# Pharmacokinetic data support 6-hourly dosing of intravenous vitamin C to critically ill patients with septic shock

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Septic shock is a leading cause of death in most countries.[1-3] There is a need for novel therapies that are effective in a variety of health care settings and that are both inexpensive and safe. It has been suggested that vitamin C (also known as ascorbic acid) may be such an intervention.[4,5]

Vitamin C is well established as an antioxidant through its electron donor attributes, and could act as a major defence against reactive oxygen species generated in sepsis.[6,7] Vitamin C is an essential water-soluble vitamin that cannot be synthesised or stored by humans, thus the human body relies on dietary intake. As a water-soluble vitamin, its plasma half-life in health is estimated to be 7–14 days.[8] While there is no established normal reference interval for plasma vitamin C concentrations, hypovitaminosis C is commonly defined by plasma vitamin C concentrations between 11–23 μmol/L, and vitamin C deficiency is defined as concentrations below 11 μmol/L.[9-11]

Critically ill patients appear to have markedly reduced plasma vitamin C concentrations when compared with a healthy population.[11-14] Furthermore, two small studies have suggested clinical benefits with exogenous vitamin C administration.[15,16] Doses used were 6 g/day and 200 mg/kg/day respectively.[15,16] These preliminary findings have stimulated a number of larger and more methodologically rigorous trials of exogenous vitamin C in septic shock,[17] including the Vitamin C, Hydrocortisone and Thiamine in Patients with Septic Shock Trial (VITAMINS).[18] However, despite the fact that multiple studies have been conducted in critically ill patients using a variety of vitamin C dosing strategies with mixed outcomes,[19] very few have included before and after dose analysis of plasma vitamin C concentrations. Therefore, the optimal dosing regimen for vitamin C administration to patients with septic shock remains unknown.[19]

The objective of this study was to estimate vitamin C pharmacokinetics in a cohort of patients with septic shock enrolled in the treatment arm of the VITAMINS trial. We hypothesised that dosing of 1.5 g intravenous vitamin C administered every 6 hours would achieve and maintain

## ABSTRACT

**Objectives:** To study vitamin C pharmacokinetics in septic shock.

**Design:** Prospective pharmacokinetic study.

**Setting:** Two intensive care units.

**Participants:** Twenty-one patients with septic shock enrolled in a randomised trial of high dose vitamin C therapy in septic shock.

**Intervention:** Patients received 1.5 g intravenous vitamin C every 6 hours. Plasma samples were obtained before and at 1, 4 and 6 hours after drug administration, and vitamin C concentrations were measured by high performance liquid chromatography.

**Main outcome measures:** Clearance, volume of distribution, and half-life were calculated using non-compartmental analysis. Data are presented as median (interquartile range [IQR]).

**Results:** Of the 11 participants who had plasma collected before any intravenous vitamin C administration, two (18%) were deficient (concentrations < 11 μmol/L) and three (27%) had hypovitaminosis C (concentrations between 11 and 23 μmol/L), with a median concentration 28 μmol/L (IQR, 11–44 μmol/L). Volume of distribution was 23.3 L (IQR, 21.9–27.8 L), clearance 5.2 L/h (IQR, 3.3–5.4 L/h), and half-life 4.3 h (IQR, 2.6–7.5 h). For the participants who had received at least one dose of intravenous vitamin C before sampling, T0 concentration was 258 μmol/L (IQR, 162–301 μmol/L). Pharmacokinetic parameters for subsequent doses were a median volume of distribution 39.9 L (IQR, 31.4–44.4 L), clearance 3.6 L/h (IQR, 2.6–6.5 L/h), and half-life 6.9 h (IQR, 5.7–8.5 h).

**Conclusion:** Intravenous vitamin C (1.5 g every 6 hours) corrects vitamin C deficiency and hypovitaminosis C and provides an appropriate dosing schedule to achieve and maintain normal or elevated vitamin C levels in septic shock.

Crit Care Resusc 2019; 21 (4): 236-42

correction of vitamin C plasma concentrations to normal or supranormal in patients with septic shock.

## Methods

### Patients

This two-centre prospective pharmacokinetic study was performed over a 9-month period. The study was approved by the Human Research Ethics Committee at the Austin Hospital (HREC/17/Austin/238), with site governance approval at the Royal Melbourne Hospital. Written informed consent was obtained from all patients or their medical treatment decision maker, unless waived by the Human Research Ethics Committee.

Participants who were randomised into the treatment arm of the VITAMINS study were eligible for the pharmacokinetics study. These patients had a diagnosis of septic shock, made according to the Sepsis-3 consensus clinical criteria, with patients requiring a suspected or documented infection and an acute increase of 2 or more points in the Sequential Organ Failure Assessment, the need for vasopressor therapy to keep mean arterial pressure at 65 mmHg or over for more than 2 hours, and a plasma lactate greater than 2 mmol/L despite adequate fluid resuscitation and receiving, or suitable to receive, vitamin C.[20] In addition, these patients required an arterial or central venous catheter in situ for blood sampling.

### Study protocol

Vitamin C (Rotexmedica, Trittau, Germany) was purchased as 5 mL vials (100 mg/mL) and stored at room temperature at both intensive care units (ICUs) in the original packaging, protected from light. Vitamin C solution was prepared within 1 hour before use by clinical staff.[21] Three vials (1500 mg) of vitamin C were dissolved in a 100 mL solution of either 0.9% sodium chloride or 5% glucose in water. The vitamin C solution was administered via standard intravenous tubing and a dedicated lumen of a central venous catheter at a continuous rate for 1 hour. Vitamin C was administered every 6 hours until the resolution of shock or up to 10 days.

### Data collection

Data including patient demographics, physiological parameters, pathology results and interventions were collected by trained staff using medical and nursing documentation. These were entered into the Research Electronic Data Capture (REDCap) system, a secure web application for managing online data collection.[22]

### Blood sample collection and analysis

Blood was collected into chilled lithium heparin 6 mL BD vacutainer tubes (Becton Dickinson, USA) from an arterial or central venous catheter at time (T) 0 hour (pre-dose), at 1 hour (at completion of infusion), and at 4 and 6 hours after infusion commencement. Lithium heparin tubes have been shown to be suitable for vitamin C analysis.[23] To facilitate data collection, there was no restriction on the number of doses required before plasma sampling. Blood samples were immediately placed on crushed ice and separated within 30 minutes of collection. Plasma was separated by centrifugation (4000 revolutions per minute [rpm] for 10 minutes at 4°C), with equal volumes of separated plasma and 0.54 M perchloric acid/diethylene-triaminepentaacetic acid (PCA/DTPA) solution aliquoted into four Eppendorf tubes (Eppendorf, Germany).[24] PCA/DTPA was added to reduce vitamin C ex vivo metabolism to 2,3-diketogulonic acid as well as precipitate proteins and chelate metal ions.[25] The Eppendorf tubes were vortexed and kept on ice before further centrifugation at 12 000 rpm for 2 minutes at 4°C. The plasma-PCA supernatant solution was stored in a –80°C freezer until further analysis.

Sample analysis for total vitamin C was performed on a Shimadzu high performance liquid chromatography system with ultraviolet detection (HPLC-UV) (Shimadzu, Japan) at the School of Health and Biomedical Sciences, Royal Melbourne Institute of Technology, Melbourne, Australia. The column, bilevel quality controls, internal standard and mobile phase were purchased through Astral Scientific (Taren Point, Australia), which were sourced from Chromsystems (Munich, Germany) with a quoted limit of quantification of 2.2 μmol/L. Samples were analysed within 2 hours of thawing. The within-assay coefficient of variation was established as 2% and the between-assay coefficient of variation as 9%. Additionally, the method was enrolled in an external quality assurance program for plasma vitamin C that achieved a coefficient of variation of 7% and bias of 6.3 μmol/L over a range of 8–159 μmol/L.

### Data analysis

Calculation of pharmacokinetic parameters employed a non-compartmental approach.[26,27] For participants with first-dose data, baseline (physiological) vitamin C concentrations were subtracted from the 1, 4 and 6-hour values. The area under the plasma concentration-time curve (AUC) was then determined using the linear trapezoidal rule, and extrapolated to infinity ($AUC_{0-\infty}$) by adding the product of the terminal slope multiplied by the last measured plasma concentration. Plasma clearance in these first-dose patients was calculated as Dose ÷ $AUC_{0-\infty}$. Where patients had received multiple doses, baseline (pre-dose) concentration was not subtracted, and AUC calculated over the 6-hour dosing period only ($AUC_{0-6}$). Plasma clearance in these patients was calculated using Dose ÷ $AUC_{0-6}$. The apparent

**Table 1. Demographic data for patients enrolled in the pharmacokinetics study**

| Demographic data | Baseline values |
|---|---|
| Number of patients | 21 |
| Sex, male | 9 (42.9%) |
| Age (years), median (IQR) | 60.3 (48.3–68.7) |
| Weight (kg), median (IQR) | 76.0 (66.0–90.0) |
| Source of ICU admission | |
| Emergency department | 10 (47.6%) |
| Transfer from ward | 4 (19.0%) |
| Transfer from another ICU | 5 (23.8%) |
| Transfer from another hospital (not ICU) | 2 (9.5%) |
| Non-surgical patients | 14 (66.7%) |
| Suspected or identified site of infection leading to septic shock | |
| Gastrointestinal/biliary | 12 (57.1%) |
| Urogenital | 3 (14.3%) |
| Primary bacteraemia, including line infections | 2 (9.5%) |
| Respiratory | 2 (9.5%) |
| Skin/soft tissue | 2 (9.5%) |
| APACHE III score, median (IQR) | 81 (67–95) |
| SOFA score,* median (IQR) | 10 (7–11) |
| Known diabetes mellitus | 2 (9.5%) |
| Blood lactate (mmol/L), median (IQR) | 4.4 (3.9–5.9) |
| White cell count ($\times 10^9$/L) (median [IQR]) | 15.1 (10.9–26.2) |
| Platelet count ($\times 10^9$/L), median (IQR) | 174 (56–231) |
| Serum creatinine (μmol/L), median (IQR) | 122 (101–186) |
| Estimated glomerular filtration rate as calculated from serum creatinine (mL/min/1.73m$^2$), median (IQR) | 81 (65–91) |
| Mechanical ventilation | 14 (66.7%) |
| Renal replacement therapy | 3 (14.3%) |
| Noradrenaline rate before vitamin C administration (μg/kg/min), median (IQR) | 0.13 (0.10–0.24) |
| Time from randomisation to first dose of vitamin C (hours), median (IQR) | 14.9 (10.6–15.6) |

APACHE = Acute Physiology and Chronic Health Evaluation; ICU = intensive care unit; IQR = interquartile range; SOFA = Sequential Organ Failure Assessment. * *n* = 9.

volume of distribution ($V_d$) during the elimination phase was calculated as clearance ÷ $k_{el}$, where $k_{el}$ is the rate elimination constant. The elimination half-life ($T_{1/2}$) was calculated as Ln(2) ÷ $k_{el}$ All analyses used PKSolver — an open-source add-in for Microsoft Excel.[28]

### Statistical analysis

Continuous data are presented as median (interquartile range [IQR]), and categorical data as counts (%). Sample size was based on similar pharmacokinetic studies, with an objective to obtain at least ten complete samples from initial and subsequent doses.[29-31]

## Results

Between May 2018 and February 2019, 21 patients who received vitamin C as part of the VITAMINS study consented to participate in this substudy analysis. At the time of sample collection, 11 patients were receiving their first dose of 1.5 g intravenous vitamin C and 10 patients were receiving a subsequent dose of vitamin C (range, 2–7). No adverse effects to vitamin C were reported.

Baseline demographics are detailed in Table 1. All patients were receiving at least one continuous vasopressor infusion. Twenty patients (95.2%) were receiving noradrenaline before the initial dose of vitamin C, with one patient (4.8%) receiving a continuous infusion of metaraminol. Nine patients (42.9%) were receiving an additional vasopressor or inotrope.

### Sampling and vitamin C concentration

Eleven patients provided first-dose vitamin C concentrations. The median time from ICU admission to baseline (T0) blood sampling was 11.5 hours (IQR, 10.6–15.8 h). The median pre-dose (T0) plasma vitamin C concentration was 28 μmol/L (IQR, 11–44 μmol/L). Of these 11 participants, two patients had concentrations in the deficient range (18%), three had hypovitaminosis C (27%), with the remaining six (55%) recording concentrations greater than 23 μmol/L (Figure 1).

An additional ten participants had sampling performed after multiple doses had been administered (median number of prior doses, 4). The median T0 concentration was 258 μmol/L (IQR, 162–301 μmol/L). None of these patients returned concentrations in the deficient or hypovitaminosis C ranges (Figure 2).

One patient died before the T6 hour collection point. Death was not deemed to be related to the study

**Figure 1. Concentration of vitamin C in plasma for patients receiving their first dose of 1.5 g intravenous vitamin C — the median is shown in red**

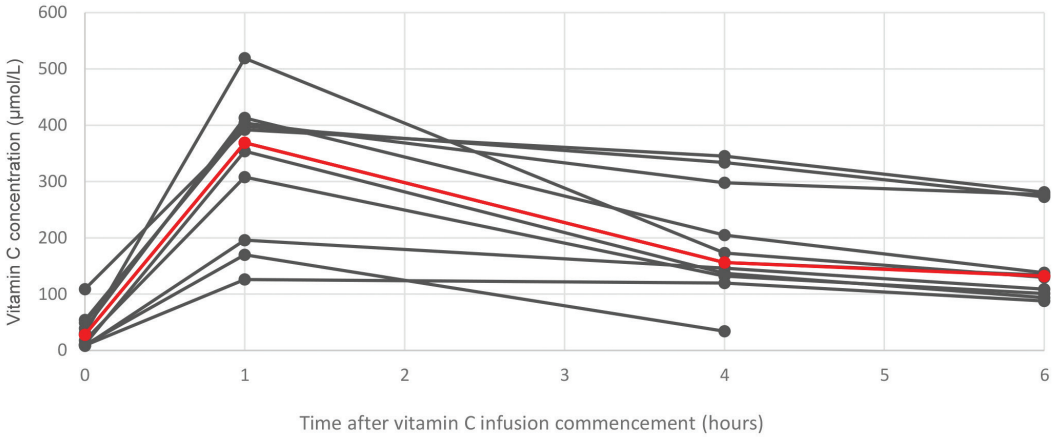
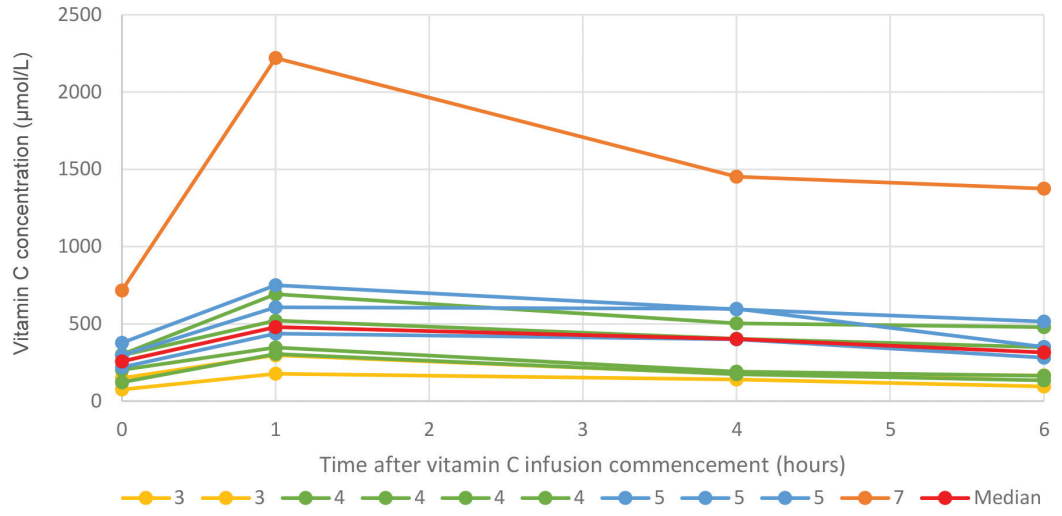



**Figure 2. Concentration of vitamin C in plasma for patients receiving a subsequent dose of 1.5 g intravenous vitamin C***




* Dose number is colour-coded: yellow, 3rd dose; green, 4th dose; blue, 5th dose; orange, 7th dose. The median is shown in red.

drug; hence, their vitamin C baseline and maximum concentrations were included in analysis.

Visualisation of vitamin C concentration versus time graphs demonstrated that plasma vitamin C concentrations peaked and subsequently declined in a linear fashion, suggesting first-order (concentration dependent) elimination. For first-dose patients, the vitamin C concentration at T1 hour was 369 µmol/L (IQR, 252–401 µmol/L), with the concentration at T4 hours 156 µmol/L (IQR, 135–252 µmol/L) and T6 hours 132 µmol/L (IQR, 103–239 µmol/L). Likewise for multiple dose patients, the vitamin C concentration at T1 hour was 479 µmol/L (IQR, 316–671 µmol/L), with T4 hours 402 µmol/L (IQR, 180–571 µmol/L) and T6 hours 315 µmol/L (IQR, 165–446 µmol/L).

### Pharmacokinetics

For patients who were receiving their first dose of 1.5 g intravenous vitamin C ($n$ = 11), the volume of distribution was 23.3 L (IQR, 21.9–27.8 L), the clearance was 5.2 L/h (IQR, 3.3–5.4 L/h), and the half-life was 4.3 h (IQR, 2.6–7.5 h).

For patients who had already received more than one dose of intravenous vitamin C ($n$ = 10), the volume of distribution was 39.9 L (IQR, 31.4–44.4 L), the clearance was 3.6 L/h (IQR, 2.6–6.5 L/h), and the half-life was 6.9 h (IQR, 5.7–8.5 h).

## Discussion

### Key findings

A major observation of this pharmacokinetic study is that vitamin C deficiency and hypovitaminosis C occurred in nearly half of the study cohort. Furthermore, 1.5 g of vitamin C was sufficient to cause a rapid and sustained increase in plasma vitamin C concentrations in all participants, with the median plasma vitamin C concentration increasing over tenfold with a single dose. Finally, pharmacokinetic data after initial and subsequent doses of vitamin C support a 6-hourly dosing regimen by showing achievement and maintenance of normal or supranormal vitamin C levels.

### Relative to other studies

There have been eight studies, including single centre observational,[11,14,32] therapeutic response,[29] prospective[33-35] or retrospective[15] interventional studies, that have reported plasma vitamin C concentrations in critically ill populations. The lowest reported percentage of patients with hypovitaminosis C was 59%.[35] Four studies restricted sampling to patients with sepsis and septic shock, with a combined total of 125 patients investigated;[11,15,33,34] however, only two reported the prevalence of hypovitaminosis C, which was present in about 80% of patients.[11,33] Our study found hypovitaminosis C in only 45% of patients. Prior data have suggested an association between hypovitaminosis C and severity of illness, with vitamin C concentration found to be significantly reduced during the early phase of multiple organ failure[36] and in patients with septic shock.[11]

Pharmacokinetic data for exogenous vitamin C use are limited. Previous studies have focused on enteral and/or parenteral administration in healthy or ambulant populations.[30,37-40] There has only been one pharmacokinetics study conducted in the critically ill.[41] This study included eight patients with severe sepsis and evaluated doses of 2 g/day and 10 g/day infused as bolus or continuous infusion.[41] Patients were excluded if they had pre-existing renal insufficiency or expected need for renal replacement therapy. Pharmacokinetic modelling was based on cohorts of five patients at each dosing regimen. It found that 2 g/day resulted in physiological plasma concentrations, whereas 10 g/day resulted in supraphysiological concentrations. This study estimated intravenous vitamin C clearance as 4.3 L/h, central volume of distribution 31.6 L, peripheral volume of distribution 39.6 L, and intercompartmental clearance 5.2 L/h.[41] These values are similar to those seen in our study.

### Implications

Our study implies vitamin C hypovitaminosis or deficiency are common in septic shock patients and that 6-hourly dosing at 1.5 g intravenous of vitamin C achieves and maintains normal or supranormal vitamin C levels in patients with septic shock. The observation of an increase in volume of distribution in the multiple dose cohort (39.9 L) compared with the first-dose cohort (23.3 L) implies either changes in volume of distribution secondary to fluid resuscitation or other septic shock-associated mechanisms such as changes in vascular permeability.[42,43] The observation that clearance was reduced in the multidose cohort implies either loss of renal clearance due to worsening function or decreased vitamin C consumption, as treatment with antibiotics decreased oxidative stress.

### Strengths and limitations

This study sampled a heterogenous group of ICU patients united by a clinical diagnosis of septic shock as defined by consensus criteria.[20] Given that this study did not exclude patients with chronic illnesses such as renal failure, this study is generalisable to most ICU patients with septic shock who may receive vitamin C. The methodology of sample collection, storage and processing was consistent with other recent studies.[11,41]

The limitations of this study include the modest size of the cohort studied ($n$ = 21), which precludes subpopulation analysis. In particular, given that vitamin C is predominantly renally excreted,[37] acute kidney injury is a coexisting event that may affect dosing regimens and deserves further analysis in future studies. Furthermore, only a single dose of vitamin C was studied, and a multiple dose protocol is needed to calculate steady state concentrations.

Limitations also include challenges inherent to the measuring of vitamin C concentrations. Vitamin C analysis in biological samples is known for its instability.[23,44,45] Delays in the samples reaching laboratory facilities or being left at room temperature for a long period can result in sample

degradation. Samples required careful handling by trained staff, with samples remaining on ice during processing and being stored in a –80°C freezer as soon as possible.[25]

## Conclusion

This pharmacokinetic study suggests that 1.5 g intravenous vitamin C is sufficient to increase plasma vitamin C concentrations almost tenfold in patients with septic shock. The estimated half-life of 4.3 hours and the plasma vitamin C concentrations achieved at 6 hours support the view that this vitamin C regimen reliably achieves and maintains normal, or supranormal, concentrations in patients with septic shock.

## Competing interests

None declared.

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