A study of associations between plasma metformin concentration, lactic acidosis, and mortality in an emergency hospitalization context

TITLE

Study on the association between metformin blood concentrations, lactic acidosis and mortality in an emergency hospitalization context.

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ABSTRACT

Objectives. To establish a blood concentration threshold of metformin significantly associated with the presence of lactic acidosis (LA) in patients on metformin treatment hospitalized in an emergency context.

Design: Retrospective, single-center, observational study.

Setting: University hospital of Amiens, France.

Patients. All consecutive patients admitted to the emergency department or intensive care unit, over a 9.7-year period, for whom lactate and pH have been analyzed up to 12h before or after metformin plasma and erythrocytes assay within 24h of admission.

Intervention: None.

Measurements and Main Results: LA was defined as arterial blood pH <7.35 and lactate ≥5mM. Of the 194 patients included, 58.2% were male and the median age was 68.6 years. There were 82 patients (42.3%) with LA, most of whom (93.9%) had acute kidney injury, and 56 patients (28.9%) died. Receiver Operating Characteristic (ROC) analysis identified a Met plasma concentration of 9.9 mg/l as a significant threshold of LA occurrence [92.9% specificity, 67.1% sensitivity, area under the Receiver Operating Characteristic (ROC) curve =0.84, p<0.0001]. Along a metformin plasma concentration ≥9.9 mg/l, a prothrombin activity (PT) <70% was another variable independently associated to LA [odds ratio (OR) with 95% confidence interval (95%CI) = 3.84 (1.47;10.04), p=0.0061]. Conversely, in the subgroup of 141 patients with available simplified acute physiology score II (SAPS II) as variable of adjustment, a metformin plasma concentration ≥9.9 mg/l was not associated with in-hospital death while a PT <70% was positively associated [OR (95%CI) = 5.87 (1.72;20), p=0.0047)], and renal replacement therapy (RRT) started at admission negatively associated [OR (95%CI) = 0.26 (0.09;0.82), p=0.0210].

Conclusion. From a threshold of 9.9 mg/l, the metformin plasma concentration, assayed in metformin-treated patients hospitalized in an emergency context, was highly associated with LA occurrence. However, this threshold was not predictive of in-hospital death, which rather depended on liver function and on a RRT starting.

KEYWORDS: Metformin; concentration threshold, hyperlactatemia; lactic acidosis; acute kidney injury; liver failure

INTRODUCTION

Lactic acidosis (LA) is the most serious adverse event reported during metformin (Met) treatment, but remains rare as its rate is estimated at about 7 cases per 100,000 patient years(1). However, the link between Met treatment and LA occurrence in type 2 diabetes patients remains unclear(2–4). The use of Met may coincide with, be the inducer of, or be co-responsible for LA (i.e., respectively, LA that is unrelated to Met (MULA); LA induced by Met (MILA); and an intermediate, more complex situation, called 'Met-associated LA' MALA)(2, 5). Although LA with fatal issue during Met treatment has been many times reported, the literature rarely provides details of the clinical context. The analysis of a large pharmacovigilance database showed that three key criteria (a high lactate concentration, a low pH and a known Met concentration) were met in just 10.4% of reported cases(6). Moreover, even when Met assay data are available, the putative association between Met and its effects on lactate metabolism cannot be reliably assessed without taking into account a large number of elements (the time interval between the last Met intake and the assay, the time interval between Met assay data and arterial blood lactate and pH data, the patient's renal status, the comorbidities etc.)(2).

Animal studies demonstrated that Met can induce a dose- or plasma concentration-dependent increase of plasma lactate concentrations and of LA frequency(7–10). Met impaired gluconeogenesis and increased lactate production in isolated hepatocytes in a concentration-dependent manner by inhibiting the complex 1 of the mitochondrial respiratory chain(9, 11, 12). In humans, US FDA label stated that "during controlled clinical trials, which served as the basis of approval for Met, maximum Met plasma concentrations did not exceed 5 mg/l, even at maximum doses". However, is this 5 mg/l Met concentration widely referred in the literature a therapeutic upper value or a toxic threshold(13)? Of note, the highest value ever reported is 432 mg/l(14), and very high Met plasma concentrations have not been necessarily

associated with hyperlactatemia(15, 16). Additionally, Met exhibits slow intracellular uptake in erythrocytes and the Met elimination half-life can be 8.7-fold longer in the erythrocytes than in the plasma(17–19). The Met erythrocytes concentration thus fluctuates to a much lesser degree than the Met plasma concentration providing a reliable indicator of deep distribution and of potential accumulation occurring over several days.

Ultimately, defining a plasma or erythrocytes concentration of Met highly associated to LA occurrence could improve the causality assessment of Met in LA. The primary objective of this study was thus to establish a concentration threshold of Met that would be significantly associated with concomitant LA in patients on Met treatment hospitalized in an emergency context. The secondary objectives were to test that threshold in multivariate logistic regression analysis of LA and of in-hospital all-cause death.

PATIENTS AND METHODS

Study design and patient selection

This is a retrospective cohort study performed in a 1600-bed university hospital. From our clinical laboratory software (DxLab, Medasys, Le Plessis-Robinson, France), we identified all adult patients, admitted to the emergency department or the intensive care units (ICU) from January 1, 2010 to August 31, 2019, who underwent analysis of arterial blood lactate and pH within up to 12h before or after the measurement of plasma and erythrocytes Met concentrations. We selected those for whom Met and arterial blood lactate and pH were assayed during the first 24h of hospital admission and at least 8h after the last supposed Met dose intake. Then, we collected other data from the medical records software (DxCare, Medasys, Le Plessis-Robinson, France). Approval was obtained from our hospital Ethics Committee. The anonymized medical data collected for the analysis has been authorized and

registered (CNIL n° CHU T205). Due to the retrospective design of the study, patient formal consent was not required.

Data collection and statistical analysis

Two trained medical investigators reviewed independently the electronic medical records of the patients included. A standardized abstraction sheet has been previously designed and tested to minimize ambiguity and maximize the completeness of the data. In case of conflicting data, a third medical abstractor adjudicated. The list of data collected is detailed in supplemental material. The median (interquartile range), and n (%) were used to express continuous data and categorical data, respectively. LA was defined as arterial blood pH <7.35 and lactate ≥5mM. Overall differences between the LA negative and LA positive groups were evaluated using student t-test or Mann-Whitney test for normally or non-normally distributed continuous data respectively and Chi square test or Fisher test for normally or non-normally distributed categorical data respectively.

The relationships between Met blood concentrations and LA were analyzed using Receiver operating characteristic (ROC) curve. The blood concentrations thresholds of Met were determined by the Youden J index and their sensitivity and specificity reported. Subsequently, univariate and multivariate logistic regression analyses were conducted to test the association between Met concentration thresholds and LA or in hospital all-cause death according to other clinical and biological variables admission. The multivariate models included variables with a p <0.20 in univariate analysis. Multicollinearity was excluded, with all the variance inflation factor (VIF) values being <2. Then, a backward stepwise procedure removed from the original model those variables that did not significantly explain LA or death according to a likelihood ratio test. The stability of the final models was assessed by bootstrapping, and odds ratio (OR) with 95% confidence interval (95% CI) for each variable was reported. Goodness-of-fit was assessed by the Hosmer and Lemeshow statistic (calibration was considered

adequate for a low $\chi 2$ value and a $p > 0.05$), and the Nagelkerke R^2 index (adjustment was considered adequate for $R^2 > 0.2$). All statistical tests were two-tailed and a p value of < 0.05 was taken to indicate statistical significance. SPSS (version 25.0, Chicago, IL, USA) was used to perform statistical analyses. Figures were drawn using GraphPad Prism software (version 7.0, San Diego, CA, USA).

RESULTS

Demographic, clinical and biological settings

During the 9.7-year study period, we performed 3359 Met plasma and erythrocytes concentration measurements for 1780 patients. Overall, 317 patients underwent concomitant (±12h) arterial blood lactate and pH, and 194 patients fulfilling inclusion criteria were included for the study (Supplemental Figure S1). In this retrospective cohort, Met plasma and erythrocytes concentrations were correlated with plasma creatinine, plasma lactate and pH levels (Supplemental Figure S2). There were 82 (42.3%) patients showing LA and 9 (4.6%) patients compensated LA as defined by pH ≥7.35 with lactate ≥5 mM and a partial pressure of carbon dioxide (PCO2)<30 mmHg, at the admission. Death during the hospital stay was reported for 56 (28.9%) patients. Patients characteristics of the whole study population or as stratified by the presence of LA ("LA positive") or not ("LA negative") are shown in Table 1. Data completeness was of 97.8%.

Associations between Met blood concentrations and LA

The median Met plasma and erythrocytes concentrations at the admission were significantly higher in LA positive patients than in LA negative patients (18.5 vs 1.7 mg/l, $p<0.0001$ and 9.0 vs. 1.9, $p<0.0001$, respectively) (Figure 1A). In LA negative patients, the median Met plasma concentration was not significantly different to the median Met erythrocytes

concentration (1.7 vs. 1.9 mg/l) while in the LA positive patients, the median Met concentration was significantly higher in plasma than in erythrocytes (18.5 vs. 9 mg/l, p=0.0217) (Figure 1A).

The ROC curves reflecting the probability for Met plasma and erythrocytes concentrations to be associated with LA are shown in the Figure 1B. Youden's index indicated that the most associated Met concentration thresholds with LA were 9.9 mg/l in plasma with 67.1% sensitivity and 92.9% specificity, and 5.8 mg/l in erythrocytes with 63.3% sensitivity and 86.5% specificity. In the context of the study, i.e. emergency hospitalization of a patient on Met treatment, the positive and negative predictive values of the Met concentration thresholds for LA were 87.3% and 79.4% in plasma and 78.1% and 75.6% in erythrocytes, respectively. When comparing patients with Met plasma concentration ≥ 9.9 mg/l (n=63/194) to those with Met plasma concentration < 9.9 mg/l (n=131/194), median eGFR was significantly lower (9 vs. 31 ml/min/1.73m² respectively, p<0.0001) (Supplemental table S1). Patients with Met plasma concentration ≥9.9 mg/l showed significantly more dehydration than patients with Met plasma concentration <9.9 mg/l, whereas less had septic or cardiogenic shock and the frequency of co-medications at risk of AKI were not significantly different (Supplemental table S1). Univariate and multivariate analysis results related to LA are reported in the Table 2. Along with a Met plasma concentration ≥9.9 mg/l, a prothrombin (PT) activity <70% was another variable positively associated with LA. Conversely, a history of chronic heart disease was negatively associated with LA. There was adequate goodness-of-fit (Hosmer and Lemeshow test $\chi^2 = 5.042$, df = 6, p=0.5385; Nagelkerke $R^2 = 0.5692$).

Associations between Met blood concentrations and in-hospital all-cause death

The median Met plasma and erythrocytes concentrations at admission in patients who died during the hospital stay were not significantly different from that of patients who did not (4.8

vs. 3.95 mg/l respectively in plasma; and 2.33 vs. 3.8 mg/l respectively in erythrocytes) (Figure 2A). The ROC curves reflecting the probability for Met plasma and erythrocytes concentrations at admission to be associated with death during the hospital stay are shown in the Figure 2B. AUROC for all-cause death was 0.54 for Met plasma concentration, and 0.56 for Met erythrocytes concentration; no Met concentration threshold significantly associated to death could be found.

In the LA positive patients, the median Met plasma and erythrocytes concentrations at admission in patients who died during the hospital stay were significantly lower than that of patients who did not (12.4 vs. 24.0 mg/l respectively in plasma, p=0.0372; and 4.1 vs. 12.9 mg/l respectively in erythrocytes, p=0.0462) (Figure 2C), while the median simplified acute physiology score (SAPS) II was significantly higher (80 vs. 54.5 respectively, p=0.0003) (Figure 2D).

When comparing patients with Met plasma concentration ≥9.9 mg/l to those with Met plasma concentration <9.9 mg/l, the in-hospital death frequency was not significantly different (33.3 vs. 26.7% respectively, p=0.4336) (Supplemental table S1). In the LA positive group, patients with Met plasma concentration ≥9.9 mg/l (n=55) showed a lower death frequency than patients with Met plasma concentration <9.9 mg/l (n=27) with a clear tendency to significance (34.5 vs. 59.3%, p=0.0563) (Supplemental table S1).

Among the 141/194 patients with SAPS II available, 63 (44.7%) were showing LA at the admission and 48 (34%) died during the hospital stay. Among LA positive patients, SAPS II was not significantly different between patients with Met plasma concentration ≥9.9 mg/l and patients with Met plasma concentration <9.9 mg/l (63.5 vs. 69, p=0.6580) (Supplemental table S1). Based on clinical and biological data of the 141 patients with SAPS II available, univariate and multivariate regression results related to in-hospital all-cause death are reported in Table 3. With a Met plasma concentration ≥9.9 mg/l at admission forced into the model,

variables positively associated with death were SAPS II and a PT activity <70%. Conversely, starting renal replacement therapy (RRT) at admission was negatively associated with death. There was adequate goodness-of-fit (Hosmer and Lemeshow test χ^2 = 7.55, df = 8, p = 0.44786; Nagelkerke R^2 = 0.37).

DISCUSSION

Association between high Met concentrations and LA

This largest retrospective cohort study yet published on LA in Met-treated patients reveals that a Met plasma concentration of 9.9 mg/l, measured in an emergency hospitalization context after at least 8h the last dose intake, would be a threshold strongly associated with LA occurrence. Compared to the steady state trough concentrations measured at Met therapeutic doses with adjustment to GFR, usually around 1 mg/l(19–21), the threshold defined here suggests that an at least 10-fold higher trough concentration would be generally required for LA to occur during Met treatment. This finding corroborates the preclinical results demonstrating that the lactate increase or the pH decrease in animals treated with Met occurred at high(7–10), instead of "normal" (i.e. at therapeutic dose) plasma concentrations(22), suggesting a threshold metabolic effect of Met. Nevertheless, as previously described, a few patients had displayed very high Met blood concentrations without significant hyperlactatemia, which may reflect inter-individual variability of the susceptibility to Met effects(15, 16).

Correlations between Met plasma concentration and arterial blood lactate and pH have been previously reported(23, 24). However, the Met plasma concentration threshold for LA has not been yet determined since all the patients included in that analyses were having LA. Our multivariable regression analysis showed that a Met plasma concentration ≥9.9 mg/l was independently associated with LA, even in the presence of chronic and acute clinical and

biological comorbidities, which may have triggered or contributed to LA. With a specificity of 92.9%, i.e. a low false positive rate, this Met concentration threshold associated with LA would be therefore a reliable discriminating factor between MULA (with Met plasma <9.9 mg/l) and MALA or MILA (with Met plasma ≥9.9 mg/l) [2]. Along with high Met plasma concentration (i.e. ≥9.9 mg/l), a PT activity below 70% without anticoagulation, suggesting liver insufficiency, was also positively and independently associated with LA. This corroborates the report of Seidoswki et al. showing significant negative correlation between PT activity and arterial blood lactate concentration in Met treated-patients with LA(24). Decreased PT activity could result from a severe underlying chronic liver disease or liver injury due to other acute comorbidities (i.e. sepsis, heart failure…), decreasing thus the liver lactate clearance capacity. Indeed, lactate production and clearance are mainly dependent from liver metabolism and, in a less extent, from kidney metabolism and excretion. Any lactate production increase, even low, may result in high lactate accumulation when liver and kidney failures are associated.

Context of high Met concentrations

Patients with Met plasma concentration ≥9.9 mg/l were not receiving larger Met doses than patients with Met plasma concentration <9.9 mg/l. They were not more suffering from CKD; but they displayed a significantly lower eGFR at admission. AKI, as from stage 1 of the KDIGO classification, has been commonly diagnosed at admission in the whole study population, but its frequency was significantly higher in patients with Met plasma concentration ≥9.9 mg/l than in patients with Met plasma concentration <9.9 mg/l. Moreover, among patients with AKI, 54/61 (88.5%) of the patients with Met plasma concentration ≥9.9 mg/l were also showing LA while they were only 23/102 (22.5%) of the patients with Met plasma concentration <9.9 mg/l (data not shown). Previously, Vecchio et al. analyzed all

cases of LA related to a Met plasma concentration > 4 mg/l, over a 5-year period(23). After exclusion of Met voluntary overdoses, all the patients they included (n=63) had AKI and a statistically significant correlation was found between Met and creatinine(23). In the Seidowsky's report, apart from cases of voluntary Met overdose, 75.9% of patients included also had AKI (24). Our results and those published are consistent with a frequent scenario of LA resulting from AKI-induced accumulation of Met. They also corroborate the results of recent case-control studies showing that the association between Met exposure and LA increases especially in AKI and as AKI is severe(25, 26).

Additionally, our cohort included 16 cases of voluntary overdose. Patients with LA (n=5/16) showed higher median Met concentrations than those without LA (7.2 vs. 2 mg/l, p=0.0092 in plasma and 2.5 vs. 0.9, p=0.0576 in erythrocytes respectively).

Interest of Met erythrocytes concentration

In case of AKI, the accumulation of Met in the plasma resulting from the Met clearance decrease is also associated with an accumulation of Met in deep compartments such as erythrocytes, but the latter accumulation process is probably slower(20, 27). This may explain why the Met concentration threshold found here for LA occurrence is higher in plasma (9.9 mg/l) than in erythrocytes (5.8 mg/l), while Met concentrations in plasma and erythrocytes are generally close when kidney function is stable and steady state has reached. Interestingly, the median Met plasma over erythrocytes concentration ratio was significantly higher in the LA positive group than in the LA negative group (2.05 vs. 1.05 mg/l, p<0.0001), suggesting a recent Met accumulation in LA positive patients (i.e. steady state not reached). However, statistical results suggested that the Met concentration threshold in erythrocytes would be less predictive of LA than that in the plasma, and some of patients with LA (3/82) showed high Met plasma concentration (≥9.9 mg/l) with low Met erythrocytes concentration (<5.8 mg/l).

This underlines the time required to objectify high levels in deep compartment under acute accumulation conditions. Nevertheless, Met erythrocytes concentration measurement remains useful i) to highlight a Met accumulation when time interval between Met intake and blood assay is unknown and, ii) to follow the Met elimination from deep compartment when Met treatment has been hold (18, 27).

Association between high Met concentration and outcome

In the study population, in-hospital death frequency was high and was about 2-fold higher in LA positive patients than in LA negative patients. However, the median Met blood concentrations were not significantly different between patients who died and those who did not. Although high Met plasma and erythrocytes concentrations (i.e. ≥ 9.9 and 5.8 mg/l respectively) were strongly associated with the presence of LA at admission, they were not associated with death neither in univariate nor multivariate analysis. This is consistent with previous results showing no correlation between Met concentration at the hospital admission and death(23, 24, 28), while in contradiction with the conclusion of a recent pharmacovigilance study(29). But the latter did not specify the time interval between Met concentration assay and the last Met dose intake, the hospital admission and the LA diagnostic. Interestingly, in LA positive patients, the median Met blood concentrations were significantly lower in patients who died than in patients who did not. Moreover, in the subgroup of LA positive patients with SAPS II score available, patients with Met plasma concentration ≥9.9 mg/l showed a lower death frequency than patients with Met plasma concentration <9.9 mg/l, even though the median SAPS II severity scores were comparable. It has been suggested that LA in Met-treated patients is associated with lower mortality compared to other forms of LA. Indeed, despite the severity of illness at admission, several reports indicated lower death frequency (10 to 30% mortality) than that predicted (50 to 60%

mortality) by severity-of-disease scores(30, 31). Friesecke et al. also compared patients with MALA (n=10) to patients with LA of other origin (LAOO) (n=31)(32). With comparable severity of disease and non-renal organ dysfunction scores, outcome was significantly better in MALA patients than in LAOO patients (50% death in MALA versus 100% in LAOO). A similar unexpected death frequency has been reported in a series of 56 cases of very serious MALA (mean arterial blood pH and lactate values of 6.75 ± 0.17 and 23.07 ± 6.94 mM, respectively)(27). Additionally, recent meta-analysis of 5 observational cohort studies (1282 patients) found an association between Met use prior to admission and lower mortality in septic adult patients with diabetes mellitus(33); and renal, cardiac and neurological protective effects of Met have been suggested many times(34–37). Met increased AMP-activated protein kinase (AMPK) and mitochondrial function in multiple tissues, effects that were associated in mice with reductions in oxidative damage, chronic inflammation and improved health span and lifespan(38). However, the protective effects of Met may be limited in sever acute multi-organ dysfunction. Here, in contrast to high Met concentrations, a reduced PT activity at admission were found as independently associated with in-hospital death, corroborating a previous report(24). Once again, deficient lactate metabolism in the liver may account for a major pejorative prognostic factor in case of LA. Nevertheless, our results also suggest that starting RRT at admission, which improves lactate clearance, would be significantly and independently associated with reduced in-hospital mortality.

Limitations

Our study is limited by its retrospective design. Moreover, the analysis is transversal with single data of lactate, pH and Met concentrations meaning that the temporal relationship between elevated Met concentrations and the generation of LA remains unknown. Therefore, one must be cautious when coining the terms MULA or MALA. Indeed, in the situation in

which Met accumulation occurs as a result of AKI caused by a severe pathology, the latter may be the true trigger of LA while high Met levels, if any, may be only secondarily associated to LA. In other words, this would be strictly a MULA to MALA sequence. Nevertheless, the present analysis is the first studying in a large cohort of patients the association, in an emergency hospitalization context, of Met plasma or erythrocytes concentration and the presence of LA in a close time window, providing a useful criterion to assess the causality of Met in case of LA. Moreover, the results highlight the importance for medical decision of an early biological assessment which includes arterial blood lactate and pH, Met blood assay, liver and kidney functions in Met-treated patients hospitalized in an emergency context.

CONCLUSION

In Met-treated patients hospitalized in an emergency context, a Met plasma concentration ≥9.9 mg/l was highly associated with the presence of LA but not with in-hospital death, which depended rather on the liver function and a RRT starting. A prospective study including standardized monitoring and treatment that compares diabetic patients not treated with Met with Met-treated patients, with or without high Met concentration, and matched for a severity of disease score, would be needed to determine accurately the metabolic and clinical impact of Met.

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FIGURE LEGENDS

Figure 1. Associations between Met blood concentrations and LA.

The Met blood concentrations have been compared according to the concomitant presence of LA (n=82) or not (n=112) (**A**). Box plots with median and 10-90 percentiles and statistical results of Kruskal-Wallis with Dunn's post-hoc analysis are shown. Thresholds of Met plasma and erythrocytes concentrations significantly associated to concomitant LA were identified through Receiver Operating Characteristic analysis using the Youden's index **(B)**.

Abbreviations: $CI_{95\%}$: 95% confidence interval; LA, lactic acidosis; Met, metformin

Figure 2. Associations between Met blood concentrations and death.

The Met plasma and erythrocytes concentrations measured at admission in the whole study population (**A)** or in the LA positive group (**C**) have been compared according to death or not during the hospital stay (n=56 and 138 respectively in the whole study population, n=35 and 47 respectively in the LA positive group). Box plots with median and 10-90 percentiles and statistical results of Kruskal-Wallis with Dunn's post-hoc analysis are shown. The association between plasma or erythrocytes Met concentration at admission and in-hospital death was studied through Receiver Operating Characteristic analysis **(B)**. The SAPS II scores measured at admission in the LA positive group have been compared according to death or not during the hospital stay (n=29 and 34 respectively) (**D**). Student t-test result is shown.

Abbreviations: Met: metformin; LA: lactic acidosis; SAPS II: simplified acute physiology score II.

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SUPPLEMENTAL DIGITAL CONTENT

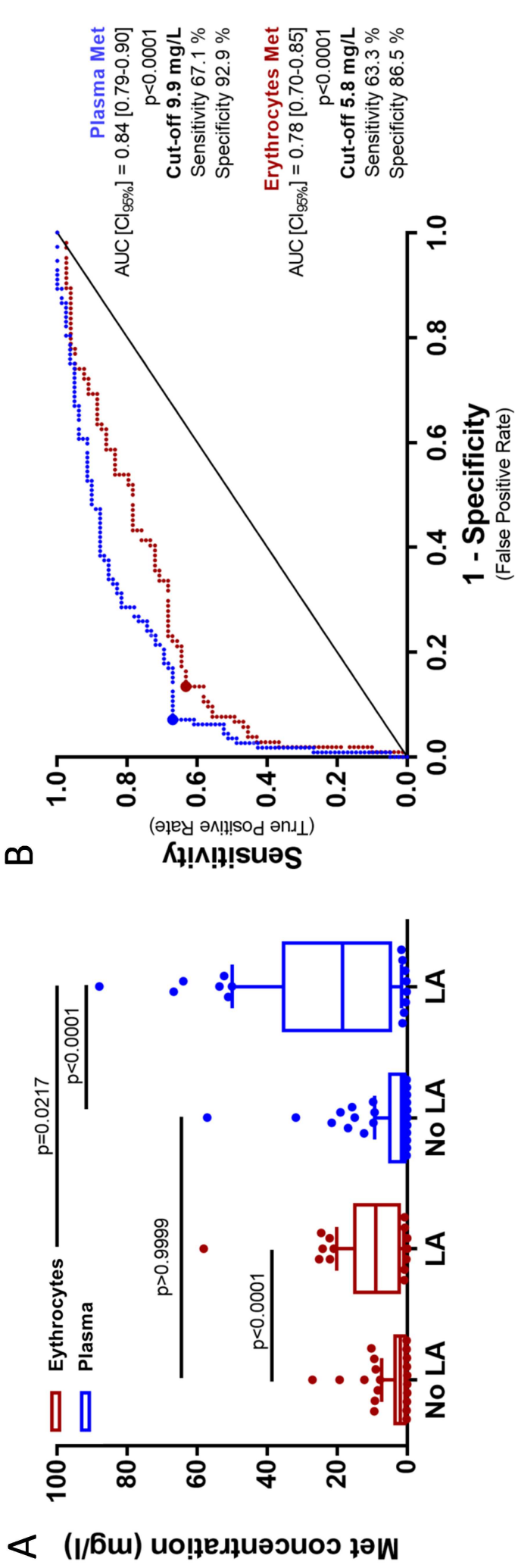
Supplemental material. Data collection

Supplemental Figure S1. Patients' selection flowchart

Supplemental Figure S2. Correlations between Met blood concentrations and plasma creatinine, plasma lactate and arterial blood pH

Supplemental Table S1. Patients' characteristics depending on whether the plasma concentration of metformin is lower or higher than 9.9.

Figure 1


A
Met concentration (mg/l)
Eythrocytes
Plasma
p=0.0217
p<0.0001
p>0.9999
p<0.0001
No LA
LA
No LA
LA
B
Sensitivity
(True Positive Rate)
1 - Specificity
(False Positive Rate)
Plasma Met
AUC [CI95%] = 0.84 [0.79-0.90]
p<0.0001
Cut-off 9.9 mg/L
Sensitivity 67.1 %
Specificity 92.9 %
Erythrocytes Met
AUC [CI95%] = 0.78 [0.70-0.85]
p<0.0001
Cut-off 5.8 mg/L
Sensitivity 63.3 %
Specificity 86.5 %

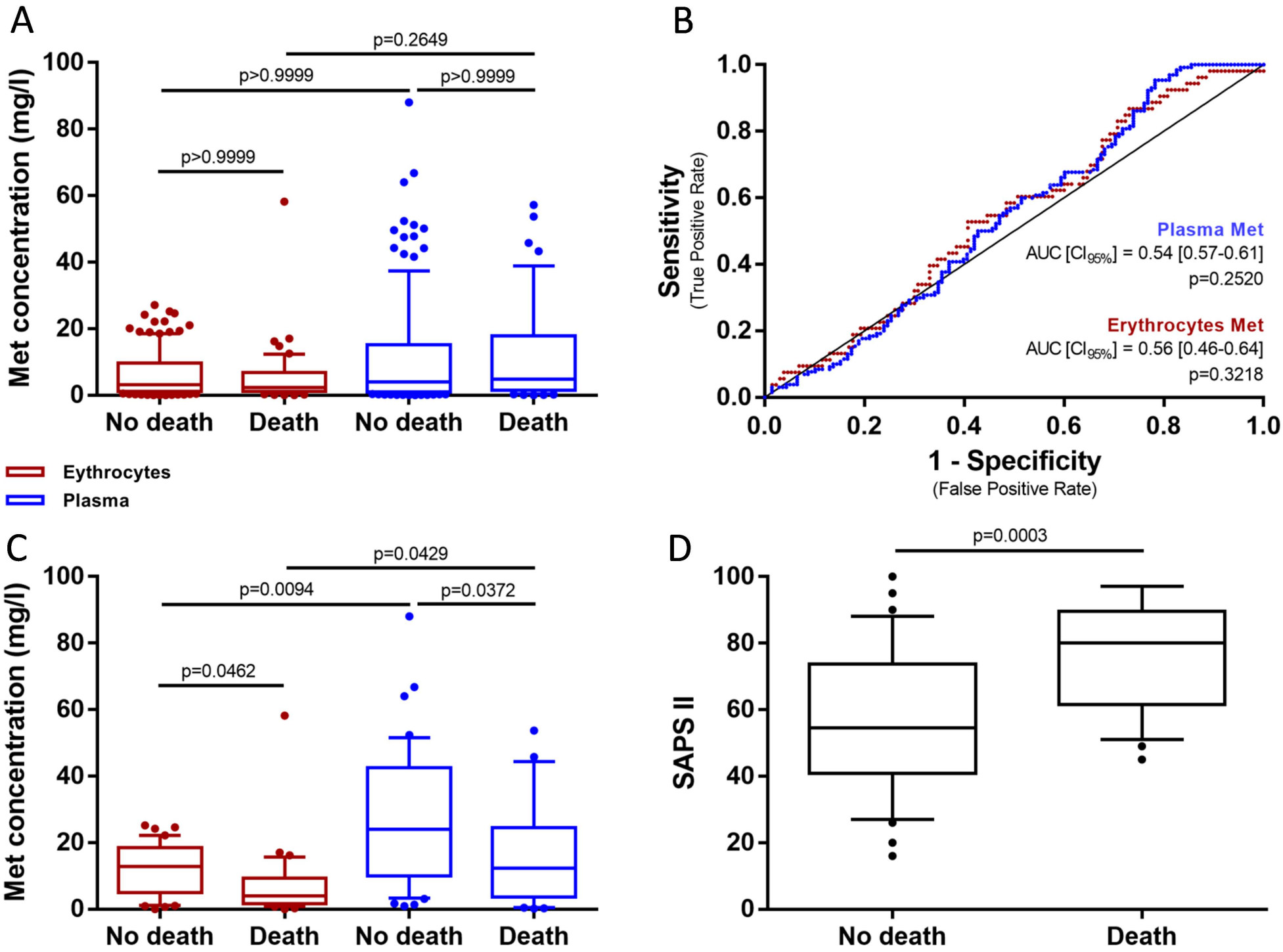

A
Met concentration (mg/l)
p=0.2649
p>0.9999
p>0.9999
p>0.9999
No death
Death
No death
Death
Eythrocytes
Plasma
B
Sensitivity
(True Positive Rate)
1 - Specificity
(False Positive Rate)
Plasma Met
AUC [CI95%] = 0.54 [0.57-0.61]
p=0.2520
Erythrocytes Met
AUC [CI95%] = 0.56 [0.46-0.64]
p=0.3218
C
Met concentration (mg/l)
p=0.0429
p=0.0094
p=0.0372
p=0.0462
No death
Death
No death
Death
D
SAPS II
p=0.0003
No death
Death

Table 1. Patients' characteristics

Characteristics	All (N=194)	No lactic acidosis (N=112)	Lactic acidosis (N=82)	p value
Background characteristics				
Sex M (%)	113 (58.2)	64 (57.1)	49 (59.8)	0.828
Age (years)	68.6 [60.2 -75.9]	69.5 [61.5 -77.1]	67.6 [59.6 -74.4]	0.4836
Weight (Kg)	87.1 [74.6 -101]	85.5 [71.5 -100]	89 [77.3 -103.6]	0.2009
BMI (Kg/m²)	29.4 [25.8 -35.2]	29.3 [25.3 -35.1]	29.5 [26.8 -35.7]	0.8231
Chronic disease				
Chronic heart disease [n(%)]	102 (52.6)	67 (59.8)	35 (42.7)	0.0267
Chronic liver disease [n(%)]	27 (13.9)	16 (14.3)	11 (13.4)	0.9706
Chronic kidney disease III to V [n(%)]	64 (33.2)	36 (32.1)	28 (34.6)	0.8429
Chronic pulmonary disease [n(%)]	48 (24.7)	29 (25.9)	19 (23.2)	0.7905
Clinical context at the admission				
Active cancer [n(%)]	28 (14.4)	14 (12.5)	14 (17.1)	0.4911
Septic shock [n(%)]	67 (34.5)	41 (36.6)	26 (31.7)	0.5781
Acute kidney injury [n(%)]	163 (84)	86 (76.8)	77 (93.9)	0.0026
Cardiogenic shock [n(%)]	40 (20.6)	23 (20.5)	17 (20.7)	0.8837
Respiratory distress syndrome [n(%)]	38 (19.6)	27 (24.1)	11 (13.4)	0.0948
Hemorrhagic choc [n(%)]	14 (7.2)	9 (8)	5 (6.1)	0.8146
Dehydration [n(%)]	75 (38.7)	36 (32.1)	39 (47.6)	0.0424
Comedication at risk of AKI [n(%)]	105 (54.1)	64 (57.1)	41 (50)	0.4007
Voluntary overdose [n(%)]	16 (8.2)	11 (9.8)	5 (6.1)	0.5046
Met dose (mg/24h)	1560 [1170 -2340]	1560 [1170 -2340]	2340 [1326 -2340]	0.0416
RRT started (%)	63 (32.5)	25 (22.3)	38 (46.3)	0.0007
SAPS II (available for 141 patients)	57 [45 -76]	54 [42 -64]	67 [50.5 -84.5]	0.0010
Biological data at the admission				
Delay between Met and arterial gazometry (h)	0.7 [0 -2.7]	0.8 [0 -3.1]	0.5 [0 -2.2]	0.3914
Arterial pH	7.3 [7.2 -7.4]	7.4 [7.3 -7.4]	7.2 [7 -7.3]	<0.0001
Arterial lactate (mmol/l)	5 [2.3 -10.3]	2.4 [1.9 -4]	11.1 [7.6 -16]	<0.0001
Arterial pO2 (mm Hg)	98.1 [78 -124.5]	91.7 [73.4 -117.3]	111 [90.7 -147]	<0.0001
Arterial pCO2 (mm Hg)	31.3 [25.5 -37.5]	33.5 [28.3 -38.8]	29.4 [19.8 -34.8]	<0.0001
Arterial SaO2 (%)	96.6 [94.2 -98]	96.6 [94 -98.3]	96.6 [94.7 -97.8]	0.8172
Arterial total CO2 (mmol/l)	17.8 [11.4 -21.5]	21 [17.8 -23.5]	10.6 [6.8 -15.7]	<0.0001
Arterial base excess (mmol/l)	-7.7 [-16 --3.4]	-4.9 [-7.3 --1.6]	-17.9 [-22.9 --11]	<0.0001
Plasma sodium (mmol/l)	137 [133 -140]	137 [134 -141]	136.5 [132 -140]	0.2567
Serum creatinine (µmol/l)	237 [130 -453]	199.5 [104 -325]	307 [187.5 -641.5]	0.0039
eGFR (ml/min/1.73m²)	24 [11 -42.3]	29.5 [16 -56.3]	16.5 [6.3 -32.8]	<0.0001
Plasma glucose (mmol/l)	9.2 [6.2 -13.9]	9.2 [6.7 -13.2]	8.9 [5.2 -15.9]	0.4704
Plasma CRP (mg/l)	37.1 [10 -117.4]	62.4 [11.6 -154.9]	28.8 [9.9 -71.4]	0.0227
Plasma ASAT (UI/l)	53 [27 -174]	42 [26 -100.3]	85 [33 -284]	0.0104
Plasma ALAT (UI/l)	34 [20 -123.5]	28 [18 -61]	47 [24 -190]	0.0018
Total plasma bilirubin (µmol/l)	8 [5 -15]	8.5 [5 -15]	7 [5 -14]	0.6346
Blood hemoglobin (g/dl)	11.2 [9.5 -12.6]	11.2 [9.8 -12.6]	10.8 [9.5 -12.4]	0.1800
PT activity (%)*	65 [46 -82.5]	73 [50.5 -88]	53 [35 -74.5]	<0.0001
Outcome				
In-hospital death (%)	56 (28.9)	21 (18.8)	35 (42.7)	0.0005

Abbreviations: ASAT: aspartate amino-transferase; ALAT: alanine amino-transferase; BMI: body mass index; CRP: c-reactive protein; eGFR: estimated glomerular filtration rate (through the abbreviated Modified of Diet in Renal Disease's equation); ICU: intensive care medicine; M: male; Met: metformin; pCO2: partial pressure of carbon dioxide; pO2: partial pressure of carbon dioxide; PT: prothrombin; RRT: Renal replacement therapy ; SaO2: oxygen saturation; SAPS II: simplified acute physiology score II.

*PT activity has been reported only for patients not anticoagulated.

Table 2. Variables associated with lactic acidosis

Univariate logistic regression			*Multivariate logistic regression*		
Background characteristics	**OR (95% CI)**	**p Value**	**Background characteristics**	**OR (95% CI)**	**p Value**
Gender (M)	1.12 (0.62;1.99)	0.7154			
Age > 65 years	0.89 (0.5;1.6)	0.6983			
BMI > 30 Kg/m²	1.07 (0.56;2.06)	0.8294			
Chronic diseases	**OR (95% CI)**	**p Value**	**Chronic diseases**	**OR (95% CI)**	**p Value**
Chronic liver disease	0.93 (0.41;2.12)	0.8625			
Chronic kidney disease	1.12 (0.61;2.04)	0.724			
Chronic heart disease	0.50 (0.28;0.89)	0.0188	Chronic heart disease	0.29 (0.11;0.75)	0.0114
Chronic pulmonary disease	0.86 (0.44;1.68)	0.6644			
Active cancer	1.44 (0.65;3.22)	0.3723			
Clinical contexts at admission	**OR (95% CI)**	**p Value**	**Clinical contexts at admission**	**OR (95% CI)**	**p Value**
Acute kidney injury	4.66 (1.70;12.73)	0.0027			
Respiratory distress	0.49 (0.23;1.05)	0.0671			
Cardiogenic shock	1.01 (0.50;2.05)	0.9734			
Septic shock	0.80 (0.44;1.47)	0.4786			
Biological parameters at admission	**OR (95% CI)**	**p Value**	**Biological parameters at admission**	**OR (95% CI)**	**p Value**
Met plasma conc. ≥ 9.9 µg/mL	26.48 (11.27;62.20)	<0.0001	MET plasma conc. ≥ 9.9 µg/mL	34.29 (12.13;96.91)	<0.0001
Met erythrocytes conc. ≥ 5.2 µg/mL	8.70 (4.38;17.28)	<0.0001			
Blood hemoglobin < 10g/dl	1.52 (0.83;2.79)	0.176			
Plasma glucose ≥ 8mM	0.69 (0.39;1.22)	0.2024			
Plasma CRP > 10 mg/l	0.75 (0.39;1.47)	0.4033			
eGFR < 15ml/min/1.73m²	3,01 (1.61;5.60)	0.0005			
PT activity < 70%	3.00 (1.51;5.98)	0.0018	PT activity <70%	3.84 (1.47;10.04)	0.0061

The association between plasma or erythrocytes Met concentration at admission and the presence of concomitant LA has been tested according to chronic and acute comorbidities. Multivariate Logistic regression analysis has been performed using backward stepwise selection of variables with p<0.2 in univariate analysis. Abbreviations: ALAT: alanine amino-transferase; BMI: body mass index; CRP: c-reactive protein; eGFR: estimated glomerular filtration rate (through

the abbreviated Modified of Diet in Renal Disease's equation); ICU: intensive care medicine; M: male; Met: metformin; pCO2: partial pressure of carbon dioxide; pO2: partial pressure of carbon dioxide; PT: prothrombin; SaO2: oxygen saturation.

Table 3. Variables associated with in-hospital death

Univariate logistic regression			*Multivariate logistic regression*		
Background characteristics	**OR** (95% CI)	**p value**	**Background characteristics**	**OR** (95% CI)	**p value**
Gender (M)	1.83 (0.97;3.86)	0.1112			
Age > 65 years	1.21 (0.58;2.51)	0.6147			
BMI > 30 Kg/m²	1.15 (0.50;2.66)	0.7367			
Chronic diseases	**OR** (95% CI)	**p value**	**Chronic diseases**	**OR** (95% CI)	**p value**
Chronic liver disease	3.47 (1.36;5.85)	0.0093			
Chronic kidney disease	0.63 (0.29;1.36)	0.2421			
Chronic heart disease	1.96 (0.95;4.04)	0.0693			
Chronic pulmonary disease	1.20 (0.54;2.66)	0.6555			
Active cancer	1.23 (0.47;3.21)	0,6713			
Clinical contexts at admission	**OR** (95% CI)	**p value**	**Clinical contexts at admission**	**OR** (95% CI)	**p value**
Acute kidney injury	2.44 (0.66;9.01)	0.1817			
Respiratory distress	1.16 (0.50;2.68)	0.7326			
Cardiogenic shock	2.19 (0.97;4.94)	0.0594			
Septic shock	1.47 (0.72;2.98)	0.2872			
RRT started	0.58 (0.27;1.26)	0.1677	RRT started	0.26 (0.09;0.82)	0.0210
SAPS II (each unit increase)	1.06 (1.03;1.08)	<0.0001	SAPS II (each unit increase)	1.05 (1.02;1.08)	0.0005
Biological parameters at admission	**OR** (95% CI)	**p value**	**Biological parameters at admission**	**OR** (95% CI)	**p value**
Met plasma conc. ≥ 9.9 µg/mL	1.52 (0.73;3.15)	0.2594	Met plasma conc. ≥ 9.9 µg/mL	1.38 (0.48;3.96)	0.5488
Met erythrocytes conc. ≥ 5.8 µg/mL	0.97 (0.46;2.04)	0.9268			
Blood hemoglobin < 10g/dl	0.95 (0.46;1.97)	0.8936			
Plasma glucose ≥ 8mM	0.97 (0.52;1.81)	0.9157			
Plasma CRP > 10 mg/l	1.38 (0.55;3.45)	0.4881			
PT activity < 70%	6.97 (2.24;27.73)	0.0008	PT activity < 70%	5.87 (1.72;20)	0.0047

The associations between plasma or erythrocytes Met concentration at admission and death during the hospital stay have been tested according to chronic and acute comorbidities. Multivariate Logistic regression analysis has been performed using backward stepwise selection of variables with $p<0.2$ in univariate analysis and a Met plasma concentration ≥ 9.9 mg/l forced to be included into the model. Abbreviations: ALAT: alanine amino-transferase; BMI: body mass index; CRP: c-reactive protein; eGFR: estimated glomerular filtration rate (through the abbreviated Modified of Diet in Renal Disease's equation); ICU:

intensive care medicine; M: male; Met: metformin; pCO2: partial pressure of carbon dioxide; pO2: partial pressure of carbon dioxide; PT: prothrombin; RRT: Renal replacement therapy; SaO2: oxygen saturation; SAPS II: simplified acute physiology score.