Biologia Futura
https://doi.org/10.1007/s42977-023-00168-1

ORIGINAL PAPER

Genes regulating oxidative-inflammatory response in circulating monocytes and neutrophils in septic syndrome

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Received: 5 August 2022 / Accepted: 13 May 2023

Abstract

Despite significant progress in the past decades, sepsis still lacks a specific treatment. Under normal conditions, leucocytes play a critical role in controlling infection and it is suggested that their activity is impaired during sepsis which contribute to the dysregulation of immune reactions. Indeed, in response to infection, several intracellular pathways are affected mainly those regulating the oxidative- inflammatory axis. Herein, we focused on the contribution of NF-kB, iNOS, Nrf2, HO-1 and MPO genes in the pathophysiology of septic syndrome, by analyzing the differential expression of their transcripts in circulating monocytes and neutrophils, and monitoring the nitrosative/oxidative status in septic syndrome patients. Circulating neutrophils of septic patients displayed a significant overexpression of NF-kB compared to other groups. In monocytes, patients with septic shock expressed the highest levels of iNOS and NF-kB mRNA. However, genes involved in cytoprotective response had increased expression in patients with sepsis, in particular, the Nrf2 and its target gene HO-1. Moreover, patient monitoring indicates that the iNOS enzyme expression and NO plasma levels may play a role in assessing the severity of septic conditions. Overall, in either monocytes or neutrophils, we pointed out the major role of NF-κB and Nrf2 in the pathophysiological process. Therefore, therapies targeted to redox abnormalities may be useful for better management of septic patients.

Keywords Sepsis · NF-kB expression · Nrf2 expression · iNOS gene expression · HO-1 gene expression

Introduction

In intensive care units, sepsis is the leading cause of death (Angus et al. 2001; Vincent et al. 2006). Sepsis pathogenesis is characterized by an excessive inflammatory response associated with the activation of the complement system and hyperactivity of cellular innate immune system. In fact, as a response to a powerful initial stimulus, neutrophils and macrophages produce and respond to cytokines, chemokines and complement-activating products. Reactive

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Published online: 08 June 2023

oxygen species (ROS) and reactive nitrogen species (RNS) are also released, further enhancing the inflammatory process (Rittirsch et al. 2008). The spectrum of cell aggression observed in septic syndrome is a consequence of a deregulated oxidative-inflammatory response to the infection (Mantzarlis et al. 2017). In this context, redox homeostasis is critical to the sepsis process. Nuclear factor (erythroid-derived)-like 2 (Nrf2), is a keystone transcription factor controlling many aspects of cell homoeostasis and inflammation (Bryan et al. 2013). It regulates the expression of a number of cytoprotective genes through its interaction with antioxidant response elements (ARE) (Innamorato et al. 2009; Jazwa and Cuadrado 2010). Some of these genes encode antioxidant enzymes such as heme oxygenase-1 (HO-1), which is a potent anti-inflammatory target, and NADP(H) quinine oxidoreductase, which participates in glutathione metabolism (Joshi and Johnson 2012; Hayes and Dinkova-Kostova 2014). Additionally, the nuclear factor-B (NF-kB) has been identified as a transcription factor whose activity is influenced by intracellular redox conditions (Sen and Packer 1996). It is an inductible transcription factor consisting of five members involved in the regulation of proinflammatory cytokines, adhesion molecules, chemokines, growth factors and inducible enzymes genes (Sun et al. 2013; Tak and Firestein 2001). It is well recognized that Nrf2 and NF-kB pathways coordinate to manage the innate immune cells' behavior and to resolve inflammation. Yet, the molecular mechanisms underlying this interaction appear to be cell-type- and tissue-specific and remain unclear (Wardyn et al. 2015). With regard to these inflammatory response and oxidative processes, Septic syndrome has also been extensively studied. However few studies have investigated the genes involved in inflammatory and oxidative connection with a cellular approach. Indeed, peripheral blood mononuclear cells (PBMCs) and polymorphnuclear neutrophils (PMNs) constitute an important component of both vascular and immune systems (Ye 2004). Hence, examining the expression patterns in cells of patients suffering from the septic syndrome would be useful for better management. Our study focus on PBMCs and PMNs, as key players in septic states, to evaluate the differential expression of genes crucial for the regulation of the oxidative-inflammatory axis, in particular NF-Kb, iNOS, Nrf2, HO-1 and MPO. Malondialdehyde (MDA) and nitric oxide (NO) measurements have been assessed as indicators of the oxidative and nitrosative stress states.

Materials and methods

Subjects

This study was approved by the institutional review board of Habib Bourguiba University Hospital, and the requirement for written informed consent was waived by the ethical committee (385/2022). We included forty patients hospitalized in the medical intensive care unit for an admission period of 24 h: 20 sepsis, 10 septic shock and 10 non-infected systemic inflammatory response syndrome (SIRS). Patients < 18 years and patients requiring intensive care stays under 24 h were excluded. All the collected data are summarized in Table 1. Fifteen subjects (HC) free from any inflammatory and/or autoimmune pathology were recruited as healthy controls for this study.

Blood samples and cells isolation

Blood samples obtained from patients, at admission (D0: within 48 h of admission) and after 7 days of treatment (D7), were drawn in tubes containing EDTA as an anticoagulant.

Table 1 Demographic, clinical and biological characteristics of recruited patients

	Sepsis $N=20$	Septic shock $N=10$	Uninfected SIRS $N=10$
Age (years)	18–82	27–67	21–75
Gender (M/F)	15/5	6/4	7/3
APACHE II score	5.22 (2–13)	14.42 (3–30)	7.93 (2–16)
SOFA score	5.28 (2–13)	10.2 (2–25)	3.81 (1–8)
Leucocytes/mm^3	3700–35,000	2500–59,900	5500–21,500
PCT (µg/L)	6.05 (0.088–100)	10.19 (0.27–56.7)	0.076
CRP (mg/L)	161.62 (40–358)	164.87 (35–309)	93.68 (8–246)
Creatinine (µmol/L)	69.95 (18–243)	136.06 (25–306)	56.36 (30–81)
Na + (mmol/L)	139.21 (126–161)	138.78 (123–154)	139.14 (134–143)
K + (mmol/L)	3.97 (2.7–4.9)	3.94 (2.1–7.4)	4 (3.3–5.5)
Treatment	Broad-spectrum ATB	High dose of catecholamines + broad-spectrum ATB	–

PCT procalcitonin, *CRP* C-reactive protein, *Na* + Natremia, *K* + Kalemia, *T* trauma, *ATB* antibiotics, *SOFA* Sequential Organ Failure Assessment, *APACHE* Acute Physiology and Chronic Health Evaluation

All samples were subjected to the same transport conditions and processing techniques.

PBMCs and PMNs cells were isolated by density gradient centrifugation (GRANULOSEP; CMSMOP01-0U, eurobio®, France) as described previously (Elloumi et al. 2017). The osmotic shock was performed to eliminate the remaining red blood cells. Using Turk staining and trypan blue dye exclusion tests, respectively, we assessed the purity and viability of the PMNs preparation to be greater than 95%.

RNA isolation and reverse transcription

Total RNA was extracted from PBMCs/PMNs cells by using the TriZol reagent. The concentration and the quality were evaluated using the bioanalyzer (Agilent®, USA). Reverse transcription of total RNA was carried out using the FIRE-Script RT cDNA Synthesis Kit cDNA (Solis Biodyne®).

Quantitative PCR analysis

Q-PCR analyses of selected genes: NF-kB (F: 5′-AACAGC AGATGGCCCATACC-3′; R: 5′- AACCTTTGCTGGTCC CACAT-3′), iNOS (F: 5′-TGCAGACACGTGCGTTAC TCC-3′; 5′-R: GGTAGCCAGCATAGCGGATG-3′, Nrf2 (F: 5′-TTCAGCCAGCCCAGCACATC-3′; R: 5′-CGTAGC CGAAGAAACCTCATTGTC-3′), HO-1 (5′-F: TCCGAT GGGTCCTTACACTC-3′; R 5′-: TAAGGAAGCCAGCCA AGAGA-3′) and MPO (F: 5′-GACAACACAGGCATCACC AC-3′; R: 5′-CAGCCCAGATATACCCCTCA-3′) were performed in duplicate using the TAKARA SYBR Green PCR Kit according to the manufacturer's recommendations in a StepOnePlus real-time PCR system (Applied Biosystems®, CA, USA). Relative quantification was performed by the comparative 2-ΔΔCT method using the endogenous gene GAPDH (F: 5′-GCTCTCTGCTCCTCCTGTTC-3′; R: 5′-CGCCCAATACGACCAAATCC-3′) as a reference.

ELISA assay

The nuclear protein extracts from PBMCs were obtained following the instructions of the nuclear extraction kit (ab 113,474, Abcam®), and the concentration was determined by the BSA calibration curve integrated into the NanoDrop (NanoPhotometer P 330, IMPLEN®). Then, 20 µg of nuclear protein extract is used to detect Nrf2 and NF-kB nuclear translocation using the transcription factor assay kit (ab207223, Abcam®) and (ab133112, Abcam®) at OD 450 nm.

Biochemical analysis

Plasma MDA level was measured by a spectrophotometric method using the thiobarbituric acid-reactive substance (TBARS). The total nitrite ($NO2^-$) content, indicative of NO production, was monitored by the Griess reaction, and the final compound was measured at 550 nm.

Statistical analysis

The results were analyzed using SPSS software 20.0. The normality assumption and the homogeneity of variance assumption are tested before choosing the suitable test. The differences in expression between groups were analyzed using the Kruskal–Wallis nonparametric tests and the Mann–Whitney independent sample test. For the results analysis of the same patient at different time points, we used the nonparametric paired-sample tests, the Friedman test and the Wilcoxon test. Spearman's test was used for correlation analysis. Statistical significance was defined as a value of $p < 0.05$. Principal component analysis (PCA) was performed using the FactoMineR package. PCA was used to help in the interpretation of the data set generated in this study with the factoextra package for data visualization. Squared Euclidean distance was used as the measure of similarity, and Ward method was applied for the agglomeration. All data matrices were auto-scaled before the analysis.

Results

Evaluation of the genes' relative expression

Our results revealed a significant difference in the NF-kB gene expression level in PBMCs and PMNs between the septic shock patients group, sepsis patients group, uninfected SIRS and HC (H (3) = 8.14, $p = 0.043$ and H (3) = 9.11, $p = 0.028$, respectively) (Fig. 1A, B). In PBMCs, septic shock patients showed a significant increase in the NF-kB gene expression compared to septic patients and HC ($Z = [-2.30]$, $p = 0.021$ and $Z = [-2.30]$, $p = 0.014$, respectively) (Fig. 1A). However, in PMNs, the sepsis group presented the highest level of NF-kB gene expression when compared to shock patients, SIRS patients and healthy controls groups ($Z = [-2.02]$, $p = [0.043]$; $Z = [-2.30]$, $p = [0.021]$ and $Z = [-2.02]$, $p = [0.043]$, respectively) (Fig. 1B).

A significant difference in the Nrf2 expression gene was also noted in both PBMCs and PMNs, between the different groups (H (3) = 8.68, $p = 0.034$ and H (3) = 9.06, $p = 0.028$, respectively) (Fig. 1C, D). Indeed, in PBMCs, HC significantly express less Nrf2 gene compared to other patient groups: sepsis, septic shock and uninfected SIRS ($Z = [-2.44]$, $p = 0.011$; $Z = [-2.12]$, $p = 0.034$; $Z = [-2.30]$, $p = 0.029$, respectively) (Fig. 1C). In PMNs, septic patients displayed an upregulation of gene encoding Nrf2 in comparison with patients with septic shock and uninfected SIRS

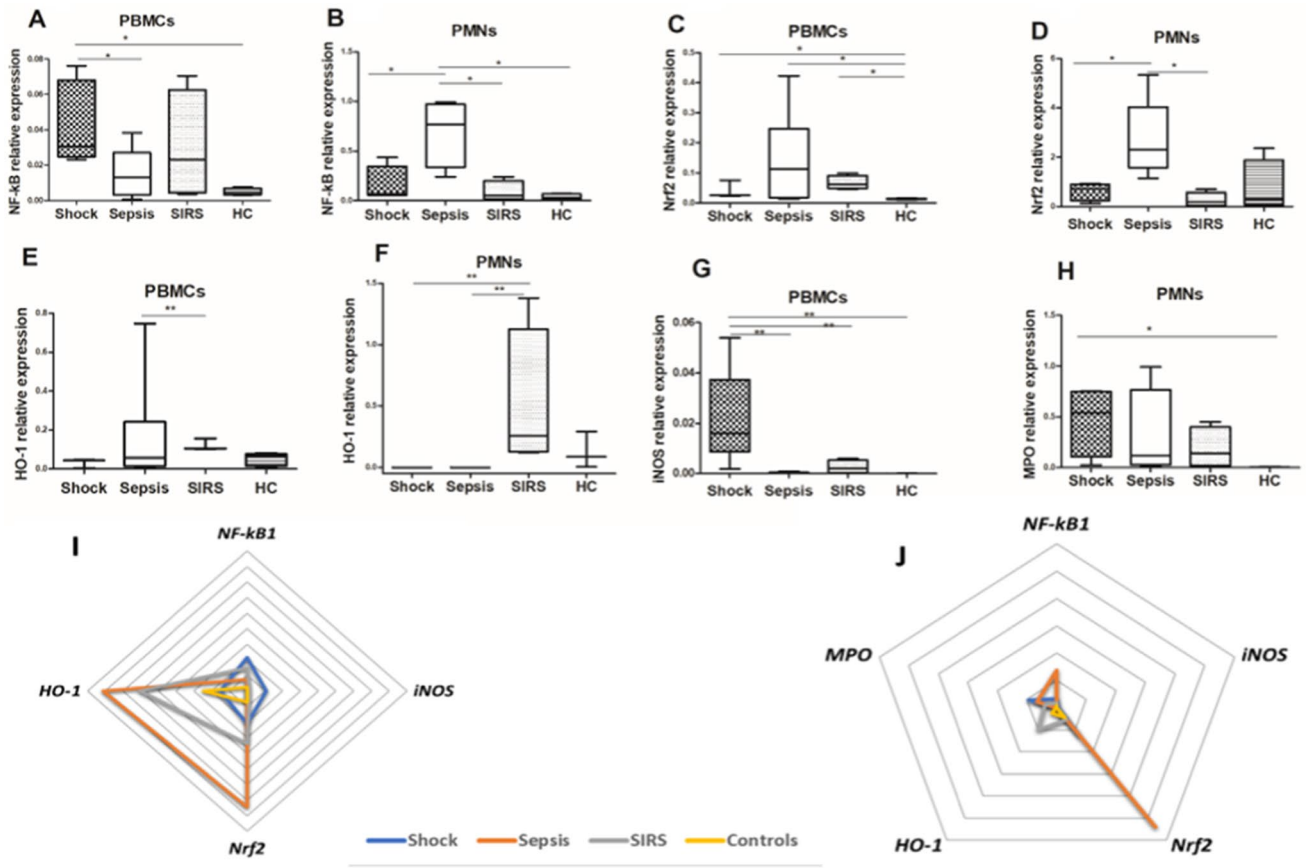

Fig. 1 Gene expression analysis of oxidative-inflammatory axis in PBMCs and PMNs. Gene relative expression was evaluated by Q-PCR of **A** NF-kB, **C** Nrf2, **E** HO-1, **G** iNOS in PBMC, **B** NF-kB, **D** Nrf2, **F** HO-1, **H** MPO in PMN. Radar plot representation of gene expression profile in **I** PBMC and in **J** PMN of each group. Sepsis patients (red line), septic shock patients (blue line), SIRS patients (gray line), control group (yellow line). Each bold line represents the average of NF-kB, Nrf2, iNOS and HO-1, MPO gene expressions. PBMC $n=45$ including sepsis $n=20$, septic shock $n=10$, uninfected SIRS $n=5$ and controls $n=10$. PMN $n=25$ including sepsis $n=10$, septic shock $n=5$, uninfected SIRS $n=5$ and controls $n=5$ (*$p<0.05$; **$p<0.01$)

($Z=[-2.44]$, $p=0.014$ and $Z=[-2.44]$, $p=0.014$, respectively) (Fig. 1D).

The HO-1 expression level assessment demonstrates a significant difference between all the patients in both cell types: PBMCs and PMNs (H (3) = 10.35, $p=0.016$ and H (3) = 12.9, $p=0.005$, respectively) (Fig. 1 E). Uninfected SIRS patients exhibited a highly significant expression level of the HO-1 gene compared to patients with sepsis in PBMCs ($Z=[-2.30]$, $p=0.01$) and to those in sepsis and septic shock in PMNs ($Z=[-2.44]$, $p=0.01$) (Fig. 1F).

The evaluation of the iNOS gene expression level revealed a highly significant difference between groups in PBMCs (H (3) = 17.34, $p=0.001$) (Fig. 1G) but not in PMNs (data not shown). Indeed, the septic shock patients group showed a highly significant increased expression, compared to patients with sepsis, HC and uninfected SIRS ($Z=[-3.46]$, $p=[0.001]$; $[Z=-2.55]$, $p=[0.01]$; and $Z=[-2.13]$, $p=[0.03]$, respectively) (Fig. 1G). The myeloperoxidase (MPO) expression in PMNs was significantly different between the four studied groups (H (3) = 8.09, $p=0.044$) (Fig. 1H). For each patients' group, the radar plot revealed distinctive gene expression profiles either in PBMCs (Fig. 1I) or PMNs (Fig. 1J).

The monitoring strategy results revealed no significant difference in all studied genes in, PBMCs and PMNs, except within iNOS gene. Indeed, iNOS gene expression level in PBMCs of septic patients was significantly lower in Day 7 than at admission (48 h) ($p=0.008$) (Fig. 2).

Evaluation of NF-kB and Nrf2 activity

Statistical analysis demonstrated that the NF-kB p65 and Nrf2 nuclear fractions differed significantly between the different groups (H (3) = 10.06, $p=0.018$ and H (3) = 14.28, $p=0.003$, respectively) (Fig. 3A). The highest NF-kB p65 nuclear fraction was observed in septic shock patients compared to uninfected SIRS, and patients with sepsis compared to HC ($Z=[-2.20]$, $p=[0.028]$ and $Z=[-2.27]$,

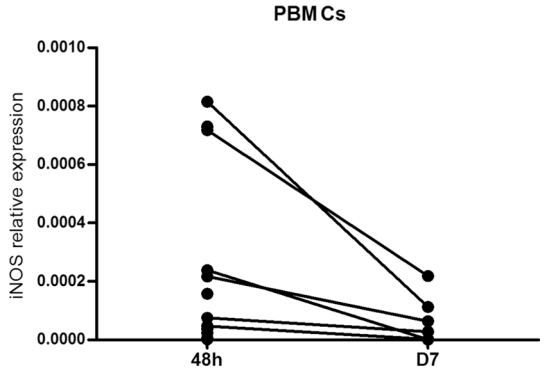

Fig. 2 iNOS gene expression follow-up. iNOS relative expression follow-up was evaluated by Q-PCR of in PBMC of septic patients $n=20$, at admission (D0: within 48 h of admission) and day 7 (D7), ($p<0.01$)

$p=[0.023]$, respectively) (Fig. 3A-(1)). Similarly, the Nrf2 nuclear fraction was highly present in the patients with sepsis and septic shock PBMCs compared to HC ($Z=[-2.61$, $p=[0.009]$) and uninfected SIRS ($Z=[-2.44]$, $p=[0.014]$) (Fig. 3A-(2)).

Biochemical results analysis

There was a highly significant difference in the total nitrite and MDA levels in the plasma between the different patient groups and HC ($H(3)=16.68$, $p=0.001$ and $H(3)=31.55$, $p=0.0001$, respectively) with the highest levels found in patients with septic shock and patients with sepsis ($p<0.001$ for all tests) (Fig. 3B-(1), B-(2)).

Principal component analysis PCA

In this study, the PC analysis was applied to a set of variables (MDA level, NO level, NF-Kb expression, iNOS expression, Nrf2 expression, HO-1 expression and MPO expression). Figure shows the stronger or weaker influence of, respectively, original variable on each of the two principal components.

In either PBMCs or PMNs, PC analysis revealed the significant influence of NF-kB ($r=98\%$ $p=8.44e^{-39}$ and $r=91\%$, $1.64e^{-09}$, respectively) and Nrf2 ($r=94\%$, $p=5.67e^{-25}$ and $r=97\%$, $1.38e^{-14}$, respectively) gene expression in the stratification variation which is notably described by their correlation with dimension 1 (Fig. 4A, B). In PBMCs, the two principal components (Dim1 and Dim2) explained 50% and 22% of the data variance, respectively (Fig. 4A). The sum of the two dimensions explains 72% of the likelihood between individuals. However, in PMNs, subgroups can rarely be distinguished by variables; indeed, the sum of the two dimensions barely exceeds 50% (Dim1 32.1% and Dim2 18.2%) (Fig. 4B).

Correlation analysis

Patients with sepsis, displayed a positive correlation between the NF-kB and Nrf2 gene expressions at

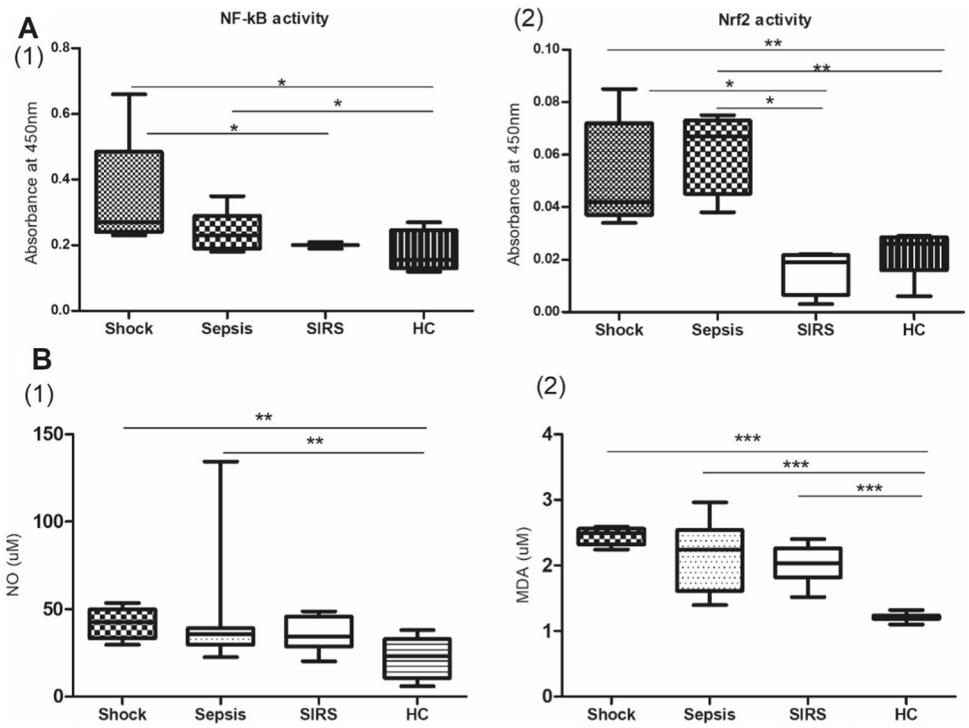

Fig. 3 Nuclear fractions evaluation and biochemical assay **A** Nuclear fraction evaluation of (**1**) NF-kB and (**2**) Nrf2 by the ELISA-SANDWICH assay in PBMC of 15 patients including 5 with sepsis, 5 with septic shock and 5 uninfected SIRS, in comparison with 5 controls. **B** Evaluation of (**1**) nitric oxide NO and (**2**) MDA levels, by Griess reaction and the TBARS method, respectively, in plasma in 40 patients including 20 with sepsis, 10 with septic shock and 10 uninfected SIRS, in comparison with 15 healthy controls. The results are expressed in µM and presented as mean values ± the standard deviation ($p<0.05$) (** *$p<0.001$)

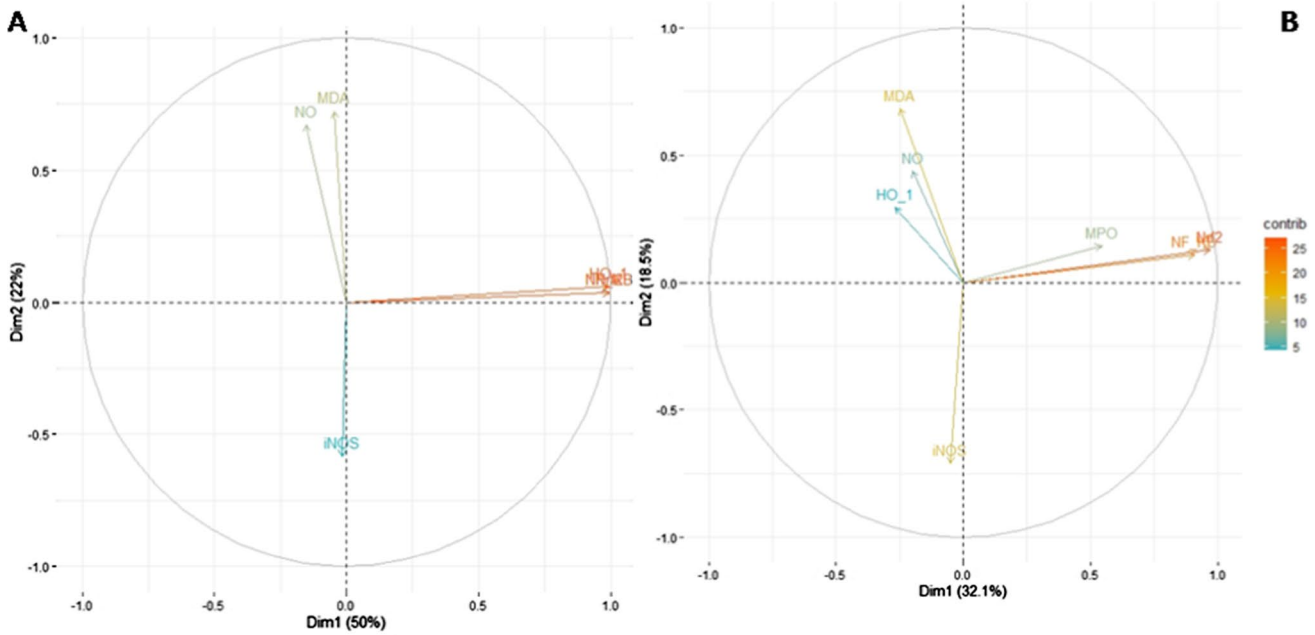

Fig. 4 Results of Principal component analysis: score plot of variables (MDA level, NO level, NF-Kb expression, iNOS expression, Nrf2 expression, HO-1 expression and MPO expression) in PBMCs-A and PMNs-B for all patients groups, uninfected SIRS and healthy controls. Dim1—Dimension 1; Dim2—dimension 3

Table 2 Correlation analysis results

Patients' group	Parameters	*rs* Spearman correlation coefficient	*p*
Sepsis (48h)	NF-kB/MDA	0.610	0.042
	NF-kB/Nrf2	0.569	0.023
Sepsis J7	NF-kB/Nrf2	0.94	0.008
Septic shock (48h)	NF-kB/HO-1	− 1	0.027
	NF-kB/SOFA	0.9	0.037

SOFA Sequential Organ Failure Assessment, *MDA* malondialdehyde

admission ($rs = 0.569$, $p = 0.023$) and on the 7th day ($rs = 0{,}94$ $p = 0.008$) (Table 2). Also, the NF-kB gene expression was positively correlated with the MDA level ($rs = 0.610$, $p = 0.042$). For septic shock patients, the correlation analysis was limited to results at admission. The NF-kB expression correlated negatively with HO-1 gene expression ($rs = -1$, $p = 0.027$) and positively with the Sequential Organ Failure Assessment (SOFA) score ($rs = 0.9$, $p = 0.037$) (Table 2).

Discussion

There is a complex interplay between different biological systems and cell types in sepsis, leading to severe dysregulation of the inflammatory network. Regulatory pathways for this network are still emerging, and we are just beginning to understand them. In this study, we focus on the molecular interactions of the oxidative-inflammatory axis that occur during septic syndrome.

Q-PCR and ELISA analysis demonstrated an increase in NF-kB gene expression and nuclear fraction in PBMCs of septic shock patients compared to those with sepsis and HC. This result is in line with existing reports in the literature indicating a significant increase in the level of activity of NF-kB in PBMC in septic shock patients (Liu and Malik 2006; Arnalich et al. 2000). Indeed, this increase is due to the exaggerated inflammatory state characteristic of the pathophysiology of septic shock, which strongly activates the NF-kB pathway in immune cells (Liu and Malik 2006). A positive correlation was also revealed between the SOFA organ dysfunction score and increased expression of the NF-kB gene supporting the impact of inflammation in tissue damage.

In the same septic inflammatory context, PBMCs in sepsis patients also had a higher expression rate and a nuclear fraction of NF-kB compared to HC and non-infected SIRS. Our results corroborate two previous works showing a high nuclear transcription factor NF-kB activity in the PBMCs of sepsis patients compared to HC (Arnalich et al. 2000) and a high expression of the NF-kB gene in PBMCs in neonatal sepsis compared to HC (AbdAllah et al. 2021). This could be explained by a functional approach to the involvement of infection in the initiation and activation of the NF-kB

pathway. Indeed, the in vitro study showed that the stimulation of macrophages, monocytes, neutrophils with LPS, a component of the membrane of Gram-negative bacteria or with the components of the Gram-positive bacterial membrane, activates the nuclear transcription factor NF-kB (Ulevitch 2000, 2001; Beutler 2000). In addition, a transgenic mouse model study showed that the administration of LPS induces gene expression of NF-kB in several organ sites (Blackwell et al. 2000; Carlsen et al. 2002).

Paradoxially, in septic shock patients PMN cells, the expression low level of the NF-kB gene is decreased. This could be a state of immunoparalysis. Indeed, in septic shock, the overactivation of the immune system leads to an initial high activation of the NF-kB pathway; however, the prolonged exposure to high level of proinflammatory cytokines and the prolonged activation of NF-kB leads to the exhaustion of this pathway, leading to decreased expression of NF-kB and the immunoparalyzed phenotype. This makes the cells less able to respond to invading pathogens and can prolong the recovery time from sepsis (Frazier and Hall 2008). This speculation is sustained by several researchers showing a phenomenon of tolerance of PBMCs, and especially PMNs, in front of bacterial toxins in an acute phase of inflammation reflected by an exhaustion state in PMNs, hence the decrease in gene expression at this stage (Sonego et al. 2016; McCall et al.1993; Adib-Conquy et al. 2000). On the other hand, the expression level of the NF-kB gene revealed in our patients in shock could be downregulated under high-dose catecholamines. Indeed, circulating catecholamines targeting adrenergic receptors on immune cells highly impact NF-κB (Bauer-Dorries et al. 2017). Particularly for neutrophils, adrenergic agents are proved to reduce PMNs responses mainly through B-adrenergic receptors (Wenischet al.1996; Abraham et al. 1999; Farmer and Pugin 2000; Scanzano and Cosentino 2015).

Subsequently, the activation of the NF-kB pathway induces the transcription of the iNOS gene, an enzyme that produces nitric oxide (NO), a molecule with potent antimicrobial properties (Cauwels 2007). Evaluation of iNOS expression in PBMC showed similarity with NF-kB gene expression profile with the highest expression level within septic shock patients as well as a high state of nitrosative stress. Actually, an increased expression of the iNOS gene in septic leukocytes is a characteristic feature of the immune response in sepsis (Welters et al. 2000; Kumar et al. 2019). In this state, the immune system over-produces NO in an attempt to fight off the infection. However, excessive NO production can lead to a phenomenon known as "NO toxicity," which can damage host tissues and contribute to the development of organ dysfunction in sepsis. Indeed, the severity of septic states is closely linked to the toxic overproduction of NO (Vincent et al. 2000; Winkler et al. 2017) and the total nitrite plasma level could be considered as a marker of septic severity and organ dysfunction (Kumar et al. 2019; Kothari et al. 2012; Yu et al. 2018). This increased oxidative stress state in patients with septic syndrome leads to an increase in free radicals and an increase in lipid peroxidation, which is obviously demonstrated by plasma measurement of a lipid peroxidation marker, the MDA in our patients. In fact, elevated MDA levels in sepsis are associated with poor outcomes and increased mortality. MDA levels can be used as a marker to evaluate the severity of sepsis and to monitor the efficacy of treatment (Lorente et al. 2013; Weiss and Deutschman 2014; Fratta Pasini et al. 2016). However, it is important to note that MDA is not a specific marker for sepsis, but a marker of oxidative stress and lipid peroxidation in general. Furthermore, a positive correlation between MDA levels and NF-kB gene expression level in septic patients was revealed confirming that activation and persistence of inflammation are associated with oxidative damage. The main cause of these oxidative damages could be due to the excessive production of prooxidants generated from microbicide activity observed in PMN, notably by the production of bactericidal granules called myeloperoxidase (MPO) (Winterbourn and Kettle 2013). Our results showed a significant difference in MPO gene expression between the different groups of patients and HC. We observed a higher relative expression of MPO in septic shock patients' PMN compared to that of sepsis and non-infected SIRS patients, but it remains statistically nonsignificant. Similarly, a previous work has shown that MPO could be a useful marker to differentiate septic states from uninfected SIRS (Schrijver et al. 2017). This nonsignificant difference can be explained, on the one hand, by the small sample size and, on the other hand, by the prescribed treatment. Indeed, all of our patients included in the sepsis and/or septic shock group received broad-spectrum antibiotics. To defend itself against oxygenated and nitrogenous toxic substances, the Nrf2 pathway is activated in the immune cells. Real-time PCR analysis and ELISA studies indicated a significant increase in Nrf2 expression in PBMC of sepsis, septic shock and SIRS patients compared to HC. Our results are consistent with several studies showing that in the presence of an imbalance redox status, Nrf2 tends to be more activated (Fratta Pasini et al. 2016; Zhang et al. 2018; Zaza et al. 2013).

Indeed, PMNs showed the same expression profile previously reported with NF-kB; the septic patient group highly express Nrf2 compared to other groups.

When evaluating expression levels in PBMC and PMN, obtained from sepsis patients and septic shock patients, a higher expression of Nrf2 was found in PMN as compared to PBMC. Existing reports in the literature demonstrate that the resultant imbalance due to the overproduction of (ROS/RNS) strongly activates the expression of the Nrf2 gene at the PMN cell type (Joshi and Werner 2017). Thimmulappa et al. (2006a,

b), reported that Nrf2 functions as a critical factor for sepsis survival in mouse models, focusing on the protective role of Nrf2 in regulating the innate immune response by mitigating the oxidative stress generated, in particular, by the oxygen-dependent microbicide activity observed in PMN. Besides, a reciprocal relationship was observed linking NF-kB to Nrf2 in monocytes, which was confirmed by the positive correlation revealed between nuclear fractions/gene expression of NF-kB and Nrf2 during the first phase of the septic syndrome (sepsis and septic shock). Indeed, several previous studies have consolidated the concept of cross talk between these two factors (Wardyn et al. 2015; Kostyuk et al. 2018; Buelna-Chontal and Zazueta 2013). This reciprocal relationship reflects the regulation of NF-kB activity by Nrf2, and vice versa, with a mechanism that is not yet well understood.

In mild or moderate stress, Nrf2 seems to be more active than NF-kB in case of sepsis. However, when stress increases, NF-kB becomes more active than Nrf2 in septic shock patients (Kostyuk et al. 2018; Buelna-Chontal and Zazueta 2013). Our results indicate that PBMC of septic shock patients expressed weakly the Nrf2 and highly NF-kB, contrary to sepsis patients. The work of Neilson et al. (2020) reported a decrease in Nrf2 expression in PBMC along with the progression of inflammatory diseases. Indeed, the ability of Nrf2 to rebalance the redox homeostasis becomes outdated in the face of a worsening state of oxidative stress. NF-kB, being the most potent in this case, is more activated and inhibits the expression of the Nrf2 gene and, subsequently, the transcription of these target genes, such as HO-1, by increasing the abundance of Keap1 nuclear levels (Yu et al. 2011). This was supported by the negative correlations revealed between the expression of the HO-1 and NF-kB genes in patients in shock and the expression of the HO-1 gene and MDA levels in non-infected SIRS patients. These data corroborate with later research reporting the inhibition of NF-kB activity by HO-1 (Brown and Jones 2004) and that the presence of ROS/RNS is accompanied by an increase in Nrf2 activity, followed by an increase in HO-1(Zhang et al. 2018). PC analysis shows that Dim-1 is in agreement with our experimental results and confirms a clinically relevant implication of NF-kB and Nrf2 transcription factors in sepsis pathogenesis which is in conjunction with many lines of evidence obtained from several experimental and clinical studies thus far reported. These preliminary results would be of a high clinical pertinence if correlated with clinical outcome, so that we may target the more useful cell type for gene analysis to predict the clinical outcome.

Conclusions for future biology

Overall, our findings confirm a failure in the antioxidant defenses and an impaired innate immune response mainly in the septic shock state. This result supports our belief that administering immunostimulatory agents to septic shock patients. We suppose that administering immunostimulatory agents to septic shock patients may be beneficial. Immune monitoring protocols are thus required in order to identify patients who may benefit from immunomodulatory trials.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42977-023-00168-1.

Acknowledgements The authors acknowledge the financial support provided by Habib Bourguiba University hospital of Sfax in Tunisia.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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