

Glutamine and glutathione at ICU admission in relation to outcome

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A B S T R A C T

Glutamine depletion is demonstrated to be an independent predictor of hospital mortality in ICU (intensive care unit) patients. Today glutamine supplementation is recommended to ICU patients on parenteral nutrition. In addition to glutamine, glutathione may be a limiting factor in ICU patients with MOF (multiple organ failure). To study the prevalence of glutamine and glutathione depletion an observational study was performed. The results were analysed in relation to mortality and the conventional predictors of mortality outcome, APACHE II (Acute Physiology and Chronic Health Evaluation II) and SOFA (Sequential Organ Failure Assessment). Consecutive patients admitted to the ICU at Karolinska University Hospital Huddinge were studied. Patient admission scoring of APACHE II and SOFA were registered as well as mortality up to 6 months. Plasma glutamine concentration and whole blood glutathione status at admittance were analysed. The admission plasma glutamine concentrations were totally independent of the conventional risk scoring at admittance, and a subnormal concentration was an independent predictor of mortality. In addition, glutathione redox status was also an independent mortality predictor, but here a normal ratio was the risk factor. In both cases the mortality risk was mainly confined to the post-ICU period. A low plasma concentration of glutamine at ICU admission is an independent risk factor for post-ICU mortality. The possible benefit of extending glutamine supplementation post-ICU should be evaluated prospectively.

INTRODUCTION

Glutamine depletion is demonstrated to be an independent predictor of mortality in a group of ICU (intensive care unit) patients when dichotomized with a cut-off at a plasma glutamine concentration of 420 $\mu\text{mol/l}$ [1].

This finding is a major rationale for the institution of extra glutamine supplementation to ICU patients. In single-centre studies of ICU patient needing parenteral nutrition, intravenous glutamine supplementation has been shown to reduce mortality [2,3]. It has been suggested that the plasma glutamine concentration may be an indicator of an insufficient availability of glutamine [4], although the endogenous production of glutamine in sepsis and MOF (multiple organ failure) is demonstrated

to be maintained [5,6]. Further knowledge of glutamine kinetics and the epidemiology of glutamine depletion are needed in order to correctly estimate the possible need for exogenous glutamine supplementation, the appropriate selection of patients and the design of studies addressing the effects of such supplementation.

It is also known that plasma glutamine concentration is fairly stable in ICU patients with MOF staying for an extended period of time in the unit regardless of being fed with conventional enteral or parenteral formulations not supplemented with glutamine [7,8]. On the other hand, when intravenous glutamine supplementation of 0.3 g of glutamine/kg of body weight per day is given as a constant infusion (separate or as a part of an amino acid mixture), plasma glutamine concentration is normalized [7,9,10].

Key words: critically ill, glutamine, glutathione, intensive care unit, mortality, redox status.

Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; ICU, intensive care unit; MOF, multiple organ failure; ROC, receiver operating characteristic; SOFA, Sequential Organ Failure Assessment.

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In addition to glutamine, glutathione may be a limiting factor in ICU patients with MOF. The possible predictive value of glutathione status has not been investigated in ICU patients. Furthermore to correctly estimate glutathione status a measure of the glutathione redox status in addition to the overall concentration is needed.

For glutamine, plasma concentrations give a fair estimate of availability and status. For glutathione on the other hand, plasma concentration is low and glutathione redox status is difficult to measure reliably in the plasma fraction, although concentration is higher in ICU patients as compared with controls or elective surgery patients [11–14]. In addition redox status in plasma may not be representative for the intracellular status. Therefore whole blood determinations give the best idea about glutathione status. In an observational study, it was demonstrated that MOF patients in the ICU were low in total glutathione, approximately 60–70% of the level seen in healthy volunteers or in stable out-patients with COPD (chronic obstructive pulmonary disease) [13], similar results are reported in paediatric ICU patients [15]. In addition, the GSH/total glutathione ratio (a reflection of glutathione redox status) was reduced. In parallel with glutamine status, the glutathione status in whole blood was demonstrated to be stable over time in the ICU.

To address these questions, we performed a pragmatic but well-controlled observational study in patients consecutively admitted to the ICU. Plasma glutamine concentration and whole blood glutathione status were determined within 48 h after ICU admission. The results were analysed in relation to mortality and the conventional predictors of mortality outcome, APACHE II (Acute Physiology and Chronic Health Evaluation II) and SOFA (Sequential Organ Failure Assessment).

MATERIALS AND METHODS

Consecutive patients admitted to the ICU at Karolinska University Hospital Huddinge during the period September 2006–March 2007 were included in the study. The unit is a mixed ICU, but without thoracic, neurosurgery and trauma cases. There are also some paediatric patients admitted to the unit. The study protocol was approved by the Regional Ethical Review Board in Stockholm and patients or their next of kin were informed orally and in writing before obtaining their informed consent.

Inclusion criteria for the study were admittance to the ICU during the study period plus informed consent and sampling within 24 h after admittance. Exclusion criteria were the absence of informed consent at 48 h after admittance, paediatric patients under the age of 18, glutamine supplementation before ICU admittance or failure of sampling within 24 h of admittance.

From the log at the unit a drop-out analysis was also performed including some basic characteristics of the non-included patients during the study period.

At the time of the study the routine in the unit was early full feeding, usually starting within 48 h, a combination of enteral and parenteral nutrition. The parenteral nutrition given did not contain any glutamine and no separate glutamine supplementation was given during this time period. The enteral nutrition contained glutamine, but was not enriched with glutamine.

Blood samples for glutamine analysis were taken in heparinized tubes and centrifuged within 30 min and the plasma was stored at -80°C pending analysis using HPLC after pre-column derivatization and with an internal standard of norvalin [8].

Blood samples for glutathione analysis were immediately precipitated with 14% HClO_4 , containing 2 mmol phenanthroline. After mixing the samples were frozen in liquid nitrogen, and thereafter stored at -80°C pending analysis, which was done in 2009 (after 18 months of storage). Just before analysis the samples were centrifuged at 12500 *g* for 15 min at 4°C . Both GSH and total [after reduction with DTT (dithiothreitol)] glutathione were then analysed in whole blood using HPLC as described previously in detail [13,16].

All results are given as means \pm S.D., unless otherwise indicated. Student's *t* test, ANOVA and Pearson's linear regression analysis were used when applicable. For mortality data, Fisher's exact two-tailed test was used. Univariate logistic regression and stepwise multiple logistic regression on predictors of mortality were performed using the software NCSS 2007.

RESULTS

The target was to include 200 patients. During the study period there were 291 admissions of patients >17 years of age. From these, 174 patients were included and 117 patients were not included (Figure 1). The characteristics of these two groups are given in Table 1. A difference in the length of ICU stay was observed. Mortality data were checked in the Swedish national registry, where all patients are identified by their unique personal number. There were no missing mortality data.

When patients were dichotomized with a cut-off at plasma glutamine concentration of $420\ \mu\text{mol/l}$, the groups were comparable in characteristics (Table 1), but a difference in 6-month all-cause mortality was observed, 33 out of 76 patients compared with 27 out of 98 patients ($P = 0.037$) for <420 and $>420\ \mu\text{mol/l}$ respectively. When patients were divided into quartiles and mortality was specified in ICU mortality and post-ICU all-cause 6-month mortality (Figure 2), it became obvious that the dichotomization at a glutamine plasma concentration of $420\ \mu\text{mol/l}$ came very close to the median value

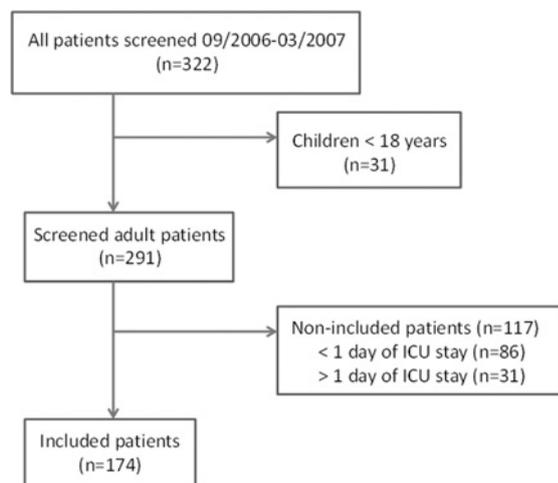


Figure 1 A CONSORT diagram illustrating the screening of all admitted patient during the study period

Non-included patients were divided into <24 h of ICU stay, with a mortality of 34% and >24 h of ICU stay with a mortality of 52%. The latter group had an ICU stay of 4 (3, 7) days (values is the median and interquartile range).

430 $\mu\text{mol/l}$. Furthermore, the 50% of patients with the lower admission plasma glutamine concentration had a higher mortality, the major part of which was post-ICU all-cause 6-month mortality. The admission plasma glutamine concentrations were totally independent of the risk scoring in terms of APACHE II scores (Figure 3) or SOFA scores (results not shown).

Whole blood total glutathione concentration was $514 \pm 136 \mu\text{mol/l}$, whereas the GSH fraction was $280 \pm 105 \mu\text{mol/l}$, resulting in a GSH/total glutathione ratio in whole blood, a reflection of glutathione redox status, of 0.55 ± 0.14 . For the total concentration of glutathione and the concentration of GSH in whole blood, as well as the GSH/total glutathione ratio in whole blood, there was no relationship with predicted mortality in terms of APACHE II scores (Figure 4) and SOFA scores (results not shown). In addition, when the

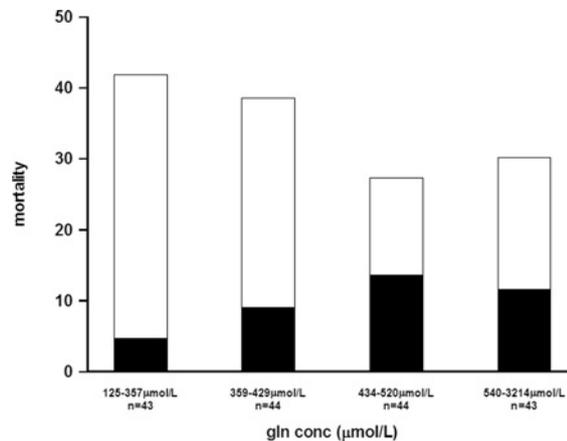


Figure 2 All-cause 6-month mortality (open bars) and ICU mortality (filled bars) of consecutive patients admitted to the general ICU ($n=174$) at Karolinska Huddinge divided into quartiles according to the admittance plasma glutamine concentration

Mortality is different in between the quartiles, where the two lower quartiles are different from the two higher ($P=0.037$).

patients were divided into quartiles according to the total concentration of glutathione and the concentration of GSH in whole blood, no relationship to the predicted or actual mortality was observed (results not shown). In contrast, when patients were divided into quartiles according to the GSH/total glutathione ratio in whole blood (Figure 5), 6-month all-cause mortality was higher for the quartile with the highest ratio (21 out of 43 patients) as compared with in the lower three quartiles (37 out of 126 patients) ($P=0.026$). The empirical ROC (receiver operating characteristic) curve of the GSH/total glutathione ratio compared with mortality [AUC (area under the curve) 0.56, $P=0.18$] confirmed the suggested cut-off for the GSH/total glutathione ratio of 0.66; characteristics of the patients according to this dichotomization is given in Table 1.

Table 1 Characteristics of the patients

Characteristics of all of the patients admitted to the ICU during the inclusion period are given in the left-hand part of the Table, characteristics of the included patients dichotomized at a plasma glutamine (Gln) concentration of 420 $\mu\text{mol/l}$ are given in the middle part of the Table, and those dichotomized at a GSH/total glutathione ratio (Redox) of 0.66 in whole blood are given in the right-hand part of the Table. Results are presented as means \pm S.D. or median (inter-quartile range) as appropriate. Statistical analysis by Student's t test*, Mann-Whitney U test† or Fisher's exact two-tailed test‡. LOS, length of study.

Characteristic	Included ($n=174$)	Not included ($n=117$)	P	Gln < 420 ($n=76$)	Gln > 420 ($n=98$)	P	Redox ≤ 0.65 ($n=126$)	Redox > 0.66 ($n=43$)	P
Age (years)	59 ± 18	54 ± 18	0.02*	63 ± 16	57 ± 18	0.03*	59 ± 18	61 ± 18	0.62*
APACHE II	19 ± 7	19 ± 9	1.00*	20 ± 7	19 ± 8	0.27*	19 ± 8	20 ± 8	0.68*
SOFA	7 ± 4	6 ± 5	0.19*	7 ± 4	7 ± 4	0.34*	7 ± 4	8 ± 4	0.007*
LOS (days)	2 (1, 5)	1 (1, 2)	<0.001†	2 (1, 6)	2 (1, 4)	0.96†	1 (1, 4)	4 (1, 8)	0.007†
Gender (male %)	59	51	0.23‡	59	58	1.00‡	60	58	1.00‡
6-Month mortality (%)	34.5	38.5	0.53‡	43	28	0.04‡	17	49	0.03‡

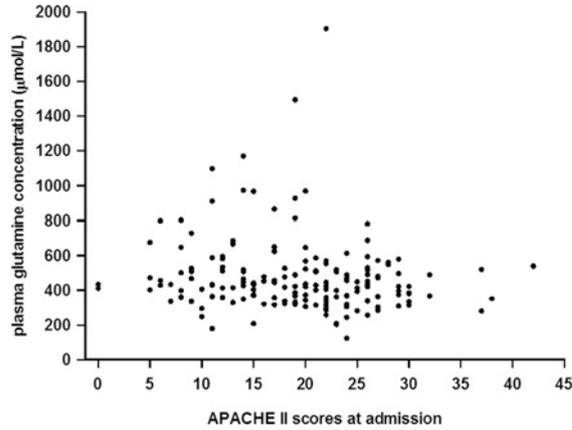


Figure 3 Relationship between the plasma concentration of glutamine of consecutive patients admitted to the general ICU ($n = 174$) at Karolinska Huddinge and their APACHE II scores

No correlation was seen ($P = 0.46$)

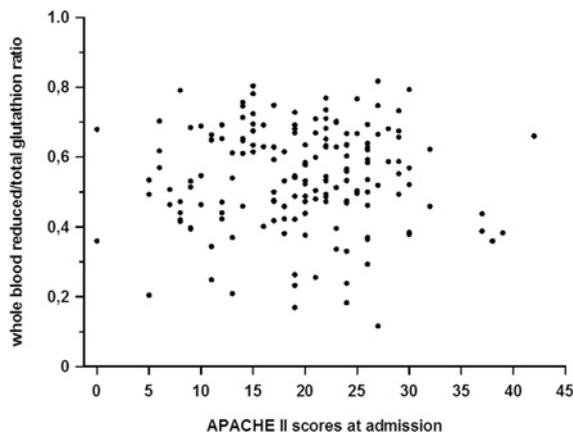


Figure 4 Relationship between the GSH/total glutathione ratio in whole blood reduced of consecutive patients admitted to the general ICU ($n = 169$) at Karolinska Huddinge compared with their APACHE II scores

No correlation was seen ($P = 0.93$).

Out of the data presented, a stepwise logistic regression analysis was performed (Table 2). In an explorative analysis, the empirical ROC curve of plasma glutamine concentration compared with 6-month mortality crossed the line of identity at a plasma glutamine concentration of $675 \mu\text{mol/l}$, suggesting a U-shaped influence of plasma glutamine concentration on mortality. ROC curves at data below ($n = 154$) and over ($n = 20$) $675 \mu\text{mol/l}$ demonstrated cut-offs (maximum of sensitivity + specificity) at 403 and $928 \mu\text{mol/l}$ respectively. These values were rounded to 400 and $930 \mu\text{mol/l}$ respectively, in the further exploration of predictors of death within 6 months. As expected APACHE II served as the major mortality predictor, but in addition age, an

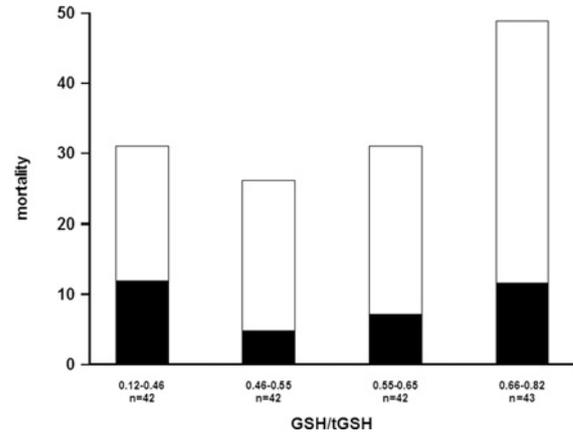


Figure 5 All-cause 6-month mortality (open bars) and ICU mortality (filled bars) of consecutive patients admitted to the general ICU ($n = 169$) at Karolinska Huddinge divided into quartiles according to the GSH/total glutathione (tGSH) ratio of whole blood at admittance reflecting glutathione redox status

Mortality is different in between the quartiles, where the highest quartile is different from the three lower ($P = 0.026$).

admission glutamine plasma concentration of <400 or $>930 \mu\text{mol/l}$ and a whole blood GSH/total glutathione ratio ≥ 0.66 could also be identified as independent predictors of mortality. In total, 82% of the total 6-month all-cause mortality was explained by these factors.

DISCUSSION

Our results reproduced the findings of Oudemans-van Straaten et al. [1] of admission plasma glutamine as an independent predictive factor for mortality also in an unselected patient data from a mixed general ICU. In that study, the endpoint was hospital mortality. An additional finding in the present study was that the mortality difference was confined to the post-ICU period of the all-cause 6-month mortality.

Related to differences in the organization of health care in different countries, ICU mortality and hospital mortality may not be immediately comparable. The Swedish health care insurance system allows for a 6-month all-cause mortality analysis with a minimal loss to follow-up. It may be a Swedish situation that the post-ICU mortality exceeds the ICU mortality, but the obvious relevance of this type of follow-up must be emphasized.

The increase in correctly predicted outcome from 70 to 75% by adding a pathological low or high glutamine concentration at admittance may not seem dramatic, but the difference may be illustrated graphically (Figure 6). This points out that this difference in terms of mortality risk for an individual patient may be dramatically different.

Table 2 Prediction of 6-month mortality in ICU patients

Univariate analysis ($n = 174$) and stepwise multiple logistic regression analysis ($n = 169$) are shown. Gln, plasma glutamine concentration ($\mu\text{mol/l}$); rGSH/tGSH, GSH/total glutathione ratio in whole blood; OR, odds ratio; CI, confidence interval.

(a) Univariate analysis

	OR (CI)	<i>P</i>
Gln < 420	2.02 (1.07–3.80)	0.029
Gln < 400	2.41 (1.26–4.59)	0.007
Gln > 930	4.11 (0.99–17.1)	0.043
Gln < 400 or > 930	3.22 (1.68–6.16)	<0.001
rGSH/tGSH > 0.65	2.17 (1.07–4.40)	0.032
Gender (male)	1.35 (0.71–2.57)	0.36
APACHE (per point)	1.14 (1.09–1.21)	<0.001
Age (per year)	1.06 (1.03–1.08)	<0.001

(b) Stepwise multiple logistic regression analysis

	β	OR (CI)	<i>P</i>	Correct (%)
Intercept	– 6.43	0.002 (0.0002–0.016)		
APACHE (per patient)	0.13	1.14 (1.07–1.22)	<0.001	70.1
Gln < 400 or > 930	1.08	2.95 (1.38–6.32)	0.005	74.6
Age (per year)	0.04	1.04 (1.01–1.07)	0.006	78.1
rGSH/tGSH > 0.65	0.85	2.35 (1.02–5.41)	<0.001	81.7

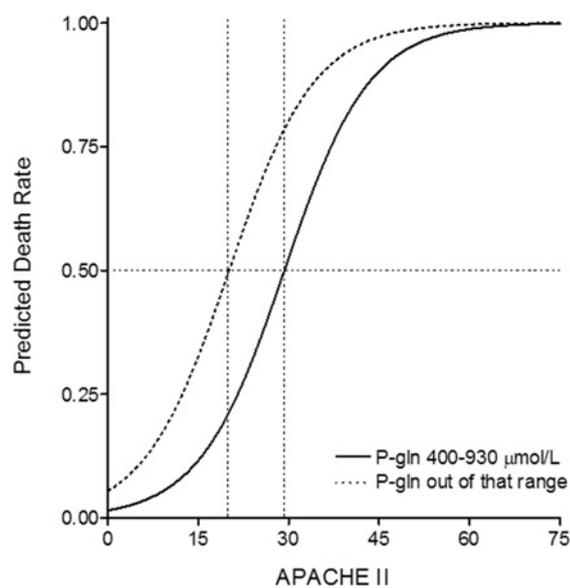


Figure 6 Simulation of the additional prediction of mortality rate from an out of range glutamine concentration at admittance

Black line is the APACHE-predicted mortality rate if the plasma glutamine concentration at admittance is within the range 400–930 $\mu\text{mol/l}$. With an admittance glutamine concentration outside of that range, the dotted curve represents the predicted mortality rate, suggesting a mortality rate of 50% at APACHE 20 in contrast with APACHE 29.5 if the glutamine concentration is not considered.

When performing the stepwise multiple regression analysis it became obvious that the admission plasma glutamine concentration compared with mortality curve had a U-shape, with an increased mortality also for very high-plasma glutamine concentrations. It has been reported previously that patients with acute fulminant liver failure (but not acute-on-chronic liver failure) may have such high concentrations [17]. Most of the small number of patients with very high plasma glutamine concentrations in this study had acute liver failure. Glutamine kinetics in this subgroup of patients is poorly understood and need to be studied further [18].

The recently presented results from the Scandinavian glutamine study suggest that intravenous glutamine supplementation may reduce ICU mortality, but that the mortality advantage is not maintained post-ICU [19]. It was hypothesized that this may be related to the discontinuation of glutamine supplementation at ICU discharge. The present observational study is also suggestive that glutamine depletion may be related to post-ICU mortality (Figure 7). The need for prospective studies extending glutamine supplementation beyond ICU stay in glutamine depleted patients is therefore obvious.

In addition our results confirm the data from earlier pilot studies in ICU patients with MOF that total glutathione and GSH concentrations in whole blood are low in ICU patients. The levels of these concentrations were not found to be related to ICU mortality outcome. Not

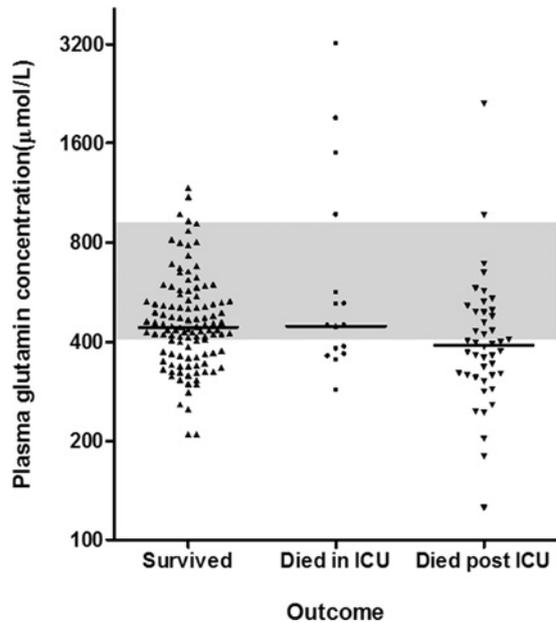


Figure 7 Individual plasma glutamine concentrations at ICU admission compared with outcome

Note the log-scale of the y-axis. Grey area represents normal values 400–930 μM . Median in each group is depicted as a line.

surprisingly the redox status of glutathione was demonstrated to be related to mortality outcome, but it was quite unexpected that the quartile with the highest ratio of GSH and total glutathione carried the highest mortality.

A possible explanation for an increased mortality risk associated with a redox ratio of glutathione closer to the normal range is that the scavenging properties of glutathione has been less utilized among these individuals. The interpretation of this quite original finding should consider a number of factors: the sampling and analysis of whole blood as an indicator of glutathione status, sampling during the initial 48 h of ICU stay, the time-course of ICU stay and the ICU mortality compared with the post-ICU mortality (Figure 4).

We know from earlier studies in selected groups of patients that the plasma concentration of glutamine, as well as the whole blood redox status of glutathione, do not change over time during an ICU stay when no specific supplementation is given [13]. Therefore should the admission values presented here be representative of the status of the particular patient during ICU stay? We know that the plasma glutamine concentration may be influenced by exogenous glutamine supplementation [7,9,10,20], but so far no treatment to influence the redox status of glutathione in whole blood in this patient group has been presented. Beside exogenous glutamine supplementation, selenium supplementation may have an effect, as the activity of glutathione peroxidase responds to selenium supplementation [21]. Controlled studies addressing these hypotheses are needed.

In the characteristics of the patient groups with a higher mortality risk according to the glutamine and glutathione status some differences were seen. It must be underlined that these rather speculative observations are merely hypothesis generating. Nevertheless, patients with a low plasma glutamine concentration tend to have high post-ICU mortality, whereas patients with a high redox ratio of glutathione in whole blood have a longer ICU length of stay and again a higher post-ICU mortality (Table 1). None of these groups stand out in terms of admittance APACHE II scores, but the risk group in terms of the high glutathione redox ratio were higher in initial 24 h SOFA score.

Observational studies always have the limitation that they basically only represent the group of patients actually studied. In the present report, we provide information of not only the studied patients, but also the patients not included in the study, in order to provide complete information. As expected, the majority of the drop-outs were short stayers (primarily during week-ends). This is reflected in that the median ICU length of stay for this group was 1 day (Figure 1). In addition, there were also a limited number of patients, where informed consent and sampling failed within the stipulated time limit of 48 h, or where withholding of treatment was decided before an informed consent was obtained.

A remarkable finding in the missing data analysis was the high APACHE II score and the high mortality in the group of mainly short stayers not included in the study. To some extent, this is probably a reflection of the rather extreme shortage of ICU beds at the time of the study. The important issue is of course to what degree our data can be generalized. Are our situations and our case-mix too special? We think that the ICU situation reflected in the patient data, with a comparatively short length of ICU stay combined with a low ICU mortality but high post-ICU mortality is special. Nevertheless, the overall outcome of our patients is probably not different from what is seen in places with a different organization of the ICU compared with the rest of the hospital.

In conclusion, low plasma concentration of glutamine at ICU admission is an independent risk factor for post-ICU mortality.

AUTHOR CONTRIBUTION

Olav Rooyackers and Jan Wernerman conceived the study. Paul Rodas, Olav Rooyackers, Christina Hebert and Jan Wernerman designed the study protocol. Paul Rodas and Jan Wernerman performed the clinical part of study. Christina Hebert and Olav Rooyackers analysed the samples. Paul Rodas, Olav Rooyackers Christina Hebert, Åke Norberg and Jan Wernerman performed the calculations and statistics. Paul Rodas and Jan Wernerman wrote the paper. Paul Rodas, Olav Rooyackers, Christina

Hebert, Åke Norberg and Jan Wernerman revised the paper.

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