

Review

Corticosteroids for alcoholic hepatitis—what's next?

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Alcoholic hepatitis (AH) is observed in approximately 20% of heavy drinkers. The treatment of AH remains controversial and will, in the near future, constitute one of the main challenges to clinicians involved in the management of severe alcoholic liver disease [1]. Individual data from the last three randomized controlled trials (RCT), prospective studies and a recent RCT constantly showed that patients with Maddrey function (DF) ≥ 32 treated by corticosteroids had a 2-month survival of 80% [2–5]. Nevertheless, the efficacy of corticosteroids is still considered as a controversial issue for some authors. Alternative therapies are required to improve the prognosis of patients with a severe form of AH [6]. Progress in understanding the pathogenesis of AH is opening up an exciting new era and is lending impetus to future evaluation of new drugs targeting the TNF pathways [7–13]. However, the classical view that considers a treatment effective only when it improves survival needs to be modified. Indeed, the sample size of future studies comparing new drugs to corticosteroids will never be sufficient to detect a difference in short-term survival [14]. Clinicians need to promote new primary endpoints in future randomized controlled trials evaluating drugs in patients with severe AH (Table 1). We suggest that a treatment should be declared successful in patients demonstrating biological improvement and who remained alive 1 or 2 months.

1. The effect of treatment on survival can be observed only in severe forms of AH

Morphological changes in alcoholic patients are classified into fatty liver, fibrosis, AH and cirrhosis. Among these lesions, AH represents one of the most

serious forms of alcoholic liver injury and is characterized by the presence of lesions, generally located in zone 3 of the lobule, such as hepatocyte necrosis or ballooning, polymorphonuclear neutrophil infiltration and intracellular Mallory inclusion bodies [15]. It is well known that the pivotal treatment in alcoholic liver disease is abstinence, since abstinent patients survive longer than non-abstinent patients [16–18]. However, to improve the survival of patients with alcoholic liver disease, pharmacological treatments aimed at controlling alcohol-induced liver injury are required [1,6].

Several investigators have given priority to the treatment of AH, as this entity is associated with significant early mortality; inpatient mortality can reach 50–75% in the most severe forms [19]. The main causes of death are liver failure, sepsis, hepatorenal syndrome and gastrointestinal bleeding. However, evaluation of any treatment effect on short-term survival requires identification of the subgroup of patients with significant risk of death at 1 or 2 months. However, until the Maddrey function became available, no reproducible objective criterion existed to predict the risk of early death. Indeed, prior to the era of the discriminant function (DF), survival in untreated control arms ranged from 0 to 81% in previous randomized controlled trials evaluating treatment in patients with AH [4,5,19–29]. Maddrey et al. described a DF [19] and later modified it [5] into its more widely used form. This modified DF, which identified patients with a high risk of early mortality, is calculated as follows: $4.6 (\text{prothrombin time} - \text{control time [in seconds]}) + \text{serum bilirubin (in micromoles per liter)}/17$ [5]. In the absence of treatment, the spontaneous survival of patients with a $DF \geq 32$ fluctuated between 50 and 65% [3–5,19]. Conversely, we showed that spontaneous survival at 28 days of patients with a $DF < 32$ was close to 90% [3]. Therefore, observation of a treatment effect on survival in patients with a $DF < 32$ is not a rational approach, and studies must include only patients with a $DF \geq 32$.

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Table 1
Characteristics of the most recent randomized controlled trials evaluating a treatment in more than 20 patients with severe AH

Author	Regimen	Patients (n)	Short-term survival
Cabre E 2000 [80]	Arm 1: Prednisolone 40 mg/day during 1 month	36	75% (at 28 days)
	Arm 2: Enteral nutrition	35	69% (at 28 days)
Akriviadis E 2000 [88]	Arm 1: Pentoxifylline 1200 mg/day during 1 month	49	75% (at 2 months) ^a
	Arm 2: Placebo	52	54% (at 2 months)
Mathurin P 2002 [3]	Arm 1 ^b : Prednisolone 40 mg/day during 1 month	113	85% (at 28 days) ^a
	Arm 2 ^b : Placebo	102	65% (at 28 days)
Sphar L 2002 ^c [40]	Arm 1: Prednisolone 40 mg/day during 1 month and one perfusion of infliximab 5 mg/kg	11	82% (at 2 months)
	Arm 2: Prednisolone 40 mg/day during 1 month with placebo	9	100% (at 2 months)
Naveau S 2004 [2]	Arm 1: Combination of Prednisolone 40 mg/day during 1 month with three perfusions of infliximab 10 mg/kg at days 0, 14 and 28	18	61% (at 2 months)
	Arm 2: Combination of Prednisolone 40 mg/day during 1 month with placebo	18	82% (at 2 months)

^a The differences of survival between the 2 arms were significant.

^b Individual data analysis of patients with DF ≥ 32 from the three randomized controlled trials [4,5,29].

^c The patients with DF > 55 were excluded.

2. We need to look beyond the controversy of corticosteroids

Thirteen randomized control trials evaluated corticosteroids in patients with AH [4,5,19–29]. These trials yielded inconsistent results. Such contradictory results can be mainly attributed to the wide variations in stringency between studies, since survival of placebo groups ranged from 0 to 81%. The last two randomized trials included only patients with either a discriminant function ≥ 32 or spontaneous encephalopathy [4,5]. In these trials, survival in corticosteroid groups was significantly higher than in placebo groups: 94% vs 65% at 28 days in Carithers' study ($P=0.006$) and 88% vs 45% at 66 days in Ramond's study ($P=0.001$). Patients with gastrointestinal bleeding or hepatorenal syndrome may be less responsive to steroid treatment than patients without these complications [30]. In such circumstances, the outcome of patients may be related to these cofactors rather than to alcoholic hepatitis itself [30]. We showed that the survival benefit due to corticosteroid treatment persisted for at least 1 year and disappeared at 2 years [31].

Previous meta-analyses observed a survival benefit in treated patients, especially in those with encephalopathy [30,32], although the latest positive randomized controlled trial was not analyzed [4,5]. However, a recent meta-analysis using multivariate statistics to adjust for confounding variables between corticosteroid and control groups concluded that corticosteroids are ineffective [33]. This method is problematic in detecting a treatment effect of corticosteroids because of the limited number of trials ($n=12$) and the inclusion of lower-quality trials in creation of the multivariate model [34]. Moreover, it must be borne in mind that the 20-year truth survival of classical meta-analysis is significantly lower than the truth survival of

randomized controlled trials or analysis of individual data: 57% vs 87% [35]. In addition, classical meta-analysis of published results is not designed to identify the effect of treatment in clinically distinct patients [36]. In the particular setting of AH, this method cannot pool the results restricted to patients with DF ≥ 32 , as most of the previous RCTs did not supply the specific survival data on this subgroup.

In order to put an end to this controversy, investigators of the three most recent randomized controlled trials (Mendenhall, Carithers and Ramond) combined their individual data [3]. The authors restricted their analyses to patients with a discriminant function ≥ 32 . The main findings of the study were: (1) At 28 days, corticosteroid patients had significantly better survival than placebo patients: $84.6 \pm 3.4\%$ vs $65.1 \pm 4.8\%$, $P=0.001$; (2) corticosteroid treatment, age and creatinine were independently associated with survival at 28 days; (3) corticosteroid treatment induced rapid improvement in hepatic function that started after 7 days of treatment [3]. The corticosteroid effect seems at least in part related to the inhibition of polymorphonuclear neutrophil activation, ICAM-1 expression and pro-inflammatory cytokines [37,38].

The final argument supporting the benefit of corticosteroids in AH with DF ≥ 32 is that all the studies, including the most recent from independent cohorts, constantly observed that 2-month survival of patients treated with corticosteroids was approximately 80% [2,4,5,39–41].

In order to make progress in the management of severe AH, our group recently proposed a simple criterion for early identification of patients who do not benefit from corticosteroids, or so-called 'non-responders to corticosteroids' [39]. This criterion, termed 'early change in bilirubin levels (ECBL)' is defined as a bilirubin level at 7 days lower than the bilirubin level on the first day of treatment. An ECBL at 7 days was observed in 73% of patients. At 7 days,

in patients with ECBL, bilirubin decreased ($-84 \mu\text{mol/l}$), whereas it increased in patients without ECBL ($76.5 \mu\text{mol/l}$, $P < 0.0001$). Ninety five percent of patients with ECBL continued to have improved liver function during the treatment period. At 6 months, survival of patients with ECBL was significantly higher than that of patients without ECBL, $82.8 \pm 3.3\%$ vs $23 \pm 5.8\%$, $P < 0.0001$. In multivariate analysis, ECBL ($P < 0.000001$), age ($P = 0.0002$), DF ($P = 0.005$) and creatinine ($P = 0.02$) were independent prognostic variables and ECBL had the most important prognostic value [39]. Recently, our group observed that in patients without ECBL, discontinuation of corticosteroids after 7 days was not detrimental (personal communication).

The current goal in the treatment of severe AH is to reduce early mortality. However, as the expected 2-month survival of patients treated with corticosteroids is around 80%, we expect that future randomized controlled trials comparing new drugs with corticosteroids, with 1- or 2-month survival as the primary endpoint, will face substantial difficulties in detecting differences in mortality [14]. It is noteworthy that clinicians consider survival alone as a primary endpoint, whereas for other liver diseases, alternative endpoints are used. It is reasonable to propose that a drug inducing a significant improvement in biological features of liver insufficiency would be considered efficient in patients with severe AH. The strategy with combined endpoints that has been used for other diseases is very attractive [42]. In the particular setting of AH. We suggest that a treatment should be declared successful in patients demonstrating biological improvement and who remained alive 1 or 2 months.

This proposition needs to be discussed by a panel of experts.

Although the survival benefit of corticosteroids in comparison to placebo was sustained at 1 year, approximately 40% of patients died in 6 months following the onset of AH [31,39]. We recently generated a model able to predict death at 6 months of treated patients with severe alcoholic hepatitis [43]. The present model is highly predictive of death at 6 months. A score above 0.5 prognosticated nearly 80% of the deaths. In the future, this powerful model should be used to identify the subgroup of patients in whom new therapeutic treatments are required. The observed rate of death at 6 months suggests that this timing may be used as a primary end point instead of 2 months. However, the 6 months timing as a survival end-point will introduce two important potential biases such as abstinence and the high drop-out rate in studies in heavy drinkers. For example, a randomized controlled trial of long-term treatment with propylthiouracil showed a 60% drop-out rate [44]. An imbalance in abstinence percentage may contribute, at least in part, to a negative or a positive result.

In conclusion, even though corticosteroids are efficient in severe AH to improve short-term survival, new treatments

are required to improve the probability of being alive within the year following the onset of the disease.

3. Insights into the mechanisms of AH are yielding new targets for future therapy

Development of effective therapies for AH depends on the understanding of the mechanisms that contribute to the disease process. Numerous arguments support the idea that an inflammatory process may contribute in the pathogenesis of AH [45–50]. Patients with AH frequently disclose features of an acute phase response, immunological disorders such as autoantibodies to liver-specific antigens, immunologically active acetaldehyde protein adducts and neutrophilia. In addition, the fact that only a minority of excessive drinkers [51] develop AH, as well as the absence of animal models of AH up until now, lend support to the role of other factors in the pathogenesis of AH. Some authors have described the pathogenic process of AH as a ‘2-hit model’ in which the second hit is related mostly to endotoxin and pro-inflammatory cytokines. Indeed, experimental and clinical findings to date suggest that lipopolysaccharide (LPS) and pro-inflammatory cytokines constitute the main effector molecules in alcohol-induced liver injury [7–13].

Activation of Kupffer cells is a key element in alcohol-induced liver injury [11,52,53]. Indeed, administration of gadolinium chloride, a selective Kupffer cell toxicant, prevents liver injury in animal models of alcoholic liver disease [11]. LPS, a major component of the outer membrane of Gram-negative bacteria, provokes excessive production of pro-inflammatory cytokines by Kupffer cells during alcohol-induced liver injury. LPS interacts with its specific carrier, lipopolysaccharide binding protein (LBP), via binding of the LPS–LBP complex to the LPS receptor complex constituted by three proteins, TLR4, CD14 and MD2. In animal models of alcohol liver disease, several studies showed that plasma endotoxin levels are elevated and that levels correlate well with pathological scores and hepatic amounts of TNF α mRNA [54–57]. Chronic acute alcohol ingestion causes an increase in intestinal permeability, leading to a substantial flow of endotoxin to the liver [55]. In support of this notion, alcoholic patients with chronic liver disease exhibit increased intestinal permeability [58,59]. Patients with alcoholic cirrhosis have higher endotoxin levels than heavy drinkers without cirrhosis and healthy controls [60]. Reduction of endotoxin levels with antibiotics or lactobacillus administration minimized early alcoholic liver injury [54,61]. Simultaneous increases in the plasma endotoxin level and mRNA expression of pro-inflammatory cytokines are noted in ethanol-fed rats [62,63]. In mice lacking CD14 or TLR4 receptors, hepatic pathology due to alcohol was largely blocked [64,65]. Taken together, these results

clearly support a critical role for endotoxin in alcohol-induced liver injury.

In animal models of alcohol liver disease and in humans affected by AH, a convincing collection of data exists supporting a pivotal role of TNF α predominantly produced by Kupffer cells [8,12,66,67]. Many studies observed that patients with alcoholic hepatitis had elevated serum TNF α [68,69]. Indeed, serum levels of IL-8 and TNF α are much higher in the plasma of patients with alcoholic hepatitis than in alcoholic patients with inactive cirrhosis or without liver disease [37], and have been correlated with the severity of liver disease [69,70].

There is increasing evidence of the participation of oxidative stress in the TNF-induced cytotoxic action caused by increased generation of reactive oxygen species during alcoholic liver injury [71–74]. Several studies demonstrated that the peroxidative capacity of TNF α in hepatocytes is restricted to the mitochondria, suggesting that this organelle is a target of TNF α . Chronic alcohol consumption induces selective depletion of glutathione in the mitochondria [74–78] that controls the fate of hepatocytes in response to TNF α , since the power of TNF α to induce cell death is observed only in cells with mitochondrial glutathione depletion [7,9].

Based on these findings, pharmacological treatments that inhibit TNF α would be effective in patients with alcoholic hepatitis.

4. New therapies

4.1. Extracorporeal liver support

Liver support devices are currently being developed to provide additional time for liver regeneration and improvement of liver function to occur. Extracorporeal liver support, consisting of a molecular adsorbent recirculating system (MARS), detoxifies patient blood of protein-bound toxins having a molecular weight of less than 50 kDa bound and water-soluble substances. The MARS system consists of hollow fiber dialysis in which the patient's blood is dialyzed across an albumin-impregnated membrane and an albumin-rich (20%) dialysate in the extracapillary compartment. In a recent pilot study, eight patients with severe AH (five with type I and two with type II hepatorenal syndrome) were treated with MARS [79]. Four patients were alive at 3 months. The serum bilirubin, serum creatinine, DF and International Normalized Ratio (INR) dropped from 385 to 197 $\mu\text{mol/l}$, 110 to 38 $\mu\text{mol/l}$ and 2.9 to 1.7 $\mu\text{mol/l}$, respectively. Sustained improvement in circulatory disturbances was observed at the end of treatment with MARS. On this basis, the investigators initiated a multicenter randomized controlled trial.

4.2. Enteral nutrition

Total enteral tube feeding was compared to corticosteroids in a randomized controlled trial [80]. The formula of the enteral diet was a low-fat diet in which medium-chain triglycerides and oleic acid accounted for most of its lipid content after considering the deleterious effects of a high-fat diet on alcoholic liver injury in animal models [81–84]. Mortality occurred earlier in the enteral group: 7 days vs 23 days, $P=0.025$. During follow-up after the treatment period, deaths were observed more frequently in the corticosteroid group (10/27) than in the enteral group (2/24, $P=0.04$). The same groups recently suggested that combined treatment with enteral nutrition and corticosteroids could improve the outcome of patients with severe AH and merited investigation in a randomized controlled trial [85].

4.3. Pentoxifylline

Pentoxifylline, an inhibitor of TNF α synthesis [86,87], was evaluated in a double-blind randomized controlled trial [88]. A total of 101 patients with severe AH (DF \geq 32) randomly received pentoxifylline ($n=49$) or a placebo ($n=52$). Clinical and biological characteristics of the two groups were similar on randomization. The mean DF in the pentoxifylline and placebo groups was 45.9 and 45.3, respectively. Twenty-four percent of pentoxifylline-treated patients and 46.1% of control patients (relative risk: 0.57, $P=0.04$) died after a mean of 29 and 33.1 days, respectively. The survival benefit of pentoxifylline appears to be related to a significant reduction in development of hepatorenal syndrome [88]. Among patients who died, hepatorenal syndrome had developed in 50% of pentoxifylline patients and 91.7% of placebo patients (relative risk 0.29, $P=0.009$). Contrary to corticosteroids, the effect of pentoxifylline was related to prevention of hepatorenal function but not to improvement of liver function. At the end of the treatment period, the two groups had similar values of DF, prothrombin time and bilirubin levels. During the treatment period, there were no significant differences between the two groups in absolute values or in changes from baseline for TNF α . In summary, these results support future evaluation of pentoxifylline in severe AH by other groups.

4.4. Anti-TNF α antibody

Production of TNF α by inflammatory cells in response to alcohol-associated oxidant stress and endotoxin is a critical mediator of alcohol liver disease. In animal models, antibodies to TNF α attenuated liver injury, and mice lacking TNF α receptor 1 did not develop alcohol-induced liver injury [12,67]. The anti-TNF α strategy is currently considered one of the most attractive approaches to developing future therapies for AH.

This strategy has been tested in two pilot studies and two randomized controlled trials [2,40,89,90]. These studies evaluated infliximab, a chimeric human/mouse antibody, administered by intravenous infusion, which binds with high affinity to TNF α . Infliximab is an effective treatment in patients with TNF α -mediated diseases such as rheumatoid arthritis and Crohn's disease. In the first study, 20 patients with biopsy-proven severe AH treated by prednisolone 40 mg/day for 28 days were randomized to receive infliximab 5 mg/kg IV ($n=10$) or placebo ($n=10$) [40]. The investigators excluded patients with DF > 55, as they sought to evaluate the tolerance of infliximab. At day 28, DF (39–12) and IL-8 levels (301–146 pg/ml) decreased significantly, whereas in the placebo group, evolution of these two parameters did not attain significance: 44–22 and 315–110 pg/ml, respectively [40]. The sample size of the study did not allow direct comparison between the two groups. Infliximab was well tolerated and there were no significant differences between the two groups in terms of side effects. These data constituted strong arguments in favor of future evaluation of infliximab, even though the study was not designed to evaluate the effects of infliximab on survival. Another study tested a single dose of 5 mg/kg of infliximab without concomitant use of steroids in 12 patients with biopsy-proven AH [90]. Despite the prophylactic use of norfloxacin, two patients died from infection within 3 weeks. The levels of pro-inflammatory cytokines IL-6 and IL-8 decreased the day after infliximab administration. At 28 days, the bilirubin level decreased from 308 to 131 $\mu\text{mol/l}$, DF from 54.1 to 37.7, and C-reactive protein from 82.1 to 38, whereas the prothrombin time did not change significantly (19.5–18.9) [90]. However, it was intriguing that bilirubin levels and DF remained stable during the 14 days following infliximab perfusion and began to decrease only after 21 days [90]. This suggests that the biological effect of infliximab is delayed compared to corticosteroids that induced a significant biological effect within 7 days [39,90]. However, in view of the small number of patients, no conclusions can be drawn. Another study evaluated the effect of infliximab on circulatory disturbances observed in patients with severe AH [89]. Twenty-four hours after a single injection of 5 mg/kg of infliximab, the mean hepatic venous pressure gradient decreased, whereas mean arterial pressure and systemic vascular resistance increased. These effects were sustained prior to discharge [89].

In order to determine the effect of infliximab on survival, French centers conducted a randomized controlled trial [2]. Investigators used interim analyses based on a group sequential test design for trial efficiency that results in lower expected sample size. The cumulative numbers of responses planned for the three analyses were 38, 78 and 116. However, the first interim analysis was not carried out; indeed, the study was stopped by the sponsor, the Assistance

Publique-Hôpitaux de Paris (AP-HP), because of the unbalanced rate of deaths prior to the initially planned enrollment of the 38 patients [2]. Indeed, after randomization of 36 patients, there were seven deaths in the infliximab group and three deaths in the steroid group. The probability of survival at 2 months was lower in the infliximab group (61%) than in group B (82%). The frequency of severe infections within 2 months increased in patients treated by infliximab (10 episodes of infection in eight patients) compared to patients treated by placebo. There were no differences between the two groups in terms of the course of DF [2].

How can we explain the difference between the French trial and the three other studies? The perfusion protocol of infliximab varied drastically. Previous studies had used a single dose of 5 mg/kg, whereas in the French trial patients were randomly assigned to receive three intravenous infusions of 10 mg/kg of infliximab. Indeed, infusions of infliximab 10 mg/kg were administered once, twice and three times in 1, 6 and 10 patients, respectively [2]. The investigators used high doses of infliximab because they hypothesized that only high doses would inhibit the high serum TNF α levels observed in patients with AH in comparison to patients with Crohn's disease. This hypothesis did not appear to be relevant for some authors, as the serum TNF α level was not indicative of a treatment response [91]. The discrepancies in disease severity between studies may also account for the differences in results. The mean DFs at the onset of treatment were 39, 54.1 and 61 in three studies, whereas the DF seemed to predict the infection [2,40,90]. Indeed, in the French study, patients with fatal infection had a DF significantly higher than patients without: 70 vs 57, $P < 0.05$ [2].

Should we consider that the French trial marks the end of the story of evaluation of anti-TNF α strategy? Definitely not, but that study does remind us of the uncertainties of hypotheses developed in animal models of alcoholic liver disease. In those models, liver injury is mild and animals never develop liver insufficiency [84]. Conversely, in humans, profound disturbances in liver function are a classical feature of AH. As an example, we fear that in patients with severe AH, the beneficial effect of anti-TNF α on alcohol-induced liver injury might be counterbalanced by its deleterious effect on liver regeneration [92]. Indeed, the crucial role of TNF α in initiating liver regeneration has been clearly established [92]. Liver regeneration after partial hepatectomy was severely impaired in mice lacking type I tumor necrosis factor receptor. This defect in liver regeneration was reversed by injection of IL-6. Other groups have confirmed the importance of the IL-6 pathway involving the STAT3 transcription factor in TNFR1 signaling [93]. Thus, clinicians must consider not only the deleterious effect of TNF α in alcohol-induced liver injury, but also its role in liver regeneration, a process that may contribute to the recovery of patients with severe AH.

5. Conclusions

The management of severe AH is challenging. Corticosteroids improve short-term survival in patients with $DF \geq 32$. ECBL is a simple, and powerful criterion for helping to identify patients who are responders to corticosteroids. Approximately 75% of patients with $DF \geq 32$ are responders to corticosteroids. At 6 months, survival of patients with ECBL is around 80%. Conversely, in current practice, we suggest that corticosteroid therapy be interrupted in non-responders after 7 days of treatment. However, the high mortality within the year following the onset of the disease highlights the need for new treatments. A meeting of a panel of experts is needed to determine whether or not a drug may be regarded as successful in patients with biological improvement and who remain alive at 1 or 2 months.

Combined treatment with enteral nutrition and corticosteroids merits investigation in a randomized controlled trial. The efficacy of Pentoxifylline needs to be confirmed by future studies and its effect is related to the prevention of hepatorenal function.

Progress in understanding the network of intercellular and intracellular signals among Kupffer cells, monocytes, and neutrophils provides new targets for therapeutic intervention. Controlling the pro-inflammatory cytokines-induced liver injury opens an exciting era for the future development of new drugs in patients with severe AH. Although studies evaluating anti-TNF α antibodies yielded inconsistent results, the TNF α -neutralizing strategy is the most attractive approach and deserves additional studies.

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