Scandinavian glutamine trial: a pragmatic multi-centre randomised clinical trial of intensive care unit patients

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Background: Low plasma glutamine concentration is an independent prognostic factor for an unfavourable outcome in the intensive care unit (ICU). Intravenous (i.v.) supplementation with glutamine is reported to improve outcome. In a multi-centric, double-blinded, controlled, randomised, pragmatic clinical trial of i.v. glutamine supplementation for ICU patients, we investigated outcomes regarding sequential organ failure assessment (SOFA) scores and mortality. The hypothesis was that the change in the SOFA score would be improved by glutamine supplementation.

Methods: Patients (n = 413) given nutrition by an enteral and/or a parenteral route with the aim of providing full nutrition were included within 72 h after ICU admission. Glutamine was supplemented as i.v. l-alanyl-l-glutamine, 0.283 g glutamine/kg body weight/24 h for the entire ICU stay. Placebo was saline in identical bottles. All included patients were considered as intention-to-treat patients. Patients given supplementation for >3 days were considered as predetermined per protocol (PP) patients.

Results: There was a lower ICU mortality in the treatment arm as compared with the controls in the PP group, but not at 6 months. For change in the SOFA scores, no differences were seen, 1 (0,3) vs. 2 (0.4), P = 0.792, for the glutamine group and the controls, respectively.

Conclusion: In summary, a reduced ICU mortality was observed during i.v. glutamine supplementation in the PP group. The pragmatic design of the study makes the results representative for a broad range of ICU patients.

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G LUTAMINE depletion reflected as a low, plasma-glutamine concentration is an independent prognostic factor for an unfavourable outcome in the intensive care unit (ICU).1 With a daily intravenous (i.v.) supplementation of 0.3 g/kg body weight, i.v. glutamine supplementation normalises the plasma concentration in virtually all ICU patients.2 Several single-centre studies demonstrate that i.v. glutamine supplementation for patients on total parenteral nutrition can improve outcome. Two classic studies reveal a lower overall 6-month mortality.3,4 In a meta-analysis, ICU mortality decreased.5 An updated meta-analysis is available on the web.*

In patients on enteral nutrition, with an enteral glutamine supplementation, the existing evidence is less conclusive. Several single-centre studies exist; some report favourable effects on morbidity6–9 but no effect on mortality.10 In general, these studies involve more heterogeneous patient materials regarding length of stay and diagnosis, and

*http://www.criticalcarenutrition.com
the patients included were not fed on their level of energy expenditure.

Given the promising evidence and the absence of any prospective multi-centric pragmatic study, a larger study was justified. We endeavoured to (1) include all possible patients who receive nutrition in the ICU – and not make an artificial distinction between patients being fed by enteral or parenteral routes, (2) use i.v. administration so that we could ensure that the prescribed dose of glutamine supplementation was administered and (3) to run a double-blind study – to avoid bias associated with non-blinded studies.

Accordingly, we conducted a multi-centric, double-blinded, controlled, randomised, pragmatic clinical trial of i.v. glutamine supplementation for ICU patients in the Scandinavian countries. The primary endpoint was the change of sequential organ failure assessment (SOFA) scores after 7 days of treatment. Pre-defined secondary endpoints were change in the SOFA score on day 10, length of ICU stay, ICU mortality and all-cause 6-month mortality.

Material and methods

Study design
This was a multi-centre study within the framework of the Scandinavian Critical Care Trials Group, which is a collaborative task-force under the umbrella of the Scandinavian Society of Anaesthesiology and Intensive Care Medicine. The study centre has been at the Karolinska University Hospital Huddinge – from where the hospital pharmacy has performed the randomisation and distributed the blinded study drug. The study protocol has been publicly available initially on the SCCTG web site and later on the Swedish Intensive Care Registry (SIR) web site. The protocol was registered at www.clinicaltrials.gov (no. NCT00922714) before unblinding.

The regional ethical committee (doc. no. 2002-250) at Karolinska Institutet, Stockholm, Sweden, approved the protocol, and then local ethical committees at sites that participated in the study approved it. In parallel, the Swedish Medical Products Agency, Uppsala, Sweden, approved the protocol (doc. no. 151:2002/31897) for Swedish sites and then authorities in Finland and Norway approved it for sites in their countries. The protocol and the risks involved were presented to the patients or their next of kin orally and in writing before obtaining informed consent.

Blinding was performed at the hospital pharmacy at Karolinska University Hospital Huddinge. Glutamine dipeptide solution and placebo were delivered in identical containers. The procedure consisted of block randomisation of six patients at the individual site. No other stratification was performed. The study drug was delivered to local sites – usually via local hospital pharmacies. Containers for the individual patients were defined by sequential numbers. Extra deliveries were organised for long-staying patients. Hospital Pharmacy at Karolinska Huddinge kept the blinding until the file was cleared in December 2009.

An electronic case report form was used with these forms: (1) a separate inclusion form that contained admission data, (2) a daily form that contained data on the study drug administration, nutrition support and SOFA scoring and (3) a separate discharge form. A study coordinator was available at Karolinska University Hospital Huddinge, Stockholm, Sweden. Mortality data were collected via national registries in Finland, Norway and Sweden.

Patients
Study inclusion criteria were as follows: (1) ICU patients who were given nutrition by an enteral and/or a parenteral route with the aim of providing full nutrition; (2) ages 18–85; (3) an APACHE II score ≥ 10 at ICU admission; and (4) inclusion in the study within 72 h after ICU admission. Exclusion criteria were (1) patients received glutamine supplementation before screening; (2) readmitted patients, who had previously participated in the study; and (3) when informed consent was not received.

Intervention
The protocol was to administer i.v. glutamine supplementation or i.v. placebo (saline) as an i.v. infusion for 12 h daily during the ICU stay. Discontinuation of <24 h was not considered to be a drop-out – if it occurred once. Supplementation started within 3 days after admission and continued throughout the ICU stay.

Glutamine was supplemented as L-alanyl-L-glutamine (Dipeptiven; Fresenius-Kabi, Bad-Homburg, Germany) 200 mg/ml. Placebo was saline in identical bottles. The dose was 0.283 g glutamine/kg body weight/12 h. Pre-ICU body weight was
used for dose calculation, and glutamine supplementation corresponding to 90 kg body weight was the maximum dose given.

**Outcome measures**

It was predeterminately approved in the original protocol by the ethical committee and the regulatory authorities to consider patients given supplementation for >3 days for per protocol (PP) analysis. In addition, all included patients were considered in the intention-to-treat (ITT) analysis, mainly as a control against any uneven distribution.

The feeding regimen – besides the glutamine supplementation – was not protocolised. Full nutrition in the inclusion criteria was recommended to be not <80% of the estimated energy expenditure. The energy-expenditure estimation and the choice between enteral and parenteral nutrition – or a combination of the two – was left to the attending doctor’s discretion. The caloric content of the administered nutrition was recorded – including the caloric content of the drugs administered without accounting for blood products.

The pre-defined primary endpoint was the change in the SOFA score from day 1 to day 7 in PP patients. The statistical power calculation showed that a difference of 0.75 scoring units may be detected or dismissed at a 5% level with 80% power, provided that the scheduled 1000 PP patients were at hand. Pre-defined secondary endpoints were: (1) change in the SOFA score from day 1 to day 10, (2) length of ICU stay, (3) ICU mortality and (4) all-cause 6-month mortality.

**Statistics**

For missing data, imputations were performed when there were existing data both on the time point preceding and the one succeeding; the mean of these two time points was imputed. The number of imputations in the two groups was compared. As SOFA scoring was incomplete even after some imputations, separate calculations were performed over the individual organ failures that built the SOFA score. Difference in mortality by the Kaplan–Meier analysis was tested using the log-rank tests.

**Results**

Eleven centres participated in the study: nine in Sweden, one in Finland and one in Norway. The original plan was to recruit 1000 patients – 500 in Sweden, 150 patients each in Denmark, Finland and Norway and 50 in Iceland. The steering group meeting terminated the study after 4 years when about 400 patients were included due to slow recruitment and insufficient funding. Patient inclusions were from October 2003 to March 2008.

Out of 418 randomised patients, no data were available for five patients; thus, they were excluded. The final database included 413 patients, 205 in the glutamine group and 208 in the control group (Table 1). This is the ITT material. Out of these patients, 284 were treated for >3 days, which is the PP material: 139 in the glutamine group and 145 in the control group.

Table 1 displays the patient characteristics of the treatment and no-treatment groups in ITT and in PP materials. No differences in age or size between the treatment groups or between the ITT and PP groups were observed. The length of glutamine supplementation was also comparable between the treatment groups: about 12 days in the PP groups and 9 days in the ITT groups. The median length of glutamine supplementation, 9 days for the PP

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td>Characteristics of patients given as medians (m) and interquartile ranges (q1,q3).</td>
</tr>
<tr>
<td>ITT</td>
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<td>---</td>
</tr>
<tr>
<td>Age (years)</td>
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<tr>
<td>Weight (kg)</td>
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<tr>
<td>Gender (m/f/u)</td>
</tr>
<tr>
<td>Diagnosis (me/su/un)</td>
</tr>
<tr>
<td>Supplementation (days)</td>
</tr>
<tr>
<td>APACHE II</td>
</tr>
<tr>
<td>SOFA day 1</td>
</tr>
</tbody>
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Gender (male, female, unidentified), diagnosis (medical, surgical, undefined).

ITT, intention-to-treat; SOFA, sequential organ failure assessment.
groups and 6 days in the ITT groups, indicates a non-parametric distribution.

For SOFA scoring of patients discharged earlier than day 7 or day 10 of the study, the SOFA scoring on the day of the discharge was used as a substitute (Table 2). In general, no detectable differences occurred between glutamine-supplemented patients and control patients. The total number of imputations was 266, 0.78% of all values, 108 in the control group and 158 in the glutamine group.

Compared with the controls in the PP group, ICU mortality was lower in the treatment arm (Fig. 1a), which was not sustained at 6 months (Fig. 2a). Parallel figures are present for the PP and the ITT materials. When examining the ITT-PP subgroup, ICU mortality was 8/11 and 28-day mortality was 14/20 for the glutamine and the control groups, respectively.

The caloric intake was comparable between the two groups (Fig. 3). The level of caloric intake was stable during the entire study period at 1800 or 22.5 kcal/kg/24 h. The fraction of calories administered by the enteral route was 30% on day 3, 50% on day 5 and stabilised at about 65% for patients staying for a longer period.

Discussion

This was a prospective randomised double-blinded multi-centric clinical trial that investigated i.v. glutamine supplementation in adequately fed ICU patients. A lower ICU mortality was observed, which was not sustained at 6 months. The SOFA scores during ICU stay revealed no differences between glutamine-supplemented patients and control patients.

This pragmatic study is the first to provide extra i.v. glutamine to an unselected ICU patient population. Inclusion criteria required patients to be adequately fed. Despite no major exclusion criteria, the participating centres may have selected patients with a probable longer course of ICU stay. Enteral nutrition was recommended as the first choice for nutritional support. In a large proportion of patients, this was, at least initially, accompanied by supplementary parenteral nutrition to reach at least 80% of the nutritional target. Still, the glutamine supplementation was administered by an i.v. infusion. The main reason for this was to ensure that the prescribed amount of glutamine was actually delivered, and therefore, it may be assumed that the glutamine concentration was normalised. No plasma glutamine concentration measurements were taken in this study. This may be seen as a shortcoming in the sense that several supplemented patients may not have required exogenous glutamine supplementation to reach the normal plasma concentration of glutamine (0.5–0.8 mM). Previous descriptive studies have shown that about 50% of mechanically ventilated ICU patients have a subnormal plasma glutamine concentration, which is suggestive of glutamine depletion. A study protocol in which plasma glutamine concentration is available at screening would make it possible to select patients with a low plasma glutamine concentration for supplementation. This would have been highly relevant as it has been demonstrated in several studies that this group of ICU patients carries a higher mortality than predicted by APACHE II scoring.

The choice of SOFA scoring as the primary endpoint was a second best choice. A study with a

Table 2

<table>
<thead>
<tr>
<th>SOFA day 1</th>
<th>Delta SOFA day 1–7</th>
<th>Delta SOFA day 1–10</th>
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<tbody>
<tr>
<td></td>
<td>Glutamine</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>m, q1, q3</td>
<td>n</td>
</tr>
<tr>
<td>Tot SOFA</td>
<td>8</td>
<td>6,11</td>
</tr>
<tr>
<td>Tot SOFA (-GCS)</td>
<td>8</td>
<td>6,11</td>
</tr>
<tr>
<td>SOFA respiration</td>
<td>3</td>
<td>3,3</td>
</tr>
<tr>
<td>SOFA coagulation</td>
<td>1</td>
<td>0,2</td>
</tr>
<tr>
<td>SOFA liver function</td>
<td>0</td>
<td>0,1</td>
</tr>
<tr>
<td>SOFA cardiovascular</td>
<td>1</td>
<td>3,3</td>
</tr>
<tr>
<td>SOFA renal function</td>
<td>0</td>
<td>0,2</td>
</tr>
<tr>
<td>SOFA GCS</td>
<td>0</td>
<td>0,0</td>
</tr>
</tbody>
</table>

Scores are also given for the individual organs systems as well as the number of patients included in each calculation(n). P-values for comparisons by Mann-Whitney U-test are given.

IIT, intention-to-treat; SOFA, sequential organ failure assessment.
The mortality endpoint would have to make an extremely brave assumption of the possible benefit of glutamine supplementation. Or with a hypothesis of a 6-month mortality reduction from 35% to 30%, approximately 2500 patients would have been needed. Reduction of SOFA scores was chosen as a direct relation between SOFA and mortality is reported. The failure in the present study to reproduce the relation between change in the SOFA score and ICU mortality is most likely related to the lack of statistical power in our study. This is unfortunately particularly true for the SOFA scoring with many missing values.

The PP group was pre-defined for the primary outcome. The failure to demonstrate a survival advantage during ICU stay also in the ITT group was not related to an opposite tendency in mortality, but rather to a general dilution effect.

In the present study, inclusion was not restricted to patients on parenteral nutrition, which has been the case in several available single-centre studies. Furthermore, glutamine supplementation was provided during the full length of the ICU stay. In the recently presented SIGNET study, no detectable effects from 7 days of i.v. glutamine supplementation on mortality and infectious morbidity were
observed. However, the protocol involved only 7 days of supplementation, and patients were withdrawn from the study when <50% of the nutritional support was provided by the parenteral route. Consequences of the protocol were that a suboptimal glutamine dose was delivered and that the number of patients in the study decreased drastically over time, because they were discharged or were fed by the enteral route or had been supplemented for 7 days.

Compared with earlier studies of ICU patients with a gastrointestinal organ failure and therefore only on parenteral nutrition, the results from the present study showed a different time pattern. We found a difference in ICU mortality not sustained at 6 months, although the study was underpowered for any conclusion of a negative finding at that time point. In the Griffiths and Goeters studies, the difference in mortality became statistically significant at 6 months. No simple explanation for this difference is evident. Patient case mix may be different, and the glutamine supplementation periods were longer in the present study as compared with the SIGNET study. In none of the studies was post-ICU glutamine supplementation included in the protocol.

The major drawback with the present study was that we could not recruit the intended 1000 PP patients. Therefore, it is not possible to conclude whether or not glutamine is effective. Other shortcomings were that the study was conceptualised and planned at a time when routines around multicentric ICU studies were not as rigorous as today. Hence, the protocol and the statistical analysis plan were not published in advance and discussed prospectively outside the SCCTG. The missing data are highly unlikely to have influenced the results, because a very conservative approach regarding imputations and data interpretation was used. In addition, the comparability of the number of missing data sets between the two groups, and the negative result in SOFA scoring, are strong arguments against the introduction of bias. Infection rate was not recorded, which would have made the study more comprehensive.

The strengths of the study are the pragmatic design, the large proportion of long-term supplemented patients, the full feeding of the patients and the i.v. glutamine delivery. This enables us to formulate several post hoc hypotheses to be addressed in future studies. As suggested above, the selection of glutamine-depleted patients for supplementation may yield a different result compared with when supplementation is given – regardless of glutamine status. Secondly, will continued glutamine supplementation after ICU discharge be advantageous for patients? At this time, we do not know when plasma glutamine is normalised and the sickness-related glutamine depletion is restored.
The pragmatic design of our study, with a strong recommendation that patients should be fed >80% of their estimated energy expenditure, resulted in a combined enteral and parenteral nutrition of about 22.5 kcal/kg/day and a larger fraction of enteral nutrition with a longer ICU length of stay. This is worth consideration because the full nutrition may not have continued during the post-ICU period—and may be a possible explanation for the non-sustained reduction in mortality.

In summary, in a prospective randomised double-blinded multi-centric clinical trial over i.v. glutamine supplementation in critically ill patients, we found a lower ICU-, but not 6-month mortality. The study’s pragmatic design makes the results representative for a broad range of critically ill patients. This is the first study to include patients on a combined enteral and parenteral nutrition, which also broadens the representativeness.

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