

Letter to the Editors

Comparison of serum and lithium-heparinate plasma for the accurate measurements of endogenous and exogenous morphine concentrations

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In addition to its presence in blood samples from morphine-treated patients and heroin addicts, morphine is endogenously synthesized from dopamine in mammals [1, 2]. However, the physiological relevance of endogenous morphine (eM) remains an open question. Several studies report an increase in eM in blood and urine samples in pathological conditions such as infection, inflammation, bulimia, anorexia and Parkinson's disease [2, 3]. For example, eM concentrations reached on average 2.5 ng ml⁻¹ in the serum of patients suffering from systemic infections (sepsis), whereas healthy donors and control patients displayed no or very low eM concentrations [4]. Therefore, quantification of endogenous and exogenous morphine is of great interest for both clinical and forensic analyses.

As both endogenous and exogenous morphine are present at low concentrations, the quantification method implemented must be very sensitive. Besides, no consensus yet exists regarding blood sample preparation for morphine measurement using ELISA. As lithium-heparinate (plasma) and serum samples are both routinely used in clinical practice, we compared the morphine concentrations using these two sampling methods.

This study was approved by our institutional review board for human experimentation (CPP no. 08/62-protocol no. IVD1 EUDRACT no. 2008-A01345-50). After informed consent, samples were collected from patients requiring clinical laboratory analyses. Blood from 80 patients was collected in two types of sampling tubes, as follows: (i) for

serum analysis, blood was collected in dry tubes (BD, Plymouth, UK; SSTII 377615), and serum was obtained after centrifugation (4°C, 1500g, 10 min); and (ii) for plasma analysis, blood was collected in lithium-heparinate tubes (BD; LH-PSTII 367378, 17 i.u. ml⁻¹) and plasma was recovered after centrifugation (4°C, 1500g, 10 min). Collected serum and plasma were immediately stored at -80°C (for 3 weeks to 3 months). Samples were thawed only once, 15 min prior to ELISA testing. In these conditions, no effect of freezing on morphine concentrations has been noticed. Both plasma and serum morphine concentrations were quantified using a morphine-specific ELISA kit (ref. 213-0480; Immunalysis, Pomona, CA, USA) [4]. No cross-reactivity has been observed for morphine metabolites (morphine-6-glucuronide, morphine-3-glucuronide and codeine) and 75 other compounds (related to eM biosynthesis pathway or not; see also kit's datasheet). The plasma or serum samples were tested in duplicates (40 µl) at 21°C. The coefficient of variation values were between 0% and 8%. All samples with a higher coefficient of variation value were retested in order to obtain a coefficient of variation below or equal to 8%. Morphine standards (0–25 ng ml⁻¹) were diluted in phosphate-buffered saline containing 1% bovine serum albumin (PBS-BSA; pH 7.2; w/v) for serum samples and in PBS-BSA 1% containing lithium-heparinate (17 i.u. ml⁻¹) for plasma samples. The detection limit for morphine was 0.1 ng ml⁻¹.

In a set of control experiments, known concentrations of morphine (0.28 and 11 ng ml⁻¹) were diluted in PBS,

PBS–lithium-heparinate (17 i.u. ml⁻¹, pH 7.2), PBS–BSA (1%; w/v) or PBS–lithium-heparinate–BSA buffer. Using the ELISA kit, no statistical differences in morphine concentrations were observed ($n = 8$, Mann–Whitney U -test, $P > 0.3$) when 0.28 ng ml⁻¹ of morphine was diluted in PBS (0.26 ± 0.03 ng ml⁻¹), PBS–lithium-heparinate [0.26 ± 0.02 ng ml⁻¹; 95% confidence interval (CI) of difference with PBS –0.03 to 0.03], PBS–BSA (0.27 ± 0.02 ng ml⁻¹; 95% CI of difference with PBS –0.04 to 0.02) or PBS–lithium-heparinate–BSA (0.28 ± 0.04 ng ml⁻¹; 95% CI of difference with PBS –0.04 to 0.0). Likewise, no statistical difference ($n = 6$, Mann–Whitney U -test, $P > 0.2$) was observed for 11 ng ml⁻¹ of morphine diluted in PBS (11.06 ± 0.58 ng ml⁻¹), PBS–lithium-heparinate (11.42 ± 0.36 ng ml⁻¹; 95% CI of difference with PBS –0.63 to 0.10), PBS–BSA (11.01 ± 0.5 ng ml⁻¹; 95% CI of difference with PBS –0.25 to 0.55) or PBS–lithium-heparinate–BSA (11.36 ± 0.55 ng ml⁻¹; 95% CI of difference with PBS –0.94 to 0.52). This experiment clearly demonstrates that lithium-heparinate and BSA do not introduce a bias in the detection of morphine using this particular ELISA kit. However, when serum and lithium-heparinate tubes were spiked with a known concentration of morphine prior to fresh blood collection (healthy donors, $n = 6$; 10 ng ml⁻¹), $86 \pm 7\%$ (95% CI 79.8–92.2%) of the initial morphine concentration was recovered in plasma samples, but only $42 \pm 3\%$ (95% CI 37.9–46.4%) in serum samples (Mann–Whitney U -test, $P < 0.01$).

Finally, 80 patients distributed in the following three groups were tested: (i) healthy donors ($n = 16$); (ii) morphine-treated patients (morphine skin patch 12–48 h before blood collection, 20 mg day⁻¹, $n = 8$); and (iii) critically ill patients ($n = 56$, naive for morphine), including patients with no infection and no inflammation ($n = 18$), systemic inflammatory response syndromes ($n = 8$), severe systemic inflammatory response syndrome ($n = 16$), sepsis ($n = 4$), severe sepsis ($n = 2$) and septic shock ($n = 8$). In healthy donors, eM was undetectable in both serum and plasma samples. In morphine-treated patients (Figure 1), the mean morphine concentration in serum samples ranged from 3.2 to 31 ng ml⁻¹ (median 8.1 ng ml⁻¹; 95% CI 5.3–20.1 ng ml⁻¹), whereas plasma values ranged from 25.7 to 114.8 ng ml⁻¹ (median 54.8 ng ml⁻¹; 95% CI 41.9–85.0 ng ml⁻¹). On average, morphine concentrations in serum were $78 \pm 15\%$ (95% CI 68–88%) lower than in plasma (Wilcoxon signed-rank test, $P < 0.05$). In critically ill patients, eM concentrations ranged between 0 (not detectable) and 23.6 ng ml⁻¹ in serum samples (median 1.1 ng ml⁻¹; 95% CI 1.9–4.8 ng ml⁻¹) and between 0.15 and 76.3 ng ml⁻¹ (median 2.8 ng ml⁻¹; 95% CI 6.7–15.5 ng ml⁻¹) in plasma samples. Again, serum concentrations of eM were lower (from +13 to –98%; mean $65 \pm 32\%$; 95% CI 57–73%) compared with plasma (Wilcoxon signed-rank test, $P < 0.01$). The presence of morphine was confirmed by liquid chromatography coupled to tandem mass spectrometry analysis performed on plasma and serum from eight patients

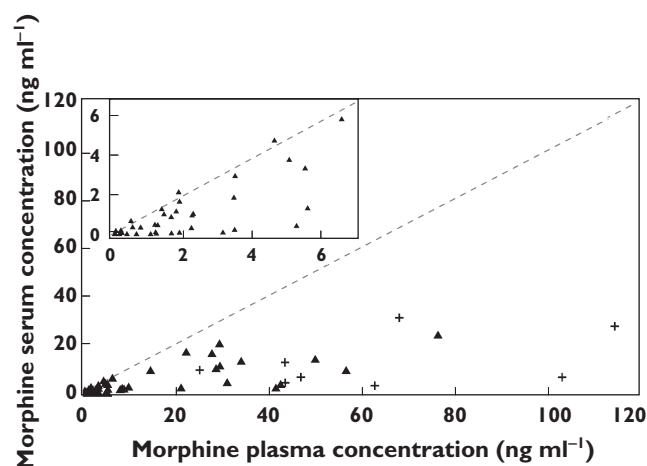


Figure 1

Concentrations of morphine in serum expressed as a function of the corresponding morphine concentrations in plasma in critically ill patients ($n = 56$, triangles) and in morphine-treated patients ($n = 8$, crosses). Inset shows a higher magnification of the lower values. All patients below the dashed line exhibited higher concentrations of morphine in the plasma than in the serum

not treated with morphine but positive for eM, whereas morphine was not detected in patients negative for eM in ELISA ($n = 4$) [4]. As expected, no morphine was detected in samples from four healthy donors.

Our results revealed that morphine concentrations were systematically higher in plasma compared with serum for 61 of 64 patients and thus indicate that lithium-heparinate samples give a more accurate quantification of morphine blood concentrations.

The cause of such a difference in morphine quantification between serum and plasma using this specific ELISA kit is currently under investigation by our group and might involve morphine binding to blood proteins [5] other than serum albumin. In line with this possibility, morphine detection in the blood has been shown to be highly dependent on urea concentrations (chaotropic agent), suggesting a link between morphine-binding proteins and morphine bioavailability [5]. Likewise, lithium is also a chaotropic/denaturing agent; therefore, it might explain why this ELISA quantification method for morphine is more efficient in the presence of lithium.

The present results clearly demonstrate that to obtain accurate ELISA determinations of exogenous and endogenous morphine concentrations in the blood, the analysis should be carried out on lithium-heparinate plasma samples rather than serum samples.

Competing Interests

There are no competing interests to declare.

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