

Bile acids are important contributors to AKI associated with liver disease: CON

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When a clear answer to a scientific question is elusive, it is sometimes valuable to take a contrarian position so as to question your original assumptions. If you had asked whether we believed bile cast nephropathy was “real” prior to writing this article, we would have answered “probably.” However, three factors suggest that interpreting bile cast nephropathy as a simple obstructive disease leading to tubular damage is incomplete: (1) absence of a clear dose-response associations between hyperbilirubinemia and kidney injury, (2) lack of convincing experimental modeling implicating bilirubin in the pathogenesis of kidney damage, and (3) the bioenergetic interactions of bile acids suggest alternate (and potentially more complicated) explanations for these histologic observations are more likely.

Renal excretion of bilirubin break-down products is a well described phenomenon. Normally, bilirubin is metabolized into colorless urobilinogen, which is renally excreted. However, in situations where serum levels are elevated, bilirubin is also excreted in the urine, thus generating its dark hue. There is no doubt that bilirubin stained casts can be detected histologically in patients with cirrhosis and acute kidney injury (AKI), as has been described in multiple autopsy series^{1,2}. However, there are a lack of convincing epidemiologic associations between this pathologic finding and clinical AKI to support its role as a causative mechanism of injury. To start, hyperbilirubinemia is incredibly common in decompensated cirrhosis, yet reports of bile cast nephropathy exist on the case report level^{3,4}. Because AKI is also so prevalent in this setting⁵, one would expect at least some dose-response relationship between serum bilirubin, bilirubin excretion, and kidney function to be present on a population level, but such data is lacking. Further, the few extant case reports of bile cast nephropathy tend to draw from autopsy series, which are subject to selection bias of unusual or severe phenotypic presentations of disease and may be compromised by post-mortem preparation artifact.

Because of the risk of bleeding, there are only a few case series of native kidney biopsies in cirrhotic populations. The largest series of native kidney biopsies in liver transplant candidates, performed by Wadei et al., examined the renal parenchyma of 128 cirrhotic patients

and did not report bile casts as a prominent histologic feature⁶. In fact, elevated serum bilirubin was associated with less tubulointerstitial damage overall. The correlation between serum bilirubin and kidney function in non-cirrhotic populations is mixed at best, with multiple studies even reporting that higher serum bilirubin is positively correlated with estimated glomerular filtration rate (eGFR)⁷⁻⁹. It is possible this association is secondary to bilirubin scavenging superoxide, resulting in increased renal bioavailability of nitric oxide and enhancement of renal blood flow¹⁰.

If bilirubin and bile casts were truly nephrotoxic, there should be some experimental models that support a mechanistic link between excess bile products and kidney injury. What is needed is evidence of AKI associated solely with a rise in bilirubin, uncomplicated by acute or chronic liver failure and their own concordant multi-factorial renal insults. However, this too is absent from the literature. We know of no animal studies where infusion of exogenous bilirubin or bile acids result in the presence of bilirubin casts, histologic tubular damage, and increased renal excretion of such products to tie together a convincing mechanistic narrative. To the contrary, multiple studies have found elevated serum bilirubin levels, achieved either through genetic manipulation or intravenous or intraperitoneal infusion, to be protective against AKI. Heme oxygenase-1 (HO-1) is induced as an adaptive and protective response to tissue injury. One of the mechanisms by which this protection is postulated to occur is through the degradation by HO-1 of heme into biliverdin and subsequently to bilirubin, known to have powerful antiapoptotic and antioxidant effects. Adin et al. utilized a bilirubin flush prior to a 20-minute period of warm ischemia in an isolated, perfused rat kidney model to examine the potential of bilirubin for nephroprotection¹¹. Bilirubin treatment as compared to control resulted in significant improvements in renal vascular resistance, urine output, glomerular filtration rate, tubular function, and mitochondrial integrity after ischemia-reperfusion injury (IRI) and the beneficial effects were found to be dose dependent, providing strong evidence of causality. A subsequent study found intravenous infusion of bilirubin before and after IRI in Sprague-Dawley

rats resulted in a dose dependent improvement in post-IRI serum creatinine and a trend towards preservation of cortical proximal tubules in rats receiving a higher dose of bilirubin¹².

Examining the interaction between elevated bilirubin and nephrotoxins, Oh et al. studied rats exposed to cyclosporine, with and without pre-treatment with bilirubin¹³. In the experimental arm, intraperitoneal infusion of bilirubin led to a 30-fold increase in the serum concentration. As compared to controls, bilirubin treated rats had significantly lower urinary levels of kidney injury molecule-1 (KIM-1) and a strong trend towards lower neutrophil gelatinase-associated lipocalin (NGAL) after exposure to cyclosporine. Histologically, bilirubin treated rats had significantly reduced afferent arteriopathy, tubulointerstitial fibrosis, and tubular injury and a significantly lower number of apoptotic proximal tubule cells. In cell culture, bilirubin exposure significantly reduced the production of intracellular reactive oxygen species. Gunn rats, deficient in the enzyme uridine diphosphate glucuronyl transferase, were used by Barabas et al. to investigate the in-vivo effects of unconjugated hyperbilirubinemia on cisplatin nephrotoxicity¹⁴. This model is critical as it assesses the effects of elevated bilirubin in isolation, outside of any injury or other intervention. Elevated serum bilirubin was found to have a nephroprotective effect, with significantly lower BUN and creatinine in homozygous Gunn rats when compared with control congenic Wistar rats at day 5. Histological grading demonstrated significant preservation of the S3 segment in the Gunn rats compared to controls. While the mechanism whereby bilirubin affords nephroprotection is not fully clear, there are suggestions it relates to the pro/anti-inflammatory balance of bile acids impacting systemic oxidative stress in the vascular compartment¹⁵.

Common bile duct ligation (CBDL) in rats and mice is a classic model of obstructive end stage liver disease where elevated serum bilirubin and abnormal kidney function parameters are noted, and AKI occurs shortly after the onset of liver fibrosis¹⁶. If the association between bilirubin and bile salts with AKI is explicitly causal, this should be a mechanistically parsimonious model to replicate purported bile cast nephropathy noted on autopsy. However,

histologic examination of the renal parenchyma of these animals at sacrifice routinely demonstrates relatively preserved tubular structure, and again, bile casts are often absent, especially early in the model timeline, when evidence of AKI is already noted on serology or via measurement of glomerular filtration rate . Kronos et al. fed animals norursodeoxycholic acid (norUDCA), a conjugated bile acid thought to promote favorable bioenergetic cellular interactions¹⁷. While they did describe evidence of kidney parenchymal injury at 8 weeks that was ameliorated by norUDCA, the degree of histologic injury was quite mild, and is confounded by the long delay between the onset of AKI (usually within 1-2 weeks of bile duct ligation) and the examination of tissue at sacrifice 8 weeks later. Their findings could alternatively be explained by systemic bioenergetic interactions of norUDCA conferring a renoprotective effect, or it simply may be that after such a long period of time following an irreversible liver insult, these animals develop some degree of ischemic kidney injury. Diffuse rarefaction of the renal arterial bed has been demonstrated following CBDL in mice (**Figure 1**) and may be expected in and of itself to foment tubular injury and fibrosis. CBDL mice have also been noted to have interstitial nephritis, but this diagnosis was not based on the actual observation of acute inflammatory cells but rather detection of increased local VCAM-1 and macrophage marker F4/80 expression in both the interstitium and glomeruli¹⁸. To any extent then that hyperbilirubinemia does play a role in renal dysfunction, it is possible this is not due to any direct tubular toxicity but instead via perturbations to renal and systemic hemodynamics. Elevated bilirubin has been shown to exert negative inotropic and chronotropic cardiac effects, sometimes referred to as “jaundiced heart”. Reduced enteral bile acids concentrations contribute to increased bacterial translocation and systemic endotoxemia, engendering splanchnic and systemic vasodilatation, reduced effective circulating volume and renal hypoperfusion¹⁹. Both of these mechanisms may contribute to the development of pre-renal azotemia and ischemic acute tubular necrosis (ATN), producing AKI absent any direct tubular pathogenic role for bilirubin, bile salts or casts.

If bilirubin casts however are not to be implicated as causal in AKI, how then does one explain the presence of such casts in autopsy series and, more commonly, in the microscopic evaluation of urine from patients with hyperbilirubinemia and AKI? Clinical conditions associated with significant elevations in serum bilirubin levels are also frequently complicated by AKI in the form of ATN. In the setting of hyperbilirubinemia, the kidneys dramatically increase bilirubin clearance via increased secretion through an organic solute transporter. When the granular casts that are the hallmark of ATN pass through the tubules in the presence of bilirubin, they become stained. It is not surprising then that utilization of a Hall or Fouchet stain would identify "bilirubin casts" in such patients with ATN. Indeed, the greater the degree of unrelated ATN, the more likely there should be bilirubin stained casts, both on examination of urine microscopy or via biopsy or autopsy. In addition, reduced filtrate in the setting of hypoperfusion and AKI will both increase cast formation and prolong their tubular transit time, increasing the likelihood they are seen on biopsy or autopsy²⁰. However, whether the ATN in these cases is itself due to the tubular toxicity of bilirubin and bile salts or to uniquely pathogenic bilirubin casts or whether the staining of granular casts is merely redecorating after the damage has been done is far from clear and the presence of such casts is by no means dispositive for pathologic culpability. The same ineluctable confusion regarding the chicken and the egg exists when appraising the significance of the widely acknowledged association between elevated urinary bile acids levels and AKI. Once filtered, bile acids are usually reabsorbed in the proximal convoluted tubule via a Na⁺-bile acid active cotransporter, ASBT, resulting in a fractional excretion of only 1-2%²¹. However, in the setting of cholestasis this is downregulated, increasing bile acid clearance²². Critically, in the presence of proximal tubule injury, the activity of virtually all local transporters is compromised and, in severe injury associated with sloughing of the brush boarder, rendered virtually non-functional. Thus, proximal tubule injury, as is seen with ATN secondary to a myriad of causes common in liver disease, will increase urinary bile acid levels, frustrating attempts to

ascribe the injury as being secondary to said elevations. Arguments against bile acids as important contributors to AKI associated with liver disease are summarized in **Table.1**.

It is important to remember that clinical attempts at diagnoses should be in service of optimizing a patient's treatment. Patients with cirrhosis and AKI are medically complicated and, by definition, suffer from multisystem organ failure. Very often, more than one renal insult is present, and patients rarely fit neatly into circumscribed diagnostic categories. Much remains left to learn about the impact of bilirubin and bile acids on overall organ function and hemodynamics and it is very possible that in some cases bile cast nephropathy may directly contribute to a multifactorial AKI, especially in cases of severe cholestatic injury and marked elevation in bilirubin^{23,24}. However, the presence of casts on urine microscopy in patients with cirrhosis and AKI, whether bile stained or not, is not ex post facto evidence that a rise in creatinine is primarily due to a structural injury nor does it speak to the presence or absence of retained tubular integrity. The treatment of patients with preserved residual renal tubular function who suffer from hypoperfusion and hemodynamic injury (such as prerenal AKI and hepatorenal syndrome) should be focused on restoring this perfusion via volume expansion, supportive care measures, and (as appropriate) splanchnic vasoconstrictors. While the historic neglect of hyperbilirubinemia as a contributing factor to AKI is lamentable, care must be taken not to expand the potential for mismanagement by diagnostically anchoring to a single culprit and ignoring other potentially readily treatable mechanisms of kidney injury.

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Tables

Table 1. Arguments against the existence of bile acid/cast nephropathy

- 1) Paucity of reported cases in a common condition

- 2) Selection bias inherent in autopsy series produces findings not generalizable to clinical settings

- 3) Population studies demonstrate elevated bilirubin associated with higher eGFR

- 4) Lack of studies showing toxicity from infused bilirubin

- 5) Bilirubin infusion shown to be protective against ischemic and nephrotoxic insults

- 6) Plausible alternative explanation for bilirubin stained casts in the setting of elevated urinary bilirubin and unrelated tubular injury

- 7) Inability to convincingly tie elevated urinary bile acids to tubular injury when levels would be expected to rise secondary to injury

- 8) Well documented systemic and renal hemodynamic effects of cirrhosis and hyperbilirubinemia provide multiple plausible physiologic alternative etiologies for AKI

Figure 1. Control rat kidney cortex vasculature and evidence of rarefaction at 21 days post-CBDL

Adopted with permission from Velez JCQ, et al. *Nat Rev Nephrol.* 2020;16(3):137-155, Courtesy of Dr. Luis Juncos

Abbreviations: CBDL, common bile duct ligation

Figure 1



CONTROL



CBDL