Selenoprotein P, Rather than Glutathione Peroxidase, as a Potential Marker of Septic Shock and Related Syndromes

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Key Words

Sepsis • Shock • Endothelium • Reactive oxygen species • Systemic inflammatory response syndrome • Free radicals • Antioxidants

Abstract

Background/Aims: Oxidative stress is involved in sepsis-related endothelium dysfunction. Selenoprotein-P (Sel-P), the main plasma selenoprotein, may have high antioxidant potential, and binds to endothelium. We hypothesize that, in septic shock, and similar syndromes such as systemic inflammatory response syndrome (SIRS), Sel-P binds massively to endothelium, causing a drop in Sel-P plasma concentration. **Methods:** Plasma Se, Sel-P and albumin concentrations, and glutathione peroxidase (GPx) activity were measured in patients with septic shock and SIRS with organ failure (S group, n = 7 and n = 3, respectively) admitted to the intensive care unit (ICU) and compared to non-SIRS patients (NS group, n = 11) and healthy volunteers (HV group, n = 7). **Results:** On ICU admission, plasma Sel-P concentrations were 70% lower in the S group than in the other groups [15 (10–26) vs. 44 (29–71) and 50 (45–53) nmol/l] and were lower in nonsurviving septic-shock patients. GPx activity did not differ between

groups. Sel-P was significantly lower before ICU death in the 3 deceased patients of the S group (septic shock) than in the 3 patients of the non-SIRS group. **Conclusions:** Early decrease in Sel-P plasma concentrations was specifically observed in septic shock and was similar in SIRS patients whereas GPx activity remained unchanged. Further studies are needed to determine whether Sel-P can be an early marker of septic shock linked to microvascular injury.

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Introduction

Septic shock – an uncontrolled systemic host response to invasive infection leading to multiple-organ failure [1] – is a public health issue due to its frequency, cost and the 45% mortality rate, which has remained stable for the

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past 20 years. Noninfectious aggressions like burn, pancreatitis, trauma, and ischemia/reperfusion can also trigger a similar activation of the host response called 'systemic inflammatory response syndrome' (SIRS) [2–4]. Today, the pathophysiology of SIRS is better understood, with increasing data supporting the key role of reactive oxygen species (ROS). ROS are involved in endothelium activation and damage to the microcirculation [5–10]. This microcirculatory endothelial dysfunction precedes organ dysfunction [8, 9]. However, the precise mechanism of endothelium protection against ROS remains poorly understood.

Most selenoproteins, including glutathione peroxidases (GPx) and thioredoxin reductase, are antioxidant selenoenzymes. Nearly all of these selenoenzymes contain a single atom of Se per protein in the form of the amino acid selenocysteine (SeCys) [11, 12]. SeCys is always found at the active site of selenoenzymes and is mandatory for antioxidant enzymatic activity due to the electronic properties of Se, compared to oxygen and sulfur [12–14]. It is well admitted that selenoenzymes like GPx play a major role in the complex defense against ROS within endothelial cells [11, 12, 15, 16]. However, in order to function, GPx enzymes require glutathione (GSH) as substrate in the millimolar range, which is the case at the intracellular level. This is not the case in plasma, where GSH concentration is at the micromolar level. This casts doubt on the role of circulating GPx as an antioxidant in plasma [11, 12, 17]. In fact, selenoprotein P (Sel-P) is the main plasma selenoprotein. Sel-P is one of the rare exceptions among selenoproteins in that it contains more than 1 SeCys per protein. It can contain up to 10 SeCys per protein, suggesting that this protein has a high antioxidant potential [18–20]. Sel-P is a liver protein with a short 3- to 4-hour plasma half-life. One of the characteristics of Sel-P is its physiological binding properties to the endothelium [19, 20]. Binding of Sel-P to the endothelium is favored by its acidic pH, which may be a mechanism for Sel-P recruitment to the endothelium at the inflammatory sites [20]. Its plasma concentration is considered as very stable in most settings except in severe Se deficiency [21, 22]. It is involved in Se transport to the tissues as demonstrated by the fact that *Sel-P* gene deletion leads to severe disorders [16, 18–20, 23, 24]. The direct and indirect participation of plasma Sel-P in antioxidant defense is supported by several studies. Firstly, Sel-P administration was shown to protect the rat endothelium against oxidative diquat-induced injury [19]. Secondly, endothelial cell death induced by oxidative stress was decreased by Sel-P through GPx induction [25]. Lastly, there is evidence of a direct

antioxidant enzymatic activity of Sel-P as demonstrated against peroxynitrite-mediated oxidation, reduction of phospholipid hydroperoxides and inhibition of oxidation of low-density lipoproteins [20, 26–30]. In septic-shock patients, plasma selenoproteins are thought to play a main role in antioxidant defense, especially in the protection of the endothelium [14, 31]. In these patients, the induction of Sel-P synthesis through Se administration in order to protect the endothelium has been proposed as a therapeutic target [32–34] based on the hypothesis of increased Sel-P binding to the endothelium for oxidative protection in these patients. However, a direct demonstration of extensive Sel-P binding to the endothelium in severe forms of sepsis or SIRS and of an endothelium-protecting activity of Sel-P has not been published until now.

In these patients, as in those with SIRS with organ dysfunction, there is an early 40% decrease in plasma Se concentrations associated with a three-fold increase in morbidity and mortality [35]. A decrease in plasma Se may be a strong mortality marker in septic surgical patients admitted to an intensive care unit (ICU) [36]. Moreover, it has been observed in an ovine model of peritonitis that plasma Se concentration decreased within 9 h after the onset of peritonitis [37], suggesting that the decrease in plasma Se concentration could appear early in human sepsis. However, the reason for this marked decrease in plasma Se remains unclear and may be related to changes in plasma concentrations of either Sel-P, GPx or albumin that account for 53, 39 and 9%, respectively, of the total plasma Se in healthy individuals [18, 38]. In patients with either septic shock (especially with multiple-organ failure), or multiple-organ failure related to SIRS, if there is major Sel-P binding to the endothelium, this binding could exceed Sel-P production, and lead to a marked early decrease in plasma Sel-P concentration, as a main causative factor responsible for the decrease in plasma Se. Moreover other mechanisms may amplify the decrease of plasma Sel-P concentration in septic-shock patients such as translocation to other tissue compartments, decreased hepatic production or degradation.

Therefore, we hypothesized that there would be a major decrease in plasma Sel-P concentration specifically in ICU septic-shock patients and in similar SIRS ICU patients and that Sel-P would be the main Se compound responsible for the decreased plasma Se.

To test this hypothesis, we compared the plasma Sel-P and GPx concentrations in septic-shock ICU patients and similar SIRS patients with organ failure to those of other ICU patients (non-SIRS) and of healthy controls.

Table 1. Baseline patient characteristics

Patient	Sex	Age years	Underlying disease	ICU admission diagnosis	SIRS type	Vent.	SAPS II	SOFA adm.	SOFA max., 10 days
S-1	M	75	cancer	abdominoperineal resection ¹	SC	Y	54	9	13
S-2	F	87	bedridden	peritonitis (on pycholecystitis) ¹	SC	Y	56	9	13
S-3	F	35	anorexia	peritonitis (on gastric ulcer)	SC	Y	44	9	14
S-4	M	77	stroke	peritonitis (postoperative) ¹	SC	Y	54	15	17
S-5	F	76	renal colic	pyelonephritis	SC	Y	69	11	14
S-6	M	51	alcoholism	pneumonia (CAP)	SC	Y	47	5	12
S-7	M	60	neurologic disorder	pneumonia (NP) ²	SC	Y	49	7	7
S-8	M	34	none	severe multiple trauma	SIRS	Y	29	6	9
S-9	M	77	CHD	aortic cross-clamping, MI ²	SIRS	N	35	4	4
S-10	F	39	obesity	respiratory distress	SIRS	Y	22	2	4
NS-1	M	71	chronic dialysis	acute coronary syndrome ²	–	N	45	7	8
NS-2	M	75	asbestosis	head trauma, acute alcoholism ²	–	N	29	4	4
NS-3	M	61	status epilepticus	epilepsy	–	Y	52	6	8
NS-4	M	74	MI	gastric hemorrhage	–	N	37	4	4
NS-5	M	55	stroke	ischemic stroke ¹	–	Y	48	7	10
NS-6	M	66	tuberculosis	respiratory distress (metastasis) ¹	–	Y	27	2	2
NS-7	F	53	depression	intoxication	–	N	18	1	2
NS-8	M	39	depression	intoxication	–	Y	28	6	6
NS-9	F	78	cardiac insufficiency	gastric hemorrhage ¹	–	N	37	6	11
NS-10	M	69	endocarditis	polyneuropathy	–	Y	38	3	6
NS-11	M	70	hemorrhage	polyneuropathy	–	N	25	3	6

Adm. = On admission; CHD = coronary heart disease; MI = myocardial infarction; CAP = community-acquired pneumonia; NP = nosocomial pneumonia; SC = septic shock; vent. = ventilated patient; Y = yes; N = no.

¹ Died in the ICU. ² Died in hospital.

Material and Methods

Patients and Study Design

Two groups of ICU patients were studied. The S group (n = 10) corresponds to patients with septic shock (n = 7) or severe SIRS with organ failure (n = 3) on admission to the ICU. The NS group (n = 11) corresponds to patients without severe sepsis or SIRS on admission to the ICU (table 1). Only patients 18 years or older were eligible for inclusion. The inflammatory and sepsis status of all patients was prospectively classified using the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) consensus conference criteria [39]. However, to identify patients without SIRS, we used the same restrictive criteria as those described in our previous study to exclude symptoms not related to acute inflammation (i.e. SIRS) but more directly related to the underlying disease or treatment side effects [35]. Patients were classified blinded to the plasma Sel-P levels. The Saint-Germain-en-Laye Ethics Committee (France) has been informed of the protocol and has waived the need to obtain individual informed consent since the plasma measurements were performed on blood samples withdrawn for other routine blood tests.

The following clinical characteristics were recorded or measured on admission: age, gender, critical illness severity scores: Simplified Acute Physiology Score II (SAPS II), Sepsis-related Organ Failure Assessment score (SOFA), ventilation, diagnosis for

admission, and documentation of infection (table 1). We recorded lactate, bilirubin concentrations and alanine transaminase (ALAT) activity, as well as prothrombin time (PT) in blood. Increased bilirubin and ALAT were defined as values above the upper limit of the reference value, i.e. $\leq 17 \mu\text{mol/l}$ for bilirubin and twice the upper limit of the reference value for ALAT, i.e. $\leq 55 \text{ UI/l}$. Increased PT was defined as a value above 2 s over the reference value (12.4 s) [40]. The main clinical outcomes (death or survival at the time of discharge from the ICU or hospital) were collected. Maximum SOFA for the first 10 days corresponding to the sum of the highest values of single-organ failure during these 10 days [41], and maximum lactate concentrations for the first 10 days were recorded as well. All S group patients survived up to 10 days in the ICU. A control group consisted of 7 healthy volunteers (HV group).

Patient characteristics in the 3 groups are summarized in tables 1 and 2. Of the 7 septic-shock patients, 5 required high doses of catecholamines and had a class 4 cardiovascular SOFA score. None of these patients had a history of malnutrition except for an anorexic patient with a body mass index (BMI) of 16 (S-3). Of the 3 S group patients who died in the ICU due to multiple-organ failure related to septic shock: 1 died from persistent shock refractory to catecholamines; the second from septic shock with multiple-organ failure, and the third from prolonged sepsis with a biliary fistula (4 interventions) and persistent shock with cate-

Table 2. Patient characteristics according to group

	S group	NS group	HV group
Patients, n	10	11	7
Age, years	68 [42–77]	69 [58–73]	27 [27–40]
Gender, M/F	6/3	9/2	4/3
SAPS II	48 [37–54]	37 [28–42]	NA
Ventilated patients, n	9 (90%)	5 (45%)	NA
Albumin, g/l	24 [21–24]	34 [28–39]	(35–48)
Lactate on admission μmol/l	4.1 [2.1–6.7]	2.1 [1.9–2.4]	(≤ 2.5)
Max. lactate (10 first days), μmol/l	4.6 [2.1–6.7]	2.3 [2.1–2.7]	(≤ 2.5)
Bilirubin, μmol/l	11 [9–15]	15 [11–20]	(≤ 17)
ALAT, UI/l	43 [21–61]	83 [78–89]	(≤ 55)
PT > 2 s over ref. value	9/10	11/11	(12.4)
SOFA on admission	8 [5–9]	4 [3–6]	NA
Max. SOFA (10 first days)	13 [8–14]	6 [4–8]	NA
Length of stay in ICU	35 [27–44]	4 [3–13]	NA
ICU mortality	3 (30%)	3 (27%)	NA
Hospital mortality	5 (50%)	5 (45%)	NA

Values are presented as medians and 25–75% interquartile ranges (in square brackets) or number of patients. All patients in the S group had an ICU stay $\geq$ 19 days, except 1 patient with an ICU stay of 3 days (S-9) and all patients in the NS group had an ICU stay $\leq$ 4 days, except 3 patients with an ICU stay of 55 days (NS-10) and 2 patients with an ICU stay of 15 days (NS-11 and NS-6). In the HV group column, the values in parentheses correspond to reference values.

choline-refractory multiple-organ failure. These patients died on days 29, 43, and 52, respectively, after the initial septic shock. Two S group patients died later, after discharge from the ICU (table 1).

The NS group patients had a wide variety of diagnoses on admission to the ICU (table 1). Three patients of the NS group died in the ICU on days 4, 9, and 15, respectively: 1 from stroke (NS-5), the second from multiple pulmonary metastases (NS-6); and the third, a 78-year-old patient with a previous history of severe cardiac insufficiency (NS-9), died from catecholamine-refractory cardiogenic shock with acute renal failure. Two NS group patients died after discharge from the ICU (table 1). One patient (NS1) died from mesenteric ischemia 3 days after discharge from the ICU.

We did not observe severe hepatic failure in S group patients on admission (table 2) as shown by the absence of increased PT or bilirubin, in all S group patients, except for 1 (S-4) and no increase in ALAT except for 2 patients (S-4 and S-8).

Blood Samples

Initial plasma samples were collected within 48 h after admission from all patients and then once a week during their stays in the ICU as in our previous study [35]. The admission plasma sample was missing for 1 septic-shock patient. Further sampling was

obtained for this patient as well as follow-up sampling. Plasma was separated from heparinized blood samples within 1 h after collection and was stored at -80°C until analysis as in our previous study [35].

Measurement of Plasma Sel-P, Albumin, and Se Concentrations, Plasma GPx Activity, and Daily Se Intake

Sel-P concentration was determined by quantitative immunoblotting using purified Sel-P as the standard [42]. The concentrations of Sel-P were expressed in nanomoles per liter, assuming a molecular weight of 61 kDa for human Sel-P. GPx plasma activity was measured using a commercial assay kit (Randox Laboratories, Ardmore Ltd., UK) with t-butylhydroperoxide as substrate. Plasma Se concentrations were measured by electrothermal atomic absorption spectrometry using a 5100 C spectrometer with Zeeman effect background correction (Perkin-Elmer, Paris, France). Albumin was measured colorimetrically with bromocresol purple (Beckman, Brea, Calif., USA). Reference values for plasma Sel-P were expected to be in the range of 40–80 nmol/l based on previous measurements in healthy individuals [18]. Reference values for plasma GPx, Se, and albumin were 450–850 U/l (according to the manufacturer), 0.8–1.2 μmol/l [35], and 35–48 g/l, respectively. In accordance with routine practice in our ICU, all patients received a supplementation of 40 μg/day Se (as sodium selenite) as part of a combination of trace elements (Nonan, Aguetant, Lyon, France).

Statistical Analysis

Nonparametric tests were used to compare values. Quantitative values (plasma Sel-P or Se concentrations) between groups (S, NS and HV groups) were compared using a Kruskal-Wallis test, and, if the differences were significant, paired comparisons were performed using the Wilcoxon test. Analyses were performed using Statistical Analysis System (Cary, N.C., USA). Data are expressed as medians, and interquartile ranges (25–75th percentiles). In all cases, differences were considered statistically significant at $p \leq 0.05$.

Results

Plasma Concentrations of Sel-P

On Admission

Figure 1a shows the individual values of plasma Sel-P concentrations in the S (n = 10) and NS groups (n = 11). Individual plasma Sel-P concentrations were homogeneous in the HV group, ranging from 39 to 65 nmol/l (table 3, fig. 1). There was no difference between the NS and the HV groups. On admission, plasma Sel-P concentrations were significantly lower in the S group compared to the NS group or the HV group (table 3, fig. 1a). The plasma Sel-P concentrations in S group patients were 70% lower than in HV patients. All the patients of the NS group had plasma Sel-P concentrations >30 nmol/l, except for 2 patients (NS-1 and NS-9). All the patients of the S group except for 1 non-septic-shock patient (S-9) had a

Fig. 1. Plasma Sel-P concentrations on ICU admission in the S, NS and HV groups. **a** Sel-P. **b** GPx. **c** Albumin. **d** Se. Values are expressed as median (middle line) and individual values. Reference plasma concentrations: Sel-P 40–80 nmol/l; GPx 450–850 U/l; Se 0.8–1.2 μmol/l and albumin 35–48 g/l. Plasma Sel-P concentrations differed significantly between the S and NS groups ($p = 0.003$). Plasma GPx activities did not differ between the S and NS or HV groups. Plasma concentrations of albumin and Se were significantly lower in the S compared to the NS and HV groups ($p = 0.002$ and $p < 0.001$).

Sel-P value <30 nmol/l on admission. Furthermore, on admission, plasma Sel-P concentrations were significantly lower in the 3 septic-shock patients who later died in hospital than in the 3 septic-shock patients who survived (1, 7 and 13 vs. 15, 26 and 28 nmol/l, respectively, $p = 0.049$). In S group patients, we did not observe any significant correlation between admission Sel-P concentration and admission SOFA or the first 10 days maximum SOFA.

During the ICU Stay

The patients' plasma Sel-P concentrations varied greatly during the ICU stay (range 0–107 nmol/l). Plasma Sel-P concentrations remained <30 nmol/l in the 5 patients with the most complicated evolution related to sepsis: 3 died in the ICU, 1 died from nosocomial pneumonia just after discharge from the ICU and patient S-3 had persistent complications, including 3 successive incidents of

peritonitis along with a persistent fistula on discharge on day 64. In patient S-8, hospitalized for severe multiple trauma and probable fat embolism, plasma Sel-P level remained <20 nmol/l until discharge from the ICU on day 19.

On Discharge from the ICU

In nonsurviving patients, plasma Sel-P concentrations before death were significantly lower in the 3 patients with septic shock compared to those of the 3 patients in the NS group (table 3, fig. 2). Plasma Sel-P concentrations were significantly lower in S group patients compared to NS group patients (table 3, fig. 2).

Plasma Sel-P concentrations did not differ significantly between nonsurviving and surviving patients from the S group [20 (9–29) and 0, 7 and 24 nmol/l, respectively, $p = 0.44$]. Plasma Sel-P concentrations were similar between nonsurviving and surviving patients from the NS

Fig. 2. Plasma Sel-P concentrations on discharge from the ICU in the S, NS and HV groups. Plasma Sel-P concentrations (reference plasma concentrations: 40–80 nmol/l) were significantly lower in S group patients than in NS group patients ($p = 0.004$). Values are expressed as median (middle line) and individual values. Plasma Sel-P concentrations were significantly lower in the 3 ICU deceased S group patients than in the 3 ICU deceased NS group patients ($p = 0.049$). ● = Deceased patients.

group [39 (29–63) and 27, 35 and 84 nmol/l, respectively, $p = 0.76$].

Comparison of Sel-P and Other Plasma Se-Containing Compounds on Admission

On admission, there was no significant difference between plasma GPx activities between the S and NS or HV groups (table 3, fig. 1b) and no correlation was found between plasma GPx activity and plasma Sel-P level.

The plasma Se concentrations in S group patients were 71% lower than in HV individuals (table 3, fig. 1d). There was a significant difference in the plasma Se concentrations between the S group and both the NS and HV groups (table 3, fig. 1d). When all patients and HVs were combined, there was a good correlation between plasma Se and plasma Sel-P levels ($r = 0.75$; $p < 0.0001$). When considering the groups separately, there was no significant correlation between plasma Se and plasma Sel-P concentrations within the S or NS group although there was a significant correlation for individuals in the HV group ($r = 0.78$, $p = 0.003$). Moreover, in the S group, no correlation was found between plasma Se and plasma Sel-P level on day 7.

On admission, plasma concentrations of albumin were significantly lower in the S group compared to the NS group and to the HV group (table 3, fig. 1c).

Table 3. Plasma Sel-P, GPx, albumin and total Se according to time and groups

	On admission to the ICU	On discharge from ICU
<i>S group</i>		
Plasma Sel-P, nmol/l	15 [10–26] ^a	17 [5–23] ^b deceased: 0, 7 and 24
Plasma GPx, U/l	371 [317–656]	576 [358–780]
Plasma albumin, g/l	24 [22–25] ^a	
Total plasma Se, μ mol/l	0.30 [0.26–0.41] ^a	0.76 [0.50–0.87]
Protein, g/l	38 [33–45]	52 [49–69]
Hematocrit, %	31 [24–32]	28 [24–29]
<i>NS group</i>		
Plasma Sel-P, nmol/l	44 [29–71]	35 [29–64] deceased: 27, 35 and 84
Plasma GPx, U/l	536 [437–827]	498 [467–827]
Plasma albumin, g/l	33 [28–39]	
Total plasma Se, μ mol/l	0.92 [0.66–1.00]	0.89 [0.69–1.00]
Protein, g/l	66 [56–70]	57 [52–62]
Haematocrit, %	37 [30–39]	31 [27–37]
<i>HV group</i>		
Plasma Sel-P, nmol/l	50 [45–53]	NA
Plasma GPx, U/l	517 [470–916]	NA
Plasma albumin, g/l	35–48	NA
Total plasma Se, μ mol/l	1.04 [0.94–1.16]	NA
Protein, g/l	61–79	NA
Hematocrit, %	37–47	NA

Values are presented as median and 25%–75% interquartile ranges (in square brackets). The 3 S group deceased patients were septic-shock patients.

^a S group versus NS or HV group ($p < 0.01$); ^b S group versus NS group ($p < 0.01$); ^c S group versus NS group ($p < 0.05$).

Regarding the physiologic plasma Se compounds, the 71% lower plasma Se concentrations in the S group compared to the HV group, matched: (1) 70% lower plasma Sel-P concentrations, (2) 40% lower albumin concentrations, (3) and no significantly lower plasma GPx activity.

Discussion

In healthy populations, the plasma Sel-P concentrations are known to be very stable [22, 43]. The variations in concentration in pathophysiological processes other than severe Se deficiency are poorly known. This is the

first description of an early, dramatic and specific decrease in plasma Sel-P concentration (70% reduction; $p < 0.003$) in ICU patients with multiple-organ failure related to septic shock or SIRS (S group) compared to other ICU patients. This decrease was also specific to Sel-P compared to the other plasma selenoprotein, GPx, whose plasma concentrations did not vary between groups. Plasma Sel-P concentrations were always <30 nmol/l in septic-shock patients on admission to the ICU whereas plasma Sel-P concentrations were similar in the NS patients and in healthy volunteers. This difference in Sel-P concentrations was observed despite high severity scores (SAPS II) and similar mortality rates in S and NS patients. Although our results support the hypothesis that during systemic inflammation in humans, Sel-P binds massively to endothelium and subsequently could be a marker of microvascular injury, further studies are required to demonstrate this and identify other mechanisms that may be involved.

Plasma Sel-P concentration is considered to be well preserved in humans [22] and has been proposed as an index of the nutritional Se status in cases of severe Se deficiency [21, 22, 38]. In our HVs, the plasma Sel-P concentrations were within the normal range, and corresponded to the relatively low Se status of the French population [11, 43]. To our knowledge, such low plasma Sel-P concentrations as we observed in our S group patients are only found in case of most severe Se deficiency [21]. As the S group patients, except for the one with anorexia, did not suffer from severe malnutrition before the onset of sepsis or inflammatory syndrome, Se deficiency is most likely not involved in the low Sel-P concentrations observed in these patients. This is in accordance with our previous study in which chronic Se deficiency in ICU patients could most likely not explain the decreased plasma Se [35].

Even though we used the same criteria for defining non-SIRS patients as in our previous work on plasma Se levels in ICU patients, the NS group exhibited an unexpected high mortality rate mostly related to isolated organ failure [35]. Nevertheless, in contrast to S group patients, NS group patients did not have low plasma Sel-P concentrations on admission. The 70% decrease in plasma Sel-P concentrations observed in our study is comparable to the decrease recently described by Hollenbach et al. [44], who assessed samples from ICU patients with sepsis from a previous study on copeptin [45], using an immunoassay. However, Hollenbach et al. did not distinguish between the degrees of sepsis (sepsis, severe sepsis or septic shock) and did not compare Sel-P concentra-

tions to those of other ICU patients, nor did they report on Sel-P kinetics and other plasma Se compounds (GPx, albumin), organ failure and mortality. Our ICU patients with the most severe septic shock showed the lowest plasma Sel-P concentrations, i.e. always <30 nmol/l, and in these septic-shock patients, Sel-P concentration was significantly lower in those who died in hospital than in those who survived. Although the sample size was small, the plasma Sel-P concentrations before death in the ICU were significantly lower in septic-shock patients of the S group than in NS group patients. This supports the hypothesis that plasma Sel-P concentration may be a severity marker for the risk of organ failure or death in septic shock.

There is no established gold standard for defining SIRS patients. In 2 patients with organ failure related to SIRS, we observed a marked decrease in plasma Sel-P concentration similar to that found in septic shock. Further studies are needed to show whether plasma Sel-P concentration can be used as a marker of organ failure related to SIRS. The S group patient with the highest plasma Sel-P concentration (S-9) (almost reaching the reference range) did not develop new organ failure, and his plasma albumin concentration did not decrease. The 2 NS patients with the lowest Sel-P concentrations (NS-1 and -9) were not classified as SIRS on admission. However, they later died from acute mesenteric ischemia (NS-1) and organ failure related to shock (NS-9). Larger series are required to analyze whether plasma Sel-P concentration could be used to predict organ failure related to SIRS.

The low plasma Sel-P concentration in patients with septic shock and severe SIRS with organ failure is in agreement with our pathophysiological hypothesis of a Sel-P decrease related to inflammation or ischemia-reperfusion at the microcirculation level. The reduced plasma Sel-P concentration observed in the S group but not in other ICU patients indicates that in the S group there is an imbalance at the plasma level between Sel-P production (mainly in the liver) and Sel-P binding, translocation or degradation. Sel-P binding to the endothelium is known to be favored by acidosis which is present at the site of inflammation [20]. Therefore, this suggests that Sel-P binding may be one of the leading mechanisms causing the plasma Sel-P decrease in case of systemic inflammation such as in the S group. However, this needs to be further demonstrated. Moreover, there is some evidence that Sel-P has an antioxidant function especially against peroxynitrite [20, 26, 28]. We observed the lowest Sel-P plasma concentrations in nonsurviving septic-

shock patients, which supports our hypothesis of a protective effect of Sel-P on the endothelium in sepsis. The results of our recent study using an experimental model of peritonitis in sheep showed that the decrease in plasma Se concentration seems to appear very rapidly in sepsis and to precede the onset of septic shock [37]. Therefore, in persistent generalized oxidative stress, as observed in the microcirculation during septic shock, a marked decrease in plasma Sel-P concentration may lead to enhanced endothelial dysfunction, microvascular injury and multiple-organ failure. Besides endothelium binding of Sel-P, other mechanisms may explain the dramatic decrease in plasma Sel-P concentration observed in ICU patients with septic shock and severe SIRS with organ failure: (1) a downregulation of its hepatic synthesis by inflammatory cytokines [18, 46], Sel-P being a negative acute-phase-response protein as recently shown in a lipopolysaccharide murine model [47]; (2) insufficient Se availability in the liver [34], or (3) liver dysfunction [40]. However, concerning this last hypothesis, hepatic injury is usually mild during sepsis [40], and we did not observe severe hepatic dysfunction in our S group patients on admission. Increased extravasation of Sel-P is another hypothesis. However, we previously observed in a larger series that water movements across the wall were insufficient to explain all the variations in plasma Se concentration [35]. Increased urine excretion is less likely to occur as we did not observe increased Se urine concentrations in our previous ICU study – in agreement with Hawker et al. [48] and Berger et al. [49]. Further *in vitro* and animal studies are needed to clarify the respective importance of these mechanisms.

In agreement with previous studies, our results in the present study highlight that plasma Se concentrations decreased in patients with septic shock in contrast to those of NS group patients [35, 36, 50]. Interestingly, we and others have shown that low plasma Se concentrations are associated with a three-fold increase in ventilator-associated pneumonia, new organ failure and mortality rate in ICU patients. They are a strong predictor of mortality in surgical septic ICU patients and were negatively correlated to SOFA in septic-shock ICU patients [35, 36, 50].

We observed that plasma GPx activity was not significantly different in septic-shock patients compared to HVs but tended to be lower (table 3). Due to the small size of our study, our results could not rule out a possible decrease in plasma GPx activity in patients with septic shock, as was recently observed by Manzanares et al. [51] in a group of 16 ICU SIRS patients with multiple-organ failure. In agreement with these authors, we found that

plasma GPx and Se concentrations in ICU non-SIRS patients were similar to those of HVs. Even with the small number of patients in our study we observed a dramatic 70% decrease of Sel-P in septic-shock and SIRS patients with multiple-organ failure whereas the plasma Sel-P concentration in ICU patients without SIRS remained similar to that of the HVs.

Taking into account that plasma Sel-P contains more Se than GPx, our results suggest that the decreased plasma Se observed in septic shock and SIRS with multiple-organ failure patients is most probably linked to the decreased plasma Sel-P rather than to GPx (table 3, fig. 1).

Moreover, in contrast to studies on healthy populations or Se deficiency [21, 22], we did not observe a correlation between plasma Se, Sel-P and GPx in ICU patients, suggesting an independent event on Sel-P in ICU patients. However, a larger study is required to demonstrate this point.

The major limitation of this study is the low number of patients. The decrease in Sel-P in severe SIRS with organ dysfunction needs to be confirmed in larger series allowing for multivariate and receiver operating characteristic curve analysis. Furthermore, the role of Sel-P as an early severity marker of sepsis, septic shock and related organ failure, or as a prognostic marker of death, remains to be determined. A second limitation could be the relatively long initial sampling time (up to 48 h after admission). This period was chosen to allow comparison with our previous study on the decrease in plasma Se concentration [35]. In addition, in a recent study, Manzanares et al. [51] also chose this time period allowing further comparison. This choice is further supported by another recent study showing that decreased plasma Se appears very early in the process of septic shock [37]. In that study using an ovine model of peritonitis, it appeared within less than 9 h after the onset of shock. Therefore in a clinical situation, it is likely that a decrease in plasma Se – and subsequently in the plasma Sel-P concentration – appears before the admission to the ICU. This strengthens the need for further studies on very early assays of plasma Sel-P concentration on admission to an ICU and even before, i.e. in the emergency room, as an early marker for septic shock.

Moreover, this study was not designed to analyze pathophysiological mechanisms that need to be studied *in vitro* and in animal studies. The objective of these studies will be to observe whether an Sel-P decrease in septic shock may be related to a main pathophysiological process involved in endothelium dysfunction, preceding multiple-organ failure.

Conclusion

In this study, we collected data in favor of a marked early specific decrease in plasma Sel-P concentration in ICU patients with organ failure related to septic shock and severe SIRS, but not in non-SIRS ICU patients suffering similar mortality. GPx, the other plasma selenoprotein, did not exhibit the same marked decrease. Further animal and in vitro studies are required to establish whether this specific decrease in Sel-P in septic shock and ICU patients with related conditions is mainly associated with early binding of Sel-P for an antioxidant protection of the activated endothelium or with other mechanisms such as negative acute-phase response that may amplify

the early Sel-P decrease in septic-shock patients. Further larger clinical studies are needed to see whether Sel-P could be an early marker of septic shock and/or organ failure related to sepsis and SIRS linked to endothelial injury.

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