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A SERUM APOPTOSIS MARKER for CHRONIC LIVER DISEASE

M30-Apoptosense[®] ELISA

Hepatocyte apoptosis causes liver fibrogenesis - use apoptosis biomarkers to monitor chronic liver disease!

Liver fibrosis results from chronic damage to the liver. The main causes of liver fibrosis include chronic hepatitis B and C virus (HBC, HCV) infection, alcoholic (ASH) and non-alcoholic steatohepatitis (NASH).

It is becoming increasingly clear that hepatocyte apoptosis contributes to liver fibrogenesis. Recent studies of chronic HBV and HCV infection as well as non-alcoholic fatty liver disease (NAFLD) show that hepatocyte apoptosis correlates with disease severity and stage of fibrosis.

The M30-Apoptosense[®] ELISA measures a protein fragment released from apoptotic hepatocytes

A number of investigators have used the M30-Apoptosense[®] ELISA to show that the serum/plasma levels of caspase-cleaved CK18 are elevated in patients with NASH and chronic hepatitis C infection. A major advantage of this approach is that the analyte measured by the M30-Apoptosense[®] ELISA is an endproduct of the apoptotic process, reflecting the extent of active disease (ongoing apoptosis).

M30-Apoptosense[®] ELISA is a blood test for liver apoptosis useful for disease monitoring in patients with HCV infection or NASH.

Caspase activity in hepatocyte apoptosis

Caspases (a form of protease) are activated during apoptosis and cleave various hepatocyte proteins. One of these substrates is cytokeratin 18 (CK18) which is degraded into fragments which are subsequently released into blood.

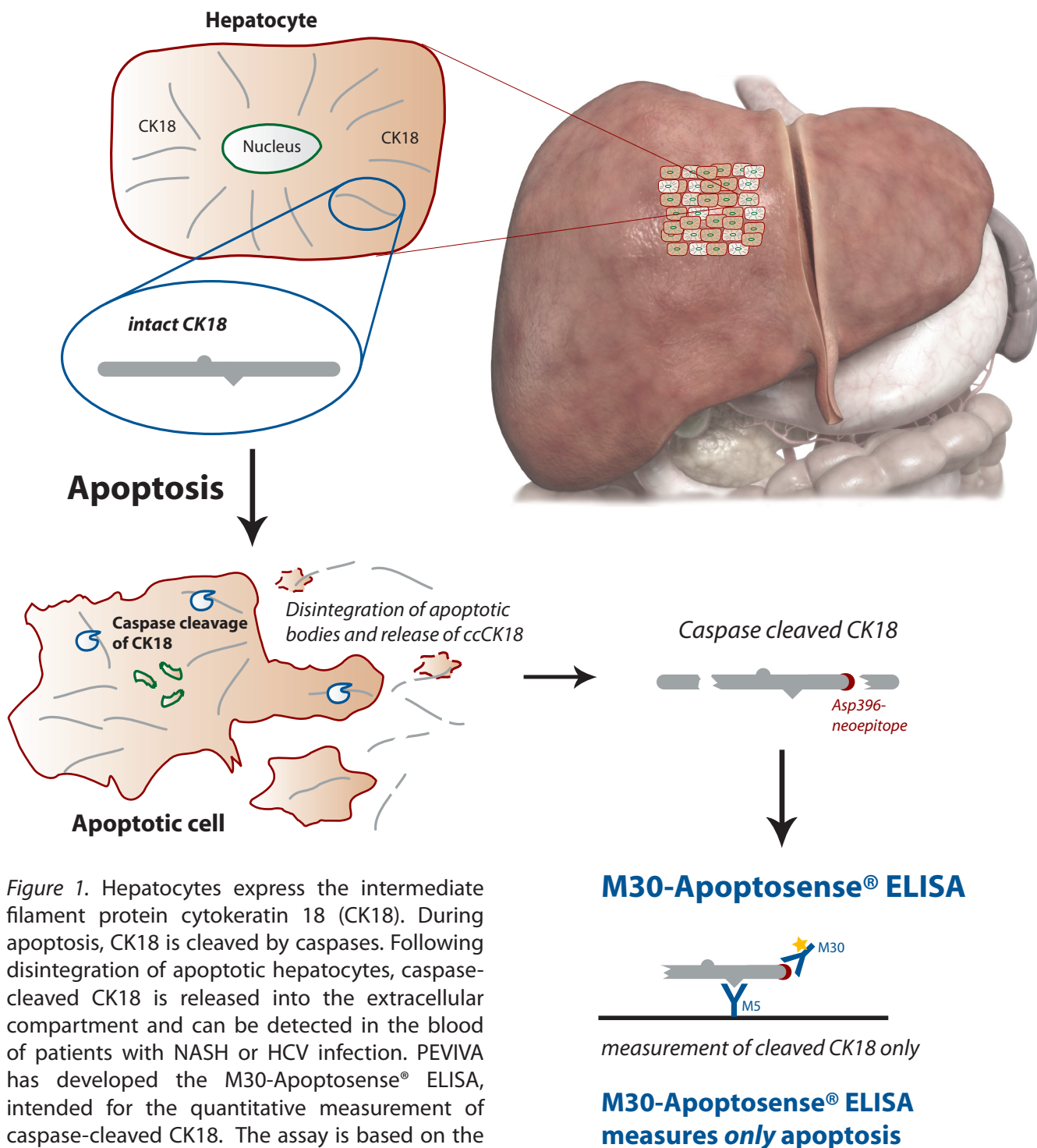


Figure 1. Hepatocytes express the intermediate filament protein cytokeratin 18 (CK18). During apoptosis, CK18 is cleaved by caspases. Following disintegration of apoptotic hepatocytes, caspase-cleaved CK18 is released into the extracellular compartment and can be detected in the blood of patients with NASH or HCV infection. PEVIVA has developed the M30-Apoptosense® ELISA, intended for the quantitative measurement of caspase-cleaved CK18. The assay is based on the monoclonal antibody M30 which recognizes a neo-epitope on CK18 formed after cleavage by caspases.

An Apoptosis Biomarker for the HCV clinic

Patients with normal serum ALT values may have active HCV disease as assessed by histopathology. Patients with normal ALT and active HCV-related liver diseases were reported to have elevated levels of caspase-cleaved cytokeratin 18 products in their serum (Bantel et al., 2004).

Available evidence suggests caspase-cleaved cytokeratin 18 to be a more sensitive marker than liver transaminases for the detection of early liver injury.

About 50 % of all HCV patients do not respond to treatment. The serum levels of caspase-cleaved CK18 were found to be higher in patients responding to anti-viral therapy compared to non-responders (Volkman et al., 2006).

M30-Apoptosense® ELISA predicts response to anti-viral therapy in HCV patients.

- » Bantel H, Luger A, Heidemann J, Volkman X, Poremba C, Strassburg CP, Manns MP, Schulze-Osthoff K, (2004). Detection of apoptotic caspase activation in sera from patients with chronic HCV infection is associated with fibrotic liver injury. *Hepatology*. (2004) 40:1078
- » Volkman X, Cornberg M, Wedemeyer H, Lehner F, Manns MP, Schulze-Osthoff K, Bantel H, (2006). Caspase activation is required for antiviral treatment response in chronic hepatitis C virus infection. *Hepatology*. (2006) 43:1311

Monitoring disease progression in patients with NASH

The dramatic increase of obesity and metabolic syndrome in many parts of the world has caused a striking increase in fatty liver disease. NASH (non-alcoholic steatohepatitis) is the most serious form of fatty liver disease and there is an increasing medical need for non-invasive markers for this condition. A number of scientific reports from well reputed institutions have established that the M30-Apoptosense® ELISA is a useful test for monitoring disease progression in patients with NASH.

References:

- » Wieckowska A, Zein NN, Yerian LM, Lopez AR, McCullough AJ, Feldstein AE, (2006). In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease. *Hepatology* 44: 27-33
- » Younossi ZM, Jarrar M, Nugent C, Randhawa M, Afendy M, Stepanova M, Rafiq N, Goodman Z, Chandhoke V, Baranova A, (2008). A novel diagnostic biomarker panel for obesity-related nonalcoholic steatohepatitis (NASH). *Obes Surg* 18: 1430-7
- » Malik R, Chang M, Bhaskar K, Nasser I, Curry M, Schuppan D, Byrnes V, Afdhal N, (2009). The clinical utility of biomarkers and the nonalcoholic steatohepatitis CRN liver biopsy scoring system in patients with nonalcoholic fatty liver disease. *J Gastroenterol Hepatol* 24: 564-8
- » Feldstein AE, Wieckowska A, Lopez AR, Liu YC, Zein NN, McCullough AJ, (2009). Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: A multicenter validation study. *Hepatology* 2009 Oct;50(4):1000-3

“CK18 fragments are expected to become an *ideal* biomarker for NASH”

Malik et al., *J Gastroenterol Hepatol* 24 (2009), 564-8

Assay characteristics

The M30-Apoptosense® ELISA is a reproducible and robust method for determination of apoptotic cell death. Typically, CVs between and within runs are <10 %. The assay includes appropriate recombinant protein standards and is ready-to-use.

Serum or plasma samples can be used for analysis. The same type of sample should be used within a specific project.

Sample stability

Cytokeratins are released into the circulation as protein complexes. These complexes are remarkably stable during sample collection and long-term storage. Furthermore, plasma/serum samples can be exposed to repetitive freeze-thaw cycles without loss of activity (Olofsson *et al.*, 2007).

Quality commitment

The M30-Apoptosense® ELISA is registered in accordance with the IVD-CE directive. PEVIVA's quality system is ISO 9001 and ISO 13485 certified.

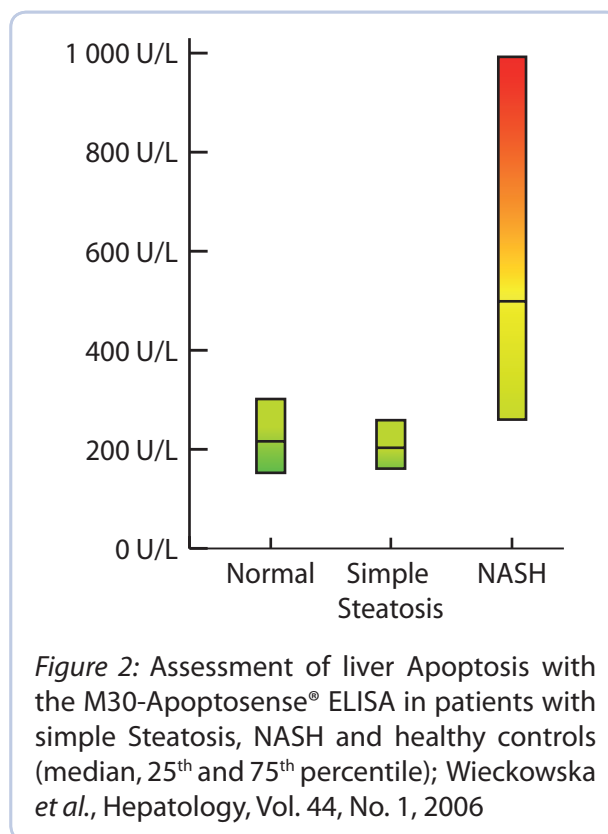


Figure 2: Assessment of liver Apoptosis with the M30-Apoptosense® ELISA in patients with simple Steatosis, NASH and healthy controls (median, 25th and 75th percentile); Wieckowska *et al.*, Hepatology, Vol. 44, No. 1, 2006

References

- Leers *et al.*, 1999, J Pathol 187, 567-572
- Hägg *et al.*, 2002, Invest. New Drugs, 20: 253-259
- Cummings *et al.*, 2005, Br J Cancer 92, 532-538
- Olofsson *et al.*, 2007, Clin Cancer Res. 13:3198-206
- Cummings *et al.*, 2008, Mol Cancer Ther. 7:455-63

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Prod. no. 10040

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