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Site-, Technique- and Time-Related Aspects of the Postmortem Redistribution of Diazepam, Methadone, Morphine and their Metabolites: Interest of Popliteal Vein Blood Sampling

Eric Lemaire,[1,4] M.D., Ph.D.; Carl Schmidt,[2] M.D.; Nathalie Dubois,[3] Ph.D.; Raphael Denooz,[3] Ph.D.; Corinne Charlier,[3] Ph.D.; and Philippe Boxho,[4] M.D., Ph.D.

[1]Department of Pathology, University Hospital - CHU Sart Tilman; Liège, Belgium.

[2]Department of Pathology, University of Michigan; Ann Arbor, MI.

[3]Medico-legal Toxicology Laboratory, University Hospital - CHU Sart Tilman; Liège, Belgium.

[4]Department of Forensic Medicine, Medico-legal Institute of the University of Liège; Liège, Belgium.

Corresponding author:

Eric Lemaire, M.D., Ph.D.; Eric.Lemaire@chu.ulg.ac.be

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ABSTRACT: Sampling site, technique and time influence postmortem drug concentrations. In 57 cases we studied drug concentration differences as follows: subclavian vein - dissection/clamping vs. blindstick, femoral vein - dissection/clamping vs. blindstick, right cardiac chamber and popliteal vein – dissection and clamping only. Cases were distributed in group #1 (all cases with both techniques), group #2 (dissection/clamping) and group #3 (blindstick). Sampled drugs were diazepam, methadone, morphine, and their metabolites. To assess PMR, mean concentrations and ratios were calculated for each group. Time-dependent variations of blood concentrations and ratios were also assessed. Results indicate that site, method and time may influence postmortem distribution interpretation in different ways. Popliteal blood seems less subject to PMR. In conclusion, our study is the first to evaluate concurrently three main aspects of PMR and confirms that the popliteal vein may represent a site that is more resistant to the changes seen as a result of PMR.

KEYWORDS: forensic science, forensic toxicology, postmortem redistribution, sampling site, sampling technique, blind stick, sissection/clamping, postmortem interval, popliteal blood

Postmortem redistribution (PMR) refers to the postmortem processes that change the distribution of drugs in tissues, resulting in blood concentration variations depending on the site where blood is sampled and the post-mortem interval elapsed at the time of sampling. This complex phenomenon is still not entirely understood but authors generally agree on the involvement of 3 main factors. First, passive drug diffusion from reservoir organs (i.e. heart, liver, lungs and proximal gastro-intestinal tract) to adjacent organs and nearby blood vessels may occur as a consequence of the non-uniform distribution of drug in body tissues during life as well as the presence of unabsorbed substances in the digestive tract, both resulting in concentration gradients. Second, cellular acidification and autolysis can lead to accumulation of basic compounds in tissues as well as disruption of the protein binding characteristics of substances. Third, basic, lipophilic, highly protein-bound drugs and those with a large volume of distribution are more prone to PMR. While almost all drugs exhibit some degree of PMR, it is almost impossible to predict the extent to which a substance will redistribute after death (1-9).

While not strictly redistribution, postmortem degradation of compounds can also lead to changes in drug concentration that can often be confused with redistribution, such as the hydrolysis of morphine glucuronides after prolonged postmortem periods (10-12) and the conversion of nitrobenzodiazepines by postmortem bacteria metabolism (13).

Sampling from central sites (subclavian vessels and heart) tends to be more affected by redistribution than peripheral sites (iliac and femoral vessels) as central blood vessels often show higher postmortem concentrations due to their proximity to reservoir organs and thoraco-abdominal viscera, which are more prone to rapid

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decomposition. Popliteal vessels are also peripheral sites, unexplored so far. We studied popliteal blood concentrations of diazepam, methadone and morphine, and showed that sampling from this site results in drug concentrations lower than those in cardiac, subclavian and even femoral sampling. This suggests that popliteal blood is less prone to PMR, probably because of its distance from the trunk and isolation from many of the factors that alter postmortem drug concentrations (14,15)

The extent to which a drug is prone to postmortem redistribution is usually described by the ratio of the central (C) to peripheral (P) concentration of a drug, or C/P ratio (1,2,6,7,16). The greater the ratio, the greater the extent of postmortem redistribution; on the contrary, a ratio less than or equal to 1 suggests absence of redistribution. However, some authors suggest that the C/P ratio is not always a reliable indicator of postmortem redistribution for a particular substance as seen with C/P ratios greater or less than 1 in cases associated with incomplete distribution or cardiopulmonary resuscitation attempts, as well as with C/P ratios greater than 1 for drugs that are theoretically not subject to redistribution (1,17,18). Hence, the liver to peripheral blood (L/P) ratio has also been evaluated as a possible alternative marker of PMR (17-21).

As mentioned above, differences in drug concentrations in postmortem blood samples taken from different sites can also arise from an incomplete distribution of the drug at the time of death, and not exclusively from postmortem redistribution; it is therefore important to keep in mind that demonstrating site-to-site differences in the blood concentrations of a particular drug does not necessarily prove that the drug undergoes postmortem redistribution (16,22,23).

How blood is sampled may also affect the measurement of drug concentrations. It has been suggested that clamping the femoral vessel before sampling may prevent possible contamination from more central sites due to the retrograde flow of blood as it can happen with a blind stick sampling. Therefore, femoral sampling done after dissection and clamping of the vein is currently considered the method of choice since it prevents the caudal flow of blood from more central sources such as iliac vessels and the inferior vena cava (1-3). However, this procedure results in added time to the external examination as well as additional incisions, and some medico-legal offices simply perform a blind stick femoral sample without tying off the femoral vein. There are only few references comparing techniques: some authors used dissection and clamping of the vein, others did a blind stick method and some did not mention which sampling method they used. Hargrove et al. concluded that the blind stick method of drawing femoral blood, the easiest, least invasive as well as least time-consuming procedure, did not have significant redistribution from central sites and was of equivalent quality to a clamped femoral sample for selected drugs (benzodiazepines-diazepam and opiates-hydrocodone), for sampling volumes up to 30 ml (24). The same authors did not observe significant changes in either clamped or unclamped femoral vein morphine concentrations over time either as well as at any period of sampling within the first 24 hours after death in bodies kept refrigerated at 4°C (25). With subclavian sampling, there are publications suggesting that the subclavian vein should not be considered a strictly central site, but rather an intermediate one (16,26), but we did not find any study addressing adequately the issue of subclavian sampling techniques. Consequently, we evaluated the sampling method in a recent study and our results showed that diazepam and methadone concentrations were lower when drawn from either clamped subclavian or femoral vein whereas subclavian morphine mean concentrations tend to be lower when drawn from a clamped subclavian vein, but not for femoral sampling (14,27). Hence, we suggested that clamping vessels and isolating them from heart or abdominal blood may result in lower concentrations depending on the drug. There was no difference between right and left popliteal samples.

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Other than sampling site and technique, the postmortem interval appears to be important. From the available data, it seems that redistribution mainly occurs in the early postmortem period, as significant increases in concentrations by passive diffusion from reservoir organs have been demonstrated during the first postmortem hours for many substances (1,28-32). However, cellular autolysis and bacterial metabolism may also result in later changes, both in central and peripheral sites (33-36). Conversely, some studies have determined that there is little evidence of time dependent variability at either central or peripheral site (5,37).

In the present study, we sampled a number of drugs from central (heart and subclavian veins) and peripheral (femoral and popliteal veins) sites and we evaluated the influence of the site of sampling, the sampling technique (for subclavian and femoral sites) as well as the time of sampling, respectively, on the blood drug concentrations and ratios.

We chose drugs more commonly abused in the jurisdiction of the Medico-Legal Institute of the University of Liège, Belgium. These were diazepam, methadone and morphine as well as their respective metabolites. Concerning their potential for postmortem redistribution, different and sometimes controversial trends are found in the literature. Diazepam may not have significant PMR; however, heart/femoral blood mean ratios greater than one are found in the literature on relatively large series, suggesting that PMR may explain at least partially site-to-site difference in diazepam concentration (38,39). Nordiazepam may not exhibit redistribution according to the mean C/P ratios found in the literature whereas oxazepam exhibits some degree of redistribution in one study (16,40-42). Methadone is thought to undergo significant redistribution (3,16,39,43-45). Morphine may (2,16,39,46,47) or may not (33,37,48) exhibit significant redistribution, whereas many animal models have shown that morphine does undergo redistribution (2,31,49,50); furthermore, studies suggested that postmortem increases in free morphine concentration could be due, at least partially, to hydrolysis of morphine glucuronides rather than postmortem redistribution and an increase in the free/total morphine concentration ratio would be seen with increasing hydrolysis (31,50,51). The chemical properties of selected substances may influence PMR: diazepam is a lipophilic weak base (pKa 3.4) with a low Vd (0.7-2.6 L/kg), nordiazepam has pKa 3.5 and Vd 0.5-2.5 L/kg and oxazepam has pKa 1.7 and Vd 0.7-1.6L/kg; methadone is a lipophilic base (pKa 8.6) with a larger Vd (4-7 L/kg); morphine is a hydrophilic amphoteric base (pKa 7.9, 9.6), but lipid soluble at physiologic pH, with an intermediate Vd (2-5 L/kg) whereas Vd is approximately 0.28 L/kg for morphine glucuronides (2,41,47). Moreover, morphine glucuronides can exist in two conformational forms, the folded conformers being more lipophilic; certain site-to-site variation could be associated with the ambiguous nature of morphine-glucuronides (47,52).

According to some authors, diazepam is stable in blood and tissues (53,54), even with putrefaction (55), unlike other benzodiazepines (9,55,56) although this can depend on specimen preservation (56), temperature (56-58) and other factors (58); nordiazepam is less stable in postmortem unpreserved blood (53). Methadone was found to be stable in postmortem blood (54,59). Concerning the stability of morphine, some authors did not see significant changes in morphine concentrations in patient samples and stored blood even when compared with admission and postmortem blood, in some cases for days after the sample was drawn (25,47,48,54,60). Other studies showed that low pH, increased storage time, temperature and degree of putrefaction resulted in greater free morphine generation (10) whereas morphine and its glucuronides were stable in sampled post-mortem blood only when stored at -20°C (12,61).

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Our study has three goals: first, to confirm the influence of sampling site; secondly, to assess the influence of sampling method by comparing blind stick with dissection/clamp technique; finally, to evaluate the influence of postmortem interval.

Methods

In this study, we included 57 cases of drugs intoxications referred to our medico-legal office in Liège during a 2,5-year period, i.e. from November 2012 to April 2015. When possible, a urine drug screen was done to assess the presence of the drugs of interest (Drug-Screen®, *nal von minden GmbH, Regensburg, Germany*). If not, the case was selected according to history and medicolegal context.

Cases were sampled as follows: subclavian blood - dissection/clamp technique (SBD), subclavian blood - blindstick technique (SBB), femoral blood - dissection/clamp technique (FBD), femoral blood - blindstick technique (FBB), intracardiac blood (ICB) and popliteal blood (PB).

Thirty cases were sampled with single specimens taken as follows:

- cardiac blood was drawn in the right atrium after a small chest incision;
- at subclavian and femoral sites, transcutaneous blind stick sampling was done on the left side of the body while a dissection with proximal clamping of the vessel was done on the right;
- popliteal blood was collected from both sides.

A second group of 27 cases was sampled twice at the same sites as above, with the first sample always done on the left side of the body and the second sample on the right side after a recorded time interval (generally greater than 24 h), along with cardiac blood taken in the right atrium for both samples. The same sampling technique was used for subclavian and femoral samples in the same case, but we alternated sampling methods from case to case.

Popliteal sampling always required dissection because of its depth in the knee; the popliteal vein was clamped as cephalad as possible to prevent any theoretical femoral blood reflux. Compression of the leg was sometimes required to obtain an adequate amount of blood for testing.

Cases were distributed in 3 groups: group #1 (n=57) included all cases, group #2 (n=42) concerned cases with dissection/clamping technique at subclavian and femoral sites and group #3 (n=45) those cases with blindstick technique at subclavian and femoral sites.

In order to assess the influence of sampling site and technique on PMR, for each substance and for each group, mean concentrations ratios were calculated as follows: [cardiac]/[subclavian], [cardiac]/[femoral], [cardiac]/[popliteal], [subclavian]/[femoral], [subclavian]/[popliteal] and [femoral]/[popliteal]. Ratios were also compared between groups #2 and #3 to assess the difference between blind stick and vein dissection techniques.

To evaluate the influence of postmortem interval, two methods were used. The first method, (method 1) studied in all cases (n=57) and for each substance, the correlation between the concentration ratios and the corresponding estimated postmortem interval. The second method (method 2) studied only in those cases sampled twice (n=27) and for each substance, the differences of concentrations and ratios between both samples. To assess the contribution of hydrolysis of morphine-glucuronides to free morphine, free morphine/total morphine ratios differences were also calculated in cases sampled twice where morphine and both morphine-glucuronides were present (n=12). Postmortem changes as well as elements collected from death scene allowed calculation of the

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estimated postmortem interval in method 1 while the precise time elapsed between both samples was recorded in method 2.

Mean sampled blood volumes were the following: ICB 7.6 ml (range 1-12 ml); SBD 6.4 ml (range 0.5-16 ml); SBB 8.7 ml (range 1-16 ml); FBD 6.3 ml (range 1-12 ml); FBB 6.9 ml (range 1-16 ml); PB 4.5 ml (range 0.5-8 ml).

Drugs concentrations were quantified, as follows: diazepam and its metabolites nordiazepam and oxazepam; methadone and its metabolite EDDP; morphine and its metabolites morphine-3-glucuronide and morphine-6-glucuronide.

Blood samples were collected in sodium fluoride/potassium oxalate (2%) vials and frozen at -20 °C prior to analysis done within the first 4 to 6 weeks after sampling.

Quantitative Analysis

The quantification of morphine and methadone was performed on an ultra-high pressure liquid chromatograph Acquity® coupled to a tandem mass spectrometer Quattro Premier® (*Waters, Zellik, Belgium*). After solid phase extraction of the sample on Oasis MCX® cartridges, the separation was performed on an Acquity HSS T3 column. The mobile phase consisted in a gradient of ammonium formate (pH 3) and acidified methanol (62).

Diazepam was analyzed in blood using a high performance liquid chromatography with photodiode array detection (Alliance®, *Waters, Zellik, Belgium*) based on a method described by Gaillard et al. (63). After a liquid-liquid extraction using a mixture of diethyl ether, dichlormethane, hexane and n-amyl alcohol, the sample was injected on a Symmetry C8 column with phosphate buffer (pH 3.8) and acetonitrile delivered according to a gradient elution as mobile phase. Considering low, intermediate and high concentration, respectively, coefficients of variation (CV) were the following: 6.02 %, 4.00 % and 3.22 % for diazepam; 5.88 %, 3.24 % and 2.60 % for nordiazepam; 6.02 %, 3.88 % and 3.66 % for oxazepam; 3.33 %, 5.08 % and 6.41 % for methadone ; 8.29 %, 15.33 % and 1.10 % for EDDP; 6.64 %, 4.24 % and 7.10 % for morphine; 5.52 %, 5.23 % and 6.02 % for morphine-3-glucuronide; 4.64 %, 6.74 % and 5.57 % for morphine-6-glucuronide.

A single quantitation of analytes was carried out for each sampling site. Quality and validation of each analysis was ensured through two levels of control (one internal, the other commercial) and by the use of a multipoint calibration curve (7 points and a blank).

Statistical Analysis

Statistical analyses were performed by using SAS software (version 9.3 for windows) and R software. Normality of the distributions was checked by using a Shapiro-Wilk test. A logarithmic transformation of concentrations was also used to normalize the distributions. Quantitative variables were summarized by the mean, standard deviation (SD), median, minimum and maximum. Qualitative variables were summarized by means number (N) and percentage (%).

Mean Concentrations and Mean ratios in All Cases (Group 1)

In Group #1 (n=57), for each substance, mean concentrations at each site were calculated for all cases and the sampling sites were compared with a non-parametric Friedman test. Results were considered as statistically significant at 5% level ($p < 0.05$). For each substance, drug concentrations differences between sites were

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calculated as follows: ICB - SB, ICB - FB, ICB - PB, SB -FB, SB -FB, and FB - PB. A non-parametric Wilcoxon signed-rank test was used to assess a significant concentration difference. For each substance, the following ratios were calculated: ICB / SB, ICB / FB, ICB / PB, SB / FB, SB / PB, and FB / PB. A non-parametric Wilcoxon signed-rank test was also utilized to assess a significant ratio, i.e. a ratio different to 1.
For the comparison of concentrations at the different sampling sites and for the comparison of mean ratios, a Bonferroni's correction (0.05/6 = 0.0083) was used to consider statistically significant results ($p<0.0083$).

Mean Concentrations and Mean Ratios in Cases with Dissection/Clamping Technique at Subclavian and Femoral Sites (Group 2)

In Group #2 (n=42), for each substance, mean concentrations at each site were calculated and the sampling sites were compared with a non-parametric Friedman test. Results were considered as statistically significant at 5% level ($p< 0.05$). For each substance, drug concentrations differences between sites were calculated as follows: ICB - SBD, ICB - FBD, ICB - PB, SBD -FBD, SBD - PB, and FBD - PB. A non-parametric Wilcoxon signed-rank test was used to assess a significant concentration difference. For each substance, the following ratios were calculated: ICB / SBD, ICB / FBD, ICB / PB, SBD / FBD, SBD / PB, and FBD / PB. A non-parametric Wilcoxon signed-rank test was also utilized to assess a significant ratio, i.e. a ratio different to 1. For the comparison of concentrations at the different sampling sites and for the comparison of mean ratios, a Bonferroni's correction (0.05/6 = 0.0083) was used to consider statistically significant results ($p<0.0083$).

Mean Concentrations and Mean Ratios in Cases with Transcutaneous Blindstick Technique at Subclavian and Femoral Sites (Group 3)

In Group #3 (n=45), for each substance, mean concentrations at each site were calculated and the sampling sites were compared with a non-parametric Friedman test. Results were considered as statistically significant at 5% level ($p< 0.05$). For each substance, drug concentrations differences between sites were calculated as follows: ICB - SBB, ICB - FBB, ICB - PB, SBB -FBB, SBB - PB, and FBB - PB A non-parametric Wilcoxon signed-rank test was used to assess a significant concentration difference. For each substance, the following ratios were calculated: ICB / SBB, ICB / FBB, ICB / PB, SBB / FBB, SBB / PB, and FBB / PB. A non-parametric Wilcoxon signed-rank test was also utilized to assess a significant ratio, i.e. a ratio different to 1. For the comparison of concentrations at the different sampling sites and for the comparison of mean ratios, a Bonferroni's correction (0.05/6 = 0.0083) was used to consider statistically significant results ($p<0.0083$).

Influence of Estimated Postmortem Interval on Mean Ratios in All Cases (n=57) (Method 1)

For each substance, non-parametric Spearman correlation coefficients were calculated to assess the correlation between mean ratios and estimated postmortem interval. A negative coefficient showed a decreasing relation between the two parameters (when one increased, the other decreased) while a positive coefficient showed an increasing relation (when one increased, the other increased too). For assessing the influence of estimated post-mortem interval, results were considered as statistically significant at 5% level ($p< 0.05$).

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Mean Concentrations and Ratios Differences in Cases Sampled Twice (n =27) (Method 2)

For each substance, mean concentrations at each site and mean ratios calculated for samples 1 and 2 were compared by using a non-parametric Wilcoxon signed-rank test. For each substance, non-parametric Spearman correlation coefficients were calculated to assess the correlation between mean concentrations and ratios differences and the time interval elapsed between samples 1 and 2. A negative coefficient showed a decreasing relation between the two parameters (when one increased, the other decreased) while a positive coefficient showed an increasing relation (when one increased, the other increased too). For assessing the influence of time interval between samples 1 and 2, results were considered as statistically significant at 5% level ($p< 0.05$). In order to assess the possible contribution of hydrolysis of morphine-glucuronides to free morphine between samples 1 and 2, free morphine/total morphine ratios differences between samples 1 and 2 were also compared by using a non-parametric Wilcoxon signed-rank test.

Results

Table 1 shows, for all cases, age, sex and average estimated postmortem interval as determined by the protocol in use by our office.

Table 2 shows, for all cases, assayed substances and their metabolites as well as their respective frequencies.

Influence of Sampling Site in All Cases (Group #1)

Figure 1 (a,b,c), Figure 2 (a,b), and Figure 3 (a,b,c), show mean blood concentrations distribution according to sampled drugs in all cases. All concentrations are expressed in microgram per liter of blood (µg/L).

For morphine (n=49), methadone (n=60) and their respective metabolites, morphine-3-glucuronide (n=47), morphine-6-glucuronide (n=39) and EDDP (n=52), mean concentrations tend to decline the further the sampling site is from the heart.

For diazepam (n=24) as well as its metabolites nordiazepam (n=26) and oxazepam (n=14), results show slightly higher femoral mean concentrations than central sites concentrations; furthermore subclavian mean concentrations are also greater than cardiac site.

For all sampled drugs, popliteal mean concentrations are lower than other three sites.

Cardiac and subclavian sites show no significant mean concentration differences for the three compounds and their metabolites except for morphine-6-glucuronide ($p=0.0011$). Cardiac and femoral sites show statistically significant mean blood concentrations differences for diazepam ($p=0.0063$), morphine ($p<0.0001$) and EDDP ($p=0.0046$) whereas cardiac and popliteal sites mean concentrations are significantly different for morphine ($p<0.0001$), methadone ($p<0.0001$) and EDDP ($p<0.0001$). For morphine ($p<0.0001$), methadone ($p<0.0001$) and EDDP ($p=0.0055$), subclavian and femoral sites also show significant mean concentration differences while all substances show significant mean concentration differences between subclavian and popliteal site ($p<0.0001$ for nordiazepam, methadone, EDDP, morphine, and morphine-6-glucuronides; $p=0.0046$ for diazepam; $p=0.0004$ for morphine-3-glucuronide), except oxazepam ($p=0.022$). Finally, for all substances, popliteal mean concentrations are significantly lower than femoral mean concentrations ($p<0.0001$ for diazepam, nordiazepam, methadone, EDDP, and morphine; $p=0.0031$ for nordiazepam; $p=0.0006$ for morphine-3-glucuronide; $p=0.0059$ for morphine-6-glucuronide).

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To assess the occurrence of postmortem redistribution, for each substance, the following average ratios of concentrations were obtained: ICB/SB, ICB/FB, ICB/PB, SB/FB, SB/PB and FB/PB as shown in Table 3.

For diazepam and its metabolites, ICB/SB mean ratios are less than or equal to 1 whereas ICB/FB and SB/FB mean ratios are consistently less than the corresponding ICB/PB and SB/PB ratios. FB/PB ratios are also consistently greater than the more usual central (cardiac/subclavian) / peripheral (femoral/popliteal) ratios.

For methadone and EDDP, we see that ICB/SB mean ratios are slightly greater than or close to 1 for methadone but show a greater difference for EDDP. For both compounds, ICB/FB and SB/FB mean ratios are consistently less than the ICB/PB and SB/PB ratios, respectively. FB/PB ratios are consistently lower than the more usual central (cardiac/subclavian) / peripheral (femoral/popliteal) ratios, except for EDDP SB/FB less than FB/PB.

For morphine and morphine-glucuronides, results show that morphine ICB/SB mean ratio is greater than 1 whereas an opposite trend is seen for both morphine-glucuronides. ICB/FB and SB/FB mean ratios are consistently less than the corresponding ICB/PB and SB/PB ratios. FB/PB ratios are also consistently lower than the more usual central (cardiac/subclavian) / peripheral (femoral/popliteal) ratios for morphine and morphine-6-glucuronide, with the exception of morphine-6-glucuronide FB/PB greater than ICB/FB. Conversely, for morphine-3-glucuronide, FB/PB mean ratio is consistently greater than (cardiac/subclavian)/(femoral/popliteal) mean ratios.

ICB/SB mean ratios are not statistically significant, i.e. different from 1, for any substances. ICB/FB mean ratios are not statistically significant, i.e. different from 1, for any substances except for diazepam ($p=0081$), EDDP ($p=0.008$), and morphine ($p<0.0001$). ICB/PB mean ratios are statistically greater than 1, methadone ($p<0.0001$), EDDP ($p<0.0001$), and morphine ($p<0.0001$), but are not statistically significant, i.e. different from 1, for diazepam, oxazepam, and both morphine glucuronides. SB/FB means ratios are statistically greater than 1, for methadone ($p<0.0001$), EDDP ($p=0.0006$), and morphine ($p<0.001$), but show no signification, i.e. different from 1, for the other sampled substances. SB/PB mean ratios are statistically greater than 1 for all substances, except for oxazepam ($p=0.022$). FB/PB mean ratios are statistically greater than 1 for all substances and their metabolites.

Influence of Subclavian and Femoral Sampling Technique on Mean Concentrations and Ratios (Group #2 and #3)

Figure 4 (a,a',b,b',c,c'), Figure 5 (a,a',b,b'), and Figure 6 (a,a',b,b',c,c'), show mean blood concentrations distribution according to sampling technique used at subclavian and femoral sites. All concentrations are expressed in microgram per liter of blood (µg/L).

For diazepam (group #2 n=20; group #3 n =18), nordiazepam (group #2 n=22; group #3 n =20), and oxazepam (group #2 n=12; group #3 n =10), mean concentrations tend to decline the further the sampling site is from the heart with dissection/clamping technique at subclavian and femoral sites (group#2), except for oxazepam with mean ICB lower than SBD. With blind stick sampling at the same sites (group #3), we see an opposite trend as mean concentrations tend to decrease the closer to the heart is the site, except for oxazepam mean ICB greater than SBB. However, for diazepam, nordiazepam and oxazepam, popliteal mean concentrations are lower than other sites for both techniques used at subclavian and femoral sites.

For methadone (group #2 n=38; group #3 n=46) and EDDP (group #2 n=34; group #3 n=42), mean concentrations tend to decline the further the sampling site is from central sites with dissection/clamping method

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(group#2) as well as with the blindstick technique (group #3). Moreover, popliteal mean concentrations are still lower than other sites for both groups.

For morphine (group #2 n=33; group #3 n=33), mean concentrations tend to decline the further the sampling site is from central sites with both sampling techniques used at subclavian and femoral sites; however, subclavian and femoral concentrations tend to be closer to cardiac concentrations with dissection/clamping method. For morphine-3-glucuronide (group #2 n=33; group #3 n=31) and morphine-6-glucuronide (group #2 n=29; group #3 n=25), with dissection/clamping technique (group#2), we see a different trend as femoral concentrations are greater than subclavian and close to cardiac concentrations; with blind stick sampling (group #3), cardiac and subclavian mean concentrations are obviously greater than femoral site whereas subclavian concentration tends also to be higher than cardiac site. Though, popliteal mean concentrations are lower than other sites for both groups for morphine and its metabolites, as observed for the other sampled drugs.

In group #2, ICB and SBD show no significant mean concentration differences for all compounds and metabolites. ICB and FBD show statistically significant mean blood concentrations differences for morphine ($p=0.003$) and EDDP ($p=0.0056$) while cardiac and popliteal sites mean concentrations are significantly different for morphine ($p<0.0001$), methadone ($p=0.0003$) and EDDP ($p<0.0001$). For morphine ($p=0.0012$) and methadone ($p<0.0001$), SBD and FBD sites also show significant mean concentration differences whereas significant mean concentration differences between SBD and PB are found for nordiazepam ($p<0.0001$), morphine ($p<0.0001$), methadone ($p<0.0001$) and EDDP ($p<0.0001$). Finally, for morphine ($p<0.0001$), methadone ($p<0.0001$) and EDDP ($p<0.0001$), PB mean concentrations are significantly lower than FBD mean concentrations.

In group #3, ICB and SBB sites show no significant mean concentration differences for the three parent drugs and their metabolites. ICB and FBB sites show statistically significant mean concentrations differences for diazepam ($p=0.0028$), morphine ($p<0.0001$) and EDDP ($p=0.0037$). For methadone ($p<0.0001$), EDDP ($p<0.0001$), and morphine ($p<0.0001$), cardiac and popliteal sites show significant mean concentration differences; so do SBB and FBB for methadone ($p<0.0001$), EDDP ($p=0.0001$), morphine ($p<0.0001$), and morphine-6-glucuronide ($p<0.0001$). SBB and PB show significant mean concentrations differences for methadone ($p<0.0001$), EDDP ($p=0.0001$), morphine ($p<0.0001$) and metabolites ($p<=0.0001$) as well as for nordiazepam ($p=0.0014$). Except for morphine-6-glucuronide ($p=0.05$) and oxazepam ($p=0.084$), PB mean concentrations are significantly lower than FBB mean concentrations for all sampled drugs.

To assess the occurrence of postmortem redistribution according to sampling technique used, for each substance, the following average ratios of concentrations were obtained as shown in Table 4: ICB/SBD, ICB/FBD, ICB/PB, SBD/FBD, SBD/PB, and FBD/PB in group #2; ICB/SBB, ICB/FBB, ICB/PB, SBB/FBB, SBB/PB, and FBB/PB in group #3.

For diazepam and its metabolites, ICB/SBD mean ratios are equal to or slightly greater than ICB/SBB ratios whereas ICB/FBD ratios are consistently greater than ICB/FBB; SBD/FBD mean ratios are also greater than SBB/FBB for the three sampled substances. Conversely, diazepam and nordiazepam show SBD/PB mean ratios slightly less than SBB/PB ratios while oxazepam SBD/PB mean ratio is greater than SBB/PB. FBD/PB mean ratios are consistently less than FBB/PB ratios for all three drugs.

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With methadone, we see only slight differences between ICB/SBD and ICB/SBB mean ratios as well as between ICB/FBD and ICB/FBB; the same trend is found when comparing SBD/FBD and SBB/FBB. However, SBD/PB and FBD/PB mean ratios are less than SBB/PB and FBB/PB mean ratios, respectively. For EDDP, cardiac/subclavian and cardiac/femoral mean ratios are consistently greater with dissection/clamping technique than with blindstick sampling at subclavian and femoral sites whereas subclavian/femoral mean ratios are lower with dissection/clamping method than with the other sampling technique. Accordingly, SBD/PB and FBD/PB mean ratios are less than SBB/PB and FBB/PB mean ratios, respectively.

For morphine and morphine-glucuronides, ICB/SBD mean ratios are greater than ICB/SBB while an opposite trend is seen with ICB/FBD mean ratios less than ICB/FBB. SBD/FBD and SBD/PB are less than SBB/FBB and SBB/PB, respectively. However, FBD/PB appears equal to FBB/PB for morphine, whereas FBD/FB is greater than FBB/PB for both morphine-glucuronides.

In group #2, ICB/SBD mean ratios are not statistically significant, i.e. different from 1, for any substances. ICB/FBD mean ratios are not statistically significant, i.e. different from 1, for any substances except for EDDP ($p=0.0016$) and morphine ($p=0.0006$). ICB/PB mean ratios are statistically greater than 1 for methadone ($p<0.0001$), EDDP ($p<0.0001$), and morphine ($p<0.0001$). SBD/FBD means ratios are statistically greater than 1 for methadone ($p<0.0001$) and morphine ($p=0.0002$). SBD/PB mean ratios are statistically greater than 1 for nordiazepam ($p<0.0001$), methadone ($p<0.0001$), EDDP ($p<0.0001$), and morphine ($p<0.0001$) whereas diazepam mean ratio is really close to significance ($p=0.0083$). FBD/PB mean ratios are statistically greater than 1 for methadone ($p<0.0001$), EDDP ($p<0.0001$), and morphine ($p<0.0001$).

In group #3, ICB/SBB mean ratios are not statistically significant, i.e. different from 1, for any substances. ICB/FBB mean ratios are statistically significant, i.e. different from 1, for diazepam ($p=0.0090$), EDDP ($p=0.0007$) and morphine ($p<0.0001$). ICB/PB mean ratios are statistically greater than 1 for methadone ($p<0.0001$), EDDP ($p<0.0001$), morphine ($p<0.0001$), and morphine-6-glucuronide ($p=0.0066$). SBB/FBB mean ratios are statistically greater than 1 for methadone ($p<0.0001$), EDDP ($p=0.0001$), morphine ($p<0.0001$), and morphine-6-glucuronide ($p<0.0001$). SBB/PB mean ratios are statistically greater than 1 for nordiazepam ($p=0.0010$), methadone (<0.0001), EDDP (<0.0001), morphine ($p<0.0001$), morphine-3-glucuronide ($p<0.0001$), and morphine-6-glucuronide ($p<0.0001$). FBB/PB mean ratios are statistically greater than 1 for all substances and their metabolites, except oxazepam ($p=0.027$) and morphine-6-glucuronide ($p=0.022$).

Influence of Estimated Postmortem Interval on Mean Ratios (Method 1)

In all cases (n=57), for each substance, in order to assess the influence of postmortem interval on mean ratios, the correlations between ratios of concentrations obtained and the estimated postmortem interval were calculated as shown in Table 5.

For diazepam, there is a significant correlation between postmortem interval and SB/FB ($r = -0.49$, $p = 0.015$) as well as FB/PB ($r = 0.61$, $p = 0.0017$). For nordiazepam, a significant correlation is found between postmortem interval and SB/PB ($r = 0.42$, $p = 0.034$) but also FB/PB ($r = 0.58$, $p = 0.0019$). There is no significant correlation observed for oxazepam.

For methadone, there is only one significant correlation observed between postmortem interval and FB/PB ($r = 0.56$, $p < 0.0001$) whereas no significant correlation is seen with EDDP.

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For morphine, there is no significant correlation observed whereas significant correlations are seen for morphine-glucuronides. For morphine-3-glucuronide, we see a significant correlation between the postmortem interval and the following ratios: ICB/PB (r = 0.38, p = 0.0079), SB/PB (r = 0.37, p = 0.011) and FB/PB (r = 0.39, p = 0.0068). For morphine-6-glucuronide, the following ratios are correlated with the postmortem interval: ICB/PB (r = 0.43, p = 0.0066) and SB/PB (r = 0.40, p = 0.011).

Comparison of Mean Concentrations and Ratios in Cases Sampled Twice (Method 2)

In order to compare mean concentrations and ratios between samples 1 and 2 in cases sampled twice (n=27), for each substance and at each site, mean concentrations and mean ratios differences were calculated according to the mean time interval elapsed between both samples as shown in Figure 7-9 (a,b,c) and Table 6, respectively. Concentrations differences are expressed in microgram per liter of blood (µg/L).

Moreover, in order to assess the possible contribution of hydrolysis of morphine-glucuronides to free morphine, only in those cases where parent drug and both metabolites were present (n=12), free morphine/total morphine (i.e. free morphine + morphine-3-glucuronide + morphine-6-glucuronide) ratios differences between samples 1 and 2 were also calculated as shown in Table 7.

Concerning concentrations differences, for diazepam (n = 5, mean time interval 27.4 h +/-9.9), nordiazepam (n = 5, mean time interval 27.4 h +/- 9.9), and oxazepam (n = 3, mean time interval 30.7 h +/-12.42), we see different trends. ICB mean concentrations tend to decrease with time except for oxazepam, whereas SB mean concentration also appears to decrease for diazepam and oxazepam but shows increase with time for nordiazepam. For all three substances, FB shows mean concentrations increase between sample 1 and 2 while PB mean concentration consistently decreases with time. Methadone (n=18, mean time interval 27.2 h +/-13.8) and EDDP (n=14, mean time interval 27.0 h +/-15.4), both show ICB mean concentrations decrease, SB mean concentrations increase as well as PB mean concentrations increase between samples 1 and 2, whereas FB mean concentration tends to increase with time for methadone but shows an opposite trend for EDDP. For morphine (n=16, mean time interval 29.0 h +/-14.3) and morphine-glucuronides (morphine-3-glucuronide n=15, mean time interval 29.6 h +/-14.6 – morphine-6-glucuronide n=12, mean time interval 30.9 h +/-16.2) we see that ICB mean concentrations decrease with time while both SB and FB mean concentrations show increase between samples 1 and 2. PB mean concentrations show marked decrease with time for morphine-glucuronides but morphine mean concentration shows only slight increase between the two samples. However, those results are only statistically significant for the following mean concentrations differences: SB2-SB1 for EDDP (p=0.0040), SB2-SB1 for methadone (p=0.0090), SB2 – SB1 for morphine (p=0.0042), PB2 – PB1 for morphine-3-glucuronide (p<0.0001) and PB2 – PB1 for morphine-6-glucuronide (p=0.0005).

When it comes to the possible contribution of morphine-glucuronides hydrolysis to free morphine, in 12 cases where morphine and both morphine-glucuronides were samples twice, we see that free/total morphine mean ratios show increases for all sampling sites; increases are statistically significant for all sampling sites except ICB. Furthermore, free morphine/total morphine ratios increases are also more important the further the sampling site is from the heart.

There are differences in the concentration ratios of diazepam and its metabolites. ICB/SB, ICB/FB, ICB/PB and SB/FB mean ratios tend to decrease with time for diazepam and nordiazepam whereas oxazepam shows decrease for ICB/FB and SB/FB mean ratios but an increase for ICB/SB and ICB/PB. SB/PB mean ratio difference with

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time is nearly null for diazepam while it shows increase for nordiazepam and decrease for oxazepam, respectively, between samples 1 and 2. FB/PB mean ratio shows increase with time for all three compounds. Methadone and EDDP both show ICB/SB, ICB/FB and ICB/PB mean ratios decreases between samples 1 and 2 while SB/FB and SB/PB mean ratios tend to increase with time for both substances. For FB/PB mean ratio difference, we see an increase with time for methadone but an opposite trend for EDDP. When it comes to morphine and morphine-glucuronides mean ratios differences, results show that all mean ratios increase with time for morphine. For both morphine-glucuronides, ICB/SB and ICB/FB mean ratios tend to decrease between samples 1 and 2 whereas ICB/PB mean ratio shows increase for morphine-3-glucuronide but decrease with time for morphine-6-glucuronide; besides, both metabolites show increase with time for other mean ratios, i.e. SB/FB, SB/PB and FB/PB. As for the study of mean concentrations differences, only a limited number of results are statistically significant, i.e. SB2/PB2 - SB1/PB1 for methadone ($p=0.039$), ICB2/SB2 - ICB1/SB1 for EDDP ($p=0.030$), SB2/PB2 - SB1/PB1 and FB2/PB2 - FB1/PB1 for morphine-3-glucuronide ($p=0.0020$ and $p=0.0042$, respectively), as well as SB2/PB2 - SB1/PB1 and FB2/PB2 - FB1/PB1 for morphine-6-glucuronide ($p=0.0005$ and $p=0.0021$, respectively).

Correlation coefficients also were calculated to assess the correlations between mean concentrations as well as ratios differences and the time interval elapsed between samples 1 and 2; correlations were not statistically significant for any substance, except for oxazepam mean concentrations differences at femoral ($r=0.99$, $p=0.0026$) and popliteal ($r= -0.1$, $p<0.0001$) sites as well as for methadone mean ICB/PB differences ($r=0.57$, $p=0.014$) and oxazepam mean ICB/FB ($r=1.0$, $p<0.0001$), ICB/PB ($r=1.0$, $p<0.0001$) and FB/PB differences ($r=1.0$, $p<0.0001$).

Discussion

Influence of Sampling Site

Evaluation of the sampling site in all cases shows that mean concentrations tend to decline the further it is from the heart for methadone and morphine, as well as their respective metabolites. Conversely, diazepam and its metabolites show slightly higher femoral blood concentrations compared to cardiac and subclavian sites, as well as mean subclavian concentrations also greater than cardiac blood; results suggest that these benzodiazepines may undergo degradation in central sites. In general, nitrobenzodiazepines (e.g. clonazepam, nitrazepam, flunitrazepam) are among the most unstable owing to bacterial reduction of the nitro group whereas benzodiazepines without the N-oxide or nitro groups appear to display greater stability in biological specimens (13,56). However, Skopp et al. showed that the concentration of 13 benzodiazepines including diazepam and nordiazepam significantly decreased in unpreserved blood (58). Hence, the more intense degradation of diazepam and metabolites in central sites may explain our findings. For all sampled drugs, popliteal mean concentrations are lower than the other three sites.

PMR average ratios find different trends. First, ICB/SB mean ratios are close to 1 and have no significant differences for any of the sampled compounds, indicating that the subclavian vein is a central site. Secondly, ICB/FB and SB/FB mean ratios are consistently less than the corresponding ICB/PB and SB/PB for all targeted substances, suggesting than PMR is more apparent when comparing central sites with the popliteal versus the

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femoral site. Third, FB/PB mean ratios are statistically greater than 1 for all drugs and their metabolites, suggesting that popliteal blood may be less prone to PMR, which we reported previously (15).

Effect of Sampling Technique

Diazepam and metabolites mean concentrations tend to decline the further from the heart with the dissection/clamp method; the opposite of the trend is seen with a blind stick, as mean concentrations tend to decrease the closer the sample is to the heart. This suggests that dissection/clamping at subclavian and femoral sites may result in isolation from both central degradation and PMR processes, respectively. Therefore, sampling techniques allow us nuancing the aforementioned discussion about degradation of diazepam and metabolites in central sites since postmortem processes of redistribution may also probably interfere. As a consequence, the blind stick method may account for drawing central blood mixed from both degradation and redistribution processes, with degradation of drugs being probably more intense than redistribution, resulting in an increase of mean concentrations the further from the heart. On the contrary, dissection/clamp technique of subclavian and femoral vessels may isolate blood from both central processes and their relative importance, resulting in an opposite trend with mean concentrations declining the further from the heart. Another hypothesis could be the blood contamination with surrounding tissues at the site of blind stick sampling, but probably to a lesser extent as diazepam and metabolites exhibit a low volume of distribution even if they are lipophilic. Moreover, the popliteal mean concentrations are lower than other three sites for all sampling methods. Study of mean ratios confirms these trends for the three sampled substances. In addition, diazepam and nordiazepam show SBD/PB mean ratios slightly less than SBB/PB while FBD/PB mean ratios are consistently less than FBB/PB ratios for all three drugs, suggesting that subclavian and femoral blood mean concentrations are closer to popliteal blood when isolated from central processes; FBD/PB mean ratios greater than 1 for all three compounds may also indicate that popliteal blood is more isolated from PMR even than femoral blood sampled from a clamped vessel.

For methadone and EDDP, with both methods used at subclavian and femoral sites, mean concentrations tend to decline the further the sampling site is from the heart. For methadone, when compared to cardiac or popliteal mean concentrations, subclavian and femoral mean concentrations do not show obvious differences attributable to sampling technique. For EDDP, with the dissection/clamp method, subclavian and femoral mean concentrations tend to be proportionally lower than intracardiac blood when compared to a blind stick. Popliteal mean concentrations are lower than the other three sites for both techniques. Mean ratios correspond with these results. For methadone and EDDP, SBD/PB and FBD/PB mean ratios are less than corresponding SBB/PB and FBB/PB mean ratios, respectively. Hence, results suggest that both the subclavian and femoral dissection/clamping techniques result in isolation from central PMR processes even if this is more evident in central sites with EDDP than with the parent drug. Moreover, FBD/PB mean ratios are significantly greater than 1 for both compounds, indicating that popliteal blood is still more isolated from PMR processes than femoral clamped vessel.

For morphine, for both techniques at subclavian and femoral sites, we still see mean concentrations declining the further the sampling site is from the central blood; however, subclavian and femoral concentrations tend to be closer to cardiac concentrations with the dissection/clamp method. This may account for the greater stability of morphine further away from central compartments. For both glucuronide metabolites, we see a different trend as

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femoral concentrations are greater than subclavian and close to cardiac concentrations with dissection/clamping method; with blind stick sampling, cardiac and subclavian mean concentrations are greater than those at the femoral site, whereas subclavian concentrations tend also to be higher than those from the heart. Hence, for morphine-glucuronides, the dissection/clamp method may result in isolation from central PMR processes, even though this is more marked at the subclavian than at the femoral site. Popliteal mean concentrations are lower than all other sampling methods for morphine and its metabolites, as seen for the other drugs. However, morphine ICB/SBD mean ratio is greater than ICB/SBB, different from the trend seen with mean concentrations, whereas morphine-glucuronides ICB/SBD mean ratios are greater than ICB/SBB, which is compatible with mean concentrations found according to sampling method. As suggested by mean concentrations, for morphine and metabolites, an opposite trend is seen with ICB/FBD mean ratios less than ICB/FBB; furthermore, SBD/FBD and SBD/PB are less than SBB/FBB and SBB/PB, respectively. For morphine, results suggest isolation from PMR processes at central sites whereas isolation from another mechanism such as greater instability of the drug in contact with central blood may account for the trend found at femoral site. Moreover, FBD/PB appears equal to FBB/PB for morphine, whereas FBD/PB is greater than FBB/PB for both morphine-glucuronides, suggesting than morphine is less sampling technique-dependent at peripheral sites than its glucuronides. Our findings concerning morphine also remind us that PMR has always to be assessed by comparison of ratios in addition to mean concentrations.

Influence of Sampling Time

The first method calculated the correlation between ratios and corresponding estimated postmortem interval in all cases. For diazepam, there is a significant negative correlation between postmortem interval and SB/FB but a positive correlation with FB/PB, indicating that SB/FB decreases whereas FB/PB increases with time, which is compatible with central degradation and peripheral redistribution with time, respectively. For nordiazepam, a significant positive correlation is found between postmortem interval and SB/PB but also FB/PB, which is still compatible with redistribution of the compound with time. There was no significant correlation observed for oxazepam. For methadone, there is only one significant correlation observed between postmortem interval and FB/PB, indicating that mean ratio increases with postmortem interval, whereas no significant correlation is seen with EDDP; this suggests redistribution of methadone increases with time at the femoral site. For morphine, there is no significant correlation observed whereas significant correlations are seen for morphine-glucuronides: morphine-3-glucuronide shows a significant positive correlation between the postmortem interval and ICB/PB, SB/PB as well as FB/PB; morphine-6-glucuronide ICB/PB and SB/PB ratios are also correlated with the postmortem interval. Hence, the first method suggests that PMR may also correlate with longer postmortem interval, and not only in the early postmortem interval as reported by many studies; popliteal blood concentrations are also reliably lower than subclavian and femoral blood.

The second method concerned the evaluation of concentrations differences at the same site as well as ratios differences in cases sampled twice.

There are different trends for postmortem concentration differences between diazepam and its metabolites: ICB mean concentrations tend to decrease with time except for oxazepam; SB mean concentrations also appear to decrease for diazepam and oxazepam but show an opposite trend for nordiazepam; for all three substances, FB

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shows mean concentrations increase whereas PB mean concentrations consistently decrease with time. There may be degradation of benzodiazepines in central sites depending on the drug, whereas the FB increase may result from redistribution process rather than degradation; conversely, PB decrease may be the consequence of degradation rather than redistribution. Concerning ratio differences, for diazepam and its metabolites, different results are seen according to the substance considered. ICB/SB, ICB/FB, ICB/PB and SB/FB mean ratios tend to decrease with time for diazepam and nordiazepam whereas oxazepam also shows decrease for ICB/FB and SB/FB mean ratios but an increase for ICB/SB and ICB/PB. SB/PB mean ratio difference with time is nearly null for diazepam while it increases for nordiazepam and decreases for oxazepam, respectively. FB/PB mean ratio shows increase with time for the three compounds. These results are in accordance with the concentrations differences but also allow clarifying their relative differences from site to site, suggesting there is less redistribution at the popliteal site as mean ratio differences are greater with PB.

Methadone and EDDP show ICB mean concentration decrease; SB and PB mean concentrations increase with time, whereas FB mean concentration tends to increase for methadone but shows an opposite trend for EDDP. Results suggest further redistribution of methadone from surroundings tissues into blood at subclavian, femoral and popliteal sites with time, whereas subsequent redistribution of methadone into cardiac tissue and/or degradation in cardiac blood may explain methadone decrease with time in ICB; the same trend is found for EDDP except at femoral site where subsequent redistribution into surrounding tissues and/or degradation may also occur. Accordingly, methadone and EDDP both show ICB/SB, ICB/FB and ICB/PB mean ratios decrease while SB/FB and SB/PB mean ratios tend to increase with time for both substances. For FB/PB mean ratio difference, we see an increase with time for methadone but an opposite trend for EDDP. The results suggest cardiac mean concentrations decrease with concomitant increase in the other sites considered as well as simultaneous mean concentrations increase in subclavian and peripheral sites, but to a lesser extent into peripheral sites, especially the popliteal site for methadone.

For morphine and morphine-glucuronides, ICB mean concentrations decrease with time while both SB and FB mean concentrations show increases. PB mean concentrations show a marked decrease with time for morphine-glucuronides but morphine mean concentration shows only a slight increase. Consequently, the ICB decrease may be explained by central degradation of morphine and morphine-glucuronides whereas redistribution may account for mean concentrations increases in SB and FB. In PB, morphine-glucuronides hydrolysis is not the only phenomenon occurring as free morphine showed only slight increase with time compared to the significant decrease in concentration of morphine-glucuronides. Hence, redistribution of morphine glucuronides into surrounding tissues may also account for their decrease with time as both glucuronides may be more lipophilic or tissue degradation allows the passive diffusion from the intravascular compartment into the surrounding tissues. Concerning mean ratio differences, results show that all mean ratios increased with time for morphine; however, the increase is greater with PB than with FB, suggesting that PB is less affected by the postmortem interval as also suggested by FB/PB increase with time. For both morphine-glucuronides, ICB/SB and ICB/FB mean ratios tend to decrease with time whereas ICB/PB mean ratio shows increase for morphine-3-glucuronide but decrease with time for morphine-6-glucuronide; both metabolites show increase with time for SB/FB, SB/PB and FB/PB. Hence, the results for morphine-glucuronides suggest cardiac mean concentrations decrease with concomitant increase in the other sites considered (except for morphine-3-glucuronide showing ICB/PB increase) as well as

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simultaneous mean concentrations increase in subclavian and femoral sites, whereas concentrations proportionally decrease in popliteal blood, suggesting different mechanism as discussed before.

Finally, to assess if the changes in the free morphine concentration between samples could be due to the hydrolysis of morphine glucuronides rather than postmortem redistribution, free morphine/total morphine ratios differences were calculated. We see that there are increases in the free/total morphine ratio at each site, statistically significant for all sites except ICB. However, the role of hydrolysis is unclear as the changes in free/total morphine ratios are only partially responsible for the changes seen in morphine with time at the same site. Therefore, although hydrolysis of morphine-glucuronides does probably occur and may have a role in the differing concentrations of free morphine and morphine-glucuronides, other factors also may influence how morphine and morphine-glucuronides specifically redistribute in the postmortem environment, i.e. pH changes modifying the equilibrium of the drug in tissue compartments and passive diffusion of the drugs down a concentration gradient from area of high concentration to areas of low free concentration, in other words, from tissue to blood or from blood to tissue.

In conclusion, our study is the first to evaluate concurrently three aspects of PMR of three selected drugs and their metabolites concomitantly sampled at 4 sampling sites, among which the popliteal site unexplored so far by other authors. Concerning sampling site, for all substances, popliteal blood mean concentrations are significantly lower than those found in femoral blood, a site commonly used for peripheral sampling, indicating that popliteal blood is probably less prone to PMR due to its greater distance from the trunk. Sampling method also appears to have an effect on subclavian and femoral mean concentrations depending on the substance considered, since dissection/clamp technique may isolate blood from central processes; however, mean concentrations still suggest that popliteal site is more isolated from PMR processes as femoral/popliteal mean ratios are greater than one even with the dissection/clamp femoral sampling technique. Finally, estimated postmortem interval as well as time interval between samples in the same case show influence on mean concentrations and mean ratios of sampled substances, depending again on the drug considered, but generally indicating that redistribution processes are progressive with time; however, PB seems less subject to redistribution with time compared to other sites, including femoral site. Hence, our results suggest that PMR is an ongoing phenomenon in central as well as in peripheral compartments, but also that popliteal blood seems more resistant to it.

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Additional information and reprint requests:

Eric Lemaire, M.D., Ph.D.
Department of Pathology
University Hospital of Liège – CHU Sart Tilman
Domaine Universitaire du Sart Tilman – B.35
B-4000 Liège
Belgium
E-mail: eric.lemaire@chu.ulg.ac.be

TABLE 1-*Sex, age and estimated postmortem interval*.

	N	Mean +/- SD	Min-Max
Sex			
Male	46		
Female	11		
Age (y)	57	39.4 +/- 9.8	22.7-58.2
Postmortem interval (h)	57	31.1 +/- 26.2	5.0-145.0

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TABLE 2—*Target substances in all cases.*

	N
Diazepam	24
Nordiazepam	26
Oxazepam	14
Methadone	60
EDDP	52
Morphine	49
Morphine-3-glucuronide	47
Morphine-6-glucuronide	39

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TABLE 3—*Mean concentrations ratios in all cases.*

Substance	Ratios	N	Mean +/- SD	Min	Median	Max	Wilcoxon p-value
Diazepam	ICB/SB	24	0.86 +/-0.28	0.45	0.90	1.41	0.034
	ICB/FB		0.79 +/-0.34	0.21	0.83	1.46	**0.0081***
	ICB/PB		1.00 +/-0.29	0.46	0.98	1.58	0.72
	SB/FB		0.93 +/-0.34	0.46	0.85	2.04	0.13
	SB/PB		1.22 +/-0.31	0.73	1.24	2.02	**0.00015**
	FB/PB		1.45 +/-0.75	0.72	1.30	4.35	**<0.0001***
Nordiazepam	ICB/SB	26	0.92 +/-0.27	0.22	0.95	1.44	0.14
	ICB/FB		0.91 +/-0.31	0.65	1.17	1.81	0.20
	ICB/PB		1.16 +/-0.30	0.51	1.50	6.24	0.017
	SB/FB		0.99 +/-0.23	0.38	0.86	1.93	0.68
	SB/PB		1.36 +/-0.54	0.78	1.26	3.48	**<0.0001***
	FB/PB		1.43 +/-0.66	0.81	1.23	3.64	**<0.0001***
Oxazepam	ICB/SB	14	1.01 +/-0.35	0.27	0.96	1.62	0.95
	ICB/FB		0.88 +/-0.33	0.41	0.86	1.65	0.14
	ICB/PB		1.38 +/-0.70	0.76	1.19	3.33	0.068
	SB/FB		0.96 +/-0.43	0.38	0.86	1.93	0.41
	SB/PB		1.52 +/-0.98	0.83	1.16	4.39	0.022
	FB/PB		1.68 +/-0.94	0.83	1.42	3.88	**0.0031***
Methadone	ICB/SB	60	1.08 +/-0.83	0.28	0.91	6.15	0.53
	ICB/FB		1.49 +/-1.25	0.33	1.09	7.37	0.015

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	ICB/PB		1.91 +/-1.46	0.47	1.51	8.67	**<0.0001***
	SB/FB		1.39 +/-0.66	0.48	1.30	5.49	**<0.0001***
	SB/PB		1.80 +/-0.78	0.89	1.64	5.72	**<0.0001***
	FB/PB		1.36 +/-0.47	0.69	1.21	3.54	**<0.0001***
EDDP	ICB/SB	52	1.25 +/-0.84	0.26	0.95	4.46	0.55
	ICB/FB		1.69 +/-1.76	0.09	1.19	11.26	**0.0008***
	ICB/PB		2.58 +/-3.42	0.36	1.64	23.78	**<0.0001***
	SB/FB		1.30 +/-0.68	0.36	1.24	4.82	**0.0006***
	SB/PB		2.03 +/-1.76	0.66	1.71	12.50	**<0.0001***
	FB/PB		1.62 +/-0.93	0.78	1.36	6.50	**<0.0001***
Morphine	ICB/SB	49	1.35 +/-1.09	0.23	1.03	6.72	0.093
	ICB/FB		1.90 +/-1.24	0.18	1.57	5.73	**<0.0001***
	ICB/PB		2.60 +/-2.05	0.73	1.94	9.33	**<0.0001***
	SB/FB		1.52 +/-0.72	0.35	1.31	4.09	**<0.0001***
	SB/PB		2.05 +/-1.18	0.65	1.65	7.47	**<0.0001***
	FB/PB		1.49 +/-1.01	0.65	1.20	6.13	**0.0061***
Morphine-3-G	ICB/SB	47	0.92 +/-0.54	0.15	0.80	2.86	0.13
	ICB/FB		1.10 +/-0.96	0.05	0.81	3.91	0.43
	ICB/PB		1.44 +/-1.35	0.21	0.84	7.33	0.36
	SB/FB		1.20 +/-0.69	0.13	1.07	4.07	0.14
	SB/PB		1.56 +/-1.03	0.40	1.22	5.73	**<0.0001***
	FB/PB		1.72 +/-2.42	0.59	1.16	17.20	**0.002***
Morphine-6-G	ICB/SB	39	0.88 +/-0.68	0.11	0.76	3.38	0.0084
	ICB/FB		1.71 +/-3.33	0.07	0.88	20.50	0.84
	ICB/PB		2.10 +/-3.51	0.11	1.07	17.57	0.17
	SB/FB		1.91 +/-2.91	0.13	1.19	17.09	0.012
	SB/PB		2.48 +/-4.92	0.50	1.43	31.33	**<0.0001***
	FB/PB		1.83 +/-2.66	0.38	1.17	13.82	**0.0014***

TABLE 4—*Mean concentrations ratios according to sampling techniques at subclavian and femoral sites.*

Substance	Ratios	N	Mean +/- SD	Min	Median	Max	Wilcoxon p-value
Diazepam	ICB/SBD	20	0.90 +/-0.30	0.45	0.89	1.35	0.18
	ICB/SBB	18	0.84 +/-0.32	0.34	0.90	1.46	0.030
	ICB/FBD	20	0.96 +/-0.40	0.42	0.91	2.07	0.35
	ICB/FBB	18	0.73 +/-0.36	0.12	0.74	1.34	**0.0048***
	ICB/PB (#2)	20	1.02 +/-0.30	0.45	0.99	1.54	0.93
	ICB/PB (#3)	18	1.04 +/-0.36	0.48	1.04	1.72	0.77
	SBD/FBD	20	1.10 +/-0.36	0.61	1.10	2.04	0.32
	SBB/FBB	18	0.86 +/-0.35	0.35	0.80	1.47	0.13

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	SBD/PB	20	1.18 +/-0.26	0.80	1.20	1.68	**0.0083**
	SBB/PB	18	1.35 +/-0.61	0.73	1.19	3.19	0.016
	FBD/PB	20	1.13 +/-0.31	0.61	1.06	1.93	0.097
	FBB/PB	18	1.95 +/-1.93	0.91	1.39	9.21	**0.0004***
Nordiazepam	ICB/SBD	22	0.95 +/-0.26	0.22	0.93	1.40	0.35
	ICB/SBB	20	0.95 +/-0.29	0.54	0.95	1.47	0.44
	ICB/FBD	22	1.06 +/-0.40	0.21	1.05	2.05	0.54
	ICB/FBB	20	0.87 +/-0.32	0.33	0.95	1.25	0.13
	ICB/PB (#2)	22	1.17 +/-0.35	0.64	1.16	2.22	0.036
	ICB/PB (#3)	20	1.20 +/-0.33	0.66	1.22	1.81	0.014
	SBD/FBD	22	1.11 +/-0.25	0.77	1.12	1.62	0.066
	SBB/FBB	20	0.94 +/-0.31	0.45	0.87	1.58	0.33
	SBD/PB	22	1.32 +/-0.55	0.89	1.19	3.48	**<0.0001***
	SBB/PB	20	1.36 +/-0.54	0.78	1.23	3.19	**0.0010***
	FBD/PB	22	1.23 +/-0.59	0.70	1.07	3.64	0.013
	FBB/PB	20	1.64 +/-1.11	0.99	1.22	5.52	**<0.0001***
Oxazepam	ICB/SBD	12	1.08 +/-0.35	0.27	1.10	1.55	0.42
	ICB/SBB	10	1.03 +/-0.36	0.63	1.06	1.68	0.85
	ICB/FBD	12	3.43 +/-8.38	0.52	0.96	30.00	0.97
	ICB/FBB	10	1.24 +/-1.58	0.41	0.80	5.70	0.13
	ICB/PB (#2)	12	3.68 +/-8.30	0.76	1.25	30.00	0.042
	ICB/PB (#3)	10	1.31 +/-0.49	0.76	1.23	2.04	0.19
	SBD/FBD	12	3.67 +/-9.24	0.38	1.05	33.00	0.70
	SBB/FBB	10	1.23 +/-1.48	0.50	0.70	5.40	0.19
	SBD/PB	12	3.94 +/-9.17	0.63	1.16	33.00	0.052
	SBB/PB	10	1.39 +/-0.64	0.73	1.18	2.71	0.16
	FBD/PB	12	1.41 +/-0.83	0.70	1.13	3.88	0.054
	FBB/PB	10	1.65 +/-0.96	0.36	1.53	3.82	0.027
Methadone	ICB/SBD	38	1.05 +/-0.57	0.36	0.97	2.59	0.91
	ICB/SBB	46	1.08 +/-0.91	0.23	0.88	6.15	0.43
	ICB/FBD	38	1.57 +/-1.20	0.33	1.19	5.81	0.018
	ICB/FBB	46	1.50 +/-1.36	0.43	1.03	7.37	0.081
	ICB/PB (#2)	38	1.86 +/-1.27	0.54	1.49	5.77	**<0.0001***
	ICB/PB (#3)	46	1.97 +/-1.62	0.47	1.45	8.67	**<0.0001***
	SBD/FBD	38	1.47 +/-0.64	0.66	1.36	4.07	**<0.0001***
	SBB/FBB	46	1.45 +/-0.93	0.48	1.26	6.98	**<0.0001***
	SBD/PB	38	1.75 +/-0.63	0.89	1.65	4.04	**<0.0001***
	SBB/PB	46	1.93 +/-1.10	0.96	1.69	7.69	**<0.0001***
	FBD/PB	38	1.25 +/-0.31	0.87	1.18	2.27	**<0.0001***
	FBB/PB	46	1.41 +/-0.51	0.69	1.27	3.54	**<0.0001***
EDDP	ICB/SBD	34	1.47 +/-1.10	0.63	1.09	5.63	0.043
	ICB/SBB	42	1.23 +/-0.83	0.26	1.01	4.23	0.66
	ICB/FBD	34	2.27 +/-3.65	0.27	1.25	21.40	**0.0016***
	ICB/FBB	42	1.74 +/-1.55	0.09	1.29	7.64	**0.007***
	ICB/PB (#2)	34	3.12 +/-4.61	0.63	1.63	26.75	**<0.0001***
	ICB/PB (#3)	42	2.77 +/-3.47	0.36	1.70	21.40	**<0.0001***

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	SBD/FBD	34	1.32 +/-0.73	0.39	1.22	3.80	0.013
	SBB/FBB	42	1.48 +/-1.06	0.36	1.26	6.87	**0.0001***
	SBD/PB	34	1.82 +/-0.91	0.66	1.61	4.75	**<0.0001***
	SBB/PB	42	2.56 +/-3.58	0.88	1.72	23.00	**<0.0001***
	FBD/PB	34	1.57 +/-0.83	0.78	1.37	5.00	**<0.0001***
	FBB/PB	42	1.65 +/-1.17	0.79	1.34	8.00	**<0.0001***
Morphine	ICB/SBD	33	1.40 +/-1.14	0.50	1.11	6.72	0.10
	ICB/SBB	33	1.26 +/-0.81	0.23	1.06	4.53	0.21
	ICB/FBD	33	1.76 +/-1.21	0.44	1.34	5.60	**0.0006***
	ICB/FBB	33	1.97 +/-1.36	0.18	1.57	7.17	**<0.0001***
	ICB/PB (#2)	33	2.37 +/-1.82	0.72	1.94	9.33	**<0.0001***
	ICB/PB (#3)	33	2.57 +/-1.99	0.86	1.60	8.50	**<0.0001***
	SBD/FBD	33	1.37 +/-0.74	0.35	1.19	4.09	**0.0002***
	SBB/FBB	33	1.60 +/-0.58	0.76	1.47	3.05	**<0.0001***
	SBD/PB	33	1.84 +/-1.18	0.65	1.55	7.47	**<0.0001***
	SBB/PB	33	2.09 +/-0.93	0.76	1.83	4.67	**<0.0001***
	FBD/PB	33	1.44 +/-0.88	0.95	1.24	6.13	**<0.0001***
	FBB/PB	33	1.43 +/-0.92	0.63	1.20	4.86	**<0.0001***
Morphine-3-G	ICB/SBD	33	1.07 +/-0.79	0.20	0.80	3.56	0.61
	ICB/SBB	31	0.97 +/-0.56	0.10	0.83	2.52	0.43
	ICB/FBD	33	1.16 +/-1.12	0.05	0.85	4.65	0.35
	ICB/FBB	31	1.29 +/-1.01	0.24	0.84	3.87	0.57
	ICB/PB (#2)	33	1.45 +/-1.55	0.21	0.81	8.25	0.38
	ICB/PB (#3)	31	1.74 +/-1.45	0.21	1.10	6.60	0.056
	SBD/FBD	33	1.09 +/-0.61	0.13	1.09	2.74	0.69
	SBB/FBB	31	1.39 +/-0.84	0.39	1.16	4.07	0.064
	SBD/PB	33	1.35 +/-0.76	0.40	1.17	3.78	0.023
	SBB/PB	31	1.86 +/-1.23	0.64	1.39	5.73	**<0.0001***
	FBD/PB	33	1.80 +/-2.84	0.50	1.13	17.20	0.018
	FBB/PB	31	1.47 +/-0.81	0.66	1.36	4.60	**0.0001***
Morphine-6-G	ICB/SBD	29	1.11 +/-1.15	0.11	0.80	5.13	0.15
	ICB/SBB	25	0.91 +/-0.64	0.09	0.76	2.74	0.14
	ICB/FBD	29	1.21 +/-1.48	0.07	0.70	6.81	0.41
	ICB/FBB	25	2.35 +/-4.01	0.33	1.18	20.50	0.12
	ICB/PB (#2)	29	1.86 +/-3.58	0.11	0.91	19.25	0.53
	ICB/PB (#3)	25	2.74 +/-3.88	0.17	1.42	17.57	**0.0066***
	SBD/FBD	29	1.05 +/-0.61	0.13	0.91	3.00	0.84
	SBB/FBB	25	2.72 +/-3.49	0.63	1.75	17.09	**<0.0001***
	SBD/PB	29	1.32 +/-0.67	0.47	1.27	3.75	0.015
	SBB/PB	25	3.48 +/-6.01	0.63	1.92	31.33	**<0.0001***
	FBD/PB	29	2.08 +/-3.09	0.47	1.17	17.20	0.019
	FBB/PB	25	1.29 +/-0.52	0.38	1.28	2.43	0.022

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TABLE 5—*Correlations between estimated postmortem interval and ratios in all cases.*

Substance	Ratios	N	Mean PMI (h) +/- SD	Correlation	Spearman p-value
Diazepam	ICB/SB	24	30.88 +/-19.12	-0.43	0.84
	ICB/FB			-0.35	0.090
	ICB/PB			0.053	0.891
	SB/FB			-0.49	**0.015***
	SB/PB			0.17	0.42
	FB/PB			0.61	**0.0017***
Nordiazepam	ICB/SB	26	29.76 +/-18.77	-0.19	0.35
	ICB/FB			-0.33	0.098
	ICB/PB			0.23	0.26
	SB/FB			-0.33	0.097
	SB/PB			0.42	**0.034***
	FB/PB			0.58	**0.0019***
Oxazepam	ICB/SB	14	35.94 +/-21.33	0.18	0.53
	ICB/FB			-0.0042	0.89
	ICB/PB			0.30	0.30
	SB/FB			-0.33	0.25
	SB/PB			0.028	0.92
	FB/PB			0.34	0.24
Methadone	ICB/SB	60	39.03 +/-33.09	0.020	0.88
	ICB/FB			-0.15	0.26
	ICB/PB			0.089	0.50
	SB/FB			-0.24	0.060
	SB/PB			0.16	0.22
	FB/PB			0.56	**<0.0001***
EDDP	ICB/SB	52	34.13 +/-23.71	-0.15	0.30
	ICB/FB			-0.21	0.13
	ICB/PB			-0.14	0.32
	SB/FB			-0.11	0.45
	SB/PB			-0.11	0.43

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	FB/PB			0.00051	0.99
Morphine	ICB/SB	49	38.92 +/-28.87	0.24	0.10
	ICB/FB			0.20	0.16
	ICB/PB			0.20	0.17
	SB/FB			0.052	0.72
	SB/PB			0.12	0.41
	FB/PB			-0.029	0.85
Morphine-3-G	ICB/SB	47	35.91 +/-25.26	0.23	0.12
	ICB/FB			0.18	0.24
	ICB/PB			0.38	**0.0079***
	SB/FB			0.17	0.26
	SB/PB			0.37	**0.011***
	FB/PB			0.39	**0.0068***
Morphine-6-G	ICB/SB	39	37.51 +/-26.74	0.25	0.12
	ICB/FB			0.23	0.15
	ICB/PB			0.43	**0.0066***
	SB/FB			0.13	0.43
	SB/PB			0.40	**0.011***
	FB/PB			0.29	0.076

TABLE 6—*Mean ratios differences between samples in cases sampled twice.*

Substance	Ratios difference	N	Mean +/- SD	Mean time interval (h) +/-SD	Min	Median	Max	Wilcoxon p-value
Diazepam	ICB 2/SB 2 – ICB 1/SB 1	5	-0.15+/-0.19	27.4+/-9.8	-0.43	-0.08	0.07	0.13
	ICB 2/FB 2 – ICB 1/FB 1		-0.49+/-0.28		-0.81	-0.51	-0.20	0.063
	ICB 2/PB 2 – ICB 1/PB 1		-0.18+/-0.09		-0.28	-0.20	-0.05	0.063
	SB 2/FB 2 – SB 1/FB 1		-0.50+/-0.47		-1.24	-0.44	0.01	0.13
	SB 2/PB 2 – SB 1/PB 1		-0.01+/-0.32		-0.36	-0.08	0.48	1.0
	FB 2/PB 2 – FB 1/PB 1		0.62+/-0.40		-0.02	0.79	0.95	0.13
Nordiazepam	ICB 2/SB 2 – ICB 1/SB 1	5	-0.36+/-0.25	27.4+/-9.8	-0.68	-0.42	-0.04	0.063
	ICB 2/FB 2 – ICB 1/FB 1		-0.42+/-0.34		-0.84	-0.52	0.08	0.13
	ICB 2/PB 2 – ICB 1/PB 1	5	-0.16+/-0.24		-0.56	-0.11	0.01	0.31
	SB 2/FB 2 – SB 1/FB 1		-0.07+/-0.44		-0.50	-0.21	0.61	0.81
	SB 2/PB 2 – SB 1/PB 1		0.67+/-0.80		0.07	0.46	2.01	0.63
	FB 2/PB 2 – FB 1/PB 1		0.84+/-0.95		-0.21	0.77	2.38	0.13
Oxazepam	ICB 2/SB 2 – ICB 1/SB 1	3	0.19+/-0.89	30.6+/-12.4	-0.44	-0.21	1.21	1.0
	ICB 2/FB 2 – ICB 1/FB 1		-0.24+/-0.25		-0.44	-0.32	0.04	0.50

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	ICB 2/PB 2 – ICB 1/PB 1		0.35+/-0.93		-0.23	-0.15	1.42	1.0
	SB 2/FB 2 – SB 1/FB 1		-0.61+/-0.82		-1.55	-0.26	-0.03	0.25
	SB 2/PB 2 – SB 1/PB 1		-0.32+/-0.89		-1.32	0.01	0.35	1.0
	FB 2/PB 2 – FB 1/PB 1		1.15+/-1.11		0.43	0.60	2.43	0.25
Methadone	ICB 2/SB 2 – ICB 1/SB 1	18	-0.19+/-0.98	27.2+/-13.8	-3.73	-0.02	0.81	0.87
	ICB 2/FB 2 – ICB 1/FB 1		-0.14+/-1.06		-3.64	-0.04	1.36	0.58
	ICB 2/PB 2 – ICB 1/PB 1		-0.11+/-1.53		-4.39	0.14	1.76	0.44
	SB 2/FB 2 – SB 1/FB 1		0.03+/-0.41		-0.57	-0.04	1.02	0.97
	SB 2/PB 2 – SB 1/PB 1		0.25+/-1.03		-2.72	0.27	2.95	**0.039***
	FB 2/PB 2 – FB 1/PB 1		0.17+/-0.91		-2.85	0.16	1.34	0.099
EDDP	ICB 2/SB 2 – ICB 1/SB 1	14	-0.33+/-0.66	27.0+/-15.4	-2.24	-0.12	0.37	**0.030***
	ICB 2/FB 2 – ICB 1/FB 1		-0.23+/-0.65		-1.33	-0.16	0.85	0.19
	ICB 2/PB 2 – ICB 1/PB 1		-0.29+/-0.88		-2.34	-0.14	0.81	0.43
	SB 2/FB 2 – SB 1/FB 1		0.04+/-0.60		-0.96	0.02	1.14	0.86
	SB 2/PB 2 – SB 1/PB 1		0.07+/-0.45		-0.77	0.00	0.83	0.76
	FB 2/PB 2 – FB 1/PB 1		-0.10+/-1.24		-2.66	-0.16	2.08	0.71
Morphine	ICB 2/SB 2 – ICB 1/SB 1	16	0.42+/-1.92	29.0+/-14.3	-2.89	0.03	6.17	0.38
	ICB 2/FB 2 – ICB 1/FB 1		0.40+/-1.70		-1.76	0.05	4.96	0.53
	ICB 2/PB 2 – ICB 1/PB 1		0.91+/-2.67		-3.23	0.33	8.60	0.25
	SB 2/FB 2 – SB 1/FB 1		0.06+/-0.78		-1.41	0.03	1.24	0.74
	SB 2/PB 2 – SB 1/PB 1		0.27+/-1.25		-2.49	0.21	3.31	0.19
	FB 2/PB 2 – FB 1/PB 1		0.27+/-1.75		-4.21	0.14	4.90	0.83
Morphine-3-G	ICB 2/SB 2 – ICB 1/SB 1	15	-0.10+/-0.50	29.6+/-14.6	-1.41	-0.09	0.59	0.60
	ICB 2/FB 2 – ICB 1/FB 1		-0.02+/-0.79		-1.89	-0.10	1.66	0.98
	ICB 2/PB 2 – ICB 1/PB 1		0.22+/-0.64		-1.03	-0.18	1.50	0.17
	SB 2/FB 2 – SB 1/FB 1		0.24+/-0.76		-0.94	0.05	2.02	0.39
	SB 2/PB 2 – SB 1/PB 1		0.69+/-1.00		-0.17	0.35	3.72	**0.0020***
	FB 2/PB 2 – FB 1/PB 1		1.54+/-4.12		-0.46	0.13	16.07	**0.0042***
Morphine-6-G	ICB 2/SB 2 – ICB 1/SB 1	12	-0.12+/-0.68	30.9+/-16.2	-1.96	0.13	0.50	0.85
	ICB 2/FB 2 – ICB 1/FB 1		-1.65+/-4.90		-17.0	-0.25	0.61	0.23
	ICB 2/PB 2 – ICB 1/PB 1		-0.59+/-3.39		-11.2	0.34	1.17	0.20
	SB 2/FB 2 – SB 1/FB 1		0.36+/-2.50		-3.00	-0.04	7.59	1.00
	SB 2/PB 2 – SB 1/PB 1		2.40+/-6.57		0.01	0.39	23.19	**0.0005***
	FB 2/PB 2 – FB 1/PB 1		2.35+/-4.46		-0.27	0.62	12.72	**0.0021***

TABLE 7—*Free morphine/total morphine mean ratios differences in cases sampled twice.*

Site		Mean +/- SD	Min	Median	Max	Wilcoxon p-value
ICB	ICB 1	0.37 +/-0.17	0.16	0.34	0.68	
	ICB 2	0.51 +/-0.19	0.18	0.52	0.80	

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	Difference	0.14 +/-0.24	-0.18	0.11	0.64	0.13
SB	SB 1	0.29 +/-0.18	0.12	0.21	0.67	
	SB 2	0.35 +/-0.20	0.13	0.29	0.81	
	Difference	0.06 +/-0.06	-0.04	0.06	0.14	**0.0021***
FB	FB 1	0.22 +/-0.13	0.09	0.16	0.47	
	FB 2	0.29 +/-0.19	0.09	0.23	0.74	
	Difference	0.07 +/-0.08	-0.03	0.05	0.26	**0.0021***
PB	PB 1	0.20 +/-0.13	0.08	0.14	0.46	
	PB 2	0.30 +/-0.17	0.09	0.26	0.61	
	Difference	0.11 +/-0.08	0.01	0.12	0.28	**0.0005***

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Figure Legends

FIG. 1—*Diazepam, nordiazepam, and oxazepam mean concentrations according to sampling sites in all cases (group #1).*

FIG. 2—*Methadone and EDDP mean concentrations according to sampling sites in all cases (group #1).*

FIG. 3—*Morphine, morphine-3-glucuronide and morphine-6-glucuronide mean concentrations according to sampling sites in all cases (group #1).*

FIG. 4—*Diazepam, nordiazepam, and oxazepam mean concentrations according to sampling techniques at subclavian and femoral sites (a,b,c = group #2; a',b',c' = group #3).*

FIG. 5—*Methadone and EDDP mean concentrations according to sampling techniques at subclavian and femoral sites (a,b = group #2; a',b' = group #3).*

FIG. 6—*Morphine, morphine-3-glucuronide, and morphine-6-glucuronide mean concentrations according to sampling techniques at subclavian and femoral sites (a,b,c = group #2; a',b',c' = group #3).*

FIG. 7—*Diazepam, nordiazepam, and oxazepam mean concentrations differences between samples 1 and 2 according to sampling sites in cases sampled twice.*

FIG. 8—*Methadone and EDDP mean concentrations differences between samples 1 and 2 according to sampling sites in cases sampled twice.*

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FIG. 9—*Morphine, morphine-3-glucuronide, and morphine-6-glucuronide mean concentrations differences between samples 1 and 2 according to sampling sites in cases sampled twice.*

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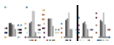

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