The Kidney in Liver Disease

The pivotal prognostic role of renal function in liver disease is reflected by the inclusion of creatinine in the Model for End-Stage Liver Disease Score (MELD Score), which is currently used for prioritization of patients on the waiting list for liver transplantation [1–3]. Acute kidney injury (AKI), which commonly occurs in patients with end-stage liver disease, represents a landmark event in the disease course. The most frequent causes for AKI in cirrhosis include (1) prerenal AKI, followed by (2) acute tubular necrosis and (3) postrenal causes, the latter representing less than 1% of cases [4]. Hepatorenal syndrome (HRS) type I, a functional and potentially reversible nonvolume responsive variant of prerenal AKI, is defined as progressive renal failure in patients with rapid decompensation of cirrhosis or acute (or chronic) liver failure in the absence of identifiable causes [4–6]. The current concept of the pathophysiology of HRS includes splanchnic vasodilatation with subsequent hemodynamic impairment and intrarenal vasoconstriction resulting in impairment of kidney function [5, 7]. Among others, recent diagnostic criteria for HRS I include the exclusion of parenchymal renal disease (proteinuria <0.5 g/day, <50 red cells/HPF and regular renal ultrasonography) [4]. Interestingly, besides previous clinical studies reporting structural kidney injury in cirrhotic patients [8], a recent

Key Words
Acute kidney injury · Cholemic nephropathy · Hepatorenal syndrome

Abstract
Kidney injury in deeply jaundiced patients became known as cholemic nephropathy. This umbrella term covers impaired renal function in cholestatic patients with characteristic histomorphological changes including intratubular cast formation and tubular epithelial cell injury. Cholemic nephropathy represents a widely underestimated but important cause of kidney dysfunction in patients with cholestasis and advanced liver disease. However, the nomenclature is inconsistent since there are numerous synonyms used; the underlying mechanisms of cholemic nephropathy are not entirely clear, and widely accepted diagnostic criteria are still missing. Consequently, the current article aims to summarize the present knowledge on the clinical and morphological characteristics, available preclinical models, derived potential pathomechanisms, and future diagnostic and therapeutic strategies in cholemic nephropathy. Furthermore, we provide a potential research agenda for this evolving field.

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histopathological study revealed vascular and predominant tubular injury in patients with liver cirrhosis and impaired renal function in the absence of proteinuria and hematuria [9]. Since comorbidities are frequently present in patients with liver cirrhosis (e.g. diabetes), the different etiologies of AKI in cirrhosis could overlap. Particularly HRS may be difficult to differentiate from other causes of AKI in clinical practice. An accurate evaluation of renal function is of utmost importance in patients with cirrhosis, but is difficult to achieve. Although creatinine is an easily measurable and widely available marker of excretory renal function, it has several limitations in assessing renal function in patients with liver cirrhosis [10–12]. As a result of malnutrition, low protein intake, decreased creatinine synthesis and increased tubular secretion of creatinine, renal function in patients with liver cirrhosis is frequently overestimated using creatinine-based methods to estimate glomerular filtration rate (GFR) [10]. GFR estimates using cystatin C, a nonglycosylated low-molecular-weight protein of the cystatin superfamily of cysteine protease inhibitors [13], have been proposed to be superior predictors of renal function than creatinine-based formulas [14, 15]. Unlike creatinine, serum cystatin C is independent of muscle mass, age and gender, and is not influenced by serum bilirubin or malignancy [10, 11, 13, 16]. In cirrhosis, cystatin C has been considered a more sensitive indicator of renal function compared to creatinine [17–24]. However, cystatin C was also shown to be influenced by other factors than GFR, such as high C-reactive protein and white blood cell count or low serum albumin, which are frequently present in cirrhosis and consequently impair the reliability of cystatin C-based equations [25]. Direct measurement of GFR (e.g. by inulin, $^{51}$Cr-EDTA, iohekol or iohamate clearance) has been considered the gold standard, but for clinical practice, direct measurement of GFR is technically demanding, time-consuming and costly.

Taken together, (1) renal function plays a pivotal prognostic role in patients with liver disease, (2) accurate differential diagnosis of AKI in cirrhosis is a clinical challenge and (3) exact measurement of GFR is limited due to low reliability of standard clinical markers of excretory renal function.

The Kidney in Cholestasis – Cholemic Nephropathy

The association between obstructive jaundice and kidney injury is a well-known clinical phenomenon and unresolved problem [26–28]. The kidney alterations in obstructive jaundice became known as ‘cholemic nephropathy’ (CN), an umbrella term describing renal dysfunction in jaundiced patients with characteristic histopathological findings on kidney histology. However, there still exists some kind of Babylonian confusion since numerous synonyms in the literature such as icteric nephrosis/nephropathy, jaundice-related nephropathy, bile cast nephropathy and (most recently) bile acid nephropathy have been proposed [29–32]. CN and its synonyms have almost disappeared from pathology textbooks and modern medical literature [33]. Nevertheless, to ignore the contribution of these phenotypical renal changes would lead to an incomplete representation of the spectrum of renal injury that may occur in the setting of liver disease [33]. The clinical phenomenon of AKI in obstructive jaundice was already described in 1911 by P. Clairmont and H. v. Haberer in a work entitled ‘Mitteilung über Anurie nach Gallensteinoperationen’ (‘Communication about Anuria following Gallstone Surgery’). In the following years, surgeons in particular were aware of perioperative renal dysfunction/failure in patients with obstructive jaundice who underwent invasive diagnostic and therapeutic procedures [28, 34]. For years, chronic cholestatic liver diseases have been reported to be associated with tubulointerstitial nephropathies such as tubulointerstitial nephritis and Fanconi syndrome (e.g. pediatric cholestatic syndromes, primary biliary cirrhosis, primary sclerosing cholangitis, drug-induced/steroid-induced cholestasis) [35–41]. Additionally, there are several reports on cholestatic liver diseases associated with progressive tubulointerstitial nephropathy as rare hepatorenal disorders and as a distinct entity in early childhood [35, 36, 42–44]. Clinical features in all these cases were comparable, including early onset of tubulointerstitial nephritis in parallel with clinical and laboratory signs of cholestasis and a renal histology characterized by tubular atrophy and dilatation, as well as interstitial and periglomerular fibrosis finally leading to end-stage renal disease [37].

Besides several clinical descriptions and former studies on CN which mainly occurred between 1920 and 1970 [30, 31, 45–48], animal experiments with regard to etiopathogenetic concepts were performed and most frequently rats and dogs were used [45, 46, 49–52]. Many pathogenetic concepts included oxidative stress, which was hypothesized to be induced by renally eliminated cholephiles, such as bilirubin, subsequently causing tubular epithelial kidney injury [53]. Besides bilirubin- and bile acid-induced oxidative stress leading to damage of tubular cell membranes, proposed mechanisms of re-
duced renal function in obstructive cholestasis to date include portal and systemic endotoxia via increased translocation from the intestine due to lack of enteral bile acids or alternative cholephiles, increased production and/or expression of vasoactive mediators such as endothelin or thromboxane and their receptors, direct tubular effects of bile, and volume depletion [49, 50, 53–63]. Bilirubin, however, has even been found to be renoprotective in experimental settings [64]. The most recent concepts revealed bile acids as key pathogenetic factors in CN [32, 65]. Cholestasis is characterized by the retention of potentially toxic cholephilic molecules such as bile acids or bilirubin, which accumulate within the liver and serum [66, 67]. Hepatocytes in human cholestatic liver diseases (e.g. obstructive jaundice, primary biliary cirrhosis, primary sclerosing cholangitis) and respective animal models (e.g. common bile duct ligation (CBDL) or bile acid-fed rodents) attempt to limit increased hepatocellular levels of bile acids and bilirubin via induction of excretory routes (i.e. basolateral hepatocellular export), facilitating their alternative renal elimination [68–72]. Despite the beneficial hepatic and systemic effects of alternative excretion of cholephiles, this may also flood the kidney ultimately causing renal injury, which was recently shown in CBDL mice, a mouse model for the most extreme form of cholestasis exhibiting all phenotypical features of CN [65].

**Definition and Characteristics of CN**

The term ‘cholemic nephropathy’ describes AKI/renal dysfunction in patients with jaundice together with typical histological changes that include a broad spectrum of renal injury, predominantly tubular epithelial injury directed to distal nephron segments together with intraluminal cast formation [29, 33]. Different names used in the literature such as bile cast nephropathy result from description of typical morphological alterations (bile casts) on the one hand and potential etiopathogenesis – as in the case of bile acid nephropathy – on the other hand. The use of the umbrella term ‘cholemic nephropathy’ might be advantageous since it neither restricts to a specific histology nor to a specific pathogenetic mechanism. This would also better fit the actual situation since up to now both are not entirely clear. Macroscopically, the renal cortex and medulla of cholemic kidneys are yellowish and become green after formalin fixation because of the conversion of bilirubin to biliverdin [33]. Due to higher concentrations of bilirubin in distal nephron segments, the green color is especially accentuated in the medulla (renal pyramids) [33]. To date, all histological kidney alterations in jaundice, including tubular epithelial changes and formation of bile casts, were reported to be predominantly present at the level of tubules and especially distal nephron segments [33, 65]. Intraluminal bile casts consist of exfoliated epithelial cells and could be easily identified and confirmed by histochemical Hall (or Fouchet) staining or periodic-acid Schiff (PAS) stains of kidney sections [33]. Besides intraluminal bile casts and tubular injury, the presence of mononuclear inflammatory cells in the vasa recta has been reported [33]. Data on glomerular alterations in obstructive jaundice and CN are scarce and limited to only a few reports on mesangial C3, glomerular C4d and IgA deposition, and hyperplasia of the parietal layer of Bowman’s capsule [45, 73, 74]. Key histomorphological characteristics of CN are shown by the example of hematoxylin and eosin-, PAS- and Sirius red-stained kidney sections of an 8-week common bile duct-ligated mouse in figure 1.

**Recent Clinical Evidence for CN**

Besides former descriptions and data from animal models, there is limited recent clinical evidence for the clinical relevance of CN in daily practice. As such, Uslu et al. [73] published a prospective study in 20 patients with short-term obstructive jaundice (mean duration of biliary obstruction of 15 days, mean total bilirubin: 10.1 ± 1.0 mg/dl), and demonstrated that those patients frequently showed acute tubular necrosis and venous dilatation upon renal biopsies despite maintenance of strict perioperative volume control and normal to only slightly impaired renal function (preoperative serum creatinine: 0.97 ± 0.1 mg/dl, mean GFR determined by MDRD equation: 81.9 ± 0.4 ml/min). Their study did not show a correlation between severity of jaundice (total bilirubin levels) and severity of the tubular and vascular changes observed.

In 2013, an autopsy study by van Slambrouck et al. [33] (41 autopsy cases and 3 patients with kidney biopsies) described bile casts in distal nephron segments in jaundiced patients with cirrhosis (e.g. due to alcohol, hepatitis C, NASH, cryptogenic), cholestasis (e.g. biliary obstruction, primary sclerosing cholangitis, cholangitis lenta) and severe acute liver dysfunction (e.g. fulminant AIH). Interestingly, and in contrast to the study by Uslu et al. [73], the presence of bile casts in these patients significantly correlated with the degree of jaundice (total and direct bilirubin levels). It is important to note that 13 of 44 pa-
tients in the study were clinically classified as having HRS; however, 11 (85%) out of these 13 patients showed bile casts upon kidney histology. In addition, bile casts were detected in all patients with alcohol-induced liver cirrhosis compared to bile casts in only 40% of patients with obstructive jaundice. The authors hypothesized that formation of bile casts contributes to kidney injury by direct toxicity of bilirubin and bile itself, as well as by tubular obstruction which would be a similar pathogenetic mechanism compared to myeloma or myoglobin cast nephropathy. In addition to patients with liver disease, 2 patients with hemolytic jaundice characterized by accumulation of indirect bilirubin were included. However, these 2 patients did not show bile casts upon kidney histology. In addition to the paper by van Slambrouck et al. [33], there have been some case reports on bile cast nephropathy [75–78]. As an example, Luciano et al. [32] reported on a case in a patient with severe cholestatic drug-induced liver injury and used the term ‘bile acid nephropathy’ for the first time. Patient characteristics and histomorphological findings of recent clinical trials and case reports on CN are summarized in table 1.

**Preclinical Models for CN – The Pathogenetic Role of Bile Acids**

By performing long-term CBDL in mice, which represents a severe form of obstructive cholestasis leading to excessive renal excretion of bile acids, we were recently able to mimic the histomorphological and functional alterations of CN. CBDL-induced tubular epithelial injury starts as early as 3 days after CBDL, predominantly at the level of collecting ducts with additional tubular epithelial injury and basement membrane defects leading to leaky tubules and obstruction of collecting ducts due to cell detritus and protein casts. This was followed by progressive interstitial nephritis and tubulointerstitial renal fibrosis in 3-, 6- and 8-week CBDL mice. Interestingly, modulation of bile acid composition significantly impacts on severity/degree of CN and renal function. Farnesoid X receptor knockout (FXR/–/) mice eliminate more hydrophilic, thus less toxic bile acids via urine during biliary obstruction [79]. In contrast to wild-type mice, FXR/–/– mice were therefore protected from CN [65]. In line with our concept that toxic bile acids might be the culprit for...
CN and a less toxic bile acid composition may prevent/reduce kidney injury, also prefeeding of the hydrophilic side-chain shortened bile acid ‘norursodeoxycholic acid’ prevents the development of collecting duct tubular epithelial lesions in CBDL mice. These findings argue for urinary excreted toxic bile acids to represent the pivotal trigger for tubular epithelial injury leading to CN in CBDL mice [65]. Taken together, cholestatic liver injury in CBDL mice results in a specific type of kidney injury with all phenotypical features of CN, and potentially toxic bile acids are a key determinant of this specific renal injury [65]. Based on the findings in CBDL mice, the following pathogenetic working model for CN has been proposed: alternative renal excretion of cholephiles in severe cholestasis leads to damage of collecting duct tubular epithelial cells, basement membrane alterations and leakage of urine into the kidney parenchyma with subsequent obstruction of collecting ducts due to cell detritus and protein casts. This leads to overexpression of proinflammatory and profibrogenetic cytokines finally resulting in interstitial nephritis and consequently tubulointerstitial kidney fibrosis [65]. However, the exact pathogenetic mechanisms of how cholestasis and in particular toxic bile acids cause tubular epithelial injury and subsequently CN remain elusive and have to be determined in detail.

Future Research Strategy and Potential Pathogenetic Concepts

Animal Models

Although recent data strongly argue for bile acids to be the main trigger of CN, there are still some open questions, and the exact mechanisms of bile acid-induced tubular epithelial injury in CBDL mice remain elusive. The fact that the CN-like renal phenotype has been to date only detectable in the CBDL mouse model [65], a model for the most extreme form of obstructive cholestasis and presumably the highest degree of alternative renal bile acid excretion, is only in part comparable to what has been found in humans. Bile cast nephropathy was also described in patients with severely impaired liver dysfunction or cirrhosis [33]. An explanation for this observation may be the more hydrophobic and thus more ag-

Table 1. Summary of recent clinical studies and case reports on CN in humans

<table>
<thead>
<tr>
<th>Author</th>
<th>Source of data</th>
<th>Subjects, n</th>
<th>Etiology of jaundice</th>
<th>Clinical/histological features</th>
</tr>
</thead>
<tbody>
<tr>
<td>van Slambrouck et al. [33]</td>
<td>Autopsy/Biopsy</td>
<td>44</td>
<td>Cirrhotic jaundice Cholestasis/obstructive jaundice Acute cholestatic hepatitis</td>
<td>Bile casts predominantly at distal nephron segments Variable degree of acute tubular injury Mononuclear inflammatory cells in vasa recta</td>
</tr>
<tr>
<td>Uslu et al. [73]</td>
<td>Biopsy</td>
<td>20</td>
<td>Obstructive jaundice</td>
<td>Acute tubular necrosis Dilatation of peritubular venules</td>
</tr>
<tr>
<td>Betjes and Bajema [29]</td>
<td>Biopsy</td>
<td>2</td>
<td>Obstructive jaundice</td>
<td>Bilirubin pigment in tubules Acute tubular necrosis Granular casts upon urine sediment</td>
</tr>
<tr>
<td>Luciano et al. [32]</td>
<td>Clinical Urine microscopy/Biopsy</td>
<td>1</td>
<td>Severe steroid-induced severe cholestatic liver injury</td>
<td>AKI Bile-stained granular casts and renal tubular epithelial cells upon urine microscopy Acute tubular injury with greenish-brown casts in distal tubular lumina</td>
</tr>
<tr>
<td>Rafat et al. [77]</td>
<td>Biopsy</td>
<td>1</td>
<td>Obstructive jaundice (cholangiocarcinoma)</td>
<td>Deterioration of kidney function Bile thrombi in dilated tubules Bile granules in cytoplasm of tubular epithelial cells</td>
</tr>
<tr>
<td>van der Wijngaart et al. [75]</td>
<td>Biopsy</td>
<td>1</td>
<td>Obstructive jaundice</td>
<td>Bile casts in tubes Reactive changes of tubular epithelial cells</td>
</tr>
<tr>
<td>Song [78]</td>
<td>Autopsy</td>
<td>1</td>
<td>Severe liver dysfunction</td>
<td>Numerous pigmented casts in distal tubules or collecting ducts</td>
</tr>
</tbody>
</table>

Bile Acid-Induced CN
gressive bile acid composition in humans compared to the relatively mild hydrophilic bile acid pool in mice. Therefore, animal models for cholestatic liver disease and liver cirrhosis should be carefully screened for a kidney phenotype (intraluminal casts, sloughing of tubular epithelial cells) by light microscopy of PAS- or Hall-stained kidney sections with a special focus on distal nephron segments (e.g. collecting ducts) and for kidney injury by the use of novel biomarkers for tubular injury such as urinary neutrophil-gelatinase-associated lipocalin. In addition, the detailed composition of the intratubular bile casts and their contribution to the pathogenesis of CN has to be determined.

Renal Autophagy as a Potential Modulator in CN

Autophagy, a highly conserved catabolic process of ‘self-eating’, is an essential mechanism to maintain cell homeostasis in eukaryotic cells [80–82]. In kidney health and disease, autophagy is an emerging biological pathway [83]. As such, autophagy was shown to regulate many aspects of normal and disease conditions in the kidney (e.g. diabetic nephropathy, tubular injuries, kidney development, aging, cancer and genetic diseases) [83]. The course of various kidney diseases can be modified by induction of autophagy by ischemic, toxic, immunological and oxidative insults [84]. Upregulation of autophagy in tubular epithelial cells in response to kidney injury caused by nephrotoxins, ischemia/reperfusion models or model of obstructive uropathy has been reported in several studies [85–88]. Regarding the different studies, however, there is a discrepancy whether autophagy supports survival or apoptosis of tubular epithelial cells [85–88]. Nevertheless, recent data argue for autophagy as an adaptive and protective mechanism for cell survival in pathological conditions or cell stress [85, 88]. As such, distal tubular deficiency of Atg5, a core component of the autophagic maturation pathway, dramatically sensitizes kidneys to ischemia/reperfusion injury as indicated by impaired kidney function, accumulation of damaged mitochondria and increased tubular cell apoptosis and proliferation [85]. Additionally, tubular deletion of Atg5 was shown to result in impaired kidney function with accumulation of p62 and oxidative stress markers already under physiological conditions [85]. Using mouse models of cisplatin- and ischemia-reperfusion-induced AKI, it was shown that blocking autophagy by the use of chloroquine, a pharmacological inhibitor of autophagy, enhanced AKI in both models [89]. In addition, Atg7-knockout mice were shown to be markedly more sensitive to cisplatin-induced AKI resulting in functional renal impairment, tissue damage and apoptosis [89]. These studies highlight an important role of autophagy in maintaining cell integrity of tubular cells during stress conditions. Taken together, autophagy is a critical mechanism for maintaining renal tissue homeostasis in stress conditions and mice defective in autophagy are prone to kidney injury. Renal accumulation of bile acids during cholestasis could represent such a stress condition. Consequently, the relevance of autophagy in the pathogenesis of CN needs clarification.

In a Clinical Setting

CN is an important and probably overlooked condition that might at least contribute to AKI and/or renal dysfunction in patients with severe liver disease. This may arise from the fact that kidney biopsy or autopsy studies are needed for the diagnosis of CN. However, conventional kidney biopsies are risky in patients with liver disease, especially those with cirrhosis with frequently significantly impaired blood coagulation. In addition, the distal nephron segments, which have been reported to be of special diagnostic importance in the setting of CN [33], would probably not be reached by kidney biopsy since this harbors a significant risk for bleeding. Transjugular kidney biopsy may represent an interesting and suitable alternative to significantly reduce such risks in this difficult to manage group of patients since this was shown to be safe in a recent study from the Clichy group [9]. With the exception of biopsy, however, there is no noninvasive diagnostic test available that specifically recognizes CN with the exception of biopsy. Future clinical research strategies should focus on development of readily available and easily applicable diagnostic tests or biomarkers for the detection of structural renal lesions in patients with liver disease. For that aim we are currently testing whether simple urine cytology analysis may represent a suitable noninvasive clinical test. Based on the fact that creatinine and its derived equations are inappropriate to accurately determine GFR in cirrhosis and the increasing number of reports on structural kidney injury in patients with liver disease [8, 9, 33], our current definition of AKI in cirrhosis and HRS probably needs to be redefined. A recent PubMed literature search using the terms cholemic nephropathy, cholemic nephrosis, icteric nephrosis and bile cast nephropathy resulted in about 18 publications compared to about 1,900 results for HRS. Therefore, the question arises whether CN is irrelevant in clinical practice or if we might have overlooked it due to limited diagnostic options as discussed above. The fact of the limited clinical effectiveness of terlipressin treatment in HRS

372 Dig Dis 2015;33:367–375
DOI: 10.1159/000371689

Krones/Wagner/Eller/Rosenkranz/Trauner/Fickert
with a reported response rate of about 34–40% [90, 91] additionally points towards the urgent need for more specific clinical tests for the differential diagnosis of impaired renal function in cirrhosis as we may probably be treating a mixed bag of patients all referred to as HRS. Such patients might have been diagnosed too late due to inappropiate accuracy of creatinine or they were already exhibiting subclinical structural kidney injury that is subsequenly prone to AKI and limits therapeutic response to vasopressin analogues since splanchnic vasodilatation might not represent the sole reason for renal impairment.

It seems obvious that AKI in patients with liver disease represents a wide group of different entities and CN could be at least one part of it. Consequently, we will have to expand our diagnostic and therapeutic tools for such patients in the future.

**Disclosure Statement**

This work was supported by grants from the Austrian Science Foundation (P24809-B19; to P.F. and F3517 to M.T.) and the Medical University of Graz (ASO2100100; to E.K.).

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Dig Dis 2015;33:367–375

DOI: 10.1159/000371689

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