M30 Apoptosense® ELISA

in paediatric NAFLD

The dead cells still count!

VLVbio

www.vlvbio.com

NAFLD and NASH

Non-alcoholic fatty liver disease (NAFLD), a condition where fat accumulates in the liver without the excess consumption of alcohol, has become the most common cause of liver disease in children. Due to the rise in obesity and diabetes, NAFLD is estimated to affect 26 % of the global population aged between 9-17 years old, with high prevalence on all continents. This increase is most likely related to the so-called Western lifestyle; fast food, and reduced physical activity. The disease begins as steatosis which is an accumulation of fat in the liver and may further develop to non-alcoholic steatohepatitis (NASH). NASH is a serious condition where the liver has become inflamed and which can progress to cirrhosis and its associated complications even during childhood.

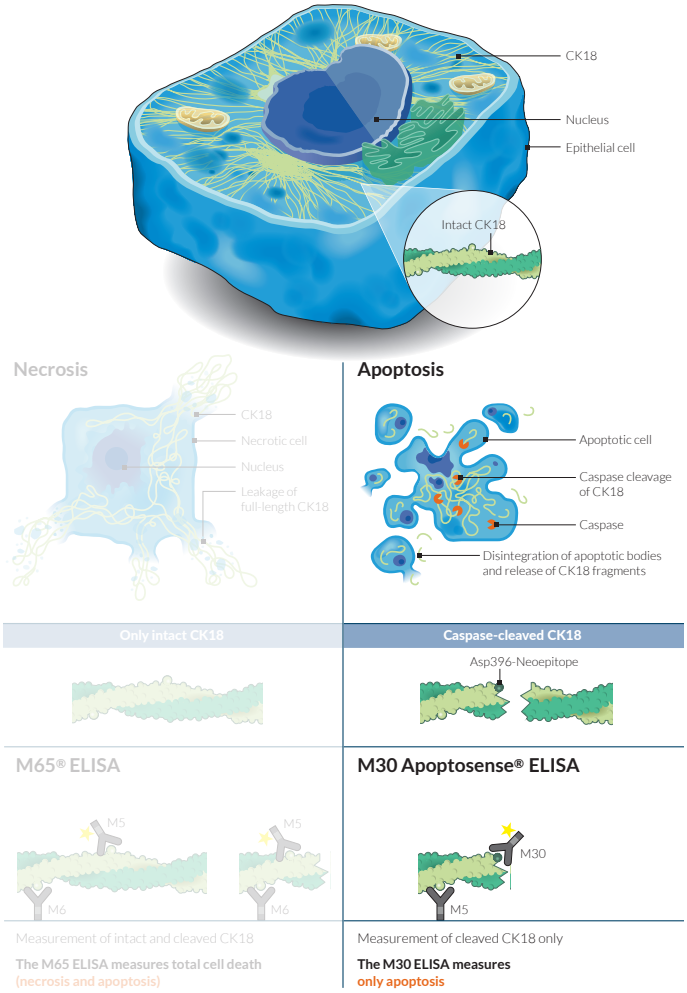
NAFLD process

healthy liver | hepatic steatosis | steatohepatitis (NASH) | liver cirrhosis

The role of keratin 18 in NAFLD

Keratin 18 (K18) is an intermediate filament protein forming part of the cytoskeletal structure expressed in epithelial cells. Hepatocytes, a form of epithelial cell, are the main tissue cells found in the liver. When simple steatosis in NAFLD is accompanied by inflammation of hepatocytes, the disease is described as NASH. This prominent characteristic of the disease is mainly caused by hepatocyte cell death due to apoptosis. Early on in the apoptosis of hepatocytes, caspases are activated which cleave the K18 protein, and the resulting fragments are subsequently released into the blood. These caspase cleaved K18 (ccK18) fragments can be efficiently quantified by the unique M30 Apoptosense® ELISA.

Since apoptosis is a well studied mechanism for NAFLD progression, the availability of a serum biomarker for hepatic apoptosis may widen the availability of the detection of NAFLD and NASH. Detection can be made using liver imaging technologies with confirmation by biopsy, but these are expensive and not readily available. A biopsy is an invasive and complicated procedure that can be traumatic for children. A non-invasive biomarker that detects the disease at an early stage and is also time- and cost efficient, while reducing patient discomfort and hospitalization, is of importance.



The M30 Apoptosense® ELISA in paediatric NAFLD and NASH:

- ✓ Is a sensitive and specific indicator of NAFLD and NASH
- ✓ Can aid in the selection of children for further investigation
- ✓ Can aid in stratifying disease severity

Example 1: ccK18 in the diagnosis of NASH in children
Feldstein *et al*[1] studied 201 children with biopsy-proven NAFLD of which 70 % were diagnosed with NASH. Plasma ccK18 levels were measured in blood samples collected at the time of biopsy using the M30 Apoptosense® ELISA.

The authors state:
"The principal findings of our study relate to the finding that measuring CK18 fragment levels in the blood, a marker of hepatocyte apoptosis, is a reliable test to diagnose NASH in children with suspected NAFLD.
Our results demonstrate that:
- *Plasma ccK18 levels are significantly higher in children with NASH compared with those with hepatic steatosis.*
- *ccK18 has excellent accuracy for diagnosing NASH on biopsy with AUC of 0.933.*
- *ccK18 levels correlated with the main histological features of NASH, NAS, and fibrosis stage."*

Example 2: ccK18 and other biomarkers in the diagnosis of NASH in children
Fitzpatrick *et al*[2]. presented the combined use of biomarkers for different pathological processes in the development of NASH and their potential for its diagnosis. Serum ccK18 measured by the M30 Apoptosense® ELISA, leptin, hyaluronic acid, adiponectin and high-sensitivity C-reactive protein were measured in 45 children with biopsy-proven NAFLD.

Findings:
- ccK18 levels were significantly higher in children with NAFLD compared with healthy controls.
- ccK18 levels were significantly higher in children with NASH compared with those with simple steatosis.
- ccK18 could differentiate those with significant fibrosis from no- or minimal fibrosis.

The authors state:
"Serum biomarkers, especially ccK18 M30, were found to be useful in stratifying disease severity in paediatric NAFLD."

Example 3: Treatment overview of NAFLD and NASH in Children, Skåne Regional Health Authority Sweden[3]
Skåne Regional Health Authority in Sweden has developed a guideline for patients with childhood obesity (iso-BMI > 30), and overweight children (iso-BMI 25-30) with abdominal obesity, to identify patients with metabolic complications. The following flow chart is based on their recommendation on how to diagnose and treat NAFLD and NASH. The authors of the guideline strongly recommend that the treatment should be intensive and controlled by a child obesity center that can be found at all regions in the country.

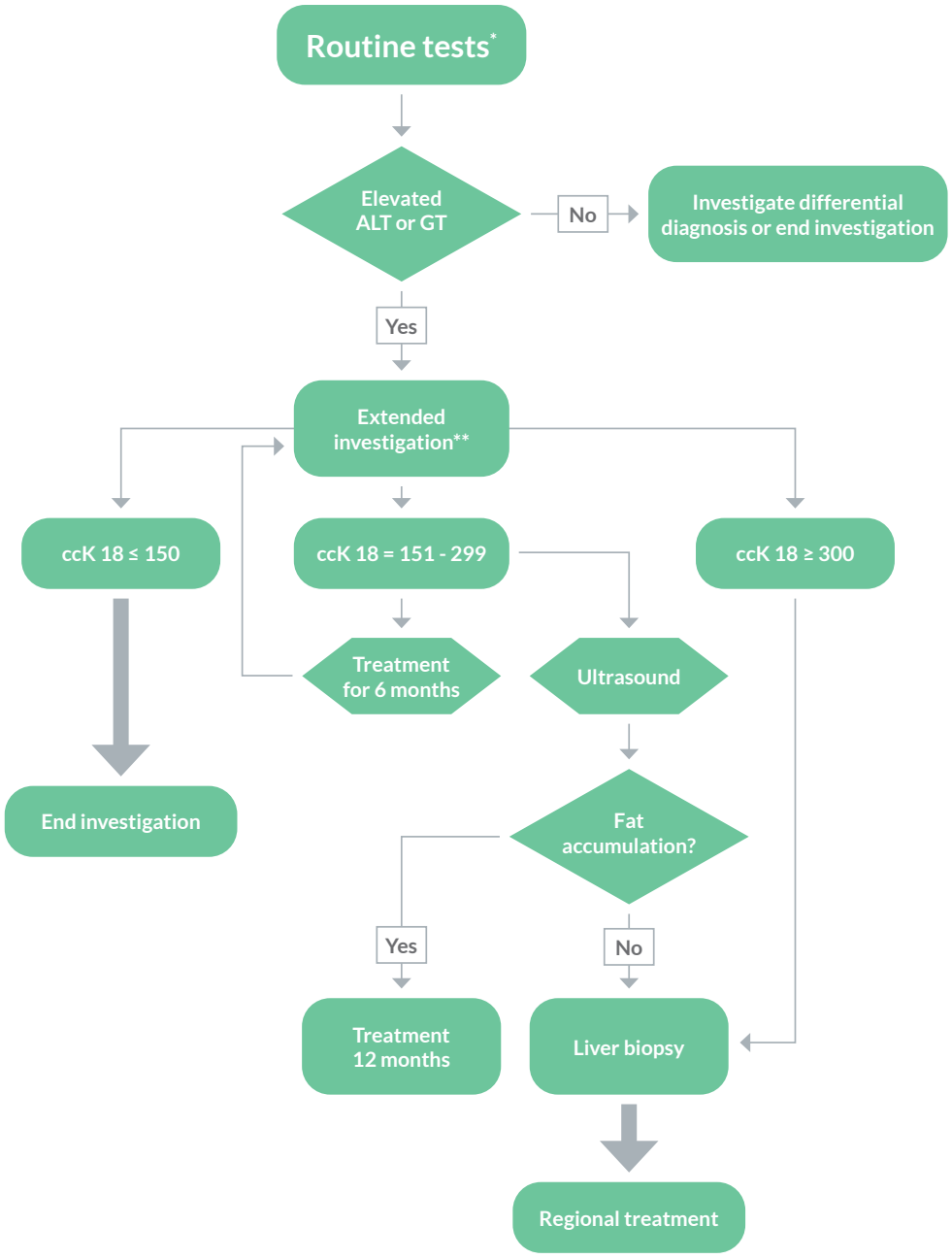
*Routine tests (fasting):

- lipid status – cholesterol, HDL cholesterol, LDL cholesterol, triglycerides
- liver function test – ALT, GT
- diabetes test – insulin, glucose, HbA1c
- thyroid test – TSH, free T4, TPO

**Extended investigation:

Further tests should be taken if ALT (≥ 0,75) or GT (≥ 0,75):

- liver function test – AST, ALT, GT, ALP, bilirubin, conjugated bilirubin
- serology – hepatitis B and C
- antibodies – ANA, smooth muscle antibodies, p-IgG, transglutaminase antibodies
- other – ccK18, p-Antitrypsin (AAT)



VLVbio™ Product Line	
ELISA Products	**Prod. No**
M30 Apoptosense® ELISA	10011
M30 CytoDeath™ ELISA	10900
M65® ELISA	10020
M65 EpiDeath® ELISA	10040
M65 EpiRat™ ELISA	10060

How to order

VLVbio is collaborating with distributors all over the world to provide fast, reliable and convenient service for you.
Please visit www.vlvbio.com or email order@vlvbio.com to find your local distributor.

References:

[1] Feldstein *et al*, 2013. Serum Cytokeratin-18 Fragment Levels Are Useful Biomarkers for Nonalcoholic Steatohepatitis in Children. American Journal of Gastroenterology. 108(9), 1526-3.

[2] Fitzpatrick *et al*, 2010. Serum Levels of CK18 M30 and Leptin Are Useful Predictors of Steatohepatitis and Fibrosis in Paediatric NAFLD. Journal of Paediatric Gastroenterology and Nutrition. 51(4), 500-6.

[3] Flodmark, 2020. Internetmedicin.se. Fettlever hos barn (Non Alcoholic Fatty Liver Disease (NAFLD) och Non Alcoholic Steatohepatitis (NASH)). https://www.internetmedicin.se/behandlingsoversikter/pediatrik/fettlever-hos-barn-non-alcoholic-fatty-liver-disease-nafld-och-non-alcoholic-steatohepatitis-nash/. 2021-07-05.

VLVbio

Hästholmsvägen 32, 131 30 Nacka, Sweden
Telephone: +46 (0) 8 122 053 00
E-mail: info@vlvbio.com • www.vlvbio.com