Received: 6 January 2019 | Revised: 19 July 2019 | Accepted: 10 August 2019

DOI: 10.1111/liv.14229

Liver INTERNATIONAL WILEY

Prevalence of and risk factors for anaemia in patients with advanced chronic liver disease

Bernhard Scheiner[1,2] | Georg Semmler[1,2] | Florian Maurer[1,2] | Philipp Schwabl[1,2] | Theresa A. Bucsics[1,2] | Rafael Paternostro[1,2] | David Bauer[1,2] | Benedikt Simbrunner[1,2] | Michael Trauner[1] | Mattias Mandorfer[1,2] | Thomas Reiberger[1,2]

[1]Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria

[2]Vienna Hepatic Hemodynamic Laboratory, Medical University of Vienna, Vienna, Austria

Correspondence
Thomas Reiberger, Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna Waehringer Guertel 18-20, 1090 Vienna, Austria.
Email: thomas.reiberger@meduniwien.ac.at

Funding information
No financial support specific to this study was received.

Handling Editor: Christophe Bureau

Abstract

Background: Anaemia is common in advanced chronic liver disease (ACLD) as a result of various risk factors.

Aims & Methods: We evaluated the prevalence and severity of anaemia as well as the impact of anaemia on clinical outcomes in consecutive patients with ACLD and portal hypertension.

Results: Among 494 patients, 324 (66%) patients had anaemia. Anaemic patients showed higher MELD (12 ± 4 vs 9 ± 3; $P < .001$), lower albumin (34 ± 6 vs 39 ± 5 g/dL; $P < .001$) and more often Child-Pugh B/C stage (56% vs 17%; $P < .001$). The prevalence of moderate-severe anaemia (haemoglobin <10 g/dL) increased with the degree of portal hypertension (HVPG: 6-9 mm Hg: 22% vs HVPG: 10-19 mm Hg: 24% vs HVPG ≥ 20 mm Hg: 36%; $P = .031$). The most common aetiologies of anaemia were gastrointestinal bleeding (25%) and iron deficiency (9%), while reason for anaemia remained unclear in 53% of cases. Male gender (odds ratio [OR]: 1.94 [95% CI: 1.09-3.47]; $P = .025$), MELD (OR: 1.20 [95% CI: 1.09-1.32]; $P < .001$), hepatic decompensation (OR: 4.40 [95% CI: 2.48-7.82]; $P < .001$) and HVPG (OR per mm Hg: 1.07 [95% CI: 1.02-1.13]; $P = .004$) were independent risk factors for anaemia. Anaemia was associated with hepatic decompensation (1 year: 25.1% vs 8.1%; 5 years: 60.3% vs 32.9%; $P < .0001$), hospitalization (73% vs 57%; $P < .001$) and a higher incidence rate of acute-on-chronic liver failure (0.05 [95% CI: 0.04-0.07] vs 0.03 [95% CI: 0.01-0.04]). Anaemic patients had worse overall survival (1 year: 87.1% vs 93.7%, 5 year survival: 50.5% vs 68.6%; $P < .0001$) and increased liver-related mortality (1 year mortality: 9.7% vs 5.7%, 5 year mortality: 38.0% vs 26.9%; $P = .003$).

Conclusion: Two-thirds of patients with ACLD suffer from anaemia. The degree of hepatic dysfunction and of portal hypertension correlate with severity of anaemia. Anaemia is associated with decompensation, ACLF and increased mortality in patients with ACLD.

Abbreviations: ACLD, advanced chronic liver disease; ACLF, acute-on-chronic liver failure; ALD, alcoholic liver disease; CLD, chronic liver disease; CPS, Child-Pugh stage; CSPH, clinically significant portal hypertension; HVPG, hepatic venous pressure gradient; INR, international normalized ratio; IQR, interquartile range; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; OR, odds ratio; SD, standard deviation; TE, transient elastography.

KEYWORDS

advanced chronic liver disease, anaemia, portal hypertension, prevalence, severity

1 | INTRODUCTION

Anaemia is a frequently observed condition with an overall prevalence of 10%-24% in the general population and up to 95% in chronically ill patients as a result of infections, cancer or autoimmune disease.[1-5] Anaemia has not only been linked to decreased quality of life and cognitive impairment but also to an increased risk of cardiovascular mortality.[6-8] Furthermore, anaemia was shown to increase morbidity and mortality in several settings which might be attributed to the generalized decrease in oxygen-carrying capacity.[2,9-11]

Anaemia is also a common finding in patients with advanced chronic liver disease (ACLD) with a reported prevalence between 50%-87% and the highest prevalence being observed in patients presenting with hepatic encephalopathy.[12-15] Several causal factors for anaemia in patients with ACLD have been described: Firstly, acute or chronic blood loss caused by gastrointestinal bleeding from gastroesophageal varices,[16] portal hypertensive gastropathy, gastric antral vascular ectasia[17] or peptic ulcers[18,19] aggravate anaemia in the setting of ACLD. Secondly, functional and structural defects in the lipid membrane of erythrocytes may lead to the formation of acanthocytes (spur cells) with a short lifespan as a result of a higher susceptibility for degradation in the spleen.[20,21] Thirdly, hypersplenism induced by splenomegaly in patients with portal hypertension can induce pancytopenia and can therefore contribute to anaemia in ACLD.[22,23] Fourthly, malnutrition and malabsorption leading to vitamin B12 and folic acid deficiencies resulting in macrocytosis and macrocytic anaemia have been acknowledged as cause especially in patients with alcoholic liver disease.[12,24] Fifthly, as the liver secretes hepcidin, the main regulator of iron homeostasis, iron deficiency is common in ACLD patients.[25] Finally, aplastic anaemia occurring especially in young men within 6 months of acute hepatitis ('hepatitis-associated aplastic anaemia') as well as after orthotopic liver transplantation has been described. While the exact underlying mechanisms remain unknown in most cases of aplastic anaemia, viral infections have been suggested to play a central causative role.[26]

The clinical consequences of anaemia in ACLD include an increased risk for hepatic encephalopathy (and is associated with serum ammonia levels[15]), fatigue [27] and a higher Child-Pugh Score.[12,13,28] Importantly, anaemia has been linked to a deteriorating kidney function and development of the hepatorenal syndrome.[29]

Although, several studies evaluated the mechanisms of anaemia in patients with liver disease, most of them did not focus on patients with ACLD and did not provide data on portal pressure. Therefore, the aim of this study was to investigate the prevalence of and risk factors for anaemia in well-characterized patients with ACLD evaluated by liver stiffness and hepatic venous pressure gradient (HVPG) measurements. Most importantly, we also aimed to evaluate the impact of anaemia on hepatic decompensation and mortality.

Key points

- Anaemia is found in two-thirds of patients with advanced chronic liver disease (ACLD) including 7% with severe anaemia defined by haemoglobin levels <8 mg/dL.
- The most common causes for anaemia in ACLD are gastrointestinal blood loss and iron deficiency anaemia (IDA).
- Severity of anaemia is closely linked to hepatic dysfunction (reflected by MELD) and severity of portal hypertension (HVPG).
- The presence of anaemia is associated with worse clinical outcomes such as a higher rate of hepatic decompensation, hospitalization, acute-on-chronic liver failure (ACLF) as well as increased overall and liver-related mortality.

2 | MATERIALS AND METHODS

2.1 | Patients and definitions

All patients undergoing simultaneous HVPG and transient elastography (TE) measurements between August 2007 and December 2015 at the Medical University of Vienna were considered for this retrospective analysis. ACLD was diagnosed by TE (liver stiffness ≥10 kPa) or HVPG measurement (HVPG ≥ 6 mm Hg).[16,30] Patient characteristics including clinical and laboratory parameters were extracted from electronic patient records. Laboratory data from the day of HVPG measurement or within 2 months prior to or after HVPG measurement were recorded. Aetiology of liver disease, the presence of varices as well as previous hepatic decompensation were documented. MELD and the Child-Pugh scores were calculated.

Severity of anaemia was graded as 'mild' if haemoglobin (Hb) level was below gender-specific lower limit of normal (<12 g/dL in female and <13.5 g/dL in male patients) but ≥10 g/dL, as 'moderate' if Hb was <10 g/dL but ≥8 g/dL and as 'severe' if Hb was <8 g/dL. Cut-offs for mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were derived from the reference values of the local laboratory. Thrombocytopenia was defined as a platelet count <50 G/L and leukopenia as a white blood cell count <4 G/L respectively. Aetiology of anaemia was determined by the most probable cause according to laboratory values and/or endoscopy findings (within 2 months prior to or

after HVPG measurement): Bleeding anaemia was defined by case of normochromic/normocytic anaemia with findings suggestive of acute or chronic bleeding on endoscopy or other forms of anaemia with clear signs of bleeding on endoscopy. Iron-deficiency anaemia was diagnosed in case of hypochromic and/or microcytic anaemia and proven iron deficiency as shown by depleted serum transferrin and/or ferritin. Patients were classified to have vitamin B12/folic acid deficiency anaemia if erythrocyte indices revealed hyperchromic and/or macrocytic anaemia and laboratory evaluation revealed vitamin B12 or folic acid deficiency. Renal anaemia was attributed to patients with normochromic and/or normocytic anaemia and chronic kidney disease (CKD) stage 3b (as indicated by an estimated glomerular filtration rate of <45 mL/min) or higher without any other apparent explanations for anaemia.[31] Haemolytic anaemia was established in patients with normochromic and/or normocytic anaemia and either decreased haptoglobin or significant elevation of unconjugated bilirubin. Anaemia was attributed to chronic inflammation in patients with hypochromic and microcytic anaemia with significantly elevated ferritin levels. If the cause of anaemia could not be determined based on the available information, anaemia was classified as 'unknown/insufficient diagnostic workup'.

2.2 | HVPG measurement

HVPG measurements were performed at the Vienna Hepatic Hemodynamic Lab according to a standardized operating procedure[32] using a 7-French balloon catheter (Pejcl Medizintechnik). Briefly, the catheter introducer sheath was placed in the right internal jugular vein after local anaesthesia and under ultrasound guidance using the Seldinger technique. The balloon catheter was advanced under fluoroscopic guidance in the middle or right hepatic vein. HVPG was calculated as the mean difference between the wedged hepatic vein pressure and the free hepatic vein pressure after three measurements.[33]

2.3 | Liver stiffness measurements and definition of ACLD

Liver stiffness measurements were performed using FibroScan® (Echosens, Paris, France) by experienced operators as previously described.[34] The M and XL-probes were used according to the recommendations of the device. Only measurements considered to be reliable as according to previously published criteria were used for this study.[35] According to the Baveno VI consensus recommendations [30] and previous studies,[36,37] patients with a liver stiffness of >10 kPa on transient elastography were considered to have ACLD.

2.4 | Hepatic decompensation, overall survival and liver-related mortality

Survival was evaluated by data obtained from the National Death Registry provided by Statistic Austria. This dataset included information on the date of death, the ICD-10 code stated on the death certificate as well as the date of the last stay in an Austrian hospital. Based on these data and additional information obtained from medical records, the cause of death was attributed to liver disease (ie liver-related) or not. Furthermore, development of acute-on-chronic liver failure (ACLF)[38] and decompensation events during follow-up (new development of ascites or the need for large-volume paracentesis, development of hepatic encephalopathy [HE] or hospital admission for HE Westhaven grade III/IV, development of spontaneous bacterial peritonitis or variceal bleeding) were assessed as previously defined.[39] ACLF was diagnosed and graded in accordance with the publication by Moreau et al[40] as well as the online available CLIF-C ACLF score calculator. Therefore, ACLF grade 1 was defined as (a) patients with single kidney failure, (b) patients with a single organ failure as according to the CLIF-SOFA score and serum creatinine between 1.5 and 1.9 mg/dL and/or mild to moderate hepatic encephalopathy, and (c) patients with single cerebral failure presenting with a serum creatinine level ranging from 1.5-1.9 mg/dL. In the presence of 2 organ failures, ACLF grade 2 and if three organ failures were present, ACLF grade 3 was diagnosed respectively.[40] Additionally, the date of first hospitalization and the number of hospitalizations during follow-up were recorded.

2.5 | Statistics

Statistical analyses were performed using IBM SPSS Statistics 25 (SPSS Inc) and GraphPad Prism 8 (GraphPad Software). Continuous variables were reported as mean ± standard deviation (SD) or median (interquartile range [IQR]), and categorical variables were shown as numbers (n) and proportions (%) of patients. Comparisons of continuous variables between patients with and without anaemia were performed using Student's t-test or Mann-Whitney–U-Test, as applicable. Comparisons of categorical variables were performed using chi-squared test or Fisher's exact test. Spearman's rank correlation was used to investigate the correlation between HVPG and Hb. Binary logistic regression analysis with backward elimination was performed to evaluate factors associated with anaemia. Kaplan-Meier Curves were used to visualize the mortality as well as the development of hepatic decompensation during follow-up and log-rank test was applied for group comparisons of patients with and without anaemia as well as between patient groups with different severity of anaemia. A two-sided $P \leq .05$ was considered statistically significant.

2.6 | Ethics

This study was approved by the ethics committee of the Medical University of Vienna (No. 2122/2016). The need for written informed consent was waived by the ethics committee for this retrospective study.

3 | RESULTS

3.1 | Patient characteristics

In total, 951 patients undergoing HVPG and TE measurement within the study period were identified (Figure 1, Table 1). About

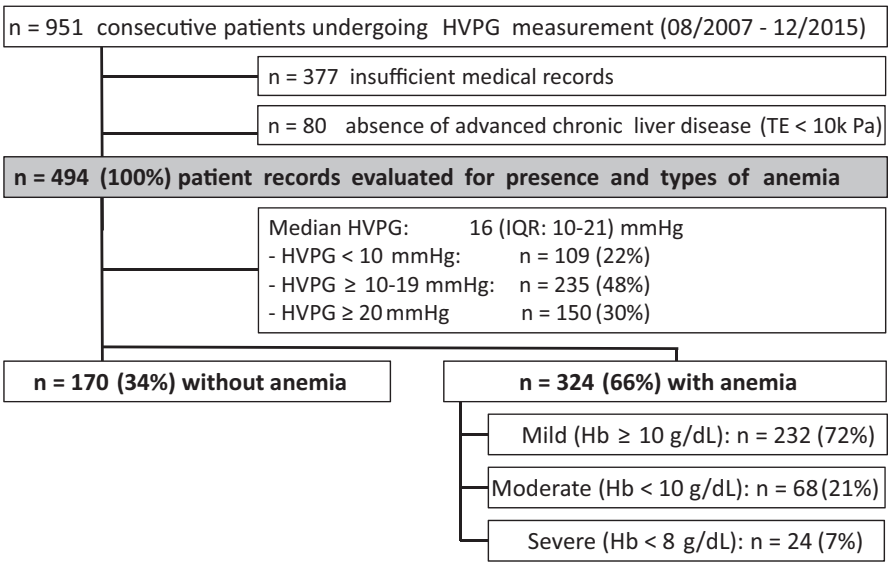

FIGURE 1 Patient flowchart. HVPG, hepatic venous pressure gradient; TE, transient elastography; Hb haemoglobin

377 patients had to be excluded because of insufficient medical records as well as 80 patients who did not fulfil the diagnostic criteria for ACLD. Finally, 494 patients were included in this retrospective analysis.

The majority of patients was male (n = 358, 72%) with a mean age of 55 ± 12 years. Chronic viral hepatitis was the most common aetiology of liver disease (n = 216, 44%), followed by alcoholic liver disease (ALD; n = 166; 34%). While 268 patients (54%) had compensated ACLD, 226 patients (46%) had experienced at least one event of previous hepatic decompensation (eg development of ascites/hepatic encephalopathy/variceal bleeding). Mean MELD score was 11.2 ± 4.1 points. While 286 patients (58%) had Child-Pugh stage (CPS) A, 176 patients (36%) presented with CPS B and 32 patients (7%) with CPS C. Varices of any size were present in 58% of patients (n = 287), whereas only 10% of patients (n = 50) had refractory ascites. Mean HVPG was 16 (IQR: 10-21) mm Hg and 385 patients (78%) had clinically significant portal hypertension (CSPH, HVPG ≥ 10 mm Hg). One hundred and fifty patients (30%) had HVPG values ≥20 mm Hg. More detailed patient characteristics are displayed in Table 1.

3.2 | Comparison of baseline characteristics between patients with and without anaemia

In total, 324 patients with ACLD presented with any kind of anaemia (66%) (Table 1). Additionally, thrombocytopenia was present in 69% (n = 342) and leukopenia in 31% (n = 151). While age and body mass index were comparable between patients with and without anaemia (55 ± 12 vs 56 ± 11 years; $P = .554$; 26 ± 6 kg × m^{-2} in both groups; $P = .587$), male gender (77% vs 65%; $P = .005$) and ALD (41% vs 19%; $P < .001$) were significantly overrepresented in the cohort of patients with anaemia. Spleen size was available in 329 (67%) patients. While splenomegaly was present in 61% of patients without anaemia, it was found in 74% of patients presenting with anaemia ($P = .020$) including 11 patients (5%) with extreme splenomegaly (>20 cm). Additionally, anaemic patients had lower serum albumin levels (34 ± 6 vs 39 ± 5 g/dL, $P < .001$), a higher international normalized ratio (INR; 1.3 ± 0.3 vs 1.2 ± 0.2, $P < .001$) as well as a significantly higher MELD score (12.2 ± 4.3 vs 9.4 ± 2.7; $P < .001$). Furthermore, patients with anaemia had more advanced liver disease as indicated by a higher CPS (56% vs 17.0% CPS B/C, $P < .001$), and a higher proportion of patients with previous hepatic decompensation (60% vs 18%, $P < .001$), as well as refractory ascites (15% vs 2%, $P < .001$) and varices (69% vs 37%, $P < .001$). Moreover, HVPG was also significantly higher in patients with anaemia (18 (13-22) vs 12 (8-17) mm Hg; $P < .001$) resulting in a higher prevalence of CSPH (84% vs 66%; $P < .001$). Furthermore, we observed a negative correlation of moderate strength between HVPG and level of haemoglobin (Spearman's rho: −0.382; $P < .001$, Figure 2).

Notably, in this real-life clinical setting, only 28 patients (9%) with anaemia received specific medication (eg iron or vitamin B12/ folic acid supplementation).

3.3 | Characteristics and aetiology of anaemia

In the cohort of patients with anaemia, 72% (n = 232) presented with mild anaemia, while 21% (n = 68) and 7% (n = 24) had moderate and severe anaemia respectively (Table 2). When studying red blood cell indices, 78% (n = 253) had normocytic, 13% (n = 42) had microcytic and 9% (n = 28) had macrocytic anaemia. Accordingly, the majority of patients (57%, n = 184) had normochromic erythrocytes whereas 27% (n = 89) and 19% (n = 61) were hypochromic and hyperchromic respectively. Thirty-two per cent of patients (n = 105) suffered from pancytopenia. When examining possible reasons for anaemia, gastrointestinal bleeding could be identified as leading cause in 80 patients (25%), iron deficiency in 28 patients (9%), vitamin B12/folic

TABLE 1 Comparison of ACLD patients with and without anaemia

	All patients, n = 494	Without Anaemia, n = 170	Any anaemia, n = 324	*P*-value
Sex, male/female (% male)	358/136 (72%)	110/60 (65%)	248/76 (77%)	**.005**
Age, years	55 ± 12	56 ± 11	55 ± 12	.554
BMI, kg×m^{-2}	26 ± 6	26 ± 6	26 ± 6	.587
Aetiology				
Alcohol, n (%)	166 (34%)	32 (19%)	134 (41%)	**<.001**
Viral hepatitis, n (%)	216 (44%)	107 (63%)	109 (34%)	
Other, n (%)	58 (11%)	16 (9%)	42 (13%)	
Cryptogenic, n (%)	54 (11%)	15 (9%)	39 (12%)	
Child-Pugh score (points)	7 ± 2	6 ± 1	7 ± 2	**<.001**
CPS A	286 (58%)	141 (83%)	145 (45%)	**<.001**
CPS B	176 (36%)	25 (15%)	151 (47%)	
CPS C	32 (7%)	4 (2%)	28 (9%)	
MELD points	11.2 ± 4.1	9.4 ± 2.7	12.2 ± 4.3	**<.001**
Compensated liver disease, n (%)	268 (54%)	140 (82%)	128 (40%)	**<.001**
Refractory ascites (n, %)	50 (10%)	3 (2%)	47 (15%)	**<.001**
INR	1.2 ± 0.3	1.2 ± 0.2	1.3 ± 0.3	**<.001**
Albumin, g/L	36 ± 6	39 ± 5	34 ± 6	**<.001**
Platelet count, G/L	126 ± 70	129 ± 59	125 ± 75	.515
Varices, n (%)[a]	287 (58%)	63 (37%)	224 (69%)	**<.001**
HVPG, mm Hg (IQR)	16 (10-21)	12 (8-17)	18 (13-22)	**<.001**
HVPG < 10 mm Hg (n, %)	109 (22%)	59 (35%)	50 (15%)	**<.001**
HVPG ≥ 10-19 mm Hg, n (%)	235 (48%)	91 (54%)	144 (44%)	
HVPG ≥ 20 mm Hg, n (%)	150 (30%)	20 (12%)	130 (40%)	
Haemoglobin levels (g/dL)	12 ± 2	14 ± 1	11 ± 2	**<.001**
Treatment for anaemia	32 (6%)	4 (2%)	28 (9%)	**.007**

P-values shown in bold indicate statistical significance.
Abbreviations: BMI, body-mass-index; INR, international normalized ratio; MELD, model for end-stage liver disease; HVPG, hepatic venous pressure gradient.
[a]Information on the presence/absence of varices was available in 356 patients (100 without and 256 with anaemia).

acid deficiency in 26 patients (8%) and renal anaemia in 16 patients (5%). Furthermore, anaemia was attributed to haemolysis in two patients and to chronic inflammation in one patient. Importantly, in 171 patients (53% the diagnostic workup was suboptimal to determine the exact type and aetiology of anaemia.

While gastroscopy is mandatory in this patient population in order to screen for gastroesophageal varices, colonoscopy is usually just performed after the age of 50 years for routine colon cancer screening or if there is the clinical suspicion of lower GI-bleeding. During the study period, 55 (17%) patients with and 14 (8%) patients without anaemia underwent colonoscopy.

Additionally, while 63 (13%) patients were diagnosed with any ongoing malignancy prior to study inclusion, 34 (7%) patients developed a new HCC during the study period. The prevalence of anaemia was comparable between patients with and without malignancy during the study period (*P* = .115).

3.4 | Comparison of anaemia characteristics between patients with different degrees of portal hypertension

In order to study the influence of portal hypertension on types and severity of anaemia, patients' characteristics were compared between the following HVPG strata: <10 mm Hg (n = 50), 10-19 mm Hg (n = 144) and ≥ 20 mm Hg (n = 130) (Table 2.). Severity of anaemia increased with rising HVPG. Moderate-severe anaemia was present in 22% (n = 11) of patients with HVPG < 10 mm Hg, 24% (n = 34) in patients with HVPG 10-19 mm Hg and 36% (n = 47) in patients with HVPG ≥ 20 mm Hg (*P* = .031). Interestingly, MCH was increasing with level of HVPG leading to hyperchromic anaemia in 4% (n = 2), 19% (n = 28) and 24% (n = 31) in the strata of patients with HVPG < 10 mm Hg, 10-19 mm Hg and ≥20 mm Hg respectively (*P* = .026). The same was observed for MCV. The prevalence of macrocytic anaemia increased numerically, yet not statistically significantly, from 2% (n = 1) to

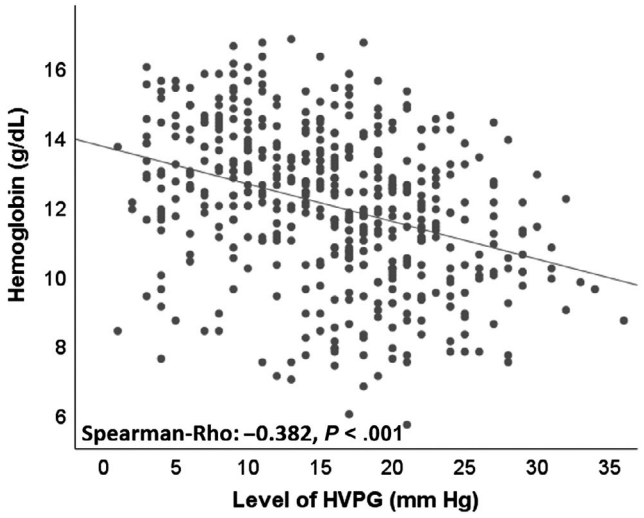

FIGURE 2 Correlation between degree of anaemia and portal pressure (HVPG). HVPG, hepatic-venous pressure gradient

9% (n = 13) and 11% (n = 14; *P* = .348). In line, prevalence of pancytopenia increased as well (18% (n = 9) vs 37% (n = 53) vs 33% (n = 43); *P* = .049). Bleeding (12 vs 26% vs 28%), iron-deficiency (4 vs 10% vs 8%) and vitamin B12/folic acid deficiency (2 vs 8% vs 11%) were overrepresented in patients with more advanced portal hypertension (*P* = .012).

3.5 | Factors associated with anaemia in ACLD patients

Binary logistic regression analysis was used to determine factors independently associated with anaemia (Table 3). Characteristics being significantly different between patients with and without anaemia in univariate analysis (sex, aetiology of liver disease, MELD score, the presence of varices, history of previous decompensation and level of HVPG) were included in the multivariate model. After backward elimination, male gender (odds ratio [OR]: 1.94 [95% CI: 1.09-3.47], *P* = .025), MELD (OR per point: 1.20 [95% CI: 1.09-1.32], *P* < .001), hepatic decompensation (OR: 4.40 [95% CI: 2.48-7.82], *P* < .001) and HVPG (OR per mm Hg: 1.07 [95% CI: 1.02-1.13], *P* = .004) were independently associated with the presence of anaemia in patients with ACLD.

3.6 | Impact of anaemia on hepatic decompensation, hospitalization, development of ACLF, overall survival and liver-related mortality

Median follow-up was 33.5 (IQR: 34.5) months (Figures 3, 4A,B, Table 4). During this follow-up, hepatic decompensation occurred significantly more often in patients with vs without anaemia (1 year: 25.1% vs 8.1%; 5 years: 60.3% vs 32.9%; *P* < .0001; Figure 3A). The

TABLE 2 Comparison of anaemia characteristics between patients with different degree of portal hypertension

	All patients, n = 324	HVPG < 10 mm Hg, n = 50	HVPG 10-19 mm Hg, n = 144	HVPG ≥ 20 mm Hg, n = 130	*P*-value
Severity of anaemia					**.031**
Mild (Hb ♀<12/♂<13.5 g/dL)	232 (72%)	39 (78%)	110 (76%)	83 (64%)	
Moderate (Hb < 10 g/dL)	68 (21%)	10 (20%)	21 (15%)	37 (28%)	
Severe (Hb < 8 g/dL)	24 (7%)	1 (2%)	13 (9%)	10 (8%)	
MCV					.348
Microcytic (<78 fL)	42 (13%)	5 (10%)	20 (14%)	17 (13%)	
Normocytic (78-98 fL)	253 (78%)	44 (88%)	111 (77%)	98 (75%)	
Macrocytic (>98 fL)	28 (9%)	1 (2%)	13 (9%)	14 (11%)	
MCH					**.026**
Hypochrome (<27 pg)	89 (27%)	16 (32%)	30 (21%)	32 (25%)	
Normochrome (27-33 pg)	184 (57%)	32 (64%)	86 (60%)	66 (51%)	
Hyperchrome (>33 pg)	61 (19%)	2 (4%)	28 (19%)	31 (24%)	
Pancytopenia, n (%)	105 (32%)	9 (18%)	53 (37%)	43 (33%)	**.049**
Aetiology of anaemia					**.012**
Bleeding	80 (25%)	6 (12%)	38 (26%)	36 (28%)	
Iron deficiency	28 (9%)	2 (4%)	15 (10%)	11 (8%)	
Vitamin B12/folic acid deficiency	26 (8%)	1 (2%)	11 (8%)	14 (11%)	
Renal anaemia	16 (5%)	6 (12%)	3 (2%)	7 (5%)	
Haemolytic	2 (1%)	0 (-)	1 (1%)	1 (1%)	
Chronic inflammation	1 (1%)	1 (2%)	0 (-)	0 (-)	
Unknown/insufficient diagnostic work-up	171 (53%)	34 (68%)	76 (53%)	61 (47%)	

P-values shown in bold indicate statistical significance.
Abbreviations: Hb, haemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin.

TABLE 3 Risk factors for the development of anaemia in ACLD patients

	First step			Last step		
Patient characteristics	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**
Sex						
Female	1			1		
Male	1.93	1.07-3.49	.030	1.94	1.09-3.47	.025
Aetiology						
Alcohol	1			—	—	—
Viral	0.55	0.27-1.10	.090			
Other	0.88	0.37-3.19	.876			
Unknown	0.73	0.27-1.99	.541			
MELD						
Per point	1.18	1.07-1.30	**.001**	1.20	1.09-1.32	<.001
Presence of varices						
No	1			—	—	—
Yes	1.61	0.80-3.23	.180			
Previous decompensation						
No	1					
Yes	3.59	1.96-6.59	**<.001**	4.40	2.48-7.82	<.001
Level of HVPG						
Per point	1.06	1.01-1.12	**.033**	1.07	1.02-1.13	.004

P-values shown in bold indicate statistical significance.
Abbreviations: 95% CI, 95% confidence interval; CAP, controlled attenuation parameter; HVPG, hepatic venous pressure gradient; MELD, model for end-stage liver disease; OR odds ratio.

incidence of hepatic decompensation was increasing with the severity of anaemia (rate of hepatic decompensation at 3 years: 66.7% in patients with severe, 60.7% in patients with moderate and 45.5% in patients with mild anaemia) compared to 24.1% in patients with normal haemoglobin levels ($P < .0001$; Figure 3B).

The percentage of patients needing hospitalization during follow-up was also associated with the severity of anaemia. While 57% of patients without anaemia were admitted, 69% of patients with mild, 81% with moderate and 92% of patients with severe anaemia, respectively, were hospitalized during follow-up ($P < .001$). Additionally, the median number (IQR) of hospital admissions during follow-up was also increasing with severity of anaemia: from one [3] hospitalization in patients without anaemia and with mild anaemia vs two [4] hospitalizations in patients with moderate and three [3] hospitalizations in patients with severe anaemia ($P < .001$) respectively.

In line, ACLF occurred in 50 (15%) patients with anaemia and 14 (8%) patients without anaemia ($P = .015$) resulting in an ACLF incidence rate of 0.05 (95% CI: 0.04-0.07) vs 0.03 (95% CI: 0.01-0.04) per patient year. Again, ACLF incidence was highest in patients with severe anaemia when compared to other severity groups (25% in severe vs 21% in moderate and 13% in mild anaemia compared to 8% in patients with normal haemoglobin levels; $P = .020$; Table 4).

Patients presenting with anaemia had a significantly worse overall survival when compared to patients without anaemia (1 year survival: 87.1% vs 93.7%, 5 year survival: 50.5% vs 68.6%; $P < .0001$; Figure 4A). Similar findings were obtained for liver-related mortality (1 year mortality: 9.7% vs 5.7%, 5 year mortality: 38.0% vs 26.9%; $P = .003$; Figure 4B). The significant prognostic impact of anaemia was also underlined by the gradual decrease in overall survival (Figure 4C) and liver-related mortality (Figure 4D) with increasing severity of anaemia.

4 | DISCUSSION

Anaemia is highly prevalent in patients with chronic diseases and not only associated with impaired mental and physical health, but also with dementia and increased mortality.[2,5,6,8] Furthermore, a sufficient oxygen supply is critical to maintain regular organ function especially under stress conditions or in critically ill patients. However, data on the prevalence of anaemia in patients with ACLD are very limited.[13-15] The largest study in ACLD patients included 110 patients with cirrhosis.[12] Our study comprises a substantially higher number of well-characterized patients with ACLD, and thus, provides more robust data on prevalence and types of anaemia. In our study, the overall prevalence of anaemia was 66% including 7% of patients with severe anaemia—these numbers are comparable to previous analyses.[12-15] We found a strong association between the presence of anaemia and indicators of more advanced liver disease such as a higher MELD and CPS scores and higher severity of portal hypertension (eg a higher prevalence of CSPH, hepatic decompensation

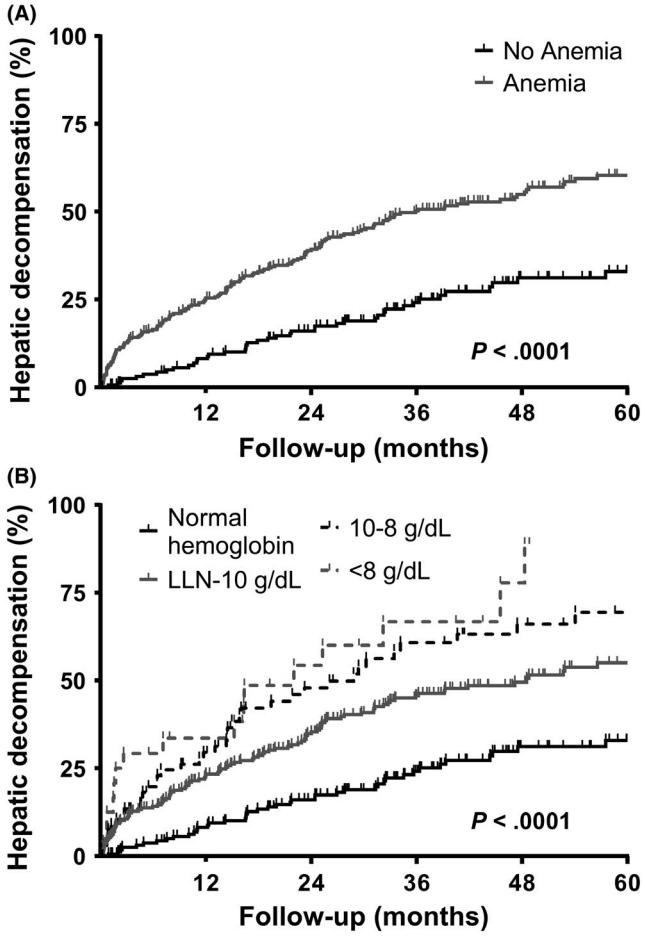

FIGURE 3 Hepatic decompensation during follow-up in (A) patients with and without anaemia and in (B) groups of patients with different severity of anaemia

and refractory ascites). These findings indicate that anaemia is closely linked to ACLD and portal hypertension and its complications in these patients.[30] The pathogenic link between CSPH and increased severity of anaemia is also supported by the negative correlation between the level of haemoglobin and HVPG, which has been previously suggested in a smaller group of patients.[14] Furthermore, we found a higher prevalence of moderate-severe anaemia in patients with more advanced portal hypertension. Pathogenic factors for portal hypertension-induced anaemia likely include gastrointestinal (congestive) bleedings and pancytopenia as a result of hypersplenism.[22,23]

Patients with ALD had a higher prevalence of anaemia in our cohort. As a matter of fact, alcohol abuse is the most common cause of vitamin B12 and/or folic acid deficiency.[24] In ALD patients with ACLD, this effect might even be amplified by other causes of anaemia resulting in 81% of patients with alcoholic ACLD suffering from anaemia. Importantly, ALD aetiology was more common in patients with more advanced portal hypertension: ALD prevalence: 8% in patients with HVPG < 10 mm Hg, 44% in HVPG 10-19 mm Hg, 48% in patients with HVPG ≥ 20 mm Hg. In line, we found increasing numbers of macrocytic and hyperchromic anaemia as well as vitamin B12/folic acid deficiency with increasing HVPG.

The role of iron-deficiency as a contributor to anaemia in ACLD patients remains controversial as decreased levels of hepcidin have been observed in patients with cirrhosis and hepatocellular carcinoma (HCC). This finding was associated with an acquired iron overload which may promote liver fibrosis and HCC development.[41-44] Furthermore, low serum transferrin and high serum ferritin levels have been observed in patients with cirrhosis. Level of serum ferritin was recently even reported to be an independent predictor of short-term mortality.[45] Interestingly, in our cohort, only approximately 10% of patients were diagnosed with iron-deficiency.[46] This rather low prevalence of iron deficiency might be explained by the fact that even a low amount of alcohol consumption seems to reduce the risk of developing iron-deficiency anaemia by 40%.[47] It remains to be determined if iron overload associated with hepatic inflammation and fibrosis may interfere with iron deficiency in cirrhosis.

Importantly, the presence and severity of anaemia was linked to adverse outcomes. Hepatic decompensation was observed at a significantly higher rate in patients with anaemia. In addition, patients with anaemia were more frequently hospitalized and developed ACLF more often. The incidence rate of ACLF was gradually increasing with the severity of anaemia. Just recently, anaemia was found to be an independent predictor for the development of ACLF – next to mean arterial pressure, the presence of ascites and the MELD.[48] Finally, the higher incidence of hepatic decompensation and ACLF also translated into higher liver-related mortality and worse overall survival.

Anaemia was reported to develop early in the course of liver cirrhosis – after a median duration of 3.3 years after diagnosis.[14] Thus, timely treatment of anaemia, which is a potentially reversible contributor to impaired physical and mental state,[27] quality of life[28,49] and even survival,[15,20,29] is likely to impact on the morbidity and mortality of patients with ACLD. In our cohort, the exact aetiology of anaemia could not be determined in 53% of patients as a result of suboptimal diagnostic workup of anaemia within 2 months prior to or after HVPG measurement. Nevertheless, in this study, the diagnostic workup for anaemia prior to presentation at our department might not have been adequately recorded. We thus believe that diagnostic testing for anaemia is often suboptimal in patients with cirrhosis, which is also represented by the low number of specific aetiological treatments for anaemia in this cohort. Both numbers emphasize that anaemia is still underappreciated and neglected in cirrhosis in clinical routine. However, optimal management of anaemia in these patients needs specific attention and proper screening and treatment strategies should be implemented.

This study has several limitations. First of all, following to the retrospective study design, some parameters were not available in all patients. However, this patient cohort represents a real-life cohort highlighting the urgent need for proper anaemia management. Nevertheless, the prevalence of specific aetiologies of anaemia might have been under- or overestimated as a result of complex evaluation criteria and missing data. Unfortunately, interesting aspects

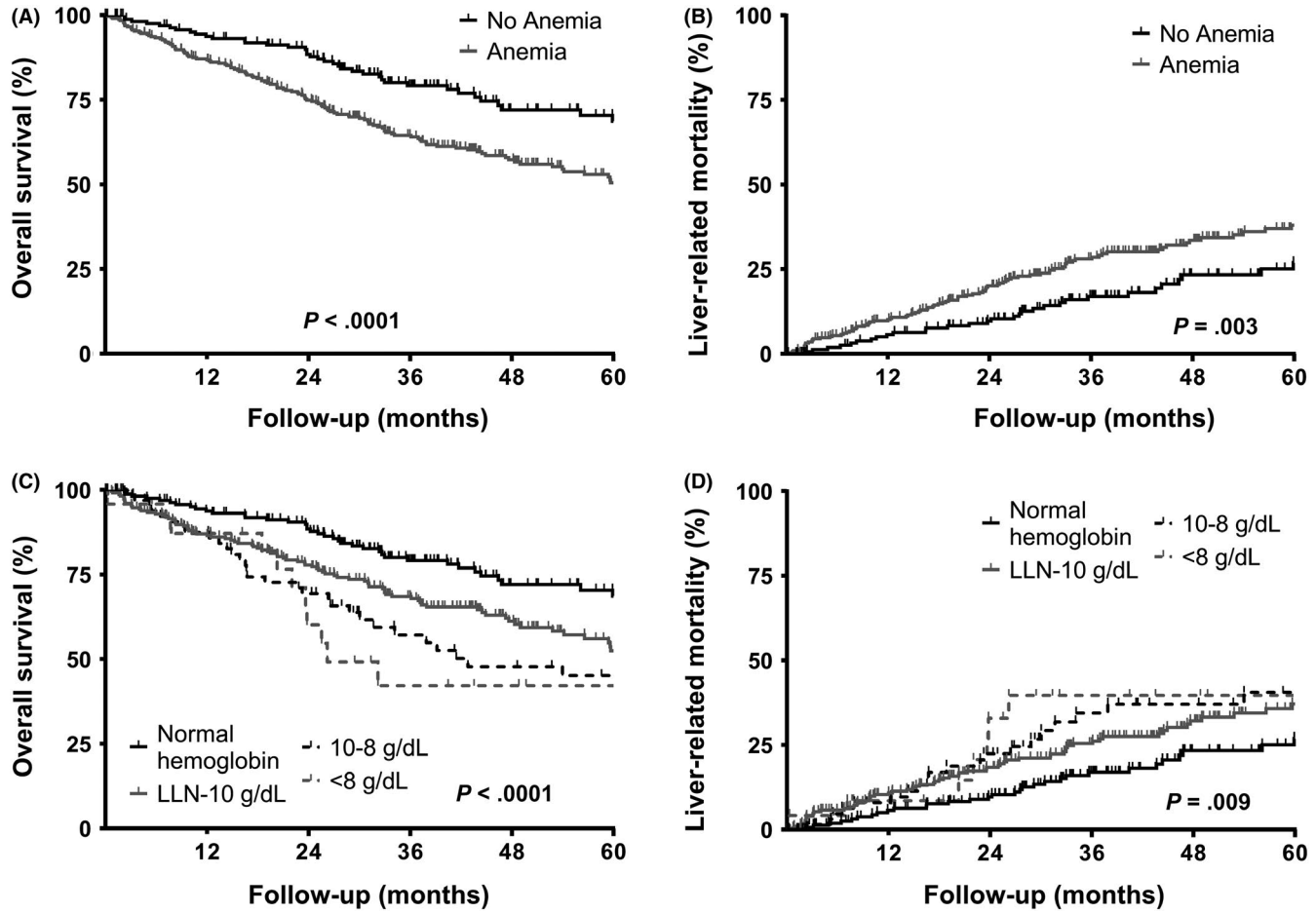

FIGURE 4 Comparison of (A) overall survival and (B) liver-related mortality between patients with and without anaemia as well as (C) overall survival and (D) liver-related survival in groups of patients with different severity of anaemia

TABLE 4 Acute-on-chronic liver failure (ACLF), hospitalizations and liver-related hospitalizations in regard to severity of anaemia

	No anaemia (Hb > LLN), n = 170	Anaemia (Hb < LLN), n = 324	*P*-value
Follow-up (months, median, range)	36.7 (33.8)	32.2 (35.1)	**.040**
Development of ACLF, n (%)	14 (8%)	50 (15%)	**.015**
Incidence-rate of ACLF (per patient year of follow-up)	0.03 (95% CI: 0.01-0.04)	0.05 (95% CI: 0.04-0.07)	—

	No anaemia (Hb > LLN), n = 170	Mild anaemia (Hb < LLN - 10 mg/dL), n = 232	Moderate anaemia (10-8 mg/dL), n = 68	Severe anaemia (<8 g/dL), n = 24	*P*-value
Development of ACLF, n (%)	14 (8%)	30 (13%)	14 (21%)	6 (25%)	**.020**
Number (%) of patients with hospitalization	97 (57%)	160 (69%)	55 (81%)	22 (92%)	**<.001**
Number of (liver-related) hospitalization during follow-up, median (range)	1 (3)	1 (3)	2 (4)	3 (3)	**<.001**

P-values shown in bold indicate statistical significance.
Abbreviations: Hb, haemoglobin; LLN, lower (gender-specific) limit of normal.

such as spleen size or the presence of acanthocytes could not be evaluated retrospectively.

In summary, this is the largest study up to date on the prevalence of and risk factors for anaemia in a thoroughly characterized cohort of patients with cirrhosis – including data on HVPG. The risk of anaemia is associated with the degree of liver dysfunction, ALD aetiology, and portal hypertension. More attention in terms of diagnostic workup and specific treatment strategies for anaemia in ACLD patients is warranted.

CONFLICT OF INTEREST

The authors have nothing to disclose regarding the work under consideration for publication. The following authors disclose conflict of interests outside the submitted work: BS received travel support from AbbVie and Gilead. PS received speaker fees from Boehringer Ingelheim and Roche as well as travel support from Boehringer Ingelheim, Gilead and Roche. TB received travel support from Gilead, BMS, Roche, Bayer and AbbVie. MT received grant support from Cymabay, Falk, Gilead, Intercept, MSD and, Takeda, honoraria for consulting from AbbVie, Gilead, Intercept, Janssen, Novartis and Regulus, speaker fees from Boehringer Ingelheim, Falk, Gilead, and MSD as well as travel support from Abbvie, Gilead and Intercept. MM has served as a speaker and consultant for AbbVie, BMS, Gilead, Gore and Janssen. TR received speaker fees from Boehringer Ingelheim, Roche, WL Gore and MSD, grant support from Boehringer Ingelheim, Boston Scientific, Cook Medical, Gilead, Guerbet, Abbvie, Phenex Pharmaceuticals, Philips, WL Gore, and MSD, served as a consultant for Abbvie, Bayer, Boehringer Ingelheim, Gilead, Intercept and MSD and received travel support from Gilead, Roche, MSD and Gore. GS, FM, RP, DB and BSi have nothing to disclose.

AUTHOR CONTRIBUTIONS

BS, MM and TR conceptualized the study; BS, FM and GS collected the data; BS and TR performed statistical analysis; BS, GS and TR involved in drafting of the manuscript; all authors revised the manuscript for important intellectual content and accepted the final version of the manuscript.

ORCID

Bernhard Scheiner https://orcid.org/0000-0002-4904-5133
Georg Semmler https://orcid.org/0000-0002-0411-166X
Theresa A. Bucsics https://orcid.org/0000-0003-3901-4111
Rafael Paternostro https://orcid.org/0000-0002-1813-5769
Benedikt Simbrunner https://orcid.org/0000-0001-8181-9146
Mattias Mandorfer https://orcid.org/0000-0003-2330-0017
Thomas Reiberger https://orcid.org/0000-0002-4590-3583

REFERENCES

1. Guralnik JM, Eisenstaedt RS, Ferrucci L, Klein HG, Woodman RC. Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. *Blood*. 2004;104(8):2263-2268.
2. Denny SD, Kuchibhatla MN, Cohen HJ. Impact of anemia on mortality, cognition, and function in community-dwelling elderly. *Am J Med*. 2006;119(4):327-334.
3. Beghe C, Wilson A, Ershler WB. Prevalence and outcomes of anemia in geriatrics: a systematic review of the literature. *Am J Med*. 2004;116(Suppl 7A):3s-10s.
4. Gaskell H, Derry S, Andrew Moore R, McQuay HJ. Prevalence of anaemia in older persons: systematic review. *BMC Geriatr*. 2008;8:1.
5. Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med*. 2005;352(10):1011-1023.
6. Terekeci HM, Kucukardali Y, Onem Y, et al. Relationship between anaemia and cognitive functions in elderly people. *Eur J Intern Med*. 2010;21(2):87-90.
7. Hong CH, Falvey C, Harris TB, et al. Anemia and risk of dementia in older adults: findings from the Health ABC study. *Neurology*. 2013;81(6):528-533.
8. Jeong S-M, Shin DW, Lee JE, Hyeon JH, Lee J, Kim S. Anemia is associated with incidence of dementia: a national health screening study in Korea involving 37,900 persons. *Alzheimers Res Ther*. 2017;9:94.
9. Caro JJ, Salas M, Ward A, Goss G. Anemia as an independent prognostic factor for survival in patients with cancer: a systemic, quantitative review. *Cancer*. 2001;91(12):2214-2221.
10. Mandorfer M, Payer BA, Scheiner B, et al. Health-related quality of life and severity of fatigue in HIV/HCV co-infected patients before, during, and after antiviral therapy with pegylated interferon plus ribavirin. *Liver Int*. 2014;34(1):69-77.
11. Vincent JL, Baron JF, Reinhart K, et al. Anemia and blood transfusion in critically ill patients. *JAMA*. 2002;288(12):1499-1507.
12. Maruyama S, Hirayama C, Yamamoto S, et al. Red blood cell status in alcoholic and non-alcoholic liver disease. *J Lab Clin Med*. 2001;138(5):332-337.
13. Mathurin SA, Aguero AP, Dascani NA, et al. Anemia in hospitalized patients with cirrhosis: prevalence, clinical relevance and predictive factors. *Acta Gastroenterol Latinoam*. 2009;39(2):103-111.
14. Qamar AA, Grace ND, Groszmann RJ, et al. Incidence, prevalence, and clinical significance of abnormal hematologic indices in compensated cirrhosis. *Clin Gastroenterol Hepatol*. 2009;7(6):689-695.
15. Kalaitzakis E, Josefsson A, Castedal M, et al. Hepatic encephalopathy is related to anemia and fat-free mass depletion in liver transplant candidates with cirrhosis. *Scand J Gastroenterol*. 2013;48(5):577-584.
16. Reiberger T, Puspok A, Schoder M, et al. Austrian consensus guidelines on the management and treatment of portal hypertension (Billroth III). *Wien Klin Wochenschr*. 2017;129(Suppl 3):135-158.
17. Ripoll C, Garcia-Tsao G. The management of portal hypertensive gastropathy and gastric antral vascular ectasia. *Dig Liver Dis*. 2011;43(5):345-351.
18. Luo J-C, Leu H-B, Hou M-C, et al. Cirrhotic patients at increased risk of peptic ulcer bleeding: a nationwide population-based cohort study. *Aliment Pharmacol Ther*. 2012;36(6):542-550.
19. Gado A, Ebeid B, Axon A. Prevalence and outcome of peptic ulcer bleeding in patients with liver cirrhosis. *Alexandria J Med*. 2014;50(2):143-148.
20. Alexopoulou A, Vasilieva L, Kanellopoulou T, Pouriki S, Soultati A, Dourakis SP. Presence of spur cells as a highly predictive factor of mortality in patients with cirrhosis. *J Gastroenterol Hepatol*. 2014;29(4):830-834.
21. Vassiliadis T, Mpoumponaris A, Vakalopoulou S, et al. Spur cells and spur cell anemia in hospitalized patients with advanced liver disease: Incidence and correlation with disease severity and survival. *Hepatol Res*. 2010;40(2):161-170.
22. Lu YF, Li XQ, Han XY, Gong XG, Chang SW. Peripheral blood cell variations in cirrhotic portal hypertension patients with hypersplenism. *Asian Pac J Trop Med*. 2013;6(8):663-666.
23. Lv Y, Yee Lau W, Wu H, et al. Causes of peripheral cytopenia in hepatitic cirrhosis and portal hypertensive splenomegaly. *Exp Biol Med (Maywood)*. 2017;242(7):744-749.
24. Lindenbaum J, Roman MJ. Nutritional anemia in alcoholism. *Am J Clin Nutr*. 1980;33(12):2727-2735.
25. Gkamprela E, Deutsch M, Pectasides D. Iron deficiency anemia in chronic liver disease: etiopathogenesis, diagnosis and treatment. *Ann Gastroenterol*. 2017;30(4):405-413.

26. Gonzalez-Casas R, Garcia-Buey L, Jones EA, Gisbert JP, Moreno-Otero R. Systematic review: hepatitis-associated aplastic anaemia–a syndrome associated with abnormal immunological function. *Aliment Pharmacol Ther*. 2009;30(5):436-443.
27. Kalaitzakis E, Josefsson A, Castedal M, et al. Factors related to fatigue in patients with cirrhosis before and after liver transplantation. *Clin Gastroenterol Hepatol*. 2012;10(2):174-181, 81.e1.
28. Les I, Doval E, Flavià M, et al. Quality of life in cirrhosis is related to potentially treatable factors. *Eur J Gastro Hepatol*. 2010;22(2):221-227.
29. Gungor G, Akyildiz M, Keskin M, et al. Is there any potential or additive effect of anemia on hepatorenal syndrome? *Turkish J Gastroenterol*. 2016;27(3):273-278.
30. de Franchis R, Baveno V. Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: stratifying risk and individualizing care for portal hypertension. *J Hepatol*. 2015;63(3):743-752.
31. McClellan W, Aronoff SL, Bolton WK, et al. The prevalence of anemia in patients with chronic kidney disease. *Curr Med Res Opin*. 2004;20(9):1501-1510.
32. Ferlitsch A, Bota S, Paternostro R, et al. Evaluation of a new balloon occlusion catheter specifically designed for measurement of hepatic venous pressure gradient. *Liver Int*. 2015;35(9):2115-2120.
33. Reiberger T, Ferlitsch A, Payer BA, et al. Non-selective beta-blockers improve the correlation of liver stiffness and portal pressure in advanced cirrhosis. *J Gastroenterol*. 2012;47(5):561-568.
34. Reiberger T, Ferlitsch A, Payer BA, et al. Noninvasive screening for liver fibrosis and portal hypertension by transient elastography—a large single center experience. *Wien Klin Wochenschr*. 2012;124(11):395-402.
35. Schwabl P, Bota S, Salzl P, et al. New reliability criteria for transient elastography increase the number of accurate measurements for screening of cirrhosis and portal hypertension. *Liver Int*. 2015;35(2):381-390.
36. Margini C, Murgia G, Stirnimann G, et al. Prognostic significance of controlled attenuation parameter in patients with compensated advanced chronic liver disease. *Hepatol Commun*. 2018;2(8):929-940.
37. Abraldes JG, Bureau C, Stefanescu H, et al. Noninvasive tools and risk of clinically significant portal hypertension and varices in compensated cirrhosis: the "Anticipate" study. *Hepatology*. 2016;64(6):2173-2184.
38. Hernaez R, Sola E, Moreau R, Gines P. Acute-on-chronic liver failure: an update. *Gut*. 2017;66(3):541-553.
39. Scheiner B, Steininger L, Semmler G, et al. Controlled attenuation parameter does not predict hepatic decompensation in patients with advanced chronic liver disease. *Liver Int*. 2019;39(1):127-135.
40. Moreau R, Jalan R, Gines P, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology*. 2013;144(7):1426–1437, 37 e1–9.
41. Girelli D, Pasino M, Goodnough JB, et al. Reduced serum hepcidin levels in patients with chronic hepatitis C. *J Hepatol*. 2009;51(5):845-852.
42. Kijima H, Sawada T, Tomosugi N, Kubota K. Expression of hepcidin mRNA is uniformly suppressed in hepatocellular carcinoma. *BMC Cancer*. 2008;8(1):167.
43. Lunova M, Goehring C, Kuscuoglu D, et al. Hepcidin knockout mice fed with iron-rich diet develop chronic liver injury and liver fibrosis due to lysosomal iron overload. *J Hepatol*. 2014;61(3):633-641.
44. Kessler SM, Barghash A, Laggai S, Helms V, Kiemer AK. Hepatic hepcidin expression is decreased in cirrhosis and HCC. *J Hepatol*. 2015;62(4):977-979.
45. Viveiros A, Finkenstedt A, Schaefer B, et al. Transferrin as a predictor of survival in cirrhosis. *Liver Transpl*. 2018;24(3):343-351.
46. Stein J, Connor S, Virgin G, Ong DE, Pereyra L. Anemia and iron deficiency in gastrointestinal and liver conditions. *World J Gastroenterol*. 2016;22(35):7908-7925.
47. Ioannou GN, Dominitz JA, Weiss NS, Heagerty PJ, Kowdley KV. The effect of alcohol consumption on the prevalence of iron overload, iron deficiency, and iron deficiency anemia. *Gastroenterology*. 2004;126(5):1293-1301.
48. Piano S, Tonon M, Vettore E, et al. Incidence, predictors and outcomes of acute-on-chronic liver failure in outpatients with cirrhosis. *J Hepatol*. 2017;67(6):1177-1184.
49. Gao R, Gao F, Li G, Hao JY. Health-related quality of life in chinese patients with chronic liver disease. *Gastroenterol Res Pract*. 2012;2012:516140.

How to cite this article: Scheiner B, Semmler G, Maurer F, et al. Prevalence of and risk factors for anaemia in patients with advanced chronic liver disease. *Liver Int*. 2020;40:194–204. https://doi.org/10.1111/liv.14229