**Supplementary Information**

**Detailed description of STOPAH study population**

Cases with severe alcoholic hepatitis were enrolled for the STOPAH trial as per the trial protocol1. All had a history of long-standing alcohol misuse; compatible clinical, laboratory, and/or liver biopsy features of alcoholic hepatitis; no other identified causes for their liver disease; and a DF ≥32. Patients were randomised to treatment with prednisolone or pentoxifylline for 28 days using a double-blind, double-dummy factorial 2 x 2 design. Outcome data were collected for the primary and secondary endpoints of mortality at 28 and 90 days, respectively. Patients were consented for follow-up via the NHS Information Centre Data Linkage service ensuring ongoing follow-up and reliable capture of mortality data. Patients presenting with either infection or gastrointestinal haemorrhage were eligible for inclusion into the trial once the condition was deemed clinically controlled by the treating physician. A detailed description is provided in the primary analysis of the trial data2.

Clinical, haematological and biochemical data were recorded at baseline in line with the trial protocol. In cases where the serum aspartate transaminase (AST) level was not recorded in the case record form these values were subsequently determined using clinical assays with the Cobas 8000 system (Roche Diagnostics, Mannheim, Germany) available at the Clinical Chemistry Department of University Hospital Aachen on the available serum samples. The test has been approved for use in clinical practice and is not altered by hemolysis or increased serum bilirubin.

**Alcoholic Cirrhosis Cohort**

Patients with alcoholic liver disease were consecutively recruited to a compensated cirrhosis cohort study, a prospective study initiated in 2010 focused on tracking liver disease progression. Here, baseline measures collected for this cohort are reported. Patients were recruited with evidence of past alcohol excess, cirrhosis (confirmed by a combination of biopsy, clinical and radiological criteria) and no evidence of decompensation (ascites, significant jaundice, hepatic encephalopathy and variceal bleeding), hepatocellular carcinoma and portal vein thrombosis. Exclusion criteria included orthotopic liver transplantation, ischaemic heart disease, alcoholic cardiomyopathy (defined by clinical evidence of systolic dysfunction) and valvular heart disease. A small cohort of patients with decompensated cirrhosis were also included as a control group for the analysis defined as Baveno 3 or 4 stage (ascites, encephalopathy or previous variceal bleed); exclusion criteria included portal vein thrombosis, the presence of hepatocellular carcinoma (HCC), and orthotopic liver transplantation3.

**Histological analyses**

All liver biopsies were obtained *via* the transjugular route by an experienced hepatologist or interventional radiologist. Biopsy material was collected in formaldehyde and left at room temperature for a minimum of 4 hours before embedding in paraffin wax. Serial 0.5 µm sections were cut. The preparation of unstained sections was carried out in the histology department of the recruiting hospital. The specimens were sent to the trial centre at St Mary’s Hospital where they were stained with haematoxylin and eosin (H&E) and Sirius red. Biopsies were considered adequate for reading if at least five portal tracts were available for assessment. Histological analyses were conducted in the subset of patients who underwent liver biopsy (n=155). Analyses reported were limited to biopsies taken within a window extending from 14 days prior to the start of treatment to 14 days after the commencement of trial therapy due to the potential impact of both time and treatment on their appearance.

After exclusions based upon an inadequate diagnostic sample (n=39) and timing greater than 14 days before or after the start of treatment (n=29), 87 samples were available for analysis.

Two experienced histopathologists (RG and AQ), blinded to patient treatment and outcomes, independently assessed the histological features of each biopsy using the alcoholic hepatitis scoring system (AHHS) (Supplementary Table 1)3. Fibrosis was graded semi-quantitatively using the Laennec system4 (Supplementary Table 1). The presence or absence of additional features associated with ASH, *viz* Mallory-Denk bodies and megamitochondria, was also recorded.

#### **Baseline demographic and histological statistical analyses**

The Jonckheere-Terpstra test was used to test for an ordered difference in medians between groups defined by the Laennec fibrosis stage. The Mann-Whitney U test was used to test for differences in the median K18 levels between groups defined by the presence or absence of histopathological features as set out in the AHHS. Area under the receiver operated characteristic (AUROC) analysis was used to test the ability of circulating K18 fragments to predict the presence of ASH and severe inflammation. Samples non-diagnostic of ASH were excluded from analyses of the prediction of severe inflammation.

Correlations were conducted in the entire cohort of patients with available serum K18 data and tested using the Spearman’s rank test. In view of the number of tests a Benjamini-Hochberg correction was applied to maintain a false discovery rate of 5%.

**Interaction testing**

It was hypothesized that the efficacy of prednisolone would increase with greater serum levels of K18 fragments but that at extreme K18 values this interaction may be non-linear. In order to model this a multiplicative interaction term was first included in the model and then an additional interaction term between prednisolone and the quadratic of K18. A likelihood ratio test implemented using the ‘lrtest’ function in the R package ‘lmtest’ was used to test the significance of the improvement in model fit associated with inclusion of these interaction terms. Finally models with significant interaction terms were further adjusted for age and MELD.

**Supplementary Table 1:** Criteria used for the histological classification of liver biopsy material.

|  |  |  |
| --- | --- | --- |
| **Feature** | **Grade** | **Criteria** |
| **Neutrophil infiltration** | None/mild | No, isolated or very few neutrophils around single or small clusters of hepatocytes, typically <15 per inflammatory focus |
| Severe | Neutrophils easily seen at low magnification, numerous in number, surrounding damaged hepatocytes |
| **Hepatocyte ballooning** | Occasional | Focal and dispersed ballooned hepatocytes, difficult to locate |
| Marked | Sizeable groups of hepatocytes all demonstrating ballooning degeneration easily locate at low magnification |
| **Fibrosis** | 0 | No definite fibrosis |
| 1 | Minimal fibrosis – no septa or rare thin septa; may have portal expansion or mild sinusoidal fibrosis |
| 2 | Mild fibrosis - occasional thin septa |
| 3 | Moderate fibrosis – thin septa; up to incomplete cirrhosis |
| 4A/4 | Mild cirrhosis – Definite or probable |
| 4B/5 | Moderate cirrhosis – at least 2 broad septa |
| 4C/6 | Severe cirrhosis – at least one very broad septum or many minute nodules |

Neutrophilic inflammation, hepatocyte ballooning and fibrosis were semi-quantitatively graded as indicated above. The criteria used for grading neutrophilic infiltration and hepatocyte ballooning were derived from Altimirano et al*.*4 in the derivation of the alcoholic hepatitis histological scoring system (AHHS). Fibrosis grades were derived from the Laennec scoring system as described by Wanless et al.5.

**Supplementary Table 2.** Control cohort characteristics.

|  |  |  |
| --- | --- | --- |
| Variables:  Median (interquartile range) | Compensated alcoholic cirrhosis cohort  [n=76] | Decompensated alcoholic cirrhosis cohort  [n=10] |
| Age, years | 61.5 (55.3-67.8) | 52.9 (46.6-59.2) |
| Females, % | 20 % | 70 % |
| Alcohol intake, units/week | 0 (max 105) [n=67] | 0 (max 320) |
| ALT, U/L | 26.0 [15.5-36.5] | 31.0 [20.0-42.0] |
| AST, U/L | 32.5 (18.0-47.0) [n=74] | 54.0 (20.0-88.0) |
| Bilirubin, umol/L | 13.0 (6.5-19.5) | 30.5 (15.5-45.5) |
| Creatinine, umol/L | 83.0 (67.5-98.5) | 61.5 (48.0-75.0) |
| Sodium, mmol/L | 138.0 (135.5-140.5) | 135.5 (132.0-139.0) |
| K18-M30, kIU/L | 0.125 (0.066-0.184) [n=74] range 0.031-0.893 | 0.168 (0.0983-0.240) range 0.069-0.989 |
| K18-M65, kIU/L | 0.119 (0.024-0.213) [n=75] range 0.031-1.208 | 0.134 (0.042-0.225) [n=9] range 0.093-1.968 |

ALT, alanine transaminase; AST, aspartate transaminase; K18, keratin-18.

**Supplementary Table 3:** Baseline demographics of populations defined by prednisolone treatment status and mortality at ninety days.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Placebo (n=417) | | Prednisolone (n=407) | |
| Variable Median (interquartile range) or ‘n’ (%) | Survivors (n=320) | Died (n=97) | Survivors (n=310) | Died (n=97) |
| Age (years) | 47.6 (41.4 – 54.3) | 50.6 (43.0 – 59.5) | 47.6 (40.9 – 54.1) | 55.0 (48.1 – 62.0) |
| Sex (male) | 191 (60%) | 65 (67%) | 195 (63%) | 69 (71%) |
| Alcohol (units/week) | 140 (90 – 210) | 105 (75 – 168) | 129 (90 – 210) | 140 (86 – 200) |
| Pentoxifylline | 157 (49%) | 56 (58%) | 160 (52%) | 42 (43%) |
| Encephalopathy |  |  |  |  |
| Grade 0 | 238 (79%) | 57 (60%) | 234 (77%) | 54 (60%) |
| Grade 1 | 45 (15%) | 27 (28%) | 50 (17%) | 22 (24%) |
| Grade 2 | 14 (5%) | 6 (6%) | 15 (5%) | 9 (10%) |
| Grade 3 | 3 (1%) | 5 (5%) | 4 (1%) | 5 (6%) |
| Haemoglobin (g/L) | 108 (95 – 121) | 100 (89 – 113) | 107 (95 – 119) | 104 (91 – 116) |
| White cell count (x106/mm3) | 8.0 (5.8 – 12.3) | 10.1 (7.0 – 14.4) | 8.2 (5.9 – 11.4) | 10.0 (7.0 – 13.6) |
| Neutrophils (x106/mm3) | 5.6 (3.8 – 9.3) | 7.8 (4.8 – 11.3) | 5.7 (4.0 – 8.8) | 7.3 (5.1 – 10.6) |
| Bilirubin (µmol/L) | 244 (164 – 374) | 361 (211 – 463) | 256 (144 – 376) | 309 (188 – 464) |
| ALT (IU/L) | 42 (30 – 60) | 43 (30 – 59) | 41 (29 – 61) | 47 (31 – 63) |
| AST (IU/L) | 115 (84 – 162) | 115 (81 – 158) | 118 (86 – 150) | 118 (94 – 160) |
| Albumin (g/L) | 26 (21 – 29) | 25 (21 – 31) | 25 (22 – 29) | 25 (20 – 29) |
| Urea (mmol/L) | 3.0 (2.1 – 4.5) | 4.2 (2.8 – 7.7) | 3.1 (2.0 – 4.8) | 5.1 (2.9 – 7.5) |
| Creatinine (µmol/L) | 61 (51 – 74) | 74 (59 – 108) | 62 (50 – 79) | 78 (59 – 111) |
| INR | 1.7 (1.5 – 2.0) | 1.9 (1.7 – 2.4) | 1.7 (1.5 – 2.0) | 1.9 (1.7 – 2.2) |
| DF | 53 (41 – 70) | 70 (54 – 91) | 52 (42 – 66) | 68 (52 – 85) |
| MELD | 23 (21 – 25) | 25 (23 – 30) | 22 (20 – 25) | 26 (23 – 29) |
| K18-M30 (kIU/L) | 1.54 (0.89 – 3.07) | 2.53 (1.11 – 6.06) | 1.50 (0.96 – 2.93) | 2.47 (1.21 – 4.34) |
| K18-M65 (kIU/L) | 3.98 (2.31 – 6.21) | 5.58 (3.19 – 9.58) | 3.94 (2.44 – 6.54) | 5.86 (3.29 – 8.46) |
| K18-M30:M65 ratio | 0.45 (0.39 – 0.53) | 0.49 (0.38 – 0.65) | 0.45 (0.38 – 0.52) | 0.47 (0.39 – 0.54) |
| High K18-M30\* | 26 (8%) | 30 (31%) | 32 (10%) | 16 (17%) |

\*High K18-M30 defined as serum K18-M30 >5 kIU/L

ALT, alanine transaminase; AST, aspartate transaminase; INR, International Normalised Ratio; DF, Maddrey’s discriminant function; MELD, Model for End-stage Liver Disease score; K18, keratin-18.

**Supplementary Table 4**. Logistic regression analyses for serum K18 fragments in relation to 28-day mortality

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Exploratory (- prednisolone) | | | Validation (+ prednisolone) | | |
|  | Univariate Analysis | | | | | |
|  | OR\* | 95% CI | *p* | OR\* | 95% CI | *p* |
| K18-M30 (kIU/L) | 1.0918 | 1.0311 – 1.1605 | 0.0032 | 1.0904 | 1.0277 – 1.1679 | 0.011 |
| K18-M65 (kIU/L) | 1.0628 | 1.0187 – 1.1075 | 0.0039 | 1.0763 | 1.0348 – 1.1247 | 0.0005 |
| M30:M65 ratio | 5.3489 | 1.1940 – 23.195 | 0.0254 | 0.5835 | 0.0585 – 4.2614 | 0.6228 |
|  | Multivariate Analysis for K18-M30 | | | | | |
| Age (years) | 1.0426 | 1.013 – 1.074 | 0.0050 | 1.1581 | 1.1039 – 1.2247 | <0.0001 |
| MELD (points) | 1.1966 | 1.1239 – 1.2806 | <0.0001 | 1.2942 | 1.2000 – 1.4090 | <0.0001 |
| K18-M30 (kIU/L) | 1.0791 | 1.0167 – 1.1469 | 0.0109 | 1.0665 | 1.0022 – 1.1600 | 0.1099 |
|  | Multivariate Analysis for K18-M65 | | | | | |
| Age (years) | 1.0438 | 1.0144 – 1.0750 | 0.0037 | 1.1617 | 1.1065 – 1.2300 | <0.0001 |
| MELD (points) | 1.1932 | 1.1206 – 1.2769 | <0.0001 | 1.2886 | 1.1946 – 1.4032 | <0.0001 |
| K18-M65 (kIU/L) | 1.0493 | 1.0015 – 1.0968 | 0.0361 | 1.0715 | 1.0181 – 1.1362 | 0.0136 |

\*Odds ratios (OR) are quoted per 1 unit increase in the predictor variable. For K18-M30 and -M65 the ORs are per 1 kIU/L increase in serum concentration, for age it is per additional year and MELD score per whole point increase. For K18 M30:M65 ratio the OR is quoted per whole number increase.

K18, keratin-18; OR, Odds Ratio; CI, confidence interval; MELD, Model for End-stage Liver Disease score.

**Supplementary Table 5**. Logistic regression interaction analyses for the K18-M30:M65 ratio with prednisolone in relation to 90-day mortality.

|  |  |  |  |
| --- | --- | --- | --- |
| Adjusted analysis prednisolone and M30:M65 ratio | | | |
|  | OR\* | 95% CI | *p* |
| Prednisolone | 0.934 | 0.645 – 1.349 | 0.714 |
| M30:M65 ratio | 3.635 | 1.206 – 10.75 | 0.0201 |
| Age | 1.065 | 1.046 – 1.086 | <0.0001 |
| MELD | 1.186 | 1.140– 1.237 | <0.0001 |
|  | | | |
| Adjusted interaction between prednisolone and M30:M65 ratio | | | |
|  | OR | 95% CI | *p* |
| Prednisolone | 2.174 | 0.696 – 6.974 | 0.185 |
| M30:M65 ratio | 7.849 | 1.800 – 34.90 | 0.0060 |
| Age | 1.065 | 1.045 – 1.086 | <0.0001 |
| MELD | 1.186 | 1.045 – 1.086 | <0.0001 |
| Prednisolone: M30:M65 ratio | 0.177 | 0.018 – 1.605 | 0.129 |

K18, keratin-18; OR, Odds Ratio; CI, confidence interval; MELD, Model for End-stage Liver Disease score. \*Odds ratios (OR) are quoted per 1 unit increase in the predictor variable. For K18-M30 and -M65 the ORs are per 1 kIU/L increase in serum concentration, for age it is per additional year and MELD score per whole point increase. For K18 M30:M65 ratio the OR is quoted per whole number increase

**Supplementary Table 6**. Logistic regression interaction analyses for the Lille score with prednisolone in relation to 90-day mortality.

|  |  |  |  |
| --- | --- | --- | --- |
| Adjusted analysis prednisolone and Lille score | | | |
|  | OR | 95% CI | *p* |
| Prednisolone | 0.724 | 0.301 – 1.724 | 0.466 |
| Lille score | 2.822 | 1.069 – 7.622 | 0.0379 |
| Age | 1.047 | 1.021 – 1.074 | 0.0004 |
| MELD | 1.121 | 1.065 – 1.183 | <0.0001 |
| Prednisolone:Lille score | 1.352 | 0.348 – 5.287 | 0.663 |

OR, Odds Ratio; CI, confidence interval; MELD, Model for End-stage Liver Disease score.

**SUPPLEMENTARY REFERENCES**

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**Supplementary Figure Legends**

**Supplementary Figure 1.** Levels of serum K18-M30 (a,d), K18-M65 (b,e) and K18-M30:K18-M65 ratio (c,f) in patients defined by the presence or absence of Mallory-Denk bodies (a-c) or megamitochondira (d-f) on liver biopsy. Data are displayed as the median (solid bar), interquartile range (box) and 95% confidence interval (whiskers).

**Supplementary Figure 2.** Levels of serum K18-M30:K18-M65 ratio in patients with different histological Laennec fibrosis stages (a), inflammation severities (b) and hepatocyte ballooning severities (c) on liver biopsy according to the alcoholic hepatitis histological scoring system (AHHS). Data are displayed as the median (solid bar), interquartile range (box) and 95% confidence interval (whiskers).

**Supplementary Figure 3.** Correlation between serum keratin 18 (K18) fragments M30/M65 and clinical scores.ALT (alanine transaminase), ASH (alcoholic steatohepatitis), AST (aspartate transaminase), DF (Maddrey’s discriminant function), GAHS (Glasgow AH score), INR (International Normalised Ratio), MELD (Model for End-stage Liver Disease score).

**Supplementary Figure 4.** Distribution of serum K18-M30 and K18-M65 values by quintile of Model for End-stage Liver Disease score (MELD; a) or Maddrey’s discriminant function (DF) score (b).

**Supplementary Figure 5.** Receiver operating characteristic (ROC) curves for the prediction of twenty-eight-day mortality by serum K18 fragments alone or combination with the MELD score in patients with clinically diagnosed alcoholic hepatitis and treated without (a) or with (b) prednisolone.

**Supplementary Figure 6.** Kaplan-Meier survival functions for (a) Lille responders (Lille score <0.45) and (b) Lille non-responders, by prednisolone treatment status. Additional Kaplan-Meier illustrating the effect of prednisolone in Lille responders and non-responders with serum K18-M30 (c,d) above or (e,f) below 5 kIU/L.