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Investigation of buprenorphine-related deaths using urinary metabolite concentrations

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Abstract

Quantitative analysis of postmortem urine, instead of blood, for buprenorphine and metabolites may provide additional evidence for the diagnosis of fatal buprenorphine poisoning. In this study, 247 autopsy urine samples, previously testing positive for buprenorphine or norbuprenorphine, were quantitatively reanalysed with a recently developed liquid chromatography–tandem mass spectrometry (LC-MS/MS) method for unconjugated buprenorphine (BUP), norbuprenorphine (NBUP), naloxone (NAL), and their respective conjugated metabolites, buprenorphine glucuronide (BUPG), norbuprenorphine glucuronide (NBUPG), and naloxone glucuronide (NALG). The cases were divided, according to medical examiners' decision, to buprenorphine poisonings and other causes of death. The groups were compared for urinary concentrations and metabolite concentration ratios of the six analytes. All median concentrations were higher in the buprenorphine poisoning group. The median concentration of BUPG was significantly higher and the median metabolite ratios NBUP/BUP, NBUPG/BUPG, and NBUPtotal/BUPtotal were significantly lower in poisonings than in other causes of death. Naloxone-related concentrations and ratios were not significantly different between the groups.

KEYWORDS
buprenorphine-related death, glucuronide, naloxone, norbuprenorphine, urine

1 | INTRODUCTION

Buprenorphine is an opioid analgesic used for opioid agonist treatment (OAT) of opioid-dependent patients. Buprenorphine is a partial agonist for the mu opioid receptors with high affinity and slow dissociation kinetics. It has a superior safety profile over methadone or morphine, particularly due to a ceiling effect with respect to respiratory depression.[1] Buprenorphine is metabolized by *N*-dealkylation to norbuprenorphine. These drugs are further conjugated primarily by glucuronidation to buprenorphine glucuronide and norbuprenorphine glucuronide, respectively.[2] The role of metabolites in the toxicity of buprenorphine in humans remains unclear.

Buprenorphine is available in different formulations.[3] In OAT, sublingual tablets and films have been the most commonly used products to date, containing either buprenorphine alone or a combination of buprenorphine and naloxone in a 4:1 ratio. The role of naloxone is to deter injection of dissolved tablets, thus reducing the product's abuse potential. Naloxone is a short-acting opioid antagonist that is poorly absorbed sublingually. The idea of the combination product is that when injected, naloxone blocks the mu-receptors and prevents

receptor activation or causes precipitated withdrawal. Newer prolonged-release buprenorphine formulations include subcutaneous implants and subcutaneous extended-release injection, and these formulations are subject to expectations of lower abuse.[4]

Buprenorphine is gaining interest worldwide because of its increasing use in OAT, the medication being an effective treatment for opioid dependence and associated with lower rates of mortality than methadone.[5] However, buprenorphine has also been associated with abuse, diversion, and poisoning deaths since the drug became more widely available in OAT during the mid-1990s.[6,7] In some countries, buprenorphine is not only the most important medicine for OAT but also among the most abused opioids. For example in Finland, some opioid users have started with buprenorphine, and the substance has become their main intoxicant.[8] There is, however, less frequent parenteral abuse with the buprenorphine–naloxone combination product than with mono-buprenorphine.[7]

Following the pioneering findings of French researchers,[9] many other groups have drawn attention to the deaths associated with buprenorphine abuse. The previous notion that buprenorphine is toxic only in specific situations has given way to more recent epidemiological data on more significant harms of this drug. Drug–drug interactions, especially with concomitant sedative-hypnotic drugs, and intravenous buprenorphine administration are currently the two most recognized circumstances that impair the buprenorphine-related ceiling effect.[10] The issue of buprenorphine abuse has been raised in many contexts, especially in Finland,[8] Sweden,[11] Australia,[12] and the United States.[13] Buprenorphine is commonly associated with polydrug use, as demonstrated for instance by studies on vulnerable populations in the United States[14] or buprenorphine deaths in Finland.[15] In Finland, most of the buprenorphine consumed in an abuse setting is not diverted from locally prescribed medication but is mono-buprenorphine smuggled from abroad.[16]

The cause of the combined toxicity from buprenorphine and concomitant drugs or alcohol has been sought through experimental animal studies.[17,18] Despite the fact that these types of studies have shed light on the synergistic effect, they were unable to explain the dramatic increase in fatal toxicity following concomitant use in humans. A recent study by Vodovar et al.[19] supported a pharmacodynamic mechanism for the buprenorphine–benzodiazepine interaction. They found that buprenorphine and diazepam interacted pharmacodynamically involving both μ-opioid receptors and $GABA_A$ receptors, resulting in a marked respiratory depression in rats. Interestingly, the buprenorphine–naloxone combination product when used parenterally does not appear to protect from fatal poisoning any better than mono-buprenorphine.[20] Naloxone has a short half-life (~60 min) and its amount in the combination product is not high enough to fully block the agonist effects of buprenorphine. Moreover, some administration ways may be used to bypass the precipitation of withdrawal after parenteral use, reducing thus the antagonist action of naloxone.[7] Much of the mechanisms of buprenorphine-related toxicity in humans remain obscure.

Buprenorphine levels in postmortem (PM) blood are difficult to interpret as they overlap antemortem plasma concentrations of maintenance dosed OAT patients.[21,22] Furthermore, buprenorphine deaths often occur delayed while the victim is asleep, as demonstrated in a study by Häkkinen et al.,[23] where delayed deaths were found in about half of the cases. Instead, metabolic ratios may be helpful in PM diagnostics. Seldén et al.[24] stated that the urinary norbuprenorphine/buprenorphine ratio better reflects acute poisoning than the corresponding ratio in blood, a low ratio in urine indicating recent intake. These researchers also speculated that the glucuronide metabolites might be helpful in determining the cause of death, although they did not investigate the matter themselves.

Recently, we described the simultaneous quantification of the six relevant analytes, unconjugated buprenorphine (BUP), norbuprenorphine (NBUP), and naloxone (NAL) and the respective conjugated metabolites buprenorphine glucuronide (BUPG), norbuprenorphine glucuronide (NBUPG), and naloxone glucuronide (NALG) in OAT patient urine samples using a liquid chromatography–tandem mass spectrometry (LC-MS/MS) method.[25] In the present study, our objective is to find diagnostic differences in the urinary concentrations of these analytes between buprenorphine poisonings and other causes of death, applying the recent LC-MS/MS method. Another aim is to differentiate between the parenteral use of the buprenorphine–naloxone combination product and the smuggled mono-buprenorphine based on naloxone-related findings in urine. Altogether, 247 autopsy urine samples from individuals who died in Finland in 2020 and tested positive for buprenorphine or norbuprenorphine at PM toxicology were reanalysed. The respective median concentrations and metabolite ratios were elaborated for the six analytes.

2 | MATERIALS AND METHODS

2.1 | Data collection

In Finland, a medico-legal autopsy ordered by the police and performed by a forensic pathologist is carried out to investigate all sudden and unexpected deaths. Approximately 16% of all deaths undergo a medico-legal investigation, and in approximately 75% of autopsy cases, a comprehensive PM toxicology investigation is conducted by request of the forensic pathologist (12% of all deaths). The Finnish Institute for Health and Welfare (THL) maintains the national PM toxicology database, in which all results of the toxicological analyses in medico-legal investigations and information from the respective death certificates are collected.

For the purpose of this study, we searched the PM toxicology database for individuals deceased in 2020 with a buprenorphine or norbuprenorphine finding in urine obtained by a previously described analytical method.[26] Subsequently, we reanalysed these urine samples using the recently published method for unconjugated BUP, NBUP, and NAL and their glucuronide conjugates BUPG, NBUPG, and NALG.[25] The total concentrations, BUPtot, NBUPtot, and NALtot, were calculated by arithmetic summation of BUP + BUPG, NBUP + NBUPG, and NAL + NALG concentrations, respectively. In addition

to the laboratory data, we utilized the forensic pathologist's referral, a brief description of the circumstances of death by the police, the main autopsy findings, and information extracted from the death certificate (time of death, age, gender, cause of death, manner of death) at our disposal. Out of the 266 buprenorphine or norbuprenorphine positive death cases in 2020, altogether 19 cases were excluded because of insufficient remaining urine for reanalysis. Reanalysis of samples was performed 4–12 months after the first analysis. Between the two analyses, the samples were stored at −20°C to ensure analyte stability, as recommended in the literature.[27,28]

2.2 | Analytical method

The LC-MS/MS method has been previously described.[25] The method involved nonpolar solid-phase extraction, separation on a C18 column, electrospray positive ionization, and mass analysis by triple quadrupole MS/MS, using multiple reaction monitoring. Quantification was based on the corresponding deuterium-labelled internal standards for each of the six analytes. The main validation results were as follows: Limit of quantification was 0.5 μg/L for BUP and NAL, 1 μg/L for NAL-G, and 3 μg/L for NBUP, BUP-G, and NBUP-G; intermediate precision (R.S.D.) was better than 20% for all analytes; expanded uncertainty *U95%* was 25% for BUP, 35% for NBUP, 24% for NAL, 23% for BUP-G, 25% for NBUP-G, and 30% for NAL-G.

2.3 | Statistical analysis

The Statistical Package for Social Sciences (SPSS version 26.0, IBM Corp., Armonk, NY) was used for all statistical analyses. The analyses were performed using the non-parametric Mann–Whitney *U* test. A *p* value of <0.05 was considered significant in all analyses.

2.4 | Ethical issues

The study was carried out on the basis of the research permit THL/1922/6.02.00/2017, issued by the Finnish Institute for Health and Welfare, Finland. According to the Finnish legislation, no separate ethical approval is needed for studies that utilize de-identified register-based data (Personal Data Act 523/1999).

3 | RESULTS

The study included 247 autopsy cases divided into two groups. In group 1, buprenorphine was implicated in fatal poisoning, that is, the forensic pathologist had recorded buprenorphine on the death certificate as the main cause or one of the main causes of the fatal poisoning ($N = 122$). In group 2, death was due to other causes, while buprenorphine or norbuprenorphine was a laboratory finding but not implicated in the cause of death ($N = 125$).

The proportion of men was 84% ($N = 102$) in group 1 and 75% ($N = 94$) in group 2. Median and average age was, respectively, 28.5 years and 31.6 years in group 1 and 48.0 years and 52.8 years in group 2. The manner of death in group 1 was accidental in 89% ($N = 109$), undetermined in 6.6% ($N = 8$), and suicide in 4.1% ($N = 5$) of cases. In group 2, the manner of death was disease in 52% ($N = 65$), accidental in 23% ($N = 29$), suicide in 13% ($N = 16$), homicide in 6.4% ($N = 8$), and undetermined in 5.6% ($N = 7$) of cases.

Table 1 compares the urinary concentrations of unconjugated BUP, NBUP, and NAL, their glucuronide conjugates BUPG, NBUPG, and NALG, and metabolite-to-parent drug concentration ratios between group 1, representing fatal buprenorphine poisonings, and group 2, representing other causes of death. For all six analytes, the median concentration was higher in group 1 than in group 2. For BUPG, the difference was significant ($p < 0.001$). Figure 1 illustrates the distribution of urinary concentration values using a box-and-whisker diagram. In four cases, including one case in group 1 and three cases in group 2, oxycodone was a concomitant finding. Because the use of an oxycodone–naloxone combination product could not be ruled out, these cases were excluded from all naloxone-related statistics, but they were included in buprenorphine-related statistics.

For metabolite-to-parent drug ratios, a significant difference was found between group 1 and group 2 in ratios NBUP/BUP ($p < 0.05$), NBUPG/BUPG ($p < 0.001$), and NBUPtot/BUPtot ($p < 0.001$).

NAL or NALG was found in 50 cases of which 28 belonged to group 1 and 22 belonged to group 2. No information was available on the possible use of naloxone for resuscitation among the naloxone-positive cases suggesting no such cases occurred. The naloxone-positive cases were divided into two concentration ranges using a cut-off value of NALtot = 100 μg/L. The high concentration range (NALtot >100 μg/L) comprised nine cases, eight (89%) of which belonged to group 1. The low concentration range (NALtot ≤100 μg/L) comprised 37 cases, 19 (51%) of which belonged to group 1. The numerical values of the parameters (median concentrations and ratios) listed in Table 1 were generally higher in the high concentration NALtot range than in the low concentration NALtot range.

Limited information was available concerning the circumstances of death in the studied cases. Among the 122 cases in group 1, use of buprenorphine before death was reported in 30% ($N = 37$). In 26 of these cases, the pharmaceutical product reported to have been used was Subutex®, in two cases Norspan®, and in one case Suboxone®. In eight cases, the product name was not mentioned. The route of administration was mentioned in 15 cases: In nine cases intravenous, in three cases intranasal, and in two cases transdermal. Among the 125 cases in group 2, buprenorphine was mentioned in two cases. In both cases, buprenorphine had reportedly been administered hours before death; in one case, the cause of death was registered as cardiomyopathy caused by long-lasting drug use and in the other as an acute severe asthma attack. The specific product name or route of administration was not mentioned in either case.

The following is a case example from group 1: A man aged 22 years was found dead at home in his bed. According to the police

TABLE 1 Concentrations of buprenorphine (BUP), norbuprenorphine (NBUP), naloxone (NAL), buprenorphine–glucuronide (BUPG), norbuprenorphine–glucuronide (NBUPG), and naloxone–glucuronide (NALG) and metabolite ratios are compared between group 1, representing fatal buprenorphine poisonings, and group 2, representing other causes of death

	Group 1			Group 2		
	N	Concentration IQR (μg/L)	Concentration median (μg/L)	*N*	Concentration IQR (μg/L)	Concentration median (μg/L)
Analyte						
BUP	111	1.7–6.3	3.38	70	1.2–6.2	2.89
NBUP	69	6.5–24	12.7	64	5.7–25	8.38
NAL	19	2.0–6.1	5.40	11	1.0–5.9	1.49
*BUPG**	*117*	*23–102*	*50.7*	*90*	*7.9–55*	*20.0*
NBUPG	91	12–93	35.0	102	8.1–65	18.7
NALG	26	5.9–115	51.5	19	4.4–41	22.9
BUPtot	119	24–106	55.9	91	9.4–59	21.2
NBUPtot	91	15–116	44.8	102	8.3–76	23.3
NALtot	27	4.1–122	49.0	19	4.4–48	23.7
Metabolite ratio						
*NBUP/BUP**	*66*	*1.3–6.4*	*2.40*	*55*	*2.2–6.9*	*3.90*
BUPG/BUP	109	9.3–26	16.9	69	6.8–23	10.8
NBUPG/NBUP	69	3.7–5.9	4.45	64	2.8–5.7	4.53
NALG/NAL	18	11–46	21.4	11	8.5–31	14.9
*NBUPG/BUPG**	*89*	*0.2–1.6*	*0.62*	*84*	*0.8–2.4*	*1.39*
*NBUPtot/BUPtot**	*90*	*0.2–1.7*	*0.73*	*85*	*0.9–2.7*	*1.53*
NALG/BUPG	25	0.2–1.8	1.18	18	0.2–1.7	0.82
NALG/NBUPG	20	0.2–1.7	0.62	17	0.1–1.3	0.26
NALtot/BUPtot	26	0.1–1.8	0.97	18	0.2–1.6	0.82
NALtot/NBUPtot	21	0.1–1.3	0.58	17	0.1–1.2	0.22
NALtot/ (BUPtot + NB UPtot)	27	0.1–1.2	0.51	19	0.1–0.8	0.29

Note: Asterisk denotes significant difference between group 1 and group 2.

report, on the previous evening the man had consumed high amounts of buprenorphine, benzodiazepines, and cannabis, after which he had gone to sleep at 10 pm. The next day at 1:30 pm his girlfriend realized he had died and called an ambulance. PM toxicology findings in femoral venous blood included buprenorphine (2.1 μg/L), norbuprenorphine (2.0 μg/L), gabapentin (80 mg/L), 7-aminoclonazepam (0.19 mg/L), alprazolam (0.024 mg/L), amphetamine (0.06 mg/L), and carboxy-THC (8.0 μg/L). Accidental poisoning due to buprenorphine, gabapentin, clonazepam, and alprazolam was determined as the cause of death. Urinary concentrations of buprenorphine, norbuprenorphine, naloxone, and their glucuronides as analysed by the LC-MS/MS method were as follows: BUP 6.8 μg/L, NBUP 15.7 μg/L, NAL 6.4 μg/L, BUPG 95.7 μg/L, NBUPG 59 μg/L, and NALG 129 μg/L. Comparison of the parameters of statistical significance with the median values of Table 1 suggested buprenorphine poisoning as can be seen in the following: BUPG 95.7 μg/L (group 1 median 50.7 μg/L vs. group 2 median 20.0 μg/L), NBUP/BUP 2.3 (2.40 vs. 3.90), NBUPG/BUPG 0.62 (0.62 vs. 1.39), and NBUPtot/BUPtot 0.73 (0.73 vs. 1.53). Similarly, the naloxone-related parameters were closer to group 1 values indicative of buprenorphine poisoning, suggesting parenteral use of the buprenorphine–naloxone combination product.

A second case example belongs to group 2: A 52-year-old woman went to the bathroom for a shower and was later found dead. She had a drug abuse history but not any other diseases. PM toxicology findings in femoral venous blood included buprenorphine (1.8 μg/L), norbuprenorphine (2.8 μg/L), amphetamine (1.4 mg/L), and carboxy-THC (6.3 μg/L). Intracerebral haemorrhage was determined as the cause of death. Urinary concentrations of buprenorphine, norbuprenorphine, naloxone, and their glucuronides as analysed by the LC-MS/MS method were as follows: BUP 1.7 μg/L, NBUP 7.2 μg/L, NAL 0.9 μg/L, BUPG 18.7 μg/L, NBUPG 23.6 μg/L, and NALG 31.6 μg/L. Comparison of the parameters of statistical significance with the median values of Table 1 suggested that the case was not a buprenorphine poisoning as can be seen in the following: BUPG 18.7 μg/L (group 1 median 50.7 μg/L vs. group 2 median 20.0 μg/L), NBUP/BUP 4.2 (2.40 vs. 3.90), NBUPG/BUPG 1.3 (0.62 vs. 1.39), and NBUPtot/BUPtot 1.5 (0.73 vs. 1.53). The naloxone-related parameters were also closer to group 2 values, not suggesting buprenorphine poisoning.

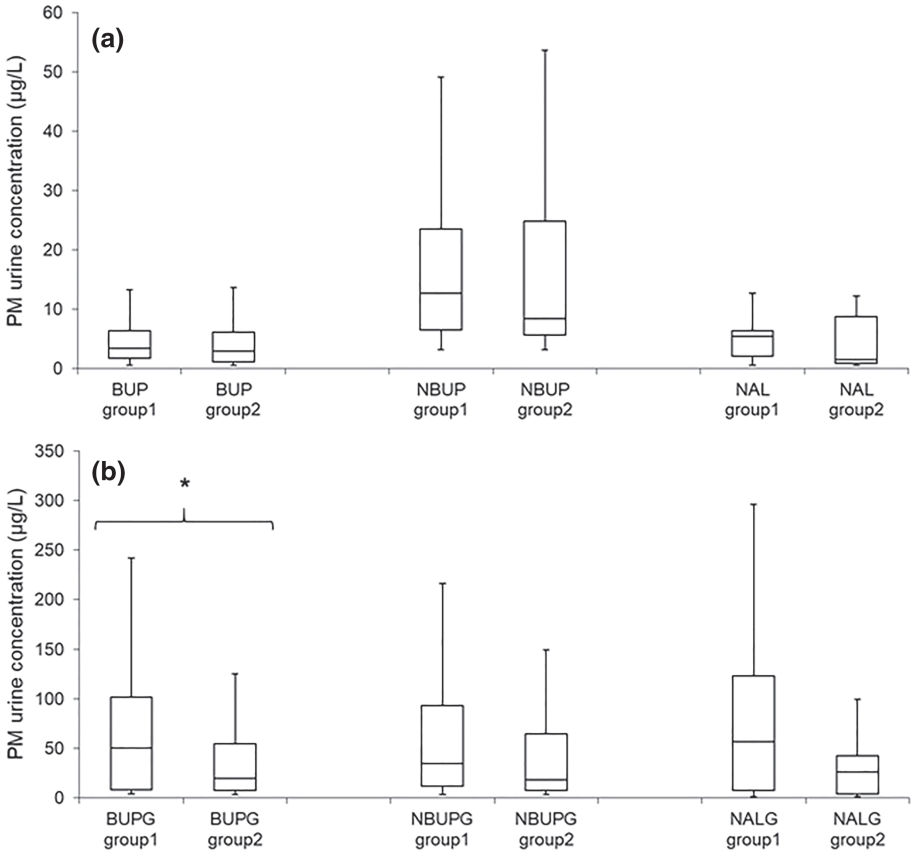

FIGURE 1 Comparison between group 1 and group 2 urine concentrations of (a) buprenorphine (BUP), norbuprenorphine (NBUP), and naloxone (NAL) and (b) buprenorphine–glucuronide (BUPG), norbuprenorphine–glucuronide (NBUPG), and naloxone–glucuronide (NALG). *Note*: Boxes represent interquartile ranges (IQRs) and bars inside boxes median values. Ends of whiskers are set at 1.5 * IQR above the third quartile and at minimum values. Outliers (extreme values) are removed for clarity. Asterisk denotes significant difference between group 1 and group 2.

4 | DISCUSSION

In this study, we identified new urinary analytical markers to aid PM diagnosis of fatal buprenorphine poisoning using the recently developed comprehensive analysis method for buprenorphine, naloxone, and metabolites. A significant difference between buprenorphine poisoning and other causes of death was found in BUPG concentration and in the concentration ratios NBUP/BUP and NBUPG/BUPG, in addition to the more established ratio NBUPtot/BUPtot. Naloxone-related concentrations and ratios were not significantly different between the groups.

As there are no previous buprenorphine-related studies with non-hydrolysed PM urine samples, comparison of our new results with previous literature is not possible. However, our median results for NBUPtot/BUPtot in buprenorphine poisonings and other causes of death (0.73 and 1.53, respectively) are consistent with the results of earlier PM studies.[24,29] Seldén et al.[24] reported median (range) norburenorphine-to-buprenorphine concentration ratios of 0.2 (0.1–5.6), 1.3 (0.1–13.8), 2.9 (0.3–9.8), and 3.2 (0.1–12.8) for fatal buprenorphine poisonings, possible buprenorphine poisonings, control cases, and unclear cases, respectively. It should be noted, however, that even though their metabolite ratios clearly indicate that low ratios are associated with fatal buprenorphine poisoning, the ranges are wide and overlap between the groups.

The urinary pharmacokinetics of buprenorphine, not to mention naloxone, in abuse situations are poorly understood. In the absence of real-life studies, an important reference for estimating the change of concentrations over time after one dose is the study by Kronstrand et al.,[30] involving 18 healthy volunteers who received a single dose of 0.4-mg buprenorphine sublingually. Urine samples were collected up to 96 h post-dose and analysed for buprenorphine and norbuprenorphine. The time when the norbuprenorphine/buprenorphine ratio exceeded one was estimated at 7 h, but the individual ratios showed higher variation with more distant collection times. This result is in line with the findings of our study and also previous reports showing that the above ratio in poisonings is often less than one, although it may be higher in delayed poisonings. Concerning naloxone, the urinary excretion has been noted to be rapid, with 24–37% of a radiolabelled naloxone dose appearing in urine during the first 6 h and little radioactivity measurable after 48 h.[31]

Diversion and abuse of OAT medication is an important issue to consider when evaluating the success of OAT. In our previous methodological study, we found the following median (maximum) concentrations in 72 urine samples collected from buprenorphine-dependent patients in different phases of OAT: BUP 4.2 µg/L (102 µg/L), NBUP 74.7 µg/L (580 µg/L), NAL 0.9 µg/L (85.5 µg/L), BUP-G 159.5 µg/L (1370 µg/L), NBUP-G 307.5 µg/L (1970 µg/L), and NAL-G 79.6 µg/L (2310 µg/L).[25] The median NBUPG/BUPG and NBUPtot/BUPtot concentration ratios were 1.8 and 2.5, respectively. These results are consistent with those of the relatively few other published studies on free and total drug concentrations in opioid-dependent patients in OAT. They regularly report NBUPG as predominant and NBUPG/BUPG and NBUPtot/BUPtot ratios in urine of well over one.[32,33]

Many clinical studies on buprenorphine and naloxone urine concentrations in opioid use disorder patients have focused on

distinguishing compliance with prescribed therapy from specimen adulteration intended to mimic compliance with prescribed buprenorphine.[34] Donroe et al.[35] examined the patterns of urine buprenorphine and norbuprenorphine levels in patients prescribed sublingual buprenorphine for opioid use disorder in an office-based addiction treatment clinic. In patients without concern for urine adulteration, they found a median norbuprenorphine/buprenorphine ratio (interquartile range) of 2.11 (1.28–3.73). Warrington et al.[36] reported a mean norbuprenorphine/buprenorphine ratio of 3.9 in cases with no obvious tampering. The median naloxone concentrations in their two practice sites were 290 and 207 μg/L, representing the whole population of 1223 patient samples studied. These types of studies do not contain detailed information on the timing between dose administration and sampling; however, they do show an enormous variation in naloxone urine concentrations in a clinical setting.

In a Finnish study from 2010–2011, Häkkinen et al.[29] divided PM cases into three groups, consisting of assumed parenteral buprenorphine–naloxone users (naloxone >100 μg/L), assumed parenteral mono-buprenorphine users (buprenorphine >50 μg/L, no naloxone), and assumed sublingual users (naloxone ≤100 μg/L; or buprenorphine ≤50 μg/L with no naloxone), reporting median norburenorphine/buprenorphine concentration ratios of 0.22, 0.26, and 1.26, respectively.

In our study, naloxone concentrations within the high concentration range (NALtot >100 μg/L) were found in nine cases (3.6%) in the whole study material, and eight cases (6.6%) were included in the group of fatal buprenorphine poisonings, as compared with the respective figures of 12% and 28% in Häkkinen et al.[29]

The proportion of buprenorphine–naloxone-related deaths from all buprenorphine-related deaths appears thus to have decreased in Finland from 2010–2014[20] to 2020. Parenteral use of buprenorphine–naloxone has also been evaluated using other methodologies. An investigation on drug residues in used syringes in Helsinki (Finland) revealed that the proportion of syringes containing naloxone changed from 11% in 2017 to 8% in 2018 and further to 5% in 2019, showing a decreasing trend.[16] A survey by Fältberg et al.[37] asking about participants' substance abuse at four time-points between 2008 and 2018 in South-Western Finland found that 66–81% of respondents had abused buprenorphine–naloxone during the last 6 months. They also noted that buprenorphine–naloxone abuse seems to be related to a more disadvantaged drug use profile than mono-buprenorphine abuse. Detection of the abuse of buprenorphine–naloxone combination product by the means of PM toxicology is therefore also a matter of societal importance.

Within the cause-of-death determination, buprenorphine may receive too little attention as a toxicological finding for several reasons. As buprenorphine is generally considered a safer medication than, for instance, methadone, medical examiners worldwide may not be aware that in certain circumstances this substance is as toxic as full opioid agonists. Looking only at drug levels in PM blood is misleading because close to zero concentrations for both BUPtot and NBUPtot are commonly found in buprenorphine poisonings.[23,24] As buprenorphine deaths are sometimes delayed, the causality between buprenorphine intake and death is not always understood, especially if other abused substances are detected in the body in apparently higher concentrations. For these reasons, the resulting national mortality figures may underestimate the rate of buprenorphine deaths in many countries.

5 | CONCLUSIONS

The contribution of buprenorphine to drug-related death is a diagnostic challenge. We applied for the first time an LC-MS/MS method developed for unconjugated and conjugated buprenorphine, norbuprenorphine, and naloxone to autopsy urine samples with the aim of improving cause-of death determination involving these drugs. The new markers, showing significant differences between poisoning and other causes of death, are likely to provide additional value to PM toxicology in the interpretation of complex cases of suspected buprenorphine poisoning.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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