ADDICTION
SSA SOCIETY FOR THE STUDY OF ADDICTION
RESEARCH REPORT
doi:10.1111/add.15211

Postmortem toxicological analyses of blood samples from 107 patients receiving opioid agonist treatment: substances detected and pooled opioid and benzodiazepine concentrations

Anne Berit Bech[1,2], Thomas Clausen[2], Helge Waal[2,3], Vigdis Vindenes[4,5], Hilde Erøy Edvardsen[4], Joachim Frost[6] & Ivar Skeie[1,2]

National Advisory Unit on Concurrent Substance Abuse and Mental Health Disorders, Innlandet Hospital Trust, Department of Mental Health, Brumunddal, Norway,[1] Norwegian Centre for Addiction Research (SERAF), Institute of Clinical Medicine, University of Oslo, Oslo, Norway,[2] National Advisory Unit on Substance Use Disorder Treatment, Oslo University Hospital, Oslo, Norway,[3] Department of Forensic Sciences, Oslo University Hospital, Oslo, Norway,[4] Institute of Clinical Medicine, University of Oslo, Oslo, Norway[5] and Department of Clinical Pharmacology, St Olav's Hospital Trondheim University Hospital, Trondheim, Norway[6]

ABSTRACT

Aims To present the substances and their concentrations detected postmortem in patients receiving opioid agonist treatment (OAT) stratified by cause of death, estimate the pooled opioid and benzodiazepine concentrations using established conversion factors for blood concentrations from the Norwegian Road Traffic Act and explore the association between drug-induced cause of death and the pooled opioid and benzodiazepine concentrations. **Design** Cross-sectional nation-wide study. **Setting** Norway. **Participants** One hundred and seven patients who died during OAT (i.e. within 5 days after the last intake of OAT medication) between 1 January 2014 and 31 December 2015, with postmortem femoral blood available for toxicology. Data were collected from hospital records, the Norwegian Cause of Death Registry and autopsy reports. **Measurements** Presence of alcohol and non-alcohol substances in the bloodstream postmortem, determined through records of toxicology of postmortem femoral blood. **Findings** A median of four substances was detected across the causes of death. At least one benzodiazepine was detected in 81 (76%) patients. The median pooled opioid concentration was significantly higher in drug-induced deaths compared with other causes of death (362 versus 182 ng/ml, $P < 0.001$), in contrast to the pooled benzodiazepine concentration (5466 versus 5701 ng/ml, $P = 0.353$). The multivariate regression analysis showed that only increasing pooled opioid concentration (ng/ml) was associated with increased odds of a drug-induced cause of death (odds ratio = 1.003; 95% confidence interval = 1.001–1.006). **Conclusions** In Norway, overall opioid concentration seems to play an important role in drug-induced deaths during opioid agonist treatment in patients prescribed methadone or buprenorphine. Patients prescribed buprenorphine tend to replace their agonist with full agonists, while patients prescribed methadone tend to have high opioid concentrations from methadone as the only opioid.

Keywords Autopsy, benzodiazepine, buprenorphine, drug-induced, forensic, methadone, opioid agonist treatment, overdose, polydrug, toxicology.

Correspondence to: Anne Berit Bech, National Advisory Unit on Concurrent Substance Abuse and Mental Health Disorders, Department of Mental Health, Innlandet Hospital Trust, P. O. Box 104, 2381 Brumunddal, Norway. E-mail: anne.berit.bech@sykehuset-innlandet.no
Submitted 26 January 2020; initial review completed 3 June 2020; final version accepted 26 July 2020

INTRODUCTION

Although opioid agonist treatment (OAT) for opioid use disorder (OUD) substantially reduces the risk of overdose [1–4], drug-induced deaths still occur among patients receiving OAT [5–7]. Methadone and buprenorphine are associated with drug-induced deaths in several countries [8–10], but the role of the OAT medications and their interaction with other substances in drug-induced deaths within OAT is little explored. Most overdoses involve multiple substances, and a median of three to four substances has been detected postmortem in patients receiving OAT [11,12]. Benzodiazepines in combination with opioids increase the risk of respiratory depression and non-fatal and

fatal overdoses [13,14]. Thus, concurrent use of benzodiazepines during OAT, whether prescribed or not, is a matter of considerable concern.

In the Norwegian Road Traffic Act, legal limits for non-alcohol drugs in blood were implemented in 2012 to evaluate driving under the influence of drugs and ensure equal jurisdiction [15]. Concentration limits corresponding to impairment comparable to blood alcohol concentrations were defined and conversion factors for concentrations of opioids and benzodiazepines were established [15,16]. Using the conversion factors from the Norwegian Road Traffic Act to estimate the pooled concentrations found in postmortem blood provides more information than only presenting the number of drugs detected. In a study from 2017, Edvardsen *et al.* [17,18] used these conversion factors to estimate and compare the pooled opioid and benzodiazepine concentrations in cases of fatal intoxication and driving under the influence of drugs. This method may expand our understanding of the total loads of opioids and benzodiazepines in fatal overdoses among patients receiving OAT. Thus, we aimed to:

1 Present substances and concentrations detected in postmortem blood from patients receiving OAT as stratified by cause of death (i.e. drug-induced cause of death compared with other causes of death during OAT).
2 Estimate pooled concentrations of opioids and benzodiazepines as stratified by cause of death using the established conversion factors from the Norwegian Road Traffic Act.
3 Explore whether pooled opioid and benzodiazepine concentrations differ in drug-induced and other causes of death.

METHODS

Study design and setting

This was a cross-sectional nation-wide study using data from hospital records, the Norwegian Cause of Death Registry and forensic and medical autopsies. In Norway, with 5.3 million inhabitants nation-wide, the national OAT programme is organized within the public specialist health-care service. At the end of 2015, 7498 patients received OAT with either buprenorphine (36%) or buprenorphine–naloxone (22%) sublingual tablets, methadone (39%, mainly syrup) and other opioids (3%) [19].

Participants

Between 1 January 2014 and 31 December 2015, 200 patients in total died during OAT in Norway (defined as within 5 days after the last reported intake of OAT medication). As reported previously [6], 90 (45%) of the 200 died of a somatic disease, 84 (42%) of a drug-induced cause of death and 23 (12%) of a violent cause of death. A forensic or medical autopsy was requested and performed in 125 (63%) of the 200 cases [6]. In the present study we included data from 107 of these patients, who were subjected to an autopsy and where femoral blood was collected for toxicological analyses. We excluded 18 autopsy reports; i.e. six medical autopsy reports where toxicological analyses were not performed, one case where the samples were unsuitable for toxicological analyses, six where toxicological analyses were performed on muscle tissue only and five cases where either the toxicology results or the whole autopsy report were missing.

The hospital trusts responsible for OAT provided information regarding age, sex and treatment (e.g. OAT status, duration of OAT, medications and coprescribing), while information regarding fatality and toxicology was obtained from the autopsy reports. The 107 patients were categorized into two groups based on the cause of death obtained from the Norwegian Cause of Death Registry. Group 1 consisted of 66 patients with drug-induced cause of death. Norway has implemented the International Classification of Diseases, 10th revision (ICD-10) coding for drug-induced deaths used by the European Monitoring Centre for Drugs and Drug Addiction [20–22]. Thus, the 66 drug-induced deaths included unintentional overdose or overdose by unknown intent ($n = 57$), intentional overdose ($n = 4$) and substance use disorder ($n = 5$). Results from both drug-induced and other causes of death were included to explore the differences between non-fatal and fatal concentrations in patients receiving OAT. Therefore, group 2 included 41 patients who died of other causes of death: i.e. 23 patients who died of a somatic disease, 17 who died of a violent cause of death [accident, homicide or suicide (except intentional overdose)] and one patient with a psychiatric diagnosis (F29) as an underlying cause of death.

Procedures

Only two laboratories in Norway perform toxicological analyses in postmortem cases: the Department of Forensic Sciences at Oslo University Hospital and the Department of Clinical Pharmacology at St Olav's Hospital Trondheim University Hospital. Details regarding the analytical procedures are described elsewhere [17].

The principle of equipotent doses, where the relative potencies of different opioids and benzodiazepines are considered, is widely acknowledged [15]. Comparable to this, we have used separate conversion factors for blood concentrations that were already implemented in the Norwegian Road Traffic Act to estimate pooled diazepam- and morphine-equivalent concentrations of opioids and benzodiazepines detected postmortem in patients receiving OAT. The principle of conversion factors for blood concentrations of alcohol and benzodiazepines assumes a linear concentration–effect relationship [15]. For opioids,

this relationship has been little investigated, but two studies [23,24] also suggested a linear concentration–effect relationship for opioids [15]. Due to the partial antagonist effect of buprenorphine and lack of evidence regarding the impairing effects of tramadol on driving, the conversion factors for concentrations of buprenorphine and tramadol are not included in the conversion table used in the Norwegian Road Traffic Act [15,16]. We consider the inclusion of buprenorphine and tramadol when investigating drug-induced deaths to be important, and we have assumed that the conversion factors for their blood concentrations are similar to the conversion factors for equipotent doses of buprenorphine and tramadol [25]. The conversion factors used in the present study are provided in the Supporting information, Appendix S1.

Substances

The following substances were detected in the present study. The detected opioids were heroin/morphine, methadone, buprenorphine, tramadol and codeine. Heroin is rapidly metabolized to 6-acetylmorphine (6-AM) in blood and further to morphine. The presence of 6-AM in blood or urine distinguishes heroin use from that of morphine [26]. If only morphine is detected, it is impossible to determine if this is a result of heroin or morphine intake. Codeine is a prodrug metabolized to the psychoactive metabolite morphine. Codeine was regarded as a trace amount/pollutant when concomitant 6-AM was detected, and was categorized as 'other medications/substances' if a concomitant morphine concentration was < 10% of the codeine concentrations or when no concomitant morphine was detected in combination with codeine.

The detected benzodiazepines were clonazepam, measured as the metabolite 7-aminoclonazepam (7-AK), diazepam and/or desmethyldiazepam (active diazepam metabolite), alprazolam, oxazepam and nitrazepam. Because of their effect similar to benzodiazepines, the Z-hypnotics zopiclone and zolpidem were added. Pregabalin was presented separately. Methamphetamine is partly metabolized to amphetamine *in vivo*; thus, concentrations of methamphetamine and amphetamine were summed and categorized as stimulants. Detection of tetrahydrocannabinol in blood was regarded as positive for tetrahydrocannabinol. Ethanol was only included if concomitant findings of its metabolites ethyl glucuronide and ethyl sulphate were present in blood or urine to exclude ethanol formed postmortem.

The detected psychotrophic medications (antipsychotics/antidepressants) were at least one of the following: quetiapine, flupentixol, risperidone, levomepromazine, olanzapine, chlorprothixene, aripiprazole, trimipramine, citalopram, mirtazapine, mianserin, sertraline, amitriptyline and fluoxetine. Other detected medications/substances were paracetamol, codeine, promethazine, dexchlorpheniramine, lamotrigine, hydroxyzine, gabapentin, valproic acid, levetiracetam, alimemazine, metoprolol, carbamazepine, 10-OH carbazepin, salicylic acid, phenytoin, *gamma*-hydroxybutyric acid and 4-fluoroamphetamine (a new psychoactive substance).

Statistical analysis

Data are presented as means, standard deviation (SD), frequencies and proportions. We used a Student's *t*-test to compare continuous data and a χ^2 or Fisher's exact test for categorical data. The concentrations of substances were not normally distributed, and were therefore presented with median, minimum and maximum values. A Mann–Whitney *U*-test was used for comparisons. Bivariate and multiple regression models were estimated to assess the association between drug-induced cause of death and pooled opioid and benzodiazepine concentrations, with other causes of death as a reference category. The results were presented as odds ratios (ORs) and 95% confidence intervals (CIs). Only cases with no missing covariate values were included in the multiple model. Because of the wide concentration range, the covariate pooled benzodiazepine concentration was re-scaled in the regression analyses (divided by 1000). All analyses were two-sided and significance was set at $P < 0.05$. The analyses were not pre-registered; therefore, the results should be considered exploratory. Results were presented in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (Appendix S3). Data were analysed using SPSS version 25 (IBM Corporation, Armonk, NY, USA).

Ethics

The study was approved by the Regional Committee for Medical and Health Research Ethics South-East (case number 2016/1204), the Cause of Death Registry, The Director of Public Prosecution, the Ministry of Justice and Public Security and the participating hospital trusts.

RESULTS

Characteristics

The mean age at the time of death was 47.4 years (SD = 8.8) for the whole group, and 79 (74%) were men (Table 1). The total duration of OAT was almost 8 years (SD = 4.3). All but two patients were prescribed methadone or buprenorphine and 71 (68%) had doses within the recommended range [19]. There were more patients prescribed methadone in those who died of a drug-induced cause of death compared with other causes of death (58

Table 1 Sample characteristics and circumstances at the time of death of 107 patients receiving opioid agonist treatment, stratified by cause of death.

	All-cause deaths	*Drug-induced deaths*	*Other causes of death*	*Missing data*
	N = 107	*n = 66*	*n = 41*	
Age and sex				
Age, mean ± SD	47.4 ± 8.8	46.9 ± 9.1	48.2 ± 8.3	0
Male, *n* (%)	79 (74)	47 (71)	32 (78)	0
OAT treatment, n (%)				
Duration of OAT in years, mean ± SD	7.7 ± 4.3	8.0 ± 4.3	7.3 ± 4.3	4
Prescribed methadone[a]	53 (50)	38 (58)*	15 (37)*	0
Prescribed buprenorphine[a]	52 (49)	26 (39)*	26 (63)*	0
Prescribed other/unknown OAT medication	2 (2)	2 (3)	0 (0)	0
Dose within recommended range[b]	71 (68)	41 (64)	30 (73)	2
Dose above recommended range[b]	13 (12)	8 (13)	5 (12)	2
Dose below recommended range[b]	21 (20)	15 (23)	6 (15)	2
Supervised intake 1–2 times a week[c]	9 (11)	4 (8)	5 (14)	22
Supervised intake 3–7 times a week[c]	74 (89)	44 (92)	30 (86)	22
Benzodiazepines/Z-hypnotics prescribed[d]	37 (40)	23 (42)	14 (38)	15
Circumstances				
OAT status described in autopsy reports	69 (65)	44 (67)	25 (61)	6
Found 0–48 hours after time of death	94 (88)	57 (86)	37 (90)	0
Median (min–max) days from death to autopsy	3 (0–14)	3 (1–14)	3 (0–11)	2
Signs of drug use[e]	64 (60)	49 (74)**	15 (37)**	4
Fresh needle marks	29 (27)	20 (30)	9 (22)	6
Median (min–max) number of substances	4 (1–11)	4 (1–11)	4 (1–8)	0
Single substance detected	7 (7)	4 (6)	3 (7)	0

*$P < 0.05$; **$P < 0.001$. [a]Median dose (min-max) prescribed at the time of death: methadone 90 mg (15–200 mg), buprenorphine 16 mg (1–28 mg). [b]Recommended dosing range methadone 80–120 mg, buprenorphine 12–24 mg [19]. [c]Supervised intake of OAT medication in the year before death. In addition to the 22 with missing data, two patients did not have supervised intake of OAT medication. [d]Benzodiazepines/Z-hypnotics prescribed at least once in the year before death according to hospital records. [e]Information in the autopsy report about substance use, drugs or drug paraphernalia detected on or close to the body, or fresh needle marks not related to medical treatment. SD = standard deviation.

versus 37%, $P = 0.025$). According to information from the hospitals, 37 (40%) patients were prescribed at least one benzodiazepine/Z-hypnotic in the year before death (mainly oxazepam, Z-hypnotics and/or diazepam).

In the autopsy reports, the pathologist had described OAT status in two-thirds (65%) of the reports. In two reports, the pathologist stated that the OAT status was unknown, and in the remainder the OAT status was not stated. Signs of drug use (fresh needle marks and/or drugs or drug paraphernalia) were described significantly more often in those who died of a drug-induced cause of death compared with other causes of death (74 versus 37%, $P < 0.001$). A median of four substances was detected postmortem.

Substances and concentrations

Methadone was detected in 60 (56%) patients, and among these, 53 had had methadone prescribed (Table 2). In contrast, buprenorphine was not detected in 12 of 52 patients (23%) prescribed buprenorphine. Methadone was detected in five patients where only buprenorphine prescription was documented in their hospital records. Morphine was detected in 30 (28%) patients and the heroin metabolite 6-AM was also found in 19 of them. In the Cause of Death Registry, an opioid was registered as the main intoxicant in 58 of the 66 drug-induced deaths; i.e. methadone in 30 cases, heroin or morphine in 21 and buprenorphine in seven cases.

The two most common benzodiazepines were clonazepam and alprazolam, which were detected in 58 (54%) and 25 (23%) of the patients, respectively. Pregabalin was detected in 19 (18%) patients, stimulants in 31 (29%) patients and tetrahydrocannabinol in 40 (37%) patients. In addition to the substances presented in Table 2, 34 (32%) patients had at least one antipsychotic and/or antidepressant medication detected, while at least one other medication/substance, as previously listed, was detected in 32 (30%) patients.

The median concentration of buprenorphine was lower in drug-induced deaths compared with other causes of death, in contrast to the concentrations of methadone, morphine and tramadol. The median concentrations of the specific benzodiazepines and other substances did not show any consistent pattern in drug-induced compared with other causes of death. There were no significant

Table 2 Substances detected post-mortem in 107 patients receiving opioid agonist treatment, with median (min–max) concentrations (ng/ml), stratified by cause of death.

	All-cause deaths, N = 107		*Drug-induced death, n = 66*		*Other causes of death, n = 41*		*Mann–Whitney U test*
	n (%)	*Median (min–max)*	*n (%)*	*Median (min–max)*	*n (%)*	*Median (min–max)*	*P*
Opioids							
Methadone	60 (56)	881.9 (22.28–4023)	44 (67)	897.4 (24.45–4023)	16 (39)	634.4 (22.28–1764)	0.273
Buprenorphine	40 (37)	5.38 (0.89–93.53)	18 (27)	3.79 (0.89–93.53)	22 (54)	6.08 (1.40–39.28)	0.366
Morphine[a]	30 (28)	228.3 (10.56–3425)	25 (38)	371.0 (11.42–3425)	5 (12)	94.18 (10.56–196.9)	0.136
Tramadol	3 (3)	421.4 (368.7–1580)	2 (3)	1001 (421.4–1580)	1 (2)	368.7 (368.7–368.7)	0.667
Benzodiazepines							
7-AK[b]	58 (54)	185.7 (8.57–3715)	39 (59)	142.9 (8.57–3715)	19 (46)	200.0 (28.57–1143)	0.506
Alprazolam	25 (23)	43.23 (3.71–176.0)	19 (29)	43.23 (3.71–176.0)	6 (15)	17.60 (3.71–61.75)	0.221
Diazepam	10 (9)	205.0 (74.02–939.5)	5 (8)	111.0 (74.02–304.6)	5 (12)	210.7 (187.9–939.5)	0.222
Desmethyldiazepam	18 (17)	188.4 (64.97–758.0)	10 (15)	215.2 (64.97–758.0)	8 (20)	188.1 (108.3–649.7)	0.897
Oxazepam	12 (11)	246.6 (174.9–630.8)	10 (15)	233.7 (174.9–630.8)	2 (5)	329.7 (258.0–401.4)	0.485
Nitrazepam	1 (1)	5.91 (5.91–5.91)	0 (0)	0	1 (2)	5.91 (5.91–5.91)	NA
Zopiclone	7 (7)	31.88 (5.83–222.8)	3 (5)	38.49 (8.94–222.8)	4 (10)	21.58 (5.83–36.16)	0.400
Zolpidem	1 (1)	768.5 (768.5–768.5)	1 (2)	768.5 (768.5–768.5)	0 (0)	0	NA
Other substances							
Pregabalin	19 (18)	5414 (1274–16082)	15 (23)	5573 (1274–16082)	4 (10)	4458 (3185–8280)	0.469
Stimulants	31 (29)	431.0 (29.74–5814)	19 (29)	365.0 (43.27–5814)	12 (29)	498.5 (29.74–4910)	0.389
THC[c]	40 (37)	4.56 (1.04–150.9)	24 (36)	3.77 (1.04–150.9)	16 (39)	5.19 (1.1–16.98)	1.0
Ethanol[d], ‰	9 (8)	1.30 (0.30–3.90)	5 (8)	1.30 (0.60–3.90)	4 (10)	1.25 (0.30–2.10)	0.556

NA = not applicable. To convert concentrations from SI units: µmol/l × molecular weight = ng/ml. Example: methadone 1.3 µmol/l × 309.4 g/mol [molecular weight (Mw) methadone] = 402.2 ng/ml. [a]Nineteen patients also had 6-AM detected in blood or urine (heroin metabolite); [b]7-aminoclonazepam (clonazepam metabolite); [c]THC = tetrahydrocannabinol, $n = 104$; [d]ethanol, $n = 106$.

differences in the median concentrations of each of the various substances according to cause of death.

Pooled concentrations

The median pooled opioid concentration was significantly higher in drug-induced deaths compared with other causes of death (362 versus 182 ng/ml, $P < 0.001$; Table 3). At least one benzodiazepine was detected in 81 (76%) of the cases, but the median pooled benzodiazepine concentrations did not differ significantly according to cause of death (5466 versus 5701 ng/ml, $P = 0.353$).

Factors associated with drug-induced death

Table 4 shows the results from a regression analysis assessing covariates associated with drug-induced cause of death compared with other causes of death during OAT. In bivariate analyses, both taking methadone as OAT medication (compared with taking buprenorphine) and increasing pooled opioid concentration were associated with higher odds of dying of a drug-induced cause of death. However, only pooled opioid concentration remained significant in the multiple-model estimation (OR = 1.003, CI = 1.001–1.006). The covariates of age, sex and pooled benzodiazepine concentration were not significant in neither bivariate nor multiple analyses.

The pooled opioid concentration was significantly higher in drug-induced cause of death compared with other causes of death in both patients prescribed buprenorphine and methadone. Figure 1 presents the pooled concentrations of the various opioids in drug-induced deaths. As illustrated, 23 (36%) of 64 patients had used more than one opioid. In patients prescribed buprenorphine, other opioids contributed substantially to the pooled opioid concentration, while patients prescribed methadone tended to have high concentrations of methadone as the only opioid (i.e. above therapeutic ranges for methadone).

DISCUSSION

In the present study, a median of four substances was detected in postmortem blood from patients receiving OAT. At least one benzodiazepine was detected in 76% of the patients. The median pooled opioid concentration was significantly higher in drug-induced cause of death compared with other causes of death, in contrast to the median pooled benzodiazepine concentration. In the multiple regression model, only increasing pooled opioid concentration was associated with increased odds of a drug-induced cause of death.

A median pooled opioid concentration of 362 ng/ml in drug-induced deaths was higher than the median of 211 ng/ml in all overdose autopsy cases and 225 ng/ml

Table 3 Pooled median (min–max) morphine- and diazepam equivalent concentrations of opioids and benzodiazepines (ng/ml) in 107 patients receiving opioid agonist treatment, stratified by cause of death.

	Total, N = 107		*Drug-induced death, n = 66*		*Other causes of death, n = 41*		*Mann–Whitney U test*
	n (%)	*Median (min–max)*	*n (%)*	*Median (min–max)*	*n (%)*	*Median (min–max)*	*P*
Pooled opioids[a]	104 (97)	314.7 (10.56–3489)	64 (97)	362.1 (20.34–3489)	40 (98)	181.9 (10.56–899.0)	< 0.001
Pooled benzodiazepines[b]	81 (76)	5466 (28.61–177652)	51 (77)	5466 (49.82–177652)	30 (73)	5701 (28.61–54794)	0.353

To convert concentrations from SI units: µmol/l × conversion factor × molecular weight for morphine or diazepam = morphine- or diazepam equivalent concentration in ng/ml. Example conversion methadone to morphine-equivalent concentration: methadone 1.3 µmol/l × 0.375 (conversion factor methadone) × 285.4 g/mol (Mw morphine) = 139.1 ng/ml. 7-aminoclonazepam, alprazolam, oxazepam, nitrazepam, zopiclone and zolpidem. All opioids and benzodiazepines as morphine- or diazepam-equivalents are presented in Supporting information, Appendix S2. [a]Pooled concentrations of opioids from Table 2: morphine and morphine-equivalent concentrations of methadone, buprenorphine and tramadol; [b]pooled concentrations of benzodiazepines from Table 2: diazepam and diazepam-equivalent concentrations of desmethyldiazepam.

Table 4 Factors associated with drug-induced cause of death versus other causes of death (reference) during opioid agonist treatment.

	Bivariate models	*Multiple model*
	OR (95% CI)	*OR (95% CI)*
Age	0.983 (0.940–1.028)	0.998 (0.942–1.057)
Gender		
Men	1	1
Women	1.437 (0.578–3.576)	1.902 (0.596–6.072)
OAT medication		
Buprenorphine	1	1
Methadone	2.533 (1.129–5.683)*	1.276 (0.436–3.733)
Pooled opioid concentration in ng/ml	1.003 (1.001–1.006)*	1.003 (1.001–1.006)*
Pooled benzodiazepine concentration[a] in ng/ml	1.009 (0.985–1.033)	1.007 (0.983–1.031)

*$P < 0.05$. Only complete cases are included in the multiple model, $n = 76$. [a]The covariate pooled benzodiazepine concentration is rescaled (divided by 1000). OAT = opioid agonist treatment; OR = odds ratio; CI confidence interval.

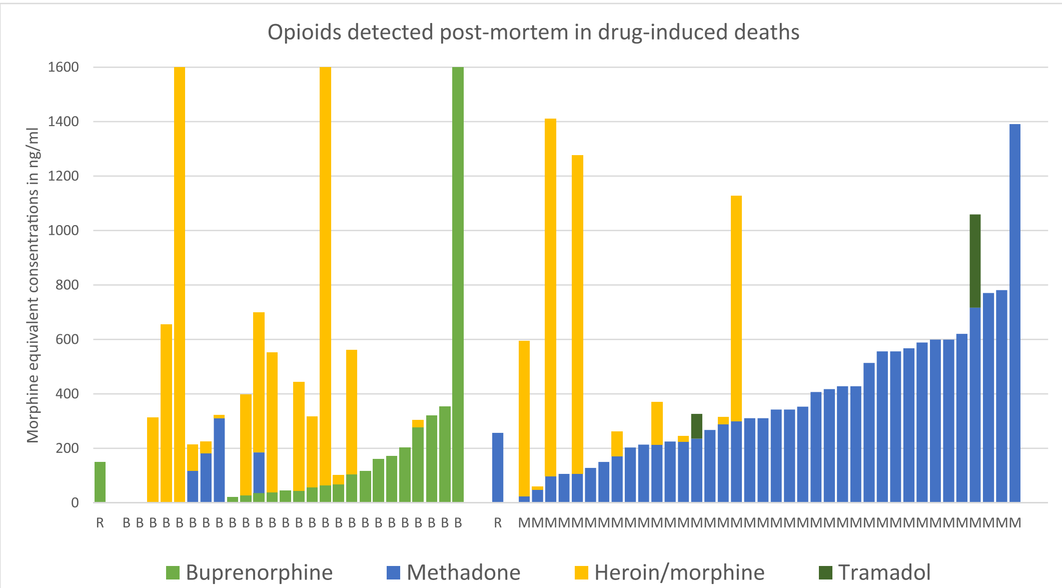

Figure 1 Pooled morphine-equivalent concentrations (ng/ml) of opioids in 64 drug-induced deaths during opioid agonist treatment. B = 26 patients prescribed buprenorphine, M = 38 patients prescribed methadone. Concentrations above 1600 ng/ml are 2140, 2483 and 3425 ng/ml. R = reference concentrations from patients in our sample who died of other causes than drug-induced death, and with the prescribed OAT medication as the only opioid detected post-mortem: 18 patients prescribed buprenorphine (morphine-equivalent concentration 150 ng/ml) and 14 patients prescribed methadone (morphine-equivalent concentration 257 ng/ml) [Colour figure can be viewed at wileyonlinelibrary.com]

in the heroin/morphine-positive autopsy cases reported by Edvardsen *et al*. [17,18]. In their study, however, the pooled opioid concentrations might have been underestimated because buprenorphine and tramadol were not included. The pooled median benzodiazepine concentrations were higher in both groups in the present study (5466 and 5701 ng/ml) compared with 1765 ng/ml in all overdose cases and 2078 ng/ml in heroin/morphine-positive autopsy cases in the same study by Edvardsen *et al*. [17,18]. The median concentrations of most substances in Table 2 in our study were higher than the corresponding findings in postmortem femoral blood from all-cause deaths in a study reported by Ketola & Ojanpera [27]. The higher median concentrations, as well as the wide concentration ranges of benzodiazepines and opioids in both groups in the present study, are probably due to variable development of tolerance. Regular intake of benzodiazepines in doses exceeding the normal therapeutic range have been reported in OAT populations [28]. Previous studies have also reported higher median/mean methadone concentrations in autopsy cases in patients receiving OAT compared with individuals not in treatment at the time of death [11,29,30], indicating, as expected, an increased opioid tolerance among patients receiving OAT.

Explanations for the high postmortem opioid concentrations in drug-induced deaths include taking extra or

'topping up' with heroin [31]. High prevalence of organ pathology (e.g. liver and kidney disease) [6,32,33] may impair metabolism and excretion and hence lead to higher blood concentrations of methadone, while lower concentrations of buprenorphine and methadone have been detected in delayed deaths compared with immediate poisonings [30,34]. Another risk factor is injecting of OAT medication instead of taking it sublingually or orally [29,35–37]. In an Italian study [38], 28% reported injecting their own OAT medication, with no differences between the different OAT medications.

Buprenorphine is considered to have a better pharmacological safety profile than methadone, and is therefore often recommended as the preferred OAT medication [39]. However, the risk of fatal overdose is increased if buprenorphine is injected or combined with benzodiazepines or alcohol [13,28,34,35,40] and, as our study suggests, if buprenorphine is replaced by other opioids. Buprenorphine is a partial agonist with antagonist properties; thus, it is likely that patients may stop taking or reduce the dose to enhance the effect from opioid agonists such as heroin [41]. When choosing between methadone and buprenorphine, it is important to consider the medications' stabilizing effect and their ability to prevent or minimize inappropriate use of the medication and other psychoactive drugs.

As expected, the pooled opioid concentration (the total opioid load) seemed to play the most important role, in line with the hierarchy of the most dangerous drug in multiple drug deaths in the ICD-10 [22]. Even though the pooled benzodiazepine concentration was comparable in drug-induced and other causes of death, we cannot draw the conclusion that benzodiazepines were not involved in these deaths. The concentration ranges were wide and the mechanisms for additive effects upon respiratory depression when opioids and benzodiazepines are combined are poorly understood [13]. Additionally, other factors not included in the present study may increase the risk of a drug-induced cause of death, such as comorbidities and the combination of opioids and substances other than benzodiazepines with central nervous system depressant effect (e.g. pregabalin).

The number of cases where an opioid, including the patient's prescribed OAT medication, was considered the main intoxicant is a cause for concern. Nevertheless, systematic reviews and meta-analyses have consistently shown higher mortality outside and after OAT [1–3], and it is imperative to keep patients with OUD in agonist treatment. The Norwegian OAT programme is low-threshold, and one-quarter of the patients had harm reduction as a treatment goal in 2015 [19]. For those who continue to use drugs during OAT, harm-reduction strategies such as information about safer use training and distribution of intranasal naloxone are essential [42,43], as well as treatment tailored to the patient's individual needs. Improved follow-up of somatic diseases and methadone dose adjustments are also important to prevent methadone toxicity as patients age.

Strengths and limitations

To our knowledge, this paper is the first to present postmortem pooled opioid and benzodiazepine blood concentrations in an OAT population, including concentrations in patients who died of causes other than overdose. We also present information concerning prescribed OAT medication. Thus, our findings broaden the understanding of the toxicology in drug-induced deaths among patients receiving OAT and complement the results from larger registry-based studies. Norway has high autopsy rates (90%), and most drug-induced deaths are based on toxicological confirmation [44]. Another strength is that the two laboratories use similar analytical methods and instruments; thus, a very low variation within the results would be expected.

The present study has some limitations. The cross-sectional design cannot address causation [45], and a higher number of participants would have allowed for more covariates in the regression analysis. postmortem re-distribution leads to site- and time-dependent changes in the measured concentrations of certain drugs [26,46,47]. Brockbals *et al.* [46] reported a median/mean +20% postmortem increase in methadone concentrations, ranging from −9 to +71%, and concluded that changes were regarded as irrelevant with respect to forensic toxicology interpretation. postmortem re-distribution will take place in both groups; thus, comparing the concentration levels will provide important information in these cases. To reduce site-dependent postmortem variation, we have included analytical results from femoral blood only. The number of days from estimated time of death to autopsy in the two groups did not differ (median = 3 days, $P = 0.517$). The Norwegian OAT population is among the oldest in Europe, and buprenorphine is the most prescribed OAT medication [19]. Thus, the results may not be fully generalizable to other countries or treatment settings. Finally, data from the Norwegian Prescription Database would have provided updated information about benzodiazepines prescribed by general practitioners, which hospital records may lack.

The conversion factors for blood concentrations are based on a limited number of studies investigating psychoactive effects among opioid-naive individuals [15]. In the present study, we have estimated and compared pooled concentrations in patients with tolerance to opioids. Tolerance is an important aspect, but the development of tolerance differs between opioids and benzodiazepines. Thus, further research is needed, and partial antagonists such

as buprenorphine should be included when this method is used to assess concentrations in drug-induced deaths. Nevertheless, the conversion factors in the present study, except those for buprenorphine and tramadol, are used to mete out legal sanctions in Norwegian driving under the influence of drugs cases.

CONCLUSIONS

The pooled opioid concentration seemed to play the most important role in drug-induced deaths during OAT in patients prescribed methadone or buprenorphine. Patients prescribed buprenorphine tended to replace their agonist with full agonists, while patients prescribed methadone tended to have high opioid concentrations from methadone as the only opioid. Deaths due to other causes had significantly lower pooled opioid concentration compared with drug-induced deaths, but comparable concentrations of pooled benzodiazepines. More research is required on the combined effect of opioids and benzodiazepines in drug-induced deaths within OAT.

Declaration of interests

None.

Acknowledgements

The study was funded by Innlandet Hospital Trust (grant no. 150351). The funder had no involvement in the design of the study or in the collection, analysis and interpretation of data or in the writing of the manuscript. We thank the Norwegian Board of Forensic Medicine for valuable contribution in finding and collecting forensic autopsy reports and Magne Thoresen, Oslo Centre for Biostatistics and Epidemiology, University of Oslo for statistical advice. We also thank all participating hospital trusts for their assistance with data collection: University Hospital of North Norway, Nordland Hospital Trust, Nord-Trøndelag Hospital Trust, St Olav's Hospital-Trondheim University Hospital, Møre og Romsdal Hospital Trust, Førde Hospital Trust, Bergen Hospital Trust, Stavanger Hospital Trust, Fonna Hospital Trust, Telemark Hospital Trust, Sørlandet Hospital Trust, Vestfold Hospital Trust, Vestre Viken Hospital Trust, Akershus University Hospital, Oslo University Hospital, Innlandet Hospital Trust, Østfold Hospital Trust and Lovisenberg Diaconal Hospital.

Author contributions

Anne Berit Bech: Conceptualisation; project administration; formal analyses; investigation, writing - original draft; visualisation. **Thomas Clausen:** Conceptualisation; writing - review and editing; supervision. **Helge Waal:** Writing - review and editing. **Vigdis Vindenes:** Methodology; formal analyses; writing - review and editing. **Hilde Erøy Edvardsen:** Methodology; formal analyses; writing - review and editing. **Joachim Frost:** Methodology; writing - review and editing. **Ivar Skeie:** Conceptualisation; investigation; writing - review and editing; supervision; project administration; funding acquisition.

References

1. Sordo L., Barrio G., Bravo M. J., Indave B. I., Degenhardt L., Wiessing L., *et al.* Mortality risk during and after opioid substitution treatment: systematic review and meta-analysis of cohort studies. *BMJ* 2017; **357**: j1550.
2. Ma J., Bao Y. P., Wang R. J., Su M. F., Liu M. X., Li J. Q., *et al.* Effects of medication-assisted treatment on mortality among opioids users: a systematic review and meta-analysis. *Mol Psychiatry* 2019; **24**: 1868–83.
3. Degenhardt L., Bucello C., Mathers B., Briegleb C., Ali H., Hickman M., *et al.* Mortality among regular or dependent users of heroin and other opioids: a systematic review and meta-analysis of cohort studies. *Addiction* 2011; **106**: 32–51.
4. Bahji A., Cheng B., Gray S., Stuart H. Reduction in mortality risk with opioid agonist therapy: a systematic review and meta-analysis. *Acta Psychiatr Scand* 2019; **140**: 313–39.
5. Clausen T., Waal H., Thoresen M., Gossop M. Mortality among opiate users: opioid maintenance therapy, age and causes of death. *Addiction* 2009; **104**: 1356–62.
6. Bech A. B., Clausen T., Waal H., Šaltytė Benth J., Skeie I. Mortality and causes of death among patients with opioid use disorder receiving opioid agonist treatment: a national register study. *BMC Health Serv Res* 2019; **19**: 440.
7. Bukten A., Stavseth M. R., Clasuen T. From restrictive to more liberal: variations in mortality among patients in opioid maintenance treatment over a 12-year period. *BMC Health Serv Res* 2019; **19**: 553.
8. United Nations Office on Drugs and Crime *World Drug Report 2019*. New York, NY: United Nations; 2019. Available at: https://wdr.unodc.org/wdr2019/ (accessed 16 September 2019).
9. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). Drug-related Deaths and Mortality in Europe: Update From the EMCDDA Expert Network. Luxembourg: EMCDDA; 2019. Available at: http://www.emcdda.europa.eu/system/files/publications/11485/20193286_TD0319444ENN_PDF.pdf (accessed 7 August 2019).
10. European Monitoring Centre for Drugs and Drug Addiction. European Drug Report. Trends and Developments. Luxembourg: EMCDDA; 2019. Available at: http://www.emcdda.europa.eu/system/files/publications/11364/20191724_TDAT19001ENN_PDF.pdf (accessed 25 November 2019).
11. Bernard J.-P., Khiabani H. Z., Hilberg T., Karinen R., Slørdal L., Waal H., *et al.* Characteristics of methadone-related fatalities in Norway. *J Forensic Leg Med* 2015; **36**: 114–20.
12. Tjagvad C., Skurtveit S., Linnet K., Andersen L. V., Christoffersen D. J., Clausen T. Methadone-related overdose deaths in a liberal opioid maintenance treatment programme. *Eur Addict Res* 2016; **22**: 249–58.
13. Lintzeris N., Nielsen S. Benzodiazepines, methadone and buprenorphine: interactions and clinical management. *Am J Addict* 2010; **19**: 59–72.

14. Votaw V. R., Geyer R., Rieselbach M. M., McHugh R. K. The epidemiology of benzodiazepine misuse: a systematic review. *Drug Alcohol Depend* 2019; **200**: 95–114.
15. Strand M. C., Morland J., Slordal L., Riedel B., Innerdal C., Aamo T., *et al*. Conversion factors for assessment of driving impairment after exposure to multiple benzodiazepines/z-hypnotics or opioids. *Forensic Sci Int* 2017; **281**: 29–36.
16. Samferdselsdepartementet (Ministry of Transport and Communications). Revidering av 'forskrift om faste grenser for påvirkning av andre berusende eller bedøvende midler enn alkohol m.m' (Revision of Legal Limits for Driving Under the Influence of Drugs). Oslo: Samferdselsdepartementet; 2015. Available at: https://www.regjeringen.no/contentassets/2b19347b73dd45d0b523f1dd19ae9154/n-0558b-revidering-av-forskrift-om-faste-grenser.pdf (accessed 7 November 2019).
17. Edvardsen H. E., Tverborgvik T., Frost J., Rogde S., Morild I., Waal H., *et al*. Differences in combinations and concentrations of drugs of abuse in fatal intoxication and driving under the influence cases. *Forensic Sci Int* 2017; **281**: 127–33.
18. Edvardsen H. E., Tverborgvik T., Frost J., Rogde S., Morild I., Waal H., *et al*. Corrigendum to 'differences in combinations and concentrations of drugs of abuse in fatal intoxication and driving under the influence cases'. *Forensic Sci Int* 2017 127–33.
19. Waal H, Bussesund K, Clausen T, Skeie I, Håseth A, Lillevold P. Statusrapport 2015. Mot grensene for vekst og nytte? (OAT Status Report 2015: Towards the Limits of Growth and Utility?). Oslo: Norwegian Centre for Addiction Research and National Advisory Unit on Substance Use Disorder Treatment; 2016. Available at: https://oslo-universitetssykehus.no/seksjon/PublishingImages/seraf-rapport-nr-1-2016-statusrapport-2015.pdf (accessed 14 January 2020).
20. World Health Organization (WHO) *International Statistical Classifications of Diseases and Related Health Problems, 10th revision (ICD-10)*. Geneva, Switzerland: WHO; 2016. Available at: https://icd.who.int/browse10/2016/en (accessed 5 December 2019).
21. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). Drug-related Deaths (DRD) Standard Protocol, version 3.2. Lisbon: EMCDDA; 2009. Available at: http://www.emcdda.europa.eu/html.cfm/index107404EN.html (accessed 14 January 2019).
22. England K. *Coding and Reporting of Drug-Related Deaths in Europe. Part 1: Codification Practices of Drug-Related Deaths Following the WHO Revision of ICD Coding Guidelines Related to DRDs*. European Monitoring Centre for Drugs and Drug Addiction: Lisbon; 2016. Available at: https://www.emcdda.europa.eu/system/files/attachments/6638/Part-I-Codification-practices-following-CD-10-updates-final-document.pdf (accessed 29 June 2020).
23. Kerr B., Hill H., Coda B., Calogero M., Chapman C. R., Hunt E., *et al*. Concentration-related effects of morphine on cognition and motor control in human subjects. *Neuropsychopharmacology* 1991; **5**: 157–66.
24. Coda B. A., Hill H., Hunt E. B., Kerr B., Jacobson R., Chapman C. Cognitive and motor function impairments during continuous opioid analgesic infusions. *Hum Psychopharmacol Clin Exp* 1993; **8**: 383–400.
25. Nielsen S., Degenhardt L., Hoban B., Gisev N. *Comparing Opioids: A Guide to Estimating Oral Morphine Equivalents (OME) in Research*. National Drug and Alcohol Research Centre: Sydney; 2014. Available at: http://www.drugsandalcohol.ie/22703/1/NDARC%20Comparing%20opioids.pdf (accessed 30 August 2019).
26. Drummer O. H. postmortem toxicology of drugs of abuse. *Forensic Sci Int* 2004; **142**: 101–13.
27. Ketola R. A., Ojanpera I. Summary statistics for drug concentrations in postmortem femoral blood representing all causes of death. *Drug Test Anal* 2019; **11**: 1326–37.
28. Jones J. D., Mogali S., Comer S. D. Polydrug abuse: a review of opioid and benzodiazepine combination use. *Drug Alcohol Depend* 2012; **125**: 8–18.
29. Iwersen-Bergmann S., Jungen H., Andresen-Streichert H., Muller A., Elakkary S., Puschel K., *et al*. Intravenous methadone application as a serious risk factor for an overdose death: methadone-related fatalities in Hamburg from 2007 to 2012. *Int J Leg Med* 2014; **128**: 751–64.
30. Gagajewski A., Apple F. S. Methadone-related deaths in Hennepin County, Minnesota: 1992–2002. *J Forensic Sci* 2003; **48**: 668–71.
31. Bloor M., McIntosh J., McKeganey N., Robertson M. 'Topping up' methadone: an analysis of patterns of heroin use among a treatment sample of Scottish drug users. *Public Health* 2008; **122**: 1013–9.
32. Darke S., Duflou J., Torok M. The comparative toxicology and major organ pathology of fatal methadone and heroin toxicity cases. *Drug Alcohol Depend* 2010; **106**: 1–6.
33. Degenhardt L., Grebely J., Stone J., Hickman M., Vickerman P., Marshall B. D. L., *et al*. Global patterns of opioid use and dependence: harms to populations, interventions, and future action. *Lancet* 2019; **394**: 1560–79.
34. Hakkinen M., Launiainen T., Vuori E., Ojanpera I. Benzodiazepines and alcohol are associated with cases of fatal buprenorphine poisoning. *Eur J Clin Pharmacol* 2012; **68**: 301–9.
35. Hakkinen M., Heikman P., Ojanpera I. Parenteral buprenorphine–naloxone abuse is a major cause of fatal buprenorphine-related poisoning. *Forensic Sci Int* 2013; **232**: 11–5.
36. Kriikku P., Hakkinen M., Ojanpera I. High buprenorphine-related mortality is persistent in Finland. *Forensic Sci Int* 2018; **291**: 76–82.
37. Larance B., Mattick R., Ali R., Lintzeris N., Jenkinson R., White N., *et al*. Diversion and injection of buprenorphine-naloxone film two years post-introduction in Australia. *Drug Alcohol Rev* 2016; **35**: 83–91.
38. Lugoboni F., Zamboni L., Cibin M., Tamburin S. Intravenous misuse of methadone, buprenorphine and buprenorphine-naloxone in patients under opioid maintenance treatment: a cross-sectional multicentre study. *Eur Addict Res* 2019; **25**: 10–9.
39. Helsedirektoratet (Norwegian Directorate of Health) *Nasjonal retningslinje for legemiddelassistert rehabilitering ved opioidavhengighet (National Guidelines on Opioid Agonist Treatment for Opioid Use Disorder)*. Oslo: Helsedirektoratet (Norwegian Directorate of Health); 2010. Available at: http://www.helsedirektoratet.no/publikasjoner/nasjonal-retningslinje-for-legemiddelassistert-rehabilitering-ved-opioidavhengighet/Sider/default.aspx (accessed 10 April 2019).
40. Selden T., Ahlner J., Druid H., Kronstrand R. Toxicological and pathological findings in a series of buprenorphine related deaths. Possible risk factors for fatal outcome. *Forensic Sci Int* 2012; **220**: 284–90.
41. Johnsen B., Richert T. Diversion of methadone and buprenorphine by patients in opioid substitution treatment

in Sweden: prevalence estimates and risk factors. *Int J Drug Policy* 2015; **26**: 183–90.
42. European Monitoring Centre for Drugs and Drug Addiction (EMVDDA) *Polydrug Use: Patterns and Responses*. Luxembourg: EMCDDA; 2009. Available at: http://www.emcdda.europa.eu/attachements.cfm/att_93217_EN_EMCDDA_SI09_polydrug%20use.pdf (accessed 15 December 2019).
43. Madah-Amiri D., Clausen T., Lobmaier P. Rapid widespread distribution of intranasal naloxone for overdose prevention. *Drug Alcohol Depend* 2017; **173**: 17–23.
44. Millar T., McAuley A. *EMCDDA assessement of drug-induced death data and contextual information in selected countries*. Lisbon: European Monitoring Centre for Drugs and Drug Addiction; 2017. Available at: https://www.emcdda.europa.eu/publications/technical-reports/assessment-drug-induced-death-data_en (accessed 29 June 2020).
45. Veierød M. B., Lydersen S., Laake P. *Medical statistics in clinical and epidemiological research*. Gyldendal Norsk Forlag: Oslo; 2012.
46. Brockbals L., Staeheli S. N., Gascho D., Ebert L. C., Kraemer T., Steuer A. E. Time-dependent postmortem redistribution of opioids in blood and alternative matrices. *J Anal Toxicol* 2018; **42**: 365–74.
47. Lemaire E., Schmidt C., Dubois N., Denooz R., Charlier C., Boxho P. 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* 2017; **62**: 1559–74.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Conversion factors for morphine- and diazepam-equivalent blood concentrations.

Appendix S2: Median (min–max) morphine-and diazepam-equivalent concentrations (ng/mL) of the various opioids and benzodiazepines presented in Table 2, in 107 patients receiving opioid agonist treatment.

Appendix S3: STROBE Statement.

