Postmortem redistribution of morphine in humans

Kamphuis, Anne Elisa Maria; Borra, Lennaert Christiaan Pieter; van der Hulst, Rogier; Kloos, Dick-Paul; Rijken, Dingeman Johannes; Bosman, Ingrid Jolanda; Touw, Daniel Johannes

Published in:
Forensic Science International

DOI:
10.1016/j.forsciint.2021.111094

Publication date:
2021

Forensic Science International 329 (2021) 111094

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Postmortem redistribution of morphine in humans: Important variables that might be influencing the central blood/peripheral blood ratio

Anne Elisa Maria Kamphuis[a,1], Lennaert Christiaan Pieter Borra[b,*,1], Rogier van der Hulst[b], Dick-Paul Kloos[b], Dingeman Johannes Rijken[b], Ingrid Jolanda Bosman[b], Daniel Johannes Touw[c,d,e]

[a] University of Groningen, Faculty of Science and Engineering, Department of Pharmacy, Nijenborgh 4, 9747AG Groningen, The Netherlands
[b] Netherlands Forensic Institute, Laan van Ypenburg 6, 2497GB The Hague, The Netherlands
[c] University of Groningen, Faculty of Science and Engineering, Pharmaceutical Analysis – Groningen Research Institute of Pharmacy, Antonius Deusinglaan 1, 973AV Groningen, The Netherlands
[d] University of Groningen, Faculty of Medical Sciences, Antonius Deusinglaan 1, 973AV Groningen, The Netherlands
[e] University Medical Center Groningen, Department of Clinical Pharmacy and Pharmacology, Hanzeplein 1, 9713GZ Groningen, The Netherlands

ARTICLE INFO

Article history:
Received 2 September 2021
Received in revised form 29 October 2021
Accepted 31 October 2021
Available online 4 November 2021

Keywords:
Postmortem
Toxicology
Pharmacokinetics
Redistribution
Morphine
C/P ratio

ABSTRACT

Introduction: In the field of forensic toxicology, many unexpected deaths are investigated as to whether toxicological substances may have caused or contributed to someone's death. One of the factors that makes interpretation of the results of quantitative analysis in postmortem toxicology challenging, is that measured postmortem drugs levels may vary according to the sampling site and the interval between death and specimen collection. These site- and time-dependent variations are caused by 'postmortem redistribution' (PMR). Literature shows that there are several factors that determine the degree of PMR, such as cell and tissue changes after death, decomposition and the physicochemical characteristics of drugs. Blood from peripheral sites seems to be less affected by PMR than cardiac blood. Therefore, the ratio of cardiac blood concentration/peripheral blood concentration (C/P) of a drug is often used as a marker of the extent of postmortem redistribution. In this study, we investigated the relationship between different potentially important variables and the C/P ratio of morphine in humans in order to provide new insights that might assist in the interpretation of quantitative results in forensic casework.
Method: Toxicological results of all morphine positive postmortem cases investigated by the Netherlands Forensic Institute between January 1, 2010 and July 31, 2020 were reviewed. Morphine was quantified in both femoral and cardiac blood in a total of 103 cases. The C/P ratios were determined for all selected cases. To collect data for this study, all corresponding files were reviewed. C/P ratios were compared between subgroups by performing either a Mann-Whitney *U* test or a Kruskal-Wallis test, followed by a post-hoc Mann-Whitney *U* test. Bonferroni correction was performed to correct for the likelihood of a significant result by chance due to multiple testing. After Bonferroni correction, a p-value < 0.004 was considered statistically significant.
Results: The data suggests a relationship between grade of decomposition at autopsy, position of the corpse at discovery, route of administration, attempted resuscitation and the C/P ratio of morphine with p-values of 0.010, 0.026, 0.035 and 0.046, respectively.
Conclusion: Grade of decomposition at autopsy, position of the corpse at discovery, route of administration and attempted resuscitation seem to be influencing the C/P ratio of morphine. Of these four variables, the route of administration seems to have the greatest impact.

1. Introduction

In the field of forensic toxicology, many unexpected deaths are investigated as to whether toxicological substances may have caused or contributed to someone's death. One of the factors that makes

* Corresponding author.
E-mail address: l.borra@nfi.nl (L.C.P. Borra).
[1] These authors contributed equally.

https://doi.org/10.1016/j.forsciint.2021.111094
0379-0738/

interpretation of the results of quantitative analysis in postmortem toxicology challenging, is that measured postmortem drug levels may vary depending on the sampling site and the time interval between death and specimen collection. These site- and time-dependent variations are caused by 'postmortem redistribution' (PMR) [1,2]. Literature shows that there are several factors that determine the degree of PMR such as cell and tissue modifications after death, decomposition and the physicochemical characteristics of the drugs [3].

Other variables that are possibly correlated with PMR are the postmortem time interval, position of the corpse, age, body mass index (BMI) and route of administration [1,3–8]. Few studies have investigated the relationship between these factors and PMR and knowledge about the exact mechanisms involved is still limited.

Blood from peripheral sites seems to be less affected by these changes than cardiac blood [7,9–11]. Therefore, the ratio of cardiac blood concentration/peripheral blood concentration (C/P) of a drug is used as a marker of PMR [12]. However, this does not necessarily mean that PMR always causes a change in C/P ratio, nor does a greater C/P ratio necessarily means that PMR has occurred to a greater extent [13].

It is now widely acknowledged that, although peripheral blood is less prone to postmortem changes in drug concentrations, these changes in fact occur [14–22].

Therefore, it is important to not simply assume that peripheral blood concentrations reflect drug concentrations at the time of death. Peripheral blood concentrations should be interpreted with caution in case of an increased or decreased C/P-ratio as this suggests that postmortem redistribution has occurred, potentially affecting peripheral blood concentrations as well.

Morphine is a widely used and misused drug [23]. Interpretation of postmortem morphine concentrations is difficult because drug response is modulated by opioid tolerance. This is reflected in a wide range of observed postmortem morphine concentrations that largely overlap with observed morphine concentrations in living persons [24].

Although the effects of morphine are largely dependent on the tolerance to opioids, knowledge regarding PMR of morphine is essential for a correct interpretation of measured postmortem morphine concentrations in relation to death. Even though morphine is one of the most extensively studied compounds postmortem, published data regarding the PMR of morphine in humans is still limited and inconsistent. Some studies merely report concentration differences of morphine in different matrices at one timepoint [25–30] while others report differences between antemortem and postmortem concentrations [31,32] or study the correlation between the extent of postmortem redistribution and the postmortem time interval [18,33–36]. In this study, we investigated the relationship between different potentially significant variables and the C/P ratio of morphine in humans, in order to provide new insights that might assist in the interpretation of quantitative results of morphine in forensic casework.

2. Methods

2.1. Ethical statement

Ethics approval is not required for this retrospective database study. The database contains descriptive data concerning forensic postmortem cases. No interventions in humans or animals were performed for this study.

2.2. Case selection and data collection

Toxicological results of all postmortem cases investigated by the Netherlands Forensic Institute between January 1, 2010 and July 31, 2020 were reviewed. All cases in which morphine was quantified in both femoral and cardiac blood (only inclusion criterium, no exclusion criteria) were selected and the corresponding data was extracted from the case files and included in the database for statistical analysis.

2.3. Sample collection and storage

Blood samples were collected during autopsy in glass Vacutainer® sodium fluoride and sodium heparin containing tubes (Becton Dickinson, Netherlands) according to a standardised protocol. Prior to internal examination, femoral blood was collected from the femoral vein with a sterile syringe, after targeted dissection of the left upper leg. If less than 2 mL femoral blood was obtained, the same procedure was performed on the right leg. During internal examination, cardiac blood was collected from either the right atrium or the inferior vena cava with the heart in situ. Samples were stored at − 20 °C until analysis. All samples were analysed within 3 months after sample collection.

2.4. Routine testing - toxicological screening and quantitative analysis of ethanol and gamma-hydroxybutyric acid (GHB)

Routine testing consists of a toxicological screening in combination with quantitative analysis of ethanol and GHB. Between January 2010 and June 2015, toxicological screening consisted of untargeted screening using Solid Phase Extraction (SPE) as sample preparation followed by both gas chromatography – mass spectrometry (GC-MS) and high-performance liquid chromatography with diode-array detection (HPLC-DAD). In June 2015 this method was replaced by a targeted screening method using ultra-performance liquid chromatography -time of flight mass spectrometry (UPLC-TOFMS). At the time of introduction this method was validated for 340 compounds. In June 2018 the method was expanded and an additional validation was performed to successfully add another 81 compounds for a total of 421 compounds. Quantitative analysis of ethanol was performed using a validated headspace – gas chromatography – flame ionisation detection (HS-GC-FID) method.

Between January 2010 and April 2016 quantitative analysis of GHB was performed using a validated gas chromatography – mass spectrometry (GC-MS) method. In April 2016 this method was replaced by a validated ultra-performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS) method for quantitative analysis of both GHB and beta-hydroxybutyric acid (BHB).

2.5. Quantitative analysis of morphine

Quantitative analysis of morphine was performed using a fully validated ultra-performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS) multi-compound method.

2.5.1. Reagents

Methanol, acetonitrile (both ULC/MS grade) and formic acid (ULC/MS, 99%) were purchased from Biosolve (Dieuze, France). Ammonium solution 25%, di-sodium hydrogen phosphate, phosphoric acid 85% (all practical grade) and ammonium carbonate (99% purity) were supplied by Merck (Darmstadt, Germany). Acetone (pico grade) was purchased from LGC Standards (Manchester, USA). Morphine and morphine-D3 were obtained from Cerilliant (Rock Round, USA). The mobile phase consisted of methanol (A) and a 10 mM ammonium carbonate pH 10 buffer solution (B).

2.5.2. Sample preparation (acetone protein precipitation)

Blood sample (75 µL), internal standard (20 µL of 100 µg/mL morphine-D3 solution) and phosphate buffer solution (20 µL, pH 8) were pipetted in a 96-well plate. After vortexing (15 min, 1250 rpm),

acetone (300 µL) was added and the plate was vortexed again (15 min, 1250 rpm), followed by centrifugation (15 min, 4500 rpm). Supernatant (250 µL) was transferred into a clean 96-well plate.

2.5.3. Instrumentation

Analysis was performed using a Xevo® TQ MS system equipped with an electrospray ionisation (ESI) source and an Acquity® Classic UPLC system equipped with a UPLC BEH C18 (100 ×2.1 mm I.D., 1.7 µm) column (Waters, The Netherlands). Data acquisition and processing was performed using Masslynx® version 4.1 software and TargetLynx® version 4.1 software (Waters, The Netherlands) respectively.

2.5.4. Method of analysis

ESI source settings were as follows: capillary voltage 0.50 kV, source temperature 150 °C, desolvation temperature 500 °C, desolvation gas flow 1000 L/Hr and collision gas flow 0.15 mL/min. The ion transitions were monitored in positive ionisation mode.

Retention time and mass-transitions *m/z* 286.2 > 201.1 and *m/z* 286.2 > 165.1, were used for identification of morphine, *m/z* 286.2 > 201.1 was used for quantification. Morphine-D3 was identified and quantified using *m/z* 289.3 > 165.1. Of the supernatant 6.0 µL was injected into the UPLC-MS/MS. Gradient elution was as follows: 95% B for 1.5 min, linear gradient from 95% to 10% B in 7.5 min, linear gradient 10–1% B in 0.01 min and maintained at 1% till 13.0 min, from 1% to 95% B in 0.01 min and maintained at 95% till 13.50 min. The flow was constant at 500 µL/min for 10.0 min, then 1000 µL/min till 12.50 min and reduced to 500 µL/min till 13.50 min. Total runtime for one injection was 13.50 min

2.5.5. Validation

The LC-MS-MS method was validated in 2009 in accordance with the EMA guideline on bioanalytical method validation (draft) [37] and Peters et al. [38]. Linearity of the calibration range for morphine was confirmed (7-point calibration curve; 0.005, 0.01, 0.05, 0.1, 0.3, 0.5, 1.0 mg/L, $r^2 > 0.999$). Overall accuracy and precision for morphine were within 9%, matrix effects within 6% and a recovery of 104%. Regarding the selectivity, there were no interfering peaks found analyzing blank samples. Long term stability was confirmed with morphine concentrations in whole blood samples stored at − 20 °C being all within ± 15% of the theoretical value after 4 months. Between January 1, 2010 and July 31, 2020, the method was regularly evaluated by participating in proficiency testing schemes. On average we participated in 8 tests per year (LGC® QUARTZ Forensic Blood Toxicology and LGC® TOX Toxicology schemes) with Z-scores within ± 1 for the entire period.

2.6. Variables

The following variables of interest were selected prior to data collection because of their relationship with pharmacokinetics of morphine and/or postmortem changes to the body and were included in the statistical analysis: grade of decomposition at autopsy, position of the corpse at discovery, route of administration, attempted resuscitation, history of morphine use based on medical files, sex, season, age, postmortem interval (PMI), co-ingestion of other substances, femoral blood concentration of morphine, Body Mass Index (BMI) determined during autopsy, and cause of death.

Grades of decomposition were scored in accordance with the decomposition staging scale in J.E. Pless et al. [39]. An adapted overview of the different grades of decomposition is provided in Table 1.

PMI was defined as the time elapsed between death and autopsy. If the exact time of death was unknown, it was approximated using the average between the moment of the last sign of life and the moment the corpse was found.

2.7. Compilation, statistical analysis and graphical representation of data

The data was initially compiled using Microsoft® Excel® version 2104 software (Microsoft, USA). Statistical analysis and subsequent graphical representation of the data was done using IBM® SPSS Statistics® version 27 software (IBM, USA).

Normal distribution of C/P ratios within groups was assessed by Shapiro-Wilk test.

The Shapiro-Wilk test showed that all the groups were non-normally distributed.

Considering the non-normality of the data, C/P ratios of subgroups were compared by either Mann-Whitney *U* test (for variables consisting of 2 subgroups) or Kruskal-Wallis test (for variables consisting of more than 2 subgroups) followed by post-hoc Mann-Whitney *U* test. For both the Mann-Whitney *U* test and the Kruskal-Wallis test an uncorrected two-sided level of significance of $\alpha = 0.05$ was used. Bonferroni correction was performed to correct for the likelihood of a significant result by chance due to multiple testing. After Bonferroni correction, a *p*-value < 0.004 was considered statistically significant.

3. Results

A total of 103 cases (206 paired specimen) were selected. All corresponding files were reviewed. Descriptive population characteristics of the cases are shown in Table 2 and Table 3.

In 60 cases (58%) the PMI could be determined with certainty, whereas in 17 cases (17%) the PMI was estimated. In another 11 cases (11%) the PMI could not be adequately estimated, however these cases could be categorized in one of the subgroups (< 24 h, 24 – 48 h, 48 – 72 h, > 72 h) based on context information.

C/P ratio distribution within groups and the results of the statistical analysis are shown in Table 4. After applying the Bonferroni correction no significant differences (*p*-value < 0.004) in C/P ratios between subgroups were observed for any of the investigated variables. However, the low *p*-values of grade of decomposition at autopsy, position of the corpse at discovery, route of administration and attempted resuscitation suggest a relationship between these variables and the C/P ratio. To rule out that the apparent influence of a variable on the C/P ratio is actually caused by another variable (e.g. all cases found on the left side administered morphine orally), the correlation between these variables was assessed. No correlation between any of these variables was observed.

In Fig. 1 C/P ratios are compared between corpses in different grades of decomposition. The C/P ratio increased with advancing decomposition, up till the stage of grade III decomposition. Post-hoc Mann-Whitney *U* testing suggests a difference in C/P ratio between corpses with grade I and grade III decomposition (*p*-value=0.010) and between corpses with grade II and grade III decomposition (*p*-value=0.034).

In Fig. 2 C/P ratios are compared between corpses in different positions at discovery. On average, the lowest C/P ratios were observed in corpses found in right side position, whilst the highest C/P ratios were observed in corpses found in left side position (between group post-hoc Mann-Whitney *U* test, *p*-value=0.025). Post-hoc Mann-Whitney *U* testing suggests a difference in C/P ratio between supine position and right side position as well (*p*-value=0.004).

In Fig. 3 C/P ratios are compared between different routes of administration.

The highest C/P ratios were observed after oral administration. Post-hoc Mann-Whitney *U* testing suggests a difference in C/P ratio between subcutaneous and intravenous administration (*p*-value=0.029), intravenous and oral administration (*p*-value=0.016) and subcutaneous and oral administration (*p*-value=0.028).

Table 1
Decomposition staging scale. J.E. Pless et al. [39].

Decomposition stage	Grade	Characteristics
Putrid	I	early putrid odor - lividity fixed - rigor waning - tissue tacky
	II	green discoloration of the abdomen - hemolysis - intense livor - no rigor - early skin slippage - drying of nose, lips and fingers
	III	tissue gas on x-rays - prominent hemolysis - tissues soft and slick - skin slips easily
Bloating	IV	early body swelling - discoloration of head - no discoloration of trunk - gas in heart - marbling - bullae
	V	moderate swelling - discoloration of head and trunk
	VI	maximal body swelling
Destruction	VII	release of gasses - exhausted putrefied soft tissues - total destruction of blood
Skeleton	VIII	partially skeletonized - adipocere – mummification
	IX	skeleton with ligaments
	X	skeleton with no soft tissues

Table 2
Population characteristics; mean, median, minimum and maximum values of age, concentration of morphine in femoral blood, concentration of morphine in cardiac blood, C/P ratio of morphine, postmortem interval (PMI), body mass index (BMI), number of comorbidities and other quantified toxicological substances. *n* represents the number of cases for which the data was available.

	n	mean	median	range
Age (years)	103	50	46	11 – 93
Concentration femoral blood (mg/L)	103	0.14	0.07	0.006 – 3.00
Concentration cardiac blood (mg/L)	103	0.40	0.14	0.006 – 10.00
C/P ratio	103	2.90	1.92	0.62 – 28.46
PMI (hours)	77	65.2	44.8	8.5–672
BMI (kg/m^2)	101	26.0	25.4	13.5 – 49.5
Comorbidities	103	1.4	1	0 – 6
Other quantified toxicological substances	103	2.6	2	0 – 7

Table 3
Population characteristics; comorbidities and other quantified toxicological substances. *n* represents the number of cases (percentage of cases) in which these comorbidities and toxicological substances were present.

Comorbidities	*n (%)*		*n (%)*
cardiovascular disease	42 (41%)	*Parkinson's disease*	3 (3%)
respiratory disease	37 (36%)	*epilepsy*	2 (2%)
liver disease	12 (12%)	*HIV*	2 (2%)
oncological disease	9 (9%)	*pancreatic disease*	2 (2%)
psychological disorder	9 (9%)	*sleep apnea*	2 (2%)
gastrointestinal disease	7 (7%)	*Hashimoto's disease*	1 (1%)
dementia	5 (5%)	*muscle disease*	1 (1%)
kidney disease	5 (5%)	*osteoporosis*	1 (1%)
diabetes mellitus	4 (4%)	*tuberculosis*	1 (1%)
Other quantified toxicological substances			
opioids		***antidepressants***	
codeine	34 (33%)	*citalopram*	6 (6%)
methadone	14 (14%)	*paroxetine*	2 (2%)
tramadol	3 (3%)	*mirtazapine*	1 (1%)
fentanyl	2 (2%)	*trazodone*	1 (1%)
oxycodone	2 (2%)	*fluvoxamine*	1 (1%)
benzodiazepines		***stimulants***	
midazolam	27 (26%)	*cocaine*	24 (23%)
diazepam	21 (20%)	*amphetamine*	5 (5%)
desmethyldiazepam	28 (27%)	*MDMA*	3 (3%)
oxazepam	11 (11%)	*MDA*	2 (2%)
temazepam	8 (8%)	*methylphenidate*	1 (%)
lorazepam	4 (4%)	***other***	
alprazolam	1 (1%)	*lidocaine*	25 (24%)
bromazepam	1 (1%)	*ethanol*	16 (16%)
clonazepam	1 (1%)	*THC*	10 (10%)
demoxepam	1 (1%)	*phenprocoumon*	3 (3%)
Z-drugs		*digoxin*	1 (1%)
zolpidem	2 (2%)	*metformin*	1 (1%)
zopiclone	1 (1%)	*modafinil*	1 (1%)
antihistamines		*paracetamol*	1 (1%)
promethazine	2 (2%)	*pentobarbital*	1 (1%)
fexofenadine	1 (1%)	*pregabalin*	1 (1%)
		quetiapine	1 (1%)

In Fig. 4 C/P ratios are compared between resuscitation and non-resuscitation cases. Higher C/P ratios were observed in the resuscitation subgroup (p-value=0.049). Although not significant (*p*-value=0.456), comparing C/P ratios between different PMI-groups reveals a positive trend as shown in Fig. 5. Groups of variables with $n = 1$ are not included in the figures because these are not a reliable representation of a group.

4. Discussion

No statistically significant differences in C/P ratios were observed between any of the subgroups of the investigated variables. However, grade of decomposition, position of the corpse at discovery, route of administration and attempted resuscitation seem to be influencing the C/P ratio. In addition, comparison of the C/P ratios between different postmortem intervals revealed a positive trend. Although statistically not significant, the *p*-values of these variables are all less than 0.05 and suggest a relationship between these variables and the C/P ratio. The large number of variables included in this study causes the *p*-value that is considered statistically significant to be dramatically lower than the 'standard' cutoff-value of < 0.05. However, the large number of variables included makes this study unique compared to previously published studies. The inclusion of a broad set of variables that might affect the C/P ratio, increases the chance to unravel potentially relevant variables influencing the postmortem redistribution. A better understanding of these mechanisms might assist in the interpretation of postmortem quantitative results in forensic casework.

4.1. Decomposition - degradation of anatomical barriers, proximity to drug reservoirs and bacterial enzymatic activity

An increase of the C/P ratio of morphine is observed with advancing decomposition, up till the stage of grade III decomposition. A possible explanation for the increase in C/P ratio with advancing decomposition, is that progressive decomposition correlates with increasing degradation of tissues and anatomical barriers, making it easier for morphine to diffuse across these barriers. The heart lies in closer proximity to potential drug reservoirs such as lung tissue and stomach content compared to the femoral veins. In addition, organs in the abdominal cavity are generally more affected by decomposition. Therefore, drugs might diffuse earlier, at a higher rate and to a greater extent towards cardiac blood than towards the femoral blood, resulting in an increased C/P ratio.

Another mechanism that might slightly contribute to this apparent increase up till grade III decomposition might be an increase in free morphine concentration in cardiac blood, due to hydrolysis of morphine-3-glucuronide to free morphine by bacterial enzymatic activity. After death, bacteria migrate from the digestive and respiratory system into the general body tissues and the blood compartment. Considering the anatomical position of the heart, bacteria might reach the cardiac blood earlier than the femoral blood,

Table 4
Statistical data. Mean, median, minimum and maximum C/P ratio are shown for the following categorical variables; grade of decomposition at autopsy, position of corpse at discovery, route of administration, attempted resuscitation, chronic or naïve user of morphine, sex, season a corpse was found, age, postmortem interval (PMI), type of intoxication, morphine concentration in femoral blood, body mass index (BMI) and cause of death.

	n (%) cases	*C/P ratio*			*p-value*
		mean	*median*	*range*	
Grade of decomposition	101 (98%)	2.93	1.97	0.62 – 28.46	0.010
grade I	56 (54%)	2.55	1.73	0.82 – 18.18	
grade II	34 (33%)	2.71	2.42	0.62 – 8.59	
grade III	6 (6%)	7.63	3.95	1.54 – 28.46	
grade IV	1 (1%)	0.80	0.80		
grade V	4 (4%)	3.40	3.67	1.36 – 4.89	
Position corpse	73 (71%)	2.84	2.11	0.62 – 18.18	0.026
supine	62 (60%)	2.98	2.37	0.62 – 18.18	
prone	3 (3%)	2.26	2.36	0.94 – 3.48	
left side	3 (3%)	3.32	3.25	1.82 – 4.89	
right side	5 (5%)	1.14	1.01	0.80 – 1.65	
Route of administration	29 (28%)	4.03	2.36	0.62 – 28.46	0.035
inhalation	2 (2%)	1.43	1.43	1.01 – 1.84	
s.c. injection	10 (10%)	3.27	1.53	0.62 – 18.18	
i.v. injection	11 (11%)	2.78	2.54	1.52 – 5.10	
i.m. injection	1 (1%)	1.43	1.43		
oral	3 (3%)	14.98	12.73	3.75 – 28.46	
rectal	1 (1%)	2.47	2.47		
epidural	1 (1%)	1.92	1.92		
Resuscitation	98 (95%)	2.92	1.94	0.62 – 28.46	0.046
resuscitation	36 (35%)	3.04	2.42	0.84 – 18.18	
no resuscitation	62 (60%)	2.85	1.69	0.62 – 28.46	
History of use	38 (37%)	2.94	1.92	0.62 – 18.18	0.221
chronic user	15 (15%)	3.17	2.40	1.01 – 12.73	
naïve user	23 (22%)	2.78	1.74	0.62 – 18.18	
Sex	103 (100%)	2.90	1.92	0.62 – 28.46	0.263
male	78 (76%)	2.94	2.00	0.80 – 28.46	
female	25 (24%)	2.74	1.67	0.62 – 18.18	
Season	103 (100%)	2.90	1.92	0.62 – 28.46	0.316
spring	28 (27%)	2.92	1.59	0.82 – 28.46	
summer	21 (20%)	3.07	1.97	1.16 – 12.73	
autumn	32 (31%)	3.14	2.38	0.80 – 18.18	
winter	22 (21%)	2.32	1.93	0.62 – 6.47	
Age	103 (100%)	2.90	1.92	0.62 – 28.46	0.375
< 25 years	12 (12%)	2.78	2.47	0.80 – 6.31	
25 – 50 years	48 (47%)	2.59	2.35	0.82 – 8.59	
50 – 75 years	24 (23%)	3.53	1.53	0.88 – 28.46	
> 75 years	19 (18%)	2.94	1.78	0.62 – 18.18	
PMI	88 (85%)	3.02	1.94	0.62 – 28.46	0.456
< 24 h	13 (13%)	2.33	1.67	0.84 – 5.20	
24 – 48 h	34 (33%)	2.65	1.89	1.08 – 12.73	
48 – 72 h	22 (21%)	4.56	2.44	1.30 – 28.46	
> 72 h	19 (18%)	2.38	2.11	0.62 – 4.89	
Intoxication	49 (48%)	2.85	2.00	0.80 – 28.46	0.457
mono-intoxication morphine	6 (6%)	1.69	1.59	1.00 – 2.73	
mixed intoxication (with morphine)	34 (33%)	3.12	2.38	0.80 – 28.46	
mixed intoxication (without morphine)	4 (4%)	2.13	2.09	0.94 – 3.41	
mono-intoxication other drug	5 (5%)	3.02	2.46	1.41 – 6.31	
Concentration	103 (100%)	2.90	1.92	0.62 – 28.46	0.558
low (< 0.01 mg/L)	7 (7%)	5.76	2.46	0.94 – 18.18	
therapeutic (0.01 – 0.1 mg/L)	57 (55%)	2.45	1.75	0.62 – 8.59	
toxic (> 0.1 mg/L)	39 (38%)	3.03	2.00	0.82 – 28.46	
BMI	101 (98%)	2.93	1.97	0.62 – 28.46	0.701
< 18.5 kg/m^2	6 (6%)	3.77	2.51	1.33 – 8.59	
18.5–25 kg/m^2	41 (40%)	2.55	1.92	0.88 – 12.73	
> 25 kg/m^2	54 (52%)	3.11	1.95	0.62 – 28.46	
Cause of death	102 (99%)	2.91	1.94	0.62 – 28.46	0.728
trauma	23 (22%)	2.94	2.55	0.84 – 8.59	
intoxication	39 (38%)	3.05	2.40	0.80 – 28.46	
other (disease, drowning, etc.)	29 (28%)	2.96	1.84	0.62 – 18.18	
intoxication and/or other	11 (11%)	2.22	1.65	1.08 – 4.36	

increasing the concentration of free morphine at a higher rate. An in vitro study of Skopp et al. [40] supports the hypothesis of bacterial conversion of morphine. In that study different degradation patterns were observed for fresh whole blood, plasma and postmortem blood with morphine glucuronides being hydrolyzed to yield free morphine in the postmortem blood. However, other studies did not observe any conversion of morphine-3-glucuronide to free morphine [18,33] or stated that the clinical relevance of this mechanism is unclear [41]. Therefore, the contribution of this mechanism to the observed increase of the C/P ratio with advancing decomposition might have been limited. The C/P ratio seems to stabilize in corpses with grade V decomposition. However, any conclusions based on this observation should be made with caution considering the small number of included grade V cases ($n = 5$). An increase in drug

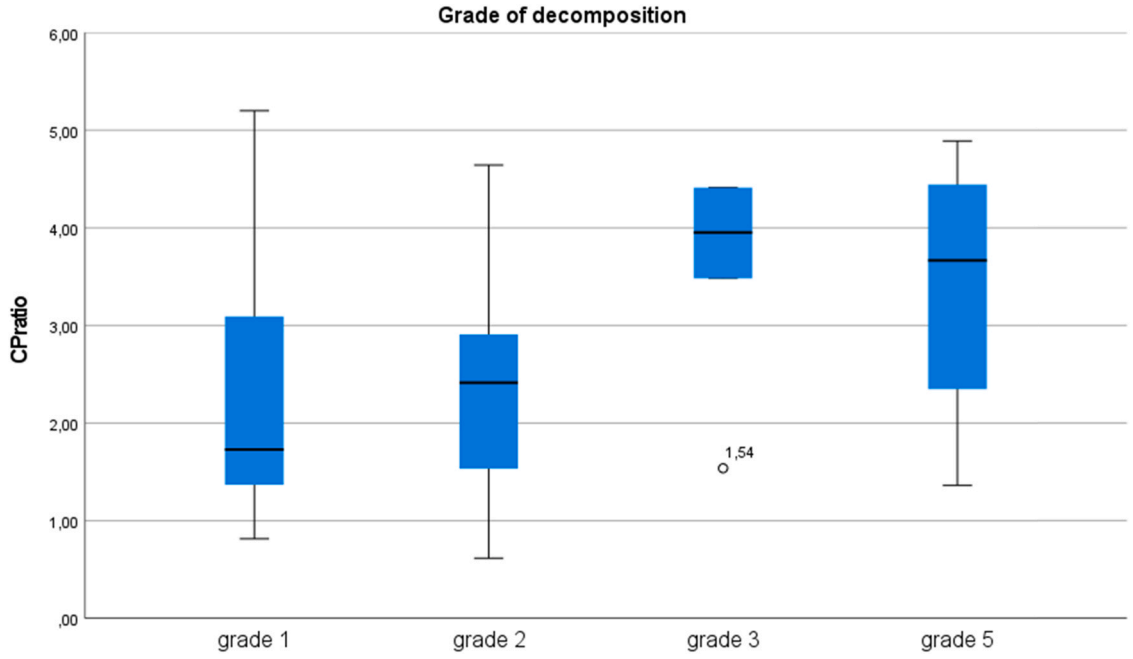

Fig. 1. Boxplots of C/P ratios of different grades of decomposition. Mean values, median values and ranges are provided in Table 4. The depicted C/P ratios in this figure are limited to a C/P ratio of 6 for ease of comparison between the different groups. Several cases have outliers outside this range and are not presented in this figure. The following C/P ratios were omitted: grade 1: 12.73, 18.18; grade 2: 6.31, 6.47, 8.59; grade 3: 28.46.

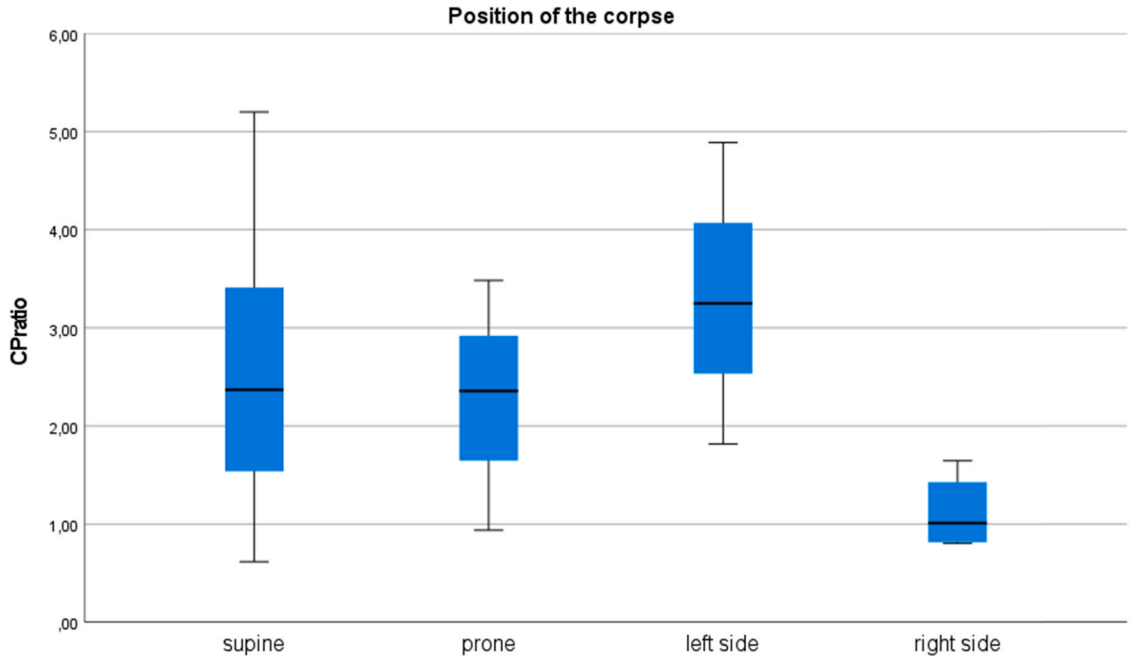

Fig. 2. Boxplots of C/P ratios of different positions of corpses. Mean values, median values and ranges are provided in Table 4. The depicted C/P ratios in this figure are limited to a C/P ratio of 6 for ease of comparison between the different groups. Several cases have outliers outside this range and are not presented in this figure. The following C/P ratios were omitted: supine position: 6.31, 8.59, 12.73, 18.18.

concentration in femoral blood at grade V decomposition might have been caused by redistribution from tissues surrounding the femoral vein such as muscle and adipose tissue. After a longer postmortem interval, it could be expected that the integrity of these tissues will be lost due to decomposition, facilitating diffusion of morphine to the femoral blood. To our knowledge, no other studies investigated the relationship between the grade of decomposition and the C/P ratio of morphine in humans. Therefore, the results of this study cannot be directly compared to other scientific publications.

Considering that the rate of decomposition is not only determined by the postmortem interval but also by other factors such as body temperature and ambient circumstances, the grade of postmortem decomposition might be a better marker for the extent of PMR than the postmortem interval. Corpses with a similar

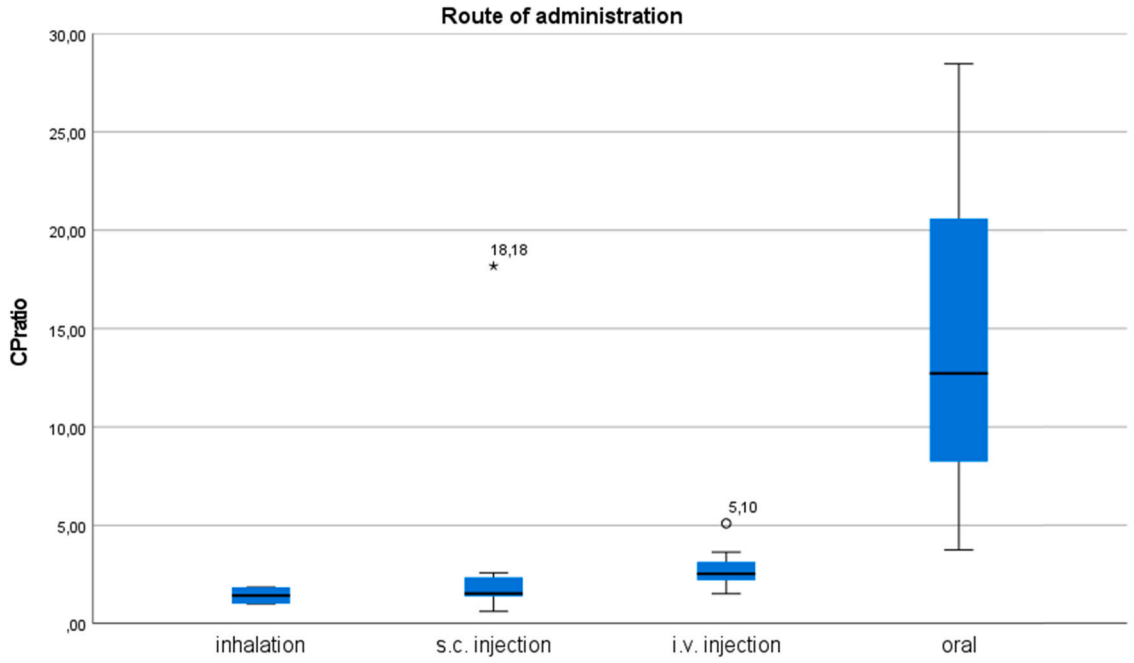

Fig. 3. Boxplots of C/P ratios of different routes of administration. Mean values, median values and ranges are provided in Table 4.

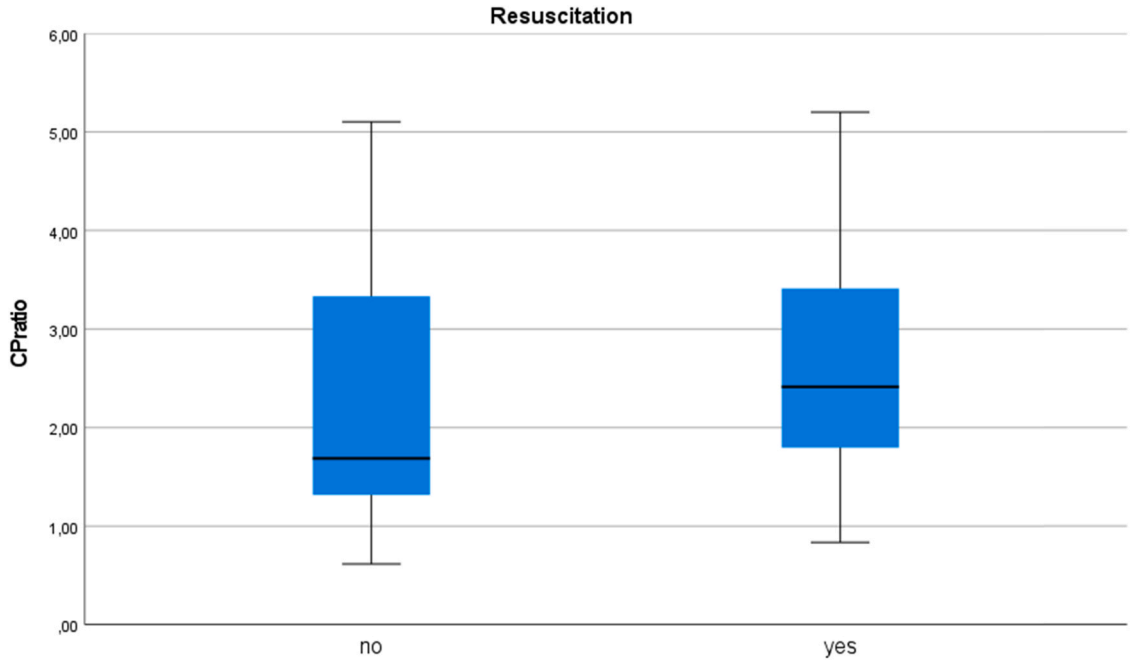

Fig. 4. Boxplots of C/P ratios of not resuscitated and resuscitated groups. Mean values, median values and ranges are provided in Table 4. The depicted C/P ratios in this figure are limited to a C/P ratio of 6 for ease of comparison between the different groups. Several cases have outliers outside this range and are not presented in this figure. The following C/P ratios were omitted: no resuscitation: 6.47, 8.59, 12.73, 28.46; resuscitation: 6.31, 18.18.

postmortem interval, but found under different circumstances, may exhibit varying grades of decomposition and therefore the C/P ratio and the extent of postmortem redistribution might be different as well.

4.2. Position corpse - hypostasis, gastroesophageal reflux and gravitational pull

A possible explanation for the C/P ratio being higher when a corpse is found on the left side is the occurrence of hypostasis. Hypostasis refers to gravitational pooling of blood and other fluids to accumulate in the lower, dependent parts of the body. Fluids containing morphine might therefore accumulate postmortem in lower, dependent parts of the body. As the heart is located in the left side of the body, morphine concentrations in cardiac blood might increase when a corpse is positioned on the left side. This hypothesis might also explain why the lowest C/P ratios are found in corpses that were positioned on the right side. In these cases, morphine containing fluids might accumulate in the right side of the body, which is more distant from the heart. This might result in lower morphine

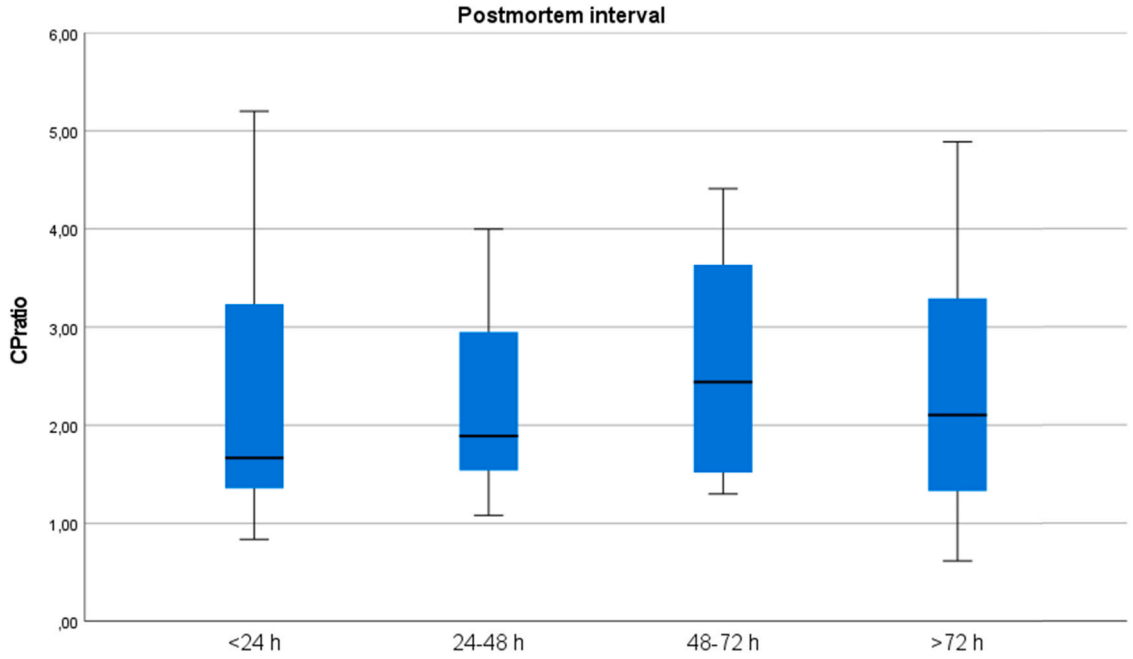

Fig. 5. Boxplots of C/P ratios of different postmortem intervals. Mean values, median values and ranges are provided in Table 4. The depicted C/P ratios in this figure are limited to a C/P ratio of 6 for ease of comparison between the different groups. Several cases have outliers outside this range and are not presented in this figure. The following C/P ratios were omitted: 24–48 h: 6.31, 6.47, 12.73; 48–72 h: 8.59, 18.18, 28.46.

concentrations in cardiac blood and thus lower C/P ratios compared to corpses positioned on the left side.

Another possible explanation for the apparent differences in C/P ratios between the different positions is the occurrence of postmortem redistribution of morphine from the esophagus. After death, relaxation of the esophagogastric sphincter might lead to gastroesophageal reflux. Hence, if morphine is taken orally, postmortem redistribution from the esophagus could occur. When a corpse is positioned on the left side, it is possible for morphine in the esophagus to diffuse towards the heart, while lying on the right side will cause the morphine to diffuse away from the heart.

Diffusion can either occur through the blood vessels or by transparietal diffusion to the surrounding organs depending on the integrity of barriers. Dryburgh et al. [42] support the hypothesis that body position affects the postmortem redistribution, by illustrating that horizontal and vertical body positions cause a different route of diffusion from the stomach, both following the direction of the gravitational pull. However, this study was performed in rats using caesium ions. As such, the results are not necessarily extrapolatable to the human population and/or substances with different physicochemical properties such as a larger molecular size. Any conclusions based on the results of our study in regard to the influence of the position of the corpse on C/P ratio should be made with caution considering the small number of included corpses positioned on the left side ($n = 3$) or the right side ($n = 5$).

4.3. Route of administration - incomplete antemortem absorption and distribution after oral administration

A possible explanation for the higher C/P ratios observed after oral intake of morphine, is that oral intake of drugs might lead to a drug reservoir in the stomach when the absorption was not completed at the time of death. As a result, continuing redistribution from the stomach and/or esophagus to the surrounding tissues after death might occur, hence increasing the concentration in cardiac blood. The overall highest C/P ratio of morphine was observed after oral administration. Moreover, the difference of C/P ratios between oral and other routes of administration seems to be higher than the difference between the subgroups of the other variables. This suggests that the route of administration might be the most important variable to take into account when evaluating the extent of postmortem redistribution. However, any conclusions based on this observation should be made with caution considering the small number of included cases with oral administration (n = 3). Unfortunately, stomach contents were not analyzed in any of the included cases. Therefore, substantiation whether the high C/P ratios were indeed caused by postmortem redistribution from an established reservoir in the stomach was not possible.

4.4. Attempted resuscitation - postmortem blood flow and internal injuries

C/P ratios found in cases were resuscitation was attempted (median=2.42) were higher than the ratios found in cases were resuscitation was not attempted (median=1.67). This might be caused by an increased diffusion to cardiac blood by postmortem blood flow or internal injuries caused by the mechanical compressions [5].

4.5. Postmortem interval

Although not significant, comparing the C/P ratios between different postmortem intervals reveals a positive trend. An explanation for the observed trend is that a longer postmortem interval provides more potential for postmortem redistribution to occur. As previously discussed, the cardiac blood is likely affected to a greater extent by postmortem changes than femoral blood, causing an increased C/P ratio over time. Gerostamoulos et al. [33] and Logan et al. [34] both found a nonsignificant trend for higher concentrations of morphine in cardiac blood compared to femoral blood in humans. However, morphine concentrations did not change significantly during the observed postmortem interval in either femoral blood or cardiac blood in these studies. The postmortem interval investigated in these studies was defined as the time between admission to the institutes and autopsy. As previously described, this method for

defining the postmortem interval might underestimate any actual PMR that occurs in time, considering redistribution could have been (partly) completed before admission to the institute. Also, in both studies the bodies were kept at 4 °C. This reduces the rate of decomposition and might therefore also reduce postmortem redistribution.

4.6. Potential explanations for the absence of a relationship between variables and the C/P ratio of morphine in this study

The results of this study did not suggest a relationship between the other investigated variables and the C/P ratio. The lack of a relationship between BMI, sex, age and the C/P ratio might be explained by the physicochemical properties of morphine. The underlying difference between subgroups of these variables is mainly a difference in fat percentage. Morphine is a weak base that is more hydrophilic than lipophilic. Since it is a weak base, it does accumulate in tissues as a consequence of lysosomal trapping [43]. The accumulation of morphine in adipose tissue seems to be limited [44]. In addition, postmortem morphine concentrations in adipose tissue seem to remain fairly stable, suggesting that little postmortem redistribution of morphine either into or from the adipose tissue occurs [45]. As such, an increasing fat compartment might not necessarily influence the C/P ratio of morphine. No relationship was observed between the femoral blood concentration and the C/P ratio. We hypothesized that a high femoral blood concentration might be a marker for a high total intrinsic concentration and therefore would be associated with a greater accumulation of morphine in lung and liver tissue that was subsequently available for postmortem redistribution. However, this hypothesis is not supported by our results.

No differences in C/P ratios are observed between the different seasons. This could be explained by the fact that almost all corpses included in this study ($n = 101$) were found inside. Whether a person was a chronic or naïve user of morphine, did not cause a significant difference in C/P ratios. However, C/P ratios found in chronic users of morphine (median=2.56) were higher than C/P ratios found in naïve users of morphine (median=1.74). This might be explained by reservoirs that are established in chronic users due to tissue accumulation of morphine in cases of chronic use.

We considered that trauma might be associated with a high C/P ratio as a consequence of accelerated invasion of bacteria. However, no such relationship was observed. This might be due to the type of trauma cases included in this study as not all cases concerned (invasive) trauma in the thoracal region. 13 out of the 23 included trauma cases died of either ballistic or blunt force trauma to the head. This sort of trauma might not influence the morphine concentration in cardiac blood, considering that it does not provide bacterial entrance into the chest cavity. Finally, no significant difference in C/P ratios was observed for different types of intoxication. A possible explanation is that the other drugs that contributed to death in these cases did not alter the antemortem pharmacokinetics of morphine and did not compete in the postmortem diffusion process.

4.7. Extrapolation of the results of this study to other compounds of interest

The results of this study hint towards the potential relevance of taking grade of decomposition at autopsy, position of the corpse at discovery, route of administration and attempted resuscitation into account when interpreting postmortem morphine concentrations. These variables might be relevant for other compounds as well, however it is important to consider the physicochemical and pharmacokinetic differences between these compounds of interest compared to morphine and their associated differences in relation to these variables. For a number of drugs fast postmortem changes in femoral blood concentrations have been described. For these drugs the influence of degradation of tissue and cell barriers, associated with decomposition and subsequent transparietal diffusion, might be clinically less relevant considering that potentially a large part of redistribution has already occurred within the first 24-hours postmortem. The impact of decomposition associated bacterial enzymatic activity differs as well for different drugs. Postmortem drug concentrations may increase for some drugs by bacterial hydrolysis of glucuronide metabolites, as described for morphine [40], while concentrations of other drugs, for example nitrobenzodiazepines, might decrease as the result of bacterial degradation [46]. The clinical relevance of the position of the corpse at discovery and route of administration is likely dependent of the drug distribution at the time of death. The pharmacokinetic properties of a drug, such as absorption rate, volume of distribution, protein binding and elimination rate influence antemortem drug distribution. It is essential to take antemortem drug distribution into account when interpreting postmortem redistribution. For example, a deceased chronic user of amitriptyline is expected to be in steady-state in which an equilibrium has been established between the blood and tissue concentrations. In this case it is likely that there are large reservoirs available for redistribution in the saturated lung and liver tissue. In contrast, after a single exposure oral overdose with amitriptyline the person might have died before blood-tissue equilibrium was achieved and the only reservoir present is unabsorbed drug present in the stomach content because of delayed gastric emptying. It is likely that these persons show different postmortem redistribution patterns because of their different initial distribution at the time of death.

5. Conclusion

Grade of decomposition, position of the corpse, route of administration and attempted resuscitation seem to be influencing the C/P ratio of morphine. Of these four variables, the route of administration seems to have the greatest impact. However, any conclusions based on this observation should be made with caution considering the small number of included oral administration cases. When interpreting postmortem toxicological results involving morphine, it might prove useful to take these variables into account. More studies concerning the postmortem redistribution of morphine have to be performed in order to elucidate the effect of different variables on the postmortem redistribution of morphine.

CRediT authorship contribution statement

Anne Kamphuis: Investigation, Formal analysis, Visualisation, Writing – original draft **Lennaert Borra:** Conceptualization, Methodology, Formal analysis, Visualisation, Writing – original draft **Rogier van der Hulst:** Conceptualization, Methodology, Writing – review & editing. **Dick-Paul Kloos:** Validation, Writing – review & editing. **Dingeman Rijken:** Investigation, Writing – review & editing. **Ingrid Bosman:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Daan Touw:** Supervision, Writing – review & editing.

Declarations of interest

D.J. Touw received a grant from Chiesi Pharmaceuticals to perform a drug-drug interaction study. D.J. Touw is a member of the Data Safety Monitoring Board of the FORMAT clinical trial. D.J. Touw is the vice-chair of the Medical Advisory Board of SANQUIN blood bank.

References

[1] A.L. Pélissier-Alicot, J.M. Gaulier, P. Champsaur, P. Marquet, Mechanisms underlying postmortem redistribution of drugs: a review, J. Anal. Toxicol. 27 (8) (2003) 533–544.
[2] D.J. Pounder, G.R. Jones, Post-mortem drug redistribution–a toxicological nightmare, Forensic Sci. Int. 45 (3) (1990) 253–263.
[3] D.J. Pounder, E. Adams, C. Fuke, A.M. Langford, Site to site variability of postmortem drug concentrations in liver and lung, J. Forensic Sci. 41 (6) (1996) 927–932.
[4] R.J. Flanagan, G. Connally, Interpretation of analytical toxicology results in life and at postmortem, Toxicol. Rev. 24 (1) (2005) 51–62.
[5] C. Sastre, C. Bartoli, V. Baillif-Couniou, G. Leonetti, A.L. Pelissier-Alicot, Post mortem redistribution of drugs: current state of knowledge, Curr. Pharm. Des. 23 (36) (2017) 5530–5541.
[6] H.J. McQuay, D. Carroll, C.C. Faura, D.J. Gavaghan, C.W. Hand, R.A. Moore, Oral morphine in cancer pain: influences on morphine and metabolite concentration, Clin. Pharm. Ther. 48 (3) (1990) 236–244.
[7] R.E. Ferner, Post-mortem clinical pharmacology, Br. J. Clin. Pharm. 66 (4) (2008) 430–443.
[8] D.J. Pounder, C. Fuke, D.E. Cox, D. Smith, N. Kuroda, Postmortem diffusion of drugs from gastric residue: an experimental study, Am. J. Forensic Med. Pathol. 17 (1) (1996) 1–7.
[9] R.W. Prouty, W.H. Anderson, The forensic science implications of site and temporal influences on postmortem blood-drug concentrations, J. Forensic Sci. 35 (2) (1990) 243–270.
[10] G. Skopp, Preanalytic aspects in postmortem toxicology, Forensic Sci. Int. 142 (2–3) (2004) 75–100.
[11] B. Zilg, G. Thelander, B. Giebe, H. Druid, Postmortem blood sampling-comparison of drug concentrations at different sample sites, Forensic Sci. Int 278 (2017) 296–303.
[12] D.S. Cook, R.A. Braithwaite, K.A. Hale, Estimating antemortem drug concentrations from postmortem blood samples: the influence of postmortem redistribution, J. Clin. Pathol. 53 (4) (2000) 282–285.
[13] F.S. Apple, A better understanding of the interpretation of postmortem blood drug concentrations, J. Anal. Toxicol. 35 (6) (2011) 381–383.
[14] K.N. Olson, K. Luckenbill, J. Thompson, O. Middleton, R. Geiselhart, K.M. Mills, J. Kloss, F.S. Apple, Postmortem redistribution of fentanyl in blood, Am. J. Clin. Pathol. 133 (3) (2010) 447–453.
[15] D. Gerostamoulos, J. Beyer, V. Staikos, P. Tayler, N. Woodford, O.H. Drummer, The effect of the postmortem interval on the redistribution of drugs: a comparison of mortuary admission and autopsy blood specimens, Forensic Sci. Med. Pathol. 8 (2012) 373–379.
[16] E. Saar, J. Beyer, D. Gerostamoulos, O.H. Drummer, The time-dependant postmortem redistribution of antipsychotic drugs, Forensic Sci. Int. 222 (1–3) (2012) 223–227.
[17] C.S. Krinsky, S.L. Lathrop, R. Zumwalt, An examination of the postmortem redistribution of fentanyl and interlaboratory variability, J. Forensic Sci. 59 (5) (2014) 1275–1279.
[18] S.N. Staeheli, D. Gascho, L.C. Ebert, T. Kraemer, A.E. Steuer, Time-dependent postmortem redistribution of morphine and its metabolites in blood and alternative matrices-application of CT-guided biopsy sampling, Int. J. Leg. Med. 131 (2) (2017) 379–389.
[19] L. Brockbals, S.N. Staeheli, D. Gascho, L.C. Ebert, T. Kraemer, A.E. Steuer, Time-dependent postmortem redistribution of opioids in blood and alternative matrices, J. Anal. Toxicol. 42 (2018) 365–374.
[20] L. Brockbals, S.N. Staeheli, T. Kraemer, A.E. Steuer, Postmortem metabolomics: correlating time-dependent concentration changes of xenobiotic and endogenous compounds, Drug Test. Anal. 12 (2020) 1171–1182.
[21] L. Brockbals, Y. Wartmann, D. Mantinieks, L.L. Glowacki, D. Gerostamoulos, T. Kraemer, A.E. Steuer, Postmortem metabolomics: strategies to assess time-dependent postmortem changes of diazepam, nordiazepam, morphine, codeine, mirtazapine and citalopram, Metabolites 11 (9) (2021).
[22] L. Brockbals, S.N. Staeheli, D. Gascho, L.C. Ebert, T. Kraemer, A.E. Steuer, Time- and site-dependent postmortem redistribution of antidepressants and neuroleptics in blood and alternative matrices, J. Anal. Toxicol. 45 (4) (2021) 356–367.
[23] S. Salmond, V. Allread, A population health approach to America's opioid epidemic, Orthop. Nurs. 38 (2) (2019) 95–108.
[24] A.W. Jones, A. Holmgren, J. Ahlner, Concentrations of free-morphine in peripheral blood after recent use of heroin in overdose deaths and in apprehended drivers, Forensic Sci. Int. 215 (1–3) (2012) 18–24.
[25] P.D. Maskell, N.E. Wilson, L.N. Seetohul, M.L. Crichton, L.J. Beer, G. Drummond, G. De, Paoli, Postmortem tissue distribution of morphine and its metabolites in a series of heroin-related deaths, Drug Test. Anal. 11 (2) (2019) 292–304.
[26] C.H. Thaulow, M.L. Øiestad Å, S. Rogde, J.M. Andersen, G. Høiseth, M. Handal, J. Mørland, V. Vindenes, Can measurements of heroin metabolites in postmortem matrices other than peripheral blood indicate if death was rapid or delayed? Forensic Sci. Int. 290 (2018) 121–128.
[27] C.S. Crandall, S. Kerrigan, R.L. Blau, J. Lavalley, R. Zumwalt, P.E. McKinney, The influence of site of collection on postmortem morphine concentrations in heroin overdose victims, J. Forensic Sci. 51 (2) (2006) 413–420.
[28] E. Lemaire, C. Schmidt, R. Denooz, C. Charlier, P. Boxho, Postmortem concentration and redistribution of diazepam, methadone, and morphine with subclavian and femoral vein dissection/clamping, J. Forensic Sci. 61 (6) (2016) 1596–1603.
[29] E. Lemaire, C. Schmidt, R. Denooz, C. Charlier, P. Boxho, Popliteal vein blood sampling and the postmortem redistribution of diazepam, methadone, and morphine, J. Forensic Sci. 61 (4) (2016) 1017–1028.
[30] J. Wyman, S. Bultman, Postmortem distribution of heroin metabolites in femoral blood, liver, cerebrospinal fluid, and vitreous humor, J. Anal. Toxicol. 28 (4) (2004) 260–263.
[31] N.J. Langford, S.R. Morley, R.E. Ferner, The relationship between antemortem and postmortem morphine concentrations, Clin. Toxicol. Philos. 57 (12) (2019) 1142–1145.
[32] S.S. Tolliver, W.L. Hearn, K.G. Furton, Evaluating the relationship between postmortem and antemortem morphine and codeine concentrations in whole blood, J. Anal. Toxicol. 34 (8) (2010) 491–497.
[33] J. Gerostamoulos, O.H. Drummer, Postmortem redistribution of morphine and its metabolites, J. Forensic Sci. 45 (4) (2000) 843–845.
[34] B.K. Logan, D. Smirnow, Postmortem distribution and redistribution of morphine in man, J. Forensic Sci. 41 (2) (1996) 221–229.
[35] E. Lemaire, C. Schmidt, N. Dubois, R. Denooz, C. Charlier, P. Boxho, Site-, technique-, and time-related aspects of the postmortem redistribution of diazepam, methadone, morphine, and their metabolites: interest of popliteal vein blood sampling, J. Forensic Sci. 62 (6) (2017) 1559–1574.
[36] V.M. Hargrove, D.K. Molina, Peripheral postmortem redistribution of morphine, Am. J. Forensic Med. Pathol. 35 (2) (2014) 106–108.
[37] European Medicine Agency, Draft guideline on bioanalytical method validation, 2009.
[38] F.T. Peters, O.H. Drummer, F. Musshoff, Validation of new methods, Forensic Sci. Int. 165 (2–3) (2007) 216–224.
[39] J.E. Pless, M. Worrell, M. Clark, Post. Chang. Soft Tissues (1996).
[40] G. Skopp, L. Pötsch, A. Klingmann, R. Mattern, Stability of morphine, morphine-3-glucuronide, and morphine-6-glucuronide in fresh blood and plasma and postmortem blood samples, J. Anal. Toxicol. 25 (1) (2001) 2–7.
[41] W.R. Sawyer, R.B. Forney, Postmortem disposition of morphine in rats, Forensic Sci. Int. 38 (3–4) (1988) 259–273.
[42] E. Dryburgh, L. Honeybun, K. Sturrock, A. Savage, P.D. Maskell, Using μX-Ray CT to observe postmortem diffusion from the stomach in a rat model, Forensic Sci. Int 312 (2020) 110310.
[43] A.C. MacIntyre, D.J. Cutler, The potential role of lysosomes in tissue distribution of weak bases, Biopharm. Drug Dispos. 9 (6) (1988) 513–526.
[44] J. Frost, T.N. Løkken, A. Helland, I.S. Nordrum, L. Slørdal, Post-mortem levels and tissue distribution of codeine, codeine-6-glucuronide, norcodeine, morphine and morphine glucuronides in a series of codeine-related deaths, Forensic Sci. Int. 262 (2016) 128–137.
[45] J.A. Levisky, D.L. Bowerman, W.W. Jenkins, D.G. Johnson, S.B. Karch, Drugs in postmortem adipose tissues: evidence of antemortem deposition, Forensic Sci. Int. 121 (3) (2001) 157–160.
[46] M.D. Robertson, O.H. Drummer, Stability of nitrobenzodiazepines in postmortem blood, J. Forensic Sci. 43 (1) (1998) 5–8.