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Review paper

Update on pathogenesis, diagnostics and therapy of nonalcoholic fatty liver disease in children

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Abstract

Nonalcoholic fatty liver disease (NAFLD) represents the most common cause of chronic liver disease. Increasing prevalence of NAFLD in children may be the cause of unfavorable metabolic implications and development of end stage liver disease. NAFLD is a "multiple-hit" disease mediated by several metabolic, environmental, genetic and microbiological mechanisms. Additionally, lipotoxicity, oxidative stress and inflammation predispose to progressive liver damage. According to current guidelines, liver biopsy is an imperfect gold standard for NAFLD diagnosis, but due to its invasive character its use is limited in children and it should be performed only in children who need exclusion of coexisting diseases. Noninvasive methods should be preferred and current research is focused on serum markers and novel imaging or elastographic techniques. Therapeutic approaches for NAFLD are currently focused on lifestyle modification, insulin resistance, dyslipidemia, oxidative stress and the gut microbiome. However, a number of clinical studies on novel therapeutic molecules are ongoing.

Key words: children, inflammation, steatosis, liver fibrosis, nonalcoholic fatty liver disease.

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Introduction

Nonalcoholic fatty liver disease (NAFLD) represents the most common cause of chronic liver disease both in adults and children. It is regarded as a spectrum of hepatic conditions, which ranges from simple steatosis through nonalcoholic steatohepatitis (NASH), with or without fibrosis, to cirrhosis and end stage liver disease [1]. According to the histologic definition, steatohepatitis requires at least 5% of liver cells containing mainly macrovesicular fatty infiltration, with simultaneously detected hepatocyte ballooning and intralobular inflammation. This clinical condition is closely associated with visceral obesity and other features of the metabolic syndrome (MS) including insulin resistance, dyslipidemia and increased cardiovascular risk [2]. Therefore, NAFLD is considered as a hepatic manifestation of MS. The worldwide increasing prevalence of NAFLD in children is a worrying phenomenon, because it may be a cause of unfavorable metabolic implications and development of end stage liver disease with the consequent need for liver transplantation in adulthood [3].

Pathogenesis

The pathogenesis of NAFLD has not been fully understood. Nowadays it is generally accepted that NAFLD is pathogenically a "multiple-hit" disease. According to this hypothesis, NAFLD is a complex disease mediated by several metabolic, environmental, genetic and microbiological mechanisms. The main role in the development of NAFLD is played by an elevated level of circulating free fatty acids (FFA) in conjunction with insulin resistance (IR) causing excessive accumulation of triglycerides in hepatocytes. Additionally it is responsible for lipotoxicity, oxidative stress and an inflammatory response, predisposing to progressive liver damage [4, 5]. Recent studies have revealed that multiple mechanisms, acting synergistically in genetically predisposed individuals, are involved in the development and progression of NAFLD [6].

Genetic factors

Some single nucleotide polymorphisms (SNPs) are identified to be associated with pediatric NAFLD through their impact on metabolic dysfunctions. Notably, the SNPs PNPLA3 rs738409, GCKR rs1260326, TM6SF2 rs58542926, as well as MBOAT7 rs626283 and rs641738, might favor progression of liver damage both in adults and children. It has been reported that genetic variants are involved in hepatic fat deposition, lipogenesis, and progression of NAFLD towards NASH through promoting inflammation, activation of stellate cells and fibrogenesis (Table 1) [7-9].

The majority of existing data supporting genetic associations with pediatric NAFLD are for the PNPLA3 polymorphism. PNPLA3 belongs to the patatin-like phospholipase domain-containing family of proteins and it encodes the insulin-regulated phospholipase adiponutrin, which is involved in lipid metabolism. Data obtained from pediatric studies showed an association of PNPLA3 rs738409 polymorphism with elevated alanine aminotransferase (ALT) and more frequent occurrence in obese subjects of all ages with metabolic syndrome [10]. Moreover, Santoro *et al*. [11] evaluated a multiethnic group of 85 children with obesity and found a positive association of hepatic fat content by magnetic resonance imaging with presence of at least 1 G allele in Caucasian and African American, but not in Hispanic children. A second gene related to pediatric NAFLD is TM6SF2. It has been proven that the variant form of the protein is misfolded, causing accelerated degradation, leading to increased intrahepatic fat accumulation and decreased secretion of very-low-density lipoprotein (VLDL) from the hepatocyte [12]. A study performed by Grandone *et al*. [13] conducted in Italian children with obesity demonstrated an association of carrying the TM6SF2 E167K variant allele with ultrasound feature of liver steatosis, higher ALT levels and lower total cholesterol, low-density lipoprotein cholesterol, triglycerides and non-high-density lipoprotein cholesterol levels, which could be a possible effect of reduced VLDL secretion.

Nobili *et al*. [14] created a genetic risk score useful to predict NASH, based on identification of PNPLA3 rs738409, SOD2 rs4880, KLF6 rs3750861 and LPIN1 rs13412852 polymorphisms in a population of obese children and adolescents with elevated liver enzymes.

Moreover, several studies have demonstrated that NAFLD is associated with deregulation of many hepatic micro-RNAs (miRNA), which are short (19-23 nucleotides) non-coding RNA molecules that modulate the expression of entire sets of genes and pathways [15].

Intestinal microbiota

In recent years, importance of the role of the intestinal microbiota in the progression of NAFLD has been highlighted. High-fat diet, environmental factors or medication can alter the normal gut microbiota and cause afterwards pathogenic effects in the liver [16]. Belei *et al*. [17] confirmed the influence of intestinal dysbiosis and diet on the gut-liver axis. According to this study, obese children with small intestinal bacterial overgrowth (SIBO) have an increased risk for NAFLD development.

Dysbiosis plays a main role in an increasing intestinal permeability with consequent passage of bacteria-derived products (lipopolysaccharides, peptidoglycan, lipoteichoic acid, flagellin, and bacterial DNA) into the portal circulation. Together they induce hepatic expression of toll-like receptor 4 (TLR4), stimulate the immune response and predispose to liver inflammation, promoting the progression of liver damage [18]. The intestinal microbiota is also able to modulate bile acid synthesis, which is crucial for absorption of fat-soluble food, preservation of the intestinal barier, prevention of bacterial translocation, as well as regulation of glucose and lipid metabolism. Analysis carried out by Mouzaki

Table 1. Selected single nucleotide polymorphisms (SNPs) associated with nonalcoholic fatty liver disease (NAFLD) [9]

Gene	Function	Clinical form
PNPLA3 rs738409	accumulation of lipid droplets in hepatocytes	steatosis, fibrosis, NASH, HCC
GCKR rs1260360	modulation of hepatic lipogenesis	fibrosis, NAFLD, NASH
TM6SF2 rs58542926	lipoprotein secretion	fibrosis, NAFLD, NASH
MBOAT7 rs626283 and rs641738	impact on glucose metabolism by modulating intra-hepatic fat content	fibrosis
ENPP1 rs1044498, IRS1 rs1801278	reduction in insulin signaling activity and promotion of insulin resistance	fibrosis
LPIN1 rs13412852 TT	synthesis of phospholipids and triglycerides/regulation of fatty acid metabolism	protective role towards NAFLD/smaller risk of NAFLD
PPARα rs1800206	lipid metabolism	steatosis, inflammation, fibrosis

NAFLD – nonalcoholic fatty liver disease, NASH – nonalcoholic steatohepatitis, HCC – hepatocellular carcinoma

et al. [19] suggested a possible role of bile acids in the progression of NAFLD. Moreover, the gut microbiota seems to be responsible for the increase of endogenous ethanol production in patients with NAFLD. This observation was also confirmed in research concurrently assessing several components of the gut-liver axis in obese children with or without liver disease. Increased permeability (evaluated on the basis of urinary lactulose/mannitol ratio) was a risk factor for the development of steatosis and it significantly correlated with ethanolemia and endotoxemia [20].

Evaluation of the gut microbiome revealed significant differences in its composition in pediatric NAFLD patients compared to healthy controls. Zhu *et al*. [21] reported that children with NASH had an increased quantity of *Bacteroidetes* and *Proteobacteria* and decreased quantity of *Firmicutes* and *Actinobacteria* compared to healthy children. On the other hand, analysis of the fecal microbiome using targeted metagenomics and metabolomics revealed significantly increased *Actinobacteria* and reduced *Bacteroidetes* compared to healthy controls [22]. Moreover, a protective role against the development of NAFLD and obesity of *Bifidobacteria* was observed in pediatric patients. In a more recent study, diagnostic usefulness of gut microbiome composition assessment was described for prediction of advanced fibrosis in adult patients with NAFLD [23].

Diagnosis

It is widely accepted that NAFLD is diagnosed in obese children with both increased transaminases and features of liver steatosis in ultrasound, after exclusion of other possible causes of chronic liver diseases (viral infections, autoimmune hepatitis, metabolic liver diseases, celiac disease). NAFLD usually does not occur in children younger than 3 years and is rare before the age of 10 years. According to the ESPGHAN Hepatology Committee guidelines liver biopsy is the preferred, but imperfect gold standard for confirmation of liver steatosis and/or NASH. Due to the invasive nature of the procedure it has important limitations in children, including risk of complications, high cost, possible sampling error and finally psychological issues, which are of particular importance in young children. Thus, liver biopsy should be performed only in children in very specific cases of NAFLD suspicion, such as advanced disease with elevated ALT activity in patients below 10 years of age, that need exclusion of coexisting diseases, before therapeutic intervention [24]. Therefore, development of new noninvasive markers, useful for prediction of hepatic steatosis and progression to steatohepatitis, represents a growing medical need. This is particularly relevant in the pediatric population. Serum markers of inflammation, apoptosis and oxidative stress have been extensively investigated in patients with NAFLD.

Adipokines

Numerous studies have demonstrated that adipokines, secreted from adipose tissue, are involved in various processes, such as inflammation, immunity, insulin sensitivity, simple liver steatosis and NASH. Adiponectin is a well-known adipokine, which is associated with an anti-inflammatory effect achieved through blocking the activation of nuclear factor κB, by stimulating secretion of anti-inflammatory cytokines such as interleukin (IL)-10 and IL-1 receptor antagonist and by suppressing the release of pro-inflammatory cytokines such as the tumor necrosis factor α (TNF-α), IL-6, and interferon γ. Thus, adiponectin deficiency is related to a pro-inflammatory condition, as demonstrated in NAFLD and other metabolic disorders [25]. Data obtained in our centre have confirmed the importance of a novel adipokine, chemerin, which seems to be a suitable noninvasive biomarker for predicting both intrahepatic lipid content in obese children and advanced liver steatosis in children with NAFLD [26]. This is in line with results from a study conducted in 101 obese children with biopsy-proven NAFLD, which demonstrated a significant association of elevated serum chemerin concentration and decreased serum adiponectin concentration with an increased possibility of NAFLD appearance. The authors found significant positive correlations of body mass index, aspartate transaminase, alanine transaminase, triglycerides, and gamma-glutamyl transferase with chemerin and significant negative correlations of these parameters with adiponectin [27].

There are also other adipokines, such as leptin, resistin or visfatin, which also correlate with severity of NAFLD and may be useful predictors of disease both in adults and children [28, 29]. Angin *et al*. [30] found that the leptin-to-adiponectin (L/A) ratio was significantly higher in children with NAFLD than in obese children without NAFLD and healthy controls. Moreover, a significant correlation of L/A ratio with weight for height, ALT, triglycerides and HOMA-IR was demonstrated and it was even stronger than that for leptin and adiponectin alone.

Hepatokines

Recently, there has also been growing interest in the role of hepatokines in pathogenesis of NAFLD. Hepa-

tokines are proteins produced exclusively or mainly by the liver and involved in regulating glucose and lipid metabolism. Fetuin-A, fibroblast growth factor 21 (FGF-21), selenoprotein P, sex hormone-binding globulin (SHBG), angiopoietin-related growth factor (also known as angiopoietin-related protein 6) and leukocyte derived chemotaxin 2 (LECT2) are considered as hepatokines possibly involved in NAFLD. Lebensztejn *et al.* [31] demonstrated that serum fetuin A concentration was significantly higher in children with NAFLD in comparison to the controls. Several publications have reported significantly higher serum concentrations of FGF-21 in a population of patients with NAFLD compared to the controls [32]. There is a suggestion that FGF-21 resistance and its hepatic expression are essential components of NAFLD development and progression to NASH. Elevated levels of FGF-21 may play a protective role against lipid and carbohydrate metabolism disorders such as metabolic syndrome or NAFLD. It has been shown that FGF-21 inhibits lipolysis, decreases circulating free fatty acid levels, and reduces insulin resistance and hepatic lipid accumulation [33].

Cytokines and other measures

The diagnostic value of plasma cathepsin D (CatD) levels to distinguish pediatric patients with hepatic inflammation from those with simple steatosis was validated by Walenbergh *et al.* [34] with an AUROC (area under receiver operating characteristic curve) of 0.94. Decreased levels of CatD correlated with pediatric NAFLD progression better than ALT and cytokeratin 18 (CK-18) with reference to severity of liver inflammation, degree of steatosis, hepatocellular ballooning and NAFLD activity score (NAS).

Manco *et al.* [35] demonstrated that TNF-α could be a specific noninvasive biomarker useful in predicting the degree of NAFLD progression. In this study TNF-α and leptin levels were significantly associated with an NAS of 5 or more in children with NAFLD. Among other markers, adropin, zonulin and retinol-binding protein 4 (RBP4) also seem to be possible indicators of liver steatosis in children, whereas plasminogen activator inhibitor 1 (PAI1) and IL-8 are particularly associated with NASH [36-38].

Recently, we evaluated IL-18 concentration in serum of 108 obese children and referred it to the degree of liver steatosis in USG or total intrahepatic lipid content assessed by magnetic resonance proton spectroscopy (^{1}HMRS). Our study demonstrated significantly higher IL-18 concentration in the group of obese children with diagnosed NAFLD compared to both healthy controls and non-NAFLD obese children. Moreover, significant positive correlations of IL-18 with alanine transaminase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), triglycerides (TG), high-sensitivity C-reactive protein (hsCRP) and the degree of liver steatosis in USG were found. ROC analysis indicated a cut-off of IL-18 concentration at the level of 326.8 pg/ml as effective for differentiation between children with or without fatty liver in ^{1}HMRS. However, combined ROC analysis of five parameters (IL-18, ALT, AST, GGT and TG) demonstrated superior AUC of 0.7826, with a sensitivity 61%, specificity 85%, and negative or positive predictive value of 38% and 94%, respectively [39].

Soluble Fas (sFas), soluble Fas ligand (sFasL) and CK-18 are considered as a markers of hepatic apoptosis, and their levels are also altered in obese children with NAFLD. Mandelia *et al.* [40] reported that cytokeratin 18 (CK-18), besides the association with apoptosis of hepatocytes, could be perceived as a noninvasive biomarker in detecting liver fibrosis in pediatric NAFLD. Moreover, Lebensztejn *et al.* [41] found that among a cohort of 52 children, 19 children who had biopsy-proven liver fibrosis had significantly higher CK-18 levels than children without fibrosis, and the AUROC value for differentiating children with fibrosis from those without fibrosis was 0.666.

Combined serum markers

Despite limited data about the natural history of pediatric NAFLD, its progression to end-stage liver diseases, such as hepatic cirrhosis or hepatocellular carcinoma, is well documented. Therefore, noninvasive methods for diagnosis of liver fibrosis in children with NAFLD can be a safe and cost-effective alternatives to liver biopsy. Liver fibrosis in children can be detected by a combination of clinical and laboratory parameters, advanced biochemical markers or imaging techniques summarized in several review articles [42]. In clinical practice, a "pediatric NAFLD fibrosis index" (PNFI), developed by Nobili *et al.* [43] in an Italian cohort of 136 children with NAFLD, seems to be useful to predict the presence of liver fibrosis in children with NAFLD. Logistic regression analysis of age, waist circumference and triglycerides were used to assess a predictive model with an AUROC of 0.85 for the detection of liver fibrosis. The "Enhanced Liver Fibrosis" (ELF) test, evaluated in 112 children with NAFLD, has been proposed as a screening method for progressive fibrosis, with an AUROC of 0.92 for any fibrosis (stage 1), 0.98 for significant fibrosis (stage 2) and 0.99 for advanced liver fibrosis. The obtained results were superior to those reported for adults [44].

Alkhouri *et al.* [45] developed a new "Pediatric NAFLD fibrosis score" based on ALT, alkaline phosphatase (ALP), GGT and platelet count, which appears relatively capable of predicting advanced liver fibrosis.

Imaging and elastography

Ultrasound (US) is the most commonly used among non-invasive imaging techniques for screening patients with suspected liver steatosis, due to its availability, lack of radiation exposure and low cost. The mean sensitivity of US for identification of steatosis ranges from 73.3% to 90.5% [46]. The major limitations of ultrasound are indirect measurement of the fat content, subjective and non-quantitative examination and finally relatively low sensitivity for detecting mild steatosis (0-10% on liver biopsy) [47]. Additionally, conventional US is not suitable to differentiate between steatosis, inflammation or fibrosis.

Considerably better for the evaluation of liver steatosis seems to be the controlled attenuation parameter (CAP). CAP is a novel noninvasive and easy to perform technique, based on the ultrasonic signal acquired by the transient elastography device (FibroScan), using the fact that fat affects ultrasound propagation. It measures the ultrasound attenuation at the center frequency of the probe expressed in decibels per meter (dB/m). The results obtained using CAP are reproducible and can assess steatosis in patients with various liver diseases. A major advantage of this method is the possibility to measure both steatosis and fibrosis [48]. Recently, Desai *et al.* [49] demonstrated that CAP can be applied easily in children and the results showed that measurements were statistically significantly higher in patients with steatosis than in those without steatosis, irrespective of whether patients were overweight, obese or normal weight. A study demonstrated a CAP cut-off point of 225 dB/m as effective (AUC = 0.93) for predicting steatosis in a pediatric population (comparably to that proposed in studies conducted in adults). Moreover, this method was able to differentiate between grades of steatosis in children. This is in line with results from the study of Ferraioli *et al.* [50], who demonstrated a better clinical value of CAP than conventional US in the diagnosis of liver steatosis in overweight and obese children. However, due to insufficient data CAP is still not recommended by international guidelines of NAFLD management.

Several elastography techniques can be useful in diagnosis of fibrosis in pediatric NAFLD patients, which include transient elastography (TE), shear wave elastography (SWE), acoustic radiation force impulse (ARFI) elastography, and magnetic resonance elastography (MRE) [51]. A study carried out in 52 children with biopsy-proven NAFLD has shown encouraging results of using TE for detection of fibrosis with AUROC values of 0.977, 0.992 and 1.0 for the prediction of "any", significant and advanced fibrosis, respectively [52]. It was also documented that the combination of pediatric NAFLD fibrosis index (PNFI) and TE can be used to accurately assess the presence of clinically significant liver fibrosis in 98% of children with NAFLD [53]. In another study the authors confirmed that SWE is an accurate and reproducible noninvasive technique integrated with the US system, which efficiently depicts the presence of significant liver fibrosis and, less accurately, mild liver fibrosis in pediatric patients with NAFLD. This method showed a very high correlation with liver fibrosis in both univariate and multivariate analyses. The AUC for the association of any and significant fibrosis were 0.92 and 0.97, respectively [54]. ARFI elastography is another promising noninvasive tool for measuring liver stiffness and assessing fibrosis. It is an ultrasound-based approach using short bursts of high-intensity acoustic pulses directed through the liver tissue. The velocity of produced shear waves (SWV) correlates with liver stiffness. ARFI can be integrated into a conventional ultrasound system, so it can be performed during routine liver US examination. However, there is evidence that ARFI elastography is modestly accurate in detecting significant fibrosis in NAFLD patients, because readings might be influenced by hepatic steatosis and inflammation. There is a limited number of studies assessing the usefulness of ARFI in estimating tissue stiffness in children. Norueges *et al.* [55] found the mean value for ARFI of 1.42 m/s in children with chronic liver disease (CLD) and/or before liver transplant and 1.11 m/s in the controls. Moreover, the study revealed that the reliability of this non-invasive tool was higher in patients with advanced fibrosis than in those with less severe fibrosis.

MRE, another elastography technique, can be useful for evaluation of the liver stiffness and differentiation stage of fibrosis. A major advantage of MRE over TE and ARFI is that MRE is less susceptible to technical interference from excess abdominal adiposity and enables one to evaluate the entire liver. On the other hand, it is an expensive technique and it requires specific hardware and software, compared to conventional magnetic resonance equipment. As a matter of fact, most of the results are from studies conducted in adults, but there are some data implying its utility in the pediatric population. A pilot study of 35 children and adolescents (median age 13 years), undergoing MRE and liver biopsy for evaluation of chronic liver disease,

demonstrated good accuracy of MRE for detecting significant hepatic fibrosis in children with an AUROC of 0.92. More recently, Schwimmer *et al.* [56] in a prospective, multicenter, cross-sectional analysis revealed the utility of MRE for estimation of hepatic stiffness in children with NAFLD. However, at the moment MRE is considered an experimental technique, and pediatric validation is necessary in further studies, which are required before introduction into clinical practice.

Magnetic resonance imaging (MRI) methods, such as proton density fat fraction (PDFF) measurement, appear to be an objective test for the quantification of liver steatosis in clinical trials and epidemiologic studies. PDFF is the ratio of MRI-visible density of mobile lipid protons and the total density of protons from water and fat in the liver. MRI-PDFF values have been shown to correlate well with steatosis grade by liver histology in pediatric patients [57]. MRI-PDFF allows fat mapping of the entire liver, whereas another available imaging modality, ^{1}HMR spectroscopy (^{1}HMRS), measures the concentration of lipids (triglycerides) within the hepatocytes in small regions of interest. The voxel is localized in such a manner that it does not include the large vessels and bile ducts. The spectral evaluation includes signals of functional groups of the lipid compounds: methyl (Lip1), methylene (Lip2), and α-methylenes for the double bond (Lip3). Total intrahepatic lipid content (calculating by summing up the content of individual lipid bands [Lip1,2,3]) is assessed in relative units (r.u.) in comparison to the unsuppressed water signal. Sensitivity and diagnostic precision in adults range from 87% to 100% and from 80% to 85%, respectively. A few studies have used ^{1}HMRS to investigate liver fat content in children and adolescents. A recently published paper suggested that ^{1}HMRS is an accurate non-invasive diagnostic technique for quantifying liver steatosis in a pediatric population and proposed the cut-off value of 6% to discriminate between patients with and without steatosis (sensitivity, 92.6%; specificity, 95.7%). Moreover, a significant correlation was found between ^{1}HMRS and histology results [58]. Although ^{1}HMRS is considered the most accurate non-invasive method for quantifying liver steatosis in obese children, it is not widely performed because it is time-consuming and requires off-scan analysis by an expert. Thus, currently it is not suitable for common use and it seems to be most appropriate for research studies at specialized centers.

Treatment

In spite of the increasing prevalence, progressive nature and unfavorable implications of NAFLD even in children, possibilities of treatment are limited. Therapeutic approaches are focused on mechanisms involved in NAFLD pathogenesis, including the role of insulin resistance, dyslipidemia, oxidative stress and the gut microbiome [59].

Lifestyle modification

The first line of intervention at all ages is lifestyle modification through changes in diet and physical activity, which should lead to/aim at weight reduction. It is well established that weight loss has a beneficial influence on both metabolic and hepatic features in obese patients with NAFLD, especially through reduction of hepatic oxidative stress and intrahepatic lipid accumulation, decrease of aminotransferases, triglycerides and cholesterol levels, as well as improvement in insulin sensitivity and glucose tolerance [60]. Ramon-Krauel *et al.* [61] compared a low-glycemic-load diet to a low-fat diet, finding that both are equally effective in decreasing hepatic lipid content measured by proton magnetic resonance spectroscopy and improvement in visceral fat accumulation, body mass index (BMI), anthropometrics, ALT activity and insulin resistance in obese children with fatty liver.

A recent meta-analysis, based on 14 clinical trials, assessing the impact of supervised exercise interventions on obesity and hepatic function in a pediatric population, revealed a significant reduction in visceral, subcutaneous and intrahepatic fat, as well as GGT activity, but without alterations in any other liver enzyme. According to this study, exercise intervention, particularly aerobic exercises in more than three sessions per week, is effective and recommended in obese youth with NAFLD [62]. Available studies have suggested that a combination of a proper balanced reduced calorie diet and moderate intensity exercise results in a significant decrease in BMI, and levels of fasting glucose, insulin, lipids, and liver enzymes activities, as well as liver steatosis determined by ultrasonography and proton magnetic resonance spectroscopy [63]. Moreover, it was proven that long-term lifestyle changes lasting for 24 months improve liver histology in terms of the grade of steatosis, lobular inflammation, hepatocyte ballooning, and NAFLD activity score in pediatric patients. However, especially in children, achieving and maintaining weight loss through compliance with recommended behavior may turn out to be very difficult and give disappointing results or prove unsuccessful in some cases. For these reasons, several studies have been conducted to develop a possible alternative pharmacological intervention based on pathogenetic targets of NAFLD.

Anti-oxidants

Oxidative stress is regarded as the initiating factor of lipid peroxidation and subsequent hepatocellular injury in NAFLD. Thus, antioxidants, such as vitamin E, have been evaluated as a possible treatment for NAFLD. Lavine *et al.* [64], who performed the first open label study in children with chronically elevated serum ALT activity and ultrasound evidence of hepatic steatosis, concluded that daily oral vitamin E administration (400-1200 IU/day) may induce a decrease of serum transaminases levels irrespective of changes in BMI and liver echogenicity in ultrasound. Unfortunately, subsequent studies have not confirmed these results. The multicenter randomized placebo-controlled TONIC trial showed that vitamin E was not superior to the placebo at attaining the primary outcome of sustained reduction in ALT levels in patients with pediatric NAFLD. However, the trial reported significant improvement in hepatocellular ballooning and NAFLD activity score in patients with NASH or borderline NASH at baseline treated with vitamin E compared with placebo. Therefore, vitamin E may offer histological benefits to children with biopsy-proven NASH. Nevertheless, vitamin E did not demonstrate significant effects on steatosis, inflammation, or fibrosis compared with placebo. Cho *et al.* [65] established that for pediatric NAFLD patients, BMI reduction (dietary therapy and exercise) in conjunction with vitamin E and UDCA treatment has a greater therapeutic effect, than BMI reduction alone, in terms of biochemical profiles. Concurrently, the authors noted that drug treatment alone, without BMI reduction, does not improve NAFLD.

Cysteamine bitartrate is another anti-oxidant, whose usefulness in pediatric NAFLD treatment is under evaluation at the moment. A study conducted in children with biopsy-proven NAFLD and elevated ALT levels revealed that 7 from 11 subjects achieved ≥ 50% reduction or normalization of serum ALT after 24 weeks of enteric-coated cysteamine therapy. There was a significant increase in serum adiponectin and reduction in leptin and CK-18 fragments without accompanying changes in BMI. The results of the first large, multi-center randomized clinical trial with cysteamine bitartrate delayed-release (CBDR) capsules have been recently published. In this manuscript, the authors found that 52 weeks of CBDR therapy did not reduce overall histologic markers of NAFLD compared to placebo in children. However, children receiving CBDR had significant reduction in serum ALT activity and improvement in lobular inflammation [66].

Probiotics

As mentioned before, growing evidence shows that the gut microbiota plays an important role in the pathogenesis of NAFLD. Based on these data, probiotics, as modulators of intestinal bacterial microbiota, seem to be an interesting and reasonable option in the treatment of NAFLD. In children, there are several randomized clinical trials evaluating the influence of probiotic supplementation on the liver function. In a study by Alisi *et al.* [67], 48 obese children with biopsy-proven NAFLD were given VSL#3 (a mixture of 8 probiotic strains) or placebo for 4 months. The results of this study showed that probiotic supplementation reduced BMI and severity of NAFLD, but it did not cause significant differences in levels of triglyceride, ALT and HOMA-IR in comparison to the placebo group. After supplementation with VSL#3, the investigators also observed increased circulating levels of the total and activated form of glucagon-like peptide 1 (GLP-1), suggesting that this molecule could be responsible for these beneficial effects. Another double-blind clinical trial demonstrated that obese children with elevated ALT levels and suspected hepatic steatosis evaluated by ultrasound, treated with *Lactobacillus rhamnosus* strain GG, reached a significant decrease in serum ALT values compared to those who received placebo. Similarly, a recent randomized trial conducted among 64 obese children with NAFLD demonstrated a significant reduction in ALT and AST activity, lipid parameters, as well as waist circumference after 12 weeks of supplementation with probiotic capsules containing a mixture of *Lactobacillus acidophilus*, *Bifidobacterium lactis*, *Bifidobacterium bifidum*, and *Lactobacillus rhamnosus* [68]. These results suggest the possible therapeutic role of probiotics in the treatment of NAFLD. However, further, longer follow-up of randomized controlled trials is needed to assess long-term probiotics' safety and effectiveness in the pediatric population.

Polyunsaturated fatty acids

In the last years, dietary supplementation with polyunsaturated fatty acids (PUFAs) has been found as a potential therapeutic strategy for NAFLD. PUFAs include essential fatty acids, such as omega-3 and omega-6 acids, which demonstrated a beneficial effect on the regulation of hepatic lipid metabolism and adipose tissue function, as well as anti-inflammatory and insulin-sensitizing properties. Their effectiveness in prevention and therapy of cardiovascular disease, dyslipidemia and metabolic syndrome is well established [69, 70].

The Nonalcoholic Steatohepatitis Clinical Research Network reported very low consumption of fish and omega-3 fatty acids in children with NAFLD, which was associated with increased inflammation in liver biopsy. Recent studies showed that supplementation with long-chain polyunsaturated fatty acids (LCPUFA) can improve liver steatosis and liver functions in children with NAFLD. Nobili *et al*. [71] in a double-blind randomized controlled trial evaluated the efficacy of 250 mg/day or 500 mg/day docosahexaenoic acid (DHA) versus placebo in 60 children with NAFLD after 6, 12, 18 and 24 months of therapy. The investigators demonstrated that 6 months of DHA administration is sufficient to reduce liver steatosis assessed on ultrasound. During a longterm follow-up patients maintained the improvement of metabolic and biochemical parameters, as well as reduction of the liver fat content evaluated by ultrasound and liver biopsy. The favorable impact of DHA on the liver may be a result of reduction of hepatic progenitor cell activation and exerting an anti-inflammatory effect through interaction with the G protein-coupled receptor-120. The benefit of dietary supplementation with PUFAs was also confirmed in the investigation by Pacifico *et al*., where the liver fat evaluated by MRI was reduced significantly after 6 months of therapy in children with biopsy-proven NAFLD. Serum ALT activity also decreased significantly in the group receiving DHA, but there was no difference compared to placebo [72].

Janczyk *et al*. [73] investigated the effect of 6 months of omega-3 fatty acid supplementation in a population of 76 overweight and obese children with NAFLD. Children were randomized to receive fish oil containing omega-3 long-chain polyunsaturated fatty acids (LC-PUFA) (a mixture of docosahexaenoic acid and eicosapentaenoic acid, 450-1300 mg/day) or placebo (omega-6 sunflower oil). There was no significant improvement in serum ALT activity, insulin resistance, lipid levels or liver hyperechogenicity on ultrasound in subjects in the omega-3 group compared with the control group. Omega-3 fatty acid supplementation resulted only in a significant decrease in AST and GGT activities and in an increase in circulating adiponectin. These divergent results obtained from omega-3 supplementation in children with NAFLD could be influenced by different genetic and epigenetic factors and heterogeneity of liver disease in NAFLD. There are also promising studies that have evaluated the effects of combination of different micronutrients compared to placebo, such as DHA plus vitamin D treatment or a mixture containing DHA, choline and vitamin E in children with NAFLD [74]. Therefore, because of the safety, tolerability and beneficial effects of DHA in the pediatric population, further studies in the management of children with NAFLD are needed.

Insulin sensitizers

Development of NAFLD is strongly associated with insulin resistance, which promotes the storage of FFA. For this reason insulin sensitizers might be considered as a potential favorable therapeutic tool. Metformin is the principal insulin-sensitizing agent evaluated in pediatric NAFLD, but there are only a few available studies about its effectiveness. The most recent research is the TONIC study – a large, multicenter, randomized clinical trial, in which metformin was not better than placebo in reducing serum ALT levels and had only a slight effect on liver histology [64]. Because of lack of evidence for the efficacy of treatment by metformin, it is not currently recommended in the treatment of pediatric NAFLD. Encouraging preliminary results are provided by new randomized, double-blind, placebo-controlled, pilot studies of the usefulness of losartan (an angiotensin II receptor blocker) and obeticholic acid (a synthetic analogue of chenodeoxycholic acid and a potent activator of farnesoid X receptor) in biopsy-proven NASH, respectively in children and adults. There are also data suggesting the potential advantageous effect of selonsertib (an apoptosis signal-regulating kinase 1 inhibitor) and cenicriviroc (a dual antagonist of C-C chemokine receptor types 2 and 5) on fibrosis observed in adults in the course of NASH [75, 76].

Bariatric surgery

Nowadays, because of insufficient effectiveness of lifestyle modification and hitherto applied treatment in patients with NAFLD, bariatric surgery is increasingly performed as an alternative option for weight reduction in morbidly obese patients. These procedures are considered to lead to sustained and successful long-term weight loss and improvement of related comorbidities, including NAFLD. Currently they are used in extremely obese adults with good effect. The mechanism by which bariatric surgery influences hepatic injury reduction is loss of fat mass, with simultaneous decrease of systemic inflammation and insulin resistance, as well as increase in beneficial adipokines and modification of the intestinal microbiome. Data from metaanalyses of limited series and follow-up of bariatric surgery in morbidly obese adolescents demonstrated effective weight loss with improvement of metabolic parameters and quality of life [77]. Recently, the Hepatology Committee of ESPGHAN proposed a society position statement about the indications and limitations of bariatric surgery in

Table 2. Current clinical trials on drugs for nonalcoholic fatty liver disease (NAFLD) [79]

Drug class	Compound	Phase	Target/Mode of action (MOA)	Company
metabolic homeostasis	RG-125 (AZD4076)	Preclin.	microRNA-103/107 ("miR103/107")/insulin sensitivity and resistance	Regulus
metabolic homeostasis	Saroglitazar (ZYH1)	1	PPAR/PPAR α/γ agonist	Zydus Cadila
metabolic homeostasis	GS-9674	1	FXR/FXR agonist	Gilead Sciences
metabolic homeostasis	PF-05231023	1	fibroblast growth factor 21 (FGF21)/FGF21 analog	Pfizer
metabolic homeostasis	IVA337	2	PPAR/PPARα-δ and γ (PanPPAR)	Inventiva Pharma
metabolic homeostasis	LJN-452	2	FXR/FXR agonist	Novartis
metabolic homeostasis	NGM-282	2	fibroblast growth factor 19 (FGF19)/FGF19 analog	NGM Biopharmaceutic
metabolic homeostasis	Aramchol	2	stearoyl-coenzyme A desaturase 1 (SCD1)/SCD1 inhibitor	Galmed Pharmaceuticals
metabolic homeostasis	LUM002	2	ASBT/ASBT inhibitor	Lumena
metabolic homeostasis	Liraglutide (NN2211)	2	glucagon-like peptide 1 (GLP-1) receptor/GLP-1 analog	Novo Nordisk
metabolic homeostasis	Elafibranor (GFT505)	3	PPAR/PPARα-δ agonist	Genfit
metabolic homeostasis	Rosiglitazone	3	PPAR/insulin sensitizer	GlaxoSmithKline
metabolic homeostasis	Pioglitazone	3	PPAR/insulin sensitizer	Takeda Pharmaceutical
metabolic homeostasis	Obeticholic acid (OCA)	3	FXR/FXR agonist	Intercept
inflammation	Cenicriviroc (CVC)	2	C-C chemokine receptor types 2 (CCR2) and 5/CCR2/CCR5 antagonist (CCR5)	Tobira Therap.
inflammation	Venlafaxine-103	2	TNF/downregulation of proinflammatory cytokines	Verlix Pharma
inflammation	Sitagliptin	Mkt	dipeptidyl peptidase 4/(DPP-4 inhibitor)	Merck Sharp & Dohme
inflammation	Evogliptin	Mkt	dipeptidyl peptidase 4/(DPP-4 inhibitor)	Dong-A ST
oxidative stress	PXS4728A	1	semicarbazide-sensitive amine oxidase (SSAO)/VAP-1 inhibitor	Boehringer Ingelheim
oxidative stress	GS- 4997	2	apoptosis signal-regulating kinase 1 (ASK1)/ASK1 inhibitor	Gilead Sciences
fibrosis	Simtuzumab	2	lysyl oxidase and lysyl oxidase- like (LOXL) enzymes	Gilead Sciences
fibrosis	GR-MD-02	2	galectin-3 protein/galectin-3 protein inhibitor	Galectin Therapeutics
apoptosis	Emricasan (IDN-6566)	2	caspase/caspase inhibitor	Conatus Pharmaceuticals

severely obese children and adolescents. According to this document, a qualification for a bariatric procedure should be considered in selected obese adolescents with BMI > 40 kg/m^2 and severe comorbidities (type 2 diabetes mellitus, moderate-to-severe sleep apnea, pseudotumor cerebri, or NASH with significant fibrosis) or with BMI > 50 kg/m^2 with mild comorbidities (hypertension, insulin resistance, glucose intolerance, a substantially impaired quality of life, or activities of daily living, such as dyslipidemia, or sleep apnea) [78]. These procedures can improve liver condition by reduction of steatosis, hepatic inflammation and fibrosis in NASH.

Innovative therapies

A number of clinical trials are ongoing, aimed at evaluation of safety and efficacy of different novel drugs, especially in the adult population with NAFLD. After their approval in adults there is a chance that some of these medications will be tested in the pediatric population. Innovative therapeutic approaches, which may deserve attention for extensive upcoming investigation, are summarized in Table 2 [79].

Disclosure

Authors report no conflict of interest.

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