



Published in final edited form as:

J Lab Precis Med. 2018 March ; 3: . doi:10.21037/jlpm.2018.03.07.

Is Keratin-18 only a Marker of Cell Death in Acute-On-Chronic Liver Failure?

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Acute liver failure (ALF) due to a variety of causes has been well established clinically for a number of decades (1). An intense research focus has recently been generated in the realm of acute-on-chronic liver failure (ACLF), especially in light of the high number of cirrhotic patients that eventually present to the clinic (2). The pathology and clinical history of ACLF is poorly understood and due to its nebulous clinical nature, there are no current mainline treatments. Cell death is a common problem and is noted in nearly every clinically relevant type of liver disease. In a recent manuscript Macdonald and colleagues attempted to clarify basic cell death mechanisms involved in ACLF by measuring a number of serum biomarkers of cell death in cirrhotic patients presenting with acute decompensation or in ACLF patients (3).

Using a broad definition of acute decompensation based on common clinical secondary morbidities in patients with cirrhosis, they succinctly demonstrated differences in two biomarkers of cell death, serum levels of Keratin-18 levels (K18) and cleaved Keratin-18 (cK18) that represent hepatic necrosis and apoptosis, respectively (3). The authors found that cK18 levels predominated in a majority of the patients they studied; the one exception being patients with severe ACLF having a substantial rise in K18, an indicator of necrosis. Moreover, patients with more severe decompensation or more severe ACLF had significantly higher cK18/K18 levels as did patients with indicators of increased inflammation such as elevated white blood cell count, and elevated interleukin-8 (IL-8) levels. These data substantiate the idea that cell death and systemic inflammation are critical players in ACLF (2,4). Perhaps most importantly, the addition of cK18 values helped predict progression to ACLF after acute decompensation in cirrhotic patients, which can assist clinicians in defining which patient populations are most susceptible to life threatening ACLF and inform clinical care.

One of the primary causes of ACLF is alcoholic hepatitis and patients with alcoholic hepatitis were a primary group included in this study (3). K18 and cK18 have also been proposed as potential prognostic and diagnostic markers in alcoholic hepatitis (5,6). Their use as biomarkers has been of special interest to the Hepatology community as there are currently no available serum biomarkers that can accurately diagnose alcoholic hepatitis, and

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Conflicts of Interest: The authors have no conflicts of interest.

thus transjugular liver biopsy remains the gold standard for diagnosis. Their inception and use as a diagnostic biomarker could potentially save a number of procedures and assist in diagnosis in tertiary units without the capacity to perform transjugular liver biopsy regularly (3,5,6). K18 and cK18 scores were elevated in non-surviving patients in this study. Moreover, there was a reduction in the cK18-to-K18 ratio indicating that the presence of necrotic cell death is associated with non-survival (3). These observations largely mirror previous data found in alcoholic hepatitis patients by our own group (5). Further studies are necessary in this area in large, multi-institutional cohorts to validate the potential for cK18-to-K18 ratio or the individual values alone to predict mortality (7). Multiple studies have now confirmed increases in K18 and cK18 in AH patients (5,6). The current study largely confirms these data but notes that due to near ubiquitous increases in K18 and cK18 in clinically relevant liver diseases, these parameters alone may not be sufficient for establishing ACLF/alcoholic hepatitis (3).

K18 and cK18 have been used extensively to classify modality of cell death in a number of liver disorders including cholestasis, ischemia-reperfusion, non-alcoholic fatty liver disease, acetaminophen overdose, alcoholic hepatitis, and more (5,8–13). Notably, in a majority of these disorders AST and ALT values correlate strongly with K18 and cK18 values (8–13). As a number of these diseases also feature substantial rises in ALT levels, commonly above 300, and up to 5,000 U/L in the case of acetaminophen overdose, this also means there are very large concurrent changes, especially in K18. Surprisingly though, the notable exception to this rule is alcoholic hepatitis (3,5). A number of papers, including the study from Macdonald and colleagues have measured K18 and cK18 in serum or plasma of alcoholic hepatitis patients either directly looking at alcoholic hepatitis or using alcoholic hepatitis as a model for ACLF. In these studies K18 and cK18 levels are >1,000 U/L despite the fact that ALT levels are rarely elevated in the context of alcoholic hepatitis (3,5,6). This is likely due to endogenous suppression of the ALT enzyme by alcohol (14). Previous work from our laboratory has noted that K18 levels can increase to as much as 30,000 U/L in the absence of increases in ALT in alcoholic hepatitis (5). As such, while K18 is likely a very good biomarker of liver injury in multiple models, K18 elevations in ACLF patients, especially those with alcoholic hepatitis, may be related to factors other than just cell death (5,7). This hypothesis is further supported by the terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) assay (3,5). TUNEL staining of purely apoptotic cells is constrained entirely to the nucleus as caspase-activated DNase generates mono- and multi-nucleosomal DNA fragments, which leak out into the cytosol but are not detected by the assay due to their small size (15). In contrast, necrosis results in diffuse TUNEL positivity within the cells indicative of nuclear breakdown and release of large DNA fragments into the cytosol, where they contribute to the TUNEL staining (15,16). This is most accurately represented in acetaminophen overdose samples, where TUNEL staining is highly diffuse, and there is no activation of caspases in humans or in the overwhelming majority of murine models (15,17–19). The nuclear DNA fragmentation under these conditions is caused by mitochondria-derived apoptosis-inducing factor and endonuclease G, which translocate to the nucleus (20,21). TUNEL staining of alcoholic hepatitis samples in the current study display the classical nuclear specific TUNEL staining in a majority of the no ACLF samples; however, a majority of ACLF patients have TUNEL staining through the cytoplasm

indicating hepatic necrosis (3). This is evidenced most clearly in the Hepatitis B samples, but is also apparent in alcoholic hepatitis samples. This stands in direct contrast to their serum data, which largely has cK18-to-K18 ratios relatively close to 1 (3). Generally, as cK18 represents the apoptotic fragment and K18 represents all cell death, this would suggest that cell death in this group is almost all apoptotic in nature, despite the fact the TUNEL staining does not necessarily recapitulate these data. Given this information, it is difficult to discern the exact modality of cell death, or whether there are other mechanisms at play. It remains a possibility that a portion of cK18 and K18 is derived from other tissues, or potentially, is released from the liver through mechanisms other than traditional cell death (7). There is a need for studies that can delineate where cK18 and K18 arise from in some diseases with systemic inflammation and multi-organ dysfunction.

Understanding the role of apoptosis and necrosis, respectively, is imperative for developing novel therapeutics for ACLF. The current studies further demonstrate the use of cK18 and K18 as potential prognostic biomarkers and biomarkers of disease progression. Future studies aimed at following K18 and cK18 values over time in patients may yield novel and highly informative data determining how cell death occurs over periods of decompensation. More studies are also needed in this area to delineate differences in K18 versus traditional markers of hepatic necrosis.

Acknowledgments

The work in the authors' laboratory was supported in part by the National Institutes of Health grant R01 AA12916, the Robert Hanlon Trust, and grants from the National Institute of General Medical Sciences (P20 GM103549 and P30 GM118247) of the National Institutes of Health.

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