

# The “Domino effect” in MASLD: The inflammatory cascade of steatohepatitis

---

Mladenić, Karlo; Lenartić, Maja; Marinović, Sonja; Polić, Bojan;  
Wensveen, Felix M.

Source / Izvornik: **European Journal of Immunology, 2024**

Journal article, Published version

Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

<https://doi.org/10.1002/eji.202149641>

Permanent link / Trajna poveznica: <https://urn.nsk.hr/urn:nbn:hr:184:864096>

Rights / Prava: [In copyright](#)/[Zaštićeno autorskim pravom.](#)

Download date / Datum preuzimanja: **2024-11-28**





Repository / Repozitorij:

[Repository of the University of Rijeka, Faculty of  
Medicine - FMRI Repository](#)



## REVIEW

# The “Domino effect” in MASLD: The inflammatory cascade of steatohepatitis

Karlo Mladenčić<sup>1</sup>, Maja Lenartić<sup>1</sup>, Sonja Marinović<sup>1,2</sup>, Bojan Polić<sup>1</sup>   
 and Felix M. Wensveen<sup>1</sup> 

<sup>1</sup> Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

<sup>2</sup> Division of Molecular Medicine, Laboratory for Personalized Medicine, Ruder Bošković Institute, Zagreb, Croatia

Metabolic dysfunction-associated steatotic liver disease (MASLD) is an increasingly common complication of obesity, affecting over a quarter of the global adult population. A key event in the pathophysiology of MASLD is the development of metabolic-associated steatohepatitis (MASH), which greatly increases the chances of developing cirrhosis and hepatocellular carcinoma. The underlying cause of MASH is multifactorial, but accumulating evidence indicates that the inflammatory process in the hepatic microenvironment typically follows a pattern that can be roughly divided into three stages: (1) Detection of hepatocyte stress by tissue-resident immune cells including  $\gamma\delta$  T cells and CD4<sup>-</sup>CD8<sup>-</sup> double-negative T cells, followed by their secretion of pro-inflammatory mediators, most notably IL-17A. (2) Recruitment of pro-inflammatory cells, mostly of the myeloid lineage, and initiation of inflammation through secretion of effector-type cytokines such as TNF, TGF- $\beta$ , and IL-1 $\beta$ . (3) Escalation of the inflammatory response by recruitment of lymphocytes including Th17, CD8 T, and B cells leading to chronic inflammation, hepatic stellate cell activation, and fibrosis. Here we will discuss these three stages and how they are consecutively linked like falling domino tiles to the pathophysiology of MASH. Moreover, we will highlight the clinical potential of inflammation as a biomarker and therapeutic target for the treatment of MASLD.

**Keywords:** Cytokinesfibrosis · Hepatocytes immune cells · Inflammation · MAFLD · MASH · MASLD · NAFLD · NASH · T cells

## Introduction

Metabolic dysfunction-associated fatty liver disease (MASLD), formerly known as nonalcoholic or metabolic-associated fatty liver disease (NAFLD or MAFLD), is considered to be the hepatic manifestation of metabolic syndrome and has an alarmingly high prevalence, affecting over 30% of the global adult population [1–3]. MASLD is diagnosed when more than 5% of hepatocytes are steatotic in the presence of a defined set

of metabolic abnormalities, such as obesity, type 2 diabetes mellitus (T2D), or dyslipidemia [4]. Many patients only have steatosis, which is associated with few if any clinical symptoms. However, a considerable fraction of patients develop metabolic dysfunction-associated steatohepatitis (MASH), formerly known as nonalcoholic steatohepatitis (NASH). MASH is characterized by an influx of proinflammatory cells, necrotic hepatocyte death, and fibrosis [5, 6]. Importantly, MASH strongly increases the risk of developing cirrhosis and hepatocellular carcinoma (HCC). Due

**Correspondence:** Dr. Felix M. Wensveen  
 e-mail: felix.wensveen@medri.uniri.hr

Bojan Polić and Felix M. Wensveen contributed equally to this study.

to its high prevalence, MASLD-induced liver pathology is therefore rapidly becoming the leading cause of liver transplantation and liver-associated death worldwide [7]. Clearly, MASLD is a major global health problem.

Notably, a key event in the pathophysiology of the disease is the transition of MASLD with only steatosis (metabolic-associated fatty liver [MAFL]) to MASH, a state in which the immune system gets activated. Various triggers have been associated with the progression of MASLD, including hypoxia, ER stress, insulin resistance, and dyslipidemia, and the underlying cause of metabolic dysfunction in the liver is therefore considered to be multifactorial [6, 8]. Various studies that analyzed cells from the liver in advanced stages of the disease defined MASH as a “Type 3” inflammatory disease, marked by the production of the cytokine IL-17A by CD4 Th17-helper T cells [9–11]. However, until recently the sequence of events leading to MASH was much less clear, particularly the earliest processes that cause inflammation to occur in some patients but not in others. In this review, we provide a brief overview of the events that lead to hepatic inflammation in the context of MASLD/MASH. The focus will be on the initial stages of immune cell activation, which represent the early toppled domino stones that lead to a cascade that ultimately causes a detrimental chronic inflammatory response. Whereas many of these events will (partially) overlap, we present them as consecutive events to facilitate understanding.

## When all tiles yet stand: The immunological state of the liver during homeostasis

The parenchyma of the liver predominantly consists of hepatocytes, structurally organized in liver lobules, which mediate most of the liver’s functions. The lobules can be subdivided into three zones, based on their oxygenation status and functionality and have recently been elucidated in high detail using technologies like single-nucleus RNA sequencing and single-molecule fluorescence in situ hybridization [12]: Zone 1 is located around the portal triads where oxygenated blood enters. Hepatocytes at this location perform more energy-intensive, anabolic tasks like protein synthesis, gluconeogenesis, cholesterol biosynthesis, and catabolic  $\beta$ -oxidation. Zone 3 is located around the central venule and contains hepatocytes more specialized in catabolic functions such as glycolysis, bile acid production, and lipogenesis [12, 13]. Zone 2 forms the transitional mid-lobule and contains cells with an intermediate function.

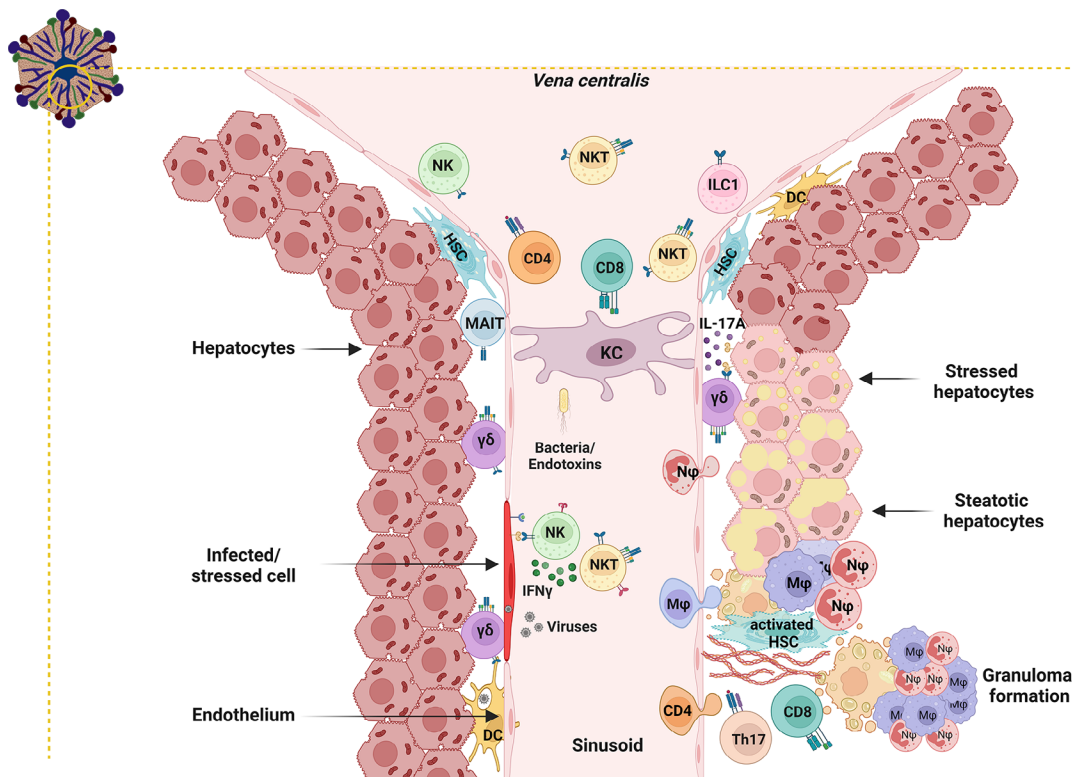
Apart from hepatocytes, the liver contains several types of non-parenchymal cells (NPCs) that play a supporting role. Hepatic stellate cells (HSC) are located in between hepatocytes. These cells contain retinoic acid and build the connective tissue matrix. In case of liver injury, these cells are activated and are responsible for the formation of fibrotic scar tissue to prevent loss of liver integrity [14]. The liver is extremely well vascularized by a network of sinusoid capillaries that lead blood from the portal triad to the vena centralis. Liver sinusoidal endothelial cells (LSECs)

therefore make up a large fraction of the nonparenchymal cells in the liver. A tubular network of vesicles lined by cholangiocytes leads bile toward the duodenum.

Finally, the liver is home to numerous innate and adaptive immune cells, which are functionally and anatomically segregated [15]. These cells are the primary sensors for immunological triggers and thus initiate inflammation and hepatic injury in disease [16]. In a metabolically healthy liver, resident immune cells maintain a tolerogenic state and suppress immune cell activation [17]. Inhibition of an overt immune response is required because the liver is drenched by blood originating for more than 70% from the portal vein and as such is enriched with gut-derived food- and commensal antigens [18, 19]. As a result, innate immune cells in the liver are desensitized to ligands of classic danger-sensing receptors such as TLR4 [15, 20]. The most abundant leukocytes in the liver are Kupffer cells (KCs). These cells comprise a considerable fraction of all the body’s macrophages and are located inside the sinusoids [16]. KCs contribute to liver homeostasis by conducting phagocytosis of dead and senescent cells through the activity of their scavenger receptors which enable them to detect and remove complement-opsonized particles from circulation [21]. Moreover, they can produce anti-inflammatory cytokines such as IL-10 [22]. In murine models, IL-10 was shown to upregulate PD-L1 on KCs which inhibits effector T-cell function and promotes Treg formation [19].

Under homeostatic conditions, the hepatic niche is enriched for growth factors that favor the development of regulatory dendritic cells. These cells are located in the space of Disse, which is a narrow region between LSECs and hepatocytes [23]. Regulatory dendritic cells were shown to promote the differentiation of CD4 T cells into Tregs [24, 25]. LSECs and HSCs also contribute to Treg development through active inhibition of Th1 or Th17 responses and secretion of retinoic acid and TGF $\beta$  which induce Foxp3, the master regulator of Treg differentiation [15, 26]. Tregs in the liver play a critical role in mediating hepatic tolerance via the production of IL-10. These cells therefore act in synergy with KCs to promote the local immunosuppressive niche [19, 27]. LSECs also appear to drive tolerance directly, as their cross-presentation of antigen resulted in CD8 T-cell anergy and deletion [15, 28, 29]. Furthermore, hepatocytes themselves were shown to induce proapoptotic genes in infiltrating CD8 T cells after antigen exposure, though the molecular mechanism remains unclear [30].

Especially in mice, CD1d-restricted natural killer T (NKT) cells are highly abundant in the liver and were shown to contribute to hepatic immune homeostasis. CD1d molecules normally present lipid- and other endogenous antigens on the surface of healthy liver cells but are downregulated in the context of various pathologies [31]. Upon CD1d engagement in the liver, NKT cells produce anti-inflammatory cytokines such as IL-10 and TGF $\beta$  [32] and promote regulatory T-cell development [33]. Moreover, NKT cells produce IL-5 following stimulation with noradrenergic neurotransmitters secreted by the sympathetic nervous system, thus supporting an anti-inflammatory milieu [34]. Finally, several immune cells including NK, NKT, and CD8 T cells mediate the



**Figure 1.** Localization of immune cells in the liver. The liver is extremely well vascularized with sinusoid capillaries which transport blood from the portal space toward the Vena Centralis. The sinusoids are populated by tissue-resident cells, including Kupffer-, NKT-, and NK cells, which scan the endothelium for threats derived from the bloodstream. In the space of Disse between hepatocytes and endothelium, a different pool of immune cells can be found, consisting of  $\gamma\delta$  T-, MAIT-, and dendritic cells. These cells are thought to screen the parenchyma for threats that have breached the endothelial barrier. During inflammation, hepatocytes in concert with tissue-resident immune cells recruit proinflammatory cells from circulation.

clearance of senescent or activated HSCs through the secretion of  $\text{IFN}\gamma$  to maintain tissue homeostasis [35–38].

In summary, in the absence of overt immune-stimulatory triggers, the liver maintains a tolerogenic state despite continuous exposure to various antigens and danger signals. This is achieved through the coordinated efforts of both immune and nonimmune cells.

## The first domino tile: Stress-sensing

Most patients with MASLD only have hepatic steatosis, which is associated with minimal clinical symptoms. However, in up to 40% of patients, MASL progresses to steatohepatitis which provides a strongly increased risk of developing cirrhosis and HCC [6]. The trigger that initiates this transition is therefore of great clinical importance. MASLD is a multifactorial disease in which several stress factors, such as hypoxia, lipotoxicity, metabolic ER stress, and endotoxins contribute to the overall pathophysiology [8]. The growing consensus is that crosstalk between immune cells, hepatocytes, HSCs, and LSECs in the liver dictates the overall inflammatory tone and mediates progression from MAFL to MASH [39]. Fibrosis, a key feature of MASH, typically arises first in the pericentral zone and extends toward the periportal zone as

the disease progresses. As zone 3 is the least oxygenated, this suggests that hypoxia plays an important role in driving stress ligands that activate tissue-resident immune cells. Moreover, the spatial distribution of immune cells within the liver lobule is not equal (Fig. 1), which may contribute to the directional progression of MASH [13, 40]. How these cumulative metabolic stress factors are translated into a signal that activates the immune system, thus converting the hepatic environment from an anti- to a proinflammatory state has long remained unclear. However, recent findings indicate that a cascade of inflammatory events needs to occur, which can be roughly divided into three phases: (1) An initial phase in which innate, tissue-resident immune cells sense hepatic stress and start producing cytokines, (2) recruitment and activation of proinflammatory cells, mostly of the myeloid lineage, which amplifies the initial inflammatory signals, and (3) escalation of the inflammatory response during which adaptive immune cells become involved and large-scale tissue damage occurs. MASLD has been marked as a type 3 inflammatory response, characterized by fibrosis and production of the cytokine  $\text{IL-17A}$  [41, 42].

The inflammatory trigger that initiates inflammation in MASLD appears to include signals that predominantly activate tissue-resident innate-like T cells.  $\text{IL-17}$ -producing  $\gamma\delta$  T cells ( $\gamma\delta^{17}$  T cells) have been uniformly proposed as an early profibrotic

immune cell population in the liver [43–45]. Metabolic disease is associated with increased gut permeability [46] and Li et al. [44] demonstrated that CD1d-dependent presentation of microbiota-derived lipid antigens on hepatocytes drives the expansion of  $\gamma\delta^{17}$  T cells in the liver. In addition to T-cell receptor (TCR) engagement, a second signal appears to be required for the full activation of hepatic  $\gamma\delta^{17}$  T cells in the context of MASLD. Accumulation of lipids, most notably cholesterol, causes metabolic stress in hepatocytes resulting in their upregulation of “stress ligands” for the activating immune receptor NKG2D [43]. In humans, the expression of MICA and MICB, key ligands of NKG2D, was shown to positively correlate with the progressive stages of MASH [43, 47]. Many immune cells express NKG2D, but of these predominantly  $\gamma\delta$  T cells are in the liver parenchyma and therefore directly able to respond to hepatocyte stress (Fig. 1). Genetic deficiency of CD1d,  $\gamma\delta$  T cells, NKG2D, or IL-17A therefore significantly reduces inflammation and liver fibrosis in animal models of MASH [43–45]. CD4<sup>-</sup>CD8<sup>-</sup> double negative TCR $\alpha\beta$ <sup>+</sup> T cells account for a small proportion of total T lymphocytes in the liver. However, an increasing number of studies have shown that this rare T-cell population has an impact on the development of fibrosis in MASLD [48, 49]. Recent findings indicate that these cells may also mediate their effect through the production of IL-17A following TCR engagement and co-stimulation via the NKG2D receptor [43].

Mucosal-associated invariant T (MAIT) cells respond to bacterial antigens presented by the MR1 molecule in response to cellular stress. Both in humans and mice, MAIT cells were shown to expand during liver fibrosis [50, 51]. Notably, in animals fed with a methionine and choline-deficient diet (MCD), a classical model for MASH, MAIT cells were shown to contribute to liver fibrosis in an IL-17A/TNF-dependent manner [51]. However, in a high-fat, fructose, and cholesterol dietary model for liver MASH, neither the absence nor the increased presence of MAIT cells resulted in a change in the level of fibrosis [43], emphasizing that the nature of the initial trigger dictates the inflammatory response. It should be noted that people have much larger numbers of MAIT cells than mice, which suggests that the role of these cells may be more prominent in human pathology [52]. iNKT cells can mediate both pro- and anti-inflammatory responses in the liver, dependent on need [53]. In MCD-fed animals, iNKT cells promoted the development of MASH, as  $\alpha 18^{-/-}$  mice, which lack this immune cell population, showed a significant reduction in liver fibrosis [54]. However, iNKT cells appear to mediate this effect through modulation of hepatic metabolism rather than inflammation, as the production of the cytokine LIGHT by these cells promoted the uptake of lipids by hepatocytes [55]. Apart from innate-like T cells, MASH patients have increased numbers of NK cells in circulation. Kahraman et al. [56] reported that hepatocytes of MASH patients can activate tissue-resident NK cells to release TRAIL and induce hepatocellular damage [57]. However, NK cells isolated from patients with MASH showed similar degranulation capacity and cytokine production as those from healthy controls and animals lacking NK cells did not show significant differences in liver fibrosis in the high-fat, fructose, and cholesterol dietary model [43, 57].

Interestingly, NK and NKT cells are mostly present in the liver sinuses, whereas  $\gamma\delta$  T, MAIT, and double negative TCR $\alpha\beta$ <sup>+</sup> T cells are predominantly in the liver parenchyma [43, 58–61]. This indicates that the former cells are more involved in the response to blood-borne triggers, whereas the latter immune cell subsets respond to threats that have breached the endothelial barrier. In the case of MASLD, blood-borne triggers are endotoxins, whereas the signal from the parenchyma is metabolic stress of hepatocytes communicated through the upregulation of stress ligands (Fig. 1).

## The next domino tile: Inflammation

Inflammation of the liver in the context of MASH requires both the local activation of yolk sac-derived KCs and the recruitment of bone-marrow-derived myeloid cells as the second domino tiles in the sequence of inflammatory events. Recent single-cell RNA sequencing studies have shed light on the identity of cells involved in this stage of MASH pathogenesis [62–66]. Due to their vascular localization, KCs are in intimate contact with LSECs and with protrusions of hepatocytes reaching through sinusoidal fenestrations [67]. This makes them highly sensitive to pathological changes in the lipid-enriched liver microenvironment in MASLD. A notable discovery was that a Western diet causes a reduction of noninflammatory liver-resident KCs expressing CD11b<sup>low</sup>, F4/80, Clec4f, and Tim-4 by inducing apoptosis of these cells in mice [62, 65, 68]. Subsequently, their niche becomes inhabited by monocyte-derived macrophages, characterized as Ccr2<sup>+</sup>, Trem2<sup>+</sup>, Cd9<sup>+</sup>, Gpnmb<sup>+</sup>, Spp1<sup>+</sup>, and Tim-4<sup>-</sup> [62, 63, 65, 68, 69]. These macrophages accumulate in liver regions between the portal and central veins, forming close connections with CD31<sup>+</sup> LSECs and Desmin<sup>+</sup> HSCs [62]. A similar population of TREM2<sup>+</sup>CD9<sup>+</sup> profibrogenic scar-associated macrophages was identified in cirrhotic human livers [64, 70]. Scar-associated macrophages were found to induce collagen expression by human hepatic stellate cells, confirming their profibrogenic role [64].

Apart from macrophages, single-cell RNA sequencing and high-parameter flow cytometry studies have shown that several other myeloid cell types also increase during the second stage of MASLD-induced liver inflammation [66, 71]. Cytokines such as IL-17A produced during the initial stage of stress sensing license hepatocytes, HSCs, and KCs to produce chemokines including CXCL1, CXCL2, CXCL10, CCL2, and CCL3. Moreover, LSECs upregulate adhesion molecules such as VCAM-1. This process recruits myeloid cells into the liver parenchyma and drives inflammation [42, 43, 71–81]. Neutrophils, known for their rapid response to tissue damage, migrate in mouse livers several weeks after initiation of a MASH-inducing diet in mice and were also detectable in the livers of human MASH patients [82–84]. Once there, these cells initiate the expulsion of neutrophil extracellular traps (NETs) thereby contributing to the early pathogenesis of MASH. Indeed, constituents of NETs, such as myeloperoxidase (MPO) and MPO-DNA complexes, were found to be elevated in the serum of MASH patients [82, 85]. Inhibition of NET formation early after initiation of a MASH-inducing diet reduced

inflammation but had no effect once inflammation was already established [83–85]. Eosinophils also increase in the livers of mice following a MASH-inducing diet [43, 86] but appear to mediate their proinflammatory effect through the secretion of IL-13 [86]. The proinflammatory profile of myeloid cells entering the liver in the context of MASH appears to be boosted by blood platelets. Activated thrombocytes are known to fuel activation of the NLRP3 inflammasome and potentiate the production of IL-1 $\beta$  by neutrophils and macrophages [87]. Elevated platelet levels were observed in both murine MASH models and MASH patients [88]. Indeed, KCs were shown to recruit GPIIb $\alpha$ <sup>+</sup> platelets to the livers of mice with MASH and exacerbate inflammation, which could be prevented by anti-platelet therapy [88, 89].

The third domino tile in MASLD-induced liver inflammation is the production of proinflammatory cytokines by proinflammatory myeloid cells. Increased intake of dietary components like toxic lipid species, danger-associated molecular patterns (s), and gut-derived pathogen-associated molecular patterns (PAMPs) activate recruited myeloid cells, leading to the secretion of effector cytokines, including TNF- $\alpha$ , IL-1 $\beta$ , TGF $\beta$ , and IL-6 [90]. Notably, saturated free fatty acids can act as proinflammatory mediators. Saturated free fatty acids stimulate infiltrating macrophages, but not Kupffer cells, by binding to and internalizing a monomeric TLR4-MD2 complex, resulting in ROS generation and increased pro-IL-1 $\beta$  expression [91–93]. Cholesterol also triggers IL-1 $\beta$  release from KCs and macrophages *in vitro*. Increased intestinal permeability in MASH promotes the translocation of bacterial products such as LPS and bacterial DNA from the gut to the liver [94, 95]. Engagement of TLR4 and TLR9 by these molecules activates the NF- $\kappa$ B signaling pathway in macrophages, leading to inflammasome activation and increased production of IL-1 $\beta$  and IL-18 [96, 97]. IgA from intestinal B cells was shown to activate pro-fibrotic Ly6C<sup>+</sup> CD11b<sup>+</sup> Fc $\epsilon$ R1g<sup>+</sup> monocyte-derived macrophages through an Fc $\gamma$ R-dependent mechanism [98]. Lipid-stressed hepatocytes can also activate proinflammatory myeloid cells. For instance, the release of TRAIL-containing extracellular vesicles by hepatocytes activates macrophages to produce IL-1 $\beta$  and IL-6 [99]. Lipid accumulation in hepatocytes can result in their necrotic death, resulting in the release of DAMPs such as high-mobility group box 1 into the extracellular space, which further promotes inflammation in a TLR4-dependent fashion [100, 101].

In summary, hepatic stress is sensed by innate tissue-resident lymphocytes, which license nonimmune cells to recruit proinflammatory cells predominantly of myeloid origin. Once there, these cells are activated by DAMPs, proinflammatory metabolites, and blood-born PAMPs which triggers their release of pro-fibrotic factors (Fig. 2).

## The cascade: Escalation

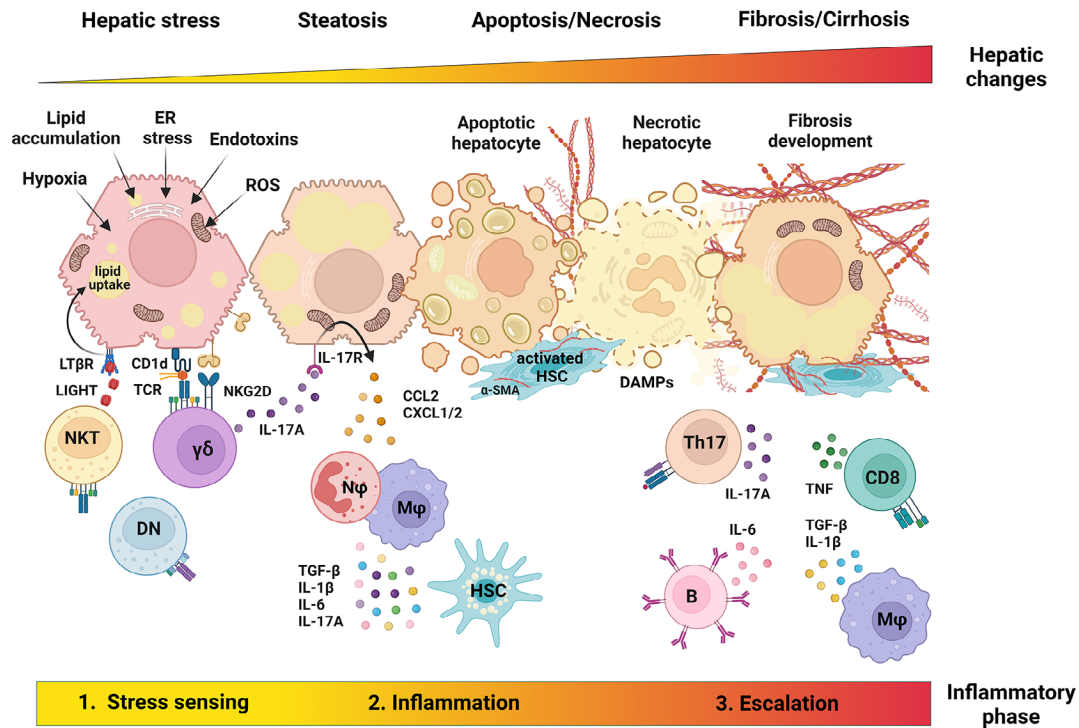
Steatohepatitis is fully established in the third stage of liver inflammation, when adaptive immune cells such as CD4<sup>+</sup> Th17 cells, CD8<sup>+</sup> T- and B cells infiltrate the liver and start contributing

to pathology [9, 55, 102]. This phase is associated with extensive hepatocyte cell death and the deposition of extracellular matrix by activated hepatic stellate cells. If a sufficient level of fibrotic scar tissue is generated, damage to the liver becomes irreversible. Recruitment and activation of adaptive immune cells requires an inflammatory environment created by myeloid cells in the liver and involves the activation of conventional type-1 dendritic cells (cDC1) [103–105]. Inflammation causes an increase of XCR1<sup>+</sup> conventional type-1 dendritic cells in the liver and blood of mice and patients with MASH. These cells were shown to promote the activation of adaptive immune cells and liver pathology, which was significantly reduced after their genetic ablation [105]. In addition, several chemokines mediate the recruitment of T cells into the liver. Hepatic expression of CXCL9 and CXCL10 is increased in mice with MASH, which is recognized by CXCR3 on inflammatory (i)Th17 cells. As a result, proinflammatory CD4<sup>+</sup> Th17 cells increase both in mice and MASLD patients during the later stages of MASH. Neutralization of Cxcl9 and Cxcl10 or deficiency of Cxcr3 on CD4 T cells therefore significantly reduces liver pathology in animal models of MASH [11, 103, 104, 106].

B cells are pivotal components of the adaptive immune system and are particularly sensitive to intestinal microbiota and oxidative stress-derived antigens [98, 102, 107]. B-cell numbers are increased in the livers of mice and patients with MASH and hepatic pathology was ameliorated in animals with B-cell deficiency [102, 107]. Activation of B cells relies on the detection of DAMPs or PAMPs by pattern recognition receptors such as TLR4 or through the engagement of microbiota-derived antigens by their B-cell receptor [102]. Activated B cells release profibrotic cytokines such as TNF and IL-6 and contribute to the formation of effector memory CD4 and CD8 T cells within the liver [102, 108]. In addition, IgA derived from metabolically activated B cells in the small intestine contributes to myeloid cell activation and inflammation in the liver [98].

Activated macrophages promote the accumulation of CD8 T cells in the livers of mice in dietary models of MASH and HCC [109, 110]. Numbers of CXCR6<sup>+</sup> CD8<sup>+</sup> T cells are increased in the livers of both mice and humans with MASH and these cells acquire auto-aggressive properties toward hepatocytes upon exposure to acetate [111]. Depletion of CD8 T cells therefore significantly reduced liver damage and lowered the incidence of HCC in the choline-deficient/high-fat diet model of liver MASH [55]. Certain CD8 T-cell subsets however may also protect against MASLD disease progression. CD69<sup>+</sup> CD103<sup>-</sup> CD8<sup>+</sup> tissue-resident memory T cells secrete CCL3, CCL4, and CCL5, which recruits HSCs in a CCR5-dependent fashion. Subsequently, these cells are killed by CD8 T cells via FasL/Fas interactions, thus reducing the development of fibrosis [112].

In the context of MASH, the immune system causes liver damage through several mechanisms, but its impact on liver fibrosis is predominantly mediated via the activation of hepatic stellate cells. These stem cell-like cells are found in the perisinusoidal space of Disse and under homeostatic conditions mainly regulate vitamin A secretion [113]. Upon activation, these cells obtain an elongated, fibroblast-like appearance and mediate extracellular



**Figure 2.** Phases of inflammation in the context of MASH. Phase 1: During MASLD, hepatocytes are subject to several stress factors, including oxidative stress, lipid accumulation, ER stress, and endotoxins. This results in the induction of stress ligands, such as those for the activating immune receptor NKG2D, which are recognized by tissue-resident immune cells. These cells secrete cytokines, predominantly IL-17A, which licenses hepatocytes to secrete chemokines. In phase 2: hepatocyte-derived chemokines recruit proinflammatory myeloid cells. These cells are activated by PAMPS and DAMPs, for example, derived from necrotic death of hepatocytes, which triggers the production of proinflammatory cytokines. These activate hepatic stellate cells, which subsequently deposit extracellular matrix. In phase 3: chemokines produced by myeloid cells recruit adaptive immune cells. These further potentiate the inflammatory response and perpetuate the pro-fibrotic effect on HSCs. As a result, inflammation becomes chronic and liver repair can no longer be accomplished by HSC, leading to large-scale extracellular matrix deposition and fibrosis.

matrix deposition and fibrosis. Activation of HSCs is regulated by multiple metabolic, epigenetic, and immune-dependent signals (34–36) of which we will focus on the latter here. TGF- $\beta$  is generally considered one of the most potent pro-fibrogenic cytokines which activate HSCs through the phosphorylation of SMAD proteins downstream of the TGF $\beta$  receptor. Hepatic macrophages are a major source of TGF- $\beta$ , but they can also be produced by other immune and non-immune cells [114–116]. In addition to TGF- $\beta$ , several other cytokines have been shown to be profibrotic, including PDGF, IL-1 $\beta$ , IL-6, and TNF, produced by macrophages and T cells [117–119]. IL-17A is produced during all stages of MASH-pathogenesis and is initially derived from innate-like T cells, whereas later in the disease Th17 cells are its major source. Originally, IL-17A was therefore proposed to directly stimulate HSC activation, which was mostly based on *in vitro* stimulation assays [44]. More recent data indicates that this cytokine signals to hepatocytes and modulates the development of CD9<sup>+</sup>TREM2<sup>+</sup> profibrotic macrophages [42, 43, 120]. Finally, hepatocytes themselves contribute to the activation of HSC. In response to cytokine stimulation and liver damage, stressed hepatocytes produce bone morphogenic proteins in patients with MASH [121, 122]. These members of the TGF- $\beta$  superfamily were shown to directly induce HSC activation. Moreover, BMP8B deficiency resulted in a significant reduction of liver fibrosis, whereas administration of

exogenous BMP9 enhanced liver pathology in mice in the CCl4 model of hepatotoxicity-induced liver damage [121, 122].

In summary, during the third phase of the immunological cascade in MASH, adaptive immune cells are recruited into the liver. These cells promote fibrosis through direct stimulation of hepatic stellate cells and sustain the inflammatory environment through the secretion of IL-17A. Thus, in the final stage of MASH, the immune response escalates into chronic inflammation, leading to fibrosis and hepatocyte cell death.

## Clinical perspectives of MASH and inflammation

Clinically, MASLD has long been underappreciated as a major health concern and was mostly regarded as a minor complication of type 2 diabetes mellitus (T2D) [1]. As a result, most drugs targeting MASLD were initially designed as antidiabetic medications, and their impact on liver pathology was only considered as a secondary outcome in clinical trials. Modern drugs impacting MASLD therefore typically target the metabolic, but not the inflammatory components of the disease. This includes incretin mimetics, which promote insulin sensitivity and reduce body weight [123]. Recently, however, studies have emerged

that describe the beneficial effects of anti-inflammatory drugs on the pathophysiology of MASLD. Treatment of patients with cenicriviroc, an antagonist of the chemokine receptors CCR2 and CCR5, showed a prolonged reduction in liver fibrosis compared with placebo-treated controls [124]. Diacerein, an inhibitor of IL-1 $\beta$ , caused a significant reduction in liver stiffness in patients with fatty liver disease after 24 months of treatment [125]. The phosphodiesterase inhibitor pentoxifylline, which inhibits inflammation and reduces TNF production [126], caused a reduction of liver enzymes in circulation, reduced hepatocyte ballooning, and lowered the histological NAS score in patients with MASH [127–129]. In addition, patients with psoriatic arthritis treated for 6 months with TNF blockers in combination with methotrexate showed a significant reduction in liver stiffness compared with people receiving methotrexate alone [130]. Psoriasis is an inflammatory disease of the skin, yet is associated with a very high prevalence of MASLD [131]. Treatment of these patients with secukinumab, a monoclonal antibody neutralizing IL-17A showed a twofold reduction in high-sensitivity C-reactive protein levels in serum [99]. Thus, anti-inflammatory drugs may be of great benefit to patients with MASLD/MASH. However, some anti-inflammatory drugs such as corticosteroids may actually aggravate MASH due to their impact on systemic metabolism [132], indicating that treatment should be chosen with care.

Apart from being therapeutic targets, inflammatory mediators have great potential as diagnostic markers of liver disease. One of the reasons why fatty liver disease long remained understudied is because it is relatively difficult to diagnose. MASLD is defined as hepatic steatosis in combination with overweight, diabetes mellitus, and/or evidence of metabolic dysregulation in lean people [4]. However, noninvasive ultrasound-based techniques to measure steatosis and fibrosis in the liver have limited sensitivity [133]. Blood parameters, such as the liver enzymes AST and ALT, or C-reactive protein are very unspecific. The gold standard for the classification of MASLD and MASH is therefore still through biopsy, an invasive technique with a relatively high chance of complications [4]. Immune responses are tailored to the threat they try to resolve. MASH appears to be the result of a well-defined sequence of type 3 inflammatory events (Fig. 2), which leave a unique immunological footprint in the blood. Indeed, TNF, IL-6, and Pentaxtrin 3 levels in the blood were shown to correlate with the severity of fatty liver disease [134–136]. Notably, a recent study showed that levels of circulating  $\gamma\delta^{17}$  T cells, which are directly associated with the pathophysiology of the disease, but not Th17 cells correlate with the severity of liver stiffness in patients with MASLD [43]. Thus, the inflammatory state in the liver is an attractive target both for the diagnosis and treatment of fatty liver disease.

## Conclusion

The prevalence of MASLD is shockingly high in the global adult population and MASH is likely to become an increasingly large burden on our health system as it strongly increases the risk of

developing cirrhosis and hepatocellular carcinoma. Fortunately, current research is rapidly uncovering the cascade of inflammatory events leading from MAFL to MASH. Not only does this research reveal potential new therapeutic targets for the treatment of MASLD it also holds promise for better and less invasive biomarkers for the diagnosis of this disease.

**Acknowledgements:** This work was supported by grants from the University of Rijeka (18-152-1301 to F.M.W. and 18-89-1224 to B.P.), a grant from the Faculty of Medicine Rijeka to M.L. (100.21.0006) and Croatian Science Foundation (IP-2016-06-9306 and IPCH-2020-10-8440 to B.P., IP-2022-10-3414, IP-CORONA-2020-04-2045 and IP-2020-02-7928 to F.M.W.), and the European Regional Development Fund (KK.01.1.1.01.0006) to B.P.

**Conflict of interest:** The authors declare no commercial or financial conflict of interest.

**Peer review:** The peer review history for this article is available at <https://publons.com/publon/10.1002/eji.202149641>

## References

- Zafrani, E. S., Non-alcoholic fatty liver disease: an emerging pathological spectrum. *Virchows Arch. Pathol. Anat. Physiol. Klin. Med.* 2004. **444**: 3–12.
- Younossi, Z. M., Golabi, P., Paik, J. M., Henry, A., Van Dongen, C. and Henry, L., The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. *Hepatology* 2023. **77**: 1335–1347.
- Riazi, K., Azhari, H., Charette, J. H., Underwood, F. E., King, J. A., Afshar, E. E., Swain, M. G. et al., The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2022. **7**: 851–861.
- Eslam, M., Newsome, P. N., Sarin, S. K., Anstee, Q. M., Targher, G., Romero-Gomez, M., Zelber-Sagi, S. et al., A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *J. Hepatol.* 2020. **73**: 202–209.
- Younossi, Z. M., Koenig, A. B., Abdelatif, D., Fazel, Y., Henry, L. and Wymer, M., Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016. **64**: 73–84.
- Huang, D. Q., El-Serag, H. B. and Loomba, R., Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention. *Nat. Rev. Gastroenterol. Hepatol.* 2021. **18**: 223–238.
- Younossi, Z. M., Stepanova, M., Ong, J., Trimble, G., AlQahtani, S., Younossi, I., Ahmed, A. et al., Nonalcoholic steatohepatitis is the most rapidly increasing indication for liver transplantation in the United States. *Clin. Gastroenterol. Hepatol.* 2021. **19**: 580–589 e585.
- Mikolasevic, I., Milic, S., Turk Wensveen, T., Grgic, I., Jakopcic, I., Stimac, D., Wensveen, F. et al., Nonalcoholic fatty liver disease - a multisystem disease? *World J. Gastroenterol.* 2016. **22**: 9488–9505.



- 9 Moreno-Fernandez, M. E., Giles, D. A., Oates, J. R., Chan, C. C., Damen, M., Doll, J. R., Stankiewicz, T. E. et al., PKM2-dependent metabolic skewing of hepatic Th17 cells regulates pathogenesis of non-alcoholic fatty liver disease. *Cell Metab.* 2021. 33: 1187–1204 e1189.
- 10 Vonghia, L., Magrone, T., Verrijken, A., Michielsen, P., Van Gaal, L., Jirillo, E. and Francque, S., Peripheral and hepatic vein cytokine levels in correlation with non-alcoholic fatty liver disease (NAFLD)-related metabolic, histological, and haemodynamic features. *PLoS One* 2015. 10: e0143380.
- 11 Rau, M., Schilling, A. K., Meertens, J., Hering, I., Weiss, J., Jurowich, C., Kudlich, T. et al., Progression from nonalcoholic fatty liver to nonalcoholic steatohepatitis is marked by a higher frequency of Th17 cells in the liver and an increased Th17/resting regulatory T cell ratio in peripheral blood and in the liver. *J. Immunol.* 2016. 196: 97–105.
- 12 Halpern, K. B., Shenhav, R., Matcovitch-Natan, O., Toth, B., Lemze, D., Golan, M., Massasa, E. E. et al., Single-cell spatial reconstruction reveals global division of labour in the mammalian liver. *Nature* 2017. 542: 352–356.
- 13 Ben-Moshe, S. and Itzkovitz, S., Spatial heterogeneity in the mammalian liver. *Nat. Rev. Gastroenterol. Hepatol.* 2019. 16: 395–410.
- 14 Kamm, D. R. and McCommis, K. S., Hepatic stellate cells in physiology and pathology. *J. Physiol.* 2022. 600: 1825–1837.
- 15 Heymann, F. and Tacke, F., Immunology in the liver - from homeostasis to disease. *Nat. Rev. Gastroenterol. Hepatol.* 2016. 13: 88–110.
- 16 Robinson, M. W., Harmon, C. and O'Farrelly, C., Liver immunology and its role in inflammation and homeostasis. *Mol. Immunol.* 2016. 13: 267–276.
- 17 Hoogerland, J. A., Staels, B. and Dombrowicz, D., Immune-metabolic interactions in homeostasis and the progression to NASH. *Trends Endocrinol. Metab.* 2022. 33: 690–709.
- 18 Gao, B., Basic liver immunology. *Mol. Immunol.* 2016. 13: 265–266.
- 19 Cheng, M. L., Nakib, D., Perciani, C. T. and MacParland, S. A., The immune niche of the liver. *Clin. Sci. (Colch)* 2021. 135: 2445–2466.
- 20 Thomson, A. W. and Knolle, P. A., Antigen-presenting cell function in the tolerogenic liver environment. *Nat. Rev. Immunol.* 2010. 10: 753–766.
- 21 Wen, Y. K., Lambrecht, J., Ju, C. and Tacke, F., Hepatic macrophages in liver homeostasis and diseases-diversity, plasticity and therapeutic opportunities. *Mol. Immunol.* 2021. 18: 45–56.
- 22 Heymann, F., Peusquens, J., Ludwig-Portugall, I., Kohlhepp, M., Ergen, C., Niemietz, P., Martin, C. et al., Liver inflammation abrogates immunological tolerance induced by Kupffer cells. *Hepatology* 2015. 62: 279–291.
- 23 English, K., Tan, S. Y., Kwan, R., Holz, L. E., Siervo, F., McGuffog, C., Kaisho, T. et al., The liver contains distinct interconnected networks of CX3CR1(+) macrophages, XCR1(+) type 1 and CD301a(+) type 2 conventional dendritic cells embedded within portal tracts. *Immunol. Cell Biol.* 2022. 100: 394–408.
- 24 Li, G. L., Kim, Y. J. and Broxmeyer, H. E., Macrophage colony-stimulating factor drives cord blood monocyte differentiation into IL-10(high) IL-12(absent) dendritic cells with tolerogenic potential. *J. Immunol.* 2005. 174: 4706–4717.
- 25 Rutella, S., Bonanno, G., Procoli, A., Mariotti, A., de Ritis, D. G., Curti, A., Danese, S. et al., Hepatocyte growth factor favors monocyte differentiation into regulatory interleukin (IL)-10++IL-12(low/neg) accessory cells with dendritic-cell features. *Blood* 2006. 108: 218–227.
- 26 Dunham, R. M., Thapa, M., Velazquez, V. M., Elrod, E. J., Denning, T. L., Pulendran, B. and Grakoui, A., Hepatic stellate cells preferentially induce Foxp3(+) regulatory T cells by production of retinoic acid. *J. Immunol.* 2013. 190: 2009–2016.
- 27 Breous, E., Somanathan, S., Vandenberghe, L. H. and Wilson, J. M., Hepatic regulatory T cells and kupffer cells are crucial mediators of systemic T cell tolerance to antigens targeting murine liver. *Hepatology* 2009. 50: 612–621.
- 28 Limmer, A., Ohl, J., Kurts, C., Ljunggren, H. G., Reiss, Y., Groettrup, M., Momburg, F. et al., Efficient presentation of exogenous antigen by liver endothelial cells to CD8(+) T cells results in antigen-specific T-cell tolerance. *Nat. Med.* 2000. 6: 1348–1354.
- 29 Limmer, A., Ohl, J., Wingender, G., Berg, M., Jungerkes, F., Schumak, B., Djandji, D. et al., Cross-presentation of oral antigens by liver sinusoidal endothelial cells leads to CD8 T cell tolerance. *Eur. J. Immunol.* 2005. 35: 2970–2981.
- 30 Holz, L. E., Benseler, V., Bowen, D. G., Bouillet, P., Strasser, A., O'Reilly, L., D'Avigdor, W. M. H. et al., Intrahepatic murine CD8 T-Cell activation associates with a distinct phenotype leading to Bim-dependent death. *Gastroenterology* 2008. 135: 989–997.
- 31 Schruppf, E., Tan, C., Karlsen, T. H., Sponheim, J., Bjorkstrom, N. K., Sundnes, O., Alfsnes, K. et al., The biliary epithelium presents antigens to and activates natural killer T cells. *Hepatology* 2015. 62: 1249–1259.
- 32 Cui, K., Yan, G., Zheng, X., Bai, L., Wei, H., Sun, R. and Tian, Z., Suppression of natural killer cell activity by regulatory NKT10 cells aggravates alcoholic hepatosteatosis. *Front. Immunol.* 2017. 8: 1414.
- 33 Hua, J., Liang, S., Ma, X., Webb, T. J., Potter, J. P. and Li, Z., The interaction between regulatory T cells and NKT cells in the liver: a CD1d bridge links innate and adaptive immunity. *PLoS One* 2011. 6: e27038.
- 34 Wong, C. H., Jenne, C. N., Lee, W. Y., Leger, C. and Kubers, P., Functional innervation of hepatic iNKT cells is immunosuppressive following stroke. *Science* 2011. 334: 101–105.
- 35 Weng, H., Mertens, P. R., Gressner, A. M. and Dooley, S., IFN-gamma abrogates profibrogenic TGF-beta signaling in liver by targeting expression of inhibitory and receptor Smads. *J. Hepatol.* 2007. 46: 295–303.
- 36 Rockey, D. C., Maher, J. J., Jarnagin, W. R., Gabbiani, G. and Friedman, S. L., Inhibition of rat hepatic lipocyte activation in culture by interferon-gamma. *Hepatology* 1992. 16: 776–784.
- 37 Radaeva, S., Sun, R., Jaruga, B., Nguyen, V. T., Tian, Z. and Gao, B., Natural killer cells ameliorate liver fibrosis by killing activated stellate cells in NKG2D-dependent and tumor necrosis factor-related apoptosis-inducing ligand-dependent manners. *Gastroenterology* 2006. 130: 435–452.
- 38 Sagiv, A., Burton, D. G., Moshayev, Z., Vadai, E., Wensveen, F., Ben-Dor, S., Golani, O. et al., NKG2D ligands mediate immunosurveillance of senescent cells. *Aging (Albany NY)* 2016. 8: 328–344.
- 39 Im, Y. R., Hunter, H., de Gracia Hahn, D., Duret, A., Cheah, Q., Dong, J., Fairey, M. et al., A systematic review of animal models of NAFLD finds high-fat, high-fructose diets most closely resemble human NAFLD. *Hepatology* 2021. 74: 1884–1901.
- 40 Takahashi, Y. and Fukusato, T., Histopathology of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J. Gastroenterol.* 2014. 20: 15539–15548.
- 41 Giles, D. A., Moreno-Fernandez, M. E. and Divanovic, S., IL-17 axis driven inflammation in non-alcoholic fatty liver disease progression. *Curr. Drug Targets* 2015. 16: 1315–1323.
- 42 Fabre, T., Barron, A. M. S., Christensen, S. M., Asano, S., Bound, K., Lech, M. P., Wadsworth, M. H. et al., Identification of a broadly fibrogenic macrophage subset induced by type 3 inflammation. *Sci. Immunol.* 2023. 8: eadd8945.
- 43 Marinović, S., Lenartić, M., Mladenčić, K., Šestan, M., Kavazović, I., Benić, A., Krapić, M. et al., NKG2D-mediated detection of metabolically stressed hepatocytes by innate-like T cells is essential for initiation of NASH and fibrosis. *Sci. Immunol.* 2023. In press.

- 44 Li, F., Hao, X., Chen, Y., Bai, L., Gao, X., Lian, Z., Wei, H. et al., The microbiota maintain homeostasis of liver-resident gammadeltaT-17 cells in a lipid antigen/CD1d-dependent manner. *Nat. Commun.* 2017. 7: 13839.
- 45 Torres-Hernandez, A., Wang, W., Nikiforov, Y., Tejada, K., Torres, L., Kalabin, A., Adam, S. et al., gammadelta T cells promote steatohepatitis by orchestrating innate and adaptive immune programming. *Hepatology* 2020. 71: 477–494.
- 46 Ray, K., NAFLD. Leaky guts: intestinal permeability and NASH. *Nat. Rev. Gastroenterol. Hepatol.* 2015. 12: 123.
- 47 Galastri, S., Zamara, E., Milani, S., Novo, E., Provenzano, A., Delogu, W., Vizzutti, F. et al., Lack of CC chemokine ligand 2 differentially affects inflammation and fibrosis according to the genetic background in a murine model of steatohepatitis. *Clin. Sci. (Lond.)* 2012. 123: 459–471.
- 48 Li, C., Du, X., Shen, Z., Wei, Y., Wang, Y., Han, X., Jin, H. et al., The critical and diverse roles of CD4(-)CD8(-) double negative T cells in nonalcoholic fatty liver disease. *Cell Mol Gastroenterol Hepatol* 2022. 13: 1805–1827.
- 49 Sun, G., Zhao, X., Li, M., Zhang, C., Jin, H., Li, C., Liu, L. et al., CD4 derived double negative T cells prevent the development and progression of nonalcoholic steatohepatitis. *Nat. Commun.* 2021. 12: 650.
- 50 Mabire, M., Hegde, P., Hammoutene, A., Wan, J., Caer, C., Sayegh, R. A., Cadoux, M. et al., MAIT cell inhibition promotes liver fibrosis regression via macrophage phenotype reprogramming. *Nat. Commun.* 2023. 14: 1830.
- 51 Li, Y., Huang, B., Jiang, X., Chen, W., Zhang, J., Wei, Y., Chen, Y. et al., Mucosal-associated invariant T cells improve nonalcoholic fatty liver disease through regulating macrophage polarization. *Front. Immunol.* 2018. 9: 1994.
- 52 Koay, H. F., Gherardin, N. A., Xu, C., Seneviratna, R., Zhao, Z., Chen, Z., Fairlie, D. P. et al., Diverse MR1-restricted T cells in mice and humans. *Nat. Commun.* 2019. 10: 2243.
- 53 Liew, P. X., Lee, W. Y. and Kubes, P., iNKT cells orchestrate a switch from inflammation to resolution of sterile liver injury. *Immunity* 2017. 47: 752–765 e755.
- 54 Syn, W. K., Agboola, K. M., Swiderska, M., Michelotti, G. A., Liaskou, E., Pang, H., Xie, G. et al., NKT-associated hedgehog and osteopontin drive fibrogenesis in non-alcoholic fatty liver disease. *Gut* 2012. 61: 1323–1329.
- 55 Wolf, M. J., Adili, A., Piotrowitz, K., Abdullah, Z., Boege, Y., Stemmer, K., Ringelhan, M. et al., Metabolic activation of intrahepatic CD8+ T cells and NKT cells causes nonalcoholic steatohepatitis and liver cancer via cross-talk with hepatocytes. *Cancer Cell* 2014. 26: 549–564.
- 56 Kahraman, A., Schlattjan, M., Kocabayoglu, P., Yildiz-Meziletoglu, S., Schlensak, M., Fingas, C. D., Wedemeyer, I. et al., Major histocompatibility complex class I-related chains A and B (MIC A/B): a novel role in nonalcoholic steatohepatitis. *Hepatology* 2010. 51: 92–102.
- 57 Stiglund, N., Strand, K., Cornillet, M., Stal, P., Thorell, A., Zimmer, C. L., Naslund, E. et al., Retained NK cell phenotype and functionality in non-alcoholic fatty liver disease. *Front. Immunol.* 2019. 10: 1255.
- 58 Geissmann, F., Cameron, T. O., Sidobre, S., Manlongat, N., Kronenberg, M., Briskin, M. J., Dustin, M. L. et al., Intravascular immune surveillance by CXCR6+ NKT cells patrolling liver sinusoids. *PLoS Biol.* 2005. 3: e113.
- 59 Nakatani, K., Kaneda, K., Seki, S. and Nakajima, Y., Pit cells as liver-associated natural killer cells: morphology and function. *Med. Electron Microsc.* 2004. 37: 29–36.
- 60 Lett, M. J., Mehta, H., Keogh, A., Jaeger, T., Jacquet, M., Powell, K., Meier, M. A. et al., Stimulatory MAIT cell antigens reach the circulation and are efficiently metabolised and presented by human liver cells. *Gut* 2022. 71: 2526–2538.
- 61 Norris, S., Collins, C., Doherty, D. G., Smith, F., McEntee, G., Traynor, O., Nolan, N. et al., Resident human hepatic lymphocytes are phenotypically different from circulating lymphocytes. *J. Hepatol.* 1998. 28: 84–90.
- 62 Remmerie, A., Martens, L., Thone, T., Castoldi, A., Seurinck, R., Pavie, B., Roels, J. et al., Osteopontin expression identifies a subset of recruited macrophages distinct from kupffer cells in the fatty liver. *Immunity* 2020. 53: 641–657 e614.
- 63 Xiong, X., Kuang, H., Ansari, S., Liu, T., Gong, J., Wang, S., Zhao, X. Y. et al., Landscape of intercellular crosstalk in healthy and NASH liver revealed by single-cell secretome gene analysis. *Mol. Cell* 2019. 75: 644–660 e645.
- 64 Ramachandran, P., Dobie, R., Wilson-Kanamori, J. R., Dora, E. F., Henderson, B. E. P., Luu, N. T., Portman, J. R. et al., Resolving the fibrotic niche of human liver cirrhosis at single-cell level. *Nature* 2019. 575: 512–518.
- 65 Seidman, J. S., Troutman, T. D., Sakai, M., Gola, A., Spann, N. J., Bennett, H., Bruni, C. M. et al., Niche-specific reprogramming of epigenetic landscapes drives myeloid cell diversity in nonalcoholic steatohepatitis. *Immunity* 2020. 52: 1057–1074 e1057.
- 66 Haas, J. T., Vonghia, L., Mogilenko, D. A., Verrijken, A., Molendi-Coste, O., Fleury, S., Deprince, A. et al., Transcriptional network analysis implicates altered hepatic immune function in NASH development and resolution. *Nat. Metab* 2019. 1: 604–614.
- 67 Chen, J., Deng, X., Liu, Y., Tan, Q., Huang, G., Che, Q., Guo, J. et al., Kupffer cells in non-alcoholic fatty liver disease: friend or foe? *Int J Biol Sci* 2020. 16: 2367–2378.
- 68 Tran, S., Baba, I., Poupel, L., Dussaud, S., Moreau, M., Gelineau, A., Marcelin, G. et al., Impaired kupffer cell self-renewal alters the liver response to lipid overload during non-alcoholic steatohepatitis. *Immunity* 2020. 53: 627–640 e625.
- 69 Scott, C. L., Zheng, F., De Baetselier, P., Martens, L., Saeys, Y., De Prijck, S., Lippens, S. et al., Bone marrow-derived monocytes give rise to self-renewing and fully differentiated Kupffer cells. *Nat. Commun.* 2016. 7: 10321.
- 70 Remmerie, A., Martens, L., Thoné, T., Castoldi, A., Seurinck, R., Pavie, B., Roels, J. et al., Osteopontin expression identifies a subset of recruited macrophages distinct from kupffer cells in the fatty liver. *Immunity* 2020. 53: 641–657 e614.
- 71 Daemen, S., Gainullina, A., Kalugotla, G., He, L., Chan, M. M., Beals, J. W., Liss, K. H. et al., Dynamic shifts in the composition of resident and recruited macrophages influence tissue remodeling in NASH. *Cell Rep.* 2021. 34: 108626.
- 72 Zhang, X., Han, J., Man, K., Li, X., Du, J., Chu, E. S. H., Go, M. Y. Y. et al., CXCR3 chemokine receptor 3 promotes steatohepatitis in mice through mediating inflammatory cytokines, macrophages and autophagy. *J. Hepatol.* 2016. 64: 160–170.
- 73 Tomita, K., Freeman, B. L., Bronck, S. F., LeBrasseur, N. K., White, T. A., Hirsova, P. and Ibrahim, S. H., CXCL10-mediates macrophage, but not other innate immune cells-associated inflammation in murine non-alcoholic steatohepatitis. *Sci. Rep.* 2016. 6: 28786.
- 74 Galastri, S., Zamara, E., Milani, S., Novo, E., Provenzano, A., Delogu, W., Vizzutti, F. et al., Lack of CC chemokine ligand 2 differentially affects inflammation and fibrosis according to the genetic background in a murine model of steatohepatitis. *Clinical Science (London, England : 1979)* 2012. 123: 459–471.
- 75 Zhou, Z., Xu, M. J., Cai, Y., Wang, W., Jiang, J. X., Varga, Z. V., Feng, D. et al., Neutrophil-hepatic stellate cell interactions promote fibrosis in experimental steatohepatitis. *Cell Mol Gastroenterol Hepatol* 2018. 5: 399–413.
- 76 Han, Y.-H., Choi, H., Kim, H.-J. and Lee, M.-O., Chemotactic cytokines secreted from Kupffer cells contribute to the sex-dependent susceptibility to non-alcoholic fatty liver diseases in mice. *Life Sci.* 2022. 306: 120846.

- 77 Liu, M., Cao, S., He, L., Gao, J., Arab, J. P., Cui, H., Xuan, W. et al., Super enhancer regulation of cytokine-induced chemokine production in alcoholic hepatitis. *Nat. Commun.* 2021. 12: 4560.
- 78 Tomita, K., Freeman, B. L., Bronk, S. F., LeBrasseur, N. K., White, T. A., Hirsova, P. and Ibrahim, S. H., CXCL10-mediates macrophage, but not other innate immune cells-associated inflammation in murine non-alcoholic steatohepatitis. *Sci. Rep.* 2016. 6: 28786.
- 79 Zhang, X., Han, J., Man, K., Li, X., Du, J., Chu, E. S., Go, M. Y. et al., CXC chemokine receptor 3 promotes steatohepatitis in mice through mediating inflammatory cytokines, macrophages and autophagy. *J. Hepatol.* 2016. 64: 160–170.
- 80 Furuta, K., Guo, Q., Pavelko, K. D., Lee, J. H., Robertson, K. D., Nakao, Y., Melek, J. et al., Lipid-induced endothelial vascular cell adhesion molecule 1 promotes non-alcoholic steatohepatitis pathogenesis. *J. Clin. Invest.* 2021. 131: e143690.
- 81 Ma, H. Y., Yamamoto, G., Xu, J., Liu, X., Karin, D., Kim, J. Y., Alexandrov, L. B. et al., IL-17 signaling in steatotic hepatocytes and macrophages promotes hepatocellular carcinoma in alcohol-related liver disease. *J. Hepatol.* 2020. 72: 946–959.
- 82 Rensen, S. S., Slaats, Y., Nijhuis, J., Jans, A., Bieghs, V., Driessen, A., Malle, E. et al., Increased hepatic myeloperoxidase activity in obese subjects with nonalcoholic steatohepatitis. *Am. J. Pathol.* 2009. 175: 1473–1482.
- 83 Zang, S., Wang, L., Ma, X., Zhu, G., Zhuang, Z., Xun, Y., Zhao, F. et al., Neutrophils play a crucial role in the early stage of nonalcoholic steatohepatitis via neutrophil elastase in mice. *Cell Biochem. Biophys.* 2015. 73: 479–487.
- 84 Zhao, X., Yang, L., Chang, N., Hou, L., Zhou, X., Yang, L. and Li, L., Neutrophils undergo switch of apoptosis to NETosis during murine fatty liver injury via S1P receptor 2 signaling. *Cell Death. Dis.* 2020. 11: 379.
- 85 van der Windt, D. J., Sud, V., Zhang, H., Varley, P. R., Goswami, J., Yazdani, H. O., Tohme, S. et al., Neutrophil extracellular traps promote inflammation and development of hepatocellular carcinoma in nonalcoholic steatohepatitis. *Hepatology* 2018. 68: 1347–1360.
- 86 Hart KM, Fabre T, Sciruba JC, Gieseck RL III, Borthwick LA, Vannella KM, Acciani TH et al., Type 2 immunity is protective in metabolic disease but exacerbates NAFLD collaboratively with TGF- $\beta$ . *Sci. Transl. Med.* 2017. 9: eaal3694.
- 87 Rolfes, V., Ribeiro, L. S., Hawwari, I., Bottcher, L., Rosero, N., Maaserwerd, S., Santos, M. L. S. et al., Platelets fuel the inflammasome activation of innate immune cells. *Cell Rep.* 2020. 31: 107615.
- 88 Malehmir, M., Pfister, D., Gallage, S., Szydłowska, M., Inverso, D., Kotsiliti, E., Leone, V. et al., Platelet GPIIb/IIIa is a mediator and potential interventional target for NASH and subsequent liver cancer. *Nat. Med.* 2019. 25: 641–655.
- 89 Fujita, K., Nozaki, Y., Wada, K., Yoneda, M., Endo, H., Takahashi, H., Iwasaki, T. et al., Effectiveness of antiplatelet drugs against experimental non-alcoholic fatty liver disease. *Gut* 2008. 57: 1583–1591.
- 90 Thibaut, R., Gage, M. C., Pineda-Torra, I., Chabrier, G., Venteclef, N. and Alzaid, F., Liver macrophages and inflammation in physiology and pathophysiology of non-alcoholic fatty liver disease. *FEBS J.* 2022. 289: 3024–3057.
- 91 Kim, S. Y., Jeong, J.-M., Kim, S. J., Seo, W., Kim, M.-H., Choi, W.-M., Yoo, W. et al., Pro-inflammatory hepatic macrophages generate ROS through NADPH oxidase 2 via endocytosis of monomeric TLR4-MD2 complex. *Nat. Commun.* 2017. 8: 2247.
- 92 Sawada, K., Ohtake, T., Hasebe, T., Abe, M., Tanaka, H., Ikuta, K., Suzuki, Y. et al., Augmented hepatic Toll-like receptors by fatty acids trigger the pro-inflammatory state of non-alcoholic fatty liver disease in mice. *Hepatology Res.* 2014. 44: 920–934.
- 93 Hung, H.-C., Tsai, S.-F., Chou, H.-W., Tsai, M.-J., Hsu, P.-L. and Kuo, Y.-M., Dietary fatty acids differentially affect secretion of pro-inflammatory cytokines in human THP-1 monocytes. *Sci. Rep.* 2023. 13: 5511.
- 94 Carpino, G., Del Ben, M., Pastori, D., Carnevale, R., Baratta, F., Overi, D., Francis, H. et al., Increased liver localization of lipopolysaccharides in human and experimental NAFLD. *Hepatology* 2020. 72: 470–485.
- 95 Harte, A. L., da Silva, N. F., Creely, S. J., McGee, K. C., Billyard, T., Youssef-Elabd, E. M., Tripathi, G. et al., Elevated endotoxin levels in non-alcoholic fatty liver disease. *J. Inflamm. (Lond)* 2010. 7: 15.
- 96 Sharifnia, T., Antoun, J., Verriere, T. G. C., Suarez, G., Wattacheril, J., Wilson, K. T., Peek, R. M. et al., Hepatic TLR4 signaling in obese NAFLD. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2015. 309: G270–278.
- 97 Miura, K., Kodama, Y., Inokuchi, S., Schnabl, B., Aoyama, T., Ohnishi, H., Olefsky, J. M. et al., Toll-like receptor 9 promotes steatohepatitis by induction of interleukin-1 $\beta$  in mice. *Gastroenterology* 2010. 139: 323–334.e327.
- 98 Kotsiliti, E., Leone, V., Schuehle, S., Govaere, O., Li, H., Wolf, M. J., Horvatic, H. et al., Intestinal B cells license metabolic T-cell activation in NASH microbiota/antigen-independently and contribute to fibrosis by IgA-FcR signalling. *J. Hepatol.* 2023. 79: 296–313.
- 99 Gerdes, S., Pinter, A., Papavassilis, C. and Reinhardt, M., Effects of secukinumab on metabolic and liver parameters in plaque psoriasis patients. *J. Eur. Acad. Dermatol. Venereol.* 2020. 34: 533–541.
- 100 Narni-Mancinelli, E., Chaix, J., Fenis, A., Kerdiles, Y. M., Yessaad, N., Reynders, A., Gregoire, C. et al., Fate mapping analysis of lymphoid cells expressing the NKp46 cell surface receptor. *Proc. Natl. Acad. Sci. USA* 2011. 108: 18324–18329.
- 101 Gan, L. T., Van Rooyen, D. M., Koina, M. E., McCuskey, R. S., Teoh, N. C. and Farrell, G. C., Hepatocyte free cholesterol lipotoxicity results from JNK1-mediated mitochondrial injury and is HMGB1 and TLR4-dependent. *J. Hepatol.* 2014. 61: 1376–1384.
- 102 Barrow, F., Khan, S., Fredrickson, G., Wang, H., Dietsche, K., Parthiban, P., Robert, S. et al., Microbiota-driven activation of intrahepatic B cells aggravates NASH through innate and adaptive signaling. *Hepatology* 2021. 74: 704–722.
- 103 Her, Z., Tan, J. H. L., Lim, Y.-S., Tan, S. Y., Chan, X. Y., Tan, W. W. S., Liu, M. et al., CD4+ T cells mediate the development of liver fibrosis in high fat diet-induced NAFLD in humanized mice. *Front. Immunol.* 2020. 11: 580968.
- 104 Moreno-Fernandez, M. E., Giles, D. A., Oates, J. R., Chan, C. C., Damen, M. S. M. A., Doll, J. R., Stankiewicz, T. E. et al., PKM2-dependent metabolic skewing of hepatic Th17 cells regulates pathogenesis of non-alcoholic fatty liver disease. *Cell Metab.* 2021. 33: 1187–1204.e1189.
- 105 Deczkowska, A., David, E., Ramadori, P., Pfister, D., Safran, M., Li, B., Giladi, A. et al., XCR1(+) type 1 conventional dendritic cells drive liver pathology in non-alcoholic steatohepatitis. *Nat. Med.* 2021. 27: 1043–1054.
- 106 Lin, S. Z. and Fan, J. G., Peripheral immune cells in NAFLD patients: a spyhole to disease progression. *EBioMedicine* 2022. 75: 103768.
- 107 Bruzzi, S., Sutti, S., Giudici, G., Burlone, M. E., Ramavath, N. N., Toscani, A., Bozzola, C. et al., B2-Lymphocyte responses to oxidative stress-derived antigens contribute to the evolution of nonalcoholic fatty liver disease (NAFLD). *Free Radic Biol Med* 2018. 124: 249–259.
- 108 Zhang, F., Jiang, W. W., Li, X., Qiu, X. Y., Wu, Z., Chi, Y. J., Cong, X. et al., Role of intrahepatic B cells in non-alcoholic fatty liver disease by secreting pro-inflammatory cytokines and regulating intrahepatic T cells. *J. Dig. Dis.* 2016. 17: 464–474.
- 109 Breuer, D. A., Pacheco, M. C., Washington, M. K., Montgomery, S. A., Hasty, A. H. and Kennedy, A. J., CD8+ T cells regulate liver injury in obesity-related nonalcoholic fatty liver disease. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2020. 318: G211–G224.

- 110 McVey, J. C., Green, B. L., Ruf, B., McCallen, J. D., Wabitsch, S., Subramanyam, V., Diggs, L. P. et al., NAFLD indirectly impairs antigen-specific CD8+ T cell immunity against liver cancer in mice. *iScience* 2022. 25: 103847.
- 111 Dudek, M., Pfister, D., Donakonda, S., Filpe, P., Schneider, A., Laschinger, M., Hartmann, D. et al., Auto-aggressive CXCR6+ CD8 T cells cause liver immune pathology in NASH. *Nature* 2021. 592: 444–449.
- 112 Koda, Y., Teratani, T., Chu, P. S., Hagihara, Y., Mikami, Y., Harada, Y., Tsujikawa, H. et al., CD8(+) tissue-resident memory T cells promote liver fibrosis resolution by inducing apoptosis of hepatic stellate cells. *Nat. Commun.* 2021. 12: 4474.
- 113 Senoo, H., Yoshikawa, K., Morii, M., Miura, M., Imai, K. and Mezaki, Y., Hepatic stellate cell (vitamin A-storing cell) and its relative—past, present and future. *Cell Biol. Int.* 2010. 34: 1247–1272.
- 114 Xu, F., Liu, C., Zhou, D. and Zhang, L., TGF- $\beta$ /SMAD pathway and its regulation in hepatic fibrosis. *J. Histochem. Cytochem.* 2016. 64: 157–167.
- 115 Hellerbrand, C., Stefanovic, B., Giordano, F., Burchardt, E. R. and Brenner, D. A., The role of TGF $\beta$ 1 in initiating hepatic stellate cell activation in vivo. *J. Hepatol.* 1999. 30: 77–87.
- 116 Cai, B., Dongiovanni, P., Corey, K. E., Wang, X., Shmarakov, I. O., Zheng, Z., Kasikara, C. et al., Macrophage MerTK promotes liver fibrosis in non-alcoholic steatohepatitis. *Cell Metab.* 2020. 31: 406–421.e407.
- 117 Kagan, P., Sultan, M., Tachlytski, I., Safran, M. and Ben-Ari, Z., Both MAPK and STAT3 signal transduction pathways are necessary for IL-6-dependent hepatic stellate cells activation. *PLoS One* 2017. 12: e0176173.
- 118 Ying, H.-Z., Chen, Q., Zhang, W.-Y., Zhang, H.-H., Ma, Y., Zhang, S.-Z., Fang, J. et al., PDGF signaling pathway in hepatic fibrosis pathogenesis and therapeutics. *Mol Med Rep* 2017. 16: 7879–7889.
- 119 Gieling, R. G., Wallace, K. and Han, Y.-P., Interleukin-1 participates in the progression from liver injury to fibrosis. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2009. 296: G1324–1331.
- 120 Ma, H.-Y., Yamamoto, G., Xu, J., Liu, X., Karin, D., Kim, J. Y., Alexandrov, L. B. et al., IL-17 signaling in steatotic hepatocytes and macrophages promotes hepatocellular carcinoma in alcohol-related liver disease. *J. Hepatol.* 2020. 72: 946–959.
- 121 Breitkopf-Heinlein, K., Meyer, C., Konig, C., Gaitantzi, H., Addante, A., Thomas, M., Wiercinska, E. et al., BMP-9 interferes with liver regeneration and promotes liver fibrosis. *Gut* 2017. 66: 939–954.
- 122 Vacca, M., Leslie, J., Virtue, S., Lam, B. Y. H., Govaere, O., Tiniakos, D., Snow, S. et al., Bone morphogenetic protein 8B promotes the progression of non-alcoholic steatohepatitis. *Nat Metab* 2020. 2: 514–531.
- 123 Gastaldelli, A., Cusi, K., Fernandez Lando, L., Bray, R., Brouwers, B. and Rodriguez, A., Effect of tirzepatide versus insulin degludec on liver fat content and abdominal adipose tissue in people with type 2 diabetes (SURPASS-3 MRI): a substudy of the randomised, open-label, parallel-group, phase 3 SURPASS-3 trial. *Lancet. Diabetes Endocrinol.* 2022. 10: 393–406.
- 124 Ratziu, V., Sanyal, A., Harrison, S. A., Wong, V. W., Francque, S., Goodman, Z., Aithal, G. P. et al., Cenicriviroc treatment for adults with nonalcoholic steatohepatitis and fibrosis: final analysis of the phase 2b CEN-TAUR study. *Hepatology* 2020. 72: 892–905.
- 125 Leite, N. C., Viegas, B. B., Villela-Nogueira, C. A., Carlos, F. O., Cardoso, C. R. L. and Salles, G. F., Efficacy of diacerein in reducing liver steatosis and fibrosis in patients with type 2 diabetes and non-alcoholic fatty liver disease: a randomized, placebo-controlled trial. *Diabetes Obes. Metab.* 2019. 21: 1266–1270.
- 126 Marques, L. J., Zheng, L., Poulakis, N., Guzman, J. and Costabel, U., Pentoxifylline inhibits TNF-alpha production from human alveolar macrophages. *Am. J. Respir. Crit. Care Med.* 1999. 159: 508–511.
- 127 Zein, C. O., Yerian, L. M., Gogate, P., Lopez, R., Kirwan, J. P., Feldstein, A. E. and McCullough, A. J., Pentoxifylline improves nonalcoholic steatohepatitis: a randomized placebo-controlled trial. *Hepatology* 2011. 54: 1610–1619.
- 128 Van Wagner, L. B., Koppe, S. W., Brunt, E. M., Gottstein, J., Gardikiotes, K., Green, R. M. and Rinella, M. E., Pentoxifylline for the treatment of non-alcoholic steatohepatitis: a randomized controlled trial. *Ann. Hepatol.* 2011. 10: 277–286.
- 129 Satapathy, S. K., Garg, S., Chauhan, R., Sakhuja, P., Malhotra, V., Sharma, B. C. and Sarin, S. K., Beneficial effects of tumor necrosis factor-alpha inhibition by pentoxifylline on clinical, biochemical, and metabolic parameters of patients with nonalcoholic steatohepatitis. *Am. J. Gastroenterol.* 2004. 99: 1946–1952.
- 130 Seitz, M., Reichenbach, S., Moller, B., Zwahlen, M., Villiger, P. M. and Dufour, J. F., Hepatoprotective effect of tumour necrosis factor alpha blockade in psoriatic arthritis: a cross-sectional study. *Ann. Rheum. Dis.* 2010. 69: 1148–1150.
- 131 Gisondi, P., Targher, G., Zoppini, G. and Girolomoni, G., Non-alcoholic fatty liver disease in patients with chronic plaque psoriasis. *J. Hepatol.* 2009. 51: 758–764.
- 132 Woods, C. P., Hazlehurst, J. M. and Tomlinson, J. W., Glucocorticoids and non-alcoholic fatty liver disease. *J. Steroid Biochem. Mol. Biol.* 2015. 154: 94–103.
- 133 Ferraioli, G. and Soares Monteiro, L. B., Ultrasound-based techniques for the diagnosis of liver steatosis. *World J. Gastroenterol.* 2019. 25: 6053–6062.
- 134 Abiru, S., Migita, K., Maeda, Y., Daikoku, M., Ito, M., Ohata, K., Nagaoka, S. et al., Serum cytokine and soluble cytokine receptor levels in patients with non-alcoholic steatohepatitis. *Liver Int.* 2006. 26: 39–45.
- 135 Garcia-Galiano, D., Sanchez-Garrido, M. A., Espejo, I., Montero, J. L., Costan, G., Marchal, T., Membrives, A. et al., IL-6 and IGF-1 are independent prognostic factors of liver steatosis and non-alcoholic steatohepatitis in morbidly obese patients. *Obes. Surg.* 2007. 17: 493–503.
- 136 Yoneda, M., Uchiyama, T., Kato, S., Endo, H., Fujita, K., Yoneda, K., Mawatari, H. et al., Plasma Pentraxin3 is a novel marker for nonalcoholic steatohepatitis (NASH). *BMC Gastroenterol.* 2008. 8: 53.

**Abbreviations:** HCC: hepatocellular carcinoma · HSC: hepatic stellate cells · KCs: Kupffer cells · LSECs: liver sinusoidal endothelial cells · MAFL: metabolic-associated fatty liver · MAFLD: metabolic-associated fatty liver disease · MAIT: mucosal-associated invariant T · MASH: metabolic dysfunction-associated steatohepatitis · MASLD: metabolic dysfunction-associated fatty liver disease · MPO: myeloperoxidase · NAFLD: nonalcoholic fatty liver disease · NETs: neutrophil extracellular traps · NKT: natural killer T · NPCs: nonparenchymal cells · T2D: type 2 diabetes mellitus · TCR: T-cell receptor

**Full correspondence:** Dr. Felix M. Wensveen, Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Braće Branchetta 20, 51000 Rijeka, Croatia  
e-mail: felix.wensveen@medri.uniri.hr

Received: 24/8/2023  
Revised: 17/1/2024  
Accepted: 17/1/2024  
Accepted article online: 22/1/2024