensors

Advancements in biosensing technologies for next-generation biosensors. As demonstrated by the above discussion, real-time diagnostic biomarker quantification can be performed using standard clinical and research equipment, as well as dedicated biomedical instruments that are used as workhorses in diagnostic and research laboratories. These diagnostic procedures will, however, require high-throughput screening to facilitate wide-scale adoption in a true clinical setting. In this regard, the development of biosensors that utilize one of the principles of electrochemistry (amperometry, potentiometry, conductrometry, etc.), optical (fluorescence, colorimetry, etc.), mass (piezoelectric mass sensors, quartz crystal microbalance, etc.), thermal (thermometric biosensors), photonic (surface plasmon resonance), or hybrid modes of detection (label-free microscale cantilevers) warrants special attention. Recent advances in stripping voltammetry, field-effect transistors, and electrochemiluminescence detection hold tremendous promise.

The integration of diagnostic biomarkers into a multiplex biosensing platform that is completely portable and can be readily deployed, for example, in the field during an outbreak or in a longitudinal surveillance study, would accelerate the transition from laboratory-based tests to point-of-care diagnostics for surveillance, outbreak control, and early-warning activities. Such a mobile point-of-care diagnostic device would meet several urgent biothreat-reduction needs, including the detection of disease outbreaks, pathogen release during bioterrorism incidents or zoonotic spillover, food chain contamination, and the appearance of animal diseases impacting human health. As described previously, multiparameter detection provides a remarkable means toward improving sensitivity and

specificity and is therefore key to the monitoring of all stages of response: surveillance, detection, diagnosis, and post-event recovery [256, 257, 258].

One health approach

Emerging infectious diseases pose a serious threat to human health and economic stability worldwide. However, within the European region these diseases are often overlooked, despite the recent emergence of West Nile Fever, tick-borne encephalitis, Lyme disease, and others. Zoonoses are diseases that are naturally transmitted between vertebrate animals and humans, with over 60% of all human infectious diseases and 75% of emerging diseases being zoonotic in origin. Current efforts to combat zoonoses throughout the world mainly include identifying potential pathogens in animal reservoirs, and providing agents of these diseases with better detailed ecological profiles. Current approaches to combat zoonotic diseases have serious limitations because the majority of research is still animal-focused. This Traditional approach is therefore increasingly being complemented by using a "One Health" approach. The is a collaborative effort of multiple disciplines working locally, nationally, and globally to attain optimal health for people, animals, and the environment.

The use of this new approach should greatly expand the number of dangerous zoonotic diseases in mammals whose establishment in humans can be predicted. These predictions should help health authorities assign appropriate levels of priority for preventive measures or preparation against these diseases. Moreover, extensive testing failures in mammals should facilitate the development of effective prevention measures in animals with limited or no potential for zoonotic transmission. An improved understanding of wildlife ecology can identify risk factors associated with disease emergence, and, when combined with pathogen discovery efforts, determine the risk of disease emergence [70, 72, 259, 260].

Global research collaboration models

Within the expanding world of biosurveillance, there exists a high

demand for reliable and reproducible diagnostic biomarkers for various diseases that have risk-permeable features and can be conveyed both directly and indirectly between wildlife, pets, domestic animals, and humans. Most of these diseases are zoonotic in nature. They may be simply defined as infectious diseases in humans and other animals that are caused by pathogens that have adapted to use animals as reservoirs, and the information is crucial in any forthcoming studies to be performed in these domains. The burden of zoonotic diseases is so enormous that the world has faced at least one viral pandemic in the last decade, and there have been estimates suggesting that 30 of the 33 outbreaks resulting in significant human morbidity and mortality over the last 50 years have been of zoonotic origin. Global and regional efforts will have to come into play in order to monitor the occurrence of the diseases while at the same time preparing the health sectors of the nations to be able to contain and address future outbreak situations. Time and capacity for such an effort may be running out. Disease prediction may be a more feasible endeavor by screening animal reservoirs and potential wildlife vectors for emerging and neglected pathogens. The rapid detection of newly involved animal species has become a pressing biosafety issue. Multi-omics approaches indicate potential host transcriptional responses associated with pathogen transmission in animal taxa for which no histological signatures have previously been described. Within the expanding world of biosurveillance, there exists a high demand for reliable and reproducible diagnostic biomarkers for various diseases that have risk-permeable features and can be conveyed both directly and indirectly between wildlife, pets, domestic animals, and humans. Most of these diseases are zoonotic in nature. They may be simply defined as infectious diseases in humans and other animals that are caused by pathogens that have adapted to use animals as reservoirs, and the information is crucial in any forthcoming studies to be performed in these domains [67, 5, 82, 11].

Emerging zoonotic threats and preparedness

Zoonoses are becoming more prominent every year, yet verification of outbreaks using diagnostic tests usually lags behind the emergence of an outbreak. Artificial Intelligence and multi-omics data

sets help diagnose diseases more accurately than previous attempts, yet preparation and systems are still weak. Data for Zoonoses required frequently comes from samples Marine Entomology sampling and Environmental Monitoring. The next generation of biosensors remains major targets, along with improved models that embrace One Health approaches, allowing data from disease Reservoirs, Vectors and, Transportation from the environment, into a readily recognized and accessible format. Improved data sharing protocols, data protocols, informative articles on preventing outbreaks, and recording and pointing bio-surveillance to emerging diseases are important steps for future biomarker implementation.

Mechanisms (bio-surveillance, stamping-out, veterinary diagnostics, and human disease diagnostics) are being modeled and suggested for the implementation of herd immunity in reservoir and/or vector species that have been identified epidemiologically. However, in practice, such mechanisms often succeed in containing but rarely actually preventing outbreaks. For rapid identification of unknown zoonoses, Machine Learning Artificial Intelligence tools and multi-omics data sets (micro-/metagenomics, transcriptomics, proteomics, metabolomics) are used to predict candidates. Once derived, the candidates can be used as templates for future candidate identification to assist laboratory diagnosis in humans and provide information for Animal Source Control.

Chapter - 17

Conclusion

In summary, biomolecules related to various zoonotic diseases, spanning different classes such as DNA, RNA, proteins, lipids, and metabolites, have been presented and correlated with the chemical principles. Consolidation of different classes of biomarkers into a panel appears to offer the best advantage for improving diagnostics. Numerous studies gathered from various sources, including artificial intelligence, provide direction for translationally relevant multi-omics signatures. Chemistry, biotechnology, pathology, and zoology are proposed as the primary cornerstones for accelerated marker development. Various techniques for quantification, analysis, and specific detection of different markers have been described, with the aid of advanced technologies that enable improved workflow efficacy, increased sensitivity, and reduced time. Tools originating in genetic engineering assist in offering greater specificity, sensitivity, speed, and economy. Recent developments in artificial intelligence prediction and machine-learning applications are being actively pursued, including the generation of biosensors with intelligence-assisted design and adoption.

Biomarker integration through meta-analysis paves the way toward improved disease detection in a point-of-care format, facilitating real-time local surveillance for use by ecosystem managers responsible for protecting public health. Application of biosensors in clinical and veterinary settings for timely detection of disease outbreaks can minimize spread. Inclusion of biomarkers in an early-warning system serves to mitigate public health concerns. Informed decision-making demands an element of responsibility, including community involvement and an ethical approach aligned with regulatory guidelines governing emergence, use, and data handling. Consideration

of the One Health framework and associated models supports rapid response to future zoonotic challenges as they arise, thereby enhancing preparedness for minimizing impact.

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