

LETTER



Improved quantification of plasma lipopolysaccharide (LPS) burden in sepsis using 3-hydroxy myristate (3HM): a cohort study

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Dear Editor,

Lipopolysaccharide (LPS) has a pivotal role in sepsis pathophysiology, but its measurement with limulus amoebocyte lysate (LAL) and other bioassays yielded conflicting results in clinical studies [1]. We developed a mass spectrometry method retaining LAL's sensitivity in plasma for LPS quantitation, using a specific compound, 3-hydroxy myristate (3HM). When LPS is spiked in plasma, 3HM concentration remains stable, whereas LAL reactivity is decreasing rapidly over time [2]. In this observational study, we used 3HM to describe LPS concentration in a cohort of critically ill patients and in healthy control subjects.

Samples of 507 patients and 49 control subjects were analysed (methods and characteristics of patients are detailed in supplementary material), independently and blindly from clinical data. Patients were classified as septic (sepsis and septic shock), or non-septic (distribution of patients is detailed in Fig. 1a, b). Septic patients had significantly higher 3HM levels when compared to non-septic patients (127 ± 75 vs 112 ± 62 pmol/ml, $p = 0.02$). Moreover, 3HM levels were significantly higher in septic shock patients compared to sepsis patients, although they were similar between non-septic patients and controls (Fig. 1a). Compared to baseline, 3HM levels decreased by 5 days in septic shock patients, and to a lesser extent in sepsis and non-septic patients (eFigure-1). In patients

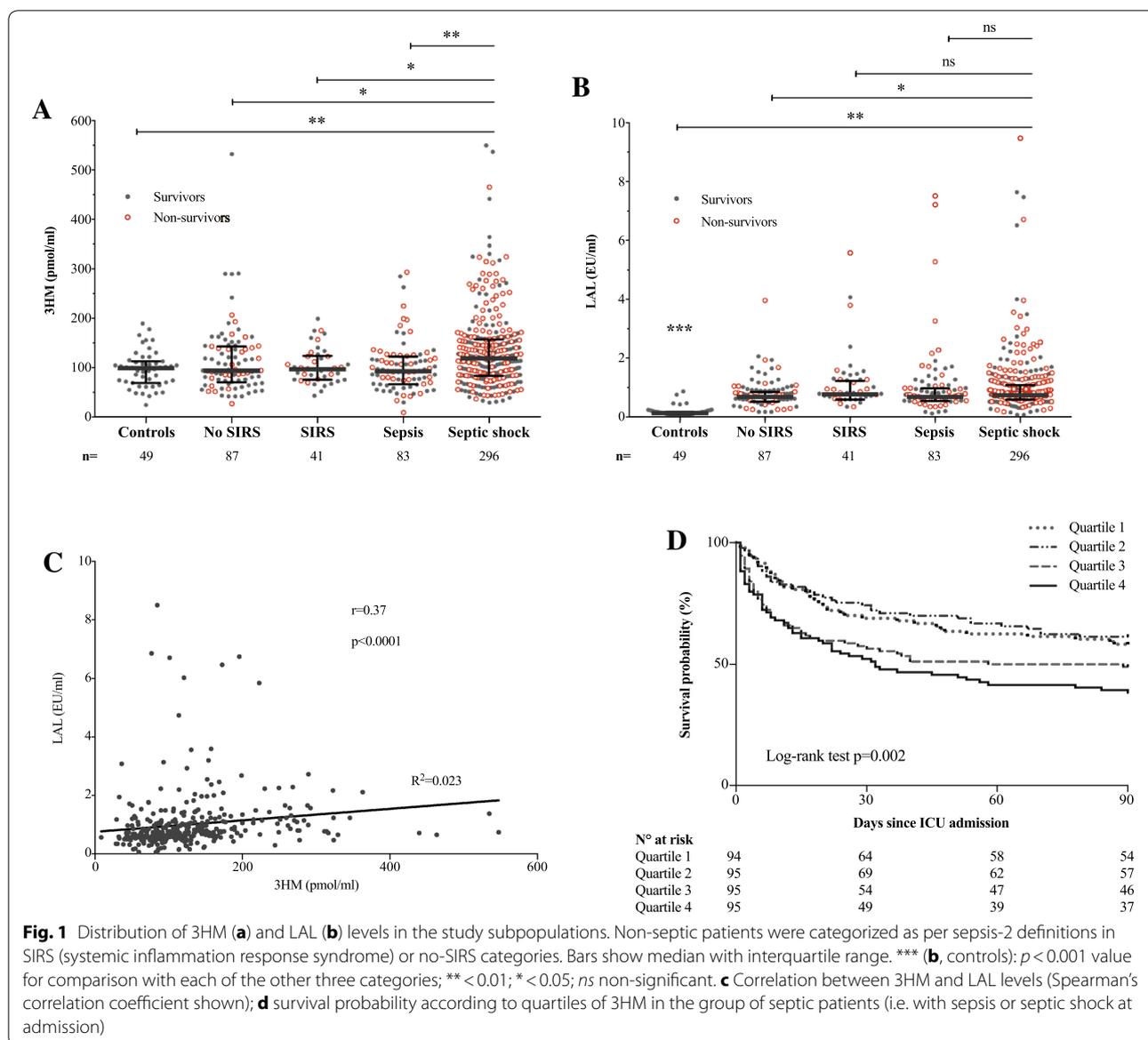
with Gram-negative bacteraemia (GNB), 3HM levels were not significantly higher than in patients without bacteraemia or with non-GNB bacteraemia. LAL levels in septic patients were not significantly different from non-septic patients (1.00 ± 0.98 EU/ml vs 0.87 ± 0.67 EU/ml, $p = 0.10$). Nevertheless, all patients' categories had significantly higher levels compared with controls, unlike 3HM (Fig. 1b). As previously demonstrated in mice [2], the correlation between 3HM and LAL levels was weak but significant in septic patients (Fig. 1c). In septic patients, 3HM levels were significantly lower in survivors compared to non-survivors (130 ± 78 vs 146 ± 71 pmol/ml, $p = 0.03$, Fig. 1a). 90 day survival of septic patients was of 58% in the first quartile and 38% in the fourth quartile of 3HM (Fig. 1d, $p = 0.002$). Our group contributed to demonstrate the pivotal role of lipoproteins [3] and phospholipid transfer protein (PLTP) [4] in the clearance of LPS. We confirmed that both HDL and LDL cholesterol levels were significantly lower in septic patients compared to non-septic patients ($p < 0.001$, eFigure-2). PLTP was positively correlated with C-reactive protein ($r = 0.64$, $p < 0.0001$) and significantly higher in septic compared to non-septic patients (eFigure-2).

3HM quantitation suggests that LPS is present in the circulation of non-septic critically ill patients and "healthy" controls. These results should be confirmed, but could be explained by diet-induced translocation from the gut [5]. We have found that this LPS burden is significantly higher in septic patients, and especially non-survivors. This could be the result of higher bacterial load in these patients, vascular-gut barrier alteration, but also of a decrease in the LPS clearance capacity, i.e. the

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failure of what we've termed the "reverse LPS transport" pathway involving lipoproteins and PLTP [4]. We believe 3HM quantitation will help to understand sepsis biology and to develop better LPS-oriented therapies.

Electronic supplementary material

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Compliance with ethical standards

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee (comité de protection des personnes (CPP), reference number 2013-A00095-40) and with the 1964 Helsinki declaration and its later amendments.

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