Intensive Care Med (2010) 36:1859–1866
DOI 10.1007/s00134-010-1962-x

ORIGINAL

Caroline Landelle
Alain Lepape
Nicolas Voirin
Eve Tognet
Fabienne Venet
Julien Bohé
Philippe Vanhems
Guillaume Monneret

Low monocyte human leukocyte antigen-DR is independently associated with nosocomial infections after septic shock

Received: 12 October 2009
Accepted: 30 May 2010
Published online: 23 July 2010

This article is discussed in the editorial available at:
doi:10.1007/s00134-010-1965-7.

Electronic supplementary material
The online version of this article (doi:10.1007/s00134-010-1962-x) contains supplementary material, which is available to authorized users.

C. Landelle · N. Voirin · P. Vanhems
Laboratoire de Biométrie et Biologie Evolutive, Epidémiologie et Santé Publique, CNRS, UMR 5558, Université Lyon 1; Université de Lyon, Lyon, France

C. Landelle · N. Voirin · P. Vanhems
Hospices Civils de Lyon, Hôpital Edouard Herriot, Service d'Hygiène, Epidémiologie et Prévention, Lyon, France

A. Lepape
Hospices Civils de Lyon, Centre Hospitalier de Lyon Sud, Service de Réanimation Chirurgicale, Lyon, France

E. Tognet · J. Bohé
Hospices Civils de Lyon, Centre Hospitalier de Lyon Sud, Service de Réanimation Médicale, Lyon, France

F. Venet · G. Monneret (✉)
Laboratoire d'Immunologie,
Hôpital E. Herriot, Hospices Civils de Lyon,
Pavillon E-5 place d'Arsonval,
69437 Lyon Cedex 03, France
e-mail: guillaume.monneret@chu-lyon.fr
Tel.: +33-4-72119758
Fax: +33-4-72119753

Abstract *Purpose:* Sepsis-induced immunosuppression is postulated to contribute to a heightened risk of nosocomial infection (NI). This prospective, single-center, observational study was conducted to assess whether low monocyte human leukocyte antigen-DR expression (mHLA-DR), proposed as a global biomarker of sepsis immunosuppression, was associated with an increased incidence of NI after septic shock. *Methods:* The study included 209 septic shock patients. mHLA-DR was measured by flow cytometry at days (D) 3–4 and 6–9 after the onset of shock. After septic shock, patients were screened daily for NI at four sites (microbiologically documented pulmonary, urinary tract, bloodstream, and catheter-related infections). A competing risk approach was used to evaluate the impact of low mHLA-DR on the incidence of NI. *Results:* At D3–4, we obtained measurements in 153 patients. Non-survivors ($n = 51$) exhibited lower mHLA-DR values expressed as means of fluorescence intensities than survivors ($n = 102$) (33 vs. 67; $p < 0.001$). The patients who developed NI ($n = 37$) exhibited lower mHLA-DR values than those without NI ($n = 116$) (39 vs. 65; $p = 0.008$). mHLA-DR ≤ 54 remained independently associated with NI occurrence after adjustment for clinical parameters (gender, simplified acute physiology score II, sepsis-related organ failure assessment, intubation, and central venous catheterization) with an adjusted hazards ratio (aHR) of 2.52 (95% CI 1.20–5.30); $p = 0.02$. Similarly, at D6–9, low mHLA-DR (≤ 57) remained independently associated with NI with an aHR of 2.18 (95% CI 1.04–4.59); $p = 0.04$. *Conclusions:* In septic shock patients, after adjustment with usual clinical confounders (including ventilation and central venous catheterization), persistent low mHLA-DR expression remained independently associated with the development of secondary NI.

Keywords mHLA-DR · Immunosuppression · Nosocomial infection · Sepsis · Septic shock

Introduction

Septic shock, the most serious clinical inflammatory response to infection, accounts for about 9% of admissions to intensive care units (ICUs), and its incidence is increasing [1]. It is characterized by high rates of nosocomial infections (NI) [2] and mortality, ranging from 40 to 60% [1, 3]. At onset, septic shock is characterized by massive release of inflammatory mediators, which are responsible for organ dysfunction and hypoperfusion [4]. In parallel, the body develops compensatory mechanisms to prevent excessive systemic inflammation. These mechanisms may rapidly become deleterious, since virtually all patients present features consistent with immunosuppression [5–7]. Such alterations might be directly responsible for worsening the outcome and may play a major role in the decreased resistance to NI in patients who survive initial resuscitation. As evidence for this, a major segment of NI in septic patients is due to commensals, which become pathogenic solely in fragile, immunocompromised hosts [8]. Consequently, in the absence of specific clinical signs of immune status in patients, biomarkers of immunosuppression are highly desirable.

Diminished monocyte human leukocyte antigen-DR expression (mHLA-DR) on the cell surface is proposed as a reflection of monocyte deactivation in critically ill patients [9, 10]. A number of studies demonstrated a correlation between decreased mHLA-DR and mortality in septic patients. More specifically, in two of them, mHLA-DR appeared as an independent predictor of 28-day mortality in septic shock patients [11, 12]. Nevertheless, none has specifically focused on a putative link between low mHLA-DR expression and NI occurrence in these patients. Interestingly, univariate analysis, in small cohorts of injured patients (trauma, burns, surgery, i.e., patients who present with similar mechanisms of immunosuppression after systemic inflammatory responses), tended to indicate that the lowest mHLA-DR values occur in patients who subsequently develop secondary NI, whereas mHLA-DR levels increase generally within 1 week in individuals who recover uneventfully [13–15].

The aim of the present study was therefore to evaluate whether low mHLA-DR expression is associated with an elevated secondary NI rate in septic shock patients who survive the initial 48 h of the syndrome.

Materials and methods

Study population

A total of 209 consecutive septic shock patients were included from 1 December 2001 through 30 April 2005. These patients were described previously in detail regarding epidemiological aspects of NI after septic shock [2]. The relationship between mHLA-DR and mortality was assessed earlier in a subgroup of patients [12]. The diagnosis of septic shock was based on classical criteria defined by the American College of Chest Physicians/Society of Critical Care Medicine [16]. Additional details regarding patients and methods are provided in the "Electronic supplementary material". The timing of septic shock onset (T0) was defined by the start of vasopressor treatment. Septic shock treatment was performed in accordance with current international guidelines. Exclusion criteria were patients younger than age 18 years old or subjects with aplasia. During follow-up, from admission to ICU discharge, clinical and biological data were collected from two sources: the national ICU NI surveillance network [17, 18] and specific data on all septic shock patients including scores. During ICU stay, patients were screened daily for exposure to invasive device (intubation, indwelling urinary catheter, and central venous line) and for four NI sites: microbiologically documented pulmonary (PI), urinary tract (UTI), bloodstream (BSI), and catheter-related infections (CRI). Definitions of NI were those used by the European Network for Surveillance of NI in ICUs.

mHLA-DR measurement by flow cytometry

The expression of cell surface HLA-DR on monocytes was assessed at D3–4 (days 3–4) and D6–9 (days 6–9) by standardized flow cytometry in peripheral whole blood collected in EDTA anticoagulant tubes. Staining and cell acquisition were undertaken as described previously [12, 19].

Statistical analysis

Baseline characteristics were described by frequencies, medians, and interquartile ranges (IQR). Groups were compared by the Mann–Whitney U test and Pearson's χ^2 test or Fisher's exact test as appropriate for continuous and categorical variables. The mHLA-DR results obtained at two time periods (D3–4 and D6–9) were analyzed; the lowest levels per period were selected. For patients who acquired NI, the expression of mHLA-DR was censored at the first NI onset. Moreover, follow-up was stopped after 28 days. mHLA-DR expression was stratified according to the best threshold indicated by receiver operating characteristic (ROC) curves at D3–4 and D6–9 (i.e., maximized sensitivity and specificity). The incidence of NI among patients according to these thresholds was compared by using cumulative incidence curves [20]. The cumulative incidence depends on both the risk of failure attributable to the event of interest (presence of NI) and the risk of failure

attributable to two competing events (discharge alive and death). A proportional subdistribution hazards model was used to compute the time to the onset of NI and to investigate risk factors for NI. The Fine–Gray extension [21] of the Cox model was adopted to take the two competing risks into account. Adjustments were made for gender, simplified acute physiology score II (SAPS II), sepsis-related organ failure assessment (SOFA), intubation, and central venous catheterization. Time 0 was the day of septic shock onset. The model was determined by a backward procedure based on the Wald test. Variables with $p \leq 0.20$ were conserved in the model. Adjusted hazard ratios (aHRs) of NI and 95% confidence intervals (95% CI) were calculated. All statistical tests were two-tailed, and $p < 0.05$ was considered to be statistically significant. Analyses were performed by SPSS (version 11.5, SPSS, Inc., Chicago, IL, USA) and R software [22].

Results

Septic shock patient characteristics

A total of 209 consecutive patients with septic shock were treated in the two ICUs. Twenty-three (11%) patients died within 48 h after the onset of shock. Thirty-three (16%) patients had no mHLA-DR measurement at D3–4 because blood samples were not collected on Saturdays and Sundays during which the laboratory did not operate. mHLA-DR expression at D3–4 was therefore available for 153 patients (Fig. 1). There were no differences in baseline characteristics between the 33 patients without and the 153 patients with mHLA-DR values at D3–4 (data not shown). Tables 1 and S1 report the characteristics of the 153 remaining patients analyzed. Thirty-seven (18%) patients of the total cohort died before D9. mHLA-DR expression at D6–9 was therefore available for 121 patients (Fig. 1).

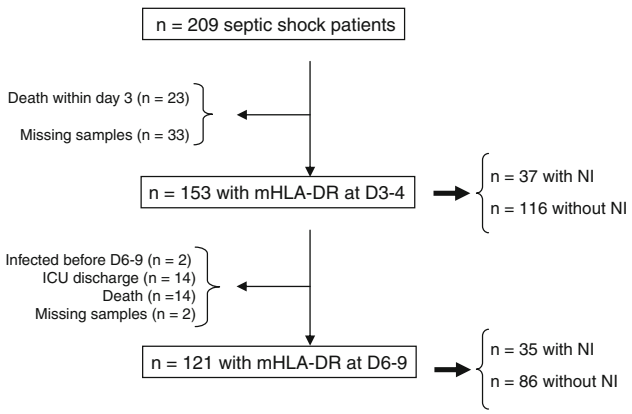

Fig. 1 Flow diagram of enrolled patients. *mHLA-DR* monocyte human leukocyte antigen-DR, *NI* nosocomial infection occurring within 28 days after the onset of septic shock, *ICU* intensive care unit, *D3–4* days 3 to 4, *D6–9* days 6 to 9

Nosocomial infections

Tables 1 and S1 depict the baseline characteristics of patients with ($n = 37$) and without NI ($n = 116$) within 28 days after septic shock onset. There was no difference regarding admission categories (medical vs. surgical, Table S1) between patients that will develop NI or not. Twenty-five patients developed NI at one site, 10 patients at two sites, and 2 patients at three sites. PI ($n = 26$) was the most frequent NI, followed by UTI ($n = 11$), CRI ($n = 7$), and BSI ($n = 7$). A total of 58 pathogens were isolated from the 51 NI. PIs were mostly caused by *Pseudomonas aeruginosa* (26%), *enterobacteriaceae* (23%), and *Staphylococcus aureus* (13%). Fungi accounted for 42% of all isolates from UTI. Forty-three percent of the BSI and 62% of the CRI were caused by gram-positive cocci. The median time (IQR) between septic shock and NI onset was 14 days (8.5–17.5). Higher SAPS II and SOFA values at onset of septic shock were recorded in patients who were going to develop NI, indicating a greater level of severity. Moreover, these patients were characterized by a lower proportion of microbiologically documented initial septic shock, greater exposure to intubation, longer duration of device exposure (urinary tract and central venous catheterization), increased proportion of renal replacement therapy, and greater median length of ICU stay after septic shock onset (Tables 1 and S1). Twenty-eight-day and hospital crude mortality rates were similar between groups, whereas ICU mortality rate was significantly higher in patients with NI (Table 1).

mHLA-DR expression

We first confirmed our previous results regarding the prediction of mortality at D3–4 [12]. In the present study and as expected, mHLA-DR at D3–4 was diminished in comparison with normal values and non-survivors ($n = 51$) exhibited lower mHLA-DR values than survivors ($n = 102$) (20 vs. 43%, $p < 0.001$). Similar conclusions were reached when mHLA-DR results were expressed as means of fluorescence intensities (MFI) (33 in non-survivors vs. 67 in survivors, $p < 0.001$). There were no difference in baseline characteristics and mHLA-DR values between the cohort of patients of the previous study and the rest of the cohort (data not shown).

At D3–4, the 37 patients who were going to develop an NI exhibited lower mHLA-DR values, expressed as MFI, than those without NI (39 vs. 65; $p = 0.008$, Table 1). From ROC curve analysis, 54 was the best cutoff value to predict NI development [area under the curve (AUC) 0.65, $p = 0.008$, sensitivity (se) 68%, specificity (sp) 62%,

Table 1 Baseline characteristics of 153 septic shock patients classified by development or not of nosocomial infection

Variable	Total ($n = 153$)	Patients with NI ($n = 37$)	Patients without NI ($n = 116$)	*P* value
Baseline descriptors				
Gender (male)	99 (64.7)	23 (62.2)	76 (65.5)	0.7
Age (years)	64.9 (49.0–75.5)	63.3 (52.0–73.8)	65.4 (47.8–75.8)	0.9
Septic shock characteristics				
SAPS II score at onset of septic shock	51.0 (41.5–62.0)	54.0 (46.5–65.5)	49.0 (39.0–61.0)	**0.04**
SOFA score at days 1–2 after onset of shock	10 (8–12)	11 (9.5–12)	9 (8–12)	**0.04**
mHLA-DR measurements (% of positive monocytes)				
Days 3–4	37.6 (20.2–52.7)	26.6 (15.8–44.3)	39.5 (23.7–54.6)	**0.01**
Days 6–9	45.3 (26.7–61.0)	34.5 (23.3–58.2)	49.9 (30.3–61.2)	**0.04**
mHLA-DR measurements (means of fluorescence intensities)				
Days 3–4	59.0 (30.1–89.8)	39.0 (27.0–61.9)	65.3 (34.5–93.9)	**0.008**
Days 6–9	60.0 (35.8–105.0)	49.3 (28.0–72.0)	67.3 (42.2–111.3)	**0.01**
Interventions				
Intubation	133 (86.9)	36 (97.3)	97 (83.6)	**0.04**
Duration of mechanical ventilation (days)	10.0 (5.0–16.3)	12.0 (7.0–20.5)	9.0 (4.5–15.5)	0.06
Central venous catheterization	149 (97.4)	37 (100)	112 (96.6)	0.6
Duration of central venous catheterization (days)	12.0 (8.0–21.0)	21.0 (15.0–28.5)	10.0 (6.0–16.8)	**<0.001**
Urinary tract catheterization	142 (92.8)	35 (94.6)	107 (92.2)	0.9
Duration of urinary tract catheterization (days)	11.0 (6.0–20.0)	19.0 (10.0–27.0)	10.0 (6.0–16.0)	**0.001**
Outcomes				
Length of hospital stay after septic shock onset (days)	29.0 (14.5–35.0)	29.0 (24.5–51.0)	29.0 (12.3–30.0)	0.07
Length of ICU stay after septic shock onset (days)	13.0 (8.0–26.0)	27.0 (16.5–39.0)	11.0 (6.3–19.0)	**<0.001**
ICU death rate within 28 days of septic shock onset	33.3%	37.8%	31.9%	0.5
ICU crude death rate	37.9%	54.1%	32.8%	**0.02**
Hospital crude death rate	45.8%	59.5%	41.4%	0.06

Characteristics are described as frequencies and percentages (%) for categorical data and medians and interquartile ranges (IQR) for continuous variables. Groups were compared by Pearson's χ^2 test or Fisher's exact test as appropriate and the Mann–Whitney *U* test for categorical and continuous variables, respectively. Bold values for $p < 0.05$

NI nosocomial infections, *SAPS II* simplified acute physiology score II, *SOFA* sepsis-related organ failure assessment, *mHLA-DR* monocyte human leukocyte antigen-DR, *ICU* intensive care unit

positive predictive value (PPV) 36%, negative predictive value (NPV) 86%]. For patients with mHLA-DR >54, the probability of developing an NI within the next 28 days was 14.3% (95% CI 7.8–22.7), whereas for patients with mHLA-DR ≤54 this probability was 36.2% (95% CI 25.0–47.5), according to cumulative incidence curves. This difference was statistically significant ($p = 0.002$, Fig. 2a). Hazard ratios adjusted for gender, SAPS II and SOFA scores, intubation, and central venous catheterization revealed that low mHLA-DR (≤54) at D3–4 was independently associated with a higher incidence of NI [aHR 2.52 (95% CI 1.20–5.30); $p = 0.02$].

Similar conclusions were reached when mHLA-DR results were expressed as percentages. We also observed significantly lower mHLA-DR percentages in patients with NI (27 vs. 39%; $p = 0.01$, Table 1). ROC curve analysis showed that 30% was the best cutoff value to predict NI development (AUC 0.64, $p = 0.01$, se 57%, sp 65%, PPV 34%, NPV 82%). For patients with mHLA-DR >30%, the probability of developing an NI within the next 28 days was 17.6% (95% CI 10.6–26.1), whereas for patients with mHLA-DR ≤30% this probability was 33.9% (95% CI 22.3–45.8), according to cumulative incidence curves. This difference was statistically significant ($p = 0.03$, Fig. 2b). In multivariate analysis, mHLA-DR and SOFA score remained in the model, with an aHR of NI of 1.80 (95% CI 0.92–3.53), $p = 0.09$ for patients with mHLA-DR ≤30% compared to >30%. However, when not adjusted on SOFA score, the model gave a significant aHR of NI of 2.04 (95% CI 1.07–3.89), $p = 0.03$.

At D6–9, we were able to measure mHLA-DR expression in 121 patients (Fig. 1) of whom 35 (29%) presented with NI. The 35 patients with NI exhibited lower MFI values at D6–9 than those without NI (49 vs. 67; $p = 0.01$, Table 1). ROC curve analysis established that 57 was the best cutoff value to predict NI (AUC 0.64, $p = 0.01$, se 66%, sp 60%, PPV 40%, NPV 81%). For patients with mHLA-DR >57, the probability of developing NI within the next 28 days was 18.8% (95% CI 10.2–29.3), and for patients with mHLA-DR ≤57 this probability was 40.4% (95% CI 27.6–52.8), according to cumulative incidence curves. This difference was statistically significant ($p = 0.008$, Fig. 2c). Hazard ratios adjusted for gender, SAPS II and SOFA scores, intubation, and central venous catheterization revealed that low mHLA-DR (≤57) at D6–9 was independently associated with a higher incidence of NI [aHR 2.18 (95% CI 1.04–4.59); $p = 0.04$].

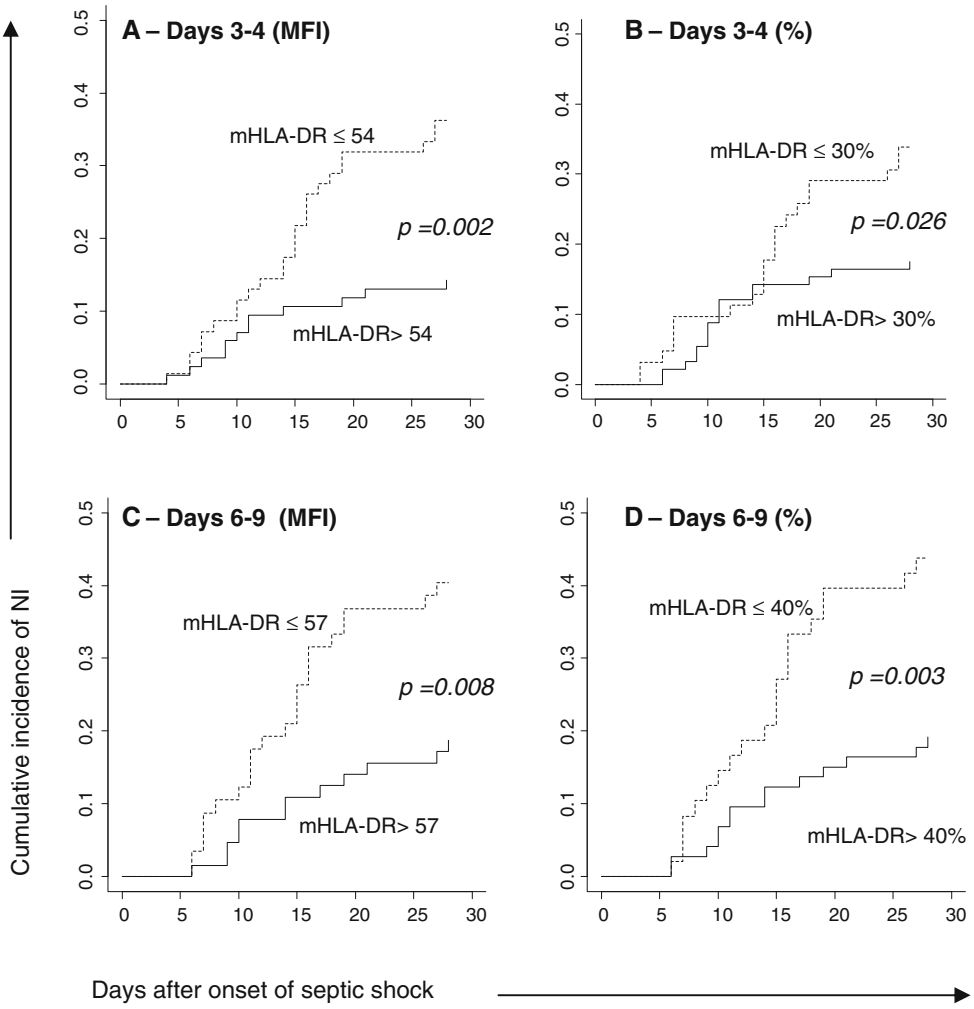

Fig. 2 Cumulative incidence curves for nosocomial infections stratified based on mHLA-DR expressions in septic shock patients. Comparison of estimated incidence of nosocomial infection (*NI*) by means of cumulative incidence curves for **a** patients with monocyte human leukocyte antigen-DR (*mHLA-DR*) values expressed as means of fluorescence intensities (*MFI*) at D3–4 of >54 (*continuous line*) and ≤54 (*dashed line*), **b** patients with mHLA-DR values expressed as percentages at D3–4 of >30% (*continuous line*) and ≤30% (*dashed line*), **c** patients with mHLA-DR values expressed as MFI at D6–9 of >57 (*continuous line*) and ≤57 (*dashed line*), and **d** patients with mHLA-DR values expressed as percentages at D6–9 of >40% (*continuous line*) and ≤40% (*dashed line*)

The difference between groups was also significant when results were expressed as percentages. The 35 patients who were going to develop an NI exhibited lower mHLA-DR values than those without NI (34 vs. 50%; $p = 0.04$, Table 1). ROC curve analysis disclosed that 40% was the best cutoff value to predict NI (AUC 0.62, $p = 0.03$, se 60%, sp 69%, PPV 44%, NPV 81%). For patients with mHLA-DR >40, the probability of developing an NI within the next 28 days was 19.2% (95% CI 11.1–29.0), and for patients with mHLA-DR ≤40 this probability was 43.8% (95% CI 29.4–57.2), according to cumulative incidence curves. This difference was statistically significant ($p = 0.003$, Fig. 2d). Hazard ratios adjusted for gender, SAPS II and SOFA scores, intubation, and central venous catheterization revealed that low mHLA-DR (≤40%) at D6–9 was independently associated with a higher incidence of NI [aHR 2.44 (95% CI 1.23–4.87); $p = 0.01$].

There were no differences in mHLA-DR values between the patients who developed only one NI and the patients who developed more than one NI (data not shown).

Discussion

Septic syndromes represent a major, albeit largely underrecognized healthcare problem worldwide characterized by persistently high mortality and increased NI rates. It is now agreed that sepsis deeply perturbs immune homeostasis by inducing severe, delayed immune alterations which are thought to play a major role in the impaired clearance of microorganisms. Together with high exposure to invasive devices [23], these alterations could explain patients' decreased resistance to secondary NI [2, 24, 25].

Decreased mHLA-DR expression has been proposed as a biomarker of monocyte deactivation/immunosuppression in ICU patients. This is supported by functional studies demonstrating a link between low levels of mHLA-DR and impairment of monocytic cellular functions, including

loss of their pro-inflammatory properties and reduction of their capacity to induce antigen-specific T-cell responses [26–29]. Previous studies measuring mHLA-DR in ICU patients reported its association with fatal outcome. However, evaluation of mHLA-DR in regard to the development of NI in septic shock patients has never been performed.

In this context, the main result of the present study is to show that septic shock patients with reduced mHLA-DR were more prone to develop NI, while patients free of NI had significantly higher mHLA-DR suggesting a better immune recovery. Importantly, after adjustment for the usual clinical confounders, mHLA-DR remained an independent predictor of NI development.

Our results are in line with data obtained in others types of ICU patients such as trauma [13], surgery [14], pancreatitis [30], and burns [15]. However, these investigations were limited by the very low number of patients included and the lack of standardization in both NI definitions and flow cytometry protocols. For example, in a work focused on the role of corticosteroids in lowering mHLA-DR during sepsis in 30 patients with septic shock or sepsis, Le Tulzo et al. [31] observed that six patients who developed NI exhibited a persistent loss of mHLA-DR expression, whereas 24 subjects free from NI had partially restored mHLA-DR expression at days 5–7. The only study which adopted a multivariate model for statistical analysis was performed in pediatric cardiac ICUs: Allen et al. [32] reported that low mHLA-DR remained an independent predictor of sepsis development after adjustment for age, gender, complexity of surgery, physiological state on arrival at the ICU, and surgeon. Although obtained in a different population, the results of Allen et al. are very close to ours. Accordingly, Grienay et al. reported that, in ICU patients with a length of stay >7 days, a lower slope of mHLA-DR recovery was associated with a higher incidence of the secondary infection [33]. Together these data suggest that the development of a state of immunosuppression and most importantly its persistence over time are to be considered as an important risk factor for the occurrence of secondary NI in ICU patients besides the usual risk factors (i.e., mainly exposure to invasive devices).

In that sense, mHLA-DR measurement may also be useful for patient stratification in clinical trials attempting to restore immune functions in ICU patients [34, 35]. Patients with low mHLA-DR should be considered as immunosuppressed, comparable to transplantation or post-chemotherapy patients, and thus they could benefit from a focused strategy aimed at dampening infectious risk: intensive screening of infections, limitation as much as possible of other risk factors (catheters, invasive devices), or strict precautions to limit cross transmission. As an illustrative example, a recent work showed that mHLA-DR-guided GM-CSF therapy in sepsis is safe and effective for restoring monocytic immunocompetence. Use of GM-CSF may shorten the time of mechanical ventilation and hospital/ICU stay [36].

Our study has some limitations. Firstly, although NI was diagnosed by two investigators (especially to eliminate extension or relapse of the initial septic episode), we cannot totally exclude the possibility that if septic shock had not been documented by pathogen isolation, the NI was a new and different infectious episode. That given, this could not account for a large number of misclassifications, since 80% of septic shock cases were confirmed microbiologically. Secondly, the selected NI presented various levels of severity (e.g., UTI) but were all microbiologically and most importantly clinically documented. These device-associated NI (except BSI), collected before post-ICU discharge, were a measure of infectious risk in ICU. Thirdly, although multivariate analysis took into account gender, severity at admission, and exposure to invasive devices—a major risk factor of NI—some other potential risk factors known to be related to NI, such as cross transmission, the ratio of nurses to patients, and level of compliance with standard precautions could not be considered in the present work. Finally, despite the fact that the study involved two ICUs with a very different case mix, the reproducibility of these cutoff values should be validated prospectively in a multicenter trial to strengthen the data. Of note, these values should be now standardized and expressed by number of antibodies bound per cell instead of MFI since the latter do not allow comparison between centers [37].

In conclusion, our findings demonstrate that after septic shock, low mHLA-DR expression was a significant predictor of NI risk in the initial phase (D3–4) of septic shock as well as later (D6–9). Most importantly, after adjustment for the usual clinical confounders (including ventilation and central venous catheterization), mHLA-DR remained an independent predictor of NI development. This may help to identify patients at increased risk of NI in whom specific preventive strategies could be developed.

Acknowledgments The authors are grateful to Professor Christian Brun-Buisson, Dr. Raphaele Girard, all the intensivists of the Centre Hospitalier Lyon Sud, CCLIN Sud Est. The study was conducted thanks to the logistic support of Centre d'Investigation Clinique (CIC 201) of INSERM and Hospices Civils de Lyon. Thanks are also due to Ovid Da Silva for editing this manuscript. The present work was supported by Hospices Civils de Lyon. In addition, C. Landelle was funded by Hospices Civils de Lyon and Centre National de la Recherche Scientifique (Contrat d'Assistant Hospitalier de Recherche HCL/CNRS). C. Landelle received the International Sepsis Forum (ISF) Sepsis Award for a part of this work during the joint ESCMID (European Society of Clinical Microbiology and Infectious Disease)/ISF Sepsis Forum on May 2009 at the 19th ECCMID (European Congress of Clinical Microbiology and Infectious Disease) in Helsinki, Finland.

Conflict of interest statement None of the authors have any financial interests or affiliations with commercial organizations whose products or services are related to the subject matter of this manuscript (i.e., no existing conflicts of interest).

References

1. Annane D, Aegerter P, Jars-Guincestre MC, Guidet B, Network ft C-R (2003) Current epidemiology of septic shock. The CUB-Réa Network. Am J Respir Crit Care Med 168:165–172
2. Landelle C, Lepape A, Français A, Tognet E, Thizy H, Voirin N, Timsit JF, Monneret G, Vanhems P (2008) Nosocomial infection after septic shock among intensive care unit patients. Infect Control Hosp Epidemiol 29:1054–1065
3. Friedman G, Silva E, Vincent JL (1998) Has the mortality of septic shock changed with time. Crit Care Med 26:2078–2086
4. Annane D, Bellissant E, Cavaillon JM (2005) Septic shock. Lancet 365:63–78
5. Reddy RC, Chen GH, Tekchandani PK, Standiford TJ (2001) Sepsis-induced immunosuppression: from bad to worse. Immunol Res 24:273–287
6. Hotchkiss RS, Karl IE (2003) The pathophysiology and treatment of sepsis. N Engl J Med 348:138–150
7. Munford RS, Pugin J (2001) Normal responses to injury prevent systemic inflammation and can be immunosuppressive. Am J Respir Crit Care Med 163:316–321
8. Pugin J (2007) Immunostimulation is a rational therapeutic strategy in sepsis. Novartis Found Symp 280:21–27; discussion 27–36, 160–164
9. Monneret G, Venet F, Pachot A, Lepape A (2008) Monitoring immune dysfunctions in the septic patient: a new skin for the old ceremony. Mol Med 14:64–78
10. Schefold JC, Hasper D, Volk HD, Reinke P (2008) Sepsis: time has come to focus on the later stages. Med Hypotheses 71:203–208
11. Caille V, Chiche JD, Nciri N, Berton C, Gibot S, Boval B, Payen D, Mira JP, Mebazaa A (2004) Histocompatibility leukocyte antigen-D related expression is specifically altered and predicts mortality in septic shock but not in other causes of shock. Shock 22:521–526
12. Monneret G, Lepape A, Voirin N, Bohé J, Venet F, Debard AL, Thizy H, Bienvenu J, Gueyffier F, Vanhems P (2006) Persisting low monocyte human leukocyte antigen-DR expression predicts mortality in septic shock. Intensive Care Med 32:1175–1183
13. Hershman MJ, Cheadle WG, Wellhausen SR, Davidson PF, Polk HC Jr (1990) Monocyte HLA-DR antigen expression characterizes clinical outcome in the trauma patient. Br J Surg 77:204–207
14. Cheadle WG, Hershman MJ, Wellhausen SR, Polk HC Jr (1991) HLA-DR antigen expression on peripheral blood monocytes correlates with surgical infection. Am J Surg 161:639–645
15. Venet F, Tissot S, Debard AL, Faudot C, Crampé C, Pachot A, Ayala A, Monneret G (2007) Decreased monocyte human leukocyte antigen-DR expression after severe burn injury: correlation with severity and secondary septic shock. Crit Care Med 35:1910–1917
16. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RM, Sibbald WJ (1992) Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest 101:1644–1655
17. Vanhems P, Lepape A, Savey A, Jambou P, Fabry J (2000) Nosocomial pulmonary infection by antimicrobial-resistant bacteria of patients hospitalized in intensive care units: risk factors and survival. J Hosp Infect 45:98–106
18. HELICS (2005) Surveillance of nosocomial infections in intensive care units. http://helics.univ-lyon1.fr/documents/icu_protocol.pdf. Accessed 15 June 2009
19. Monneret G, Elmenkouri N, Bohe J, Debard AL, Gutowski MC, Bienvenu J, Lepape A (2002) Analytical requirements for measuring monocytic human lymphocyte antigen DR by flow cytometry: application to the monitoring of patients with septic shock. Clin Chem 48:1589–1592
20. Gray RJ (1988) A class of K-sample tests for comparing the cumulative incidence of a competing risk. Ann Stat 16:1141–1154
21. Fine J, Gray RJ (1999) A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc 94:496–509
22. R Development Core Team (2009) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. http://www.R-project.org
23. Vincent JL (2003) Nosocomial infections in adult intensive-care units. Lancet 361:2068–2077
24. Brawley RL, Weber DJ, Samsa GP, Rutala WA (1989) Multiple nosocomial infections. An incidence study. Am J Epidemiol 130:769–780
25. Alberti C, Brun-Buisson C, Burchardi H, Martin C, Goodman S, Artigas A, Sicignano A, Palazzo M, Moreno R, Boulmé R, Lepage E, Le Gall R (2002) Epidemiology of sepsis and infection in ICU patients from an international multicentre cohort study. Intensive Care Med 28:108–121
26. Wolk K, Docke WD, von Baehr V, Volk HD, Sabat R (2000) Impaired antigen presentation by human monocytes during endotoxin tolerance. Blood 96:218–223
27. Astiz M, Saha D, Lustbader D, Lin R, Rackow E (1996) Monocyte response to bacterial toxins, expression of cell surface receptors, and release of anti-inflammatory cytokines during sepsis. J Lab Clin Med 128:594–600
28. Manjuck J, Saha DC, Astiz M, Eales LJ, Rackow EC (2000) Decreased response to recall antigens is associated with depressed costimulatory receptor expression in septic critically ill patients. J Lab Clin Med 135:153–160
29. Piani A, Hossle JP, Birchler T, Siegrist CA, Heumann D, Davies G, Loeliger S, Seger R, Lauener RP (2000) Expression of MHC class II molecules contributes to lipopolysaccharide responsiveness. Eur J Immunol 30:3140–3146
30. Satoh A, Miura T, Satoh K, Masamune A, Yamagiwa T, Sakai Y, Shibuya K, Takeda K, Kaku M, Shimosegawa T (2002) Human leukocyte antigen-DR expression on peripheral monocytes as a predictive marker of sepsis during acute pancreatitis. Pancreas 25:245–250
31. Le Tulzo Y, Pangault C, Amiot L, Guilloux V, Tribut O, Arvieux C, Camus C, Fauchet R, Thomas R, Drénou B (2004) Monocyte human leukocyte antigen-DR transcriptional downregulation by cortisol during septic shock. Am J Respir Crit Care Med 169:1144–1151
32. Allen ML, Peters MJ, Goldman A, Elliott M, James I, Callard R, Klein NJ (2002) Early postoperative monocyte deactivation predicts systemic inflammation and prolonged stay in pediatric cardiac intensive care. Crit Care Med 30:1140–1145
33. Lukaszewicz AC, Grienay M, Resche-Rigon M, Pirracchio R, Faivre V, Boval B, Payen D (2009) Monocytic HLA-DR expression in intensive care patients: interest for prognosis and secondary infection prediction. Crit Care Med 37:2746–2752

34. Döcke WD, Randow F, Syrbe U, Krausch D, Asadullah K, Reinke P, Volk HD, Kox W (1997) Monocyte deactivation in septic patients: restoration by IFN-gamma treatment. Nat Med 3:678–681
35. Nakos G, Malamou-Mitsi VD, Lachana A, Karassavoglou A, Kitsiouli E, Agnandi N, Lekka ME (2002) Immunoparalysis in patients with severe trauma and the effect of inhaled interferon-gamma. Crit Care Med 30:1488–1494
36. Meisel C, Schefold JC, Pschowski R, Baumann T, Hetzger K, Gregor J, Weber-Carstens S, Hasper D, Keh D, Zuckermann H, Reinke P, Volk HD (2009) Granulocyte-macrophage colony-stimulating factor to reverse sepsis-associated immunosuppression: a double-blind randomized placebo-controlled multicenter trial. Am J Respir Crit Care Med 180:640–648
37. Döcke WD, Höflich C, Davis KA, Röttgers K, Meisel C, Kiefer P, Weber SU, Hedwig-Geissing M, Kreuzfelder E, Tschentscher P, Nebe T, Engel A, Monneret G, Spittler A, Schmolke K, Reinke P, Volk HD, Kunz D (2005) Monitoring temporary immunodepression by flow cytometric measurement of monocytic HLA-DR expression: a multicenter standardized study. Clin Chem 51:2341–2347