Gastroenterología y Hepatología xxx (xxxx) 502207

Gastroenterología y Hepatología

www.elsevier.es/gastroenterologia

SPECIAL ARTICLE

Proceedings of the 5th Meeting of Translational Hepatology, organized by the Spanish Association for the Study of the Liver (AEEH)

V Reunión de Hepatología Traslacional, organizada por la Asociación Española para el Estudio del Hígado (AEEH)

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https://doi.org/10.1016/j.gastrohep.2024.502207

Please cite this article as: E. Alvarado-Tapias, D. Maya-Miles, A. Albillos et al., Proceedings of the 5th Meeting of Translational Hepatology, organized by the Spanish Association for the Study of the Liver (AEEH), Gastroenterología y Hepatología, https://doi.org/10.1016/j.gastrohep.2024.502207

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Introduction

Recent estimations suggest that approximately 1.5 billion people around the world are suffering from a chronic liver condition associated to diverse etiopathological agents.[1] Among them, hepatic viral infections are highly prevalent and account for significant morbidity and mortality. About 257 million people live with chronic hepatitis B virus (HBV) and 71 million people with chronic hepatitis C virus (HCV) infection, despite effective treatments for HCV, especially direct-acting-antivirals (DAAs), and a better control of HBV infection through vaccination programs and new therapeutic options (Entecavir and Tenofovir).[1] Besides, the incidence of metabolic-dysfunction associated steatotic liver disease (MASLD), previously known as non-alcoholic fatty liver disease (NAFLD), has alarmingly increased in the last decade, particularly in obese[2] and diabetic patients,[3] and is estimated to affect 20–30% of the global population.[4,5] Moreover, alcohol-associated liver disease (ALD) is also a major cause of liver-related morbidity and mortality worldwide, with an incidence of around 2–3% of the global population.[1] Additionally, even less prevalent diseases like cholestatic, autoimmune and genetic liver diseases have shown an increasing trend in prevalence in the last years. However, the management of these chronic liver conditions is limited by the lack of reliable diagnostic biomarkers and the few therapeutic options available.[6] These chronic liver conditions can eventually lead to cirrhosis, one of the main causes of death in this population, being responsible for 2.4% of global deaths in 2019[7] or, in the latest stages, to liver cancer, which accounts for more than 800,000 deaths per year worldwide and is among the top three causes of cancer-related deaths in many developed countries.[8]

Thus, hepatology is becoming a rapidly evolving field with ongoing research developments and advances in diagnostic techniques, therapeutic interventions, and a better understanding of liver diseases at the molecular level. In this sense, translational hepatology aims to bridge the gap between laboratory research and clinical practice, with the ultimate goal of improving patient outcomes by advancing in preclinical research, clinical trials, translation of new interventions into routine clinical practice, and boosting the field of precision medicine. This field aims to tailor medical treatments to individual patients based on their unique characteristics, such as genetic traits, lifestyle factors, and disease phenotypes. The aim of this article is to provide an update on the most recent advances in the field of basic and translational hepatology, covering a broad range of molecular, cellular, and pathophysiological aspects of the most relevant clinical needs in liver pathologies.

Biomarkers and diagnostic tools in chronic liver diseases

Biomarkers, or biological markers, are molecules that can be measured in a biological sample and play a relevant role in some physiological processes. Thus, its detection can serve as helpful tools in the decision-making process in clinical practice. Biomarkers play a pivotal role in current medical research and practice. These measurable indicators enable early disease diagnosis, prognosis assessment, treatment monitoring, and identification of genetic susceptibility. Their application has transformed medical practice and fueled advances in personalized medicine, tailoring treatments to individuals based on their unique molecular characteristics. The study and utilization of biomarkers continue to shape the healthcare landscape, providing precise and personalized insights for improved health management. Since personalized medicine is still under development in the context of liver diseases, the use of biomarkers for both in- and outpatients is increasingly wide. From a clinical perspective, an optimal biomarker, in addition to being accurate, should meet various features including, non-invasiveness, repeatability, simplicity (based on simple variables), and inexpensiveness. The type of sample used for the detection of the biomarker is of particular importance. Serum or urine samples are preferable compared to liver tissue, to avoid the liver biopsy and consequently, the development of soluble biomarkers for clinical practice is strongly encouraged.

Liver fibrosis is a key outcome in chronic liver diseases. Strategies able to screen fibrosis in general population are a must, given its strong impact on liver-related and unrelated outcomes.[9,10] At risk metabolic-dysfunction associated steatohepatitis (MASH), defined as the presence of a fibrosis stage $\geq$F2 and a NAFLD activity score (NAS) $\geq$4, might also be important to follow in patients at risk of suffering from MASLD, as it seems to have a stronger correlation with progression to cirrhosis and major adverse liver outcomes than fibrosis per se. Current available non-invasive tools and scores are quite efficient to rule out the presence of advanced liver fibrosis, but still entail a significant number of false positives, leading to unnecessary referrals, and a lower but not neglectable amount of patients with false negatives, who could benefit from specialized care but remain undiagnosed.[11,12] Finding new algorithms able to improve the specificity and the sensitivity of the current clinical circuits used to estimate risk is essential, especially in diseases with a high prevalence like MASLD and ALD.[13] Liver Investigation: Testing Marker Utility in Steatohepatitis (LITMUS) project and the Non-Invasive Biomarkers of Metabolic Liver Disease (NIMBLE) project have recently unveiled their primary research findings in the search for biomarkers able to improve disease stratification in MASLD.[14,15] Analyses in these large multinational cohorts indicated that NIS4, SomaSignal and the FAST algorithm were all good tools to discriminate patients with at-risk MASH. Other promising new biomarkers for at-risk MASH include NIS2, a simplified version of NIS4,[16] the promising MRI-aspartate aminotransferase score (MAST) (AUC: 0.93 [0.88–0.97]),[17] or emerging alternative combinations of routine analytics with circulating proteins or metabolites.[18] Further studies will be required to determine if these new biomarkers can be useful for the screening of fibrosis independently of etiology in general population.

The validation of the usefulness and/or accuracy of the biomarker in different scenarios are of pivotal importance. Indeed, the operator characteristics curve of the same biomarker can vary depending on its application (stratifying, predicting, or assessing the response to treatment). Biomarkers need to prove their repeatability, especially in those contexts in which they are used to monitor disease

progression and regression, in order to set cut-offs able to differentiate meaningful improvements in patients from spurious stochastic changes. Further, biomarkers need to validate their utility there where they can become useful tools (e.g., general population for liver screening tools).[19,20] It is also of utmost importance that we facilitate the reuse of published datasets that could be used to identify potential biomarkers, as the validation of any biomarker aiming to reach the market will require a large-scale analysis before approval. This strategy can sometimes highlight simple approaches that can be used to perform a first estimation of the risk of severe liver disease in patients independently of liver etiology, as recently proved by the LiverScreen project.[21] This is of special importance in liver diseases due to the low accuracy of current diagnostic techniques, which results in a high percentage of diagnosis of severe liver pathologies (such as hepatocellular carcinoma or cholangiocarcinoma) at advanced stages, when therapeutic options are much more limited.[22] Moreover, although the variety of treatments has expanded significantly over recent years primarily due to the surge in immunotherapy, the lack of prognostic and predictive biomarkers hampers the putative benefits associated to early application of specific treatment. In this dismal scenario, there is a joint effort for the search and development of new non-invasive biomarkers based on liquid biopsy strategies. This could improve the management of patients with various chronic liver diseases, including liver cancer.[23–25] In this sense, the development of new biomarkers for different clinical applications, particularly in liver diseases, has been boosted by the emergence and advancement of new technologies for liquid biopsy analysis.[26] For instance, DNA methylation analysis in cell-free DNA (cfDNA) has shown promising results on early hepatocellular carcinoma diagnosis.[27,28] Lapitz et al. identified specific proteins in plasmatic extracellular vesicles from cholangiocarcinoma patients with utility in prediction, early diagnosis, and prognosis.[29] Arechederra et al. demonstrated an exceptional sensitivity of the mutational analysis of bile cfDNA to detect malignancy in patients with strictures initially classified as nonmalignant yet developing tumors after follow-up.[30]

Transferring the knowledge from basic and translational research to the clinical practice is essential to improve the management of patients with liver disorders. This can be achieved through the development of in situ diagnostic systems such as point-of-care (POC) assays. An example of this translational research has been shown in the detection of portal hypertension, which constitutes a significant advancement in the field of hepatology and cutting-edge healthcare. Portal hypertension, characterized by an increase in pressure within the porto-sinusoidal vascular system, is a serious complication of various chronic liver diseases, such as cirrhosis.[31] This condition can lead to devastating clinical consequences, including potentially life-threatening gastrointestinal bleeding and other systemic complications. Therefore, early and precise identification of portal hypertension is crucial for facilitating timely therapeutic intervention, which can be translated into a significant improvement in the quality of life for affected patients. The development of a POC for portal hypertension has needed comprehensive research in molecular biology and medical technology to firstly identify specific biomarkers that exhibit a close correlation with portal hypertension. Such biomarkers were identified in a recent study using a liver-on-a-chip asset where hydrodynamic pressure can be modulated,[32,33] and the response of liver sinusoidal endothelial cells was determined.[34] Two potential biomarkers, E-Cadherin and Spink1, were identified and preliminary validated. The creation of this POC assay for the detection of portal hypertension promises to revolutionize the diagnostic and treatment landscape in this critical medical area. By enabling early diagnoses and prompt therapeutic decisions, this technology has the potential to substantially enhance patient outcomes, thus demonstrating the capacity of translational research for the identification and development of novel biomarkers and diagnostic tools.

Immune response and inflammation in liver diseases

The most prevalent causes of liver diseases (alcohol intake, hepatitis viral infections and accumulation of fat in the liver) can lead to hepatocyte necrosis with significant consequences. In particular, damaged hepatocytes release cell damage signals that stimulate innate immune system cells to produce inflammatory mediators. This leads to subclinical systemic inflammation with hemodynamic implications. Repeated exposure to these stimuli exhausts the immune system leading to a compensatory anti-inflammatory response. Changes in the phenotype and function of innate immune system cells have been described, which are associated with reduced immune efficacy.[35,36] In addition, multiple experimental evidence has associated altered levels of pro- and anti-inflammatory cytokines with clinical manifestations of severe disease.[37] This sequence of events generates a vicious cycle of inflammation and immunocompromise that exacerbates chronic liver disease progression.

Neutrophils, the most abundant leukocytes in the body, are emerging as significant players in this pathological process. Traditionally viewed as short-lived cells involved in the initial inflammatory response, neutrophils are now understood to be a heterogeneous population with multifaceted roles in regulating liver homeostasis.[38,39] In circulation, neutrophils become primed due to an inflammatory environment, with Damage-Associated Molecular Pattern (DAMPs) and Pathogen-Associated Molecular Pattern (PAMPs) leading to reduced phagocytic and oxidative burst capacity, and the consequent inefficient clearance of infection and debris.[38] Moreover, immature populations are present due to early egress from the bone marrow. In the liver tissue, there is an increased recruitment of neutrophils due to the production of chemokines and DAMPs, which is accompanied by enhanced neutrophil extracellular trap (NET) formation. In these conditions, neutrophils exert a dual effect, while participating in the clearance of debris they also induce hepatocyte and vascular injury and inflammation due to increased degranulation and NET formation. In early disease stages, neutrophils are present in the liver parenchyma associated to necrotic areas or damaged cells, participating in debris, clearing and modulation of inflammatory response. However, in advanced conditions, a substantial number of

neutrophils are found in the periportal area, immobilized to biliary cells and the ductular reaction, which shows a pro-inflammatory profile and establish a direct crosstalk with neutrophils.[38,40,41] Ductular reaction associated-neutrophils (DRANs) show specific phenotypic and functional features and have an extended lifespan. Intrahepatic neutrophils directly participate in both injury progression and wound-healing response.[42] While neutrophils directly promote the proliferation and inflammatory profile of ductular reaction cells, the depletion of neutrophils and the absence of neutrophil elastase reduce the progression of ductular reaction, fibrosis and angiogenesis.[40] However, in experimental conditions of injury reversion, neutrophils drive tissue injury resolution, as neutrophil depletion prevents wound-healing.[43] These findings indicate the complex role of neutrophils in the liver, participating in the regulation of liver homeostasis, injury and wound healing. Overall, neutrophils are plastic cells capable of adapting their phenotype and function to the environment and to the organ they infiltrate. Moreover, neutrophils not only interact with immune cells, but also with parenchymal and non-parenchymal liver cells, establishing mutual crosstalk that regulates the response to injury.

In parallel, during the development and progression of chronic liver diseases, the permeability of the intestinal epithelium increases, facilitating infections and bacterial translocation. This serves as the source of new stimulatory signals from innate immune system cells in the form of PAMPs that amplify inflammation.[35] Particularly, during the progression of chronic liver diseases and cirrhosis, there is a change in the composition of the intestinal microbiota, termed dysbiosis.[44] Various aspects such as decreased bile acids flow, intestinal hypomotility, and compromised hepatic immunosurveillance contribute to this change. The dysbiotic microbiota, in turn, plays a fundamental role in the development of many complications of microbial origin that aggravate the disease course. The expansion of pathogenic species leeads to an increase in secondary bile acids at the intestinal level, reducing the activity of Farnesoid X receptor (FXR), which is involved in the stimulation of the mucus layer, the production of antimicrobial peptides and the expression of intercellular junctions in the epithelial barrier. As a consequence, the permeability of the intestinal barrier increases, facilitating bacterial translocation. On the other hand, the increase in immunogenic microbial antigens and the loss of symbiotic products such as short-chain fatty acids (SCFAs) alters the interaction between the microbiota and the local immune system. The homeostatic activity mediated by secreted IgA, the predominance of regulatory T cell activity and the presence of anti-inflammatory cytokines such as IL-10 or TGF-β, among others, depict a local immune activity characterized by an expansion of the pro-inflammatory T response.[45]

In this pathological crosstalk between the diseased liver, the immune system and gut microbiota, the extracellular vesicles (EVs) are gaining increasing relevance, especially in the most prevalent liver diseases worldwide, namely MASLD and ALD. EVs are nano-sized structures delimited by a lipid bilayer, released from all types of cells in the extracellular space in healthy and pathological conditions, in all biological fluids. The most accepted classification of EVs currently is based on their physical properties such as size, i.e., large and small EVs.[46,47] EVs participate in cell-to-cell communication in liver diseases. It is known that stressed/damaged hepatocytes release high quantities of EVs that contribute to the occurrence of inflammation, fibrogenesis, and angiogenesis, which are key pathobiological processes in liver disease progression. EVs can be generated as waves from different origins, favoring the progression of liver disease. In MASLD, lipotoxic stimuli generate hepatocyte damage [endoplasmic reticulum (ER) stress, MLK3, TRAIL] and enhance the release of EV enriched with S1P, ceramides, mtDNA, CsCL10. These hepatocyte-derived lipotoxic EVs mediate macrophage-monocyte accumulation in the liver with an increase secretion of TNFα and IL-1β.[48,49] In ALD, alcohol injury provokes an increased EVs release, from different origins; EVs from intestinal epithelial cells favor liver lipid accumulation and hepatocyte-derived EVs are enriched with proteins and coding and non-coding miRNA. These EVs are taken by monocytes (miR-122), macrophages (CD40L) and neutrophils (mtDNA), overall contributing to increased liver inflammation. EVs enriched with miRNAs (miR-27a and miR-181) can activate hepatic stellate cells (HSCs) and contribute to fibrosis deposition.[48,49] On the other hand, EVs have been evaluated as peripheral liquid biopsy markers in the study of hepatocellular carcinoma (HCC) and cholangiocarcinoma.[29,50] Specific molecular signatures of released EVs in biofluids have allowed EVs to be considered promising candidates to serve as disease biomarkers. Additionally, EVs may have potential for therapeutic use as a liver-specific delivery method of different agents, taking advantage of their hepatocellular uptake through interactions with specific receptors.

Reversion of liver disease in the era of etiological treatments

The liver is a large organ with an exceptional functional reserve and regeneration capacity. However, these mechanisms may be exhausted during severe acute injury or in a persistent chronic liver disease, which eventually would produce liver cirrhosis and hepatocellular carcinoma. Most chronic liver diseases share a natural history of asymptomatic progressive damage for more than 10–20 years which involves different overlapping histological dimensions, such as inflammation, steatosis, vascular injury, cholestasis, and fibrosis. The predominance of one or another would depend on the etiology of liver disease. For instance, in viral hepatitis inflammation predominates, while in ALD and MASLD there is a mixture of inflammation and steatosis. In the absence of an effective therapy, this variety of liver damage profiles would converge into subsequent stages of progressive fibrosis, compensated cirrhosis, decompensated cirrhosis and end-stage liver disease or HCC. Etiological therapies may slow the progression or even reverse liver damage at some extent, mostly at the expense of inflammation, cholestasis, and steatosis.[51,52] For example, lifetime alcohol abstinence in alcoholic liver disease, bariatric surgery in MASH, antiviral therapies against HBV-HCV, or biliary acids in primary biliary cholangitis, have shown to slow the progression of liver disease or even to reverse the histological grade of inflammation, steatosis and/or fibrosis.[53–57] However, liver cirrhosis, par-

ticularly when decompensated, is generally considered a point of no return, and efforts should be driven to ameliorate the risk of decompensations and to decrease the incidence of HCC.[58,59] For patients with end-stage liver disease with severe decompensations, etiological therapies may avoid liver transplantation temporarily in 8%–10% of patients, but the risk of eventual worsening remains significant.[60,61] Emerging ''pan-etiological therapies'' targeting liver fibrosis,[62] bone marrow-derived stem cells[63] or even perfusion devices[64] could probably allow full reversibility of advanced liver damage in the future.

In this sense, several drugs are being tested in clinical trials, especially in the context of MASLD, and are mainly focused on inhibiting or reverting the activation of hepatic stellate cells, the main matrix-producing cells.[65] These pharmacological strategies target the interaction of HSC with activating mediators and inflammatory cells, and cellular processes required for transdifferentiation to myofibroblasts, as well as the elimination of activated HSC and the degradation of extracellular matrix.[66,67] Besides the development of new compounds, some drug repurposing candidates have arisen for this disease. For instance, statins may reduce fibrosis progression and incidence of cirrhosis in some populations,[68] and the anti-HIV drug rilpivirine is reported to induce hepatoprotective effects in preclinical models of CLD and HIV/HCV-coinfected patients.[69,70] Furthermore, pirfenidone and its derivate hydronidone, indicated for pulmonary fibrosis, have shown some efficacy against MASH-induced fibrosis and are currently under investigation in viral hepatitis.[71,72]

In fact, one of the liver diseases whose management and prognosis have completely changed within recent years is hepatitis C due to the discovery of DAAs. DAAs have also increased our knowledge on the impact of successful etiological therapy in reversion of liver damage since HCV patients with advanced stages of liver disease also achieve sustained virological response (SVR).[73–75] The accurate management of patients after viral eradication with DAAs, the most reliable non-invasive tools to predict (or exclude) liver-related complications, or to what extent viral eradication reduces the risk of liver disease progression in the long term are some of the current lines of research.[76] Liver stiffness measurement by transient elastography (alone or in combination with other blood tests) is the most studied prognostic factor and has proven to be useful in determining post-SVR prognosis as well as guiding clinical management.[74] Regarding hepatocellular carcinoma, screening in patients with pre-SVR advanced liver disease is cost-effective.[77] Although patients with advanced fibrosis (F3) have a low hepatocellular carcinoma risk, more prospective studies are needed to exclude them from screening programs.[75,78] Hepatocellular carcinoma risk stratification models are needed to identify patients with a particularly high hepatocellular carcinoma incidence following SVR.[79,80] Finally, for patients with some extrahepatic manifestations such as cryoglobulinemic vasculitis it is relevant to consider that relapse after SVR may occur although more studies on the associated risk factors to guide clinical follow-up are needed.[81,82]

On the other hand, it is well known that aging represents an important risk factor for chronic liver diseases.[83–85] Several forms of energy limitation including caloric restriction and intermittent fasting, promote lifespan and delay the onset and severity of several age-associated metabolic diseases, including MASLD and obesity.[86–89] Body weight loss, improvement of insulin sensitivity, reduction of intrahepatic triglyceride and chronic inflammation, as well as prevention of cardiovascular and neurological diseases, are among the physiological mechanisms boosted by energy restriction.[90,91] Noteworthy, the architecture of eating modulates the response to energy restriction beyond calorie intake, likely by the interaction between fundamental components such as fasting durations, timing of feedings, circadian alignment and/or dietary compositions.[92–95] In this scenario, it is imperative to gain knowledge on the physiological and metabolic mechanisms that underlie the enhanced cellular and organismal health observed under the temporal regulation of energy restriction.

In summary, the growing number of promising drug candidates, new nutritional interventions, and potential therapeutic targets for liver diseases, together with continuous advances in the understanding of its pathogenic mechanisms, may lead to the future approval of single or combined effective treatments for reversion of liver fibrosis, regardless of the etiology.

Beyond omics approaches in liver diseases

The rapid advances in omics technologies, including genomics, transcriptomics, proteomics, metabolomics, or lipidomics, among others, have contributed to the generation of a wealth of mineable biological ''big data'', revolutionizing biomedical research and enabling detailed molecular studies, moving a step forward toward personalized medicine. These methodologies offer the possibility to underpin the pathophysiological mechanisms of specific liver diseases from a global perspective, allowing a comprehensive understanding of the biological system that can serve to guide omics-based molecular measurements toward a clinical outcome.[96] Moreover, integration of multi-omics data provides a global picture of the processes within cells at multiple levels, leading to novel discoveries that otherwise would not have been found from a single omics dataset. By integrating the big data and complementary tools, the biomedical community has been able to identify novel accurate diagnostic/prognostic modalities and to unravel key aspects in the pathogenesis of liver diseases. In addition, most of the data generated through omics approaches is deposited in public repositories and freely accessible, allowing their subsequent analysis by all the scientific community.

The cBioportal (https://www.cbioportal.org) aggregates genomic mutational profile data from multiple studies, encompassing more than 20,000 cases across 33 different cancer types. Consequently, individual study analysis or meta-analysis can be conducted, correlating specific cancer mutations with demographic, clinical, and histopathological variables. The Cancer Genome Atlas (TCGA: https://www.cancer.gov/ccg/research/genome-sequencing/tcga) houses transcriptomic data (mRNA, miRNAs, lncRNAs) from studies on 33 different cancers, including hepatocellular carcinoma and cholangiocarcinoma, featuring tumor and healthy tissues from over 11,000 cases. It includes annotated information on demographics and clinical features for further multivariate analysis. The Human Protein Atlas

(https://www.proteinatlas.org) incorporates multi-omics data (e.g., transcriptomic, proteomic, and immunohistochemistry images) and single-cell RNA sequencing data from 61 tissues and organs, as well as various cancer types, with annotated demographic and clinical information. The GEO (Gene Expression Omnibus) database (https://www.ncbi.nlm.nih.gov/geo/) encompasses raw RNA profiling from multiple disease tissues and controls. However, omics studies face additional challenges such as the complexity of liver disease etiology, confounding factors that are difficult to control for, and distinguishing causal changes from reactive ones in the context of the disease.[97] Despite these challenges, omics approaches have the potential to provide meaningful insights into liver disease and to uncover so far unmet different aspects of liver pathophysiology.[98] Therefore, careful planning and execution are required to realize the full clinical potential of omics research in the study of liver diseases.[97] Indeed, new applications and methods of analysis of certain omics are discussed below, as well as examples of the usefulness of multi-omics integration in specific liver pathologies.

The advent of affordable next-generation sequencing has facilitated the generation of high-resolution snapshots of the entire transcriptome of a given cell or tissue type, capturing every RNA molecule and their relative abundance. This has allowed to search for genetic elements that had not been identified before. Examples of these successful expeditions include circular RNAs or other non-coding elements, but also unexpected coding bits of the genome, such as microexons, tiny exons that encode as few as one or two amino acids. Microexons are tightly regulated across cell types, being often expressed only in specific cell types, such as neurons or endocrine cells,[99] where they modulate the way proteins interact with each other and play crucial physiological roles. By investigating their functional, regulatory, and evolutionary impact, their roles in human diseases have been defined, particularly in metabolic disorders.[100] While the role of microexons in liver disease is not yet fully understood, their potential impact on many cellular pathways suggests that they may play a role in the pathophysiology of liver diseases, directly or indirectly, through physiological cross-talks between endocrine pancreas and liver. Further research is needed to fully elucidate the role of microexons in certain liver pathologies and explore their potential applicability.

In the same line, an increasing knowledge about mRNA metabolism regulation has proven that the proteome is not a static reflection of the transcriptome[101,102] and might therefore be considered as a useful tool for precision medicine in liver diseases. Proteomics is the study of the proteome, which is the complete set of proteins that are produced in a biological system. Proteomics focuses on the study of proteins, their structures, interactions, and functions and can, thus, provide insights into cellular functions, post-translational modifications, movement of proteins between different subcellular compartments or involvement of proteins in metabolic pathways. Among proteomic approaches, quantitative proteomics have been used to characterize liver disease progression and to identify novel molecular alterations. However, different limitations, including an incomplete coverage of the proteome, especially in targeted proteomics where only a pre-defined subset of proteins is quantified[103] or etiology biases toward viral hepatitis[104–106] or MASLD-related hepatocellular carcinoma[107] have hampered a wider recognition of the proteomic studies in hepatocellular carcinoma. In a recent study, some of these issues were addressed through a multi-omics analysis of hepatocellular carcinoma derived from different etiologies, in which some cellular pathways (i.e., RNA processing) were consistently dysregulated at all levels.[108] These studies demonstrate the usefulness of quantitative proteomics for the identification of tumoral subgroups, novel pathogenic pathways, dysregulated actionable targets [Platelet Derived Growth Factor Receptor Beta (PDGFRB), Fibroblast Growth Factor Receptor 4 (FGFR4), Erb-b2 Receptor Tyrosine Kinase 3 (ERBB2/3), Cyclin-Dependent Kinases (CDKs), Microfibril Associated Protein 5 (MFAP5), Hemicentin 1 (HMCN1), Heat Shock Proteins (HSPs), and Aurora Kinase A (AURKA)] or prognostic biomarkers [Pyrroline-5-Carboxylate Reductase 2 (PYCR2) and Alcohol Dehydrogenase 1A (ADH1A)] and illustrate the necessity of developing more ample proteomic approaches capable to integrate the wide spectrum of liver diseases together with more comprehensive data analysis, alone or in combination with other omics approaches.

Recently applied multi-omics approaches to investigate the pathophysiology of acute-on-chronic liver failure (ACLF), a complex syndrome that develops in patients with acutely decompensated cirrhosis, is an example of the success of these integrative analyses. By using omics approaches in a large cohort of patients with acutely decompensated cirrhosis, it was identified that dysbalanced immune function and excessive systemic inflammation are the main drivers of extrahepatic organ failure and high short-term mortality in these patients.[109] Indeed, each one of the -omics technologies, especially metabolomics, lipidomics and transcriptomics has contributed to the characterization of the hyperinflammatory state in patients with acutely decompensated cirrhosis developing ACLF. Likewise, these -omics facilitated the identification of the main triggers (PAMPs and DAMPs) and effector molecules (cytokines, chemokines, growth factors and bioactive lipid mediators) that lead to activation of the innate immune system. Furthermore, the use of integrated multi-omics data has been an invaluable tool to accelerate the identification of novel biomarkers that could guide the implementation of novel therapies/interventions aimed at protecting these patients from excessive systemic inflammation and organ failure.

Other examples of applicability of multi-omics approaches have been recently published in cholangiocarcinoma, a rare cancer with very dismal prognosis arising from the bile ducts. Particularly, the applications of proteomics, transcriptomics, and metabolomics for the discovery of novel predictive, diagnostic, and prognostic non-invasive biomarkers will be firstly under the spotlight while their usefulness for the understanding of cholangiocarcinogenesis. As example, certain RNAs and proteins present in serum EVs show promising values for the early and differential diagnosis of primary sclerosing cholangitis, cholangiocarcinoma, and hepatocellular carcinoma.[29,110–113] Furthermore, the analysis of serum metabolomics is providing selective lipidomic profiles with diagnostic capacity for primary sclerosing cholangitis, cholangiocarcinoma, hepatocellular carcinoma and

pancreatic ductal adenocarcinoma.[114–116] These circulating metabolite changes may result from cell metabolic rewiring, as observed in cholangiocarcinoma.[114]

The application of omics approaches in the study of liver diseases is leading to a growing demand for new methodologies to manage, share, and reuse existing datasets. This trend is driven to align with the Open Data strategies, which favor releasing raw data with none or very limited restrictions.[117] Data recycling strongly relies on the ability of datasets to remain sustainable beyond initial funding periods, ensuring data and samples are shared and reused. In this context, it is key that Findable, Accessible, Interoperable, Reusable (FAIR) principles shift from a bunch of sentences incorporated to scientific proposals to real good practices for scientific data and data resources.[118] Recently, several resources as fairsharing.org have been created to facilitate the Findability (F) and Accessibility (A) of biomedical datasets and avoid the siloing of datasets and, consequently, a limited reuse.[19,119] Data repurposing strongly relies also on the quality of metadata, which is essential to understand the dataset associated to it. A number of articles, common regulations and community-driven suggestions regarding minimal requirements of metadata have been published during the last decade but there is still work to do to facilitate data harmonization and promote an appropriate use of data.[120] Overall, the omics field is rapidly evolving and constitutes an extremely useful tool to understand the pathogenesis of both, common and rare liver diseases. To leverage all this potential and combine clinical information together with omics data, the implementation of artificial intelligence (AI) can become an important tool in the study of liver diseases, improving diagnostic accuracy and enhancing predictive decision-making roadmaps.[121,122] Although there are several hurdles that need to be overcome, such as the need for large datasets and the potential for bias in the data, multi-omics analysis and AI present the potential to revolutionize the diagnosis and treatment of liver diseases.

Impact of toxic and metabolic factors on the progression of chronic liver disease

As mentioned above, MASLD has emerged as a prominent global cause of chronic liver disease characterized by the presence of steatosis in more than 5% of hepatocytes associated with metabolic risk factors, particularly obesity and type 2 diabetes in the absence of excessive alcohol consumption.[123] Metabolic factors and toxic exposure appear associated with perturbations of key hepatic metabolic pathways, thus harmful effects of diet, alcohol, and commonly used drugs strongly impact on the progression of chronic liver disease.

MASLD is a complex medical condition closely linked to disturbances in lipid metabolism, insulin resistance, and diet. Obesity is a significant risk factor for MASLD.[124,125] Excess adipose tissue, especially abdominal or visceral fat, releases fatty acids into the bloodstream and subsequently transports them to the liver for storage. The excess of fat overwhelms the liver's capacity to process them efficiently, leading to the accumulation of triglycerides, which can trigger inflammation and liver damage.[126,127] Insulin resistance is another critical player in this intricate crosstalk of interrelated factors. Obesity and poor dietary choices can lead to insulin resistance. This can lead to elevated blood glucose levels, increased fat storage in the liver, and inflammation, all contributing to MASLD's development.[128] Diet plays a pivotal role in both the development and management of MASLD. A diet rich in whole foods, including fruits, vegetables, lean proteins, and healthy fats, can help prevent and manage MASLD by supporting healthy lipid metabolism and reducing inflammation. Conversely, a diet high in processed foods, saturated fats, sugary beverages, and excessive calorie intake exacerbates this condition.[129] In conclusion, MASLD has strong connections to biological processes closely associated to the metabolic syndrome, including lipid metabolism, insulin resistance, and diet. Lifestyle modifications, including a balanced diet and regular exercise, are essential for both preventing and managing MASLD. Understanding these connections is crucial for effective interventions.

On the other hand, whereas overwhelming evidence undoubtedly concludes that consumption of alcoholic beverages is harmful to human health, yet it is possible to find in the scientific literature, a great number of studies supporting its beneficial effects.[130–132] Alcohol toxicity is caused due to its metabolism and metabolites (acetaldehyde). Mechanistically, oxidative and nitrosative stress mediators trigger changes in lipid metabolism and fat accumulation, ER stress, cell death (necrosis, apoptosis, necroptosis), alterations in the immune system, inflammation, and fibrosis, oral and intestinal bacterial translocation, fungal dysbiosis, and DNA damage.[132] The clinical manifestations of the above-mentioned cellular and molecular mechanisms produce deleterious effects in the cardiovascular system (hypertension, ischemia, stroke, cardiomyopathy, myocarditis and arrythmias), the nervous system (depression and neurobehavioral impairment), the renal system (glomerulonephritis, acute nephropathy and kidney graft failure), the reproductive system (fertility, premature birth, fetal alcohol syndrome), the pancreas (pancreatitis and fibrosis), and most importantly, in the gastrointestinal system where it triggers different subtypes of steatohepatitis, cirrhosis and hepatocellular carcinoma. However, alcohol-related carcinogenesis is not liver-centered and tumor in the oral cavity, pharynx, larynx, esophagus and colorectum, and breast cancer have been also associated to alcohol intake.[132,133] Importantly, the beneficial effects of alcohol consumption have exclusively been attributed to moderate consumption, usually defined as 1–2 servings/day or 14–28 g of alcohol[131]; however, those doses have recently been challenged as detrimental.[134] These supposedly 'healthy' effects have been related to the anti-inflammatory, antioxidant and hypotensive properties of several components of alcoholic beverages (polyphenols, xanthohumol).[135] Specifically, ethanol regulates cytokine production and the inhibition of the NLRP3 inflammasome in macrophages when taken moderately.[136] Therefore, protective effects of moderate consumption have been linked to a decrease rate of myocardial infarction and atherosclerosis, lower risk of developing type 2 diabetes mellitus,[137] and immunomodulation in autoimmune diseases such as rheumatoid arthritis.[138] Altogether, the individual's susceptibility to alcohol toxicity is determined by genetics (SNPs in target genes),[139] gender

– women more susceptible, higher rate of death in men –, lifestyle habits (smoking, drug abuse), dietary patterns (high fat, alcohol intake), environmental and other co-morbidities (obesity, synergistic effect with other drugs).

Finally, drug-induced liver injury (DILI) is a complex, unexpected adverse reaction, in which commonly used drugs cause liver damage. DILI has been classified into intrinsic and idiosyncratic types; being the idiosyncratic type -dependent on the patient's genetic and non-genetic factors – unpredictable, difficult to diagnose and investigated due to the lack of reliable preclinical models, which are restricted to the intrinsic dose-dependent type of DILI, typically acetaminophen (APAP) overdose.[140] As for the mechanism involved, drugs metabolized through phase I and II enzymatic reactions where reactive oxygen species (ROS) will also be generated, are converted into reactive metabolites that create adducts with proteins able to generate mitochondrial and/or reticulum stress. These reactive metabolites might also inhibit the bile transporter BSEP, hindering its outflow and biliary excretion. The generation of drug-protein adducts, the accumulation of reactive metabolites and the generation of cellular stress, among others, lead to the activation of an immune response.[140] A hallmark of DILI is the mitochondrial dysfunction with high ROS formation.[141] The preclinical mechanistic studies carried out mostly in animal models of APAP overdose showed that the mitochondrial dysfunction leads to difficulties in managing energetic sources and obtaining energy through the electron transport chain (ETC), resulting in inhibition of mitochondrial fatty acid oxidation (FAO).[142,143] Thus, pharmacological activation of PPAR, which target genes involved in FAO and the ETC, alleviates the hepatotoxicity induced by APAP overdose[144,145] in a mechanism mediated, at least in part, through the mitochondrial uncoupler protein 2 (UCP2).[146] Dysregulated mitochondrial function with deficient FAO, altered activation of PPARs and impaired autophagy are also hallmarks of certain chronic liver diseases such as MASLD[147]; therefore, it is foreseeable that these patients are more susceptible or have an increased risk for DILI. However, the evidence supporting this is weak. Whereas existing prospective DILI registries have not identified, neither MASLD, nor the features of metabolic syndrome as a risk factor for DILI, retrospective analysis of clinical databases of patients with biochemical hepatic alterations suggesting MASLD showed a significant increase of the risk of DILI with the drugs mostly involved in this adverse effect in USA.[148] On the other hand, several drugs due to its mechanism of action that include enhancement of lipid synthesis or FFA hepatic uptake, inhibition of mitochondrial β-oxidation, lipophagy, as well as impairment of lipid degradation or lipid exportation via VLDL, are inducers or aggravators of pre-existing fatty liver and/or steatohepatitis, which is now called indirect type of DILI.[149] Although there is no strong evidence on whether MASLD and other chronic liver disorders generate vulnerability to DILI in general, it has become clear that patients with previous liver disease show a higher risk of mortality than those without, in the American DILIN Registry[150] or more recently in of the Spanish DILI Registry (7.5% vs. 1.8%).[151] Hence, MASLD might increase the risk of idiosyncratic DILI and chronic liver disease, which poses the patient to a higher risk of serious outcome including death.

Interorgan crosstalk

It is well known that chronic liver diseases are multisystem disorders that go beyond the liver, negatively affecting a wide range of organs. The other way around is also true, as the (mis)communication between the liver and other organ systems contributes to the development and progression of liver disease. Improved knowledge of the pathogenic interorgan crosstalk between the liver and peripheral organs is crucial to identify risk factors for chronic liver disease onset and progression, whilst highlighting novel therapeutical approaches. Recent advances in the role of the gut, adipose tissue, heart, and muscle in chronic liver diseases are explored herein.

Disruption in mitochondrial function, along with oxidative stress and alterations in gut microbiota, contributes to the development of MASLD.[152] Indeed, the gut-liver axis is known to be disturbed in MASLD, although the specific microbial signature associated with MASH remains to be fully understood.[153] Recent research has revealed a potential bidirectional interaction between mitochondrial dysfunction and gut microbiota.[154] Mitochondria are believed to have evolved from a bacterial phylum, either *Alphaproteobacteria* or an ancestor related to *Rickettsiales*, leading to the sharing of several proteins for parallel metabolic pathways.[155] Dysregulation of mitochondrial functionality and the resulting increase in ROS can impact the gut microbiota by modulating the integrity of the intestinal barrier, thereby triggering the immune response. Apart from regulating the innate immune response against pathogen infection, mitochondria also play a role in controlling the intestinal epithelial barrier.[156] Studies on models of mitochondrial dysfunction have demonstrated that variations in mitochondrial genetics alter the composition of the microbial community, which is linked to the production of reactive species by the host.[157] Conversely, the gut microbiota is known to regulate mitochondrial biogenesis, and microbial metabolites can also affect mitochondrial respiration.[158] Therefore, addressing mitochondrial dysfunction could serve as a protective measure to maintain a proper gut-liver axis, potentially delaying or even preventing the progression of MASH. Indeed, recent results show that deficiency in methylation-controlled J protein (MCJ), an endogenous negative regulator of the mitochondrial complex I, and the consequently improved mitochondrial activity reshape a specific and protective microbiome signature able to delay the disease progression in a dietary mouse model of MASH. Overall, these results highlight the importance of mitochondria–microbiota crosstalk in MASH paving the way for therapeutic strategies based on microbiota transplantation.[159]

The intricate connection between the liver and adipose tissue plays a crucial role in regulating energy balance, impacting metabolic disorders such as obesity and MASLD. This dynamic interplay relies on a complex network of hormones, metabolites, and signaling molecules. Within this intricate framework, the metabolite succinate emerges as a potential key actor in mediating communication between these two organs. Beyond its conventional function as an energy source, succinate assumes the additional role of an extracellular signaling molecule, actively engaged

by its receptor, SUCNR1.[160,161] Notably, SUCNR1 exhibits heightened expression in adipose tissue, where it effectively inhibits the process of lipolysis.[162–164] In the liver, its role was previously associated mainly with stellate cells, promoting fibrosis development.[162,163,165–167] Indeed, its potential inhibition was proposed as a therapeutic target for MASH treatment.[168] However, this line of research has not advanced significantly. This lack of progress might be attributed to the prior oversight of its potential roles in other liver cell types. Indeed, recent research presents a contrasting perspective. Novel data reveals that SUCNR1 deficiency reduces fibrosis and ER stress in diet-induced MASLD. Nonetheless, this deficiency concurrently exerts adverse effects on steatosis, inflammation, and glycogen content. Global SUCNR1 deficiency negatively impacts glucose homeostasis, underscoring the complex interplay of its effects on metabolic regulation. Intriguingly, SUCNR1s functional scope appears to be primarily confined to hepatocytes, where it actively fosters the maintenance of lipid and glycogen equilibrium during MASLD progression. Clinical evidence substantiates these findings, underscoring the utility of SUCNR1 expression as a valuable marker for tracking MASLD progression. Furthermore, elevated circulating succinate levels correlate with fatty liver, making succinate and hepatic SUCNR1 expression potential diagnostic markers for fatty liver and MASH, respectively.[169] These insights shed light on a novel regulatory role for SUCNR1 in hepatocyte metabolism and offer promising diagnostic avenues for liver-related conditions.

The heart, previously regarded simply as a blood pump, is now recognized as a metabolic and endocrine organ. Its function is tightly regulated by a variety of metabolic processes, while it also acts as an endocrine organ, releasing bioactive molecules that influence systemic metabolism.[170] In recent years, research has revealed the interaction between the heart and other metabolic organs, such as adipose tissue, liver, and skeletal muscle. The metabolic flexibility of the heart and its ability to utilize different energy substrates play a crucial role in maintaining cardiac function and overall metabolic homeostasis. Understanding how metabolic disorders disrupt cardiac metabolism is fundamental, as it plays a major role in the development and progression of heart disease. The growing recognition of the heart as a metabolic and endocrine organ highlights its essential contribution to whole-body metabolic regulation and provides new insights into the pathogenesis of metabolic diseases such as obesity, diabetes, and MASLD.[171] Cardiac metabolism is significantly influenced by adipose tissue. Adipokines and metabolites secreted by fat can affect cardiac metabolism and thus the functionality of the heart. In turn, the heart may also regulate the metabolism of other tissues, including the liver and fat. Thus, cardiac dysfunction can trigger global metabolic disturbance, including insulin resistance and metabolic alterations in distant tissues. In addition, liver disorders may impact cardiovascular health, as liver fibrosis is associated with an increased risk of cardiovascular disease.[172] Although the underlying molecular mechanisms are not yet fully elucidated, a better understanding of this interplay between cardiac metabolism and the liver may contribute to the development of new therapeutic strategies for chronic liver diseases.

Finally, sarcopenia, which refers to the reduction of skeletal muscle mass and strength, and myosteatosis (ectopic fat accumulation in the skeletal muscle) are common in subjects with chronic liver disease. Thus, the bidirectional muscle-liver axis could play a significant pathophysiological role across the full spectrum of chronic liver disease. In cirrhotic patients, sarcopenia is a key component of malnutrition and has been strongly associated with several poor clinical outcomes and increased mortality.[173,174] Remarkably, sarcopenia has also been involved in the pathogenesis of MASH, increasing the risk of progression to MASH and fibrosis, and mortality.[175,176] In addition to sarcopenia, myosteatosis has been also related to MASH development and progression, although its implication in MASH pathogenesis is still poorly understood.[177,178] Alterations in muscle protein turnover due to anabolic resistance are an important contributor to muscle loss in patients with chronic liver disease.[179] Furthermore, other factors such as hyperammonemia critically contribute to the development of sarcopenia in patients with cirrhosis, thus reducing the muscle mass available to remove excess ammonia.[124,180] In cirrhotic patients, hyperammonemia has been associated with increased myostatin expression, increased ROS, mitochondrial dysfunction, and induced autophagy in the skeletal muscle, resulting in reduced muscle protein synthesis and, consequently, muscle loss.[179] In MASH, insulin resistance and chronic inflammation are key contributors to the development and progression of the disease, negatively impacting skeletal muscle by inducing skeletal muscle atrophy and inhibiting myogenesis.[176] Emerging evidence recommends the management of sarcopenia through a multifaceted approach, including nutrition, exercise, and pharmacological interventions in chronic liver disease patients.

Conclusions

Chronic liver disease represents a significant clinical and public health challenge. Our understanding of the pathology of these diseases has evolved significantly, emphasizing the complex interplay between genetic, metabolic, and environmental factors in disease development and progression. Research into etiological factors, immune system involvement, organs crosstalk, and the application of emerging technologies (such as -omics) is revealing the underlying molecular pathways. In addition, the integration of artificial intelligence systems in the management of chronic liver disease offers a promising approach for optimizing screening and risk stratification. However, significant challenges remain in the field. Further translational research and the implementation of clinical trials incorporating robust and validated early and late response markers are essential. Multidisciplinary collaboration and support for basic and translational research will remain cornerstones in the fight against chronic liver disease, with the ultimate goal of improving clinical outcomes for patients with chronic liver disease.

Conflicts of interest

None of the authors have conflicts of interest related to this work.

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