**Online-only supplement of Atkinson\*, Hamesch\* et al. – “Serum transferrin is an independent predictor of mortality in severe alcoholic hepatitis.”**

# **SUPPLEMENTARY METHODS**

### **Study population (continued)**

Ethical approval was granted for this study by the Wales Research Ethics Committee (REC 09/MRE09/59). The study was conducted according to the Declaration of Helsinki (Hong Kong Amendment) and Good Clinical Practice (European guidelines). All participants, or their legally appointed representatives, provided written informed consent.

### **Measurement of laboratory parameters**

All blood samples were drawn at baseline, i.e. prior to the initiation of therapy but after confirmation of eligibility for entry to the STOPAH trial by screening and subsequent randomization. These pre-treatment serum samples were successfully collected from 866/1092 patients. In 828 of them (96%), a sufficient volume of serum was available to carry out the described analyses. Serum C-reactive protein (CRP; turbidimetry), ferritin (electrochemiluminescence), transferrin (TF; turbidimetry), iron (photometry), TSAT, and soluble transferrin receptor (sTfR; turbidimetry) were measured with routine assays using the Cobas 8000 system (Roche Diagnostics, Mannheim, Germany) available at the Clinical Chemistry Department of University Hospital Aachen. All tests have been approved for use in clinical practice and were not altered by hemolysis or increased serum bilirubin. Serum hepcidin was quantified by a commercially available enzyme-linked immunosorbent assay kit (EIA-5782; DRG Instruments, Marburg, Germany). Non-transferrin bound iron (NTBI) was measured using the FeROS eLPI kit, a fluorescence-based assay intended for the in vitro semiquantitative detection of both overt and cryptic redox active forms of NTBI (Aferrix Ltd, Tel Aviv, Israel). LPI levels less than 0.4 were considered to be negative.

# **SUPPLEMENTARY FIGURES**



**Supplementary Figure 1. Application of Youden’s index to ROC curve for serum transferrin in relation to 28-day mortality.**

The cut point (sensitivity 71%, specificity 64%) corresponded to serum transferrin level of 102 mg/dL.

 **Supplementary Figure 2. Correlation heat map for serum biomarkers and clinical scores.**

Cells are shaded according to the Spearman’s rank Rho value and annotated with the Benjamini-Hochberg (false discovery rate) adjusted p-value. Serum transferrin demonstrated statistically significant moderate negative correlations with serum C-reactive protein (rho=-0.33, p<0.01) and ferritin (rho=-0.40, p<0.01) as acute phase reactants. Weak negative correlations were also observed between serum transferrin and serum bilirubin (rho=-0.20, p<0.01) and the international normalised ratio (rho=-0.19, p<0.01). Abbreviations: CRP: C-reactive protein; TSAT: transferrin saturation; GAHS: Glasgow alcoholic hepatitis score; MELD: model for end-stage liver disease; DF: discriminant function; Creat: Creatinine; ALT: alanine transaminase; AST: aspartate transaminase; INR: international normalised ratio; WCC: white cell count; HB: haemoglobin.

# **SUPPLEMENTARY TABLES**

**Supplementary Table 1. Iron indices in patients with severe alcoholic hepatitis, separated by sex.**

|  |  |  |  |
| --- | --- | --- | --- |
| Characteristic | Exploratory population (n=200) | Replication population (n=628) | Reference ranges |
| Ferritin (ng/ml) | Male: 862 (307 – 1574)Female: 497 (260 – 853)p=0.01 | Male: 827 (353 – 1577)Female: 692 (275 – 1310)p=0.02 | Male: 30 – 400Female: 13 – 150 |
| Transferrin (mg/dL) | Male: 93 (72 – 124)Female: 99 (69 – 129) | Male: 126 (93 – 171)Female: 120 (90 – 168) | 200 – 360 |
| TSAT (%) | Male: 69 (38 – 87)Female: 69 (43 – 82) | Male: 58 (35 – 82)Female: 58 (34 – 83) | 25 – 45% |
| Serum iron(µmol/L) | Male: 13.8 (10.2 – 18.3)Female: 13.8 (9.0 – 18.6) | Male: 16.5 (11.4 – 22.8)Female: 15.9 (11.1 – 22.5) | 5.8 – 35 |
| Hepcidin (ng/ml) | Male: 9.78 (3.69 – 23.6)Female: 8.53 (2.98 – 21.1) | Male: 14.1 (5.4 – 29.4)Female: 10.4 (3.48 – 21.3) | NA |

Data are presented as median and interquartile range. Baseline serum parameters were available in 828 patients, who were randomly divided into the exploratory and replication subgroup. Male vs. female comparisons for serum ferritin were made with the Mann-Whitney U test. Abbreviations: TSAT: transferrin saturation

**Supplementary Table 2. Logistic regression model assessing interaction between serum transferrin and prednisolone therapy in relation to Lille response.**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **OR** | **95% CI** | **P** |
| **Prednisolone** | 1.49 | 0.65 – 3.44 | 0.346 |
| **Transferrin** | 1.00 | 0.996 – 1.004 | 0.840 |
| **Prednisolone\*Transferrin** | 1.002 | 0.996 – 1.008 | 0.544 |

**Supplementary Table 3. Iron indices and their predictive power for 28-day mortality in subgroups with/without infection or gastrointestinal bleeding.**

|  |  |  |  |
| --- | --- | --- | --- |
| Characteristic | No infection or GI bleeding (n=663) | Infection(n=107) | GI bleeding(n=76) |
| Transferrin (mg/dL) | 117 (84 – 156) | 105 (78 – 149)\* | 138 (100 – 176)\* |
| OR 0.985 (95% CI 0.980 – 0.991), p<0.01 | OR 0.983 (95% CI 0.968 – 0.998), p=0.03 | OR 0.982 (95% CI 0.967 – 0.997), p=0.02 |
| TSAT (%) | 63 (39 – 84) | 56 (34 – 81) | 35 (20 – 65)\*\*\* |
| OR 1.024 (95% CI 1.02 – 1.03), p<0.001 | OR 1.014 (95% CI 0.994 – 1.033), p=0.17 | OR 1.020 (95% CI 0.999 – 1.041), p=0.06 |
| Ferritin (ng/ml) | 792 (350 – 1535) | 541 (204 – 1317) | 328 (169 – 960)\*\*\* |
| OR 1.000 (95% CI 1.000 – 1.000), p=0.05 | OR 1.001 (95% CI 1.000 – 1.001), p=0.02 | OR 1.001 (95% CI 1.000 – 1.001), p=0.05 |
| Serum iron(µmol/L) | 16.5 (11.6 – 22.5) | 13.5 (9.9 – 18.6)\*\* | 11.7 (8.2 – 18.0)\*\*\* |
| 0.98 (95% CI 0.95 – 1.01), p=0.13 | OR 0.96 (95% CI 0.88 – 1.04), p=0.28 | OR 1.008 (95% CI 0.93 – 1.09), p=0.85 |
| Hepcidin (ng/ml) | 12.4 (4.6 – 25.6) | 11.1 (4.7 – 28.4) | 5.4 (2.4 – 14.6)\*\*\* |
| OR 1.01 (95% CI 1.00 – 1.02), p=0.05 | OR 1.01 (95% CI 0.98 – 1.04), p=0.56 | OR 1.02 (95% CI 0.989 – 1.06), p=0.19 |

Data are shown as medians and interquartile ranges. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 for pairwise comparison between those presenting with infection or GI haemorrhage and those without. Odds ratios and 95% confidence intervals are referring to association with 28-day mortality. Abbreviations: CI: confidence interval; GI: gastrointestinal; OR: odds ratio; TSAT: transferrin saturation

**Supplementary Table 4. Serum iron parameters in exploratory and validation cohorts, dichotomized by the diagnosis of gastrointestinal bleeding at presentation**

|  |  |  |
| --- | --- | --- |
|  | **Exploratory** | **Validation** |
|  | **No baseline GI bleed (n=187)** | **Baseline GI bleed (n=13)** | **p**  | **No baseline GI bleed (n=565)** | **Baseline GI bleed (n=63)** | **p** |
| **Hepcidin (ng/ml)** | 9.12 (3.15 – 22.7) | 5.64 (3.03 – 32.39) | 0.799 | 13.45 (5.25 – 27.6) | 5.23 (2.28 – 14.3) | <0.0001 |
| **Iron (µmol/l)** | 14.1 (9.75 – 18.3) | 11.1 (8.4 – 15.5) | 0.148 | 16.8 (11.9 – 23.1) | 12.6 (7.5 – 18.0) | <0.0001 |
| **Transferrin (mg/dl)** | 93 (70 – 125) | 105 (72 – 153) | 0.157 | 123 (90 – 167) | 138 (102 – 180) | 0.138 |
| **TSAT(%)** | 70.7 (42.2 – 86.2) | 35.5 (27.0 – 75.7) | 0.098 | 59.5 (36.5 – 82.9) | 35.2 (18.1 – 64.8) | <0.0001 |
| **Ferritin (ng/ml)** | 643 (298 – 1414) | 611 (187 – 1500) | 0.754 | 815 (357 – 1521) | 313 (130 – 929) | <0.0001 |

Data are shown as medians and interquartile ranges.

**Supplementary Table 5. Serum iron parameters in exploratory and validation cohorts, dichotomized by the diagnosis of infection at presentation.**

|  |  |  |
| --- | --- | --- |
|  | **Exploratory** | **Validation** |
|  | **No baseline infection (n=179)** | **Baseline infection (n=21)** | **p**  | **No baseline infection (n=542)** | **Baseline infection (n=86)** | **p** |
| **Hepcidin (ng/ml)** | 8.62 (2.94 – 22.46) | 9.76 (6.21 – 32.8) | 0.334 | 12.43 (4.78 – 25.92) | 14.24 (4.18 – 26.05) | 0.998 |
| **Iron (µmol/l)** | 14.4 (9.3 – 18.45) | 12.0 (9.75 – 14.1) | 0.173 | 16.8 (11.7 – 23.1) | 14.6 (9.83 – 19.7) | 0.14 |
| **Transferrin (mg/dl)** | 96 (72 – 131) | 78 (61 – 99) | 0.016 | 123 (93 – 169) | 114 (84 – 168) | 0.165 |
| **TSAT(%)** | 69.8 (38.7 – 85.6) | 64.6 (47.6 – 87.5) | 0.667 | 59.1 (34.9 – 82.7) | 50.9 (31.1 – 79.3) | 0.200 |
| **Ferritin (ng/ml)** | 647 (294 – 1391) | 526 (233 – 1448) | 0.973 | 793 (342 – 1529) | 556 (199 – 1278) | 0.036 |

Data are shown as medians and interquartile ranges.

**Supplementary Table 6. Multivariable logistic regression analysis for 28- and 90-day mortality considering transferrin and prognostic scoring systems.**

|  |  |  |
| --- | --- | --- |
| Characteristic | 28-day mortality | 90-day mortality |
|  | OR (95% CI) | P | OR (95% CI) | P |
| DF |
| DF | 1.02 (1.01 – 1.02) | <0.001 | 1.02 (1.01 – 1.02) | <0.001 |
| Transferrin (mg/dL) | 0.987 (0.982 – 0.992) | <0.001 | 0.993 (0.989 – 0.996) | <0.001 |
| MELD |
| MELD | 1.16 (1.11 – 1.21) | <0.001 | 1.15 (1.10 – 1.20) | <0.001 |
| Transferrin (mg/dL) | 0.990 (0.984 – 0.995) | <0.001 | 0.995 (0.991 – 0.998) | 0.005 |
| GAHS |
| GAHS | 1.94 (1.61 – 2.33) | <0.01 | 1.80 (1.55 – 2.08) | <0.001 |
| Transferrin (mg/dL) | 0.989 (0.984 – 0.994) | <0.001 | 0.994 (0.991 – 0.998) | 0.002 |

Abbreviations: CI: confidence interval; DF: Discriminant function; GAHS: Glasgow alcoholic hepatitis score; MELD: model for end-stage liver disease; OR: odds ratio

Parameters of scores: DF = prothrombin time, bilirubin; GAHS = age, white blood cell count, blood urea nitrogen, bilirubin, prothrombin time; MELD = creatinine, bilirubin, INR, presence of dialysis.

**Supplementary Table 7. Serum soluble transferrin receptor (sTfR) levels, by 28- and 90-day mortality status.**

|  |  |  |
| --- | --- | --- |
|  | 28-day mortality  | 90-day mortality  |
|  | Alive (n=530) | Died (n=62) | Alive (n=473) | Died (n=119) |
| sTfR (mg/L) | 4.79 (3.12 – 7.60) | 3.99 (2.65 – 7.53) | 4.71 (3.08 – 7.34) | 4.56 (3.03 – 8.61) |
|  | OR 1.02 (95% CI, 0.996 – 1.042), p=0.10 | OR 1.02 (95% CI 0.999 – 1.04), p=0.07 |

Data presented as median (interquartile range)

Abbreviations: CI: Confidence interval; OR: Odds ratio; sTfR: soluble transferrin receptor

**Supplementary Table 8. Correlation of non-transferrin bound iron (NTBI) with selected laboratory parameters and prognostic scoring systems.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Marker | Iron | TF | Ferritin | TSAT | Hepcidin | sTfR | PCT | MELD | GAHS | DF |
| Rho | -0.45 | -0.23 | 0.34 | 0.61 | 0.07 | 0.05 | 0.06 | 0.08 | 0.05 | 0.17 |
| P | <0.001 | 0.001 | <0.001 | <0.001 | 0.31 | 0.94 | 0.41 | 0.26 | 0.49 | 0.01 |

The values are based on Spearman’s rank correlation test. Abbreviations: TF, transferrin; TSAT, transferrin saturation; sTfR, soluble transferrin receptor; PCT, procalcitonine; MELD, model for end-stage liver disease; GAHS, Glasgow alcoholic hepatitis score; DF, Discriminant function.