BBA - Molecular Basis of Disease 1865 (2019) 577–586

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

Review article

Prognostic molecular markers in pediatric liver disease – Are there any?

Jon Nielsen[a], Vibeke Brix Christensen[a], Lise Borgwardt[b], Allan Rasmussen[c], Olga Østrup[d,*,1], Mette Skalshøi Kjær[e,1]

[a] Department of Paediatrics and Adolescent Medicine, Copenhagen University Hospital Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen Ø, Denmark
[b] Department of Clinical Physiology, Nuclear Medicine and PET, Copenhagen University Hospital Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen Ø, Denmark
[c] Department of Surgical Gastroenterology, Copenhagen University Hospital Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen Ø, Denmark
[d] Center for Genomic Medicine, Copenhagen University Hospital Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen Ø, Denmark
[e] Department of Hepatology, Copenhagen University Hospital Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen Ø, Denmark

ARTICLE INFO

Keywords:
Hepatobiliary disease
Children
Diagnostics
Outcome prediction

ABSTRACT

Pediatric liver disease (PLD) is a major cause of severe morbidity and prolonged hospitalizations in children. Stratifying patients in terms of prognosis remains challenging. The limited knowledge about molecular mechanisms causing and accompanying PLD remains the main obstacle in a search for reliable prognostic biomarkers. A systematic search of MEDLINE via PubMed and Embase via OVID was conducted on studies published between August 2007 and August 2017. Molecular markers with a prognostic potential in terms of survival, need for liver transplantation or disease progression/regression were selected. In general, identified studies were single center smaller case-control studies or case series with a low level of evidence and a high risk of bias. Only 23 studies comprising 898 patients could be included, mostly focusing on biliary atresia, non-alcoholic fatty liver disease, viral hepatitis, and LT; and markers related to morphogenesis and fibrosis. Furthermore, molecular markers in metabolic pathways and inflammation shown to be relevant, however requiring further validation. Hence, further biological and clinical studies are needed to gain greater molecular insight into PLD.

1. Introduction

Pediatric liver disease (PLD) is a serious condition causing repeated hospitalizations and long-term morbidity and has a potentially fatal outcome without curative or supportive treatment. The true incidence and prevalence are unknown, but an estimated 15,000 children are hospitalized for PLD in the US annually [1,2]. Based on the clinical pictures, PLD is essentially comprised of two main groups, i.e. acute liver failure [3] and chronic liver disease, although etiologies can overlap. Pediatric chronic liver disease has a more insidious course of varying degrees of inflammation and changes in the extracellular matrix often leading to fibrosis/cirrhosis [4].

Prognostication based on disease severity or etiology is difficult in PLD. Seemingly comparable children can present markedly different outcomes. Although some prognostic scoring systems appear promising, addressing prognosis in PLD remains challenging [5–7]. Prognostic markers range from general ones such as age, sex, and clinical appearance to clinical scoring systems, histology, functional liver tests, and biomarker analysis. Prognostication is especially important for PLD patients with a potential need for liver transplantation (LT). Liver transplantation can be lifesaving for PLD patients and should be reserved for patients who have lost their capacity for liver regeneration; however, a shortage of donor organs still exists [8]. Prognostic markers enabling children requiring rapid and extensive medical attention possibly involving LT to be differentiated from patients with a favorable prognosis continue to be sought by physicians.

The exponential development of precision medicine and accordingly related molecular methods has shifted the focus of PLD research onto

Abbreviations: AUROC, area under receiver operating characteristic; BA, biliary atresia; CAR, constitutive androstane receptor; CD, cluster of differentiation; CK, cytokeratin; COX, cyclooxygenase; CTGF, connective tissue growth factor; EMT, epithelial to mesenchymal transition; FAS, first apoptosis signal; Gli-2, glioblastoma associated protein; GLP, glucagon-like peptide; HBV, hepatitis B virus; HCV, hepatitis C virus; HIF, hypoxia-inducible factor; HSC, hepatic stellate cell; IHC, immunohistochemistry; LB, liver biopsy; LT, liver transplantation; MeSH, medical subject headings; miR, microRNA; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PCR, polymerase chain reaction; PF, portal fibroblast; PLD, pediatric liver disease; PXR, pregnane X receptor; SHH, sonic hedgehog; SMA, smooth muscle actin; TGF, transforming growth factor; TLR, toll like receptor

* Corresponding author at: Center for Genomic Medicine, Copenhagen University Hospital, Blegdamsvej 9, 2100-CPH, Denmark.
E-mail address: Olga.oestrup@regionh.dk (O. Østrup).
[1] Authors contributed equally and share last authorship.

https://doi.org/10.1016/j.bbadis.2018.12.018
Received 14 September 2018; Received in revised form 6 December 2018; Accepted 19 December 2018
Available online 22 December 2018
0925-4439/

molecular biomarkers [9,10]. Biomarkers evaluated in liver biopsy (LB) have demonstrated the strongest diagnostic and prognostic values among various PLD diagnoses [11]. Hence, despite being an invasive procedure, LB remains the gold standard for PLD diagnostics. Of note, while prognostic molecular liver tissue markers are well described in malignant PLD [12–14], there is only limited knowledge about markers in non-malignant PLD.

The aim of this systematic review was to summarize, and present liver-specific molecular markers associated with the prognosis and spontaneous course of non-malignant PLD.

2. Methods

The databases MEDLINE via PubMed and Embase via OVID were searched in August 2017. A systematic strategy based on the PICO criteria [15] was used. This approach included MeSH terms and text words for the PubMed search and Subject Heading and text words for the Embase search. Briefly, three main aspects for the search were defined: i) patient and diagnosis; ii) molecular marker; and iii) the prognostic outcome. For each of these aspects, the queries were MeSH term/Subject Heading search combined with a text word-based search combined with "OR". All three aspects of the search were combined by "AND" to ensure identification of articles fulfilling all aspects. Studies published between August 2007 and August 2017 were included in the review. Applied search filters were "human studies", "child 0–18 years", "English or Danish language".

COVIDENCE systematic review software (Veritas Health Innovation, Melbourne, Australia. Available at www.covidence.org) was used for the abstract and full text screening process. All articles from the initial search were independently screened for study title and abstract by 2 authors (JN, OØ). 10,996 articles were excluded after title and abstract screening. Full text of remaining 74 articles (Table 1_online) was studied for inclusion or exclusion by 4 authors (JN, VB, MSK, OØ), and subsequently 23 articles fulfilled the inclusion criteria for the final review. An overview of the search is found in Fig. 1 and in the supplementary material (Method 1_online and Method 2_online). Decision concerning in- or exclusion were blinded between authors. Disagreements were solved by joint discussion of the article. Papers on molecular markers in liver tissue addressing the prognostic course of pediatric liver disease were included. Case reports were excluded and *neoplasia*, in vitro *studies*, *polymorphism*, *germline mutation/variant*, *non-child*, *non-human*, *non-liver tissue* were all exclusion criteria. Only studies identifying a significant up- or downregulation of marker(s) were included. Assessment of prognosis across PLD diagnoses is not a well-defined entity. Hence, overall survival, native liver survival, liver transplant free survival and jaundice free survival were all used to evaluate prognosis of liver disease. Moreover, hepatic fibrosis was used as prognostic surrogate [11,16–18], due to the proven risk of progression to cirrhosis dependent on fibrosis stage. In non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) inflammation was included as a prognostic surrogate since NASH is the inflammatory progression of NAFLD. Other disease specific scoring systems were excluded when evaluating prognosis.

We conducted the review according to PRISMA guidelines and the levels of evidence of included articles were graded according to those of the Oxford Centre for Evidence-Based Medicine [19,20]. The risk of bias was evaluated independently by two authors (JN, MSK) according to predefined criteria (Table 2_online). Results from the included trials are

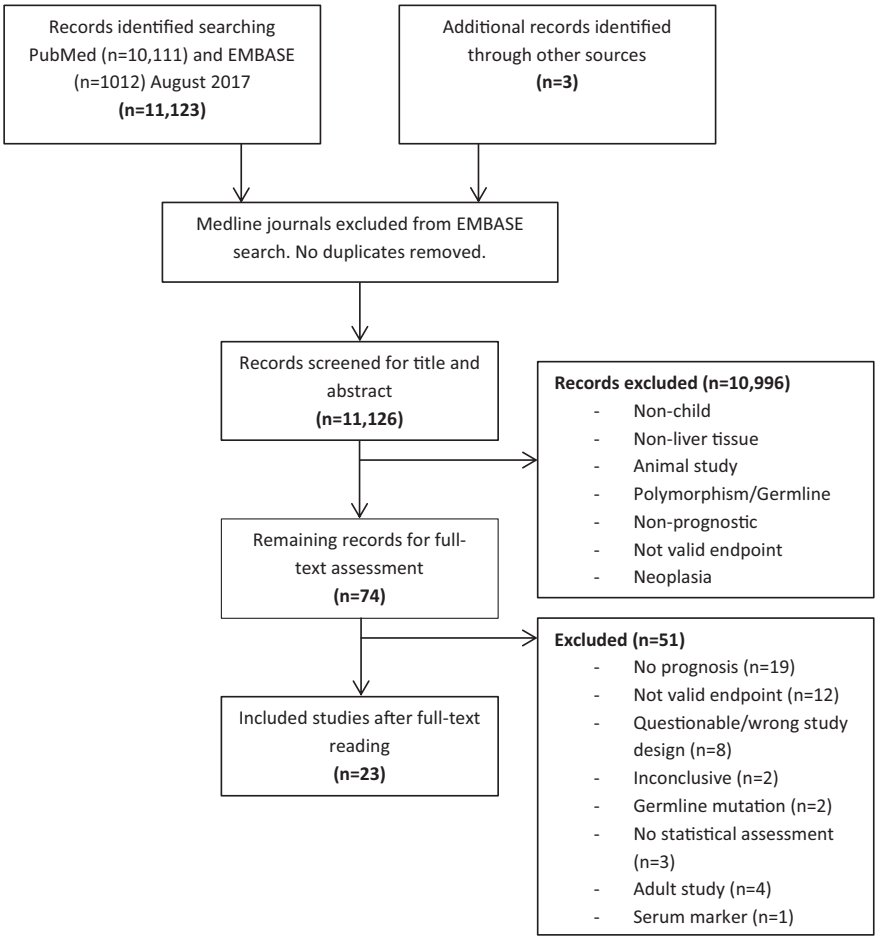

Fig. 1. PRISMA search flowchart.

Table 1
Overview of included tudies.

Study	Year	Diagnosis	Study design	Patients (n)	Ctrl ped (adult)	Endpoints - prognosis	Method	Markers
Moyer K et al.	2010	BA	CS	47	0	TFS, need for LT	GeneSpring, RT-qPCR	Gene signature
Saito T et al.	2011	BA	CC	19	30	Need for LT	RT-qPCR	*TLR2*, *TLR3*, *TLR4*, *TLR7*, *TLR8*
Zhao R et al.	2011	BA	CS	49	20	CJ, cholangitis	Array, RT-qPCR	Transcriptome
Xiao Y et al.	2014	BA	CS	18	0	FS	RT-qPCR	*miR-200b*, *pp85*, *p-Akt*(*S473*), *MMP-2*
Chen H-L et al.	2008	BA	CC	34	8 (4)	NLS/need for LT	IHC, RT-qPCR	17 markers
Chen W et al.	2013	BA	CC	55	5	Need for LT, FS, EV	IHC, RT-qPCR	*Apelin*, *apelin Receptor*
Huang L et al.	2008	BA/CBD	CC	33	8	Hepatic fibrosis	IHC, WB, RT-qPCR	*OPN*, *NFkB*, *TGF-β1*
Jung HY et al.	2015	BA	CC	57	7	JFS, LT free survival	IHC	*SHH*, *PTCH*, *Gli-2*
Longo-Santos LR et al.	2015	BA	CS	35	0	Progression time curve to LT	IHC	*Collagen type I*, *III*, *IV and V*
Santos JL et al.	2009	BA	CS	47	0	1 year NLS	IHC	*CK-7*
Suominen JS et al.	2014	BA	CC	33	0 (35)	NLS, development of EV, FS	IHC	*Collagen-1*, *a-SMA*, *CD34*
Honsawek S et al.	2009	BA	CC	28	9	Liver function; LT 6 months post-KP	IHC	*COX-2*
Hill R et al.	2015	BA	CC	37	12	NLS, CJ	IHC	*Tbet*, *GATA3*, *FOXP3*, *IL17* (*Th1*, *Th2*, *Tregs*, *T17cells*)
Dong R et al.	2012	BA	CC	21	15	FS, direct bilirubin 3 months post-KP	IHC	*α-SMA*, *CD68*
Li FB et al.	2016	BA/NeoHep	CC	36	2	FS	IHC	*TGF-β1*, *CTGF*
Swiderska-Syn M et al.	2013	NAFLD	CS	56	0	FS	IHC	*SHH*, *Vim*, *Gli2*, *a-SMA*, *CK-7*
De Vito R et al.	2012	NAFLD	CS	34	0	FS, NAFLD activity score	IHC	*CD45*, *CD3*, *CD163*
Nobili V et al.	2012	NAFLD/NASH	CC	30	6	FS, NAFLD activity score	IHC	*CK-7*, *adiponectin*, *resistin*, *GLP-1*, *p21waf1*, *cleaved caspase-3*
Valva P et al.	2008	NASH	CC	25	8	FS	IHC	*CK18*(*M30*), *CASP-3a*
Valva P et al.	2010	cHCV	CS	23	0	FS	IHC, TUNEL	*CK-18*(*M30*), *CASP3a*, *NS3*
Lotowska JM et al.	2015	cHBV	CS	70	0	FS	IHC	*α-SMA*
El-Araby HA et al.	2015	cHCV	CS	80	0	FS	IHC	*CK-7*
Miyagawa-Hayashino A et al.	2007	LT	CC	31	5 (6)	Graft rejection activity	IHC, RT-qPCR, TUNEL	*Fas*, *FasL*,

Included Studies. Abbreviations: BA, biliary atresia; CBD, congenital biliary dilatation; CC, case control; cHBV, chronic hepatitis B virus; cHCV, chronic hepatitis C virus; CJ, clearance of jaundice; CS, case series; EV, esophageal varices; FS, fibrosis stage; IHC, immunohistochemistry; JFS, jaundice free survival; LT, liver transplantation; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NeoHep, neonatal hepatitis; NLS, native liver survival; ped, pediatric; RT-qPCR, reverse transcription quantitative polymerase chain reaction; TFS, transplant free survival; WB, western blot.

presented and discussed in a descriptive manner and subdivided into specific disease categories.

3. Results

Twenty-three studies comprising 898 patients were included in this review (Table 1). Most of the studies addressed patients with biliary atresia (BA; $n = 15$), while the remaining ones focused on NAFLD and NASH ($n = 4$), viral hepatitis ($n = 3$), and LT (n = 1). From a methodological viewpoint, most of studies (n = 15) used conventional immunohistochemistry (IHC) in isolation for marker detection. Only two studies applied a broader transcriptomic screening for potential marker identification [21,22], rather than solely relying on conventional PCR methods ($n = 2$) [23,24], while four studies used a combination of PCR and IHC [25–28]. The identified studies were small case-control studies or case series of a descriptive nature, with an inherent high risk of bias, although recall and observer biases are minimized due to the objective nature of molecular factor analysis. Due to small cohort size, study design, and limitations in the availability of appropriate controls, all studies were assigned low levels of evidence (=4). Such studies show considerable variation in outcome measures, patient population, and estimated prognostic factors.

3.1. Biliary atresia

Biliary atresia, an inflammatory, sclerosing cholangiopathy occurring in early infancy, is manifested by fibrous obliteration of extrahepatic bile ducts, leading to biliary cirrhotic transformation and progressive intrahepatic bile duct damage with subsequent cholestatic liver failure. Despite early hepatoportoenterostomy, up to 70–80% of those affected eventually need LT [29]. Table 2 and Table 3_online present an overview of identified markers in BA studies. Only three of the markers (collagen-1, α-SMA, and TGF-β1) were evaluated in more than one study.

Fibrosis is the main pathological process that occurs in BA. Consequently, eight studies chose to study the prognostic value of individual fibrosis-related markers. Interestingly, six of these studies selected well-known markers of fibrogenesis and bile ductular proliferation, such as collagen-1, CK-7, α-SMA, TGF-β1, and CTGF [27,30–34]. The markers showed to have a prognostic value, i.e. collagen-1, CK-7, and α-SMA correlated to mortality [32], need for LT and/or native liver survival [30–32]. Indeed, early deposition of collagen-1 indicates faster progression to LT as shown in survival analysis, and a high percentage of CK-7 positive cells reflected decreased 1 year native liver survival with an area under the receiver operating characteristic (AUROC) curve of 0.845 [30,32]. TGF-β1 and CTGF correlated to fibrosis [27,34] and one study found a moderate correlation of α-SMA with fibrosis and

Table 2
Studies of prognostic molecular markers in biliary atresia.

Biliary atresia marker	Finding	Method	References
Inflammatory and fibrotic gene signatures	Fibrotic signature relates to lower **TFS** ($p = 0.04$)[a] Fibrotic compared to inflammatory gene signature relates to OR (8.2) for **death** or **LT** ($p = 0.04$[b])	KM	[21]
TLRs *Toll like receptors*	↑TLR3 in patients with **LT** vs. non-LT ($p = 0.02$[a]) ↑TLR7 in patients with **LT** vs. non-LT ($p = 0.01$[a])		[23]
PXR *Pregnane X receptor*	↓PXR in poor **prognosis** vs. good prognosis ($p = 0.014$[a]) ↓PXR relates to lower **survival rate** ($p = 0.008$[a])	KM	[25]
CAR *Constitutive Androstane receptor*	↓CAR in patients with poor **prognosis** compared to good prognosis ($p = 0.025$[a]) ↓CAR relates to lower **survival time** ($p = 0.041$[a])	KM	[25]
SHH & Gli-2 *Sonic Hedgehog* *Glioblastoma associated protein*	↑SHH on stromal cells associated to poor **JFS** (HR: 1.16, $p = 0.01$[a] & HR: 1.20, $p = 0.032$[b]) ↑Gli-2 associated to poor **JFS** (stromal (HR: 1.14, $p = 0.003$[a] & HR: 1.35, $p = 0.01$[b]) cholangiocytes (HR: 1.05, $p = 0.032$[a])) ↑SHH on stromal cells associated with poor **JFS** on Kaplan-Meier ($p = 0.036$[a]) ↑Gli-2 on cholangiocytes and stromal cells associated with poor **JFS** on Kaplan-Meier ($p = 0.006$ & $p = 0.002$[a])	COX	[38]
Collagen-1	↑collagen-1 associates with worse Kaplan-Meier **progress-time** curve to **LT** ($p = 0.04$[a]) ↑collagen-1 associate with higher need for **LT** ($p = 0.024$[a])		[30] [31]
CK-7 *Cytokeratin-7*	↑CK-7 relates to **mortality** and need for **LT** ($p < 0.001$[a]) ↑CK-7 relates to 1 year **NLS** ($p < 0.001$[a]) (sensitivity 0.71; specificity 0.88) ↑CK-7 relates to lower 1-year NLS ($p < 0.001$[a]) ↑CK-7 associates with OR (1.5) for **death/LT** ($p = 0.002$[b])	AUROC KM	[32]
Apelin	↑Apelin in high **fibrosis** stage ($p < 0.05$[a] & $p < 0.01$[a] stage 4 vs. stage 0–1; stage 4 vs. stage 2–3)		[26]
COX-2 *Cyclooxygenase-2*	↑COX-2 in severe **liver dysfunction** post HPE ($p < 0.005$[a] vs. satisfactory liver function)		[37]
T-cell subsets	↓Th-17 relates to **CJ** six months post HPE ($p = 0.008$[a])		[36]
RRAS	↓RRAS relates to better **CJ** ($p = 0.007$[a]) ↑RRAS relates to occurrence of **cholangitis** ($p = 0.028$[a])		[22]
α-SMA *α-smooth muscle actin*	↑α-SMA associates with higher need for **LT** ($p = 0.009$[a]) α-SMA moderate positive correlation to fibrosis ($p = 0.022$[a]) α-SMA moderate negative correlation to conjugated bilirubin 3 months post HPE ($p = 0.029$[a])		[31] [33]
miR-200b	↑miR-200b in severe **fibrosis** ($p < 0.05$[a])		[24]
CD34 *Cluster of differentiation 34*	↑CD34 associates with higher need for **LT** and **NLS** ($p = 0.029$[a]) ↑CD34 relates to **esophageal varices** ($p = 0.019$[a])		[31]
OPN *Osteopontin*	OPN correlates to **fibrosis** ($r = 0.97$, $p < 0.01$[a])		[27]
TGF- β1 *Transforming growth factor-β1*	TGF- β1 correlates to **fibrosis** ($r = 0.96$, $p < 0.01$[a] & $r = 0.584$, $p < 0.01$[a])		[27] [34]
CTGF *Connective tissue growth factor*	CTGF at diagnosis correlates to BA and hepatic **fibrosis stage** ($r = 0.741$, $p < 0.01$[a])		[34]

Studies in bold applied survival statistical methods, i.e. Kaplan-Meier (KM; log rank), Cox proportional hazards regression and/or information on performance or accuracy measures (AUROC curve, sensitivity, specificity, positive or negative predictive values).
Abbreviations: AUROC, area under receiver operating characteristics; CJ, clearance of jaundice; JFS, jaundice-free survival; LT, liver transplantation; NLS, native liver survival; TFS, transplant-free survival; ↑, upregulation; ↓, downregulation.
[a] Univariable test/unadjusted comparison.
[b] Multivariable test/adjusted comparison.

Table 3
Studies of prognostic molecular markers in non-alcoholic fatty liver disease and non-alcoholic steatohepatitis.

NAFLD Marker	Finding	References
SHH & Gli-2	↑SHH correlates with **fibrosis** stage ($p = 0.0008$[a]; $p = 0.002$[b])	[43]
Sonic Hedgehog	↑SHH correlates with **steatosis** grade ($p = 0.022$[a])	
Glioblastoma associated protein	↑Gli-2 correlates with **fibrosis** stage ($p = 0.0013$[a]; $p = 0.045$[b])	
	↑Gli-2 correlates with **portal inflammation** grade ($p = 0.0012$[a]; $p = 0.022$[b])	
CK-7	↑CK-7 in **NASH** compared to NAFLD biopsies ($p < 0.05$[a]) [41]	[44]
Cytokeratin-7	CK-7 expression correlates with degree of fibrosis ($p = 0.033$[b]) [41]	[43]
	↑CK-7 in NAFLD with **fibrosis** compared to NAFLD biopsies without fibrosis ($p < 0.01$[a]) [41]	
	↑CK-7 positively associated with severity of **portal inflammation** ($p = 0.0185$[a]) [40]	
Vim	↑Vim in advanced **fibrosis** stages ($p = 0.038$[a])	[43]
Vimentin		
Casp-3a	↑Cleaved Casp-3 in **NASH** compared to NAFLD biopsies ($p < 0.05$[a]) [41]	[44]
Caspase-3a	↑Casp-3a relates to low **inflammation** grade ($p = 0.03$) [42]	[45]
CD45	↑CD45 in **fibrosis** vs. no fibrosis ($p = 0.035$[a])	[46]
Cluster of differentiation 45	↑CD45 positive cells in **NAS ≥ 5** compared to NAS < 5 ($p < 0.0001$[a])	
	CD45 correlates with **lobular inflammation** and **steatosis** ($r = 0.555$, $p = 0.001$[a] & $r = 0.842$, $p < 0.001$[a])	
CD3	↓CD3 in **fibrosis** vs. no fibrosis ($p = 0.047$[a])	[46]
Cluster of differentiation 3	↓CD3 positive cells in **NAS ≥ 5** vs. NAS < 5 ($p = 0.003$[a])	
	CD3 correlates with **lobular inflammation** and **steatosis** ($r = -0.618$, $p < 0.001$[a] & $r = -0.702$, $p < 0.001$[a])	
CD163	↑CD163 in **fibrosis** vs. no fibrosis ($p = 0.019$[a])	[46]
Cluster of differentiation 163	CD163 correlates with **lobular inflammation** and **steatosis** ($r = 0.489$, $p = 0.003$[a] & $r = 0.755$, $p < 0.001$[a])	
Adiponectin	↓Adiponectin in **NASH** compared to NAFLD ($p < 0.01$[a])	[44]
	% of adiponectin positive HPCs/HPF correlates with **inflammation** ($r = -0.487$, $p < 0.01$[a])	
	% of adiponectin positive HPCs/HPF correlates with **NAS and steatosis** ($r = -0.792$, $p < 0.001$[a]; $r = -0.769$, $p < 0.001$[a])	
Resistin	↑Resistin in NAFLD with **fibrosis** vs. NAFLD without fibrosis ($p < 0.05$[a])	[44]
	↑Resistin in **NASH** compared to NAFLD ($p < 0.05$[a])	
GLP-1	↑GLP-1 in **NASH** compared to NAFLD ($p < 0.01$[a])	[44]
Glucagon-like peptide-1		
$P21^{waf1}$	↑$p21^{waf1}$ in **NASH** compared to NAFLD ($p < 0.05$[a])	[44]
M30	↑M30 in low **fibrosis stages** vs. higher fibrosis stages ($p = 0.02$[a])	[45]
Cytokeratin-18		

Abbreviations: NAS, NAFLD Activity Score.
↑, upregulation; ↓, downregulation.
[a] Univariable test/unadjusted comparison.
[b] Multivariable test/adjusted comparison.

bilirubin [33]. Three studies explored the prognostic value of novel markers – a complex fibrotic-inflammatory gene signature [21], CD34 [31], and miR-200b [24] – which were found to relate to mortality, need for LT, and risk of hepatic decompensation manifested by esophageal varices or to fibrosis grade, respectively. The first study [21] was designed to differentiate between patients having inflammatory or fibrotic liver features. In transplant free survival analysis, inflammatory gene signature proved favorable when compared to a fibrotic gene signature. However, lower age in the molecular inflammatory group could reflect diagnosis of the disease at an earlier stage, which potentially affects outcome. Among other markers, the second study [31] looked at CD34 a cell-surface glycoprotein expressed in vascular endothelium which might be crucial for interplay between angiogenesis, neovascularization, and fibrogenesis, and thereby serve as an indicator for response to hepatic injury in chronic liver disease [35]. Similarly, apelin, known for its function in angiogenesis, was found to be positively correlated with fibrotic stages [26], further confirming the role of angiogenesis in BA pathophysiology.

Inflammation and related markers were investigated in six BA studies. Four studies selected known inflammation-related factors, i.e. osteopontin [27], T-cell subsets [36], toll-like receptors (TLRs) [23], and COX-2 [37], to validate their prognostic value in BA. Osteopontin strongly correlated to fibrosis [27], while Th-17 related to clearance of jaundice [36]. The absence of clearance of jaundice was also related to high RRAS expression [22]. Both markers, Th-17 and RRAS, however, did not translate into improved native liver survival. The analyses of COX-2 and TLRs found that COX-2 was elevated in patients with severe liver dysfunction and need for LT [37], while TLR3 and TLR7 were elevated in patients needing LT [23], in concordance with the role of TLRs as biomarkers of innate immune response. Host immunological reactions may trigger progressive inflammatory biliary destruction manifesting as BA in infants. Hence, unregulated activation of immune response mechanisms would relate to the prognosis of patients.

BA is naturally related to lower liver performance. Upon investigation of **metabolic** markers in LBs from BA patients, survival analysis showed that low expressions of pregnane X receptor (PXR) and constitutive androstane receptor (CAR) were associated with significantly lower survival [25]. Similarly, a dysregulated Sonic Hedgehog (SHH) pathway (PTCH, Gli-2) proved to be associated with poor jaundice-free survival in a Cox regression analysis combined with Kaplan-Meier analysis [38].

In summary, increased expression of SHH, Gli-2, collagen-1, CK-7, α-SMA, CD34, and TLR3/7 or decreased expression of PXR and CAR may serve as markers of poor transplant-free survival in BA patients. However as depicted in Table 2 and Table 3_online, the temporal aspect of survival was only assessed by Kaplan Meier and/or Cox regression analysis for SHH, Gli-2, collagen-1, CK-7, PXR and CAR, and assessment of the accuracy of the method by AUROC curve analysis was only reported for CK-7. Furthermore, a fibrotic gene signature may predict lower transplant-free survival estimated by the Kaplan-Meier method. Increased Th-17 or RRAS expression negatively relates to clearance of jaundice at six months but should be interpreted cautiously due to the statistical methods applied.

Nevertheless, potential prognostic markers in BA require further clinical validation. Methodologically strongest evidence can be found on markers involved in ECM remodelling, fibrillar collagen deposition, ductular reactions and in tissue injury and repair.

3.2. Non-alcoholic fatty liver disease and non-alcoholic steatohepatitis

Childhood NAFLD is the most common cause of chronic PLD with a prevalence of 9.6–38% in the US [39]. Mediated by accumulating fatty

Table 4
Studies of prognostic molecular markers in viral hepatitis B/C.

Hepatitis-B/C marker	Finding	References
α-SMA α-smooth muscle actin	↑ α-SMA correlates with **fibrosis** in cHBV ($r = 0.518$, $p < 0.001$[a])	52
Casp-3a Caspase-3	↑Casp-3a relates to **fibrosis** in cHCV ($p = 0.03$[a]) Casp-3a correlates with number **of HCV positive** cells ($r = 0.83$, $p < 0.0001$[a])	54
HCV NS3 viral protein	↑NS3 relates to **fibrosis** in cHCV ($p = 0.03$[a])	54
TUNEL late apoptosis	↑apoptosis correlates with number of **HCV infected** hepatocytes ($r = 0.61$, $p = 0.0017$[a])	54
CK-7 Cytokeratin-7	↑CK-7 relates to **fibrosis** stage in cHCV ($p = 0.029$[a])	53

↑, upregulation.
[a] Univariable test/unadjusted comparison.

acids, mitochondrial dysfunction, increased oxidative stress, membrane lipid peroxidation, and pro-inflammatory cytokine production, NAFLD is described as the hepatic manifestation of metabolic syndrome [40]. NAFLD ranges from mild steatosis to accompanying ballooning and necrotic inflammation known as NASH, with progression to liver cirrhosis in some subjects [41,42].

Using IHC, four studies assessed the expression of markers associated with the **fibrosis** stage, activity, and disease progression in pediatric NAFLD and NASH applying simple hypothesis testing without survival analysis (Table 3 and Table 4_online). The SHH pathway represented by PTCH and Gli-2 was found to be activated in NAFLD and positively correlated with fibrosis, steatosis, and portal inflammation [43]. Similarly, CK-7 was found to be overexpressed in NAFLD biopsies displaying fibrosis compared to NAFLD biopsies without fibrosis, and in biopsies displaying NASH compared to non-NASH [44].

During NAFLD, progressive **cell death** with expansion of the progenitor cell compartment develops. Per se, the outcome is degenerative liver disease with cirrhotic transformation and a high risk of hepatocellular carcinoma. Hence, markers of apoptosis and cell cycle arrest may serve as potential prognostic indicators. Two studies assessed these cellular processes by testing activated and cleaved caspase-3, $p21^{waf}$, and cleaved CK-18 (M30) as markers [44,45]. One of the studies observed increased cytoplasmic levels of cleaved caspase-3 and nuclear accumulation of $p21^{waf}$ in the hepatic tissue of children with NASH compared to children with NAFLD [44]. However, in contrast to this and the findings of many adult studies, the other study found increased expression of activated caspase-3 in a low inflammatory state [45]. Moreover, increased M30 expression was found to be associated with low fibrosis stages [45], thus indicating possible markers of a better prognosis.

The **inflammatory** aspect of NAFLD was addressed using markers of inflammatory cells. CD45 and CD163 were increased while CD3 was decreased in fibrotic NAFLD patients [46]. As expected from the biological nature of these markers, CD45 and CD163 were positively correlated with inflammation and steatosis, whereas CD3 displayed a negative correlation. Association of increased vimentin with advanced fibrosis stages was also found [43]. This is in accordance with the finding of upregulated vimentin during the inflammatory process of NAFLD [47,48].

A role of the insulin-triglyceride-fatty-acid axis in **metabolic** syndrome and hepatic steatosis with insulin resistance is evident. Many other peptides and hormones including adipokines are believed to modulate important biological actions in the progression of NAFLD through and after the “first hit”. These actions involve effects on proliferation, inflammation, hepatocyte injury, and fibrogenesis. Leptin, adiponectin, and resistin are known effectors of this pathway. However, their role and prognostic value during the transition from simple steatosis to NASH and to potential cirrhosis are still not fully elucidated. Indeed, a protective role of an adipokine in one stage of hepatic fat accumulation can shift towards a negative influence in a later stage of the disease [49]. One study looked at adiponectin, GLP-1, and resistin to evaluate the insulin-triglyceride-fatty-acid axis. Decreased expression of adiponectin accompanied progression to NASH and correlated with NAFLD activity score, inflammation, and steatosis [44], illustrating an inverse relation of hepatic fat mass and levels of adiponectin [49]. Resistin and GLP-1 were related to advanced stages and fibrosis in NAFLD [44].

Morphogenic, apoptotic, and inflammatory pathways as well as the insulin-triglyceride-fatty-acid axis may serve as markers of disease progression and fibrosis in pediatric NAFLD. The effect on survival, however, has not yet been investigated. Hence, methodologically robust studies of prognostic tissue markers in pediatric NAFLD are lacking.

3.3. Viral hepatitis B and C

Viral hepatitis B and C (HBV/HCV) can cause decompensated PLD, cirrhosis, and possibly hepatocellular carcinoma [50]. In HBV, viral genotype, seroconversion to the immune inactive state, and more rarely resolution of disease are important prognostic events in terms of the risk of cirrhosis, but less is known about the risk of developing carcinoma [50]. Knowledge on prognostic factors for sustained virologic response, mortality, and decompensation in pediatric HCV is scarce at present, but sustained response to treatment has been shown to depend on HCV genotype [51].

All three identified studies on chronic HBV/HCV used IHC to detect and compare markers to **fibrosis** stage using simple hypothesis testing with no survival analysis (Table 4 and Table 5_online). α-SMA and CK-7 which identify stellate cell-induced fibrogenesis and progenitor cell expansion in chronic HBV and chronic HCV were both related to fibrosis stage [52,53]. In another study, apoptosis in childhood chronic HCV was evaluated by caspase-3a and TUNEL. Increased caspase-3a expression was found in higher stages of fibrosis, while both apoptotic markers correlated to number of infected hepatocytes [54].

In conclusion, markers of fibrogenesis and apoptosis may serve as markers of fibrosis stage in pediatric viral hepatitis. However, the effect on survival has yet to be investigated.

3.4. Pediatric liver transplantation

Liver transplantation constitutes the outcome of various childhood conditions affecting the liver and biliary tree. Pronounced improvements in patient and graft survival reaching a level of 90% have been seen in both the short- and long-term [55,56].

Only one identified study on pediatric LT fulfilled the inclusion criteria for this review. Miyagawa-Hayashino et al. explored the role of Fas-mediated **apoptosis** in pediatric liver allografts and showed FasL expression to be a marker of acute cellular rejection ($p = 0.0045$) [28]. Furthermore, an elevated ratio of FasL-positive Kupffer cells was found to help distinguish acute rejection from chronic rejection ($p < 0.01$). Increased apoptosis was observed in portal areas in acute cellular rejection compared to chronic rejection and stable grafts ($p < 0.001$).

3.5. Methodology in PLD molecular marker studies

In the field of diagnostics, the last decade can be defined as an era of transformation from single marker detection to complex molecular profiling by high-throughput technologies. Despite this advancement, most of identified studies were based on standard IHC or conventional PCR-based technologies (Table 1; Fig. 2). Moreover, rather than large screening of several potential biomarkers, single or only a few potential markers were selected and tested (Fig. 2). Among these potential markers, predominance was found for known markers of fibrogenesis,

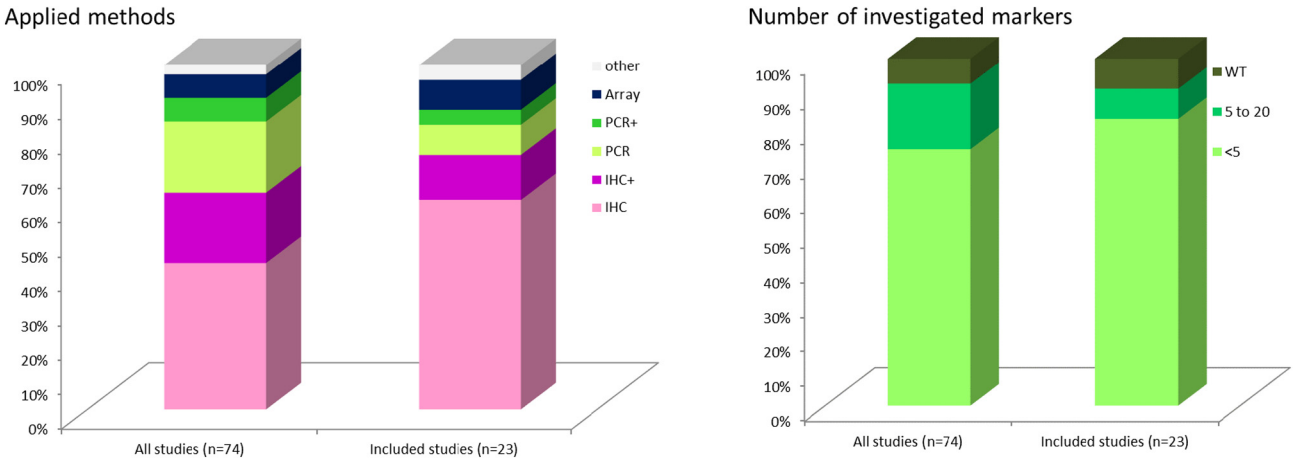

Fig. 2. Methodology in full-text screened studies.
Graphs showing percentage of studies applying methods ("Applied methods"), and percentage of studies investigating under 5 markers, 5 to 20 markers or providing whole transcriptome (WT) screening ("Number of investigated markers"). Legend: Array – array-based technology; PCR+ - polymerase chain reaction analysis combined with other analytical methods; IHC+ - immunohistochemistry combined with other analytical methods; WT – whole transcriptome investigation.

inflammation, and apoptosis. Only two studies applied transcriptomic profiling and succeeded in defining potential prognostic markers [21,22]. Interestingly, three more studies applied array-based transcriptomic profiling in PLD [57–59]. However, one of these studies failed to prove the prognostic power of potential markers [57], while another did not separate adult and pediatric patients in the analysis [58]. The remaining study attempted to define expressional signatures for fibrogenic and inflammatory types of BA, but proved inconclusive for potential clinical application at this stage [59].

4. Discussion

This systematic review summarizes the prognostic value of tissue-specific genetic and molecular markers in PLD across various diagnoses. Presently, there is a small number of reviews describing the roles of various markers in PLD (e.g. [10]). However, for the first time, this review systematically addresses the prognostic role of biologically relevant markers in liver tissue. An ideal prognostic marker in PLD would be non-invasive, sensitive and specific, reproducible among laboratories, easily measured, and inexpensive [60,61]. However, to identify a non-invasive or minimally invasive biomarker, knowledge about the marker's biology is of crucial importance. Therefore, a marker originating in the diseased organ undoubtedly has the highest potential to possess prognostic value, which could then hopefully be extrapolated to non-invasive diagnostics.

4.1. Study design

Despite the number of studies on PLD, only a small number provides a clinically considerable prognostic marker. Most of the studies are retrospective, small size, single center studies with a low level of evidence combined with a high risk of bias (e.g. lacking information on recruitment and patient selection). Furthermore, the rare occurrence of PLD and apparent limitations in establishing suitable control groups represent additional obstacles. Additionally, a small sample size limits the ability to prove significant associations, as illustrated by, for example, the apelin study [26].

BA represents an ideal condition for studying organ-specific prognostic markers during PLD, and repeated liver tissue sampling is often performed. In this review, BA was the most frequently studied condition. The natural history of BA with extensive and rapid liver damage poses a clinically significant challenge in terms of predicting patients needing early listing for LT and ones with a favorable prognosis. Nonetheless, only one study was designed to follow a presumptive marker in repeated biopsies from the same patients at hepatoportoenterostomy and LT. However, the study was inconclusive in terms of intra-individual gene expression during disease progression, likely due to the limited number of patients [26].

A possible prognostic biomarker, as opposed to a predictive biomarker, can be identified through relatively small retrospective studies. However, validation and translation into a clinically valid prognostic marker is much more complicated. Validation of a surrogate endpoint is even more complex as it also demands evidence for a correlation with the outcome and evidence that treatment effect on the surrogate correlates with effect on the outcome. The evidence should preferably be established through randomized trials or meta-analysis, though large trials can sometimes be accepted [62]. None of these criteria are met in the selected studies, which are all in the early pre-clinical phase of identification. Moreover, several studies used surrogate endpoints, further complicating the validation of identified markers.

Native liver survival, jaundice-free survival, transplant-free survival, overall survival, and LT were assessed as prognostic endpoints in a total of seven studies. In five of these studies Kaplan-Meier analysis was used [21,25,30,32,38], with only one study applying additional multivariable Cox regression analysis [38], whereas two studies used multivariable logistic regression analysis [21,32]. Most of studies testing the surrogate endpoints as fibrosis and/or inflammation used correlation analysis or simple comparisons of two or more independent samples, often without adjusting for multiple comparisons. This approach, however, ignores the temporal aspect, a key feature of prognostication. Survival statistics using Kaplan-Meier analysis and Cox regression incorporate progression over time and censor for dropout, and these methods along with assessment of the accuracy of the applied method using AUROC curve are important for evaluation of disease outcome. A lack of temporal assessment, adjustment for possible confounders and information on e.g. sensitivity and specificity as well as a small population size are limiting factors of most of the included studies. Nevertheless, even though most of the PLD studies fail to provide substantial prognostic evidence, many of them could serve as a basis for generating hypotheses for further research.

4.2. Marker selection

Every biological marker reflects a process accompanying disease. In PLD, markers relate to fibrosis, inflammation, cell cycle arrest and apoptosis, metabolism, epithelial to mesenchymal transition (EMT), and angiogenesis (Fig. 3). Despite etiological differences, some of these processes may be general markers of liver damage [63] and thereby

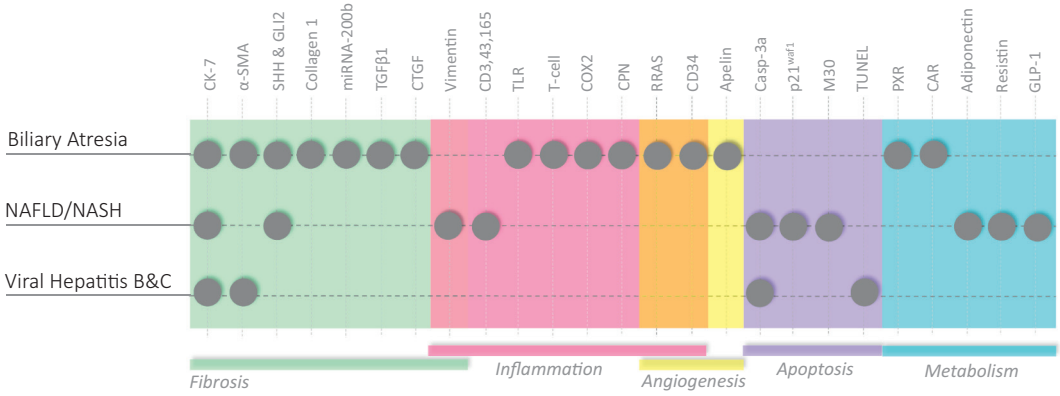

Fig. 3. Overview of identified PLD markers with prognostic values.

might serve as a good starting point for prognostic marker detection in PLD. In addition, defining the exact etiology of PLD can be challenging [3]. Hence, prognostic markers do not necessarily need to be disease specific, and indeed, markers are demanded across the entire spectrum of PLD diagnoses.

Activation of the hepatic stellate cell (HSC) and portal fibroblast (PF) compartment with myofibroblast formation is one of the most essential events in hepatic fibrogenesis [64–67]. The list of possible prognostic markers from this pathway is extensive, including, for example, α-SMA, fibronectin, vimentin, TGFs, HIF, and interleukins. The role of EMT and ductular reactive epithelial cells as additional myofibroblast precursors in liver fibrogenesis has also been widely debated [64,68]. Pediatric studies have evaluated the relation between EMT, Hedgehog signaling, and fibrosis, and demonstrated that Hedgehog signaling may possess a valuable prognostic potential [69].

Due to a crucial role in hepatic fibrogenesis, myofibroblast activation may also represent a tool for assessment of fibrosis formation and chronic liver impairment. However, manifested fibrosis is regarded as a relatively late event in liver decompensation. Hence, processes occurring prior to fibrosis (e.g. the origin of HSCs and PFs and factors involved in their regulation during liver injury) would be of much greater clinical use as both prognostic indicators and a focus for antifibrotic treatment [69–72]. Activation of morphogenic and organogenic pathways during myofibroblastic activation is therefore an area of extensive biological investigations. Among these, the SHH pathway is likely to play an important role in the onset of fibrosis and its activation relates to poor jaundice-free survival in BA [38]. Similarly, growth factor-derived activation and proliferation of HSCs and PFs is a central part of the fibrotic process in liver decompensation. Furthermore, collagen and α-SMA continue to be of interest as they have proved to have prognostic implications for LT and native liver survival [30,31,33].

From morphogenic processes, angiogenesis and neovascularization are closely correlated to progression of liver fibrosis. Pericytes from proliferating capillaries accumulate in injured hepatic tissue and actively contribute to extracellular matrix formation [73]. Consequently, anti-angiogenic drugs have become an area of interest in treating hepatic fibrosis. In PLD, angiogenic markers are related to LT and native liver survival, although the statistical strength of a possible prognostic role remains questionable.

Xenobiotic metabolizing properties are key features of hepatic function. Therefore, they offer comprehensive pathway analysis, but appear to be less well investigated in terms of prognosis. In cholestasis, failure to clear toxic biliary derivatives leads to progressive liver injury. PXR and CAR are receptors possessing bile acid-detoxifying properties [74,75] and both demonstrate prognostic properties in the neonatal cholestasis of BA [25].

Cellular immunity is known to play a pivotal role in liver graft rejection, but a mediatory role of humoral immunity likely contributes to the process [76,77]. Early and late apoptotic markers may possess prognostic implications in pediatric LT and viral hepatitis [78,79]; however, further clarification is needed.

4.3. Marker discovery

The rapid advances in high-throughput gene technologies present new and interesting perspectives for prognostication. Though single markers may prove to serve prognostically, the complexity of many diseases, including PLD, implies that gene signatures would potentially predict the course of a given disease more accurately. Two studies applied expression arrays to investigate the expressional changes accompanying fibrosis progression in BA [21] or specific for BA [22]. Moyer et al. distinguished between a fibrotic and an inflammatory gene signature and found that a fibrotic gene signature associates with overexpression of genes tightly connected to extracellular matrix formation. BA patients with this profile were found to have lower transplant-free survival compared to patients showing enrichment of binding sites for transcription factors of the immune response [21]. A typical explorative study was performed by Zhao et al. As a result, a new prognostic marker was identified, RRAS, which correlated to clearance of jaundice and occurrence of cholangitis. However, the number of samples studied was rather limited and there was no subsequent appropriate clinical validation [22]. Despite these limitations, findings of this type provide not only new prognostic perspectives but also potentially new knowledge on BA and other PLD pathophysiology.

5. Conclusions

Numerical measures and an overall summary of the results were not possible due to the designs of the studies, differences in outcome measures and the quality of prognostic assessment. Hence, the results of these studies do not translate directly into clinical practice. Furthermore, based on this review, changes to the clinical guidelines or prognostic workup of individual pediatric patients are not recommended. However, several markers and pathways show a great potential for further investigation and perhaps even future clinical application. Markers of manifest fibrogenic processes and ECM remodelling should be further explored in terms of prognosis and seem promising, however pathways of morphogenesis, angiogenesis, metabolic state and inflammation possibly reflect early prognostic markers and need additional focus. Large scale transcriptomic screening can increase our knowledge on critical steps in disease progression thus opening new avenues of research on the prognostic assessment of PLD.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbadis.2018.12.018.

Funding

We thank the research fund of Copenhagen University Hospital,

Rigshospitalet for financial support of the project.

Transparency document

The Transparency document associated with this article can be found, in online version.

References

[1] W. Kim, R.S. Brown, N.A. Terrault, H. El-Serag, Burden of liver disease in the United States: summary of a workshop, Hepatology 36 (1) (2002 Jul) 227–242.
[2] G. Arya, W.F. Balistreri, Pediatric liver disease in the United States: epidemiology and impact, J. Gastroenterol. Hepatol. 17 (5) (2002 May) 521–525.
[3] R.H. Squires, B.L. Shneider, J. Bucuvalas, E. Alonso, R.J. Sokol, M.R. Narkewicz, et al., Acute liver failure in children: the first 348 patients in the pediatric acute liver failure study group, J. Pediatr. 148 (5) (2006 May) 652–658.
[4] V. Hernandez-Gea, S.L. Friedman, Pathogenesis of liver fibrosis, Annu Rev Pathol Mech Dis. 6 (1) (2011 Feb 28) 425–456.
[5] V. Sundaram, B.L. Shneider, A. Dhawan, V.L. Ng, K. Im, S. Belle, et al., King's College Hospital Criteria for non-acetaminophen induced acute liver failure in an international cohort of children, J Pediatr. 162 (2) (Feb 2013) 319–323.e1 Mosby, Inc..
[6] B.R. Lu, S. Zhang, M.R. Narkewicz, S.H. Belle, R.H. Squires, R.J. Sokol, et al., Evaluation of the liver injury unit scoring system to predict survival in a multinational study of pediatric acute liver failure, J Pediatr. 162 (5) (May 2013) 1010–1016.e4.
[7] M.E.M. Tessier, S. Harpavat, R.W. Shepherd, G.S. Hiremath, M.L. Brandt, A. Fisher, et al., Beyond the pediatric end-stage liver disease system: solutions for infants with biliary atresia requiring liver transplant, World J. Gastroenterol. 20 (32) (Aug 28 2014) 11062.
[8] R.H. Squires, V. Ng, R. Romero, U. Ekong, W. Hardikar, S. Emre, et al., Evaluation of the pediatric patient for liver transplantation: 2014 practice guideline by the American association for the study of liver diseases, American society of transplantation and the North American society for pediatric gastroenterology, hepatology and nutrition, Hepatology. 60 (1) (Jul 2014) 362–398.
[9] M.R. McGill, V.S. Staggs, M.R. Sharpe, W.M. Lee, H. Jaeschke, Acute liver failure study group. Serum mitochondrial biomarkers and damage-associated molecular patterns are higher in acetaminophen overdose patients with poor outcome, Hepatology 60 (4) (Oct 2014) 1336–1345.
[10] D. Calvopina, M. Coleman, P. Lewindon, G. Ramm, Function and regulation of MicroRNAs and their potential as biomarkers in paediatric liver disease, Int. J. Mol. Sci. 17 (12) (2016 Oct 27) 1795.
[11] A. Dezsőfi, U. Baumann, A. Dhawan, O. Durmaz, B. Fischler, N. Hadzic, et al., Liver biopsy in children: position paper of the ESPGHAN hepatology committee, J. Pediatr. Gastroenterol. Nutr. 60 (3) (2015 Mar) 408–420.
[12] J. Gödeke, E. Luxenburger, F. Trippel, K. Becker, B. Häberle, J. Müller-Höcker, et al., Low expression of N-myc downstream-regulated gene 2 (NDRG2) correlates with poor prognosis in hepatoblastoma, Hepatol. Int. 10 (2) (2016 Mar 8) 370–376.
[13] H.-H. Chua, D.-J. Tsuei, P.-H. Lee, Y.-M. Jeng, J. Lu, J.-F. Wu, et al., RBMY, a novel inhibitor of glycogen synthase kinase 3?, increases tumor stemness and predicts poor prognosis of hepatocellular carcinoma, Hepatology 62 (5) (2015 Nov) 1480–1496.
[14] P. Czauderna, D. Lopez-Terrada, E. Hiyama, B. Häberle, M.H. Malogolowkin, R.L. Meyers, Hepatoblastoma state of the art: pathology, genetics, risk stratification, and chemotherapy, Curr. Opin. Pediatr. 26 (1) (2014 Feb) 19–28.
[15] J.P.T. Higgins, GS (Eds.), Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0, 2011 updated March 2011.
[16] R.N. Lopez, C.Y. Ooi, U. Krishnan, Early and peri-operative prognostic indicators in infants undergoing hepatic portoenterostomy for biliary atresia: a review, Curr. Gastroenterol. Rep. 19 (4) (Apr 3 2017) 16.
[17] M. Pokorska-Śpiewak, B. Kowalik-Mikołajewska, M. Aniszewska, M. Pluta, M. Marczyńska, Is liver biopsy still needed in children with chronic viral hepatitis? World J. Gastroenterol. 21 (42) (Nov 14 2015) 12141–12149.
[18] M. Ekstedt, H. Hagström, P. Nasr, M. Fredrikson, P. Stål, S. Kechagias, et al., Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up, Hepatology 61 (5) (2015 May) 1547–1554.
[19] D. Moher, A. Liberati, J. Tetzlaff, D.G. Altman, PRISMA group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement, Ann. Intern. Med. 151 (4) (Aug 18 2009) 264–269 (W64).
[20] Oxford Centre for Evidence-based Medicine - Levels of Evidence, CEBM, March 2009 [Internet]. [Cited 2017 Nov 7]. Available from: http://www.cebm.net/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/.
[21] K. Moyer, V. Kaimal, C. Pacheco, R. Mourya, H. Xu, P. Shivakumar, et al., Staging of biliary atresia at diagnosis by molecular profiling of the liver, Genome Med. 2 (5) (May 13 2010) 33.
[22] R. Zhao, H. Li, C. Shen, S. Zheng, RRAS: a key regulator and an important prognostic biomarker in biliary atresia, World J. Gastroenterol. 17 (6) (Feb 2011) 796–803.
[23] T. Saito, T. Hishiki, K. Terui, T. Mitsunaga, E. Terui, M. Nakata, et al., Toll-like receptor mRNA expression in liver tissue from patients with biliary atresia, J. Pediatr. Gastroenterol. Nutr. 53 (6) (Dec 2011) 620–626.
[24] Y. Xiao, J. Wang, Y. Chen, K. Zhou, J. Wen, Y. Wang, et al., Up-regulation of miR-200b in biliary atresia patients accelerates proliferation and migration of hepatic stallate cells by activating PI3K/Akt signaling, Cell. Signal. 26 (5) (May 2014) 925–932.
[25] H.-L. Chen, Y.-J. Liu, H.-L. Chen, S.-H. Wu, Y.-H. Ni, M.-C. Ho, et al., Expression of hepatocyte transporters and nuclear receptors in children with early and late-stage biliary atresia, Pediatr. Res. 63 (6) (Jun 2008) 667–673.
[26] W. Chen, T. Oue, T. Ueno, S. Uehara, N. Usui, M. Fukuzawa, Apelin is a marker of the progression of liver fibrosis and portal hypertension in patients with biliary atresia, Pediatr. Surg. Int. 29 (1) (Jan 2013) 79–85.
[27] L. Huang, M.-F. Wei, J.-X. Feng, Abnormal activation of OPN inflammation pathway in livers of children with biliary atresia and relationship to hepatic fibrosis, Eur. J. Pediatr. Surg. 18 (4) (Aug 2008) 224–229 ([et al] = Zeitschrift fur Kinderchirurgie. United States).
[28] A. Miyagawa-Hayashino, T. Tsuruyama, H. Egawa, H. Haga, H. Sakashita, T. Okuno, et al., FasL expression in hepatic antigen-presenting cells and phagocytosis of apoptotic T cells by FasL+ Kupffer cells are indicators of rejection activity in human liver allografts, Am. J. Pathol. 171 (5) (Nov 2007) 1499–1508.
[29] P.-Y. Hung, C.-C. Chen, W.-J. Chen, H.-S. Lai, W.-M. Hsu, P.-H. Lee, et al., Long-term prognosis of patients with biliary atresia: a 25 year summary, J. Pediatr. Gastroenterol. Nutr. 42 (2) (Feb 2006) 190–195.
[30] L.R. Longo-Santos, W.R. Teodoro, E.S. de Mello, A.P.P. Velosa, E.R. Parra, V.L. Capelozzi, et al., Early type I collagen deposition is associated with prognosis in biliary atresia, J. Pediatr. Surg. 51 (3) (Mar 2016) 379–385.
[31] J.S. Suominen, H. Lampela, P. Heikkila, J. Lohi, H. Jalanko, M.P. Pakarinen, Myofibroblastic cell activation and neovascularization predict native liver survival and development of esophageal varices in biliary atresia, World J. Gastroenterol. 20 (12) (Mar 2014) 3312–3319.
[32] J.L. Santos, C.O. Kieling, L. Meurer, S. Vieira, C.T. Ferreira, A. Lorentz, et al., The extent of biliary proliferation in liver biopsies from patients with biliary atresia at portoenterostomy is associated with the postoperative prognosis, J. Pediatr. Surg. 44 (4) (Apr 2009) 695–701.
[33] R. Dong, Y. Luo, S. Zheng, Alpha-SMA overexpression associated with increased liver fibrosis in infants with biliary atresia, J. Pediatr. Gastroenterol. Nutr. 55 (6) (Dec 2012) 653–656.
[34] F.B. Li, H. Zhao, K.R. Peng, Z.G. Gao, S.J. Huang, J.F. Tou, et al., Expression of transforming growth factor-beta1 and connective tissue growth factor in congenital biliary atresia and neonatal hepatitis liver tissue, Genet. Mol. Res. 15 (1) (Feb 2016).
[35] A.D. Amarapurkar, D.N. Amarapurkar, S. Vibhav, N.D. Patel, Angiogenesis in chronic liver disease, Ann. Hepatol. 6 (3) (2007) 170–173.
[36] R. Hill, A. Quaglia, M. Hussain, N. Hadzic, G. Mieli-Vergani, D. Vergani, et al., Th-17 cells infiltrate the liver in human biliary atresia and are related to surgical outcome, J. Pediatr. Surg. 50 (8) (Aug 2015) 1297–1303.
[37] S. Honsawek, N. Klaikeaw, P. Vejchapipat, V. Chongsrisawat, P. Ruangvejvorachai, Y. Poovorawan, Cyclooxygenase-2 overexpression is associated with clinical outcome in biliary atresia, Eur. J. Pediatr. Surg. 20 (3) (May 2010) 164–168.
[38] H.Y. Jung, J. Jing, K.B. Lee, J.-J. Jang, Sonic hedgehog (SHH) and glioblastoma-2 (Gli-2) expressions are associated with poor jaundice-free survival in biliary atresia, J. Pediatr. Surg. 50 (3) (Mar 2015) 371–376.
[39] J.B. Schwimmer, R. Deutsch, T. Kahen, J.E. Lavine, C. Stanley, C. Behling, Prevalence of fatty liver in children and adolescents, Pediatrics 118 (4) (Oct 1 2006) 1388–1393.
[40] M. Malaguarnera, M. Di Rosa, F. Nicoletti, L. Malaguarnera, Molecular mechanisms involved in NAFLD progression, J. Mol. Med. 87 (7) (Jul 8 2009) 679–695.
[41] P. Angulo, Nonalcoholic fatty liver disease, N. Engl. J. Med. 346 (16) (Apr 18 2002) 1221–1231.
[42] M. Krawczyk, L. Bonfrate, P. Portincasa, Nonalcoholic fatty liver disease, Best Pract. Res. Clin. Gastroenterol. 24 (5) (Oct 2010) 695–708.
[43] M. Swiderska-Syn, A. Suzuki, C.D. Guy, J.B. Schwimmer, M.F. Abdelmalek, J.E. Lavine, et al., Hedgehog pathway and pediatric nonalcoholic fatty liver disease, Hepatology 57 (5) (May 2013) 1814–1825.
[44] V. Nobili, G. Carpino, A. Alisi, A. Franchitto, G. Alpini, R. De Vito, et al., Hepatic progenitor cells activation, fibrosis, and adipokines production in pediatric nonalcoholic fatty liver disease, Hepatology 56 (6) (Dec 2012) 2142–2153.
[45] P. Valva, E. De Matteo, M. Galoppo, A. Pedreira, G. Giacove, C. Lezama, et al., Apoptosis markers in liver biopsy of nonalcoholic steatohepatitis in pediatric patients, Hum. Pathol. 39 (12) (Dec 2008) 1816–1822.
[46] R. De Vito, A. Alisi, A. Masotti, S. Ceccarelli, N. Panera, A. Citti, et al., Markers of activated inflammatory cells correlate with severity of liver damage in children with nonalcoholic fatty liver disease, Int. J. Mol. Med. 30 (1) (Jul 2012) 49–56.
[47] S.J. Lee, Yoo J. Do, S.Y. Choi, O.-S. Kwon, The expression and secretion of vimentin in the progression of non-alcoholic steatohepatitis, BMB Rep. 47 (8) (Aug 2014) 457–462.
[48] N. Mor-Vaknin, A. Punturieri, K. Sitwala, D.M. Markovitz, Vimentin is secreted by activated macrophages, Nat. Cell Biol. 5 (1) (Jan 16 2003) 59–63.
[49] S.A. Polyzos, J. Kountouras, C.S. Mantzoros, Adipokines in nonalcoholic fatty liver disease, Metabolism 65 (8) (Aug 2016) 1062–1079.
[50] E.M. Sokal, M. Paganelli, S. Wirth, P. Socha, P. Vajro, F. Lacaille, et al., Management of chronic hepatitis B in childhood: ESPGHAN clinical practice guidelines, J. Hepatol. 59 (4) (Oct 2013) 814–829.
[51] E.M. Sokal, A. Bourgois, X. Stéphenne, T. Silveira, G. Porta, D. Gardovska, et al., Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection in children and adolescents, J. Hepatol. 52 (6) (Jun 2010) 827–831.
[52] J.M. Lotowska, D.M. Lebensztejn, Immunoreactive hepatic stellate cells in biopsy material in children with chronic hepatitis B. The first report in pediatric patients, Pol. J. Pathol. 66 (3) (Sep 2015) 224–230.

[53] H.A. El-Araby, N.A. Ehsan, H.A. Konsowa, B.M. Abd-Elaati, A.M. Sira, Hepatic progenitor cells in children with chronic hepatitis C: correlation with histopathology, viremia, and treatment response, Eur. J. Gastroenterol. Hepatol. 27 (5) (May 2015) 561–569.
[54] P. Valva, E. De Matteo, M.C. Galoppo, M.I. Gismondi, M.V. Preciado, Apoptosis markers related to pathogenesis of pediatric chronic hepatitis C virus infection: M30 mirrors the severity of steatosis, J. Med. Virol. 82 (6) (May 2010) 949–957.
[55] J.C. Magee, S.M. Krishnan, M.R. Benfield, D.T. Hsu, B.L. Shneider, Pediatric transplantation in the United States, 1997–2006, Am J Transplant. 8 (4p2) (Apr 2008) 935–945.
[56] K.A. Soltys, G.V. Mazariegos, R.H. Squires, R.K. Sindhi, R. Anand, SPLIT research group. Late graft loss or death in pediatric liver transplantation: an analysis of the SPLIT database, Am. J. Transplant. 7 (9) (Sep 2007) 2165–2171.
[57] T.N. Pereira, P.J. Lewindon, R.M. Greer, A.C. Hoskins, R.M. Williamson, R.W. Shepherd, et al., Transcriptional basis for hepatic fibrosis in cystic fibrosis-associated liver disease, J. Pediatr. Gastroenterol. Nutr. 54 (3) (Mar 2012) 328–335.
[58] S. Salehi, H.C. Brereton, M.J. Arno, D. Darling, A. Quaglia, J. O'Grady, et al., Human liver regeneration is characterized by the coordinated expression of distinct microRNA governing cell cycle fate, Am. J. Transplant. 13 (5) (May 2013) 1282–1295.
[59] T. Iordanskaia, M.J. Hubal, E. Koeck, C. Rossi, K. Schwarz, E.P. Nadler, Dysregulation of upstream and downstream transforming growth factor-beta transcripts in livers of children with biliary atresia and fibrogenic gene signatures, J. Pediatr. Surg. 48 (10) (Oct 2013) 2047–2053.
[60] Biomarkers Definitions Working Group, Biomarkers and surrogate endpoints: preferred definitions and conceptual framework, Clin. Pharmacol. Ther. 69 (3) (Mar 2001) 89–95.
[61] P.C. Ng, T.P.Y. Ma, H.S. Lam, The use of laboratory biomarkers for surveillance, diagnosis and prediction of clinical outcomes in neonatal sepsis and necrotising enterocolitis, Arch. Dis. Child. Fetal Neonatal Ed. 100 (5) (Sep 2015) F448–F452.
[62] M. Buyse, D.J. Sargent, A. Grothey, A. Matheson, A. de Gramont, Biomarkers and surrogate end points—the challenge of statistical validation, Nat. Rev. Clin. Oncol. 7 (6) (Jun 6 2010) 309–317.
[63] W.M.C. Rosenberg, M. Voelker, R. Thiel, M. Becka, A. Burt, D. Schuppan, et al., Serum markers detect the presence of liver fibrosis: a cohort study, Gastroenterology 127 (6) (Dec 2004) 1704–1713.
[64] J. Xu, X. Liu, Y. Koyama, P. Wang, T. Lan, I.-G. Kim, et al., The types of hepatic myofibroblasts contributing to liver fibrosis of different etiologies, Front. Pharmacol. 5 (Jul 22 2014) 167.
[65] J.M. de C. Maia, H. de S. Maranhao, L.V. de Sena, L.R.M. da Rocha, I.A. de Medeiros, A.M. de O. Ramos, Hepatic stellate cell activation and hepatic fibrosis in children with type 1 autoimmune hepatitis: an immunohistochemical study of paired liver biopsies before treatment and after clinical remission, Eur. J. Gastroenterol. Hepatol. 22 (3) (Mar 2010) 264–269.
[66] G.A. Ramm, A.C. Hoskins, S.A. Greco, T.N. Pereira, P.J. Lewindon, Signals for hepatic figrogenesis in pediatric cholestatic liver disease: review and hypothesis, Comp Hepatol. 3 (Suppl. 1) (Jan 14 2004) S5.
[67] B.L. Copple, S. Bai, L.D. Burgoon, J.-O. Moon, Hypoxia-inducible factor-1α regulates the expression of genes in hypoxic hepatic stellate cells important for collagen deposition and angiogenesis, Liver Int. 31 (2) (2011 Feb) 230–244.
[68] R. Diaz, J.W. Kim, J.-J. Hui, Z. Li, G.P. Swain, K.S.K. Fong, et al., Evidence for the epithelial to mesenchymal transition in biliary atresia fibrosis, Hum. Pathol. 39 (1) (Jan 2008) 102–115.
[69] A. Omenetti, L.M. Bass, R.A. Anders, M.G. Clemente, H. Francis, C.D. Guy, et al., Hedgehog activity, epithelial-mesenchymal transitions, and biliary dysmorphogenesis in biliary atresia, Hepatology 53 (4) (Apr 2011) 1246–1258.
[70] J. Mann, D.C.K. Chu, A. Maxwell, F. Oakley, N. Zhu, H. Tsukamoto, et al., MeCP2 controls an epigenetic pathway that promotes myofibroblast transdifferentiation and fibrosis, Gastroenterology 138 (2) (Feb 2010) 705–714.e4.
[71] Y. Sekiya, T. Ogawa, K. Yoshizato, K. Ikeda, N. Kawada, Suppression of hepatic stellate cell activation by microRNA-29b, Biochem. Biophys. Res. Commun. 412 (1) (Aug 19 2011) 74–79.
[72] A. Omenetti, A.M. Diehl, The adventures of sonic hedgehog in development and repair. II. Sonic hedgehog and liver development, inflammation, and cancer, Am. J. Physiol. Gastrointest. Liver Physiol. 294 (3) (Mar 2008) G595–G598.
[73] Q.T. Lemos, Z.A. Andrade, Angiogenesis and experimental hepatic fibrosis, Mem. Inst. Oswaldo Cruz 105 (5) (Aug 2010) 611–614.
[74] M. Wagner, E. Halilbasic, H.-U. Marschall, G. Zollner, P. Fickert, C. Langner, et al., CAR and PXR agonists stimulate hepatic bile acid and bilirubin detoxification and elimination pathways in mice, Hepatology 42 (2) (Aug 2005) 420–430.
[75] E. Halilbasic, A. Baghdasaryan, M. Trauner, Nuclear receptors as drug targets in cholestatic liver diseases, Clin. Liver Dis. 17 (2) (May 2013) 161–189 Elsevier.
[76] H. Yamada, H. Kondou, T. Kimura, K. Ikeda, M. Tachibana, Y. Hasegawa, et al., Humoral immunity is involved in the development of pericentral fibrosis after pediatric live donor liver transplantation, Pediatr. Transplant. 16 (8) (Dec 2012) 858–865.
[77] S. Varma, J. Ambroise, M. Komuta, D. Latinne, P. Baldin, R. Reding, et al., Progressive fibrosis is driven by genetic predisposition, Allo-immunity, and inflammation in pediatric liver transplant recipients, EBioMedicine 9 (Jul 2016) 346–355.
[78] X. Zhao, Y. Li, H. Ohe, H. Nafady-Hego, S. Uemoto, G.A. Bishop, et al., Intragraft Vdelta1 gammadelta T cells with a unique T-cell receptor are closely associated with pediatric semiallogeneic liver transplant tolerance, Transplantation 95 (1) (Jan 2013) 192–202.
[79] Y. Li, X. Zhao, D. Cheng, H. Haga, T. Tsuruyama, K. Wood, et al., The presence of Foxp3 expressing T cells within grafts of tolerant human liver transplant recipients, Transplantation 86 (12) (Dec 2008) 1837–1843.