Effect of RBC Transfusion on Sublingual Microcirculation in Hemorrhagic Shock Patients: A Pilot Study

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Objectives: The effects of RBC transfusion on microvascular perfusion are not well documented. We investigated the effect of RBC transfusion on sublingual microcirculation in hemorrhagic shock patients.

Design: Prospective, preliminary observational study.

Settings: A 28-bed, surgical ICU in a university hospital.

Patients: Fifteen hemorrhagic shock patients requiring RBC transfusion.

Intervention: Transfusion of one unit of RBCs.

Measurements and Main Results: The sublingual microcirculation was assessed with a Sidestream Dark Field imaging device before and after RBC transfusion. After transfusion of one unit of RBC, hemoglobin concentration increased from 8.5 g/dL (7.6–9.5 g/dL) to 9.6 g/dL (9.1–10.3 g/dL) g/dL ($p = 0.02$) but no effect on macrocirculatory parameters (arterial pressure, cardiac index, heart rate, and pulse pressure variations) was observed. Transfusion of RBC significantly increased microcirculatory flow index (from 2.3 [1.6–2.5] to 2.7 [2.6–2.9]; $p < 0.003$), the proportion of perfused vessels (from 79% [57–88%] to 92% [88–97%]; $p < 0.004$), and the functional capillary density (from 21 [19–22] to 24 [22–26] mm/mm^2; $p = 0.003$). Transfusion of RBC significantly decreased the flow heterogeneity index (from 0.51 [0.34–0.62] to 0.16 [0.04–0.29]; $p < 0.001$). No correlations were observed between other macrovascular parameters and microvascular changes after transfusion. The change in microvascular perfusion after transfusion correlated negatively with baseline microvascular perfusion.

Conclusions: RBC transfusion improves sublingual microcirculation independently of macrocirculation and the hemoglobin level in hemorrhagic shock patients. The change in microvascular perfusion after transfusion correlated negatively with baseline microvascular perfusion. Evaluation of microcirculation perfusion is critical for optimization of microvascular perfusion and to define which patients can benefit from RBC transfusion during cardiovascular resuscitation. (*Crit Care Med* 2016; XX:00–00)

Key Words: capillary density; hemorrhagic shock; red blood cells; sublingual microcirculation; transfusion; vessel perfusion

The effect of RBC transfusion on microcirculation is not well documented in hemorrhagic shock patients. In septic patients, microvascular effects of RBC transfusions were quite variable with a considerable interindividual variation and were dependent of baseline microvascular perfusion (1). In addition, when basal microcirculation was preserved, transfusion could lead to a deterioration of the microcirculation in septic patients.

RBC transfusion is the cornerstone of the management of bleeding trauma patients to improve oxygen delivery and to restore as soon as possible tissue oxygenation to limit tissue hypoxia and organ dysfunction. However, alterations of microcirculation in hemorrhagic shock resulting from the interplay among hemorrhage-induced tissue hypoperfusion, trauma injuries, and inflammatory response have been reported (2). Thus, despite restoration of macrocirculation and hemoglobin concentration, the sublingual microcirculation could still be altered without any beneficial effect of transfusion. In addition, during storage, RBCs undergo numerous changes with reductions in RBC functionality and viability. There is a progressive increase in the concentrations of lactate, K+, cytokines, free hemoglobin, and free iron, as well as a decrease in adenosine triphosphate, 2,3-diphosphoglycerate, and S-nitrosohemoglobin (3, 4). Furthermore, RBC membrane integrity is progressively compromised with a reduction of RBC deformability (5, 6). The goal of the present study was to

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Dr. Hamada's institution received support for the development of educational presentations from LFB (on hemorrhagic shock). Dr. Harrois received payment from the LFB (French Laboratory of blood drugs) for the creation of an educative software dealing with severe trauma care. The remaining authors have disclosed that they do not have any potential conflicts of interest.

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DOI: 10.1097/CCM.0000000000002064

assess the microcirculatory impact of RBC transfusion during the early phase of hemorrhagic shock.

The main questions addressed by the present study were the following ones:

Is transfusion associated with improvement of microvascular blood flow and capillary density during hemorrhagic shock as assessed by the Sidestream Dark Field technique?
Is the change in microvascular perfusion after transfusion correlated to changes in macrovascular parameters?

MATERIALS AND METHODS

Patients

This study was approved by our local ethics committee (Comité de Protection des Personnes de l'Université Paris VII no SC 13–025). It is a prospective, observational, single-center study performed from February 2013 to February 2014 in a 28-bed surgical ICU in a tertiary university hospital. Hemorrhagic shock patients were prospectively included in the study before or after after partial or complete surgical or embolization bleeding control within the first 12 hours of their admission. Hemorrhagic shock was defined as the presence of a systolic arterial pressure less than or equal to 90 mm Hg requiring use of vasopressors in need of infusion of vasopressors with an acute bleeding requiring RBC transfusion. Fluid resuscitation and vasopressors (norepinephrine) were infused to preserve arterial pressure. All patients were mechanically ventilated and equipped with an arterial and central venous catheter. Cardiac output (CO) and cardiac index (7) were monitored either by a thermodilution catheter used in combination with a central venous catheter above the diaphragm (PiCCO; Pulsion Medical Systems, Munich, Germany) or by an esophageal Doppler (Deltex Medical, Chichester, UK). Exclusion criteria were age less than 18 years, shock with any other cause (septic shock or cardiogenic shock), and patients with maxillo-facial trauma and oral injuries hampering the realization of the sublingual microcirculation video.

Transfusion Protocol

Transfusion of 1 U of deleucocyted RBC was performed in 10–15 minutes during the transfusion procedure. Systemic hemodynamic and microcirculatory indices were assessed immediately before and after the transfusion. We define T_0 as the time of the assessment before transfusion of one RBC and T_1 as the period just after the transfusion. The ventilator settings and sedative and vasoactive drugs infusion rates were kept constant throughout the study. The new Simplified Acute Physiology Score II was recorded on admission and the Sequential Organ Failure Assessment score at inclusion.

Macrocirculatory Measurements

Systemic parameters included measurement of heart rate (HR); mean, systolic, and diastolic arterial pressures (MAP, SAP, and DAP, respectively); cardiac index; pulse pressure variation (ΔPP); and hemoglobin concentration (Hb). These measurements were performed before and after the RBC administration. The ΔPP, as previously described (8), was automatically calculated by the monitoring device (IntelliVue MP70; Philips, Suresnes, France). Oxygen delivery was calculated using standard formula.

Microcirculatory Measurements

For the measurements of sublingual microcirculation, we used a Sidestream Dark Field imaging device (Microscan; MicroVision-Medical, Amsterdam, The Netherlands) derived from the orthogonal polarized spectral imaging technology. The device was gently applied without pressure under the tongue after removal of secretions with gauze. Five sequences of 20 seconds each from different adjacent areas were recorded and stored under an alphanumeric code and later analyzed semiquantitatively and quantitatively. All sequences were acquired by the same investigator, and analysis was performed by a second blinded investigator. Images acquisition and analysis were performed following international recommendations with dedicated software analysis (Automated Vascular Analysis version 1.0; Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands). According to guidelines, semiquantitative analysis with AVA provided the microcirculatory flow index (MFI), the proportion of perfused vessels (PPV, %) and the flow heterogeneity index (HI) (9). Regarding quantitative analysis, the functional capillary density (FCD), which quantifies capillary perfusion, was calculated.

Statistical Analysis

Baseline data for a power calculation were not available; therefore, we decided to include n equal to 15 patients based on a feasibility approach and so to have at least 80% to detect a large effect size according to Cohen's criteria (i.e., difference in means/SD = d/SD = 0.8). Baseline data for usual power calculations were not available. Therefore, we decided to use the method proposed by Cohen based on power calculation to detect a standardized effect size (10). In this pilot study, we included $n = 15$ patients to allow a 80% power to detect a large effect size that according to Cohen's criteria corresponds to a ratio of the Delta between the studied parameter before versus after transfusion on the SD of the difference equal to d/SD = 0.8 and considering a 5% two-sided significance level. Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA). For continuous variables, all data are presented as median and interquartile range. Comparison between groups of non-normally distributed microvascular parameters (MFI, FCD, PPV, and HI) was performed with a Mann-Whitney U test. Comparison against baseline of macro- and microcirculatory parameters was performed with a paired t test or with a Wilcoxon signed rank test in case of non-normally distributed variables. Correlations between variables were investigated by Spearman ρ, and a linear regression line was computed with 95% confidence interval. p value of less than 0.05 was considered statistically significant.

RESULTS

Patient's Characteristics

The main characteristics of the 15 patients are presented in **Table 1**. All the patients had mechanical ventilatory support

TABLE 1. Values Are Presented as Median [25–75% Percentile]

Variables	
Age (yr)	46 [28–58]
Sex, *n* (M/F)	10/5
Sequential Organ Failure Assessment	8 [7–10]
Simplified Acute Physiology Score II	41 [35–50]
Diagnosis at inclusion, *n* (%)	
Severe trauma	11 [73]
Gastro-intestinal hemorrhage	1 [7]
Massive bleeding during surgery	3 [20]
Norepinephrine (μg/kg/min)	0.24 [0.13–0.70]
Hb before the inclusion (g/dL)	8.5 [7.6–9.4]
Number of RBC before the inclusion	3 [1–5]
Volume of RBC (mL)	296 [271–311]
Age of RBC (d)	19 [11–22]
Lactate before the inclusion (mmol/L)	3.2 [1.5–4.0]
Length of stay in ICU (d)	8 [6–25]
Death in ICU, *n* (%)	1 [7]

at the time of microvascular assessment. Patients received a median value of 3 U (1–5 U) of RBC before inclusion. Fourteen patients were studied before bleeding control (surgery or embolization). Only one patient was studied after bleeding control. No one received renal replacement therapy during his stay in ICU. One patient (7%) died before 28 days.

Impact of Transfusion on Macrocirculation

Macrohemodynamic parameters are represented in **Table 2**. There was no significant difference in macrovascular parameters (MAP, SAP, DAP, CO, HR, and ΔPP) before and after the transfusion of one RBC. Hemoglobin concentration was significantly increased after transfusion (from 8.5 [7.6–9.5] to 9.6 [9.1–10.3] g/dL; $p < 0.0001$). Transfusion induced a significant increase in oxygen delivery (from 327 [245–433] to 425 [302–525] mL/min M^2; $p < 0.0001$). No significant change in lactate concentration was observed after RBC transfusion (Table 2).

Impact of Transfusion on Microcirculation

Transfusion of one RBC improved microcirculatory parameters (**Figs. 1** and **2**). Transfusion of RBC significantly increased MFI (from 2.3 [1.6–2.5] to 2.7 [2.6–2.9]; $p < 0.003$), PPV (from 79% [57–88%] to 92% [88–97%]; $p < 0.004$) and FCD (from 21 [19–22] to 24 [22–26] mm/mm^2; $p = 0.003$). Transfusion of RBC significantly decreased HI (from 0.51 [0.34–0.62] to 0.16 [0.04–0.29]; $p < 0.001$). No correlation was observed between baseline Hb (T_0) and the change in MFI following transfusion: Delta MFI ($\rho = 0.22$), Delta PPV ($\rho = 0.28$), Delta FCD ($\rho = 0.14$), and Delta HI ($\rho = 0.22$). When Delta Hb (%) was defined as the difference between Hb after and before the transfusion, no correlation was found between between Delta Hb and Delta MFI ($\rho = –0.46$), Delta PPV ($\rho = –0.44$), Delta FCD ($\rho = –0.31$), and Delta HI ($\rho = –0.06$). No correlations were observed between other macrovascular parameters and microvascular changes after transfusion. Delta MFI ($\rho = –0.90$; $p = 0.0001$), Delta PPV ($\rho = –0.95$; $p = 0.0001$), Delta FCD ($\rho = –0.54$, $p = 0.04$), and Delta HI ($\rho = –0.79$, $p = 0.0007$) were negatively correlated with pretransfusion MFI, PPV, FCD, and HI, respectively (**Fig. 3**).

DISCUSSION

In the present study, transfusion of one RBC in hemorrhagic shock patients induced a significant improvement of sublingual microvascular blood flow and vascular density with an increase of MFI, PPV, and FCD and a decrease of capillary heterogeneity without any modification of macrovascular parameters. No correlations were observed between macrovascular parameters and microvascular changes after transfusion. The change in microvascular perfusion after transfusion correlated negatively with baseline microvascular perfusion. To our

TABLE 2. Values Are Presented as Median [25–75% Percentile]

Variables	T_0	T_1	*p*
Mean arterial pressure (mm Hg)	71 [61–78]	78 [70–84]	0.06
Systolic arterial pressure (mm Hg)	118 [108–136]	127 [119–138]	0.08
Diastolic arterial pressure (mm Hg)	65 [53–71]	65 [58–77]	0.12
Heart rate, beats/min	101 [85–118]	97 [80–117]	0.14
Cardiac index (L/min M^2)	3.1 [2.7–3.5]	3.2 [2.6–4.0]	0.14
ΔPP, %	18 [9–19]	12 [8–17]	0.12
Lactate (mmol/L)	3.2 [1.5–4.0]	2.9 [1.5–3.8]	0.16
Hb (g/dL)	8.5 [7.6–9.5]	9.6 [9.1–10.3]	< 0.05
Oxygen delivery (mL/min M^2)	327 [245–433]	425 [302–525]	< 0.05

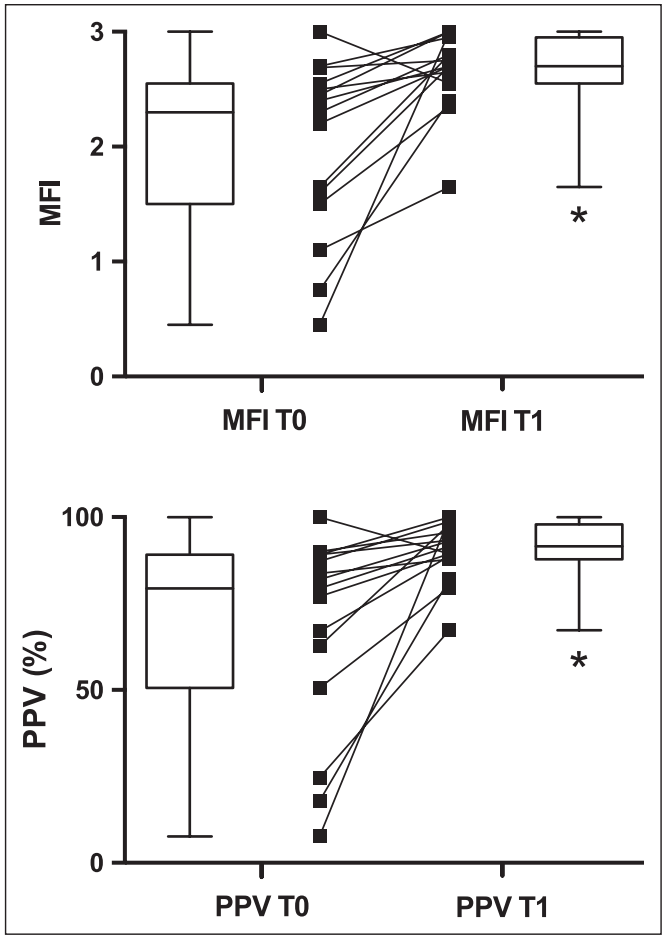

Figure 1. Microcirculatory flow index (MFI) and proportion of perfused vessels (PPV, %) before (T_0) and after (T_1) 1 U of RBC transfusion. Median (min to max) and individual values. *$p < 0.05$ versus T_0.

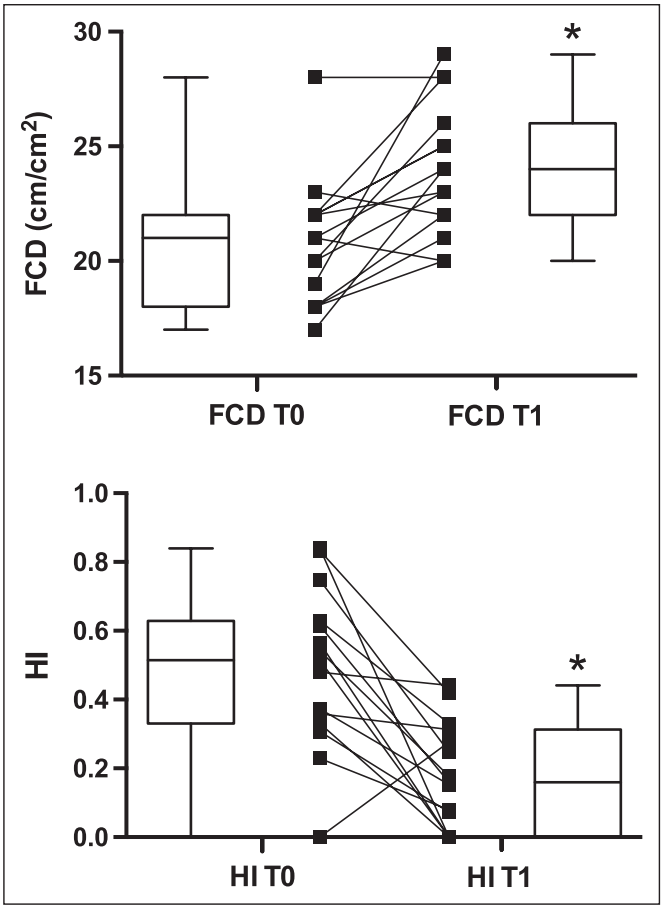

Figure 2. Functional capillary density (FCD) and flow heterogeneity index (HI) before (T_0) and after (T_1) 1 U of RBC transfusion. Median (min to max) and individual values. *$p < 0.05$ versus T_0.

knowledge, it is the first work reporting the impact of RBC transfusion on sublingual microcirculation in human hemorrhagic shock.

Animal experimental studies have reported these effects showing an increase of capillary density and RBC velocity after transfusion (11, 12). For example, during an experimental procedure of hemorrhagic shock interesting 63 hamsters, Kerger et al. have investigated microcirculation of skeletal skin muscle and subcutaneous connective tissue with a dorsal skinfold chamber. In this model, hemorrhagic shock led to significant decreases in blood flow and vessel density. whole-blood transfusion after a 4 hours lasting hemorrhagic shock period increased both macrovascular parameters and microcirculation with an increase of FCD and RBC velocity (11). However, microvascular normal values were not re-established after the resuscitation. In a pig model of hemorrhagic shock with isovolemic resuscitation with autologous blood, van Iterson et al (12) reported that macrocirculation and gut and heart microcirculations variables were profoundly affected and closely related to each other during hemorrhagic shock and isovolemic autologous whole-blood resuscitation.

Regarding human studies, the effect of RBC transfusion on microcirculation is not well documented in ICU patients. In septic patients, Sakr et al (1) have emphasized that transfusion of one or two deleucocyted RBC did not have any effect on sublingual microcirculation (Orthogonal Polarization Spectral imaging) despite increases in Hb concentration, MAP, and oxygen delivery. But they observed a considerable interindividual variation. In addition, the change in capillary perfusion after transfusion was negatively correlated to baseline capillary perfusion. Capillary perfusion was significantly lower at baseline in patients who increased their capillary perfusion by more than 8% than in those who did not, whereas hemodynamic and global oxygen transport variables were similar in the two groups. When basal microcirculation was preserved, transfusion could lead to a deterioration of the microcirculation (1). Thus, in these severe septic patients, the microvascular effects of RBC transfusions were quite variable and were dependent on baseline microvascular perfusion. These results were confirmed by Sadaka et al (13) who reported that muscle oxygen saturation (near-infrared spectrometry [NIRS]), oxygen consumption (NIRS), microvascular reactivity (StO_2 recovery slope after vascular occlusion test), and sublingual microcirculation were globally unaltered by RBC transfusion in severe septic patients. However, muscle oxygen consumption improved in patients with low baseline and deteriorated in patients with preserved baseline (13).

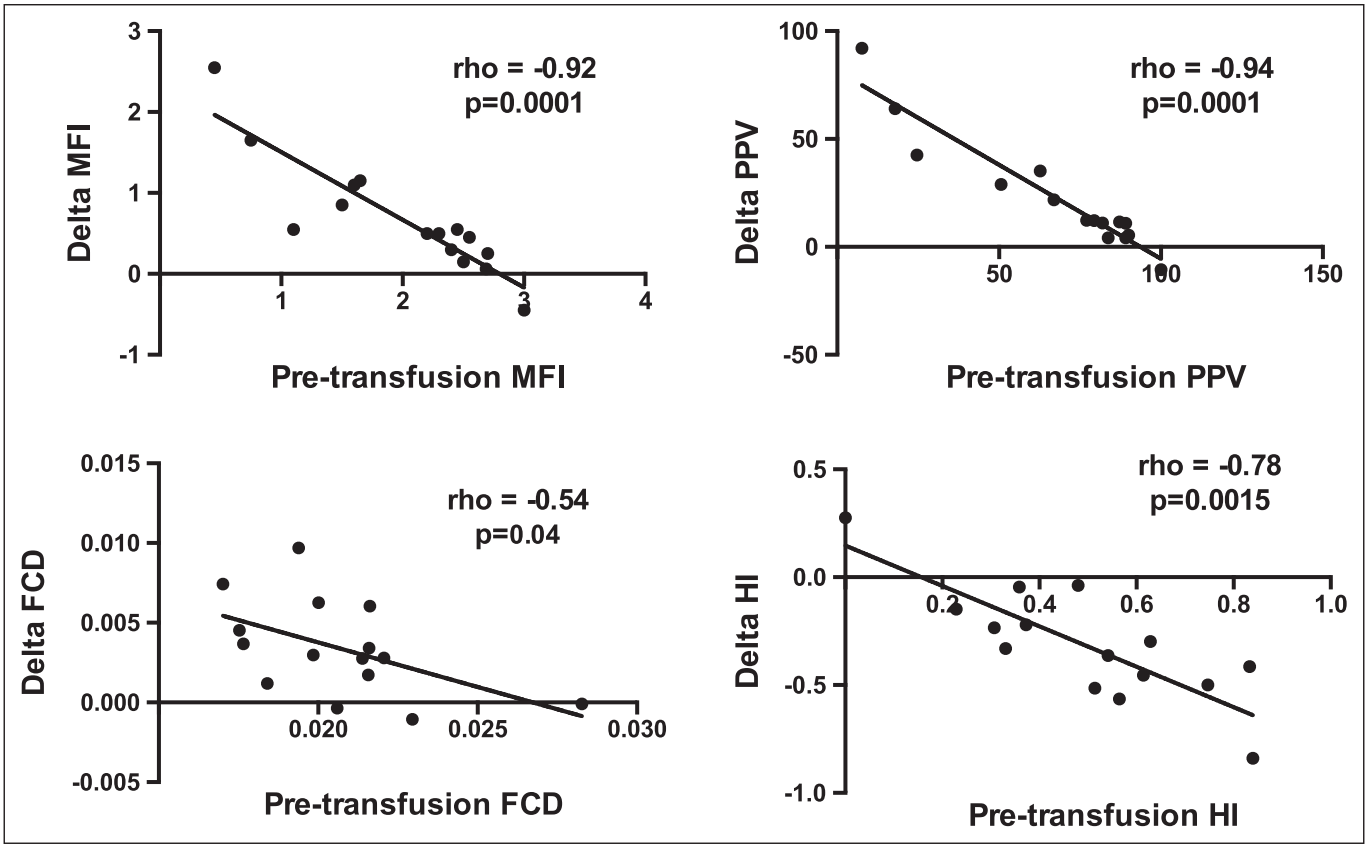

Figure 3. Correlation between changes in microcirculatory flow index (MFI), proportion of perfused vessels (PPV), functional capillary density (FCD), and heterogeneity index (HI) following transfusion (Delta MFI, Delta PPV, Delta FCD, and Delta HI) and pretransfusion MFI, PPV, FCD, and HI.

If transfusion of RBC has no significant beneficial effects on microcirculation perfusion in septic patients, the result is quite different in other populations of ICU patients. In 12 patients during extracorporeal circulation in cardiac surgery, Yuruk et al (14) have observed that blood transfusion improves sublingual microcirculatory density (SDF imaging) and oxygen saturation (sublingual spectrophotometry). No change in MFI was reported (from 2.97 ± 0.03 to 2.96 ± 0.03), but the basal MFI value before transfusion was high. More recently, Ayhan et al (15) demonstrated that transfusion of RBC in mixed surgical patients increased FCD without effect on MFI and macrovascular parameters. Creteur et al (16) in 44 consecutive medico-surgical ICU patients reported that RBC transfusion did not globally affect NIRS-derived variables and has observed a considerable interindividual variation. Finally, Weinberg et al (17) have studied sublingual microvascular perfusion (SDF imaging) before and after transfusion of 1 U of RBC in hemodynamically stable, anemic trauma patients. Following transfusion, changes in proportion of perfused capillaries were significantly inversely correlated with pretransfusion proportion of perfused capillaries. Patients with relatively altered baseline proportion of perfused capillaries tend to demonstrate improvement in perfusion following transfusion, whereas those with relatively normal perfusion at baseline tend to demonstrate either no change or, in fact, a decline in proportion of perfused capillaries (17).

Thus, independently of the hemodynamic macrovascular status and the hemoglobin level, interindividual variations in the microvascular response to RBC transfusion were reported with improvement in some patients and deterioration in others with a potential negative correlation to baseline capillary perfusion.

In the present study, transfusion of 1 U of RBC induced a capillary recruitment with the opening of initially non-perfused capillaries and an improvement of capillary flow after the transfusion. This positive microcirculatory response to RBC transfusion was not coupled with an increase of macrovascular parameters, and notably, with baseline Hb concentration, the daily parameter in clinical practice for deciding to transfuse RBC. Only baseline microvascular perfusion parameters were able to predict the microcirculatory response to RBC transfusion. These results reinforce the fact that evaluation of microcirculation perfusion is critical as a clinical index of the adequacy of resuscitation in individual patients in shock. It is remarkable that most of the patients studied (8 of the 15 patients) had Hb concentrations within the recommended target Hb concentrations in hemorrhagic shock (7 to 9 g/dl) (18). However, for these patients the transfusion of 1 U of RBC was capable to improve sublingual microvascular blood flow and vascular density. It is well demonstrated that microvascular hematocrit is lower than macrovascular hematocrit (19). This difference is dependent on network topography and local flow rates. In addition, several factors in hemorrhagic shock patients are able

to increase the difference between systemic Hb and microvascular Hb. Indeed, hemorrhage can induce alterations in the microcirculation and oxygen delivery by affecting microvascular flow, microvascular viscosity, microvascular endothelium, leukocyte adherence, glycocalyx, interstitial and endothelial edema, and coagulation activation (20–22). Accordingly, systemic Hb is not a good index of microvascular Hb. Thus, the results of the present study reinforce the fact that clinicians do not have to focus exclusively on the hemoglobin concentration as a transfusion trigger and that evaluation of microcirculation perfusion is critical for optimization of microvascular perfusion and to define which patients can benefit of RBC transfusion during cardiovascular resuscitation. It could be interesting to investigate in future studies whether monitoring the sublingual microcirculation, in association with other factors, may be useful to guide transfusion policy in hemorrhagic patients.

In the present study, the improvement of sublingual microcirculation by RBC transfusion could in hemorrhagic shock patients was not related to an improvement of systemic hemodynamic. The observed positive microvascular response probably involve local microvascular mechanisms with an important role of RBC. Indeed, erythrocyte is a key factor in the regulation of microvascular tone and plays a fundamental role in matching microvascular oxygen supply with local tissue oxygen demand (23–27). Indeed, Ellsworth et al (23) have reported that the erythrocyte could be a mobile sensor of oxygen and is able to control vascular tone with the liberation of ATP. Erythrocyte can liberate ATP in response to a deformation of its membrane, an exposition to a low vascular Po_2 or a stimulation of β-adrenergic receptor and prostacyclin. For example, in case of exposition to a low Po_2 level, the erythrocyte liberates ATP that interacts with endothelial purinergic receptors and induces a microcirculatory vasodilatation (by the liberation of NO and non-NO mediators) with an increase of oxygen delivery. Stamler et al (25) demonstrated that erythrocyte regulates TaO_2 by the delivery of S-nitrosothiol, an arteriolar vasodilator, which is liberated by hemoglobin in case of decrease of oxygen saturation At last, the hemoglobin in deoxyhemoglobin state is able (nitrite reductase function) to transform nitrite to NO that leads to an arteriolar vasodilatation in response to an arteriolar desaturation.

Our study has several limitations. First, we only included 15 patients. However, assessment of sublingual microcirculation during the early phase of the management of hemorrhagic shock is difficult because the patient remains unstable with often more important priorities than the protocol (i.e., urgent need for surgery or embolization for instance). The second difficulty was the availability of an expert operator to assess the sublingual microcirculation. However, the microcirculatory differences before and after transfusion were significant with this small number of patients, confirming the strong effect of transfusion on microcirculation on these patients. Second, it is an observational study with no feasible blinding, although analysis of microvascular video was performed by a blinded investigator. Third, the sublingual microcirculation may not reflect perfusion in other microcirculatory beds. Nevertheless, sublingual microcirculation alterations are considered to be a strong predictors of outcome (28). Thus, looking at this microcirculation has clinical relevance. Fourth, we have studied the effect of only 1 U of RBC, whereas transfusion of several units of RBC could have different effects on microcirculation. However, It would be very difficult to distinguish between specific effects of the massive transfusion and the impact of fluids, vasopressors, or inflammation on sublingual microcirculation. It should however be noted that patients in the present study had a median of 3 U (1–5 U) of RBC before starting the study.

CONCLUSION

This preliminary study demonstrated that RBC transfusion in hemorrhagic shock patients improves sublingual microcirculation independently of any change in macrocirculation. An improvement of microvascular perfusion and density with capillary recruitment associated with a decrease in microcirculation heterogeneity was observed after 1 U of RBC transfusion. The change in microvascular perfusion after transfusion correlated negatively with baseline microvascular perfusion. This result suggests that RBC transfusion improves microcirculatory perfusion in ways that are not entirely explained by macrocirculatory effects. This microcirculatory improvement could involve microvascular local mechanisms in which the erythrocyte could have a central role. The present study reinforces the fact that clinicians should not exclusively focus on the hemoglobin concentration as a transfusion trigger and that evaluation of microcirculation perfusion is critical for optimization of microvascular perfusion and to define which patients can benefit of RBC transfusion during cardiovascular resuscitation.

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