

Vitamin C Improves Microvascular Reactivity and Peripheral Tissue Perfusion in Septic Shock Patients

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Research

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Abstract

Background

Vitamin C has potential protective effects through anti-oxidant and anti-inflammatory properties. However, the effect of Vitamin C supplementation on microvascular function and peripheral tissue perfusion in human sepsis remains unknown. We aimed to determine vitamin C effect on microvascular endothelial dysfunction and peripheral tissue perfusion in septic shock patients.

Methods

Patients with septic shock were prospectively included after initial resuscitation. Bedside peripheral tissue perfusion and skin microvascular reactivity in response to acetylcholine iontophoresis in the forearm area were measured before and 1 hour after intravenous Vitamin C supplementation (40 mg/kg). Norepinephrine dose was not modified during the studied period.

Results

We included 30 patients with septic shock. SOFA score was 11 [8–14], SAPS II was 66 [54-79] and in-hospital mortality was 33%. Half of these patients had vitamin C deficiency at inclusion. Vitamin C supplementation strongly improved microvascular reactivity (AUC 2263 [430-4246] vs 5362 [1744-10585] UI, $p=0.0004$). In addition, Vitamin C supplementation improved mottling score ($p=0.06$), finger-tip ($p=0.0003$) and knee capillary refill time (3.7 [2.6-5.5] vs 2.9 [1.9-4.7] s, $p<0.0001$), as well as and central-to-periphery temperature gradient (6.1 [4.9-7.4] vs 4.6 [3.4-7.0] °C, $p<0.0001$). The beneficial effects of Vitamin C were observed both in patients with or without Vitamin C deficiency.

Conclusion

In resuscitated septic shock patients, vitamin C supplementation improved peripheral tissue perfusion and microvascular reactivity whatever plasma levels of vitamin C.

Introduction

Sepsis is a common and life-threatening condition that develops in response to bacterial injury. Around 50 millions of incident cases of sepsis are recorded worldwide every year. In the United States, around 535 cases of sepsis occur annually per 100,000 people, accounting for more than USD 23 billion in annual U.S. hospital expenditures [1]. Despite improvement in early resuscitation, sepsis-related disability and mortality remain unacceptably high [2]. Therefore, in association with symptomatic correction of acute circulatory failure and infection source control, there is urgent need for novel therapies to limit sepsis-induced tissue damage and organ failure.

Sepsis pathophysiology is complex, with immune response dysregulation, coagulation activation and oxidative burst affecting cardiac and endothelial cell function, resulting in impaired microvascular blood flow, tissue hypoperfusion and ultimately life-threatening organ failure [3]. Several studies have reported that the severity [4] and persistence [5] of microvascular blood flow alterations are closely correlated with patient prognosis. At bedside, impaired peripheral tissue perfusion evaluated using mottling score [6], capillary refill time [7] or temperature gradient [8] has been associated with poor outcome.

Recently, vitamin C supplementation (Ascorbic acid) has been proposed as a potential “pleiotropic” form of therapy, interacting with multiple pathologic pathways in sepsis. Several potential beneficial effects of vitamin C have been reported in both animal and human studies, including 1/ antioxidant properties (scavenging of reactive oxygen species) [9, 10] 2/ downregulation of pro-inflammatory gene expression (cytokines, chemokines) 3/ restoration of immune cell activity [11–13] 4/ downregulation of coagulation gene expression [14]. Experimental studies have also reported that vitamin C could modulate endothelial function [15, 16]. However, the *in vivo* effect of vitamin C on microvascular blood flow and tissue perfusion in sepsis patients with severe infections has never been investigated before.

In this study, we aimed to prospectively explore the effects of vitamin C supplementation on both endothelial-dependent microvascular reactivity and bedside peripheral tissue perfusion in septic shock patients.

Material And Methods

Included patients

We conducted a prospective study in an 18-bed intensive care unit (ICU) in a tertiary teaching hospital. During a 6-month period, patients older than 18 years admitted for septic shock were included. Septic shock was defined according to the Third International Consensus Definitions for Sepsis and Septic Shock [17]. We included resuscitated patients within the first 24 hours of vasopressor initiation. Exclusion criteria were pregnancy, forearm skin lesions, important soft tissue edema and agitation.

After initial resuscitation, including antibiotic administration, fluid infusion (30 mL/Kg) and norepinephrine infusion to maintain a MAP>65 mmHg, as well as infection focus control when available, patients received intravenous (IV) vitamin C (40 mg/Kg) over 30 minutes. We compared global hemodynamic and tissue perfusion parameters before and 1 hour after vitamin C supplementation, as well as skin microvascular endothelial reactivity (see below). Vasopressor dose was not modified during microvascular investigations.

Assessment of skin microcirculation reactivity

The skin microvascular reactivity was measured in the forearm area by transdermal iontophoresis of acetylcholine (Ach) [18]. This non-invasive technique allows local transfer of Ach across the skin, which produces vasomotor action on subcutaneous capillaries [19, 20]. Ach solution and a weak electrical

current are applied onto the skin, creating local differences in electrical potential and active migration of ions and molecules bearing a net electrical charge through epithelial layers. The direction and speed of migration can be adjusted using polarity and the current's magnitude. The total amount of Ach delivered into the skin is related to the current and duration of application (ie. electrical charge). Acetylcholine acts as an endothelium-dependent vasodilator [21], which induces the production of nitric oxide (NO) after stimulation of the endothelial NO-Synthase. Next, NO induces smooth muscle cells relaxation by activating guanylate cyclase that is responsible for vasodilation and increased blood flow.

The iontophoresis drug delivery chamber was attached to the flexor surface of the non-dominant forearm. The negative lead of the current source was attached to the drug delivery chamber, and the positive lead (ie., reference electrode) to a conductive hydrogel pad fixed onto the wrist. After measurement of baseline blood flow for 60 seconds, three successive applications of Ach were made, every 60 seconds, using anodal current (0.12 mA for 12 seconds each). The drug delivery chamber was loaded with 80 μ L of Ach (Miochol®). Variations of blood flow in the skin were assessed by Laser-Doppler Flowmetry technique. A Laser-Doppler Flowmeter probe (Periflux 5000, Perimed), embedded within a heating drug delivery chamber, was used in combination with a current-controlled delivery device (Perilont, Perimed). Laser-Doppler Flowmeter signals were recorded continuously using an interfaced computer with acquisition software (Perisoft, Perimed).

Baseline blood flow (BF) and area-under-the-curve (AUC) of BF recorded during a standardized 10-minute period were recorded (**Additional file 1**). Skin blood flow monitoring and analysis were performed by an independent physician who did not participate in patient care.

Data collection

Patients' characteristics were prospectively collected: age, sex, previous chronic illness, severity of illness evaluated by the Sequential Organ Failure Assessment score (SOFA score) at inclusion [22], source of sepsis, mode of mechanical ventilation, and vasopressor dose. Biological parameters, global hemodynamic parameters [mean arterial pressure (MAP), heart rate (HR)] and cardiac output measured using echocardiography were recorded at 2 time points. In addition, several tissue perfusion parameters were collected at baseline and 1 hour after Vitamin C supplementation: arterial lactate level, index and knee capillary refill time and mottling score, skin temperature and central-skin temperature gradient.

Plasma levels of Vitamin C

Vitamin C plasma levels were measured by a high performance liquid chromatography (HPLC) method adapted from Speek et al. [23]. Briefly, heparinized plasma is stabilized by diluting samples (1/10; v/v) with 5% (w/v) metaphosphoric acid solution. The samples remain frozen at -80°C until assayed. After alcalinization of samples with sodium acetate 4,5 mM (respectively 1 mL and 0,2 mL) and action of ascorbate oxydase (25 μ L solution 62 U/mL in Na H₂PO₄ 4mM pH 5,6; 5 minutes at 37°C), the total vitamin C of the sample is converted into acid L-dehydroascorbate. This compound is derivatized with ortho-phenylenediamine (300 μ L OPDA 100 mM-water solution, 30 minutes 37°C) giving a fluorescent

quinoxaline. The vitamin C assay is performed by HPLC in reverse phase with fluorimetric detection. The column is an Intersil C18 ODS2 5µM 4,6x 150 mm. The mobile phase (H2PO4 50mM / methanol (500/214 ; v/v) pH 7,4) flow is 1,15 mL / minute and the injection volume 20 µL. The excitation is done at 346 nm and emission at 424 nm. All the reagent are from Sigma-Aldrich, the column is from Interchim, the HPLC system is a Summit Dionex-Thermo.

Statistics

Continuous variables were presented as median and 25th–75th interquartile ranges (IQRs). Discrete variables were presented as percentages. Comparisons before and after vitamin C injection were made with a paired non-parametric test. Statistical analysis and graphical representations were performed using GraphPad Prism 8.4.1 software (Graph Pad Software Inc., La Jolla, CA). A two-sided p value of less than 0.05 was considered statistically significant.

Ethics

The protocol was approved by an institution's ethical committee -*Comité de Protection des Personnes (CPP Ile de France France, 2019-A03199-48)*. All patients or their families gave their consent for the study (ClinicalTrials.gov Identifier: NCT04778605).

Result

Characteristics of included patients

During the study period, 30 septic shock patients were included. Median age was 67 [57-74] years with a higher proportion of men (70%). The main sources of infection were respiratory (43%) and abdominal (33%). Included patients had severe disease with high SOFA scores (11 [8-14]), high SAPS II (66 [54-79]) and frequent organ support therapy such as invasive mechanical ventilation (67%). In-ICU mortality was 33 % (N=10/30) (**Table 1**). Global hemodynamic and tissue perfusion parameters were measured after initial resuscitation. All patients received crystalloids (2.5 [2-3] liters) and norepinephrine to maintain MAP >65 mmHg (dose 0.6 [0.3-1.2] µg/kg/min).

Biological parameters of included patients are depicted in **Table 1**.

Microvascular blood flow parameters

Endothelial-dependent microvascular reactivity was measured in the forearm area after acetylcholine challenge before and 1 hour after vitamin C administration. We observed that skin microvascular reactivity, evaluated using the area under the curve (AUC) during a 10-minute monitoring period, strongly increased after vitamin C supplementation (AUC 2263 [430-4246] vs 5362 [1744-10585] UI, p=0.0004) (**Figure 1A-B**). Vitamin C improved microvascular reactivity in patients with and without peripheral tissue hypoperfusion (**Additional file 2**).

Global hemodynamic and tissue perfusion parameters.

Parameters were recorded after initial resuscitation, before and 1 hour after vitamin C supplementation. Following vitamin C infusion, cardiac output significantly decreased (4.1 (3.3-4.5) vs 4 (3.1-4.3) L/min, $p=0.0376$) and MAP tended to increase (71 (66-75) vs 72 (66-77) mmHg; $p=0.07$) despite no change in vasopressor dose (0.60 [0.30-1.10] vs 0.60 [0.30-1.20] $\mu\text{g/kg/min}$; $p=0.46$) (**Table 2**). Interestingly, we observed that vitamin C supplementation quickly improved peripheral tissue perfusion with a trend to a decrease of mottling score ($p=0.06$), and a significant decrease in finger-tip CRT (2.1 (1.7-3.5) vs 2 (1.2-3) sec, $P=0.0003$), Knee CRT (3.7 (2.6-5.5) vs 2.9 (1.9-4.7) vs sec, $p<0.0001$), skin temperature and central-to-skin temperature gradient (6.1 (4.9-7.4) vs 4.6 (3.4-7.0) $^{\circ}\text{C}$, $p<0.0001$) (**Figure 2C & Table 2**).

Tissue perfusion parameters and microvascular parameters according to plasma levels of vitamin C

Plasma levels of vitamin C were measured in 24/30 septic shock patients at admission. Half of them ($N=12/24$) had vitamin C deficiency ($< 5 \text{ mg/L}$) (**Figure 2A**). We did not observe any difference between No deficiency and Deficiency groups in terms of age, gender and co-morbidity, but time between hospital admission and ICU admission was longer in Deficiency group patients. SOFA and SAPS II were not different between groups, but vasopressor doses trended to be lower in the vitamin C deficient group (0.6 [0.2-0.7] $\mu\text{g/kg/min}$) vs 1.1 [0.4-1.4] $\mu\text{g/kg/min}$, $P=0.06$) (**Additional file 3**).

We found that vitamin C supplementation significantly improved microvascular reactivity in patients with and without vitamin C deficiency (**Figure 2B**), as well as bedside evaluated peripheral tissue perfusion (**Table 3**).

Discussion

Our study prospectively investigated the impact of vitamin C infusion on microvascular function in septic shock patients. We found that vitamin C supplementation quickly improved microvascular reactivity and peripheral tissue perfusion, a benefit observed in patients with or without vitamin C deficiency.

Vitamin C supplementation was performed after initial resuscitation within the first 24 hours of ICU admission. We found that vitamin C strongly increased skin microvascular blood flow after acetylcholine challenge, supporting an improvement in endothelial-dependent microvascular function. This finding is of great interest because microvascular reactivity is highly correlated with both septic shock severity and outcome: the lower the reactivity, the higher the mortality [24]. Acetylcholine specifically targets endothelial cells and promotes NO release, inducing vascular smooth muscle relaxation and *in fine* vasodilatation [18]. The beneficial effects of vitamin C on endothelial- and nitric oxide-dependent vasodilation have also been previously observed in patients with chronic endothelial dysfunction due to atherosclerosis [25], hypertension [25] or diabetes [26]. Such rapid effect observed one hour after vitamin C injection may be mediated by increased NO availability, either through enhanced synthesis mediated by BH4 recycling, direct reduction of nitrite to NO, release of NO from nitrosothiols, or by scavenging

superoxide that would otherwise react with NO to form peroxynitrite [27]. Other protective effects of vitamin C on endothelial cell biology have been reported but these take longer time to develop. For instance, vitamin C promotes endothelial cell proliferation, capillary-like structures formation [28, 29] and prevents apoptosis both *in vitro* [30] and *in vivo* [31]. Vitamin C treatment limits Intercellular Adhesion Molecule (ICAM)-1 production by human umbilical endothelial cell line [32] and also decreases endothelial glycocalyx shedding *in vivo*, as assessed by plasma Syndecan-1 levels [33].

In our study, the beneficial effect of vitamin C supplementation was also observed clinically at the bedside with a decrease in mottling score, capillary refill time and temperature gradient, all markers of peripheral tissue perfusion. Mottling extension, which reflects impaired skin microvascular blood flow [34], has been identified as a strong independent predictive factor of mortality in sepsis [35] and septic shock patients [6]. Prolonged CRT measured either on the finger-tip or on the knee area is also associated with poor outcome in studies performed in the emergency ward [36] and the ICU [7]. Some criticisms have been raised about the reproducibility of these bedside parameters, but intra-rater concordance is excellent after standardization and training [37]. Skin temperature (and gradient) changes [8, 38], which were quantified with an accurate and reliable probe, also support the beneficial effect of vitamin C supplementation on peripheral perfusion.

In our cohort, around half of included patients had vitamin C deficiency, which is in line with previous works reporting that low plasma vitamin C concentrations are common in critically ill patients, and in particular in patients with sepsis [39, 40]. Vitamin C levels might be correlated with higher incidence of organ failure in septic patients [41], but in our study, SOFA score was not different patients with and without vitamin C deficiency. Several combined mechanisms may be responsible for vitamin C deficiency, such as pro-inflammatory cytokines regulating endothelial sodium-dependent vitamin C transporters activity [42] and increased vitamin C consumption by leukocyte turnover in the context of sepsis [43]. In our study, vitamin C deficient group was characterized by longer in-hospital length of stay before ICU admission, with potential decreased Vitamin C intake during hospital stay and also prolonged Vitamin C consumption because of subacute sepsis.

The beneficial effects of vitamin C were not restricted to vitamin C deficient patients, since supplementation was also beneficial in septic shock patients without deficiency.

Overall, the beneficial impact of vitamin C in sepsis patients is still into debate [44]. In a recent meta-analysis including eleven randomized controlled trials and more than 1700 patients, high-dose IV vitamin C did not improve short-term survival, but was associated with a significantly shorter duration of vasopressor use, as well as a significantly greater decline in the SOFA score at day 3 [45]. Based on our results, we believe that future trial testing high-dose IV vitamin C treatment should be proposed in selected septic shock patients with peripheral tissue hypoperfusion, a subset of patients with very poor outcome [46].

Finally, we did not observe any adverse effect after vitamin C injection, confirming previous work showing that pharmacologic ascorbic acid administration is safe. It is noteworthy that Sartor et al. reported that

point-of-care blood glucose measurements may become inaccurate after ascorbate injection, since the molecular structures of vitamin C and glucose are somewhat similar [47]

Our study has several limitations. It is a monocentric study and the results need to be confirmed in a larger population. Nevertheless, we found significant difference despite limited number of patients. Vitamin C improved vascular parameters in septic shock patients under vasopressor support, but we cannot affirm that the protective effect would be still observed in sepsis patients without vasopressor. Finally, we observed beneficial effect of vitamin C early after infusion but we did not analyze microvascular function and peripheral tissue perfusion at later stages.

Conclusion

In resuscitated septic shock patients, vitamin C supplementation improved microvascular reactivity and peripheral tissue perfusion whatever plasma levels of vitamin C

Abbreviations

ACH, acetylcholine

AUC, Area under the curve

CRT, capillary refill time

ICU, intensive care unit

MAP, mean arterial pressure

SOFA, sequential organ failure assessment

SAPS II, simplified acute physiologic score II

Declarations

Ethical approval

The protocol was approved by an institution's ethical committee - Comité de Protection des Personnes (CPP Ile de France France, 2019-A03199-48). All patients or their families gave their consent for the study (ClinicalTrials.gov Identifier: NCT04778605).

Consent to participate

Yes

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Competing Interests

None

Availability of data and materials

Not applicable

Consent for publication

Not applicable

Authors Contributions

Study concept and design, all authors. Acquisitions of data, all authors. Drafting of the manuscript, J.R.L. L.R., A.T, B.G and H.A.O. Critical revision of manuscript, all authors. Statistical analysis, J.R.L and H.A.O.

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Tables

Table 1 General characteristics of included patients.

Patients' characteristics	n (%) or Med. [IQR]
Age	67 [57-74]
Body mass index (kg/m ²)	22 [20-26]
Male gender	21 (70)
Simplified Acute Physiology Score 2	66 [54-79]
Sequential Organ Failure Assessment	11 [8-14]
<i>Comorbidities</i>	
Diabetes	7 (23)
Hypertension	14 (46)
Cardiovascular disease	10 (33)
Tobacco use	6 (20)
Cirrhosis	3 (10)
Cancer/hematologic malignancies	5 (17)
<i>Septic shock sources</i>	
Lung	13 (43)
Abdomen	10 (33)
Urinary tract	3 (10)
Catheter	2 (7)
Others	2 (7)
<i>Organ support therapy</i>	
Invasive mechanical ventilation	20 (67)
Norepinephrine dose (µg/kg/min)	0.6 [0.3-1.2]
Crystalloid infusion prior Vitamin C (L)	2.50 [2.10- 3.20]
<i>Biological parameters at inclusion</i>	
Leucocytes (Giga/L)	11 (1.5-24)

Hemoglobin (g/dL)	10.6 (8.3-15.2)
Platelets (Giga/L)	132 (50-208)
Serum creatinine (μmol/L)	119 (83-182)
Procalcitonin (ng/mL)	12 (2.7-30)
Bicarbonate (mmol/L)	21 (17-24)
Arterial lactate (mmol/L)	3.9 (2.8-5.1)
Protidemia (g/L)	57 (46-63)
Serum albumin (g/L)	25 (22-31)
Vitamin C (μmol/L)	5.3 (2-17)

Table 2. Global hemodynamic and tissue perfusion parameters before and 1 hour after Vitamin C infusion

Parameters, median (IQR)	H0	H1	P value
Blood pressure (mmHg)			
Systolic	107 (100-120)	111 (102-121)	0.43
Diastolic	54 (51-61)	58 (53-62)	0.18
Mean	71 (66-75)	72 (66-77)	0.07
Heart rate (/min)	107 (101-111)	107 (101-110)	0.67
Mottling score	1 (0-3)	1 (0-2)	0.06
Capillary refill time (sec)			
Index	2.1 (1.7-3.5)	2 (1.2-3)	0.0003
Knee	3.7 (2.6-5.5)	2.9 (1.9-4.7)	<0.0001
Cardiac output (L/min)	4.1 (3.3-4.5)	4 (3.1-4.3)	0.0376
Skin temperature	31.2 (30.2-32.3)	32.2 (31-33.7)	<0.0001
Central-to-skin gradient temperature	6.1 (4.9-7.4)	4.6 (3.4-7.0)	<0.0001
Norepinephrine dose (μg.kg ⁻¹ .min ⁻¹)	0.6 (0.3-1.2)	0.6 (0.3-1.1)	0.46

Table 3. Clinical and hemodynamic parameters at admission and 1 hour after Vitamin C infusion of included patients according to Vitamin C deficiency

Variables, median (IQR)	H0		H1		P value (H1 vs H0)	
Vitamin C (μmol/L)	< 5 μmol/L	³ 5 μmol/L	< 5 μmol/L	³ 5 μmol/L	< 5 μmol/L	³ 5 μmol/L
Blood pressure (mmHg)						
Systolic	103 [100-115]	108 [101-122]	109 [96-124]	110 [101-120]	0.26	0.8
Diastolic	56 [53-64]	53 [45-59]	58 [52-63]	56 [51-62]	0.9	0.11
Mean	71 [66-77]	67 [61-71]	74 [67-79]	68 [64-75]	0.29	0.05
Heart rate (/min)	105 [100-111]	107 [101-124]	105 [100-110]	107 [100-124]	0.8	0.9
Mottling score	1 [0-3]	2 [0-3]	1 [0-2]	2 [0-3]	0.6	0.9
Capillary refill time (sec)						
Index	1.8 [1.6-3.9]	2.2 [1.6-3.1]	1.7 [1.2-3.5]	1.9 [1.1-2.7]	0.04	0.02
Knee	3.8 [2.5-5.8]	4.1 [3.4-6]	2.7 [1.9-4.7]	3.5 [2.3-5.6]	0.001	0.005
Cardiac output (L/min)	3.3 [3-4.2]	4.3 [3.8-5.2]	3.5 [3.1-4.2]	4.3 [3-4.7]	0.5	0.04
Temperature (°C)						
Skin	31.7 [30.2-32.7]	31.2 [30.5-32.8]	32 [31.1-34.3]	32.2 [31.2-33.6]	0.06	0.008
Central-to- skin gradient	6.5 [4.8-7.3]	5.3 [4.7-6.8]	4.9 [3.5-6.9]	4.5 [3.3-6.6]	0.06	0.001
Norepinephrine infusion (μg.kg-1.min-1)	0.6 [0.2-0.7]	1.1 [0.4-1.4]	0.6 [0.2-0.7]	1.0 [0.4-1.4]	0.9	0.5

Figures

Figure 1

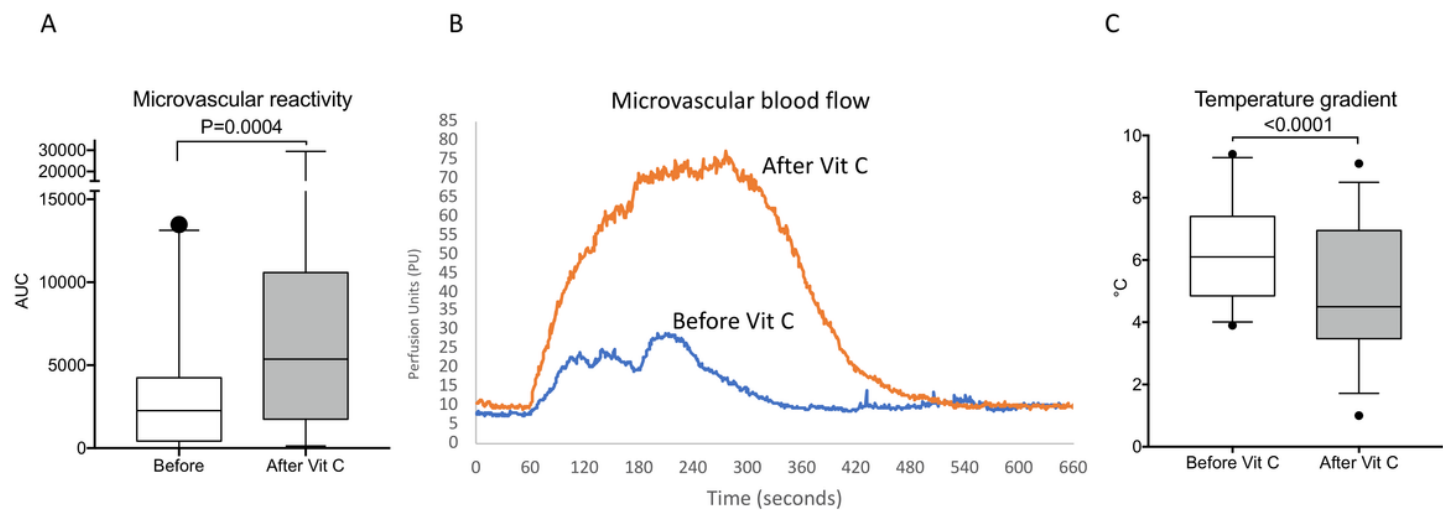


Figure 1

A. changes of forearm skin microcirculatory reactivity in response to acetylcholine challenge before and after Vitamin C supplementation in patients with septic shock. B, Example of skin microcirculatory blood flow change in response to acetylcholine iontophoresis before (blue) and after (Orange) vitamin C injection. C, central-to-Knee skin temperature gradient before and after vitamin C infusion. PU for Perfusion units.

Figure 2

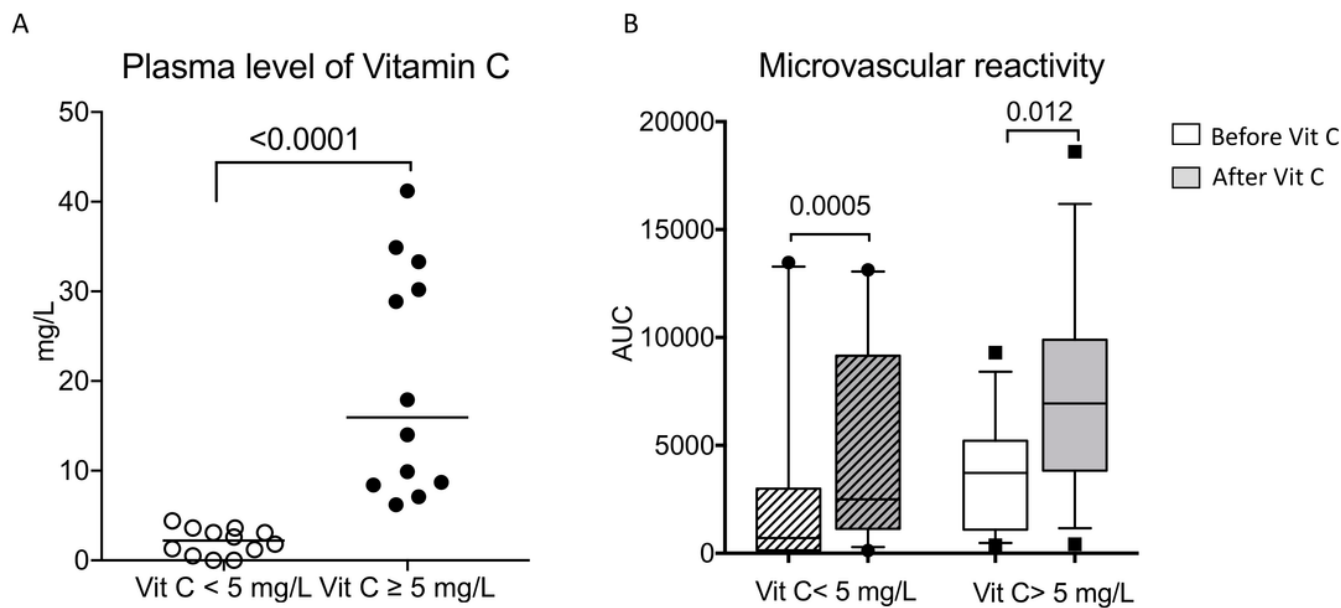


Figure 2

A. plasma levels of vitamin C in patients with and without deficiency in patients with septic shock. B, changes of microcirculatory reactivity before and after vitamin C supplementation in septic shock patients with and without vitamin C deficiency.

Supplementary Files

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