Baseline neutrophil-to-lymphocyte ratio predicts response to corticosteroids and is associated with infection and renal dysfunction in alcoholic hepatitis

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Baseline neutrophil-to-lymphocyte ratio predicts response to corticosteroids and is associated with infection and renal dysfunction in alcoholic hepatitis.

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Abbreviations: MELD: Model of End-Stage Liver Disease; ABIC: Age Bilirubin, INR and Creatinine Score; NLR: Neutrophil-to-Lymphocyte ratio; GAHS: Glasgow Alcoholic Hepatitis Score; ACLF: Acute-on-Chronic Liver Failure; WCC: White Cell Count; AKI: Acute Kidney Injury; DF: Discriminant Function; AUC: area under the curve; HR: Hazard ratio; OR: Odds ratio; CI: confidence interval; SIRS: Systemic Inflammatory Response Syndrome; mGAHS: modified Glasgow Alcoholic Hepatitis Score; INR: International Normalised ratio.

SUMMARY

Background and Aim: Treating severe alcoholic hepatitis involves the exposure of patients to corticosteroids for 7 days to assess 'response'. The aim of this study was to assess the prognostic and therapeutic implications of baseline Neutrophil-to-Lymphocyte ratio (NLR) in patients with severe alcoholic hepatitis.

Methods: NLR was derived retrospectively from STOPAH patients and an independent validation group. Area under the Receiver Operating Curve (AUC) analysis was performed. Kaplan-Meier analysis was used to assess survival. Log-Rank test and Odds Ratio (OR) were used for comparative analysis.

Results: Baseline NLR was available for 789 STOPAH patients. The AUC for NLR was modest for 90-day outcome (0.660), but it was associated with infection, acute kidney injury (AKI) and severity of alcoholic hepatitis. 90-day survival was not affected by prednisolone treatment with NLR<5 but mortality was reduced with prednisolone treatment when NLR 5-8: 21.0% *cf.* 34.5%; p=0.012. prednisolone treatment increased the chance of Lille response if the NLR≥5 (56.5% *cf.* 41.1%: p=0.01; OR 1.86) but increased the risk of Day 7 infection (17.3% *cf.* 7.4%: p=0.006; OR 2.60) and AKI (20.8% *cf*. 7.0%: p=0.008; OR 3.46) if NLR>8. Incorporation of NLR into a modified Glasgow Alcoholic Hepatitis Score (mGAHS) improved the AUC to 0.783 and 0.739 for 28-day and 90-day outcome respectively.

Conclusion: The NLR is associated with AKI and infection in severe alcoholic hepatitis. The NLR identifies those most likely to benefit from corticosteroids

at baseline (NLR 5-8). The mGAHS has a good predictive value for 28- and 90-day outcomes.

INTRODUCTION

Treatment for severe alcoholic hepatitis remains problematic. Whilst corticosteroids appear to have a favourable effect upon short-term (28-day) mortality, this advantage is not sustained to 90 days or beyond[1,2]. Corticosteroid treatment is also associated with a higher risk of infection[2]. Therefore, not all patients with alcoholic hepatitis benefit from such an approach. The difficulty is in identifying those for whom such treatment is useful, but avoiding unnecessary exposure to corticosteroids for those whom such treatment is ineffective or even detrimental. Various strategies have been suggested. A recommended approach is to treat alcoholic hepatitis patients but to assess 'response' after 7 days of therapy, often using the Lille score[3]. An unfavourable response would indicate an early discontinuation of corticosteroids, thereby limiting treatment exposure. It has been suggested that Lille score 'response' at Day 4 may be indicative of an early response and so further curtail corticosteroid exposure[4]. A more recent strategy has been to only treat those with more severe disease as indicated by high MELD, ABIC (Age, Bilirubin, INR, Creatinine score) or GAHS (Glasgow Alcoholic Hepatitis Score) values, so limiting the number of patients treated[5]. However even with this approach, benefit beyond 28 days is only seen in those with a Day 7 response. Thus current treatment strategies rely upon exposure to potentially ineffective, or even harmful, corticosteroid therapy. The optimal approach would be to identify patients at baseline for whom corticosteroid treatment would be beneficial.

Recently several studies have found a high Neutrophil-to-Lymphocyte Ratio (NLR) to be indicative of a worse prognosis in a variety of different liver

disease contexts: patients on the liver transplant waiting list[6], those with low MELD scores[7], hospitalised patients with cirrhosis[8], patients with acute-on-chronic liver failure (ACLF)[9] and patients with decompensated liver disease without ACLF[10]. The prognostic value of NLR in these studies appears additional to standard scores of liver function and to that of total white blood cell count (WCC) alone. Relative lymphopenia plays an important role as well as an inflammatory neutrophilia; lymphopenia along with an increased CD4+/CD8+ ratio has been noted in alcohol-related liver disease previously[11]. The aim of this study was to assess whether baseline NLR might be used to identify steroid responders among a cohort of patients with severe alcoholic hepatitis.

METHODS

STOPAH Group

Patients recruited to the STOPAH trial were studied. The characteristics of these patients have been described in detail previously[12]. Inclusion was based upon a clinical diagnosis of severe alcoholic hepatitis with recent onset of jaundice (serum bilirubin>80μmol/l) and heavy alcohol misuse and no other aetiology of liver disease. All patients had a baseline Discriminant Function (DF) greater than or equal to 32. Patients were randomised by a factorial design to receive prednisolone and placebo, pentoxifylline and placebo, prednisolone and pentoxifylline or double placebo. The overall STOPAH results showed no therapeutic effect with pentoxifylline at any time point and so patients randomised to pentoxifylline were analysed as per untreated (placebo) patients. Thus, the therapeutic comparison was for those treated with prednisolone or not given at a dose of 40mg per day for 4 weeks. Analysis was performed on an intention to treat basis. As data on blood lymphocyte count was not collected in the original STOPAH dataset, this information was obtained retrospectively in order to calculate the NLR. The trial was approved by the Multicentre Research Ethics Committee (09/MRE09/59) and under the oversight of University of Southampton Clinical Trials Unit. All participants, or their legally appointed representative, provided written informed consent.

Mortality at 28 and 90 days after randomisation was analysed. Outcome beyond 90 days was not assessed as other factors, such as continued alcohol use, were felt to influence outcome beyond that point. Patients were

consented for follow-up using the National Health Service data linkage service so that even if lost to direct follow-up their outcomes could be captured.

Baseline Acute Kidney Injury (AKI) was defined by an initial creatinine ≥133μmol/l and incident AKI was defined as either an increase of serum creatinine by 26.5μmol/l or by 50% by Day 7 in those without baseline AKI. All patients were screened for infection and active infections were treated prior to participation. A pre-determined analysis excluding those who presented initially with either gastro-intestinal bleeding or sepsis was carried out. Surveillance for infection continued after randomisation and rates of infection noted. MELD (pre-2016 UNOS variant), GAHS and Lille Scores were calculated with prognostic thresholds of 21, 9 and 0.45 respectively as previously described. The Chronic Liver Failure (CLIF) - Organ Failure and CLIF-Consortium ACLF scores and grades were calculated with the limitation of the lack of respiratory data managed as previously described[13,14].

Validation Group

A Validation Group was derived from patients assessed and treated for alcoholic hepatitis from four United Kingdom centres outside of the STOPAH trial: Leeds, Bristol, Brighton and Plymouth. Data was collected in keeping with local Clinical Governance practice. All patients had a serum bilirubin greater than 80μmol/l and a DF greater than or equal to 32. Treatment with corticosteroids (prednisolone 40mg/day) and their continuation or discontinuation in the light of an assessment of response after 7 days was based on local clinical discretion.

Statistical Analyses

Analyses were performed using MedCalc Statistical Software version 17.6 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2017). Comparison of the discriminatory strength of scores was performed by area under the Receiver Operating Curve (AUC) analysis. Optimal cut-offs were identified by calculating the Youden Index (*J*). Kaplan-Meier analysis was used to assess survival and survival curves were compared using the Log-Rank test with Hazard Ratio (HR) described where relevant. Results are presented with 95% confidence intervals (95% CI). Direct comparisons between data were performed using t-tests and between proportions using Odds Ratio (OR) calculation. Pearson's correlation co-efficient (r) was calculated for associations between variables.

RESULTS

STOPAH Patients

Of the 1103 patients recruited to STOPAH, it proved possible to obtain data to calculate the baseline NLR in 789 (71.5%). Of these patients 393 were treated with prednisolone. The overall mortality at Day 90 was 25.1% for those treated with prednisolone and 25.5% not treated with prednisolone (p=0.682). The mean NLR at baseline for all patients was 6.51 (6.14, 6.88). Patient characteristics are described in Table 1 with details of the STOPAH patients included and excluded from the analysis, and those of the Validation Group. All groups were well matched at baseline with the exception of a difference in total white blood cell count between the STOPAH patients with NLR values and those for whom the lymphocyte count was not available.

Associations of NLR in Alcoholic Hepatitis

Severity of alcoholic hepatitis and NLR were related (Supplementary Table 1). Survivors at Day 90 had having a lower NLR than those who had died: 5.7 (5.44, 6.09) *cf.* 8.70 (7.67, 9.72): p<0.0001. Patients with a low GAHS (less than 9) had a mean NLR of 5.05 (4.74, 5.37); those with a high GAHS (greater than or equal to 9) had a mean NLR of 8.12 (7.47, 8.77): p<0.0001. Similarly a MELD less than 21 was associated with a mean NLR of 4.89 (4.27, 5.49); MELD greater than or equal to 21 the mean NLR was 6.72 (6.29, 7.14): p<0.0001. The NLR correlated with the Lille score (r=0.212 (0.13, 0.29); p<0.0001): patients with a favourable Lille Score (<0.45) having a lower NLR than those with an unfavourable score (≤0.45): 5.78 (5.33, 6.22) *cf.* 7.71 (6.90, 8.53): p<0.001. A higher NLR was associated with higher ACLF grade (Supplementary Figure 1).

The NLR was significantly greater in those patients who presented with infection and in those with AKI at baseline. It was also greater in those who developed new infection in the first 7 days and 28 days after randomisation (Table 2). There was a strong correlation between the total WCC and NLR: r=0.564 (0.52, 0.60); p<0.0001.

Predictive Discrimination for Mortality of NLR and Outcome of Prednisolone Treatment (Figure 1 and Supplementary Table 2)

Overall the baseline NLR had a modest discriminatory capacity with an AUC of 0.660 (0.626, 0.693) with an optimal cut-point of 5 (*J*=0.247) for 90-day mortality. Mortality at Day 90 was 16.1% for those with a NLR<5 and 33.6% for those with a NLR≥5 (p<0.0001; HR 2.39 (1.81, 3.15)). With regards to 90-day outcome, in those patients with a NLR<5 (n=372, 47.1% of the population), prednisolone had no effect upon outcome, although there was an insignificant trend towards higher mortality with treatment: 19.0% *cf*. 13.2%; p=0.176.

For those with a NLR≥5 who were treated with prednisolone, the optimal cut-point was 8 (*J*=0.272). For these patients, mortality at Day 90 was 21.0% for those with a NLR<8 and 41.8% for those with a NLR≥8 (p=0.0003: HR 2.50 (1.52, 4.12)). The mortality of the 199 patients (25.2%) with a NLR>8 was unaffected by prednisolone treatment: 41.8% *cf.* 37.6%; p=0.604. However, for the 218 patients (27.6%) with NLR 5-8, prednisolone treatment was associated with a significant reduction in 90-day mortality: 21.0% *cf.* 34.5%; p=0.012; HR 0.52 (0.31, 0.86) (Figure 3). This pattern was repeated with exclusion of those who presented initially with either GI bleeding or sepsis.

Day 7 Response and Risk of Infection and AKI Relative to NLR Range (Figure 2)

For those with an NLR<5, prednisolone treatment did not increase the chance of a favourable Lille score: 63.7% *cf.* 64.5% for those untreated. The proportion of patients achieving a favourable Lille score with prednisolone treatment was increased for those with an NLR≥5: (56.5% *cf.* 41.1%: p=0.01; OR 1.86 (1.16, 2.99)). NLR≥5 patients were more likely to achieve a 25% fall in bilirubin from baseline with prednisolone treatment compared with those not treated: 60.8% *cf.* 27.4% (p<0.0001; OR 2.76 (1.72, 4.43)). This was not so for those with NLR<5: 47.8% *cf*. 40.3% (p=0.243 OR 1.36 (0.81, 2.26)). The significance of the Lille score regarding 90-day outcome varied depending upon NLR category. Those with an unfavourable Lille score whether they received corticosteroids or not and an NLR<5 had a lower mortality (26.2%: p=0.0021) compared with NLR 5-8 (46.3%) and NLR>8 (51.9%). prednisolone treatment reduced the 90-day mortality of NLR 5-8 patients with a favourable Lille score compared with those not treated: 8.9% *cf.* 25.0%: p=0.046; HR 0.32 (0.11, 0.97). However even for those with an NLR 5-8 and treated with corticosteroid, a Lille non-response was associated with a higher mortality: 48.2% *cf*. 8.9%: p=0.0001; HR 6.55 (2.39, 17.98).

The risk of developing infection within 7 days after prednisolone treatment was greater for those with an NLR>8 compared with an NLR<8: 17.3% *cf.* 7.4%: p=0.006; OR 2.60 (1.32, 5.14). This difference was also seen with incident infections until 28 days after prednisolone treatment: 30.6% *cf.* 20.0%: p=0.031; OR 1.76 (1.05, 2.96). For those with an NLR<8, prednisolone treatment was associated with a lesser chance of infection by

Day 7 compared with untreated patients (7.4% *cf.* 18.4%: p=0.013; OR 2.00 (1.16, 3.46)) but this was not so for those with an NLR>8 (17.3% *cf.* 13.9%: p=0.499; OR 0.77 (0.36, 1.66)).

Similarly, the risk of developing incident AKI after prednisolone treatment was greater for those with a NLR>8 compared with a NLR<8: 20.8% *cf*. 7.0%: p=0.008; OR 3.46 (1.39, 8.62). For those with NLR<8, prednisolone treatment was associated with a lesser chance of incident AKI compared with untreated patients (7.0% *cf.* 17.1%: p=0.005; OR 2.72 (1.35, 5.47)) but again this was not so for those with NLR>8 (20.8% *cf.* 20.0%: p=0.913; OR 0.95 (0.38, 2.39)).

Incorporation of NLR into GAHS

In view of the expected correlation of NLR with total WCC, the NLR was incorporated into the GAHS with an NLR threshold of 5 replacing the WCC threshold of 15, creating a modified GAHS (mGAHS) (Table 3). The AUCs for mGAHS for 28-day and 90-day outcomes were 0.783 (0.752, 0.812) and 0.738 (0.705, 0.769) respectively. For 28-day outcome the mGAHS AUC was superior to that of the DF (0.683: p<0.0001; 0.06, 0.14), the original GAHS (0.762: p=0.027; 0.002, 0.04), the MELD (0.738: p=0.0013; 0.009, 0.08) and the CLIF-OF Score (0.730: p=0.022; 0.007, 0.08) (Figure 3). At a threshold of 9, the mGAHS had greater sensitivity than the original GAHS (82.3% *cf.* 71.2%) but lesser specificity (49.4% *cf.* 60.8%) for 90-day outcome. The corresponding sensitivity and specificity for MELD relative to a threshold of 21 were 92.4% and 20.0% (Supplementary Table 3). A mGAHS≥9 was present in 460 patients (58.6%) with a 90-day mortality of 35.4% compared with a mortality of 10.8% for those with mGAHS<9 (p<0.0001 HR 3.91 (2.96, 5.17))

(Supplementary Figure 2). The corresponding figures for the original GAHS were 373 patients (47.4%) ≥9 with 90-day mortality of 38.3% compared with a mortality of 13.8% for those with GAHS<9 (p<0.0001; HR 3.34 (2.52, 4.42)). For MELD, 630 patients (83.6%) had a score ≥21 with 90-day mortality of 28.4% compared with a mortality of 8.9% for those with MELD<9 (p<0.0001; HR 3.66 (2.54, 5.29)).

The proportions of NLR categories relative to MELD, GAHS and mGAHS category are shown in Supplementary Figure 3. Within NLR categories, mGAHS did not affect the potential benefit from prednisolone: those with an NLR 5-8 showed benefit with prednisolone, and those with NLR<5 or >8 showed no prednisolone benefit, irrespective of whether the mGAHS was less than or greater than 9 (Table 4).

Validation Group

There were 237 patients in the validation group, 138 of whom were treated with prednisolone. The overall mortality at Day 90 was 26.3% for those treated with prednisolone and 32.6% not treated with prednisolone (p=0.20). The mean NLR at baseline for all patients was 8.56 (7.23, 9.84). Patient characteristics are described in Table 1.

As with the STOPAH cohort the NLR was greater in those who presented with AKI (10.22 *cf.* 7.37: p=0.014; 0.57, 5.14) and those who presented initially with sepsis (10.06 *cf.* 7.36: p=0.023; 0.38, 5.01).

<u>NLR Category and Prednisolone Treatment</u>

There was no reduction in mortality with prednisolone treatment for those with NLR<5 or >8 at either 28 days or 90 days. At Day 28 those with NLR 5-8 treated with prednisolone had a reduced mortality compared with those

untreated: 2.7% *cf.* 28.6%: p=0.0023; HR 0.084 (0.03, 0.27). The trend continued to Day 90 but was not significant: 21.6% *cf.* 37.1%: p=0.097; HR 0.484 (0.20, 1.14). However, for those with mGAHS≥9 and NLR 5-8 there was a reduction in 90-day mortality with prednisolone treatment: 23.3% *cf.* 46.4%: p=0.036; HR 0.268 (0.10, 0.71). (Table 5). There was no evidence of benefit for those with NLR<5 or >8 in relation to mGAHS category.

Performance of mGAHS

The AUC for 90-day outcome was highest for mGAHS (0.716: 0.652, 0.774) compared with the original GAHS (0.698: 0.633, 0.757), DF (0.655: 0.589 0.717) and MELD (0.702: 0.637, 0.761). At a threshold of 9, the mGAHS had almost identical sensitivity (83.4%) and specificity (49.1%) as the STOPAH cohort for 90-day outcome. Again, similar to the STOPAH cohort mGAHS≥9 was present in 144 patients (61.0%) with a 90-day mortality of 39.4% compared with 12.0% for those with mGAHS<9 (p<0.0001 HR 3.87 (2.39, 6.28)).

Treatment Strategies

We assessed the effect of different treatment strategies in patients from the STOPAH cohort who had NLR available. A comparison of these is shown in Table 6. Those approaches which applied a baseline prognostic score to determine treatment exposed a greater proportion of patients to prednisolone: DF 100%; MELD (threshold of 21) 84%; GAHS (threshold of 9) 47%. Using NLR 5-8 to determine those who should have treatment, only 28% of patients would have received prednisolone. Despite the reduced number of patients exposed to prednisolone treatment, the overall mortality of the NLR strategy was less than that of any of the baseline prognostic score strategies. In

addition, the rate of reported infection by Day 28 was reduced with the NLR strategy compared with any of the baseline score strategies.

DISCUSSION

The decision to treat severe alcoholic hepatitis with corticosteroids has been a contentious issue for many years. There is some short-term improvement in outcome with such treatment when given to patients with a DF greater than 32[1,2,15]. However this benefit does not extend beyond 28 days and it has not been possible to define which of these patients will respond before starting treatment. There is also an increased risk of infection with corticosteroid treatment potentially limiting any beneficial impact of this therapy[16]. The recommended approach is to look for evidence of response after 4-7 days of exposure to corticosteroids and to continue treatment if such a response is favourable. This response is usually indicated by a fall in serum bilirubin which is incorporated into the Lille score[3]. A more recent approach to reducing corticosteroid exposure is to only treat those with more severe disease as indicated by a high GAHS, ABIC or MELD value[4,5], however even with this targeted approach benefit beyond 28 days depends upon response after initiating treatment. The optimal treatment strategy would be to identify those likely to benefit from treatment at baseline rather than having to give a trial of therapy. Not only would this target those likely to benefit, it would lessen the risk to those for whom such treatment could be deleterious.

Several recent reports suggested means to identify patients who might benefit from corticosteroids before such exposure. Higher density of Mallory bodies and more severe hepatocyte ballooning on biopsy may be associated with non-response[17]. Alternatively, high circulating levels of bacterial DNA may predict the development of infection within the first seven days of corticosteroid therapy and identify non-responders[16]. A further study found

that circulating microvesicles, reflective of oxidative stress, are more numerous and larger in patients with a Lille non-response[18] as are higher levels of urinary albumin[19]. Higher levels of lipopolysaccharide, procalcitonin and the presence of the systemic inflammatory response syndrome (SIRS) have also been shown to indicate a worse outcome at 90 days in corticosteroid treated patients[20]. Unfortunately it was not possible to calculate the SIRS for our patients as the respiratory component was not recorded. However the NLR is a simple, readily accessible score that has been applied to liver disease previously as prognostic indicator. The current observations indicate the NLR has at best modest overall prognostic discrimination in alcoholic hepatitis. However, with appropriate calibration it can identify those who may benefit from treatment. Those with low (less than 5) or high (greater than 8) NLR values do not appear to benefit from prednisolone treatment. However, for those with NLR values between 5 and 8, an improvement in 90-day outcome was observed with corticosteroid treatment. The previously observed improvement in 28-day survival for those with high GAHS (greater than or equal to 9) is probably explained by the lower number of those with NLR<5 in this poor prognostic group. However even this poor prognostic group is heterogeneous with regards to NLR categories which may explain the failure to predict a favourable outcome with corticosteroids after 28 days. The mGAHS has a higher proportion of those with a favourable NLR 5-8 category for corticosteroid effect when greater than or equal to 9, though the predictive capacity of NLR 5-8 was also seen in those with a lower mGAHS. Using the NLR to target treatment would substantially reduce those exposed to corticosteroids with the expectation of a sustained improvement in outcome

for those so treated. It should also be noted that a high Lille score was still indicative of worse outcome even for those with NLR 5-8, therefore steroid exposure could be curtailed even further for those with an unfavourable Lille score. For those with NLRs too low or too high for corticosteroids, the options for management are for effective supportive treatments or the investigation of alternative or novel approaches.

The association of NLR with AKI has been noted before with increased values found in patients with hepatorenal syndrome and those with refractory ascites[6,21]. Similarly NLR has been associated with risk of infection in patients with cirrhosis[8] and severity of sepsis in critical care[22]. It is interesting to note that in these studies the prognostic value of NLR appears to be independent of standard scores of prognosis in liver disease such as the MELD and Child-Turcotte-Pugh scores[9,23]. The degree of neutrophilia may be reflective of the degree of inflammation but the relative lymphopenia may reflect a more subtle alteration of immune regulation[7,10, 24]. In addition the degree of lymphopenia may also be reflective of sarcopenia as has been noted in other clinical contexts[25]. Protein energy malnutrition is a frequent finding in alcoholic hepatitis and is strongly related to outcome[26] and sarcopenia is increasingly recognised as an important factor in liver disease prognosis independent of traditional laboratory-based markers of liver function[27]. It is possible that the NLR is simply another marker of SIRS, and SIRS, especially that associated with infection, is an indicator of overall prognosis[20]. The presence of SIRS on admission has also been previously associated with a greater risk of developing AKI[28,29]. In the current study baseline NLR has been shown to be reflective of the presence of AKI and infection at presentation, and also

predictive of the development of these complications subsequently. However, the presence of SIRS has not previously been shown to identify those who will most likely benefit from corticosteroid therapy. In the current study the NLR provides information relating to therapeutic options as well as prognosis and complications. The SIRS criteria are binary, whereas the NLR is a continuous measure which may reflect the complex interaction between pro- and anti-inflammatory factors in alcoholic hepatitis. It may be an indicator of where a patient is in the fine balance between inflammatory hepatic injury and the development of sepsis and/or multi-organ failure (AKI). Those with NLR less than 5 derived no benefit from corticosteroids with no greater chance of a Lille response compared with untreated patients. Those with higher NLR were more likely to have a Lille response with corticosteroids, but the potential benefit of this was offset for those with NLR greater than 8 probably because of failure to reduce the chance of developing sepsis and/or AKI.

The application of the NLR to treatment demonstrated that it could be used to target prednisolone treatment more effectively without any compromise in overall outcome. The absolute risk of 28-day infection and 90-day mortality were actually reduced by a NLR approach. Current guidelines suggest the use of a baseline score to determine a trial of prednisolone treatment and then use of the Lille Score at Day 7 to identify those whose treatment should continue for a further 3 weeks. The European Association for the Study of the Liver guidelines suggest a DF greater than or equal to 32 or a GAHS greater than or equal to 9 as the threshold for treatment[30]; the American College of Gastroenterology suggest a MELD greater than or equal to 21[31]. An NLR

approach would substantially reduce prednisolone exposure and may reduce the 90-day mortality in comparison to these recommendations.

Incorporation of the NLR into the GAHS instead of the WCC to create the mGAHS improved the discriminatory power of the score. However there was evidence of benefit from corticosteroids even for those with mGAHS<9 whose NLR was 5-8. This suggests that whilst the mGAHS has greater prognostic implications, the NLR is more 'theragnostic', that is, predictive of therapeutic response. Prognosis is important to manage patient expectations and to anticipate the course of illness, whereas theragnosis advises the clinician towards specific interventions. The Validation Group confirmed the prognostic usefulness of the mGAHS as well as identifying the NLR 5-8 group as those most likely to benefit from corticosteroid therapy. It is important to note that this group validated mGAHS and NLR in severe alcoholic hepatitis despite the non-randomised allocation of corticosteroid therapy and the liberty of clinicians to discontinue corticosteroids in the event of 'non-response' after seven days of treatment. This implies that these measures are clinically robust and applicable in practice as well within the restrictions of a clinical trial. The inclusion of patients on clinical criteria without the need for biopsy might raise concern that some patients without histological alcoholic hepatitis might have been included in the study. This is possible, however the clinical criteria used are in keeping with those proposed for clinical trials[32] and biopsy is infrequently used to diagnose alcoholic hepatitis in the United Kingdom[33]. Therefore the inclusion criteria are reflective of current standard practice. Whilst the mGAHS may be criticised for being a categorical score, even continuous scores such as the DF and MELD require a threshold to be

defined to guide clinical management. Therefore, categorisation is inevitable if a score is to be clinically applied. The current study indicates the effectiveness and robustness of the mGAHS and NLR in both clinical practice and clinical research.

This study indicates that for some alcoholic hepatitis patients there are characteristics evident before corticosteroid treatment that could be used to avoid a trial of therapy to assess response. Whilst further validation in other patient cohorts is justified, this analysis from the STOPAH cohort with subsequent validation shows that mGAHS and NLR could help stratify risk and likelihood of corticosteroid response in patients with alcoholic hepatitis.

REFERENCES

1. Singh S, Murad MH; Chandra AK, *et al*. Comparative Effectiveness of Pharmacological Interventions for Severe Alcoholic Hepatitis: A Systematic Review and Network Meta-analysis. *Gastroenterology* 2015;149: 958-70
2. Thursz MR, Richardson P, Allison M, *et al*. Prednisolone or pentoxifylline for alcoholic hepatitis. *N Engl J Med* 2015; 372: 1619–1628.
3. Louvet A, Naveau S, Abdelnour M, *et al*. The Lille model: a new tool for therapeutic strategy in patients with severe alcoholic hepatitis treated with steroids. *Hepatology* 2007; 45: 1348–54.
4. Garcia-Saenz-de-Sicilia M, Duvoor C, Altamirano J, Chavez-Araujo R, Prado V, Candolo-Martinelli A et al. A Day-4 Lille Model Predicts Response to Corticosteroids and Mortality in Severe Alcoholic Hepatitis. *Am J Gastroenterol* 2017; 112: 306–315.
5. Forrest EH, Atkinson SR, Richardson P, et al. Application of prognostic scores in the STOPAH trial: Discriminant function is no longer the optimal scoring system in alcoholic hepatitis. *Journal of Hepatology* 2018; 68(3): 511-518.
6. Leithead JA, Rajoriya N, Gunson BK et al. Neutrophil-to-lymphocyte ratio predicts mortality in patients listed for liver transplantation. *Liver Int* 2015; 35: 502-509.
7. Kalra A, Wedd JP, Bambha KM et al. Neutrophil-to-lymphocyte ratio correlates with proinflammatory neutrophils and predicts death in low

Model for End-stage Liver Disease patients with cirrhosis. *Liver Trans* 2017; 23: 155-165.

8. Kwon JH, Jang JW, Kim TW et al. The usefulness of C-reactive protein and neutrophil-to-lymphocyte ratio for predicting the outcome in hospitalized patients with liver cirrhosis. *BMC Gastro* 2015; 15: 146.
9. Chen L, Lou Y, Chen Y et al. Prognostic value of the neutrophil-to-lymphocyte ratio in patients with acute-on-chronic liver failure. *Int J Clin Prac* 2014; 68: 1034-1040.
10. Cai Y-J, Dong J-J, Dong J-Z et al. A nomogram for predicting prognostic value of inflammatory response biomarkers in decompensated cirrhotic patients without acute-on-chronic liver failure. *Aliment Pharm Ther* 2017; 45: 1413-1426.
11. Matos LC, Batista P, Montiro N et al. Lymphocyte subsets in alcoholic liver disease. *World J Hepatol* 2013; 5:46-55.
12. Forrest EH, Mellor J, Stanton L, et al. Steroids or pentoxifylline for alcoholic hepatitis (STOPAH): study protocol for a randomised controlled trial. *Trials* 2013; 14: 262.
13. Arroyo V, Moreau R, Jalan R, Ginès P; EASL-CLIF Consortium CANONIC Study. Acute-on-chronic liver failure: A new syndrome that will re-classify cirrhosis. *Journal of Hepatology* 2015; 62 (1 Suppl): 131-143.
14. Forrest EH, Atkinson SA, Richardson P et al. Prevalent acute on chronic liver failure and response to corticosteroids in alcoholic hepatitis. *Journal of Hepatology* 2018; 69(5): 1200-1201.

15. Louvet A, Thursz M, Kim DJ et al. Corticosteroids Reduce Risk of Death Within 28 Days for Patients With Severe Alcoholic Hepatitis, Compared With Pentoxifylline or Placebo—a Meta-analysis of Individual Data From Controlled Trials. *Gastroenterology* 2018; 155: 458-468.
16. Vergis N, Atkinson SR, Knapp S, Maurice J, Allison M, Austin A et al. Patients with severe alcoholic hepatitis given prednisolone therapy who have high circulating levels of bacterial DNA are at increased risk for developing infections. Gastroenterology 2016; 152: 1068-1077.
17. Shasthry SM, Rastogi A, Bihari C, et al. Histological activity score on baseline liver biopsy can predict non-response to steroids in patients with severe alcoholic hepatitis. *Virchows Arch* 2018;472:667-675.
18. Sukriti S, Maras JS, Bihari C, et al. Microvesicles in hepatic and peripheral vein can predict nonresponse to corticosteroid therapy in severe alcoholic hepatitis. *Aliment Pharmacol Ther* 2018; 47:1151–1161.
19. Das S, Hussain M, Maras J, et al. Modification Patterns of Urinary Albumin Correlates With Serum Albumin and Outcome in Severe Alcoholic Hepatitis. *J Clin Gastroenterol* 2018 [accessed 25 Jan 2019]. Epub ahead of print available from: https://journals.lww.com/jcge/Abstract/publishahead/Modification_Patterns_of_Urinary_Albumin.97926.aspx
20. Michelena J, Altamirano J, Abraldes JG, et al. Systemic Inflammatory Response and Serum Lipopolysaccharide Levels Predict Multiple

Organ Failure and Death in Alcoholic Hepatitis. Hepatology 2015; 62: 762-772.

21. Nand N, Malhotra P, Dhoot DK. Clinical Profile of Alcoholic Liver Disease in a Tertiary Care Centre and its Correlation with Type, Amount and Duration of Alcohol Consumption. *J Assoc Phys India* 2015; 63: 14-20.
22. Liu X, Shen Y, Wang H et al. Prognostic Significance of Neutrophil-to-Lymphocyte Ratio in Patients with Sepsis: A Prospective Observational Study. *Med Inflam* 2016: 8191254. DOI: 10.1155/2016/8191254.
23. Biyik M, Ucar R, Solak Y, *et al*. Blood neutrophil-to-lymphocyte ratio independently predicts survival in patients with liver cirrhosis. *Eur J Gastro Hep* 2013; 25:435-441.
24. Markwick LJL, Riva A, Ryan JM et al. Blockade of PD1 and TIM3 Restores Innate and Adaptive Immunity in Patients With Acute Alcoholic Hepatitis. *Gastroenterology* 2015; 148: 590-602.
25. Kim EY, Kim YS, Seo J-Y et al. The relationship between sarcopenia and systemic inflammatory response for cancer cachexia in small cell lung cancer. *Plos One* 2016; 11: e0161125. DOI:10.1371/journal.pone.0161125
26. Mendenhall CL, Anderson S, Weesner RE, Goldberg SJ, Crolic KA. Protein-calorie malnutrition associated with alcoholic hepatitis. Veterans Administration Cooperative Study Group on Alcoholic Hepatitis. *Am J Med* 1984; 76(2): 211-22.
27. Dasarathy S, Merli M. Sarcopenia from mechanism to diagnosis and treatment in liver disease. *Journal of Hepatology* 2016; 65: 1232-1244.

28. Altamirano J, Fagundes C, Dominguez M, Garcia E et al. Acute Kindey Injury is an Early Predictor of Mortality for Patients with Alcoholic Hepatitis. Clin Gastroent Hepatol 2012; 10: 65-73.
29. Ravi S, Cruz-Lemini M, Altamirano J, Simonetto DA et al. A Validated Score Predicts Acute Kidney Injury and Survival in Patients with Alcoholic Hepatitis. Liver Transplantation 2018; 24: 1655-1664.
30. Thursz M, Gual A, Lckner C, Mathurin P, Moreno C, Spahr L, Sterneck M. EASL Clinical Practice Guidelines: Management of alcohol-related liver disease. *Journal of Hepatology* 2018; 69: 154-181.
31. Singal A, Bataller R, Ahn J, Kamath PS, Shah VH. ACG Clinical Guideline: Alcoholic Liver Disease. *Am J Gastro* 2018; 113: 175-194.
32. Crabb DW, Bataller R, Chalasani NP, et al. Standard Definitions and Common Data Elements for Clinical Trials in Patients With Alcoholic Hepatitis: Recommendation From the NIAAA Alcoholic Hepatitis Consortia. *Gastroenterology* 2016;150:785-790.
33. Potts JR, Goubet S, Heneghan MA, Verma S. Determinants of long-term outcome in severe alcoholic hepatitis. *Aliment Pharmacol Ther* 2013; 38: 584-595.

Appendix:

STOPAH NLR Group: Ewan Forrest, Glasgow; Natasha Storey, Glasgow; Rohit Sinha, Edinburgh; Stephen Atkinson, London; Paul Richardson, Liverpool; Steven Masson, Newcastle; Stephen Ryder, Nottingham; Mark Thursz, London, Michael Allison, Cambridge; Andrew Fraser, Aberdeen; Andrew Austin, Derby; Anne McCune, Bristol; Ashwin Dhanda, Plymouth; Dev Katarey, Brighton; Jonathon Potts, Brighton; Sumita Verma, Brighton; Richard Parker, Leeds; Peter Hayes, Edinburgh; Debbie Shawcross, London, Dermot Gleeson, Sheffield; Steve Hood, Aintree; Mark Wright, Southampton; John Dillon, Dundee; David Patch, London; Jennifer Ryan, London, Graham Foster, London; Toby Delahooke, Leicester; Sulleman Moreea, Bradford.

Table 1: Characteristics of the patients studied from the STOPAH Cohort and the Validation Group. Characteristics of those patients from STOPAH not included in the analysis (for whom NLR was not available) are also shown for comparison.

	STOPAH Cohort		Validation Cohort n=237
	NLR available n=789	**No NLR available n=314**	
Age	49 (48, 50)	49 (48, 50)	49 (47, 50)
Bilirubin (μmol/l)	307 (296, 318)	302 (284, 319)	288 (268, 307)
INR	1.9 (1.8, 1.9)	1.9 (1.8, 1.9)	1.9 (1.8, 2.0)
WCC (10^9/l)	9.8~ (9.4, 10.2)	10.7~ (10.1, 11.3)	10.9 (10.1, 11.8)
Neutrophils (10^9/l)	7.5 (7.4, 7.9)	7.5 (7.2, 7.8)	8.3 (7.6, 9.0)
Lymphocytes (10^9/l)	1.3 (1.3, 1.4)	-	1.2 (1.1, 1.3)
Creatinine (μmol/l)	79 (75, 82)	79 (76, 82)	88 (76, 100)
DF	63 (61, 65)	63 (60, 66)	66 (62, 70)
GAHS	8 (6-12)	8 (6-11)	8 (6-12)
MELD	24 (24, 25)	24 (24, 25)	25 (24, 26)
Lille Score	0.44# (0.41, 0.47)	0.49# (0.44, 0.54)	0.40 (0.35, 0.45)
GAHS≥9	47%	49%	42%
MELD≥21	84%	84%	83%
Lille≥0.45	45%*	51%*	40%

WCC: Total White Blood Cell Count
GAHS: Glasgow Alcoholic Hepatitis Score
MELD: Model of End-Stage Liver Disease
DF: Discriminant Function
INR: International Normalised Ratio
Mean values shown; in parentheses: 95% Confidence Intervals or percentage of available data
Exception: GAHS: median and range shown
*p=0.074
#p=0.078
~p=0.018

Table 2: Association of Neutrophil-to-Lymphocyte ratio (NLR) with Acute Kidney Injury (AKI) (incident and at baseline) and presence of Infection at baseline and the development of incident infection (7 and 28 days after randomisation).

		Baseline NLR	
Baseline AKI	Present n=63	11.12 (8.64, 13.59)	p=0.0001 (2.64, 7.62)
	Absent n=691	5.99 (5.66, 6.31)	
Incident AKI	Present n=67	7.53 (6.37, 8.69)	p=0.0056 (0.46, 2.65)
	Absent n=403	5.98 (5.58,06.38)	
Infection at Baseline	Present n=99	7.84 (6.60, 9.08)	p=0.021 (0.23, 2.82)
	Absent n=690	6.32 (5.94, 6.70)	
Incident Infection Day 7	Present n=94	7.75 (6.30, 9.20)	p=0.035 (0.12, 3.11)
	Absent n=695	6.14 (5.76, 6.52)	
Incident Infection Day 28	Present n=185	7.14 (6.29, 7.99)	p=0.025 (0.14, 2.02)
	Absent n=604	6.06 (5.64, 6.48)	

Mean values shown; in parentheses: 95% Confidence Intervals

Table 3: Integration of Neutrophil-to-Lymphocyte ratio (NLR) in to Glasgow Alcoholic Hepatitis Score (GAHS) to create the modified GAHS (mGAHS):

mGAHS	Score Given		
Variable	**1**	**2**	**3**
Bilirubin (µmol/l)	<125	125-250	>250
Prothrombin Time ratio/ INR	<1.5	1.5-2.0	>2.0
Blood Urea (mmol/l)	<5.0	≥5.0	
Age	<50	≥50	
Neutrophil-Lymphocyte ratio (NLR)	<5.0	≥5.0	

INR: International Normalised Ratio

Table 4: Day 90 Mortality by Neutrophil-to-Lymphocyte ratio (NLR) Category and modified Glasgow Alcoholic Hepatitis Score (mGAHS) Category in STOPAH patients

	NLR<5		NLR 5-8		NLR>8	
Prednisolone	**Untreated**	**Treated**	**Untreated**	**Treated**	**Untreated**	**Treated**
mGAHS<9	7.3%	12.6%	24.1%	4.2%	12.5%	0%
n=325	P=0.197		P=0.042 HR 0.15 (0.04, 0.61)		P=0.379	
mGAHS≥9	25.9%	31.8%	38.1%	26.2%	58.3%	56.0%
n=460	P=0.538		P=0.052 HR 0.58 (0.34, 1.00)		P=0-822	

HR: Hazard Ratio

Table 5: Mortality Relative to Neutrophil-to-Lymphocyte ratio (NLR) Category, modified Glasgow Alcoholic Hepatitis Score (mGAHS) Category and Prednisolone Treatment in all patients in the Validation Cohort.

		NLR<5		NLR 5-8		NLR>8	
		Untreated n=39	**Treated n=50**	**Untreated n=35**	**Treated n=37**	**Untreated n=25**	**Treated n=51**
mGAHS<9 n=92	**28 Day Mortality**	8.3%	7.5%	0%	0%	0%	30.0%
		P=0.881		- -		P=0.246	
	90 Day Mortality	8.3%	10.0%	0%	14.3%	25.0%	30.0%
		P=0.858		P=0.317 -		P=0-756	
mGAHS≥9 n=144	**28 Day Mortality**	0%	20.0%	35.7%	3.3%	30.0%	21.9%
		P=0.075		P=0.0016 HR 0.080 (0.024, 0.26)		P=0.337	
	90 Day Mortality	37.5%	44.4%	46.4%	23.3%	50.0%	41.5%
		P=0.555		P=0.036 HR 0.39 (0.16, 0.95)		P=0.391	
TOTAL n=237	**28 Day Mortality**	5.1%	10.0%	28.6%	2.7%	28.0%	23.5%
		P=0.416		P=0.0023 HR 0.084 (0.026, 0.27)		P=0.469	
	90 Day Mortality	18.4%	16.3%	37.4%	21.6%	48.0%	39.2%
		P=0.843		P=0.097 HR 0.48 (0.20, 1.14)		P=0.362	

HR: Hazard Ratio

Table 6: Comparison of Treatment Strategies derived from the STOPAH patient group.

DF/Lille Strategy: all patients with DF≥32 treated with prednisolone with Lille Response assessed at Day 7

GAHS/Lille Strategy: only patients with GAHS≥9 treated with prednisolone with Lille Response assessed at Day 7

MELD/Lille Strategy: only patients with MELD≥21 treated with prednisolone with Lille Response assessed at Day 7

NLR Strategy: NLR<5 and NLR>8 not treated; NLR 5-8 treated with prednisolone

DF/Lille Strategy			
	Proportion	**28-Day Infection**	**90-Day Mortality**
No Prednisolone	0%	-	-
Prednisolone: Lille (+)	60%	22.6%	15.1%
Prednisolone Lille (-)	40%	56.1%	41.8%
TOTAL	100%	36.1%	25.9%
GAHS/Lille Strategy			
	Proportion	**28-Day Infection**	**90-Day Mortality**
No Prednisolone (GAHS<9)	53%	24.3%	15.0%
Prednisolone (GAHS≥9): Lille (+)	22%	25.4%	22.3%
Prednisolone (GAHS≥9): Lille (-)	25%	48.6%	47.3%
TOTAL	100%	29.6%	24.7%
MELD/Lille Strategy			
	Proportion	**28-Day Infection**	**90-Day Mortality**
No Prednisolone (MELD<21)	16%	10%	8.5%
Prednisolone (MELD≥21): Lille (+)	47%	21.7%	17.5%
Prednisolone (MELD≥21): Lille (-)	37%	44.1%	43.0%
TOTAL	100%	30.5%	25.5%
NLR Strategy			
	Proportion	**28-Day Infection**	**90-Day Mortality**
No Prednisolone (NLR<5))	47%	22.5%	13.2%
No Prednisolone (NLR>8))	25%	23.8%	37.6%
Prednisolone (NLR 5-8)	28%	20.9%	21.0%
TOTAL	100%	22.4%	21.5%

MELD: Model of End-Stage Liver Disease

DF: Discriminant Function

Glasgow Alcoholic Hepatitis Score (GAHS)

Neutrophil-to-Lymphocyte ratio (NLR)

Lille (+): Lille Responder

Lille (-): Lille Non-Responder

Figure 1: Survival Curves Assessing Effect of Prednisolone using Baseline Neutrophil-to-Lymphocyte ratio (NLR): **A:** NLR<5; **B**: NLR 5-8; **C**: NLR>8

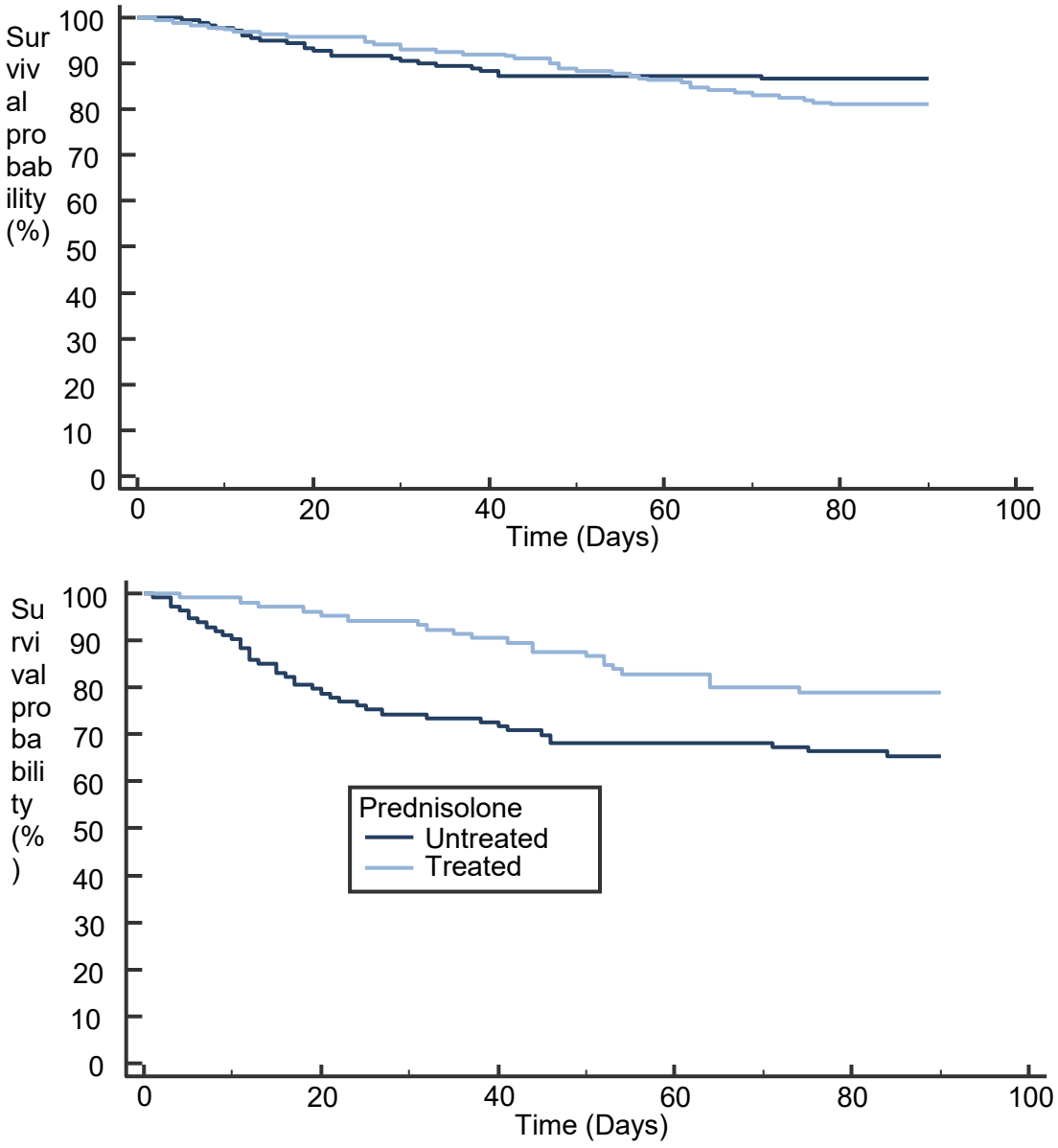
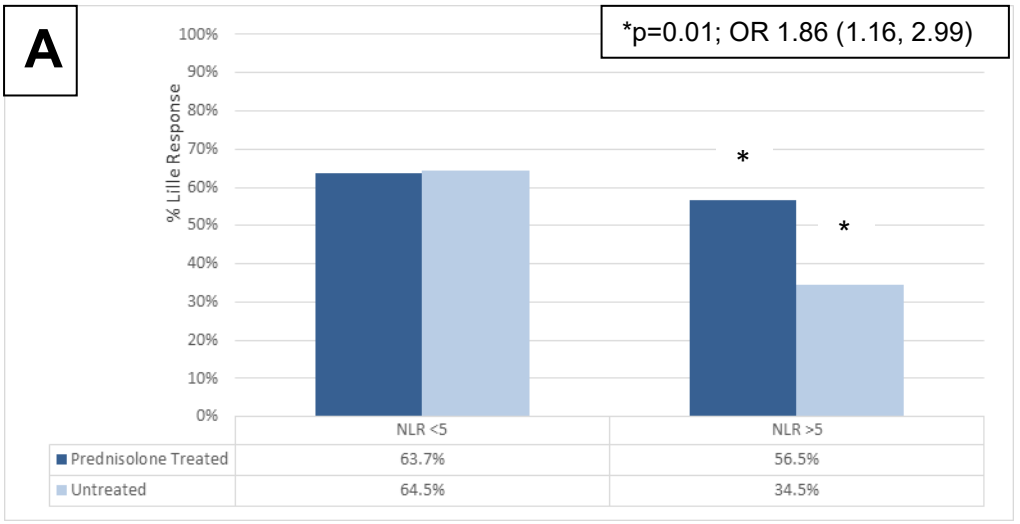
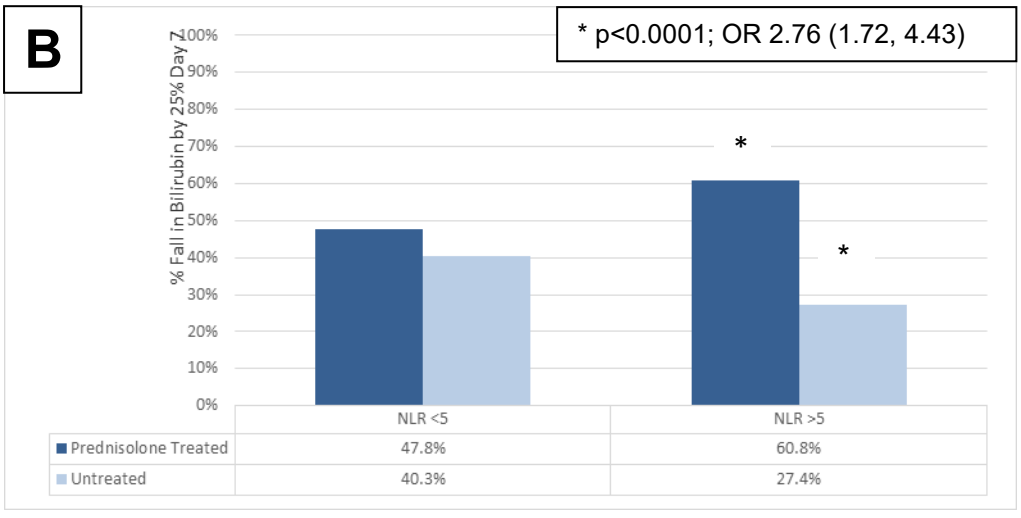
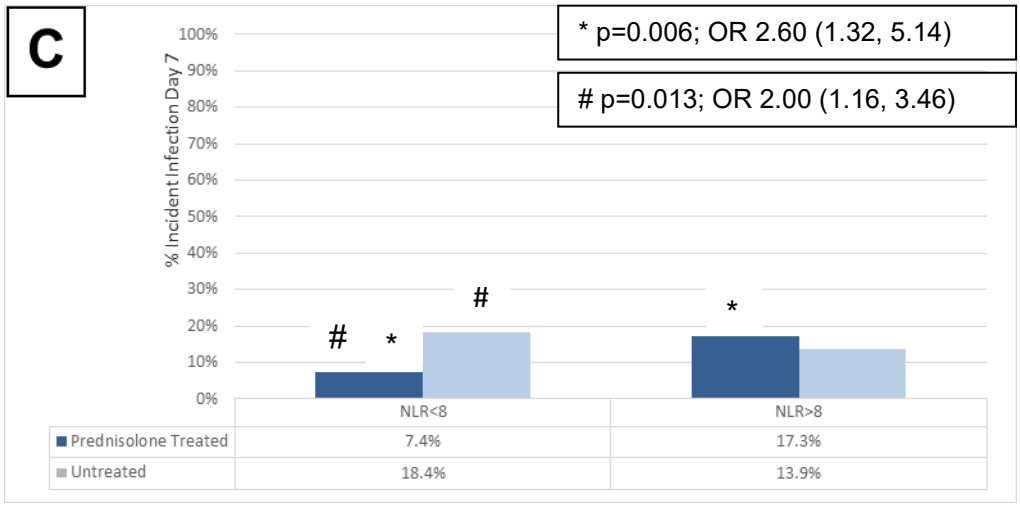


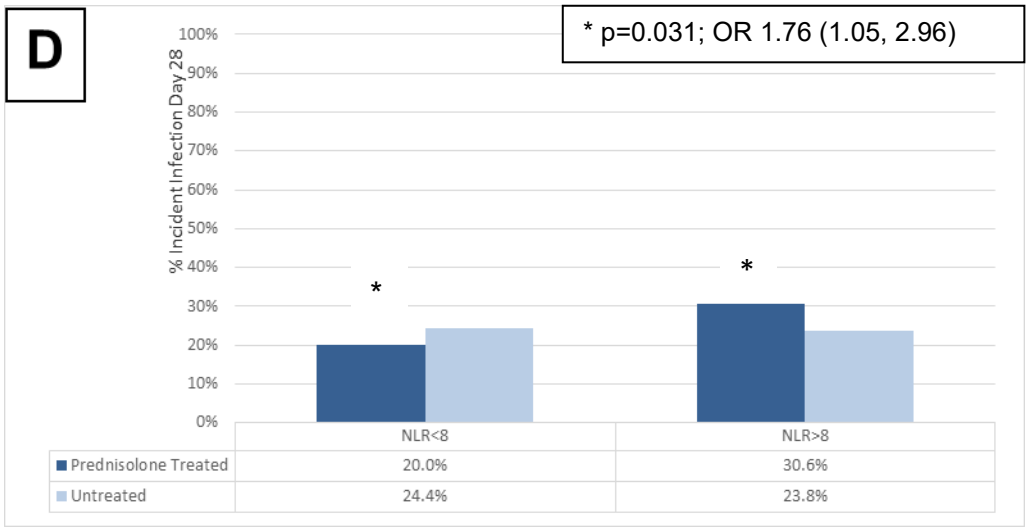
Figure 2: Effect of Prednisolone Relative to Neutrophil-to-Lymphocyte ratio (NLR) Category. Differences between marked columns noted.

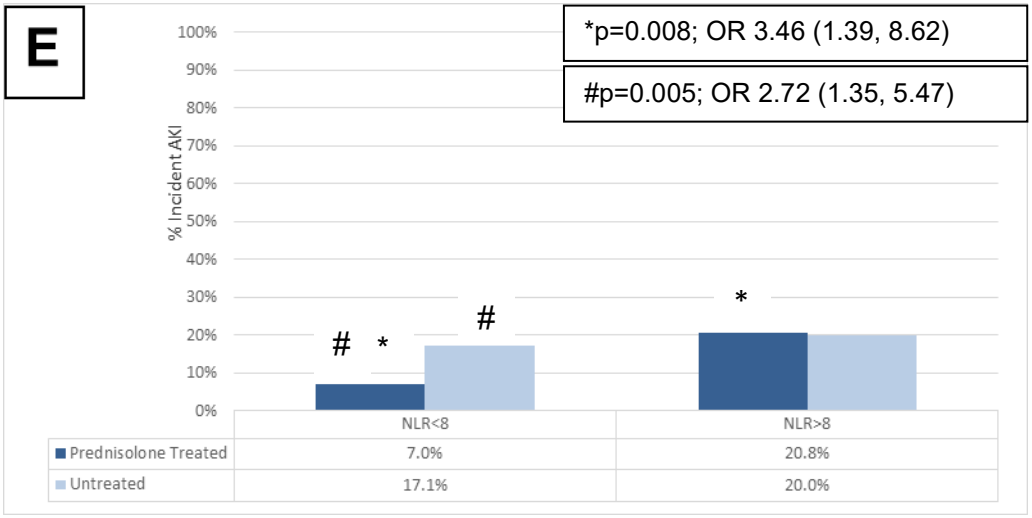
A) Chance of Lille Response
B) Chance of 25% Fall in Bilirubin by Day 7
C) Development of Infection by Day 7
D) Development of Infection by Day 28
E) Development of Incident Acute Kidney Injury (AKI)
F) Effect on 90 Day Survival








D
* p=0.031; OR 1.76 (1.05, 2.96)
% Incident Infection Day 28
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
*
*
NLR<8
NLR>8
Prednisolone Treated
20.0%
30.6%
Untreated
24.4%
23.8%


E
*p=0.008; OR 3.46 (1.39, 8.62)
#p=0.005; OR 2.72 (1.35, 5.47)
% Incident AKI
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
*
#
*
NLR<8
NLR>8
Prednisolone Treated
7.0%
20.8%
Untreated
17.1%
20.0%

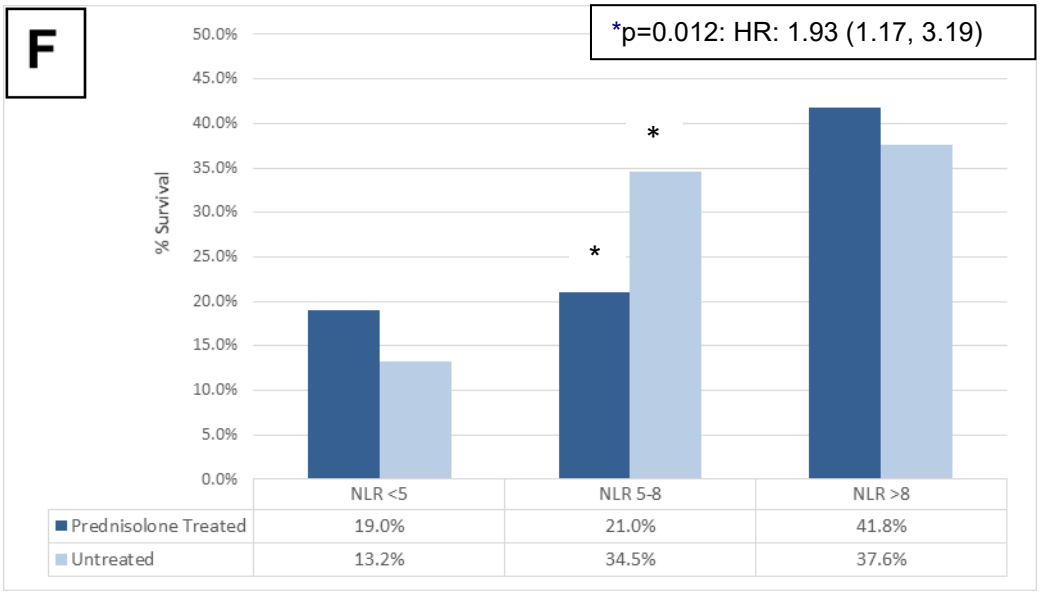
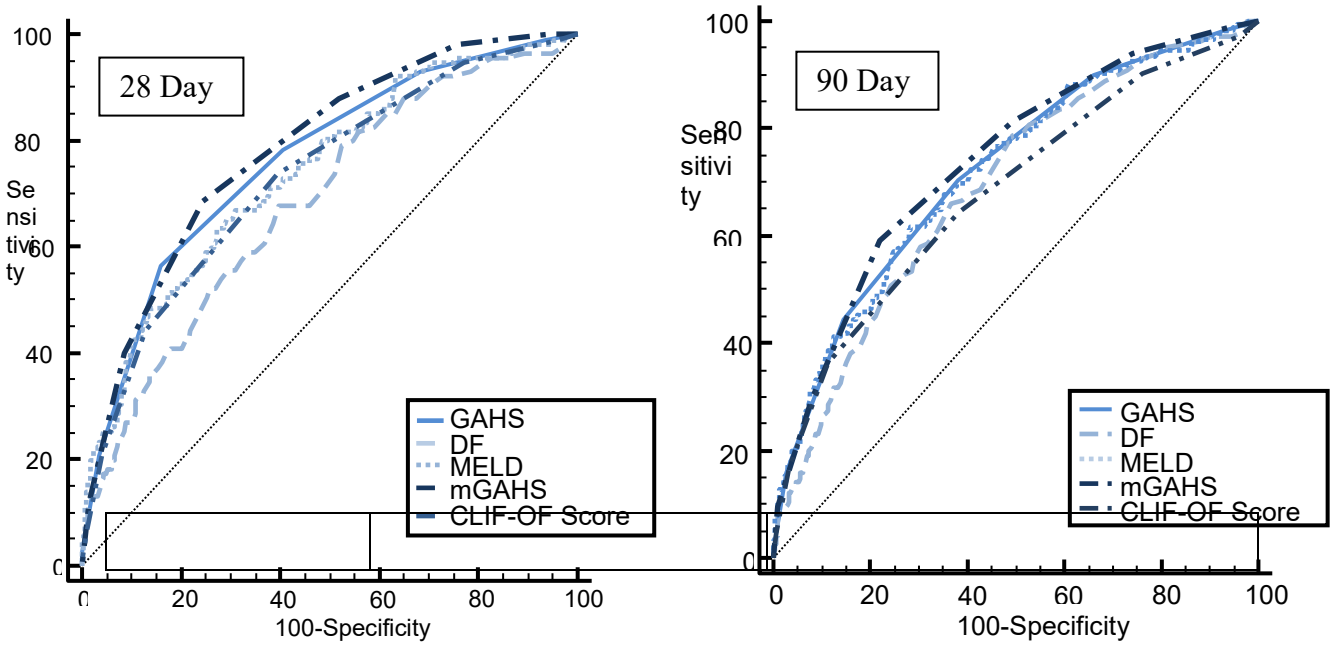

F
*p=0.012: HR: 1.93 (1.17, 3.19)
% Survival
50.0%
45.0%
40.0%
35.0%
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
*
*
NLR <5
NLR 5-8
NLR >8
Prednisolone Treated
19.0%
21.0%
41.8%
Untreated
13.2%
34.5%
37.6%

Figure 3: Area under the Receiver Operating Curve for MELD, GAHS, DF and mGAHS and CLIF-OF Scores



	28-Day Outcome	90-Day Outcome
mGAHS	0.783 (0.752, 0.812)	0.738 (0.705, 0.769)
DF	0.683 (0.649, 0.717)	0.690 (0.655, 0.723)
GAHS	0.762 (0.730, 0.792)	0.719(0.686, 0.751)
MELD	0.738 (0.705, 0.769)	0.718 (0.684, 0.750)
CLIF-OF Score	0.730 (0.697, 0.762)	0.680 (0.645, 0.713)

95% Confidence Intervals in parentheses

MELD: Model of End-Stage Liver Disease

DF: Discriminant Function

Glasgow Alcoholic Hepatitis Score: GAHS

Neutrophil-to-Lymphocyte ratio: NLR

Modified Glasgow Alcoholic Hepatitis Score: mGAHS

Chronic Liver Failure- Organ Failure: CLIF-OF

Supplementary Tables and Figures:

Supplementary Table 1: Differences in Patient Characteristics between Neutrophil-to-Lymphocyte ratio (NLR) Categories in STOPAH patients

	NLR<5 (n=372)	**NLR 5-8 (n=218)**	**NLR>8 (n=199)**
Age	48 (47,49)	49* (48, 51)	51* (50, 52)
Bilirubin (µmol/l)	257 (243, 271)	330* (310, 350)	375*# (353, 398)
INR	1.8 (1.8, 1.9)	1.8 (1.8, 1.9)	2.0 (1.9, 2.1)
WCC (10^9/l)	7.4 (7.0, 7.7)	10.2* (9.6, 10.7)	14.8*# (13.1, 15.0)
Neutrophils (10^9/l)	4.8 (4.5, 5.0)	7.8* (7.3, 8.2)	12.4*# (11.5, 13.3)
Lymphocytes (10^9/l)	1.5 (1.4, 1.6)	1.3* (1.2, 1.3)	1.0*# (1.0, 1.1)
Creatinine (µmol/l)	68 (65, 72)	79* (74, 85)	98*# (89, 108)
DF	58 (56, 59)	62 (59, 66)	73*# (68, 77)
GAHS	8 (6-11)	8* (6-12)	9*# (6-12)
MELD	23 (23, 24)	24* (24, 25)	27*# (26, 28)
Lille	0.37 (0.33, 0.41)	0.48* (0.42, 0.53)	0.53* (0.47, 0.59)

WCC: Total White Blood Cell Count
GAHS: Glasgow Alcoholic Hepatitis Score
MELD: Model of End-Stage Liver Disease
DF: Discriminant Function
INR: International Normalised Ratio

Mean values shown; in parentheses: 95% Confidence Intervals or percentage of available data
Exception: GAHS: median and range shown
*all $p<0.05$
#all $p<0.0005$

Supplementary Table 2: Mortality Relative to Neutrophil-to-Lymphocyte ratio (NLR) Category and Prednisolone Treatment in all patients (A) and with exclusion of those patients presenting with Infection or Gastro-intestinal Bleeding (B) in the STOPAH Cohort.

A) All Patients

	NLR<5		**NLR 5-8**		**NLR>8**	
Prednisolone	**Untreated n=182**	**Treated n=190**	**Untreated n=113**	**Treated n=105**	**Untreated n=101**	**Treated n=98**
28 Day Mortality	8.2%	5.8%	25.7%	5.7%	29.7%	30.6%
	P = 0.359		P=0.0001 HR 0.20 (0.10, 0.38)		P=0.923	
90 Day Mortality	13.2%	19.0%	34.5%	21.0%	37.6%	41.8%
	P = 0.176		P=0.012 HR 0.52 (0.31, 0.86)		P=0-604	

B) Excluding Patients Presenting with Infection or GI Bleeding

	NLR<5		**NLR 5-8**		**NLR>8**	
Prednisolone	**Untreated n=146**	**Treated n=161**	**Untreated n=98**	**Treated n=85**	**Untreated n=80**	**Treated n=71**
28 Day Mortality	7.5%	5.0%	26.5%	2.3%	30%	31%
	P = 0.349		P<0.0001 HR 0.077 (0.037, 0.16)		P=0.961	
90 Day Mortality	11.6%	18.6%	34.7%	17.6%	38.8%	42.2%
	P=0.125		P=0.0035 HR 0.42 (0.24, 0.73)		P=0.717	

Supplementary Table 3: Comparison of Cut Offs for A) MELD, B) GAHS, C) mGAHS

A) MELD

	Cut Off	Sensitivity	Specificity	+LR	95% CI	-LR	95% CI
28-Day Outcome	≥21	95.7	18.6	1.18	1.1 - 1.2	0.23	0.10 - 0.6
90-Day Outcome	≥21	94.2	20.0	1.18	1.1 - 1.2	0.29	0.2 - 0.5

B) GAHS

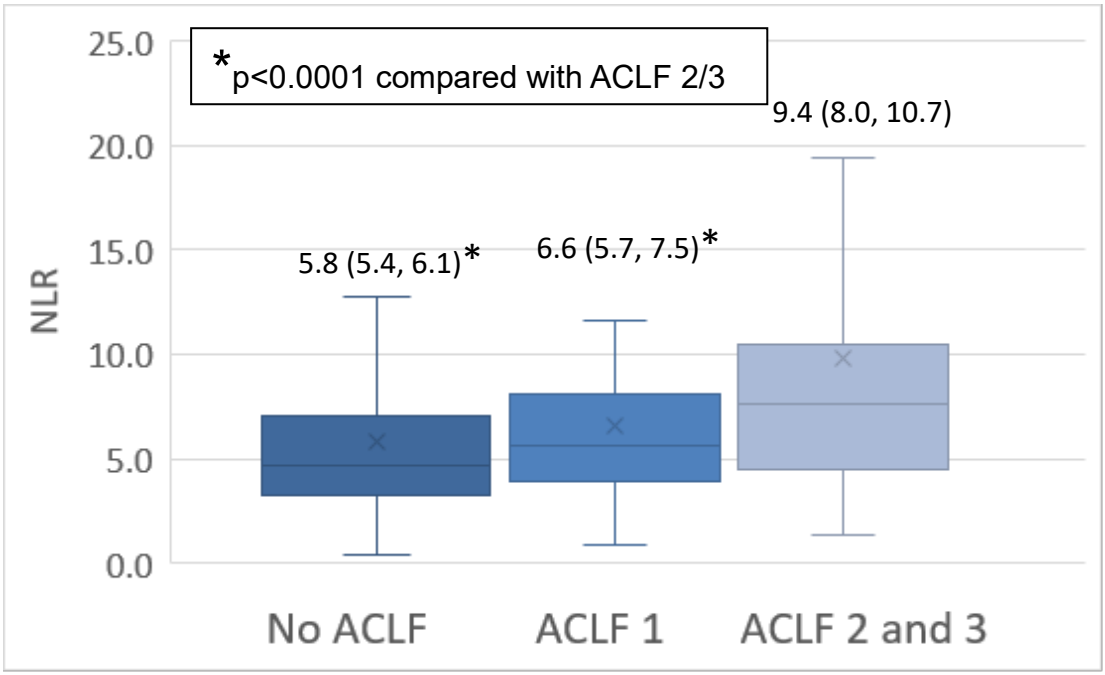
	Cut Off	Sensitivity	Specificity	+LR	95% CI	-LR	95% CI
28-Day Outcome	≥9	78.2	58.3	1.87	1.6 - 2.1	0.38	0.3 - 0.5
90-Day Outcome	≥9	71.2	60.8	1.82	1.6 - 2.1	0.47	0.4 - 0.6

C) mGAHS

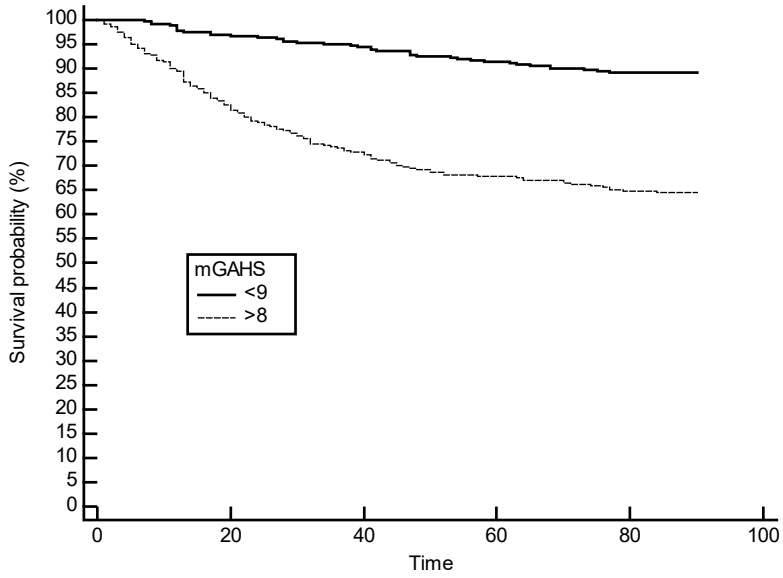
	Cut Off	Sensitivity	Specificity	+LR	95% CI	-LR	95% CI
28-Day Outcome	≥9	88.2	46.7	1.66	1.5 - 1.8	0.25	0.2 - 0.4
90-Day Outcome	≥9	82.3	49.4	1.63	1.5 - 1.8	0.36	0.3 - 0.5

LR: Likelihood Ratio
GAHS: Glasgow Alcoholic Hepatitis Score
MELD: Model of End-Stage Liver Disease
mGAHS: modified Glasgow Alcoholic Hepatitis Score

Supplementary Figure 1: Neutrophil-to-Lymphocyte ratio (NLR) and Acute-on-Chronic Liver Failure (ACLF) Grade. Grades 2 and 3 merged on account of numbers: No ACLF (ACLF 0) n=543, ACLF 1 n=113, ACLF 2 n=82, ACLF 3 n=13.



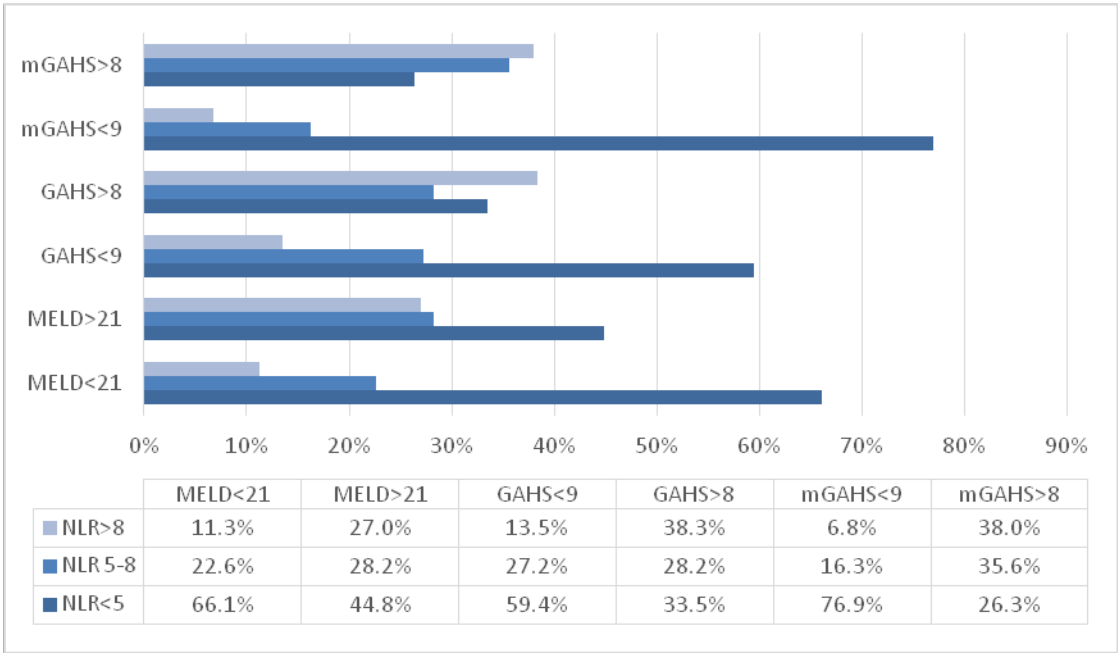
Supplementary Figure 2: Survival Relative to the modified Glasgow Alcoholic Hepatitis Score (mGAHS) (less than 9/ greater than or equal to 9)



p<0.0001 HR 3.91 (2.96, 5.17)

mGAHS	Survival (%)	Total sample
<9	290 (89.2%)	325
>8	297 (64.6%)	460
Overall	587 (74.8%)	785

Supplementary Figure 3: Proportions of NLR Category Relative to Baseline Prognostic Score Category: mGAHS, GAHS and MELD.



	MELD<21	MELD>21	GAHS<9	GAHS>8	mGAHS<9	mGAHS>8
NLR>8	11.3%	27.0%	13.5%	38.3%	6.8%	38.0%
NLR 5-8	22.6%	28.2%	27.2%	28.2%	16.3%	35.6%
NLR<5	66.1%	44.8%	59.4%	33.5%	76.9%	26.3%

GAHS: Glasgow Alcoholic Hepatitis Score
MELD: Model of End-Stage Liver Disease
mGAHS: modified Glasgow Alcoholic Hepatitis Score

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were

		categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.