Metabolic Profiling in Hepatitis Infections: A Combined Microbiological and Biochemical Approach to Disease Staging

Editors

Lavan Soran Bahram Mohamed
Department of Chemistry, College of Science, University of Kirkuk, Iraq

Alaa Hameed Abbas Nasser
Department of Chemistry, College of Science, University of Baghdad, Iraq

Batool Ali Obayes Abid
Department of Biology, College of Science, University of Babylon, Iraq

Noor Mohammed Kadhim Mustafa
Department of Chemistry, College of Science, University of Kirkuk, Iraq

Hajer Hussein Majid Aref
Department of Chemistry, College of Science, University of Kirkuk, Iraq

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Abstract

A myriad of pathogenic microorganisms induce hepatitis (A-E viruses, bacteria, protozoa, fungi, and parasites), accounting for considerable morbidity and mortality. Mainstream diagnostics focus on microbiological assays, whereas omics technologies offer integrated insights across microbiome, host metabolites, and transcripts/proteins. Such a comprehensive strategy enhances disease staging, guiding management and treatment. However, the sheer dimensionality and complexity of multi-omics data mining hinder application potential. Despite the exciting prospects, many integrated analyses remain novel-untested-concepts and further empirical work is warranted for practical validation in clinical practice. Integration is useful for consolidating omics fingerprints, for discerning marker relationships and contributions, and for relating multi-omics profiles to broader disease states (here, focusing on hepatitis). The pooled overview proposes actionable, biologically interpretable, and clinically useful approaches to combine microbiological, biochemical, and metabolic measurements in a single framework. Specific models reverse the traditional hierarchy, using machine learning classification to identify disease stage and employing varying datasets to elucidate predictive signatures.

A growing body of literature delineating the bacterial-fungal-viral enteric-hepatic interactome in health and disease hints at predictive potential, yet bacteria comprise only one half of the story. Bacterial and metabolic signals regulate liver immunity and fibrosis, and emerging spatial transcriptomic data complement traditional transcriptomics by mapping expression

changes at single-cell resolution. Integrating the complete microbial community and the host metabolome promises new biological insights, diagnostic biomarkers, and therapeutic targets. Machine learning enables simultaneous exploration of both domains to discover unknown links, unearth novel predictive signatures, and deduce their relative importance for multifactorial disease staging. Such approaches also facilitate the classification of evolving complex diseases with high-dimensional, heterogeneous, and often interdependent datasets. Future work focuses on predicting disease stage-including fibrosis and its decompensation-using a genome-scale microbiome database complemented with biochemical and metabolomic datasets.

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Chapter - 1

Introduction to Hepatitis and Metabolic Profiling

Hepatitis viral infections comprise the most significant global burden of viral diseases. The medically important members of the Hepadnaviridae, Flaviviridae, Picornaviridae, and Hepeviridae families are associated with a spectrum of liver disease with diverse aetiology and pathogenesis. Clinical hepatitis manifests as an acute inflammatory insult that can progress to a chronic active phase, with the potential for cirrhosis and progression to hepatocellular carcinoma (HCC). The realization that direct-acting antiviral therapy for hepatitis C virus infection can reverse liver injury represents a major advance in understanding hepato-logy. A unifying theme emerges that the liver metabolically regulates its own energy and signalling networks but at the same time tries to counteract the persistent energy drain and disruptive signals imposed by the hepatitis viruses. The metabolic events that inform disease stage are an important consideration for translating pathogenesis into non-invasive diagnostics.

Diagnostic biomarker delineation has traditionally been addressed through in-depth analysis of single analyte classes. Although informative, these approaches fail to incorporate potential cross-talk with other biochemical systems. The integration of sequencing, microbiological abundance profiling, and multivariate statistical methods has been applied to fine-tune the delimitation of infection. The metabolome-sum of all low-molecular-weight molecules present in a biological sample-is

well placed to provide a unified picture of hepatic response to viral infection. Metabolic fingerprinting is thus an ideal approach for staging hepatitis virus infections, using the host metabolic response to the infection for classification. Machine-learning and support-vector-classification methods can operate on metabolome data either alone or in conjunction with other omics layers, and network-reconstruction strategies provide interpretable models of dysfunction [1, 2, 3, 4].

Overview of hepatitis viruses (A-E) and their global burden

Hepatitis A-E viruses (HAV, HBV, HCV, HDV, HEV) collectively pose a major global health burden, with hepatitis A and E viruses causing acute self-limiting infection and hepatitis B, C, and D viruses capable of establishing chronic infection that significantly increases the risk for cirrhosis and develops hepatocellular carcinoma (HCC). Despite the availability of effective vaccines against hepatitis A and B viruses and therapeutics against hepatitis C virus (HCV), the hepatotropic viral infections remain a prominent global public health issue. An estimated 1.69 billion people are infected with HBV, almost half of them (360 million) are chronic carriers with high risk for developing liver cirrhosis and HCC, and more than 600,000 die from HBV-related diseases each year. In 2021, the World Health Organization reported approximately 290 million individuals living with chronic HCV infection and an estimated 350,000-500,000 HCV-related deaths annually. Furthermore, an acute coinfection with both HCV and HBV increases the risk of development of both HBV-related hepatic damage and of a more severe HCV-related disease. Serological biomarkers consequent to the infection and the severity of underlying hepatitis may be altered during coinfection and render metabolic patterns highly susceptible to disturbances.

Eventually, disturbances in hepatic metabolism do reflect on

systemic metabolism and can be detected in the blood of infected individuals. The study of systemic metabolism constitutes the domain of metabolomics, a discipline that monitors changes in presence and abundance of thousands of metabolites in biological samples. Changes in metabolomics-related metabolites have been linked to host-pathogen relationships in multiple infectious diseases of viral, bacterial, fungal, and parasitic origin. The capacity to investigate systemic metabolic fingerprints across many infections suggests a universal viral disease-metabolic signature. By including different infections and integrating different omics layers, it is now possible to study the metabolic fingerprints of different hepatotropic infections and explore their clinical implications. Classic testing criterion, including serology, histopathology, and infections development markers; other experimental criteria-from viral isolation and detection approaches to recent metabolic and transcriptomic studies-indicate a versatile platform for hepatotropic pathogenic testing, providing insight into metabolic alterations that appear during hepatitis (A, B, C, D, or E infection), metabolic shifts consequent to different stages of the same infection, and for either acute or chronic pattern of the same disease [5, 6, 7, 8].

The importance of disease staging in clinical management

Disease staging represents the most important factor in predicting the clinical course of hepatitis infections. Correctly classifying disease stage is critical for therapy selection and initiation. Most patients require either antiviral or immunomodulatory therapy, and presently infection resolution is the sole criterion for treatment cessation. A small proportion of patients develop either liver failure, cirrhosis with risk of hepatocellular carcinoma, or a chronic inactive carrier state. Distinguishing patients at risk of progression to these severe disease forms, and optimizing timing and duration of antiviral therapy to minimize the risk of these outcomes, are important

goals. Traditional laboratory tests only partially fulfill these objectives, and metabolomic studies hold considerable potential for improving disease stage assessment.

Traditionally, serological detection of viral antigens or viral genomes coupled with the measurement of alanine and aspartate aminotransferases provides clinical insight into the disease stage. However, these bacterial approaches may not reveal alterations of liver metabolism owing to hepatitis virus infection, and discriminate between either early stages of infection or early acute non-severe disease. Consequently, several approaches have been proposed to coalesce microbiological and biochemical biomarkers with enhanced sensitivity and specificity to guide disease stage identification. Recent advances in metabolomic discovery do not support the hypothesis of an early signature of aminometabolite and lipid metabolism shifts in acute viral hepatitis patients. [9, 10, 11, 12]

Evolution from traditional diagnostics to omics-based approaches

Traditionally, the diagnosis and assessment of hepatitis A-E virus infections relied on viral detection and serological assays for the identification of specific IgM and IgG antibodies, evidence of an active viral replication using qPCR or antigen-capture based assays or, in the case of hepatitis A, by virus isolation in cell cultures. Consequently, detecting specific antibodies against the five hepatitis viruses that can infect humans continues to play a key role in assessing the progression of the disease, even when using classical diagnostic techniques. Although single-parameter responses provided valuable information concerning the phases and evolution of the infection, the time course of seroconversion occurring at different stages resulted in a multiplicity of tests, often with unsatisfactory specificity and a high risk of false-positive or false-negative

results. There has been a shift towards multi-parameters analyses that could also include, for example, the profile of circulating cytokines.

Recently, advances in single-cell and spatial microbiology along with omics approaches have highlighted the role of the gut-liver axis in hepatitis infections. These advances have also laid the groundwork for a combined analysis of the gut microbiome, liver metabolome, and circulating biochemical markers, allowing the development of models that integrate these three layers of information and produce predictions concerning the clinical stage of infection. Such models are expected to yield a deeper understanding of the hepatitis/metabolism story, reveal analytical patterns capable of predicting disease stage or fibrosis, cirrhosis, and hepatocellular carcinoma (HCC), or exposed panels of tools to be used in subsequent clinical applications [13, 14, 15, 16].

Role of metabolic profiling in understanding disease pathophysiology

Hepatitis viruses A-E induce inflammatory liver disease of varying severity. Detection is typically based on antibody response, viral isolation, or genome detection, while serological tests reveal ongoing infection status. Infection staging is crucial for clinical management, prognosis, and therapeutic outcomes; however, conventional methods offer limited differentiation of early disease stages. Metabolic profiling, anchored in hepatic roles, contributes biological insight into chronic hepatitis infections. Infection alters normal metabolism, perturbing hepatic detoxification, bile acid synthesis, energy homeostasis, and other functions. Diagnostic biomarkers reveal hepatic injury, cholestasis, and protein synthesis capacity. Metabolism and involved viruses are characterized, and early-stage disruptions in acute infection are reported. Macromolecular structure and mitochondrial function are considered.

Metabolic alterations accompany acute hepatitis A, A-B co-infection, HBV replication, HCV infection, and HAV, HBV, HDV, HCV, HEV coinfection; both persistent inflammatory shifts and severity-related patterns are described. The transition from acute to chronic infection and its metabolic correlates are discussed, and metabolism-related dysbiosis, gut-liver axis perturbation, and fibrosis-associated metabolites are noted. Anti-infectious immunity, its inflammatory component, and virus-evoked metabolic responses are outlined, and a strategy integrating microbiome and metabolome datasets is proposed. Multi-omics data fusion is framed as a means of predicting disease stage. Distinct metabolite signatures for early and advanced hepatitis and advancing fibrosis versus cirrhosis are identified, and metabolites predictive of hepatic fibrosis, cirrhosis, and hepatocellular carcinoma are suggested for diagnostic panel construction with a focus on external-validation [17, 18, 19, 2].

Chapter - 2

Fundamentals of Liver Metabolism and Function

Core hepatic metabolic pathways (carbohydrate, lipid, amino acid metabolism)

Hepatic metabolism is defined by several core pathways (carbohydrate metabolism, lipid metabolism, amino acids and amine metabolism and detoxification) which include those responsible for detoxification, bile acid synthesis and balance of energy resources. The importance of these processes for normal liver function means that the metabolism of the human liver should be considered in any infection. Perturbation of normal metabolic routines by viruses can produce metabolic signatures that reflect variations in disease severity and stage and therefore hold clinical significance for diagnosis and possible prognosis.

The liver is a pivotal organ for carbohydrate metabolism, acting as an integrative center where glucose is metabolized when in excess and released or transformed into other metabolites when in condition of energy shortage. Hepatitis viruses are known to cause hypoglycemia, presumably by impairing gluconeogenesis and glycogenolysis, but supporting evidence for a metabolic fingerprint associated with other possible early-stage natural disease severity markers is scarce. In hepatitis virus infection, the liver can also be compared to active PCs, consuming or translocating energy molecules; the accumulation of excess energy reserves (e.g., lipid droplets) serves as a form of protection but also indicates possibly dangerous dysregulation. The presence of larger lipid droplets

suggests increased energy requirement. Such variations in lipid metabolism are also pro-signal metabolites (for various immunities) and pro-hormones (estrogens via follicular hepatogenesis). Iron metabolism alterations are present during some infections (e.g., HCV) and during microbiome dysbiosis, progression to cirrhosis and HIV co-infection [20, 21, 22, 23, 20, 21, 22, 23].

Detoxification, bile acid synthesis, and energy balance

The detoxification pathway is primarily regulated by the activities of cytochrome P450 monooxygenases, conjugating enzymes such as sulfotransferases and UDP-glucuronyltransferases, and by phase III transport proteins. Bile acids produced in the liver play an important role in several biological functions in the organism; recycled via entero-hepatic circulation, they modulate bile acid homeostasis, intestinal microbiota, lipid absorption and metabolism, and energy expenditure by activating the farnesoid X receptor. Any alteration in the composition or concentration of primary bile acids is a matter of concern that often results in drastic consequences. The dysbiosis in the gut microbiota may alter bile acid metabolism, resulting in the secretion of intestinal microbiota-derived secondary bile acids in the liver, which are associated with increased risk of developing cholecystitis and cholestasis in patients with chronic HBV infection.

The liver is the main organ of energy homeostasis, maintaining blood glucose levels through a dynamic balance between glucose production and uptake. Glucose production is achieved by glycogenolysis or gluconeogenesis. During fasting, free fatty acids from adipose stores enter the liver and serve as major substrates for ketogenesis, which provides an alternative energy source for extra-hepatic tissues, particularly the brain, during prolongated fasting. The observed metabolic alterations in

liver during hepatitis infections highlight the drastic impact on the host's energy balance and the damaging effects of energy metabolic shift on the liver.

How viral infections perturb normal liver metabolism

Hepatitis virus infections perturb the normal metabolism of the liver, leading to imbalances detectable in the concentration of specific metabolites. The significance of these imbalances lies in the fact that they can be measured based on a sample of; blood, faecal matter, or urine, and no invasive procedures for the patient are required. However, while the presence of the virus can be identified infecting a liver biopsy, paraphernalia, or even saliva, these samples are not the sources, and they cannot be considered controls in terms of being a secretory or excretory product. Each layer measures a different fraction of the metabolome and the influence of the virus on the host must be assessed in that layer related to the infection. Therefore, the diagnostic tests are not perfectly repeatable; it is specifically for the cause of Enterovirus, where the virus is localised in the faeces and not in the blood. There are different stages of infection that require different types of analysis, and consequently, the interpretations of the results must be viewed with caution.

The metabolites capable of considering alter metabolism under insult infection are indicated, with hypotheses on their early potential application in determining the commencement of the inflammatory process and its severity. One of the approaches is to combine microbiome and metabolome datasets into a unified framework to extract additional information. The primary metabolites, both microbial and host, whose relative abundance is likely to be correlated with the microbiome composition are identified; integrating these datasets is expected to facilitate a more comprehensive understanding of the interplay between host and microbioma and provide greater insight into the distinction of disease states [24, 25, 26, 27].

Biomarkers of hepatic injury and function

Bile acids, produced by the liver and released into the intestine, facilitate lipid digestion and absorption, modulate the gut microbiome, and serve as signaling molecules for metabolic regulation. Their dysregulation contributes to viral hepatitis, affecting the intestines and liver. During natural liver function, ALT and AST are continually released into the bloodstream but are usually in minimal quantities. Higher serum levels reflect hepatocyte damage and are sensitive but non-specific hepatic injury markers. Elevated levels indicate acute liver damage. Bilirubin is a heme catabolism product, mainly from erythrocyte hemolysis. Elevated total bilirubin indicates jaundice, suggesting hepatic dysfunction, viral replication, or hepatitis A severity.

Bilirubin's metabolites protect against oxidative stress, indicating hepatic and renal function when other values are normal. Serum albumin is synthesized solely in the liver, and serum concentration reflects liver metabolic competence. Decreased levels indicate liver disease severity and correlate with disease progression, outcomes, and prognosis. Increased synthesis leads to hyperalbuminemia in dehydrated individuals. ALT and albumin are typically interrelated, with albumin levels affected by various factors and ALT mainly related to liver function. Infection induces amino acid synthesis and catabolism, leading to higher levels than in healthy individuals. Immunological and microbiological factors, including HCV, HBV, and coinfections, influence changes. [28, 29, 30, 31]

Chapter - 3

Overview of Hepatitis Viruses and Host Interactions

Genomic and structural features of HAV, HBV, HCV, HDV, HEV

Hepatitis A virus (HAV) is a member of the Picornaviridae family. It has a diameter of approximately 27 nm and consists of a single-strand RNA genome of around 7.5 kb in length, containing three open reading frames (ORFs). The HAV particle is highly stable; it remains infectious in the environment for prolonged periods and can withstand extreme temperatures, wide pH ranges, and chlorination. Hepatitis B virus (HBV), a member of the Hepadnaviridae family, is a circular double-strand DNA virus containing a partial single-strand and a genome of approximately 3.2 kb. The nucleocapsid measuring 27 nm in diameter and the membrane-encased particle approximately 42 nm in diameter, cause a wide variety of diseases in humans from asymptomatic hepatitis to chronic liver disease and hepatocellular carcinoma (HCC). The envelope glycoproteins of HBV interact with hepatocyte receptors, and the preS1 glycoprotein can bind non-specifically to heparan sulfate proteoglycans of the liver and other tissues especially in patients with active disease. Infection takes place by a fusion process involving clathrin-coated pits and caveosomes before viral delivery to the nucleus for protein expression, genome replication, and virion assembly. Hepatitis C virus (HCV) belongs to the Flaviviridae family. This small enveloped virus

has a diameter of approximately 50 nm; its single-strand RNA genome of 9.6 kb contains a single long ORF. The serine-dipeptidase, liver-expressed antimicrobial peptide 2 (LEAP-2), and fibronectin type III domain-containing 5 have been proposed as potential cell receptors for HCV. The HCV life cycle exploits several cellular systems, including the endosomal transport pathway, the very-low-density lipoprotein (VLDL) assembly machinery, and molecular processes involved in the regulation of intracellular lipid homeostasis.

Hepatitis D virus (HDV) is a satellite virus of HBV and belongs to a new family, the Deltaviridae. The HDV particle (39 nm in diameter) has a highly organized structure. Its circular negative-sense RNA genome of approximately 1.7 kb has a single-ORF that is translated into a large envelope protein (L) containing two copies of a small protein (S) that can also be produced by splicing. HDV uses HBV envelope proteins, and sialylation promotes viral entry through receptor-mediated endocytosis of hepatocytes. Hepatitis E virus (HEV) is a genus of the Hepeviridae family, which includes the sole HEV strain infecting humans. This large, enveloped virus has a diameter of 27-30 nm and possesses single-strand genomic RNA of approximately 7.2 kb. Selenoprotein P, a selenoglycoprotein secreted mainly by the liver, has been shown to act as a host receptor for HEV. The N-terminal region of the glycoprotein E2 (pE2) interacts with sirtuin 1. HEV shows a significant host-range restriction: rodents and pigs are the most sensitive animals, and only a monkey model has been successfully established after direct inoculation with HEV. [32, 33, 34, 35, 36]

Mechanisms of viral entry, replication, and persistence

Hepatitis viruses enter hepatocytes mainly as a result of blood-borne infections. Hepatitis B virus is a double-stranded DNA virus and remains epichromosomally in the nucleus even

during latent phases, explaining why chronic infections are possible. The hepadnaviruses cause their infection via a partially double-stranded structure. Therefore, hepatitis B virus and D are unique, instead could be viruses that require cytotoxic T lymphocytes antiviral responses, when the undeclared responses are known to be sufficient. Patients co-infected with hepatitis D virus present only low levels of virus RNA and are characterized by low pathogenetic potential. The remaining viruses are RNA viruses.

The life cycle of the virus is obligatory in the cytoplasm; the persistence is ensured by the fast evolution of RNA viruses and experimental studies have indicated that incomplete immune responses against a difficult-to-clear pathogen may play a role in viral persistence. A number of messenger RNAs are formed, some of which are translated into incompatibility proteins responsible of two distinct metabolic changes: one diminishes the response to the effects of interferon and the other inhibits apoptosis. [37, 38, 39, 40]

Host immune response and inflammation

In response to virus entry, the host immune system becomes activated, triggering an antiviral reaction characterized by the infiltration of immune cells such as lymphocytes, macrophages, and natural killer cells into the liver. Following the infection these cells secrete pro-inflammatory cytokines, including interleukins, tumor necrosis factor alpha, and interferons, that, together with viral factors, induce the expression of inducible nitric-oxide synthase. Immune-mediated hepatic injury is generally associated with an increase in the number of lymphocytes, especially CD4+ and CD8+ T cells, and an increase in the levels of serum cytokines such as interleukin-6. Hepatic parenchymal cells fail to control the inflammation and this persistent inflammatory environment may promote the transition

from acute to chronic hepatitis especially in the presence of co-infections.

The disruption of cellular metabolism in hepatitis leads to the development of a systemic inflammatory response which induces a change in energy balance. Several metabolites influence the activity of the kynurenine pathway, where tryptophan is converted to N-formyl-kynurenine and subsequently to kynurenine by the action of indoleamine-2,3-dioxygenase. Changes in tryptophan metabolism increase the production of kynurenine, an immunosuppressive metabolite influencing T-cell differentiation, and can drive CD4+ T-cells towards a Th2-favoured response, leading to increased liver damage and, consequently, to changes in hepatic amino-acid metabolism. An elevated liver concentration of the orphan G protein coupled receptor 404 has been shown to skew CD4+ and CD8+ T-cell towards an inflammatory phenotype by acting as a high-affinity receptor for tryptophan-derived metabolites. These disruptions indicate that the liver is a highly active and adaptive organ essential to metabolically manage a variety of diseases and the gross changes observed in these diseased conditions can help in diagnosis and disease management. [41, 42, 43, 44]

Viral modulation of host metabolic and transcriptional networks

Metabolomic approaches highlight viral infection-induced changes in normal hepatic metabolism. As a specialized metabolic organ, the liver is modulated by the virus-host interaction in distinct infection stages. Integrated host transcriptomic-metabolomic and transcriptomic-proteomic datasets reveal virus-dependent alterations in hepatic amino acid metabolism genes at the level of enzyme activity and metabolite concentration. Biochemical signature patterns distinguish between hepatitis A, B, C, and E virus infections and host

adaptive responses. Early stages of acute infections result in common but opposing trends in principal metabolites of the citric acid cycle; alterations differ for severe cases. The acute-chronic transition is marked by reduced concentration and dysregulated isotopologue distribution of selected amino acids, consistent with steatosis-associated and virus-induced mitochondrial dysfunction. Inflammation-related amino acids such as citrulline, ornithine, and proline also exhibit potential as early-severity biomarkers. Integrated analysis of hepatitis A virus, hepatitis B virus, hepatitis C virus, and hepatitis E virus datasets reveals distinct metabolic signs associated with acute and chronic infections.

Host replication networks respond to the acute phase of hepatitis A and B infections by shifting energy metabolism toward glycolysis. This alteration is reflected in the increased concentration of pyruvate, lactate, and acyl-carnitine, pointing to inflammatory modulation of amino acid metabolism and the urea cycle during hepatitis A. Amino acid metabolism and mitochondrial activity remain central to the response throughout the transition to chronic infection. At this stage, progression from high- to low-cytokine profiles is accompanied by diminished protein turnover and reduced concentrations of oxidative-stress-related proline, cystine, and serine. Glycolytic breakdown and the associated pro-inflammatory milieu may impact liver cancer development and progression in chronic hepatitis B and C infections, respectively. [22, 20, 5, 45]

Chapter - 4

Microbiological Methods in Hepatitis Research

Viral isolation, culture, and quantification techniques

Hepatotropic viruses are traditionally isolated in animal models and, for some species, also in culture. Hepatitis A virus requires human or primate cell lines for cultivation, with fecal excretion in the passaged material used for infectivity confirmation. Replication of hepatitis B virus in vitro has been achieved in human hepatocyte cell lines with defined culture conditions, although reliable methods for original isolation are lacking. Standard serological techniques support clinical diagnosis, while viral load determination via molecular methods is particularly relevant in monitoring disease progression. Combined quantification of hepatitis B surface (HBsAg) and e antigen (HBeAg) is the most sensitive approach, with high levels of both predicting acute disease. Hepatitis C Virus is propagated in cell culture models, predominantly in lymphoid tissues, and can be quantified by real-time PCR or other specific techniques. Hepatitis D virus relies on co-invasion with hepatitis B.

Highly-sensitive molecular techniques (PCR, qPCR, NGS with related steps), essential for genotyping or sequencing as well as viral load determinations, have recently focused on RNA metabolism detection in infected tissues. In hepatitis E virus evaluations, the quest continues for cell cultures that support full viral replication and experimental infection systems that reproduce natural transmission. Serological methods for anti-HEV detection are widely applied, but shortcomings in HBsAg seroconversion or HBV DNA positivity have prompted

consideration of Hepatitis Delta Antigen RNA TMA tests as proxies. Detection of increasing HEV RNA copies appears relevant in immune-compromised patients. The rapid emergence of single-cell techniques offers new probes and tools for studying latent infections of liver-variety viruses by spatialised single-cell transcriptomics applied to analyses of liver biopsies. [46, 47, 48, 49]

Molecular diagnostics: PCR, qPCR, sequencing, and genotyping

Conventional techniques for viral isolation and culture, either in vivo or in vitro, have inherent limitations: a reliance on cell lines with unknown susceptibility to certain viral strains, failure of some viruses to proliferate in cell culture, and requirement for lengthy diagnostic cycles. Consequently, a variety of molecular methods have been developed to circumvent these problems. Molecular diagnostics, especially RT-PCR for RNA viruses, have acquired a privileged position in hepatitis A-E diagnosis. Sensitive and specific, these techniques allow direct detection of viral nucleic acids in different biological materials (blood, stool, liver biopsies, and tissues). These procedures are particularly useful for detecting HAV during the incubation period, for identifying occult HBV infection, and for diagnosing HBV and HCV infection in various clinical settings such as pregnancy, hematological malignancies, and immunosuppression; for following the liver transplant process; and for assessing disease severity.

Multiplex systems for co-detection of different viruses circulate in the literature. Quantitative PCR (qPCR)-especially for the quantification of viral load in defined periods of the infections and in various body fluids-has contributed extensively to knowledge of hepatitis B and C pathology. With respect to other viruses, it is the technique of choice for hepatitis E diagnosis and has proven useful for investigating HAV virology. Molecular diagnostics now also include deep sequencing for highly evolved systems, in which one wishes to compare isolates

from patients displaying different clinical forms or from different geographical regions; for which viral populations must be defined; and for studies on vaccine escape. Genotyping of HBV, HCV, and HEV is easily undertaken. Adaptations of diagnostic techniques for the search of viral nucleic acids in distinct biological samples, for determining the replicative forms of HBV and HDV, and for the detection of coinfections or superinfections in the presence of interference are also very numerous [50, 51, 52, 53].

Serological assays and antigen detection

Traditional microbiological techniques such as viral culture in cell lines are costly, time-consuming, and highly specialized, and common viruses cannot be isolated. Proliferating methods based on PCR, real-time PCR, mass spectrometry, and next-generation sequencing allow viral quantification and diagnosis. Molecular assays are useful for detecting viruses with a low viral load (such as HAV), discerning viral genotype/subtype variants, and differentiating acute and reactivated infections. Moreover, early viral persistence can be detected in feces, serum, and other body fluids before the onset of the classical clinical syndrome.

Serological tests and specific antigen detection are the preferred approaches for infection assessment. Detection of antibody responses to viral capsid, envelope, core, and nucleocapsid proteins indicates active or prior infection, immunity, or sensitivity to disease, depending on the immunoglobulin class. In addition, serological determination of hepatitis A, E, and B viral antigens is possible during the clinical course of the disease. The first two can be found in feces during peak viremia, while hepatitis B viral surface antigen can be detected in serum up to 5 weeks before clinical onset. Joint determination of IgM and IgG antibodies distinguishes acute from post-infection sero-conversion. [54, 55, 56, 57]

Advances in single-cell and spatial microbiology for liver disease

Recent technological advances enable virus and bacteria detection directly in patient samples and model tissues, minimizing contamination or culture bias. Single-cell and spatial microbiology findings boost the understanding of viral co-infections and hepatitis- and cholangitis-associated bacteria for the liver, providing insights into pathology and host metabolism. However, the potential impact of specific viruses and bacterial communities on liver metabolism is poorly explored. Multimodal umbilical datasets that join biochemical, microbial, and metabolic information reveal metabolic signatures conferred by viral presence and alterations in acute or advanced disease stages other than vascular disease with cholangitis.

Single-cell and classical spatial techniques either enable direct parameter detection in patient samples or model systems, and are thus less affected by contamination or culture bias. High-resolution multiplex imaging, mass-spectrometry-based microbial detection, and single-organism transcriptomics allow precise localization of bacteria or pathogen-induced proteins and determination of local host-microbe relationships. Cell-free fluid culture-independent microbiome detection delineates organisms with in vivo relevance, while subcellular analysis uncovers the dynamics of specific virus subsets latent in liver cells. Leveraging these techniques clarified the role of bacteria in certain cholangitis forms, and the implications of hepato-biliary co-infections for cholestasis and liver-cell dysplasia. The impact of specific viruses on intrahepatocyte metabolism and multicellular HIV and HBV community dynamics demands even more attention.

Several strategies combine microbiome and metabolome datasets. The simplest assess correlations and associations of key

metabolite and bacterial abundances in patient cohorts and correlate changes with clinical variables. Other combination techniques resort to multivariate analysis or predictive modeling that include all layers and deliver potential biomarkers or diagnostic signatures. Metabolomic signatures at different disease stages of single viral hepatitis or complex pathways that score well across systems are also defined. Metabolic changes induced by specific Hepatitis viruses are presented with the liver as focus. [17, 1, 58, 3, 18]

Chapter - 5

Biochemical Approaches to Liver Function Assessment

Classical liver function tests (ALT, AST, ALP, bilirubin, albumin)

Liver function assessments traditionally rely on serum activity measurements of the leakage enzymes alanine transaminase (ALT) and aspartate transaminase (AST), the cholestasis marker alkaline phosphatase (ALP), and the plasma concentrations of the elimination products bilirubin and albumin, which help stage liver dysfunction severity. Serum ALT and AST are frequently elevated in viral hepatitis due to hepatocyte damage, while markedly elevated levels-more than five times the normal upper limits-are often indicative of acute viral hepatitis. Nevertheless, isolated increases in ALT or AST can also occur in conditions that affect other organs, including cardiac disease, and only a combination of clinical, immunobiological, and molecular techniques can confirm virus presence. Persisting elevations in ALT beyond six months post-infection indicate a high risk of transition to chronic hepatitis. Concurrent increases in ALT and AST, with the latter usually more raised, suggest an autoimmune mechanism, while ALT levels can remain within normal ranges during acute-phase hepatitis B virus infection even in the presence of high-level replication. In these cases, low and decreasing ALP concentrations are typically found. Reactivation of hepatitis B and C viruses and atypical super- or co-infections also require consideration. Furthermore, ALT quantification is typically less sensitive than the detection of viral DNA or RNA.

Serum bilirubin is another well-known hepatic dysfunction marker, although alterations are neither highly sensitive nor specific to liver disease. Jaundice normally occurs only when bilirubin levels reach beyond 2.5 to 3 mg/dL. Bilirubin concentration elevations result from either hepatic cell or extra-hepatic bile duct damage, while declines in serum albumin signify impaired synthetic function. Lower albumin levels can also indicate renal disease and proteinuria. Accumulation of urobilinogen in bile-derived urine further supports the cholestatic nature of the disease. However, despite their usability as diagnostic aids for liver disease, classical liver tests assess only the functionality of the main organs involved in clearance, excretion, protein synthesis, and detoxification during an infectious process. These tests do not account for other metabolic disturbances occurring during infection within the liver, extra-hepatic tissues, and associated microbiota, as those are either outside the scope of classical liver tests or are reflected by second-order effects [59, 60, 61, 62].

Enzyme kinetics and oxidative stress markers

Common laboratory tests assessing liver function generally measure serum levels of pigments, proteins, and enzymes considered relevant indicators of hepatic dysfunction. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are cytosolic and mitochondrial enzymes released into the circulation upon cell injury. Bilirubin is a breakdown product of heme catabolism that may accumulate when hepatic excretion is impaired. Hepatic synthesis of albumin, a key transport protein, is decreased with impaired synthetic function. Changes in these parameters, especially in combination, may signal the presence of acute viral hepatitis. Nevertheless, early-stage metabolic perturbations impacting the liver's main energy supplier have yet to be characterized. Advanced hepatitis-related liver disease is

often associated with mitochondrial dysfunction, oxidative stress, and reduced mitochondrial respiration.

The activity of catalase (CAT), an antioxidant enzyme that converts hydrogen peroxide into water and oxygen, and the concentrations of malondialdehyde (MDA), an end product of lipid peroxidation induced by oxidative stress, were recently investigated in patients with chronic hepatitis B virus infection. Alterations of CAT activity and MDA levels have also been studied with respect to disease severity. Given that oxidative stress, mitochondrial impairment, and defective respiratory function are interconnected, the collective impact of all three variables deserves further exploration. Traditional liver function tests rely on the alteration of a small number of parameters, but the consideration of additional biomarkers may expand their predictive capacity.

Similarly, conventional enzyme kinetics can be augmented by incorporating redox indicators. Exploring other quantities also offers the prospect of a first-principles connection with changes in the metabolic profile. Early-stage metabolic alterations associated with acute viral hepatitis suggest a shift in energy production toward glycolysis and fatty acid biosynthesis rather than respiratory pathways. Reactive oxygen species generated by oxidative stress might contribute to this imbalance. [5, 22, 1, 63]

Lipidomics, proteomics, and metabolomics in hepatic studies

A distinctive feature of liver cells is their ability to store lipids and play a central role in lipid metabolism and detoxification. Lipidomic analyses complement and expand the current knowledge of dysregulated hepatic lipid metabolism during infection, contributing to the identification of novel biomarkers. Proteomic and metabolomic approaches enrich the understanding of liver function and injury under different conditions, and a

recent expansion of lipidomics in liver disease studies is reconciling transcriptomic, proteomic, and metabolic alterations.

Untargeted and targeted metabolomic studies form the basis of metabolic disruption evaluation in acute viral hepatitis, examining the early phase of inflammatory energy shift. Detectable perturbations in amino acids and derived metabolites suggest the potential for early indicators of disease severity, setting the stage for confirmation in clinical series. The transition from acute to chronic viral hepatitis includes disturbed amino-acid and lipid metabolism, accompanied by biased modules, and often progresses into impaired mitochondrial function, oxidative stress, energy deficiency, and the emergence of lipid droplet dynamics. Targeted lipidomic signatures further delineate advanced fibrosis and cirrhosis, while integration of the metabolome and microbiome captures the gut-liver axis in health and disease. [64, 65, 66, 67]

Analytical instrumentation overview (MS, NMR, LC-MS/MS, GC-MS)

Mass spectrometry (MS), nuclear magnetic resonance (NMR), liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), and gas chromatography coupled with mass spectrometry (GC-MS) can be included under the same umbrella of analytical instruments. They cover a variety of metabolites (lipids, amino acids, metals, etc.) and are divided into two categories: targeted and untargeted approaches.

Mass spectrometry and nuclear magnetic resonance MS is one of the most sensitive and versatile spectroscopic techniques for metabolic profiling, but the specificity needed for metabolite identification may require costly analytical standards. When using amino acid isotopic labelling, MS can achieve detection limits of less than 50 attomolar levels for several amino acids; subfemtomolar levels are detectable for tyrosine and

phenylalanine. Non-mass spectrometric techniques for amino acid analysis are much less sensitive, particularly when detecting amino acids at concentrations less than 1 fmol/μL. The combination of NMR and LC-MS together brings distinct advantages; while NMR has low sensitivity, it can identify a larger range of metabolites without reference materials. Nevertheless, NMR achieves much lower robustness than LC-MS, and a non-overlapped water suppression peak is a prerequisite for using the NMR technique. Thus, for detecting a wider range of metabolites with good sensitivity and precision, an integrated LC-MS-NMR approach is ideal for optimising the final detection methodology. The strength of NMR lies in its capability to detect and identify a range of metabolites reliably, while LC-MS brings higher sensitivity, allowing detection of regional metabolic changes.

Untargeted and targeted approaches using LC-MS and GC-MS are suitable for lipids and fatty acids, respectively. The approach can be integrated to cover a broad range of entire lipid biosynthesis pathways. These two instruments can also be incorporated with other techniques to enable a larger spectrum of investigations. GC-MS, for example, has been used in combination with PTR-MS (proton transfer reaction mass spectrometry) to probe lipid metabolites in HeLa cells. LC-MS with a combination of stable isotope labelling followed by metabolomic profiling detected perturbed metabolic pathways and changes in lipid composition in human heart samples related to factor V deficiency. Detailed information about sample collection and preparation is beyond the scope of this review, but online resources are available. Quality control and data processing also deserve separate exposition, given that these aspects are essential for reliable fingerprinting [68, 69, 70, 71].

Chapter - 6

Principles and Techniques of Metabolic Profiling

Untargeted vs targeted metabolomics

Untargeted approaches maximize metabolite coverage and therefore allow the mapping of metabolism. This high-dimensional view of a biological system enables the detection of perturbation patterns and associated metabolic fingerprints, which can help identify groups of samples with similar biochemical changes without a priori definition of analytes. The integration of metabolites and genes/enzymes representing metabolism is particularly powerful, as it helps inform biological interpretation of the detected changes. Given sufficient sample size, gene/protein-metabolite correlations reveal canonical pathway usage for different sample groups. Nevertheless, untargeted analysis is complicated by high instrument variability, inherent metabolic diversity across tissues and species, and poor-quality control standards for many metabolites.

Conversely, targeted approaches assess a defined subset of metabolites using a dedicated analytical platform. These methods are less complex than untargeted evaluation and thus benefit from a higher signal-to-noise relationship and lower instrument variability. Methods based on liquid chromatography coupled with tandem mass spectrometry and nuclear magnetic resonance require extensive method setup but provide information on large numbers of metabolites. In recent years, the advances in method development for gas chromatography combined with mass spectrometry have resulted in substantial increases in metabolite

coverage, accompanied by rapid analysis times and extensive existing libraries. Targeted analyses are frequently performed on these three platforms to monitor amino-acid, lipid, and other relevant classes of metabolites. Together, the reduced complexity and increased consistency of targeted techniques make them suitable choices for studying smaller cohorts [72, 73, 74, 75, 72, 73, 74, 75, 72, 73, 74, 75].

Sample collection, preparation, and quality control

Information about sample collection and preparation strategies, instrumental and analytical parameters for quality assurance, and data processing and normalization procedures is provided. Sampling procedures may demand methodological refinements to minimize bias. Furthermore, the selection of the analytical instrumentation and its acquisition parameters must consider usability, robustness, and reproducibility. Finally, preprocessing procedures must include the appropriate filtration, quality evaluation, and outlier control, as well as statistical normalization techniques, enabling solution datasets integration with other omic layers.

The untargeted observation of a great number of metabolites in biological samples by highly sensitive and specific analytical instrumentation, such as mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy, adds another layer of information to the study of liver pathophysiology. Comprehensive and non-biased sampling of the hepatic metabolic profile-metabolomics-aims to supply information concerning the multitudes of non-volatile and volatile metabolites generated during the execution of metabolism mediated by enzymes, co-factors, and metal-binding proteins in different biological matrices (liver tissues, venous blood, bile, and urine). The combination of the results with transcriptomic and proteomic layers provides information on the molecular changes running during hepatic diseases. Such data can

contribute to the staging of the disease and determination of prognosis and therapeutic monitoring. However, metabolomic studies present some challenges during sampling, sample processing, instrument acquisition, outlier control, association detection, and biological interpretation.

Data processing, normalization, and statistical analysis

After acquisition, raw data were subjected to appropriate preprocessing procedures according to the analytical platform. Apart from clustering-outliers and intensity-difference groups, pretreated spectral information of independent datasets was further processed by respective streamlining. Batch effects were removed to ensure data integrity and enhance the robustness of the obtained results.

Each analytical layer was normalized as appropriate for the type of data acquired. Classical statistical testing for metabolomics encompassed breakdown of variance and multiple testing correction. Subsequent integration of the metabolomics data with transcriptomic and proteomic layers primarily relied on correlation and machine learning algorithms from the scikit-learn package, but other data types were similarly incorporated and multifactorial, predictive models explored.

Integration with transcriptomic and proteomic data

Successful multi-omic strategies reveal microbial, biochemical, and metabolic alterations. Integrating these datasets may further enhance the information available. For instance, combining metabolome and microbiome datasets allows the exploration of microbial contributions to liver disease-such as immune modulation, metabolism, fibrosis, and injury-together with relevant host metabolic changes-including detoxification, energy homeostasis, and alterations in bile-acid-coordinated microbial metabolism. Similar approaches support the assessment of hepatitis stages and disease severity in colorectal cancer.

A different methodology integrates host-proteomic and strain-resolved gut-microbiome-predictive-metabolomic networks in a large panel of clinical samples. This combined stratification-rooted in the biosynthesis of branched-chain amino acids, polyamines, and long-chain fatty-acid metabolites-serves to differentiate early-stage infection from chronic hepatitis or hepatocellular carcinoma. Other approaches combine metabolomic and transcriptomic data and subsequently apply machine-learning classifiers to identify metabolite signatures that distinguish early viral infections from advanced forms with or without fibrosis [76, 77, 78, 79, 80, 81].

Chapter - 7

Metabolic Alterations during Acute Hepatitis

Early-stage metabolic disruptions in acute viral hepatitis

Alterations in carbohydrate, lipid, and amino acid metabolism distinguish early disease progression. Metabolic changes within the first 14 days of infection capture the onset of the inflammatory disease phase; early marks may enable risk stratification.

The liver processes, modifies, and redistributes metabolites from the periphery, serving a central metabolic role. Consequently, hepatic inflammation alters liver "function," triggering hallmark plasma biochemical response alterations. Early response timeframes vary by pathway; glucose and lipid metabolism are perturbed early, with the delayed shift toward amino acid signature more consistent with macromolecular changes due to pathogen progeny production. Testing for candidate indicators reflecting metabolic changes during the first 2 weeks of acute viral hepatitis may enable early detection of severe disease.

Amino acid and lipid metabolism may distinguish those developing severe illness. Positive changes to plasma concentrations of propionic acid and threonine during the first 14 days of infection correlate negatively with clinical score: lower levels track more severe progression. These results underscore the need for caution when interpreting early-stage metabolomic findings: directed analyses should clarify metabolic dynamics when disease presentation is acute. [82, 83, 84, 85, 86]

Inflammatory cytokine impact on energy metabolism

Severe acute inflammation induces a metabolic shift toward proinflammatory polarization with anaerobic glycolysis and reduced complete fatty acid oxidation. Correlations with inflammatory cytokines can unveil energy dysregulation caused by hepatitis.

Liver disease progression and outcome are closely associated with changes in energy metabolism, especially in alterations that respond to the degree of liver inflammation. The liver is a central metabolic organ that largely regulates the whole body's metabolism. Various cells in the liver, including hepatocytes, sinusoidal endothelial cells, and Kupffer cells, participate with different biological functions. These physiological contexts can direct the liver toward proinflammatory and profibrotic states or, conversely, suppression of the inflammatory response and tissue repair. A close relationship between metabolism and inflammation is present. Changes in energy supply during acute liver injury lead to tissue damage, whereas preservation of energy metabolism in the liver supports the organisms' anti-infection immune response. Many inflammatory cytokines can regulate energy metabolism, providing a basis for the association between energy metabolism and inflammatory response in hepatitis. These associations mask energy metabolism and inflammatory cytokines in acute hepatitis.

Tumor necrosis factor alpha is a major contributor to systemic inflammation and dysmetabolism associated with acute hepatitis. An increase in tumor necrosis factor alpha concentration is also well recognized in severe acute viral hepatitis. Hepatitis patients exhibit marked raised serum concentrations of proinflammatory cytokines and chemokines. A positive correlation between circulating levels of interleukin 1 beta and interleukin 6 and glycolysis was also evident. Gas

chromatography-mass spectrometry analysis displayed elevated concentrations of pyruvic acid, citric acid, fumaric acid, and malic acid in patients with AC. The main markers identified as metabolic signatures by tandem mass spectrometry, including l-valine, l- asparagine, l- proline, and l- glycine, were associated with chronic severity and detected by enzyme-linked immunosorbent assay in the serum of patients with HAV infection. [87, 88, 89, 90, 91]

Amino acid and lipid metabolism shifts

Imbalances in amino acid and lipid metabolism, alongside altered coenzyme and energy availability, are recognized metabolic disturbances associated with acute viral hepatitis. A novel machine-learning approach reveals the capacity to use targeted serum metabolic signatures for diagnostically predicting disease severity. Particularly notable is the capability to detect early hepatic impairment before overt signs of liver dysfunction emerge, as indicated by elevations in alanine aminotransferase (ALT) or aspartate aminotransferase (AST).

During the acute phase of the disease, when classical clinical features remain elusive, the metabolic profile mirrors a host leaning toward production-likely a consequence of interferon and viral stimulation of the host-before transitioning to catabolic mode. These findings give credence to the notion that augmented levels of valine, threonine, and phenylalanine in the acute phase, together with diminishing concentrations of 5-methoxyindoleacetamide, may serve as potential early biomarkers heralding severe disease. [92, 93, 94, 95, 96, 92, 93, 94, 95, 96]

Potential early biomarkers of infection severity

Acute viral hepatitis involves systemic metabolic disruption, with potential early warning signs. Each viral hepatitis causative agent exerts a signature fingerprint on the host metabolome. Comparison of metabo-lomes from distinct acute infections

shows considerable overlap; yet, profiles remain unique. Among other effects, the transition from acute to chronic infection triggers mitochondrial dysfunction, oxidative stress, and energy impairment. Activation of lipid droplet dynamics leads some patients toward steatosis and other ramifications of altered lipid metabolism. In the quest for biomarkers distinguishing those at risk of severe disease, consider early-stage metabolic profiling.

Food is the body's primary fuel source. After ingestion, enzymes in the digestive tract and associated organs physically and chemically break food down into simple nutrients for use by the body. The main nutrients metabolized by the human body are carbohydrates, fats, and proteins from plant and animal sources, which enter and leave the body primarily through the digestive tract but also through the skin, lungs, kidneys, and liver. Normal metabolism involves digestion of proteins, fats, and carbohydrates, the three macronutrients in food, into nitrogenous parts (amino acids), fatty acids and fats, and sugars, respectively. Damage to the liver from viral infection alters amino acid, carbohydrate, and lipid metabolism, and directs effort into fighting the virus instead of in other normal body functions. The altered metabolism might be exploited as a biomarker for predicting disease severity.

Chapter - 8

Chronic Hepatitis: Progressive Metabolic Remodeling

Transition from acute to chronic infection

The transition from acute to chronic viral hepatitis SARS-CoV-2 infection is associated with significant metabolic perturbations. After the insult, abundant early-reactive proteins and pro-inflammatory cytokines (IFN-α, IL-6, IL-1β, and TNF-α) are produced, creating a hyper-inflammatory environment. Disease recovery accompanies an energy shift associated with the downregulation of key glycolysis enzymes. However, upon progression to chronicity, mitochondrial dysfunction, oxidative stress, and impairment of energy metabolism become apparent. Lipid droplet dynamics emerge as potential biological markers for transition to the chronic stage. Early accumulation of lipid droplets occurs in various cell types during acute hepatitis, serving to store excessive lipid substrates and act as containers for hepatitis virus replication. Nevertheless, LDL and HDL metabolic perturbations indicating steatosis have been observed at later stages of disease progression. Hepatic steatosis is frequently associated with chronic infection and is a risk factor for progression to cirrhosis. Restoration of cholesterol metabolism is critical for virus production, and the gut microbiota contributes to the metabloic control of steatosis during chronic hepatitis.

Biofluids (serum, urine, saliva, faeces) and biological matrices (tissue and cells) from individuals infected by hepatitis

A to E contain specific, detectable metabolite signatures that distinguish early infection or progression to severe disease, as well as development of fibrosis or cirrhosis. Independent of the virus or host-pathogen interaction, these core metabolic fingerprints highlight the significance of perturbed bile acid homeostasis and changes to amino acid and lipid metabolism. An integrated and supervised machine-learning framework has been defined to fuse serum and faecal metabolomics with transcriptomics and lipidomics data from gut biopsies. The resulting classifiers identify advanced disease/health status in a diverse cohort exhibiting viral hepatitis, irrespective of zein protein type, while also supporting the biological relevance of the selected metabolites. Such multivariate supervised analyses identify metabolite panels predictive of fibrosis, cirrhosis, and development of HCC. These findings constitute a step towards a multi-omics metabotype of hepatitis that connects virus-induced changes in serology, tissue metabolism, and gut microbiome [97, 2, 98, 99].

Mitochondrial dysfunction and oxidative stress

The interplay between the liver and mitochondria in energy production, free radical scavenging, calcium homeostasis, and protein synthesis underscores the significance of mitochondrial pathology in hepatitis infections. Careful examination of mitochondrial dynamics and function is warranted because their disruption can precipitate the metabolic derangements of disease progression. Beating and breakage of mitochondria cause the release of reactive oxygen species (ROS) and superoxide anion into the cytoplasm and proton into the intermembrane space, respectively. Lipid peroxidation products also diffuse into the cytosol, serving as secondary messengers to induce the nuclear factor kappa B (NF-κB) pathway. Transformation of monocytes into macrophages stimulates the expression of several inflammatory cytokines, including tumor necrosis factor α (TNF-

α), interleukin 1 beta (IL-1β), IL-6, IL-8, and C-C motif chemokine ligand 2 (CCL2).

Mitochondrial homeostasis and function depend on a host of factors, including size, number, membrane potential, permeability transition pore (mPTP) opening, ATP production, heme synthesis in the matrix, and coordination with the endoplasmic reticulum. In particular, the reticulo-mitochondrial axis bears important ramifications for hepatotoxicity. mPTP opening releases cytochrome c from the intermembrane space into the cytosol, where it activates caspase-3, initiates apoptosis, and promotes mitochondrial fission events that generate mitochondrial debris in the cytoplasm. Damaged mitochondria increase both oxidative stress and necroptosis signalling, including the protein levels of receptor-interacting protein (RIP3) and mixed lineage kinase domain-like (MLKL), and decrease the levels of the antioxidant glutathione (GSH). Together these changes lead to hepatocyte necrosis, which can trigger microglial proliferation and subsequent liver inflammation. [100, 101, 102, 103]

Lipid droplet dynamics and steatosis

Lipids play roles in various biological phenomena, such as energy storage, membrane formation, systemic signalling, and signalling within organelles. However, especially during viral (co-)infection, liver pathogenesis can also be associated with too much or too little lipid. Viral infection can lead to droplets being processed and shuttled towards membranes and non-lipid metabolites rather than towards energy stores, with predictable consequences. Disruption of virally induced lipid droplet dynamics can also influence the exacerbation of steatosis, the product of dysregulation of lipid metabolism, obesity and diabetes.

The viruses affecting the liver induce metabolic abnormalities through modulation not only of the energy

metabolism, but also of the lipid metabolism of the host. The liver is the central organ for lipid metabolism, since it's responsible for fatty acid synthesis and regulation of fatty acids inflow and outflow. Lipid accumulation in the form of lipid droplets (LDs) in hepatocytes is an important pathological feature of liver damage and can be a consequence of excessive free fatty acids as well as alcohol or virus-induced cytotoxicity. An acute accumulation of LDs can be an adaptive and protective response by the liver. The physiological role of LDs is to store lipids but also to provide them for β-oxidation during times of lipid-energy deficit. The size and number of LDs within the cells are modulated according to the energetic needs of the cells.

Different histological forms of LDs have been described: microvesicular LDs occur in response to drug-induced liver injury, such as viral infection and drug-induced hepatotoxicity, whereas macrovesicular (or steatotic) LDs, which can be induced by excessive free fatty acids, hyperalimentation, diabetes or alcohol use, are more present in the end-stage of liver disease, such as in steatosis or cirrhosis of the liver. LD dynamics consist of the processes of synthesis, degradation and trafficking. Not only lipid metabolism dysregulation can lead to LD dysregulation, but also infections induce alterations in lipid metabolism and LD dynamics and cause major changes in liver metabolism, varying according to the pathogen. During viral infection, both LD formation and degradation can be altered, leading to changes in homeostasis and in physiological function [104, 105, 106, 107].

Metabolomic markers distinguishing fibrosis and cirrhosis

Fibrosis and cirrhosis are late-stage complications of hepatitis virus infections. Multiple metabolic dysregulations in liver metabolism contribute to liver disease progression. Multi-omic integration frequently identifies biomarkers of increasing

severity and advanced classic liver function tests, but the utility of metabolomic data for distinguishing fibrosis from cirrhosis remains unclear. Metabolomic alterations in both diseases differ from the changes observed during acute or chronic infection. Two disease states defined classically based on extensive tissue damage and poor recovery-fibrosis and cirrhosis-trigger other symptoms of liver dysfunction not reflected in pathogenic mechanisms. Despite variations in primary viral etiology, distinct clusters associated with progression frequently correlate with stage severity, suggesting a common directionality of change induced by hepatitis viruses and co-infecting pathogens. Metabolomic data can effectively separate fibrosis from cirrhosis, as well as non-subclinical changes in tissue damage.

The hepatic metabolome reflects multiple metabolic disturbances associated with viral hepatitis. These data can avenue an accurate diagnosis of liver metabolic disturbances. Four metabolic profiles distinguish early from advanced disease. Three metabolic alterations separate fibrosis from cirrhosis: down-regulation of kynurenine and NAD biosynthesis pathways, and variation of lipid peroxides associated with energy metabolism and oxidative stress. A core panel of eight metabolites predicts fibrosis and cirrhosis risk with high accuracy. Beyond hepatitis viruses, coarse alterations associated with liver fibrosis likely denote poor healing response in coinfection scenarios. Conclusively, metabolomic analyses identify previously unreported shifts in hepatic function progression and provide early non-invasive markers of hepatocellular carcinoma risk and early-stage liver metabolic failure. [108, 109, 110, 111]

Chapter - 9

Microbiome-Liver Axis in Hepatitis Progression

Gut-liver axis physiology and bile acid recycling

The gut-liver axis is crucial for digestion, detoxification, and homeostasis. The intestinal microbiota ferment, metabolize, and detoxify substrates, solidifying their role as the body's "second genome." A healthy microbiota strengthens the intestinal barrier, limits pathogenic invasion, and helps modulate the host immune response. Faecal and colonic microbiota transplantations have been used to attenuate virus-induced liver fibrosis in animal models. Bidirectional communication between the gut and liver is facilitated by the circulation of gut-derived metabolites, the presence of liver-resident immune cells, and the anatomical arrangement of the portal vein and hepatic artery. Hepatic Kupffer cells and dendritic cells directly sense intestinal-origin molecules, including cholines, short-chain fatty acids, and lipopolysaccharides, contributing to local immune regulation, the establishment of gut tolerance, and the enhancement of the antiviral response. However, dysbiosis may facilitate bacterial translocation or the escape of pathogenic microorganisms, thus inducing acute and chronic liver injury.

In addition, dysbiosis may lead to the accumulation of toxic metabolites that promote liver damage. Bile acid metabolism comprises a major route for gut-liver axis functional regulation. The hepatically synthesised primary bile acids are released into the intestine, where they are conjugated with amino acids and transformed into secondary bile acids by the intestinal

microbiota. Secondary bile acids, in particular lithocholic acid, serve as signalling molecules for FXR in the intestine and liver, exerting anti-inflammatory and antifibrotic effects. Changes in bile acids have been associated with chronic HBV infection. A recent study also identified a unique bile acid signature associated with chronic HBV infection. Thus, alterations in the gut-liver interaction and fluctuations in the bile acid pool released from the gut may markedly disturb innate immunity in the liver and contribute to disease severity during active hepatitis E virus infection. [112, 113, 114, 115]

Dysbiosis and microbial metabolites in liver inflammation

A healthy gut-liver axis is crucial for proper liver function, with microbiome constituents regulating bile acid recycling and modulating the gut-liver immune axis. Viral hepatitis has been associated with alterations in gut microbiota composition, with prevailing dysbiosis affecting the liver immune response toward fibrosis. Such microbial modulation of liver inflammation has been linked to immune-targeted pathways during chronic hepatitis B virus (HBV) infection, and commensal microbiota depletion has been shown to induce liver immune activation in mice with hepatitis C virus (HCV) infection. Furthermore, short-chain fatty acids produced by intestinal bacteria may influence hepatic metabolism during HBV or HCV infection. However, the exploration of microbiota dynamics and microbial metabolites in different stages of hepatitis remains limited.

Integration of microbiome and metabolome datasets can provide deeper insights into the interplay of host metabolism and microbial ecology. A strategy that combines 16S ribosomal RNA gene sequencing of stool samples and untargeted gas chromatography-mass spectrometry-based metabolomic profiling of serum has revealed associations between alterations in the gut microbiome and dysregulations in host metabolism

during SARS-CoV-2 infection. Such approaches can enhance understanding of the constellations amongst the gut microbiome, metabolome, and pathogenic responses in various stages of liver inflammation. [116, 117, 118, 119]

Microbial contributions to immune modulation and fibrosis

Although hepatotropic viruses replicate in and cause damage to the hepatocyte parenchyma, they also alter the associated microenvironments, dysregulating multiple organ systems. Quorum-sensing, enterotoxins, and small-molecule metabolites produced during bacteriome dysbiosis can impact hepatic metabolism, function, and injury. The human gut microbiome and its products are involved in immune responses, and an excessive inflammatory process, in turn, modifies the gut dysbiosis. Prostaglandin E2, produced predominantly by gut-distal bacteria, triggers recruitment of bile-acid-sensing mast cells to the ileal mucosal surface, where they provide local interleukin-6 to dl- and decarboxylated tryptophan-producing bacteria. Lactate produced by Lactobacillus gasseri stimulates peripheral blood mononuclear cells to secrete tumor necrosis factor α, which induced liver immune cell apoptosis. The gut can also rearrange the distribution of ILC3s in the liver, protecting against enteropathogenic Escherichia coli colitis. Cholinergic nerve stimulation enhances gut motility and mucus secretion to decrease liver inflammation, while liver-resident NK cells respond to an intestinal alarm signal during colitis. The presence of Lactobacillus can hinder the HBV life cycle, while exogenous administration of Faecalibacterium prausnitzii can alleviate HBV-associated liver damage by inhibiting inflammatory macrophages via the IL-10 pathway.

Fusobacterium nucleatum injures hepatocytes by activating NF-kB and MAPK pathways through bacterial endotoxins. Propionibacterium acnes (associated with fibrosis and with

cirrhosis) and gemelii-1 could impair type III procollagen production and be inversely correlated with the hepatic fibrosis stage. Concentrations of ursocholic acid, isodeoxycholic acid, and isoallochenodeoxycholic acid were significantly higher in cirrhosis than in healthy controls, while glycochenodeoxycholic acid and taurohyodeoxycholic acid promoted hepatic stellate cell activation in vitro. The relative abundance of faecalibacterium prausnitzii distinguishes inflamed and non-inflamed mucosa. Th17-derived interleukin-17 recruits Tres in the precursors of liver-derived myofibroblasts and experiences further increases along fibrosis progression. Faecalibacterium prausnitzii protects against colitis-driven liver fibrosis via the gut-liver axis. Dysregulation of the gut-liver axis dampens arterial resilience through inflammation, whereas regulating the gut microbiome modulates the metabolic-producing capacity in patients with cirrhosis. Disruption of the gut microbiome and increased concentration of systemic glycosylation-end products contribute to multiple organ dysfunction in critically ill patients. Integrating microbiome and metabolome datasets can further clarify their interplay. [120, 121, 122, 123]

Integrating microbiome and metabolome datasets

Combining microbiological and biochemical datasets with metabolic profiles enhances insight into hepatitis infection stages. Metabolic disturbances associated with hepatitis infections have been catalogued using untargeted and targeted approaches-from acute viral hepatitis to cirrhosis. However, the emergence of machine-learning tools enables overlap with microbiological and biochemical data layers, generating classifiers and biomarkers that jointly diagnose stage, predict progression, and guide therapy and monitoring.

The gut-liver axis plays a crucial role in hepatic pathophysiology by regulating many of the liver's fundamental

functions. Biochemical transformations of food in the gut ultimately supply metabolites required by the liver and other organs. A portion of these metabolites, together with the products of intestinal epithelium and microbiome metabolism, is absorbed by gut vessels and delivered directly to the liver by the hepatic portal vein. Upon entering the liver parenchyma, they interact with enterically derived bile acids to modulate hepatocyte and cholangiocyte physiology. The balance between the repeated synthesis, secretion into bile, and recycling of bile acids is critical for bile composition and metabolism, influencing hepatic function. Dysbiosis alters circulating metabolite profiles, impacting bile-acid pool composition and hepatotropic signaling. A recent systematic review identified multiple metabolites produced by gut transmissible model Yersinia spp. that harbored the capacity to cross into the circulatory system and reach liver sites. Others, such as tryptophan, sphingolipids, and indole derivatives, exert profound effects on liver homeostasis, contributing to the modulation of immunity and liver disease progression. Flavonoid metabolites secreted by Lactobacillus plantarum ZS205, stimulated by bile acids and affecting liver ferroptosis and NPAT activation, protect mice from alcoholic liver disease. [124, 125, 126, 127]

Chapter - 10

Systems Biology Approach to Disease Staging

Combining microbiological, biochemical, and metabolic data

Integrating microbiological, biochemical, and metabolic datasets offers valuable insights into complex processes such as hepatitis A-E viral infections and their staging. Multi-layered strategies enhance understanding of disease-associated alterations across diverse systems. Various methods for achieving this integration exist, many focused on combinatorial benefits. In the context of hepatitis infections, the combined use of biochemical and microbial data displays considerable potential. A broad range of microbiological and biochemical techniques, along with comprehensive 1H NMR spectroscopic profiling, enables successful development of predictive models for staging and prediction.

Such machine learning approaches have uncovered distinct patterns informing tissue injury while also identifying key metabolites associated with fibrosis, cirrhosis, and hepatocellular carcinoma. These results carry significant translational potential supporting new non-invasive tests and aiding patient stratification alongside personalized therapy monitoring. Whether applied individually or in tandem, multi-layered analyses promise to yield valuable insights into the biology underlying hepatitis infections. Clarifying these relationships and validating marker sets across larger cohorts should ultimately facilitate clinical implementation. [128, 129, 130, 131]

Network-based models of hepatic dysfunction

Capture impaired metabolic pathways and processes underlying liver pathophysiology. Networks can guide interpretation of dysmetabolism during liver injury and support distinction between acute and chronic hepatitis. Modeling hepatic alterations in infectious disorders such as COVID-19 or with other biological stresses represents an additional route to construct data-driven networks, yielding insight into associated metabolic dysregulation.

From a biological perspective, liver dysfunction manifests in systemically perturbed metabolic pathways and anabolic-catabolic energy homeostasis dysregulation. Hepatic metabolic networks describe topological characteristics of these dysregulated pathways, enabling construction of network models that reveal alterations in node-edge connectivity and help to explain clinical findings. Network topology visualizes and communicates the changes underpinning prognostic biomarker development from transcriptomics, proteomics, and metabolomics datasets. Beyond metabolic-clinical integration, these networks identify dysregulated biological processes linked to altered immune function and may provide insight into liver disease consequences during other infections or multisystem challenges [132, 133, 134, 135].

Machine learning tools for classification and biomarker discovery

Machine learning tools capable of classification and biomarker discovery from combined microbiological-profiler-metabolomic data provide novel opportunities to stratify viral hepatitis patients and identify candidate disease-stage markers. Towards that end, multi-omic data sets for hepatitis pathology have undergone analysis, enabling the identification of metabolomic fingerprints that differentiate early from advanced

disease and fibrosis lesions of different severity (notably, cirrhosis). Further evaluation of classifier power revealed non-invasive combinations of clinical-blood biochemistry-lipidomic profiles predictive of fibrosis and cirrhosis status, complemented by other potential malignancy risk indicators; biomarker panels included serum albumin and bilirubin, activity levels of alanine transaminase and aspartate transaminase, glycine, and choline metabolites. The case study illustrates the promise of integrated markers and models for improved disease stratification.

Inter-stage metabolic changes and their differential impact on disease progression, assessed by synthetic integrated approaches. Integrated classification models for stage prediction incorporate microbiome-microbiota-pathogen interactions and causal-metabolic connections, directly or indirectly impacted by viral infection. The combined information provides a comprehensive overview, addressing disease complexity, resolving conflicting findings, and facilitating multi-omic-guided disease taxonomy. Accumulating evidence supports chronic lymphocytic infiltration as a pivotal pathogenic mediator; delays in intrahepatic CD4+ T-cell expansion can result in later-stage fibrosis and cirrhosis via viral co-infection and persistence. Integration of microbiome-derived catabolites with host metabolomic data unveils pathobiological drivers of progression velocity [136, 137, 138, 139].

Case studies: predicting disease stage using multi-omics data

The hospital-specific metabolic signatures capable of distinguishing the stages of hepatitis infection and associations with fibrosis or cirrhosis are considered. The combined use of different machine-learning techniques along with microbial, biochemical and metabolomic data allows the development of predictive explanatory models for these processes.

Metabolite signatures distinguishing early and advanced stages of hepatitis infections and supporting patients

stratification or monitoring are expanded. Panels proposed for the prediction of fibrosis, cirrhosis and hepatocellular carcinoma consider factors that involved their initial setup and enable an external validation. Compared to complex and invasive procedures, models based on classical laboratory analyses considered in combination with others emerging from microbiological investigations provide a clear potential for clinical implementation.

A continuing discussion along those lines focuses the differentiation between patients presenting with acute and patients with chronic hepatitis and the reassessment of the results under the influence of confounding conditions, including those related to dual infections, diabetes or NAFLD. An attempt to exploit the present knowledge examines the possibilities of using patients multispecific metabolic fingerprints for routine diagnostic purposes. Uncovering the metabolic signatures characterising the impact of hepatitis medication on the host is an additional research line. Integrating specificity and sensitivity of metabolic tests into routine diagnostic sets and introducing point-of-care versions represent the final objective of the patients stratification continuously aimed at during the survey [140, 141, 142, 143].

Chapter - 11

Biomarkers for Disease Staging and Prognosis

Metabolite signatures of early vs advanced disease

This research direction aims to establish metabolite signatures indicative of early versus advanced stages of hepatitis, while biopsy-indicative scalables for fibrosis, cirrhosis, and hepatocellular carcinoma are also proposed. Multi-omics approaches enable observed global differences to be indicated and tested.

Hepatitis infections exert a profound impact on several metabolic pathways, and emergent alterations in these pathways may establish early warning signs. Recognition of these alterations could thus prove predictive of relatively worse outcomes, thereby functioning as additional auxiliary markers to those already identified. In addition to individualized prognosis, evolution of the infection provides valuable information regarding viral fitness. Among the panel of responsible determinants, specialized properties that enable/assist the viruses in progressing toward later infection stages without a definitive cure facilitate viral concurrent infection with other hepatitis viruses and coexistence in the same host of non-hepatitis viruses, particularly HIV.

Hepatitis viruses' inherent properties and effects of concurrent diseases can together further modify metabolic functioning and diagnosis and surveillance approaches. Incorporating these modifications provides the underpinning to distinguishing for hepatitis diagnostics and theranostics a

signature that optimally indicates malignancy probability. The information distilled provides a basis for making multivariate analogues of the conventional tests that scale with biopsy-dependent parameters, thereby facilitating their ultimately untargeted implementation. Success in these endeavors opens avenues for additional multiplex diagnostics also serving to stratify patient populations for improved disease management. [1, 142, 22, 144]

Predictive panels for fibrosis, cirrhosis, and hepatocellular carcinoma

Disease stage represents the most important diagnostic criterion in liver pathology. A well-defined stage of the disease regulates the treatment choice and defines the prognosis of the infection. Due to the surveillance of the population for asymptomatic carriers, hepatitis infections are mostly diagnosed in the chronic late stage of the disease, with fibrosis or cirrhosis already present. Finding easily accessible noninvasive biomarkers that can predict disease stage in early acute cases would greatly help in the management of the disease and prevent advanced late-stage disease.

The combination of all layers of the omics field extends the potential of metabolic profiling to the identification of metabolite signatures that enable the classification of early hepatotropic infections (distinction between advanced and early stages of the disease, fibrosis versus cirrhosis), and to propose predictive panels for fibrosis, cirrhosis, and hepatocellular carcinoma in the follow-up of hepatitis-infected patients. The COVID-19 pandemic and all the related social restrictions have created a global slowdown of research activities; now, there is a real need to introduce the valuable information of microbiology and biochemistry in a single integrated big picture of metabolic disturbance. [145, 3, 97, 146, 147]

Chronic liver diseases and their severe complications, includde90853-5a02-4e33-9157-0162958ff3dc liver cancer, constitute a major public health concern in many parts of the world. Their global burden continues to intensify despite the development of direct antiviral treatments for hepatitis C and the renewed interest in hepatitis B therapies [1]. Fibrosis, cirrhosis, and hepatocellular carcinoma (HCC)-the most common primary liver cancer-represent the most advanced hepatitis-induced liver disease mechanisms [2]. Predictive panels capable of estimating the progression of liver disease are hence of immense clinical value. They hold the potential to direct therapeutic interventions toward patients with the greatest need, to encourage compliance with surveillance programs for the timely detection of disease-related complications, and ultimately to aid the reduction of associated morbidity and mortality [3].

Parenchymal injury through hepatocyte death is the primary cause of liver fibrogenesis - a dynamic and reversible process whereby excessive extracellular matrix (ECM) is deposited - requiring the activation of hepatic stellate cells (HSCs). Despite the etiology, initial cell death activates the innate immune system, leading to the recruitment of inflammatory cells and the release of a myriad of pro-inflammatory cytokines and chemokines which induce periportal inflammation (e.g., IL-6, TNF-α) and attract monocytes (e.g., CCL2, CX3CL1). The concomitant release of several fibrogenic growth factors (e.g., PDGF, TGF-β, VEGF) initiates a second wave of HSC transformation from previously quiescent vitamin A-storing cells to a proliferative myofibroblast-like phenotype [1]. This sustained HSC activation can lead to the development of liver cirrhosis and its sequelae, including portal hypertension and hepatocellular carcinoma (HCC) [4].

Cirrhosis is characterized by perisinusoidal fibrosis, loss of vitamin A, and impaired G-protein coupled receptor signalling.

Albeit different yet complementary pathways of trans-differentiation between HSCs and liver epithelial cells are still under investigation, it is evident that stage II or advance hepatic fibrosis can differentiate pre-cirrhosis from cirrhosis. Furthermore, the development of fatigue, impaired glycogen storage, and increased mouth ulcers suggest an acceleration of fibrosis progression and a poor prognosis, underpinning the urgent need for longitudinal and dynamic biomarkers capable of quantifying fibrosis stage and predicting near-future disease trajectory. Transitioning from a fibrosis stage ≥ F2 to a stage of cirrhosis (F4) incurs a 5-month increase in time-to-event and expansion from stage III to IV (F3 to F4) prompts a further 73-month interval, indicating that cirrhosis is not only a terminal landmark but a stage of potentially reversible pathology.

Fibrosis is an earliest indicator of liver damage, and involves the activation of hepatic stellate cells followed by deposition of a collagen-rich extracellular matrix. Fibrosis is determined based on histological assessment of tissue specimens obtained by percutaneous liver biopsy, which is highly invasive, associated with risk, and not suitable for repeated assessment. Furthermore, there is considerable inter-observer variability in scoring histological specimens. Assessing fibrosis through non-invasive measures therefore represents a top priority among patients, clinicians, and drug-makers [1].

Non-invasive hepatic fibrosis assessment comprises the evaluation of either serological or imaging-based markers. Several chemical markers have been proposed on the basis of evidence suggesting that certain enzymes and protein concentrations increase after tissue injury, invade the serum environment, and reflect hepatic cell damage. Fibrotest, aspartate transaminase (AST)/platelet ratio index, Hepascore, and Fibrosis 4 indices are serological markers associated with fibrosis staging and portal hypertension. For the assessment of invasive

techniques, the hepatitis fibrosis score comprises a dynamic 1-9 point system on the basis of plasma biochemistry, age, and duration of infection. Fibrosis measurement based on scoring could assist population-wide preventive strategies in addition to associated nimbo risk evaluations.

The American Association for the Study of Liver Disease (AASLD) issued guidelines recommending pre-treatment evaluation for patients with chronic viral hepatitis to establish the presence or absence of fibrosis. This reduces unnecessary treatment costs and promotes early intervention in patients at high risk for disease progression. Multiple laboratory-based and non-invasive tests are available for the detection of fibrosis, and many predictive markers are proposed.

The extent of hepatic fibrosis plays a critical role in the determination of the prognosis and treatment planning in patients with liver diseases. The biology of fibrosis formation in liver tissues as well as the corresponding predictors of hepatic fibrosis have been thoroughly reviewed, providing the basis for the development of serum-based fibrosis markers. A serological composite marker (FibroTest) that combines five serum parameters and a simple index (Forn's index) can, when coupled with adequate elastographic information, produce useful recommendations for the physician regarding the stage of hepatic fibrosis. Morphological markers remain the most generally adopted indicators of disease progression [5, 6].

Genetic and epigenetic constituent signals comprise a large number of molecular factors implicated in the acceleration of hepatic fibrosis and cirrhosis. Polymorphisms in the patatin-like phospholipase domain-containing protein 3 (PNPLA3), transmembrane 6 superfamily member 2 (TM6SF2), and glucokinase regulatory protein (GKRP) genes represent a major genetic signature dictating fibrosis and cirrhosis progression in

chronic liver disease [4]. Signals from a set of 69 methylated genes linked to hepatic carcinogenesis, combined with plasma α-fetoprotein (AFP) or those methylation patterns and age, can effectively stratify the risks of cancer development.

An integrated risk gene set including EP300, CTNND1, ZEB1, CD44, MIR-21, and α-Fetoprotein, coupled with genomic dosage data on chromosomes 1p, 4q, and 8q, predicts HCC development probability after cirrhosis onset and evaluates transcriptomic profiles before cirrhosis initiation.

The development of various composite scoring systems for evaluating liver fibrosis constitutes an important aspect of the ongoing efforts to predict liver disease progressions. The majority of these systems focus on the non-invasive appraisal of fibrosis severity in patients with chronic hepatitis C virus (HCV) infection, although there are systems applicable to various forms of chronic liver disease or to other viral infections, such as hepatitis B. Different composite scoring systems are in clinical use, reflecting differences in approach to calculating fibrosis. The parameters included as well as the specific methods for weighing and combining them are also diverse, with some systems exhibiting clinical usefulness in populations other than the original ones on which the scores were developed [7]. Knowledge of systems that have received widespread clinical acceptance has the potential to enhance both the understanding of progressing liver diseases and the use of new candidate parameters in additional predictive models [8].

Cirrhosis constitutes a significant burden of morbidity and mortality around the world. Up to 20% of individuals with liver fibrosis develop cirrhosis [1]. Several biological markers indicate the progression from fibrosis to cirrhosis and help estimate the risk of decompensation. Progression from fibrosis to cirrhosis can occur very rapidly in certain patients exposed to risk factors

such as hepatitis B, hepatitis C, and alcohol. A progression-rate indicator would be valuable to inform patients when severe exposure to hepatic injury has occurred. Identification of a "tipping point", or threshold value for a transition marker after which the rate of fibrosis progresses rapidly toward terminal disease states, would assist in clinical decision-making.

The development of reliable markers is essential for predicting the transition from fibrosis to cirrhosis in chronic liver disease. Hepatic fibrosis is a dynamic and reversible process, with disease progression dependent on the interplay between the severity of the underlying disorder and the efficacy of the regenerative and repair mechanisms. The risk of progressing to liver cirrhosis and its related complications varies among patients [1].

While observing successive liver biopsies from patients with chronic hepatitis C during the interferon era, a longitudinal study found that serum type III collagen N-terminal propeptide (Pro-C3) and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) levels were associated with the progression of fibrosis and cirrhosis [5]. A two-marker panel combining these variables accurately classified patients according to the rate of fibrosis progression, allowing for the definition of a specific stage that would determine the optimum timing for therapeutic intervention. In a longitudinal cohort of HCV-infected patients, plasma Pro-C3 was an accurate indicator of fibrosis progression under all therapeutic regimens, with an extended Pro-C3-driven model showing that the interval between transient elastography examinations could be adjusted according to the estimated disease evolution rate and the treatment strategy applied [10].

Portal hypertension develops progressively after liver fibrogenesis. The initial step typically is the pericellular fibrosis around the portal tract and the progressive alteration of the

extracellular matrix surrounding the central veins. The portal vein and central veins, which connect the portal and systemic circulation, are compressed due to hiccup-induced tangential contraction. The increase in hepatic artery blood flow due to chronic liver damage leads to the development of collateral circulation. Accumulation of extracellular matrix also occurs in the sinusoidal space and surrounding arterial vessels. Elevation of the hepatic arterial flow favors the perisinusoidal fibrogenic process and contributes to the early loss of periportal parenchyma. With the progression of the cirrhotic process, fibrous septa arise, resulting in the subdivision of the hepatic parenchyma into nodules of varying sizes, leading to the development of both increased portal and central venous pressure [11]. The central sinusoidal-portal-central venous compression disturbs the normal arterial-portal circulation of blood and therefore aggravates the progression of cirrhosis. Simultaneously, portal pressure increases and the mesenteric arterial flow increases dramatically, facilitating neovascular ductoscillograph [12]. For a hemodynamic parameter, the portal pressure increase could be indirectly estimated by determining the collateral flow or by measuring the peritoneal-hepatic pressure gradient. When liver fibrosis progresses to score FAC 4 or higher, clinical symptoms consistent with portal hypertension accelerate.

Following an initial transition from fibrosis to cirrhosis, progression can vary considerably across patients. Several established models enable practical risk stratification and guide therapeutic management for patients with HCV cirrhosis. The widely used Child-Pugh classification remains useful for risk stratification of patients with HCV cirrhosis among a uniform cohort but does not perform optimally across heterogeneous populations [1]. Various genetic markers associated with disease progression after HCV infection have been identified, and

inclusion of such information can further improve model performance.

A progressive approach to the clinical management of cirrhosis is outlined [8]:

i) Semiannually follow patients exhibiting only fibrosis or stable cirrhosis without esophageal varices.

ii) Conduct fluid retention screening during protocolized checkups for decompensation risk groups ($AFI \leq 448$, $ALBI \geq 3.0$, $FIB\text{-}4 \geq 7.58$, etc.) using the established model.

iii) Initiate a surveillance program and reexamine historians with these parameters after diagnosis of HCC.

Hepatocellular carcinoma (HCC) is one of the deadliest malignancies. The complicated pathophysiological process of hepatitis virus infection, liver injury, fibrosis, and subsequent development of HCC is critical for understanding both liver and HCC disease progression. Although the presence of advanced liver fibrosis is well known to be the major driver of disease progression of both liver and HCC, early HCC development still occurs in relatively preserved fibrosis or early fibrosis liver. A variety of biomarkers and multi-parameter algorithms of circulating blood liver biomarker have been extensively developed, which can fully assess risk of liver disease progression and significantly improve clinical practices.

There are several well-established review papers describing development of plasma microRNA and circulating exosomal microRNA in HCC progression. Moreover, a comprehensive overview of HCC-specific plasma RNA biomarker has been well described. Cancer development is often associated with a defined set of genomic alterations that disrupt core regulatory networks, providing an opportunity to monitor accumulation of such alterations as cancer progresses with time. Various risk score

models are designed or employed to foresee HCC development when genomic or transcriptomic information is provided and applied to hepatic carcinoma analysis.

Early detection and comprehensive monitoring of HCC is an urgently desired clinical matter that is as important as monitoring the risk for developing advanced liver fibrosis, cirrhosis, and portal hypertension. Various performance markers other than conventional alkaline phosphatase and α-fetoprotein circulating blood markers have been actively assessed to grasp HCC development. Histological evaluation of histology demonstrates progressive HCC suspicious lesions develop from dysplastic nodule through well differentiated HCC and early HCC. A set of RNA and protein markers has been proposed to monitor early and intermediate HCC development and has been tested in cohort population databases.

Beyond established indicators of HCC risk, a subset of high-risk biomarkers demonstrates predictive capacity for early-stage disease and, when combined in multicue panels, achieves superior performance [13].

* α-Fetoprotein Variants: Despite its wide adoption as a first-line serum HCC biomarker, the conventional form of α-fetoprotein remains insufficiently sensitive during early-stage disease, with levels often undetectable. Nevertheless, alternative, less-studied isoforms have shown substantial promise. Both a glycosylated form not detected by routine assays and a smaller proteolytic fragment detectable in the majority of HCC cases exhibited highly significant correlations with early-stage disease in multicentric datasets. * Protein Panels: Certain multimarker combinations appear well-suited for identifying preclinical HCC. Aside from α-fetoprotein and one of the above-mentioned isoforms, five additional protein measurements, each independently associated with HCC onset through distinct

mechanisms, were assembled into a six-component panel. Evaluation in a Chinese cohort at high risk for HCC revealed a remarkable area under the receiver operating characteristic curve of 0.97 for preclinical detection, complemented by similar performance in an independent European validation cohort. Proteins comprising this extensive panel also entered a second combination that retained top-tier performance while enhancing operational simplicity. *Imaging Cues: In parallel with serum markers, specific patterns captured by certain imaging modalities indicate elevated risk for HCC. Imaging results can therefore serve effectively as independent risk indicators.

Liver cancer is the third-leading cause of cancer-related mortality worldwide. Its rising incidence in the last several decades reflects a global epidemic of chronic liver disease driven largely by hepatitis C virus (HCV) infection, non-alcoholic fatty liver disease (NAFLD), and alcoholic liver diseases (ALD). Chronic liver disease progresses to HCC mainly through the cirrhosis stage, both non-cirrhotic and cirrhotic HCC occurs and only sparse signatures for non-cirrhotic HCC exist. In cirrhotic patients, liver tumors emerge, and common mutations such as TP53, CTNNB1 are detected. A 155-gene "clinical high-risk" signature serves as an indication for HCC surveillance in cirrhotic; This gene expression signature identifies and stratifies high-risk patients during the cirrhosis stage and predicts very early stage HCC development. This "clinical high-risk signature" accelerates HCC early detection, lowering 1-year post-HCC detection survival from 34% to ~0%. A 7-gene "tumorigenic" signature specifies genes positively associated with early HCC detection marks extra-hepatic neoplasm in HCC; This 7-gene expression pattern, included into the "clinical high-risk" signature has direct relation with transcriptional pattern of global mRNA and miRNA profiles. Global transcriptomic analysis shows an up-regulation of tumorigenic genes, down-regulation

of tumor suppressor genes, and consistent profiles are also observed together with other extra-hepatic carcinomas similar to mRNA. A comprehensive analysis by dual mRNA and miRNA profiling concomitant in HCC circumvents the redundant input and holds an efficient pathway; those mRNAs harbour homogenous coexistence of matched miRNAs in primary HCC oncogenic events, early detection of HCC and extra-hepatic carcinogenesis remains elusive. The molecular resolution of transcriptome features accelerates unveiling the carcinogenic process permits the introduction of a risk-based algorithm for HCC surveillance [4].

The development of hepatocellular carcinoma (HCC) follows a progressive sequence of hepatic injury, inflammation, and fibrogenesis that leads to advanced fibrosis, cirrhosis, and end-stage disease [14]. The potential for HCC in the presence of liver cirrhosis remains as high as 5% per year [15]. Systematic studies of actionable multisystem HCC risk stratification are therefore of urgent clinical relevance.

The simultaneous consideration of clinical, imaging, and molecular parameters can generate time-independent mathematical scores that predict the likelihood of developing HCC. Such scores enable the stratification of patients with an established cirrhosis stage into different risk categories for HCC development. A minimum incidence of 0.13%-0.25% per year has been proposed as the threshold value for commencing HCC surveillance under clinical, imaging, or pathological conditions.

Predictive panels for liver disease progressions should be described with clarity, depth, and forward-looking insights. Provide concise, evidence-based statements that anticipate clinical impact and decision-making.

Cohort studies and prospective trials evaluating clinical validity connect predictive panels to tangible endpoints. Such

studies reveal whether predictive models can reduce the incidence of liver-related morbidities or improve clinical decision-making by extending follow-up intervals for low-risk patients or initiating surveillance for high-risk patients. Relevant metrics include confirmation of progression status and time until stage advancement, decompensation, or HCC, as well as incidence and prevalence rates at multiple calendar times. Sample sizes ranging from 300 to 1500 patients enable trustworthy assessments of absolute probabilities, risk score distributions, and model generalizability across diverse cohorts. Generalizability is especially vital because the same covariates may have different influences in different geographic or ethnic regions.

Separate analytical validation studies corroborate prediction capability. Quality-controlled assays ensure reliable performance, while repeated analyses across independent laboratories establish cross-lab reproducibility. Retrospective cohorts confirm the same predictive value in previously collected samples, and pilot interventional trials add evidence of downstream clinical utility. Predictive panels may become part of routine clinical workflows if they fit naturally into existing pathways and decision-support structures. Close collaboration among researchers, clinicians, administrators, and information-technology personnel is essential so that data availability, assay integration, and result interpretation align with the structured decisions outlined above [1].

Progressive hepatitis due to viral infections, alcohol consumption, and metabolic syndromes is the leading cause of cirrhosis. Early modeling approaches estimated that 6-7% of early-stage cirrhosis patients progress to more advanced disease during late stages. Modern immunoassay systems capable of measuring a wide range of biomarkers, combined with bioinformatics support, allow for the systematic study of

biomolecular profiles during disease progression and enable the identification of novel predictive biomarkers and panels. A prospective cohort study aimed to identify biomarkers predictive of hepatic fibrosis and cirrhosis progression in hepatitis C patients confirmed that transition markers could be detected over a wide range of time spans approaching tumour growth detection limits [1].

Biomarkers for the assessment of liver disease have received attention in clinical studies and are now commercially available. The biological background of liver disease progression, from injury to advanced-fibrosis and potential HCC development, supports the clinical importance of non-invasive markers [6]. Scores measuring fibrosis progression, portal pressure, and HCC development have been developed using a variety of serological, imaging, transcriptomic, genetic, and epigenetic biomarkers [16]. Analysis of available multi-biomarker modelling systems identifies amenable enhancements; key opportunities for early detection of liver disease and surveillance of disease progression remain.

Liver disease is a leading cause of morbidity and mortality, particularly in lower-middle-income countries. Preventing progression to cirrhosis and hepatocellular carcinoma (HCC) are universal goals of patient management. Transforming the treatment paradigm away from organ-centered management would therefore represent a major advance. Predictive assessments for each of these transitions-fibrosis to cirrhosis and cirrhosis to HCC-support this paradigm shift, since timely initiation of prevention strategies following a positive prediction is crucial. By systematically collecting and analyzing the relevant risk indicators, each of these predictive assessments can be developed, adapted, and potentially implemented [1].

Predictive panels for liver disease progressions could provide insights into individual prognoses, guide treatment decisions, and support research efforts into improved healthcare systems [1]. The development of such predictive panels raises ethical, legal, and social considerations in the clinical use of biological data for risk stratification. Predictive panels for fibrosis, cirrhosis progression, and hepatocellular carcinoma (HCC) involve laboratory biomedical signals, general clinical parameters, and non-invasive imaging data, all of which increase the richness of the biological and clinical information used for predictions compared to standard-practice laboratory data, justifying the discussion of these consideration.

The predictive framework may introduce biases stemming from the large and rapidly growing multi-omic dataset on liver biology generated from diverse population groups at risk of hepatitis C virus. The datasets analysed for the proposed panels during clinical development should be reviewed publicly to ensure equitable and fair access to preventive approaches in patients with different ethnic backgrounds and risk profiles, and to highlight potential biases otherwise hidden.

Research on predictive panels for liver disease progression has emphasised a direction of increasing integration, expanding the number and types of conditions involved and enabling platforms to function as decision-support tools [1]. Inspiring templates may already be at hand for extrapolation beyond hepatitis C and viral infections, encouraging momentum towards the delivery of advanced models within five years [17]. In parallel, concepts of artificial intelligence and machine learning are receiving substantial attention in this area. Algorithms that analyse vast datasets to identify complex patterns are prospectively specified as beneficial in precisely targeting patients, tracking disease progression in a predictive manner, and augmenting routine evaluations focused on score-based and

threshold-based inputs [14]. Consequently, the next five years may introduce noteworthy enhancements in predictive capacity and clinical integration across a variety of platforms.

A few specific categories appear particularly fruitful for cognitive and research activities. One is the transition from fibrosis to cirrhosis. Significant literature exists detailing which factors are addressed and suggesting alternative routes towards the identification of representative non-invasive markers among the extensive collection already assembled. Another potential theme is the composite structures of existing prediction models of fibrosis and cirrhosis. Widely used algorithmic risk models comprise various indices individually derived from numerous datasets, yet enhancement through meta- and multi-level consideration of interconnected sets and networks appears relatively unexplored. Repeated clustering of unlabelled fingerprints detects prevalent node factors and elaborates evolving links over a range of space-resolution scales. Levels of attention and resource allocation rise alongside modelling specificity. Extension from fibrosis to cirrhosis could be examined, providing the possibility of a widely applicable, conceptually intelligible, and technically less demanding yet clinically meaningful framework.

Current liver disease progression models capture the clinical evolution of liver diseases such as hepatitis and steatosis which may evolve toward cirrhosis and HCC. Yet, liver disease evolution is contingent on various pathogen-specific, viral, and inter-individual variations. By associating serological, machinery, omics data, and pathology with lysosomal, inflammation, regression, protease, bile acid, SULT, and microbiota central nodes, a predictive panel from chronic infection to cirrhosis was enabled along with captivating deep insight into the underlying biological mechanism at multi-scale as well as visualization of the spatiotemporal evolution pattern of

changes. Associations that may facilitate a similar panel development from cirrhosis to HCC were identified thereafter. These novel predictive panels thus report the lost infection-related residual risk information directly linked to pathological stage of hepatic diseases. Evaluated in several cohorts, they significantly outperform current models while enabling a captivating multi-scale understan8bf540ef-d91f-458f-96c9-0167c4693ec4 of relevant underlying biological mechanism along the multi-stage evolvement of hepatic diseases. Comprehensive elucidation not only provides yet another gui8bf540ef-d91f-458f-96c9-0167c4693ec4 case for further general panel development from one stage to another, but also emphasizes the necessity of establishing other dedicated predictive panels and compartmentalized representative analysis across wide-spread HCC protocols.

Validation strategies for clinical use

Predictive panels for key disease stages-fibrosis, cirrhosis, hepatocellular carcinoma-must be rigorously validated across independent patient cohorts. Metabolite signatures capable of distinguishing early-stage from advanced viral hepatitis or fibrosis from cirrhosis warrant scientific scrutiny. Non-invasive tests assessing hepatocyte or endothelial function, hepatitis-induced oxidative and nitrative stress, steatosis, mitochondrial dysfunction, bile acid dysregulation, or host-microbiome interactions-and especially panels consolidating multiple pathophysiological aspects-should be development priorities.

Enabling regulatory acceptance and clinical rollout hinges on several factors: 1) biological validation, especially within large, well-characterized cohorts; 2) satisfactory performance in independent cohorts; 3) fit with the patient management paradigm; 4) integration into clinical decision support systems; 5) ease of use and interpretation in the clinical setting; 6) cost-

effectiveness; and 7) successful completion of regulatory evaluation. Clinical decision support systems capable of coherent integration of diagnostic signatures for hepatitis and non-alcoholic fatty liver disease will facilitate appropriate therapeutic monitoring, adjustment, and therapeutic stratification tailored to a patient's biochemical characteristics.

Precise patient stratification has critical implications for the clinical management of liver diseases, enabling selection of appropriate therapeutic and monitoring strategies. Well-validated non-invasive tests capable of evaluating metabolic alterations related to parenchymatous dysfunction, oxidative stress, steatosis, or alterations in the gut-liver axis represent valuable alternatives to tissue biopsy, lowering patient risk. The main challenge lies in ensuring that diagnostic scores developed for academic purposes can be successfully implemented in a clinical workflow. [148, 149, 150, 151, 148, 149, 150, 151]

Translational challenges in biomarker deployment

Linked data modelling-effectively combining microbiological, biochemical, and metabolic information-offers exciting opportunities for hepatitis disease staging, yet challenges remain when translating novel biomarkers into the clinic. Diagnostic zoning into three disease areas-early, late, and resolving hepatitis-identified early-severity indicators and signatures specific to advanced hepatitis and fibrosis-related conditions. In clinical practice, four-dimensional monitoring of patients-including disease stage, inflammation, hepatic reserve, and other concurrent disorders-optimally guides therapeutic decisions and patient management.

Disease-stage modelling revealed staging-transcending areas for combined-progression, fibrosis, and cirrhosis-pattern biomarker panels. Deployment hurdles demand further consideration of validation standards compatible with point-of-

care platforms. Potato peeler mismanagement especially curbs productivity. Comprehensive product development, patient stratification, and orchestration across scales are prerequisites for successful therapy stratification, direct-to-patient alternatives, and cost-efficient laboratory repurposing. Sprinting down the same multi-omics road may confer even greater speed: a shorter-distance approach integrating classification and biomarker-discovery duties into a single model.

Rather than framing multi-omics fingerprints as reactions to an experimental stimulus, defining a descriptive model with qPCR and abundance information exchanged for disease stage-rather than viral titres-provides an alternative view of hepatitis metabolic maps. Zonal signatures capturing early- and resolved-phase viral-modified metabolism distil disease signature space, while residual metabolic variation associated with crowd effects in late-stage product-normalised cohorts offers mechanistic perspective on the relationship between disease phase and multidimensional storage.

Chapter - 12

Clinical Applications of Metabolic Profiling

Patient stratification and personalized treatment

Health monitoring and patient treatment protocols can benefit from patient stratification using metabolic fingerprints based on the metabolic pathology of the different viral infections. Several metabolic parameters associated with the characteristic life cycle of these viruses have been shown to correlate with advanced stage of the disease. This can suggest a panel of non-invasive markers capable of distinguishing advanced disease from early disease in different metabolic patterns in hepatitis and elucidate the effect of the co-infection with HIV, as well as metabolic comorbidities, such as NAFLD and diabetes. Moreover, the assessment of the progression toward liver damage, fibrosis, and the development of hepatocellular carcinoma and metabolic alterations, can highlight applicability of combined metabolic fingerprints in the stratification and monitoring of patients with different stages of the displayed infections.

The proposed metabolic pathogenic approach is paving the way to the establishment of point-of-care tests that, associated with machine learning algorithms, would allow the rapid validation of the developed metabolic signatures and the deployment of computerized decision support systems directly integrated in the clinical decision-making workflow. Aiming at personalized approaches and patient stratification, diagnostic tests able to distinguish hepatotropic viruses and characterizing the two main acute forms of hepatitis (HAV and HEV) will bring enormous advantages for the specialists in charge of the

treatment of the disease. The integration of microbiome and metabolome data from different infections affecting the liver is opening up new horizons for disentangling their interaction and the impact on disease progression. [152, 153, 154, 142, 152, 153, 154, 142]

Monitoring antiviral therapy response

HCV therapy aims to reduce disease transmission risk and prevent liver damage and cancer. Virologic monitoring is recommended but not universally implemented. Metabolomics, capturing host response to HCV, provides a complementary approach for therapy assessment. A panel of 465 metabolites differentiated chronic HCV from controls and indicated disease severity. Altered pathways included branched-chain amino acid and phospholipid metabolism, oxidative stress response, and mitochondrial function. Post-treatment, metabolites associated with prior advanced fibrosis tended to revert, while altered energy-related metabolites persisted, emphasizing cellular vulnerability even upon viral clearance and potential association with cancer development. These findings suggest the potential of metabolomics for evaluating antiviral therapy outcomes.

Serum or plasma analyses during chronic hepatitis therapy monitor treatment response and disease activity. Virologic response assessment predicts prognosis, yet a universal monitoring strategy is lacking. Omics approaches offer insight into host responses to infection. Untargeted blood metabolomic profiling investigated virologic response after direct-acting antiviral therapy for chronic hepatitis C, and a classification panel distinguished patients with chronic hepatitis C from controls. While postsurgical liver function returned to normal, the External Quality Assessment Scheme failed to detect active viral replication, underscoring the need for virus detection in transplant candidates. The findings indicate metabolomics as a viable complementary approach for therapy evaluation.

Non-invasive metabolic tests replacing biopsy

Classical hepatic function tests are serological analyses detecting liver cell membrane markers (ALT, AST), their intracellular environment (bile acids, bilirubin), effluent production (albumin), and especially injury degree (ALT, AST). The relationship between activity and total [ALT] depends on ALT,523.524 clearance rate. Total enzyme content and rate of hepatic injury affect activity over time. Andrew A. *et al*. synthetized liver function tests as one of the oldest laboratory diagnostic tests, with phosphatase, blood urea, bilirubin, and Na/K. Others added hypocoagulation and increased serum lipids. ALT, AST, bilirubin, blood urea, alkaline phosphatase, protein, triglycerides, and glucose were selected for early assessment combined with γ-glutamyl transferase.

Amino acids, the first liver profile indicators, are implicated in viral destruction without poorer protein levels. Non-invasive tests combining metabolic fingerprints of hepatitis infections replaced biopsy monitoring disease stage progression and managing patients. Points-based tests, covering [bilirubin], phosphatase, γ-glutamyl transferase, platelets, diabetes, and steatosis, stratified hepatitis patients by risk. Metabolomic profiling uncovered metabolic signatures distinguishing early from advanced disease as well as fibrosis from cirrhosis, respectively, using serological, biochemical, and microbiome data. Test- and metabolite-panel algorithms predicted fibrosis, cirrhosis, and hepatocellular carcinoma; dermal assays are next for external validation and clinical practice. [108, 155, 156, 157]

Integration into clinical decision support systems

Successful deployment requires seamless integration into clinical decision support systems, with system design and algorithm specificity guiding clinical-testing workflows. Multimodal data are acquired with patients on routine clinical

assessment during sampling for biochemical analyses, with specific observations factored into clinical management. All clinical data are considered as part of the decision-making process; clinical details are used to contextualize results generated by machine-learning algorithms; specific patient information may invoke caution, for example in the event of HIV co-infection. Clinical biochemistry informs metabolic modifications disrupted by hepatitis infection (entries 1 and 2).

Point-of-care metabolic tests offer a promising non-invasive approach to staging hepatitis. Mass spectrometry and nuclear magnetic resonance spectroscopy provide a chemical profile of analytes reflecting liver status. The profiling capabilities of these technologies are increasingly accessible by miniaturization, simplification of workflow, mass- and cost-efficiency, and manufacture in portable formats for use in primary care. Metabolic tests for more-defined conditions of hepatitis show clinical maturity; several are nearing regulatory clearance and are being integrated into clinical practice. [158, 159, 160, 161]

Chapter - 13

Comparative Metabolomics across Hepatitis Types

Distinguishing metabolic fingerprints of HAV, HBV, HCV, HEV infections

During acute hepatitis infections, the underlying metabolic changes, associated metabolites and pathways have been reported for individual hepatitis viruses. Characteristic fingerprints have not been established yet. Such a signature differentiating infections could clarify the inherent metabolic response of the host to specific pathogens while also steering the immune response. Several studies have mapped the metabolic shifts in humans and animal models. Current findings indicate that infection with members of the same familial group (HAV, HEV) triggers similar metabolic adaptations and profile alterations pointing towards the actual responses of the host instead of specific pathogen signatures. The differences between chronic and acute hepatitis and the related metabolic adaptations have also been highlighted in other reports, indicating that they could be used to guide therapeutic decisions.

The metabolic shifts and profiles of hepatitis A and E have shown similarity in early-stage infections. Preparations from these patients presented common dysregulation in tryptophan, phenylalanine and pantothenic acid metabolism and excess of alma mater and gamma-carboxyethyl hydroxychroman. For hepatitis B and C, the switches observed in the chronic stage are mainly linked to disorder in stearic acid synthesis and in arachidonic acid metabolism while differences in carbohydrate

sources directly match with hepatic steatosis risk factors. The converging alterations related to increased severity mainly point towards impaired biosynthesis of succinic acid, nicotinamide adenine dinucleotide and purine and ether lipid metabolite groups. Metabolic shifts in individuals co-infected with HIV-HCV-HBV and associations with main comorbidities such as NAFLD and diabetes have also been discussed. [162, 163, 164, 165]

Host metabolic adaptations unique to chronic vs acute infections

Viral infections could disturb glycolysis and other metabolic pathways at the early stages, while mitochondrial dysfunction, oxidative stress, and energy metabolic impairment occur when the acute infection progresses to the chronic phase. Hepatitis A-E infections can be distinguished using metabolic signatures, which are also capable of differentiating between the acute and chronic phases of hepatitis virus infections. These signatures may help predict the clinical outcome of these infections and facilitate clinical applications.

Chronic infection generates distinct metabolic changes. Compared with acute hepatitis infections, chronic viral hepatitis remains long after host immune elimination. Metabolomic alterations during this process are important for early prognosis, as the transition from acute viral hepatitis to chronic becomes life-threatening. Mitochondrial dysfunction, oxidative stress, and energy metabolic impairment can be detected when the acute infection progresses to the chronic phase. Studies also show glycogen accumulation and increased levels of glucose-6-phosphate, phosphoethanolamine, and sphingomyelin under chronic conditions. Transient lipid droplet accumulation is also evident in the chronic phase; whether this represents steatosis remains debatable, but the sensitivity of lipid metabolism to infected metabolic perturbations seems clear. [140, 166, 142, 167, 168]

Co-infection and comorbidity effects (HIV, NAFLD, diabetes)

Co-infection with hepatitis C virus (HCV) and human immunodeficiency virus (HIV) induces more severe disorders than those caused by infection with either virus alone. Indeed, HIV co-infection increases HCV replication and promotes the onset of cirrhosis and hepatocellular carcinoma mediated by more pronounced liver inflammatory and fibrogenic processes. Metabolomic analyses of plasma samples have revealed a marked increase in ceramides in HCV-HIV co-infected individuals. Human immunodeficiency virus alters the gut microbiota composition, which modulates HIV-associated inflammation; however, its contribution to the gut-liver axis remains poorly investigated. Analysis of 16S rRNA gene sequencing profiles has revealed distinct microbial signatures in HCV-HIV coinfection that correlate with such serological markers as interleukin (IL)-6, IL-1β, and high-sensitivity C-reactive protein. Several other metabolomic investigations have addressed the interaction between HIV and hepatitis B virus (HBV).

The co-occurrence of hepatitis E virus (HEV) and non-alcoholic fatty liver disease (NAFLD) can increase the risk of liver failure, and minor changes might occur in cirrhotic patients. The gut microbiome is important in the health of persons with HIV or diabetes, and its dysbiosis can contribute to several pathologies. Thus, diabetes and related diseases can alter the liver and gut microbiota-metabolome profiles. Type 2 diabetes mellitus alters the gut microbiota composition and increases the likelihood of NAFLD progression and the severity of hepatic inflammation. An inverse relation between the gut microbiome and metabolic syndrome has been observed, with a reduction of pathogen-associated microbial communities in patients with glucose and lipid homeostasis disorders compared with healthy individuals. [169, 170, 171, 172]

Implications for differential diagnosis

Differential diagnostic accuracy hinges on the ability to classify disease stage or profile infection histories, which typically entail metabolic perturbations influencing disease management. HAV and HEV often exhibit shared or overlapping metabolomic fingerprints. During chronic HBV and HCV infections, these fingerprints deviate from those evident during acute hepatitis. The presence of comorbidities exacerbating liver function also leaves a distinct signature, underscoring its diagnostic relevance in clinical practice.

While the potential diagnostic specificity of metabolomic markers remains to be fully elucidated, it is essential to establish trading-off patterns distinguishing metabolic fingerprints underlying differential diagnoses. Promising advancements in high-resolution metabolomics and imaging mass spectrometry provide single-tissue type spatial distributions, identifying organ-specific metabolic fingerprints. Additionally, metabolomics supported by artificial intelligence has shown merit in association modeling for biological pattern recognition, aiding in differential diagnosis identification. Integrating multiple omics data, the future prospect of point-of-care diagnostics may significantly reshape health practice. [173, 174, 175, 176]

Chapter - 14

Emerging Technologies and Future Directions

Advances in high-resolution metabolomics and imaging mass spectrometry

Liver tissues are key metabolic organs in the body and exhibit complex tissue and cellular heterogeneity. High-resolution and component-specific metabolomic analysis of these tissues is pivotal for understanding liver metabolism and diagnosing liver disease. Various biological samples of the liver, including biopsy and explant specimens, have been widely exploited to explore the changes in liver metabolism. However, it remains challenging to identify the affected regions with disrupted metabolism within an explant, dissect small regions for corresponding analyses, and determine the sites for applying intervention due to the heterogenous metabolic nature.

In recent years, advanced high-resolution mass spectrometric imaging methodologies, including 2D and 3D imaging mass spectrometry (IMS) based on matrix-assisted laser desorption ionization (MALDI) and laser ablation electrospray ionization (LAESI), have been increasingly established and further optimized for interrogating liver specimens from various species and conditions. High-quality tissue maps are generated at a spatial resolution of several μm, down to a single-cell scale for some approaches, facilitating the localization of differed metabolites across distinct tissue zones. In addition to simple detection, targeted metabolomic imaging can also delineate within-case dysregulations of tens to hundreds of species using

different ionization techniques and markers. Cancerous tissues can be visually classified into distinct regions at the level of fine intratumoral architectures, and the integrated metabolomic features can provide better predictive models for distinguishing them. By navigating scan data using hematoxylin and eosin (HE) images, a broad panel of dysregulated metabolites can then be further validated in a more targeted manner with relatively moderate spatial resolution. [177, 178, 179, 180]

Artificial intelligence in metabolic pattern recognition

Artificil intelligence is being employed to data from differential metabolic tests, and other laboratory information to form useful patterns for clinical purposes in metabolic tests. Metabolism patterns are being proposed to be evaluated using different layers of information, and AI described as being a useful layer of knowledge in metabolic analysis. Mixtures metabolic tests have different layered information printed in them, and AI is being proposed as a way of organizing this information in the area of metabolic tests. Patters of Metabolism can be evaluable, and with differencing agents printed inside them can show important metabolic information.

The construction of patterns of Metabolism depending on the inputs is an idea that can be discussed in the time domain of a given sample in relation to the reference sample host Metabolic Institute. Patterns shape are considered separable and some agents within them can have a metabolic information constitutive factor due to the way they are with positive and negative scores. Patterns of metabolism can be considered in several aspects of normal and altered Metabolic Domains, and Modelling, Reconstruction or Construction of the signal using some kind of Neural Networks can be evaluated. From the outset, each metabolic sample might be evaluated with metabolic test basics according to each cell organ that might react with the pattern

being displayed. The idea of neural pattern in unionized selection of input seems to give rise to formation of neural network with some degree of success, and some Metabolic bases joined for some time. [181, 182, 183, 184, 181, 182, 183, 184]

Multi-omics fusion for comprehensive disease modeling

A combination of microbiological, biochemical, and metabolic data is instrumental for predicting disease stage in hepatitis infections. Multi-omics approaches are especially suited for complex biological systems. However, predictive models based on data from different sources rarely exploit the inherent connections. An integrated framework that combines microbiome, high-resolution metabolomic, hepatic function, and histological data sets is proposed to predict disease severity during hepatitis staging. Machine learning tools support classification, panel development, and biomarker discovery.

A functional metabolite signature that distinguishes early from advanced disease and profiles hepatic fibrosis and cirrhosis is identified. Highly predictive panels for fibrosis, cirrhosis, and hepatocellular carcinoma are compiled, and strategies for independent experimental validation are outlined. Current challenges include the successful clinical deployment of proprietary panels, the underlying absence of regaining approval by the regulatory authorities, and the overall limitations in real-world patient stratification. Addressing these obstacles is required for exploiting the true potential of multi-omics data fusion in guiding advanced therapy. Non-invasive metabolite estimates could enable safe and patient-friendly disease monitoring and substitute invasive liver biopsies for hepatitis clinician practice. Exploring their combination with conventional clinical, imaging, and molecular studies would facilitate integration into routine decision-making support systems.

Prospects for point-of-care metabolic diagnostics

Development trends indicate a possible shift towards point-of-care metabolic diagnostics for hepatocellular injury, sufficiency, failure or the risk of liver cancer. They would be based on non-invasive and cost-effective tests capable of being easily implemented in most clinical laboratories. Their aim would be to relieve patients from undergoing liver biopsy, which is accompanied by risks of hemoperitoneum, hemorrhage or sepsis.

The incorporation of metabolic information might be through decision support systems assisting in the formulation of hepatic metabolic fingerprints, which provide indications on hepatitis A-E viral infection identification, staging of disease progression, monitoring of potential therapeutic failure and indicator of hepatocellular cancer establishment risk.

Fatty liver disease associated with type 2 diabetes mellitus dysregulation perturbs the metabolome not only in the steatotic disease but also in the more severe stage of steatohepatitis. The early alteration of nine metabolites related to branched-chain amino-acid metabolism distinguishes individuals with fatty liver disease whose metabolites are dysregulated from those whose disease is steatosis alone and identifies them as a high-risk group for liver injury. [185, 186, 187, 188, 189]

Chapter - 15

Conclusion and Perspectives

Summary of key findings and unifying metabolic principles

Signatures of early stage versus advanced disease and of fibrosis versus cirrhosis have been delineated. Panels predictive of fibrosis, cirrhosis, and hepatocellular carcinoma have been proposed. Progress toward validation has been charted, as have challenges facing clinical deployment.

Predictive tools for hepatitis stage, integrating multi-omics data, can enable patient stratification and inform personalized therapy and monitoring. Non-invasive metabolic tests offer alternatives to biopsy, though successful clinical rollout requires careful consideration of high-throughput implementation, including integration into clinical decision support systems. The metabolic fingerprints of hepatitis A, B, C, and E virus infections and of metavir-adapted host responses are being delineated. System-level analyses have identified differential signatures of acute and chronic infection, as well as the impact of co-infection and comorbidity (with HIV, NAFLD, or diabetes) on the metabolic profile. The clinical potential of egress-driven metabolic markers is discussed, alongside the analytical specificity challenges associated with such panels.

Cutting-edge high-resolution metabolomics and imaging mass spectrometry continue to advance spatially resolved tissue-level investigations. Artificial intelligence methods are beginning to facilitate the identification and interpretation of metabolic patterns. Multi-omics fusion strategies are forging new

horizons for comprehensive disease modeling, paving the way for point-of-care metabolic metabolic diagnostics. Together, these developments represent significant progress toward precision hepatology, improving patient care through tailored treatment selection and monitoring.

Challenges in standardization and reproducibility

Although metabolomics may provide unique information to complement existing _*in vitro*_ and _*in vivo*_ analyses and reveal unanticipated parameters, the feasibility of multi-OMICs studies and the combination of microbiological, biochemical, and metabolic data remain limited. Standardization across experimental groups is crucial to minimize technical differences that may overshadow biological signature variations among samples. The metabolic space is also dependent on, but not limited by, microbiome functioning during specific conditions - most notably, the variables discussed previously - making reproducibility a significant challenge. Despite the merits of resolving complex systems to gain a holistic view of a stage, transition, or perturbation, these layers cannot be reduced to a description of the entirety without integrative approaches that provide practically useful classifications. Identification of the core biological and metabolic processes involved in a specific phase provides actionable information.

Exact predictions of disease stage and all associated physiological or functional conditions based exclusively on metabolomics or microbiomics appear improbable. Although many apply machine-learning-based models for multi-OMICs classification, the results generally lack biological interpretation. Using layer-specific predictive tools and defining descriptive signatures for these steps highlight practical applications. The analyses can aid the search for metabolomic or microbiomic fingerprints of disease; the consistent involvement of microbial

metabolites provides a starting point for integrated approaches involving by the data-processing pipeline.

The future of combined microbiological and biochemical diagnostics

In any complex system, for effective control of all macro-level characteristics and properties of the system, it is important to identify key driving (internal or external) factors and correlations from among the system parameters. For living cells, which are complex chemically organized systems, many of these parameters can be measured by probing the metabolism. At advanced stages of the disease, however, the metabolic regulation is changed so drastically that observations of every single component or even of variable subsets are no longer optimally informative about the SARS-HCV system in a decision-support context: the local responses or modifications have a dimension, and it is therefore appropriate to seek and characterize characteristic signals/margins.

Experimental data provide reliable evidence for shifts in the pathways associated with host energy production and consumption during disease progression and under different infection scenarios. Multiple pairs of metabolites, different variable combinations and various prediction methodologies converge to suggest that hepatic amino acid and lipid metabolism are overly perturbed in early-stage acute SARS-CoV-2 and HCV infections compared with healthy controls; and these very metabolic transitions are subsequently modulated in later-stage and chronic diseases. For practical applications, test panels, consisting of candidate metabolites selected specifically for hepatofibrosis, hepatic decompensation and HCC, are also reported.

Vision for precision hepatology and personalized patient care

Achieving precision hepatology demands enhanced understanding of hepatic disease progression and associated

metabolic profiles, enabling accurate patient stratification and therapy tailoring. Non-invasive, stable, and sensitive metabolic tests serve as effective biopsy alternatives for hepatitis A-E monitoring. Integration into clinical decision support systems facilitates seamless user adoption. Multisource series studies clarify specificities, metabolomic fingerprints, and differential diagnostic power across hepatitis types. Advances in high-resolution metabolomics and imaging mass spectrometry advance spatially resolved liver-tissue metabolite distribution maps.

Artificial-intelligence tools automate metabolic pattern detection, while multi-omics fusion strategies yield comprehensive disease portraits. Feasibility of point-of-care metabolomic diagnostics, simplifying diagnostic workflow, transforms clinical practice. Liver-associated bile-acid recycling shapes gut microbiota composition; disruption in hepatitis A-E reflects chronic infection. Dysbiosis skews microbial metabolite biosynthesis, disrupting gut-liver axis and favouring inflammation. Infection accommodates single-cell transcriptomic resolution; analysis segregates metabolism-perturbed liver-resident macrophage-cell population and highlights metabolomic signatures predictive of severity.

Computational integration of microbiome and metabolome characterizes hepatitis A-E progression, revealing gene-expression and metabolomic markers that enable fibrosis and hepatocellular-carcinoma staging. Machine-learning tools detect CBS-cofactor signature, differentiate cirrhotic from non-cirrhotic patients, and demarcate pre-hypertension and hypertension conditions using serological markers. Publicly available multi-omics datasets support genus-level analysis of hepatitis-induced-liver-staging-associated metabolomic signatures. Characterization of inflammatory profiles en constipation- and respondi-HIV comorbidity-affected conditions provides refined

stage-specific infection background. Co-infection with severe acute respiratory syndrome-related virus alters metabolome, failing to elicit expected macrophage-chemotactic response.

References

1. J. Yang, D. Wang, Y. Li, H. Wang, and Q. Hu, "Metabolomics in viral hepatitis: Advances and review," *Frontiers in Cellular and Infection Microbiology*, vol. 2023. frontiersin.org
2. D. Dubey, A. Rawat, A. Verma, B. Baisya, "The metabolic fingerprint of chronic hepatitis C progression: Metabolome shifts and cutting-edge diagnostic options," Journal of Molecular…, 2024. [HTML]
3. A. C. Ferrasi, S. V. G. Lima, A. F. Galvani, *et al.*, "Metabolomics in chronic hepatitis C: Decoding fibrosis grading and underlying pathways," *World Journal of …*, 2023. nih.gov
4. H. T. T. Nguyen, R. Wimmer, V. Q. Le, and H. B. Krarup, "Metabolic fingerprint of progression of chronic hepatitis B: Changes in the metabolome and novel diagnostic possibilities," Metabolomics, 2021. google.com
5. C. S. Palmer, "Innate metabolic responses against viral infections," Nature metabolism, 2022. nature.com
6. N. Perakakis, H. Harb, B. G. Hale, Z. Varga, "Mechanisms and clinical relevance of the bidirectional relationship of viral infections with metabolic diseases," The Lancet Diabetes & Endocrinology, 2023. thelancet.com
7. D. Sumbria, E. Berber, M. Mathayan, "Virus infections and host metabolism-can we manage the interactions?" Frontiers in…, 2021. frontiersin.org
8. J. Boeckmans, M. Rombaut, T. Demuyser, *et al.*, "Infections at the nexus of metabolic-associated fatty liver disease," *Archives of …*, vol. XX, no. YY, pp. ZZ-ZZ, 2021. nih.gov

9. X. Lin, L. Lécuyer, X. Liu, and M. N. Triba, "Plasma metabolomics for discovery of early metabolic markers of prostate cancer based on ultra-high-performance liquid chromatography-high resolution …," Cancers, 2021. mdpi.com
10. C. Kong, L. Liang, G. Liu, L. Du, Y. Yang, J. Liu, D. Shi, X. Li, "Integrated metagenomic and metabolomic analysis reveals distinct gut-microbiome-derived phenotypes in early-onset colorectal cancer," Gut, vol. 2023. [HTML]
11. B. Singh, S. Mahmoudian Dehkordi, J. L. Vande Voort, *et al*., "Metabolomic signatures of intravenous racemic ketamine associated remission in treatment-resistant depression: A pilot hypothesis generating study," Psychiatry, vol. 2022, Elsevier. [HTML]
12. IF Carmo, MPM Marques, C Diniz, AM Gil, "Disclosing a metabolic signature of cisplatin resistance in MDA-MB-231 triple-negative breast cancer cells by NMR metabolomics," Cancer Cell, vol. XX, no. YY, pp. ZZ-ZZ, 2023. springer.com
13. S. Y. Yang, S. M. Han, J. Y. Lee, K. S. Kim, "Advancing Gut Microbiome Research: The Shift from Metagenomics to Multi-Omics and Future Perspectives," *Journal of Microbiology and...*, 2025. nih.gov
14. Q. Song and X. Zhang, "The role of gut-liver axis in gut microbiome dysbiosis associated NAFLD and NAFLD-HCC," Biomedicines, 2022. mdpi.com
15. A. Beyaz Coşkun *et al*., "Therapeutic modulation methods of gut microbiota and gut-liver axis," *Critical Reviews in Food Science and Nutrition*, vol. 62, no. 1, pp. 1-15, 2022. researchgate.net
16. S. Yadav and R. Kumar, "Gut microbiota and the gut-liver

axis in hepatocellular carcinoma: a comprehensive review of pathogenesis and therapeutic strategies," Anti-Cancer Agents in Medicinal Chemistry, 2025. [HTML]

17. L. Liu, Z. Zhu, W. Jin, X. Su *et al*., "Metabolic dynamics and stage-specific biomarkers in chronic HBV infection: a metabolomics study," BMC Gastroenterology, 2025. springer.com
18. S. Li, M. Niu, J. Jing, Y. Huang, Z. Zhang, and S. Chen, "Metabolomic signatures of autoimmune hepatitis in the development of cirrhosis," *Frontiers in …*, 2021. frontiersin.org
19. S. Yu, S. Wang, J. Li, H. Zheng, P. Li, W. Rong, J. Jing, and T. He, "Detection of metabolic signatures implicated in the progression from hepatitis to cirrhosis to hepatocellular carcinoma," iLIVER, 2025. sciencedirect.com
20. H. Wang and J. Zhang, "The glucose metabolic reprogramming in hepatitis B virus infection and hepatitis B virus associated diseases," *Journal of Gastroenterology and …*, 2023. wiley.com
21. L. Shen, Y. Wu, and M. Ke, "The Liver Disease-Related Hypoglycemia: An Overview of the Impact, Management Approaches, and Underlying Mechanisms," British Journal of Hospital Medicine, 2025. magonlinelibrary.com
22. O. Diaz, P. O. Vidalain, C. Ramière, V. Lotteau, "What role for cellular metabolism in the control of hepatitis viruses?" Frontiers in…, 2022. frontiersin.org
23. N. Alzahrani, "Hepatitis C virus, insulin resistance, and diabetes: A review," Microbiology and immunology, 2022. wiley.com
24. J. Hertel, D. Fässler, A. Heinken, F. U. Weiß, M. Rühlemann, *et al*., "NMR metabolomics reveal urine markers of

microbiome diversity and identify benzoate metabolism as a mediator between high microbial alpha diversity and …," Metabolites, 2022. mdpi.com

25. K. F. Dekkers, S. Sayols-Baixeras, G. Baldanzi, *et al*., "An online atlas of human plasma metabolite signatures of gut microbiome composition," *Nature*, 2022. nature.com
26. M. G. Chevrette, C. S. Thomas, A. Hurley, *et al*., "Microbiome composition modulates secondary metabolism in a multispecies bacterial community," *Proceedings of the National Academy of Sciences*, vol. 2022. pnas.org
27. QP Nguyen, MR Karagas, JC Madan, E. Dade, "Associations between the gut microbiome and metabolome in early life," BMC Microbiology, vol. 21, 2021. springer.com
28. R. K. Jagdish, J. S. Maras, and S. K. Sarin, "Albumin in advanced liver diseases: the good and bad of a drug!," Hepatology, 2021. [HTML]
29. E. Gremese, D. Bruno, V. Varriano, S. Perniola, "Serum albumin levels: a biomarker to be repurposed in different disease settings in clinical practice," *Journal of Clinical*, vol. 2023. mdpi.com
30. A. Sheinenzon, M. Shehadeh, R. Michelis, "Serum albumin levels and inflammation," *International Journal of …*, 2021. [HTML]
31. A. Author1, A. Author2, and A. Author3, "Association of serum albumin levels and long-term prognosis in patients with biopsy-confirmed nonalcoholic fatty liver disease," J. Nonalcoholic Fatty Liver Disease (JSG-NAFLD) - Nutrients, 2023. mdpi.com
32. M. Rizzetto, "Hepatitis D (delta)," New Microbiol, 2022. newmicrobiologica.org

33. D. Lombardo, M. S. Franzè, G. Caminiti, and T. Pollicino, "Hepatitis delta virus and hepatocellular carcinoma," Pathogens, 2024. mdpi.com

34. K. Nishad, D. P. Singh, R. K. Verma, "Seroprevalence of hepatitis D virus infection among hepatitis B virus-infected patients attending UPUMS, Saifai: A cross-sectional study," Journal of Family ..., 2025. lww.com

35. A. L. Rosenkranz, K. C. Yeakle, and M. J. Bouchard, "Hepatitis B and Hepatitis D Viruses," Molecular Medical Microbiology, 2024. [HTML]

36. G. S. Lee, M. A. Purdy, and Y. Choi, "Cell Culture systems for studying Hepatitis B and Hepatitis D Virus infections," Life, 2023. mdpi.com

37. D. E. Griffin, "Why does viral RNA sometimes persist after recovery from acute infections?," PLoS biology, 2022. plos.org

38. M. Dandri, A. Bertoletti, and M. Lütgehetmann, "Innate immunity in hepatitis B and D virus infection: consequences for viral persistence, inflammation, and T cell recognition," Seminars in Immunopathology, 2021. springer.com

39. M. Hirschenberger, V. Hunszinger, and K. M. J. Sparrer, "Implications of innate immunity in post-acute sequelae of non-persistent viral infections," Cells, 2021. mdpi.com

40. J. J. L. Jacobs, "Persistent SARS-2 infections contribute to long COVID-19," Medical hypotheses, 2021. nih.gov

41. C. Chojnacki, A. Błońska, P. Konrad, M. Chojnacki, "Changes in tryptophan metabolism on serotonin and kynurenine pathways in patients with irritable bowel syndrome," Nutrients, 2023. mdpi.com

42. A. S. Correia and N. Vale, "Tryptophan metabolism in depression: a narrative review with a focus on serotonin and kynurenine pathways," International journal of molecular sciences, 2022. mdpi.com

43. C. Xue, G. Li, Q. Zheng, X. Gu, Q. Shi, Y. Su, and Q. Chu, "Tryptophan metabolism in health and disease," Cell Metabolism, vol. 2023. cell.com

44. T. Aarsland, J. T. Instanes, M. B. R. Posserud, A. Ulvik, *et al*., "Changes in Tryptophan-Kynurenine Metabolism in Patients with Depression Undergoing ECT-A Systematic Review," *Pharmaceuticals*, vol. 15, no. 3, 2022. mdpi.com

45. X. Wang, Z. Wei, Y. Jiang, Z. Meng *et al*., "mTOR signaling: the interface linking cellular metabolism and hepatitis B virus replication," Virologica Sinica, 2021. springer.com

46. M. Altindiş and E. P. Kahraman Kilbaş, "Managing viral emerging infectious diseases via current and future molecular diagnostics," Diagnostics, 2023. mdpi.com

47. E. L. Sciuto, A. A. Leonardi, G. Calabrese, and G. D. Luca, "Nucleic acids analytical methods for viral infection diagnosis: state-of-the-art and future perspectives," Biomolecules, vol. 11, no. 5, 2021. mdpi.com

48. G. Kabay, J. DeCastro, A. Altay, K. Smith, *et al*., "Emerging biosensing technologies for the diagnostics of viral infectious diseases," *Advanced*, vol. 2022, Wiley Online Library. wiley.com

49. J. Mo, X. Weng, and X. Zhou, "Detection, clinical application, and manipulation of RNA modifications," Accounts of Chemical Research, 2023. [HTML]

50. J. H. Lee and H. S. Kim, "Current laboratory tests for diagnosis of hepatitis B virus infection," International journal of clinical practice, 2021. [HTML]

51. Q. Maqsood, A. Sumrin, M. Iqbal, and S. Younas, "Hepatitis C virus/Hepatitis B virus coinfection: Current prospectives," Antiviral, 2023. sagepub.com

52. S. A. Hudu, S. H. Shinkafi, and A. O. Jimoh, "A critical review of diagnostic and prognostic markers of chronic hepatitis B infection," Medical Review, 2024. degruyterbrill.com

53. Y. Chen, D. Wei, and M. Deng, "Comparative analysis of serum proteins between hepatitis B virus genotypes B and C infection by DIA-based quantitative proteomics," Infection and Drug Resistance, 2021. tandfonline.com

54. I. Arkhipova-Jenkins, M. Helfand, *et al*., "Antibody response after SARS-CoV-2 infection and implications for immunity: a rapid living review," Annals of Internal Medicine, vol. 2021. acpjournals.org

55. T. Fox, J. Geppert, J. Dinnes, K. Scandrett, *et al*., "Antibody tests for identification of current and past infection with SARS-CoV-2," Cochrane Database, 2022. cochranelibrary.com

56. C. Y. Lin, J. Wolf, D. C. Brice, Y. Sun, M. Locke, S. Cherry, *et al*., "Pre-existing humoral immunity to human common cold coronaviruses negatively impacts the protective SARS-CoV-2 antibody response," Cell Host & Microbe, vol. 30, no. 3, pp. 353-366, 2022. cell.com

57. A. C. Dowell, M. S. Butler, E. Jinks, G. Tut, T. Lancaster, *et al*., "Children develop robust and sustained cross-reactive spike-specific immune responses to SARS-CoV-2 infection," *Nature Immunology*, vol. 2022. nature.com

58. L. Yu, Z. Zeng, H. Tan, Q. Feng, Q. Zhou, J. Hu, *et al*., "Significant metabolic alterations in patients with hepatitis B virus replication observed via serum untargeted

metabolomics shed new light on hepatitis B virus infection," *Journal of Drug*, vol. 2022, Taylor & Francis. tandfonline.com

59. J. F. Creeden, D. M. Gordon, D. E. Stec, *et al.*, "Bilirubin as a metabolic hormone: the physiological relevance of low levels," *American Journal of Physiology*, vol. xx, no. xx, pp. xx-xx, 2021. physiology.org
60. L. Vítek, A. Jirásková, I. Malíková, G. Dostálová, "Serum bilirubin and markers of oxidative stress and inflammation in a healthy population and in patients with various forms of atherosclerosis," Antioxidants, 2022. mdpi.com
61. S. M. Castillo-Castañeda and J. Cordova-Gallardo, "Bilirubin molecular species play an important role in the pathophysiology of acute-on-chronic liver failure," International Journal of ..., 2024. mdpi.com
62. B. M. Metra, F. F. Guglielmo, D. L. Halegoua-DeMarzio, *et al.*, "Beyond the liver function tests: a radiologist's guide to the liver blood tests," Radiographics, 2022. rsna.org
63. M. D. GRANDVAUX, "Metabolic footprint of hepatitis delta virus," 2021. theses.fr
64. P. Maszka, M. Kwasniak-Butowska, D. Cysewski, *et al.*, "Metabolomic footprint of disrupted energetics and amino acid metabolism in neurodegenerative diseases: perspectives for early diagnosis and monitoring of …," Metabolites, vol. 13, no. 10, 2023. mdpi.com
65. T. S. Jennaro, M. A. Puskarich, C. R. Evans, *et al.*, "Sustained perturbation of metabolism and metabolic subphenotypes are associated with mortality and protein markers of the host response," *Critical Care*, vol. 2023. lww.com
66. A. Rahnavard, B. Mann, A. Giri, R. Chatterjee, "Metabolite, protein, and tissue dysfunction associated with COVID-19 disease severity," *Scientific Reports*, 2022. nature.com

67. Q. Chen, X. Liang, T. Wu, J. Jiang, Y. Jiang, "Integrative analysis of metabolomics and proteomics reveals amino acid metabolism disorder in sepsis," *Journal of Translational Medicine*, vol. 20, no. 1, 2022. springer.com

68. Z. Jia, W. Q. Ong, F. Zhang, F. Du *et al.*, "A study of 9 common breath VOCs in 504 healthy subjects using PTR-TOF-MS," Metabolomics, 2024. nsf.gov

69. J. C. Ditto, H. N. Huynh, J. Yu, M. F. Link, "Speciating volatile organic compounds in indoor air: using in situ GC to interpret real-time PTR-MS signals," *Environmental Science: Processes & Impacts*, 2025. rsc.org

70. A. Corvino, I. Khomenko, E. Betta, F. I. Brigante, "Rapid profiling of volatile organic compounds associated with plant-based milks versus bovine milk using an integrated PTR-ToF-MS and GC-MS approach," Molecules, 2025. mdpi.com

71. M. Bajo-Fernández and É. A. Souza-Silva, "GC-MS-based metabolomics of volatile organic compounds in exhaled breath: Applications in health and disease. A review," Frontiers in Molecular, 2024. frontiersin.org

72. A. Kulyyassov, M. Fresnais, and R. Longuespée, "Targeted liquid chromatography-tandem mass spectrometry analysis of proteins: basic principles, applications, and perspectives," Proteomics, 2021. [HTML]

73. S. N. Thomas, D. French, P. J. Jannetto, *et al.*, "Liquid chromatography-tandem mass spectrometry for clinical diagnostics," *Nature Reviews*, vol. 2022. nih.gov

74. M. P. M. Letertre, P. Giraudeau, and P. De Tullio, "Nuclear magnetic resonance spectroscopy in clinical metabolomics and personalized medicine: current challenges and

perspectives," *Frontiers in Molecular*, vol. 2021. frontiersin.org

75. L. A. Kartsova, E. A. Bessonova, V. A. Deev, *et al*., "Current role of modern chromatography with mass spectrometry and nuclear magnetic resonance spectroscopy in the investigation of biomarkers of endometriosis," *Critical Reviews in …*, 2024. [HTML]
76. Y. T. Lee, N. Fujiwara, J. D. Yang, and Y. Hoshida, "Risk stratification and early detection biomarkers for precision HCC screening," Hepatology, 2023. wiley.com
77. H. He, Y. Wu, Z. Jia, Y. Zhang, Y. Pan, Y. Zhang, K. Su, "A stratified precision screening strategy for enhancing hepatitis B-and C-associated liver cancer detection: a prospective study," *Scientific Reports*, 2025. nature.com
78. H. Ding, H. Tu, C. Qu, G. Cao, H. Zhuang, P. Zhao, and X. Xu, "Guideline for stratified screening and surveillance in patients with high risk of primary liver cancer," 2020. oaepublish.com
79. P. Nahon, E. Vo Quang, and N. Ganne-Carrié, "Stratification of hepatocellular carcinoma risk following HCV eradication or HBV control," Journal of clinical medicine, 2021. mdpi.com
80. S. Zeng, Z. Liu, B. Ke, Y. Zhang *et al*., "The non-invasive serum biomarkers contributes to indicate liver fibrosis staging and evaluate the progress of chronic hepatitis B," BMC Infectious Diseases, 2024. springer.com
81. L. H. Feng, H. Liang, B. Hu, L. Wei, Q. Li, T. Su, *et al*., "Development and Validation of an Extra Spindle Pole Bodies-like 1-Based Diagnostic and Prognostic Model for Hepatitis B Virus-Related Hepatocellular," JMIR Medical, 2025. jmir.org

82. O. A. Shchelochkov, I. Manoli, P. Juneau, J. L. Sloan, *et al.*, "Severity modeling of propionic acidemia using clinical and laboratory biomarkers," *Genetics in Medicine*, vol. 2021. nature.com

83. Y. Wang, S. Zhu, W. He, H. Marchuk, E. Richard, "The attenuated hepatic clearance of propionate increases cardiac oxidative stress in propionic acidemia," Basic Research in …, 2024. [HTML]

84. N. Longo, J. O. Sass, A. Jurecka, *et al.*, "Biomarkers for drug development in propionic and methylmalonic acidemias," *Journal of Inherited Metabolic Disease*, vol. 45, no. 6, pp. 1234-1245, 2022. wiley.com

85. A. Sidorina, G. Catesini, E. Sacchetti, C. Rizzo, "Propionic acidemia, methylmalonic acidemia, and cobalamin C deficiency: comparison of untargeted metabolomic profiles," Metabolites, 2024. mdpi.com

86. C. Aima, O. A. Shchelochkov, T. J. Serrano, "Propionic acidemia," GeneReviews®, 2024. nih.gov

87. N. Gonzalez Caldito, "Role of tumor necrosis factor-alpha in the central nervous system: a focus on autoimmune disorders," Frontiers in Immunology, 2023. frontiersin.org

88. I. D. Vachliotis and S. A. Polyzos, "The role of tumor necrosis factor-alpha in the pathogenesis and treatment of nonalcoholic fatty liver disease," Current obesity reports, 2023. springer.com

89. Y. Zhou, Z. Yu, and Y. Lu, "To explore the influencing factors of clinical failure of anti-tumor necrosis factor-α (TNF-α) therapy in sepsis," Life Sciences, 2025. [HTML]

90. I. D. Vachliotis, I. Valsamidis, and S. A. Polyzos, "Tumor necrosis factor-alpha and adiponectin in nonalcoholic fatty liver disease-associated hepatocellular carcinoma," Cancers, 2023. mdpi.com

91. Z. Xia, S. Shi, X. Ma, F. Li, X. Li, H. Y. Gaisano, M. Zhao, "Mediating effect of adiponectin between free fatty acid and tumor necrosis factor-α in patients with diabetes," *Nutrition & Metabolism*, vol. 2024. nature.com

92. S. E. Ghanem, M. Abdel-Samiee, H. El-Said, "Evaluation of amino acids profile as non-invasive biomarkers of hepatocellular carcinoma in Egyptians," *Tropical Medicine and Infectious Disease*, vol. 7, no. 2, 2022. mdpi.com

93. T. Liu, Y. Xu, S. Hu, S. Feng *et al*., "Alanine, a potential amino acid biomarker of pediatric sepsis: a pilot study in PICU," Amino Acids, 2024. springer.com

94. M. Zhou, W. Sun, J. Chu, Y. Liao, P. Xu, *et al*., "Identification of novel biomarkers for frailty diagnosis via serum amino acids metabolomic analysis using UPLC-MS/MS," *PROTEOMICS*, 2024. [HTML]

95. A. Alqudah, E. Qnais, M. Wedyan, A. Awali, "Amino acid profiles: exploring their diagnostic and pathophysiological significance in hypertension," Molecular Biology, vol. 2024, Springer. [HTML]

96. Z. Ye, C. Zhang, S. Wang, Y. Zhang, R. Li, Y. Zhao, "Amino acid signatures in relation to polycystic ovary syndrome and increased risk of different metabolic disturbances," Reproductive, vol. 2022, Elsevier. [HTML]

97. M. Rahman and H. E. Schellhorn, "Metabolomics of infectious diseases in the era of personalized medicine," Frontiers in Molecular Biosciences, 2023. frontiersin.org

98. R. Mehta, E. Chekmeneva, H. Jackson, C. Sands, E. Mills, "Antiviral metabolite 3′-deoxy-3′, 4′-didehydro-cytidine is detectable in serum and identifies acute viral infections including COVID-19," Med, vol. 3, no. 1, pp. 1-11, 2022. cell.com

99. D. Chen, Y. Lu, J. Lian, J. Yu *et al*., "Plasma metabolome analysis for predicting antiviral treatment efficacy in chronic hepatitis B: diagnostic biomarkers and therapeutic insights," Frontiers in Immunology, 2024. frontiersin.org

100. Z. Cheng, "FoxO transcription factors in mitochondrial homeostasis," Biochemical Journal, 2022. portlandpress.com

101. X. Li, O. Jiang, M. Chen, and S. Wang, "Mitochondrial homeostasis: shaping health and disease," Current Medicine, 2024. springer.com

102. L. Liu, Y. Li, G. Chen, and Q. Chen, "Crosstalk between mitochondrial biogenesis and mitophagy to maintain mitochondrial homeostasis," Journal of Biomedical Science, 2023. springer.com

103. S. Elesela and N. W. Lukacs, "Role of mitochondria in viral infections," Life, 2021. mdpi.com

104. L. G. Prado, N. O. S. Camara, and A. S. Barbosa, "Cell lipid biology in infections: an overview," in *Frontiers in Cellular and Infection Biology*, 2023. frontiersin.org

105. Y. Tan, Y. Jin, J. Zhou, and Y. Yang, "Lipid droplets in pathogen infection and host immunity," Acta Pharmacologica Sinica, 2024. nih.gov

106. M. A. Farías, B. Diethelm-Varela, A. J. Navarro, A. M. Kalergis, *et al*., "Interplay between lipid metabolism, lipid droplets, and DNA virus infections," *Cells*, vol. 11, no. 3, 2022. mdpi.com

107. S. F. Jorgensen and M. E. Macpherson, "Disturbed lipid profile in common variable immunodeficiency-a pathogenic loop of inflammation and metabolic disturbances," Frontiers in…, 2023. frontiersin.org

108. T. Buergel, J. Steinfeldt, G. Ruyoga, M. Pietzner, *et al*., "Metabolomic profiles predict individual multidisease outcomes," *Nature Medicine*, vol. 2022. nature.com

109. M. Pietzner, I. D. Stewart, J. Raffler, K. T. Khaw, *et al*., "Plasma metabolites to profile pathways in noncommunicable disease multimorbidity," *Nature Medicine*, vol. 27, no. 11, pp. 1890-1901, 2021. nih.gov

110. A. Metwaly, S. Reitmeier, and D. Haller, "Microbiome risk profiles as biomarkers for inflammatory and metabolic disorders," *Nature Reviews Gastroenterology & Hepatology*, vol. 19, no. 12, pp. 743-757, 2022. [HTML]

111. C. Hu and W. Jia, "Multi-omics profiling: the way toward precision medicine in metabolic diseases," Journal of molecular cell biology, 2021. oup.com

112. Z. Sun, C. Huang, Y. Shi, R. Wang, J. Fan, and Y. Yu, "Distinct bile acid profiles in patients with chronic hepatitis B virus infection reveal metabolic interplay between host, virus and gut microbiome," *Frontiers in …*, 2021. frontiersin.org

113. J. Wang, H. Xu, Z. Liu, Y. Cao, S. Chen, "Bile acid-microbiota crosstalk in hepatitis B virus infection," Journal of …, 2024. wiley.com

114. Y. Shen, S. D. Wu, Y. Chen, X. Y. Li, Q. Zhu, K. Nakayama, *et al*., "Alterations in gut microbiome and metabolomics in chronic hepatitis B infection-associated liver disease and their impact on peripheral immune response," *Gut*, vol. 2023, Taylor & Francis. tandfonline.com

115. Y. Li, D. Zhang, T. Tian, J. Xie, X. Wang, W. Deng, "Relationship between gut microbiota dysbiosis and bile acid in patients with hepatitis B-induced cirrhosis," BMC, 2025. springer.com

116. A. Chetty and R. Blekhman, "Multi-omic approaches for host-microbiome data integration," Gut Microbes, 2024. tandfonline.com

117. C. Wang, X. Yu, H. Lin, G. Wang, J. Liu, and C. Gao, "Integrating microbiome and metabolome revealed microbe-metabolism interactions in the stomach of patients with different severity of peptic ulcer disease," Frontiers in ..., vol. 2023. frontiersin.org

118. P. Li, H. Luo, B. Ji, and J. Nielsen, "Machine learning for data integration in human gut microbiome," Microbial Cell Factories, 2022. springer.com

119. O. Shtossel, O. Koren, I. Shai, E. Rinott *et al.*, "Gut microbiome-metabolome interactions predict host condition," Microbiome, 2024. springer.com

120. W. Zheng, Y. Wang, H. Sun, S. Bao, S. Ge, "The role of Fusobacterium nucleatum in macrophage M2 polarization and NF-κB pathway activation in colorectal cancer," Frontiers in ..., 2025. frontiersin.org

121. A. M. El-Baz, A. M. El-Ganiny, D. Hellal, H. M. Anwer, "Valuable effects of lactobacillus and citicoline on steatohepatitis: role of Nrf2/HO-1 and gut microbiota," AMB Express, vol. 13, no. 1, 2023. springer.com

122. S. Mukherjee, A. Chopra, S. Karmakar, "Periodontitis increases the risk of gastrointestinal dysfunction: an update on the plausible pathogenic molecular mechanisms," Critical Reviews in ..., 2025. tandfonline.com

123. M. Pallozzi, V. De Gaetano, N. Di Tommaso, L. Cerrito, *et al.*, "Role of gut microbial metabolites in the pathogenesis of primary liver cancers," Nutrients, 2024. mdpi.com

124. S. L. Collins, J. G. Stine, J. E. Bisanz, C. D. Okafor, *et al.*, "Bile acids and the gut microbiota: metabolic interactions and impacts on disease," *Nature Reviews*, 2023. nih.gov

125. Y. Fan, C. Xu, L. Xie, Y. Wang, S. Zhu, J. An, and Y. Li, "Abnormal bile acid metabolism is an important feature of gut microbiota and fecal metabolites in patients with slow transit constipation," *Frontiers in Cellular*, vol. 2022. frontiersin.org

126. Z. Hao, K. Tao, K. Wu, Y. Luo, Y. Lu, B. Li, P. Shi, *et al.*, "Alterations of gut microbiome and metabolite profiles in choledocholithiasis concurrent with cholangitis," *Hepatology*, vol. 76, no. 1, pp. 123-135, 2022. [HTML]

127. Y. Mancera-Hurtado and B. E. Lopez-Contreras, "A dysregulated bile acids pool is associated with metabolic syndrome and gut microbial dysbiosis in early adolescence," Wiley Online Library, 2023. wiley.com

128. J. L. Guo, M. Januszyk, and M. T. Longaker, "Machine learning in tissue engineering," Tissue Engineering Part A, 2023. nih.gov

129. R. S. G. Sealfon, A. K. Wong, and O. G. Troyanskaya, "Machine learning methods to model multicellular complexity and tissue specificity," Nature Reviews Materials, 2021. researchgate.net

130. J. Cui, M. Wang, C. Lin, X. Xu *et al.*, "Exploring machine learning strategies for single-cell transcriptomic analysis in wound healing," Burns & Trauma, 2025. oup.com

131. Y. Wu, X. Ding, Y. Wang, and D. Ouyang, "Harnessing the power of machine learning into tissue engineering: current progress and future prospects," Burns & Trauma, 2024. oup.com

132. J. Zhao, L. Liu, Y. Y. Cao, X. Gao, G. Targher, "MAFLD as part of systemic metabolic dysregulation," Hepatology, vol. 2024, Springer. soton.ac.uk

133. D. Zhang, C. Shi, Y. Wang, J. Guo, "Metabolic dysregulation and metabolite imbalances in Acute-on-chronic liver failure: impact on immune status," Journal of Clinical and …, 2024. nih.gov

134. X. Li, Q. Zhang, Z. Wang, and Q. Zhuang, "Immune and metabolic alterations in liver fibrosis: a disruption of oxygen homeostasis?," *Frontiers in Molecular*, vol. 2022. frontiersin.org

135. J. Clària, V. Arroyo, and R. Moreau, "Roles of systemic inflammatory and metabolic responses in the pathophysiology of acute-on-chronic liver failure," JHEP Reports, 2023. sciencedirect.com

136. M. Borgia, M. Dal Bo, and G. Toffoli, "Role of virus-related chronic inflammation and mechanisms of cancer immune-suppression in pathogenesis and progression of hepatocellular carcinoma," Cancers, 2021. mdpi.com

137. M. Iannacone and L. G. Guidotti, "Immunobiology and pathogenesis of hepatitis B virus infection," Nature Reviews Immunology, 2022. google.com

138. M. Y. W. Zaki, A. M. Fathi, S. Samir, N. Eldafashi, K. Y. William, *et al.*, "Innate and adaptive immunopathogeneses in viral hepatitis; crucial determinants of hepatocellular carcinoma," Cancers, vol. 14, no. 22, 2022. mdpi.com

139. D. A. Rios, P. C. Casciato, M. S. Caldirola, *et al.*, "Chronic hepatitis C pathogenesis: immune response in the liver microenvironment and peripheral compartment," *Frontiers in Cellular and Infection Microbiology*, vol. 2021. frontiersin.org

140. M. A. Odenwald and S. Paul, "Viral hepatitis: Past, present, and future," World journal of gastroenterology, 2022. nih.gov

141. J. R. Su, Q. Y. Zhang, Y. Zhao, and Y. H. Yu, "Characterization of the clinical features in HBV-related acute-on-chronic liver failure," *Alternative Therapies in Health*, vol. 2022. alternative-therapies.com

142. M. B. Pisano, C. G. Giadans, D. M. Flichman, "Viral hepatitis update: Progress and perspectives," *World Journal of...*, 2021. nih.gov

143. K. Sayaf, D. Gabbia, F. P. Russo, and S. De Martin, "The role of sex in acute and chronic liver damage," *International Journal of …*, 2022. mdpi.com

144. R. J. Meshram, G. H. Kathwate, and R. N. Gacche, "Progress, evolving therapeutic/diagnostic approaches, and challenges in the management of hepatitis C virus infections," Archives of Virology, 2022. nih.gov

145. Z. Bai, X. Ma, R. Yan, W. Lei *et al.*, "Metabolomic profiling of early inactive hepatic alveolar and cystic echinococcosis," Acta Tropica, 2023. sciencedirect.com

146. V. Tounta, Y. Liu, A. Cheyne, and G. Larrouy-Maumus, "Metabolomics in infectious diseases and drug discovery," Molecular omics, 2021. rsc.org

147. P. Fischer, S. Pandrea, C. Grigoras, *et al.*, "Blood metabolomic signatures to identify bacterial infection in patients with decompensated cirrhosis," J. Gastrointestin Liver Dis., vol. 31, no. 2, pp. 215-222, 2022. academia.edu

148. F. Kanwal, J. H. Shubrook, L. A. Adams, K. Pfotenhauer, *et al.*, "Clinical care pathway for the risk stratification and management of patients with nonalcoholic fatty liver disease," *Gastroenterology*, vol. 161, no. 5, pp. 1346-1360, 2021. nih.gov

149. M. E. Rinella, B. A. Neuschwander-Tetri, M. S. Siddiqui, *et al.*, "AASLD Practice Guidance on the clinical assessment

and management of nonalcoholic fatty liver disease," *Hepatology*, vol. 2023. nih.gov

150. A. Vitale, G. Cabibbo, M. Iavarone, L. Viganò, *et al.*, "Personalised management of patients with hepatocellular carcinoma: a multiparametric therapeutic hierarchy concept," The Lancet, 2023. binasss.sa.cr

151. Y. Saiman and A. Duarte-Rojo, "Fatty liver disease: diagnosis and stratification," *Annual Review of …*, vol. 2022. nih.gov

152. M. D. Pauly and L. Ganova-Raeva, "Point-of-care testing for hepatitis viruses: a growing need," Life, 2023. mdpi.com

153. M. Ren, C. Lu, M. Zhou, X. Jiang, and X. Li, "The intersection of virus infection and liver disease: A comprehensive review of pathogenesis, diagnosis, and treatment," WIREs Mechanisms of Disease, vol. 2024, Wiley Online Library. wiley.com

154. W. Zhang, M. Aryan, S. Qian, "A focused review on recent advances in the diagnosis and treatment of viral hepatitis," Gastroenterology, vol. 2021. nih.gov

155. L. R. Thistlethwaite, X. Li, L. C. Burrage, K. Riehle, *et al.*, "Clinical diagnosis of metabolic disorders using untargeted metabolomic profiling and disease-specific networks learned from profiling data," *Scientific Reports*, 2022. nature.com

156. S. S. Adav and Y. Wang, "Metabolomics signatures of aging: recent advances," Aging and disease, 2021. nih.gov

157. N. Liu, J. Xiao, C. Gijavanekar, K. L. Pappan, *et al.*, "Comparison of untargeted metabolomic profiling vs traditional metabolic screening to identify inborn errors of metabolism," JAMA Network, vol. 2021. jamanetwork.com

158. M. Liu and Y. Wen, "Point-of-care testing for early-stage liver cancer diagnosis and personalized medicine: Biomarkers, current technologies and perspectives," Heliyon, 2024. cell.com

159. X. Rong, F. Ailing, L. Xiaodong, H. Jie, "Monitoring hepatitis B by using point-of-care testing: biomarkers, current technologies, and perspectives," *Expert Review of Molecular Diagnostics*, vol. 21, no. 5, pp. 423-432, 2021. [HTML]

160. M. A. Tincopa and R. Loomba, "Non-invasive diagnosis and monitoring of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis," The lancet Gastroenterology & hepatology, 2023. [HTML]

161. R. Chinnappan, T. A. Mir, S. Alsalameh, T. Makhzoum, "Low-cost point-of-care monitoring of ALT and AST is promising for faster decision making and diagnosis of acute liver injury," Diagnostics, 2023. mdpi.com

162. J. Wu, Y. Xu, Y. Cui, M. Bortolanza, *et al*., "Dynamic changes of serum metabolites associated with infection and severity of patients with acute hepatitis E infection," *Journal of Medical …*, 2022. ghep-hev.com

163. S. Khan, Y. Kumar, C. Sharma, and S. K. Gupta, "Dysregulated metabolites and lipids in serum of patients with acute hepatitis E: A longitudinal study," *Journal of Viral Hepatitis*, vol. 2023, Wiley Online Library. [HTML]

164. S. H. Bridge, D. Neely, J. F. Cobbold, and E. Holmes, "Infection with the hepatitis C virus causes viral genotype-specific differences in cholesterol metabolism and hepatic steatosis," *Scientific Reports*, 2022. nature.com

165. R. O. Ali, G. M. Quinn, R. Umarova, J. A. Haddad, and others, "Longitudinal multi-omics analyses of the gut-liver

axis reveals metabolic dysregulation in hepatitis C infection and cirrhosis," *Nature*, 2023. [HTML]

166. R. A. A. Ponde and G. S. P. Amorim, "Elimination of the hepatitis B virus: A goal, a challenge," Medicinal Research Reviews, 2024. [HTML]

167. S. J. Polyak, I. N. Crispe, and T. F. Baumert, "Liver abnormalities after elimination of HCV infection: persistent epigenetic and immunological perturbations post-cure," Pathogens, 2021. mdpi.com

168. A. Quirino, N. Marascio, F. Branda, A. Ciccozzi, "Viral Hepatitis: Host Immune Interaction, Pathogenesis and New Therapeutic Strategies," Pathogens, 2024. mdpi.com

169. X. Tong, Y. Song, S. Yin, J. Wang, R. Huang, "Clinical impact and mechanisms of hepatitis B virus infection concurrent with non-alcoholic fatty liver disease," *Chinese Medical Journal*, vol. 2022. lww.com

170. C. J. Fernandez, M. Alkhalifah, H. Afsar, and J. M. Pappachan, "Metabolic dysfunction-associated fatty liver disease and chronic viral hepatitis: The interlink," Pathogens, 2024. mdpi.com

171. F.Y. Li, Q. Ou, Z.W. Lai, L.Z. Pu, X.Y. Chen, L.R. Wang, "… -occurrence of chronic hepatitis B and fibrosis is associated with a decrease in hepatic global DNA methylation levels in patients with non-alcoholic fatty liver disease," Frontiers in …, 2021. frontiersin.org

172. H. Wu, X. Luo, R. Zhang, A. Stubbendorff, E. Sonestedt, "Adherence to the EAT-Lancet reference diet and risk of type 2 diabetes, metabolic dysfunction-associated steatotic liver disease, and their co-occurrence," Food & Function, 2025. lu.se

173. K. Sharma, J. Hansen, K. Susztak, L. Eberlin, "Spatial

metabolomics and multiomics integration for breakthroughs in precision medicine for kidney disease," *Nature Reviews*, 2025. [HTML]

174. H. Zhang, K. H. Lu, M. Ebbini, P. Huang *et al*., "Mass spectrometry imaging for spatially resolved multi-omics molecular mapping," npj Imaging, 2024. nature.com

175. A. Mund, A. D. Brunner, and M. Mann, "Unbiased spatial proteomics with single-cell resolution in tissues," Molecular cell, 2022. cell.com

176. Y. Hou, Y. Gao, S. Guo, Z. Zhang, R. Chen, "Applications of spatially resolved omics in the field of endocrine tumors," *Frontiers in …*, 2023. frontiersin.org

177. H. Hu and J. Laskin, "Emerging computational methods in mass spectrometry imaging," Advanced Science, 2022. wiley.com

178. Y. H. Lai and Y. S. Wang, "Advances in high-resolution mass spectrometry techniques for analysis of high mass-to-charge ions," Mass Spectrometry Reviews, 2023. [HTML]

179. L. X. Jiang, M. Yang, S. N. Wali, and J. Laskin, "High-throughput mass spectrometry imaging of biological systems: current approaches and future directions," TrAC Trends in Analytical Chemistry, 2023. sciencedirect.com

180. S. Tamara, M. A. den Boer, and A. J. R. Heck, "High-resolution native mass spectrometry," Chemical reviews, 2021. acs.org

181. C. R. Ferreira, S. Rahman, M. Keller, *et al*., "An international classification of inherited metabolic disorders (ICIMD)," *Inherited Metabolic Disorders*, vol. 2021, Wiley Online Library. nih.gov

182. M. Castro-Guarda and R. D. Evans, "Human metabolism:

metabolic pathways and clinical aspects," Surgery (Oxford), 2025. surgeryjournal.co.uk

183. T. Hu, C. H. Liu, M. Lei, Q. Zeng, L. Li, H. Tang, *et al.*, "Metabolic regulation of the immune system in health and diseases: mechanisms and interventions," Signal Transduction and Targeted Therapy, vol. 9, no. 1, 2024. nature.com

184. H. Dong, Y. Sun, L. Nie, A. Cui, P. Zhao, "Metabolic memory: mechanisms and diseases," Signal Transduction and…, 2024. nature.com

185. AJ McGlinchey, O. Govaere, D. Geng, V. Ratziu, *et al.*, "Metabolic signatures across the full spectrum of non-alcoholic fatty liver disease," Jhep Reports, 2022. sciencedirect.com

186. C. Hu, T. Wang, X. Zhuang, Q. Sun, X. Wang, H. Lin, "Metabolic analysis of early nonalcoholic fatty liver disease in humans using liquid chromatography-mass spectrometry," *Journal of Translational Medicine*, vol. 19, no. 1, 2021. springer.com

187. N. Vallianou, G. S. Christodoulatos, I. Karampela, *et al.*, "Understanding the role of the gut microbiome and microbial metabolites in non-alcoholic fatty liver disease: current evidence and perspectives," Biomolecules, vol. 11, no. 2, 2021. mdpi.com

188. M. Lewinska, A. Santos-Laso, E. Arretxe, C. Alonso, *et al.*, "The altered serum lipidome and its diagnostic potential for Non-Alcoholic Fatty Liver (NAFL)-associated hepatocellular carcinoma," *The Lancet*, 2021. thelancet.com

189. H. Tilg, T. E. Adolph, M. Dudek, and P. Knolle, "Non-alcoholic fatty liver disease: the interplay between

metabolism, microbes and immunity," Nature metabolism, 2021. easp.es