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# Human deaths from drug overdoses with carfentanyl involvement—new rising problem in forensic medicine

## A STROBE-compliant retrospective study

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**Abstract**

Carfentanyl, an ultra-potent synthetic opioid, is approved for use only in veterinary medicine as a tranquilizing agent. However, many cases of human poisoning with carfentanyl have recently appeared in the news with limited information given and scientific literature provides only 1 case of documented human exposure to carfentanyl.

Fifteen cases of death from drug overdoses with carfentanyl involvement are being presented. Fifteen blood and urine samples have been taken for alcohol and drug testing. Headspace gas chromatography was used for alcohol detection. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) and liquid chromatography–time-of-flight mass spectrometry (LC/MS TOF) system was used for drug detection.

Sixty-three cases of death from poisoning with drugs have been tested for carfentanyl in the State Forensic Medicine Service. Fifteen of them were positive for carfentanyl.

The cases mentioned above show that carfentanyl exposure causes signs and symptoms similar to other opioid toxicity. Carfentanyl intoxication may even be fatal if appropriate treatment is not available. Therefore, nowadays it is very important to draw forensic medicine expert's attention to new substances in drug trade.

**Abbreviations:** EOB = edema of the brain, LVH = left ventricular hypertrophy, SFMS = State Forensic Medicine Service.

**Keywords:** autopsy, carfentanyl, drugs, forensic science, opioids, overdose, toxicology



## 1. Introduction

Although heroin use in drug market is decreasing, 14 new synthetic opioids have been reported to the EU Early Warning System since 2005, among which are several highly potent uncontrolled fentanyls.[1] New substances may not be detectable, are difficult to identify or poorly researched and contribute to the undetermined deaths. Since the spring of 2015, numerous outbreaks of deaths from drug overdoses have been reported in Vilnius region, Lithuania.[2] The drugs, which were found in the scene of the accidents, contained carfentanyl. Lately, there have been numerous stories of carfentanyl poisoning reported in the news all over the world,[3–5] however, only background information is given. Scientific literature provides few cases of documented human exposure to carfentanyl.[6] The effect of carfentanyl to humans is barely known. Therefore, 15 cases of death from drug poisoning, containing carfentanyl, have been presented including autopsy and toxicology results.

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## 2. Methods

### 2.1. Study design and data source

There has been performed a retrospective study, which involved 15 patients from Lithuania, Vilnius county in particular, whose cause of death was drug overdose, including carfentanyl. This research was designed as a retrospective cohort study and written in accordance to the Strengthening the Reporting of Observation studies in Epidemiology (STROBE) guideline. The research was approved by the Ethics Committee of Vilnius regional biomedical research. An informed consent was signed by the research subjects' representatives. The data, regarding postmortem investigation of the decedents between 2015 and 2017 years was obtained from Lithuanian State Forensic Medicine Service (SFMS) database. The data concerning the patients with drug overdose as the cause of death was collected from the central health e-database of Lithuania (www.esveikata.lt). All decedents received full autopsies including toxicological tests for alcohol

and drugs. In every case, there was information provided from the law enforcement agencies, including: the possible crime place, time of death, and the presumable death mechanism.

### 2.2. Identification of cases

The research involved 15 subjects dead from drug overdose, including carfentanyl. All the cases were diagnosed as sudden deaths and defined according to the International Classification of Diseases. Drug overdose including carfentanyl involving/not involving ethanol was chosen as enrollment criteria. 4 cases included both, intoxication with ethanol and drug overdose, including carfentanyl.

### 2.3. Statistical analysis

The data collected was processed using R software. Where the variables found to have significant difference 2-independent sample tests were performed to test for significance within 2 relations. In addition, 95% confidence interval was calculated. Difference with $P$ values less than .05 was considered significant.

### 2.4. Methods

After performing each forensic dissection blood and urine samples were taken from all subjects for alcohol and drug testing. Headspace gas chromatography was used for alcohol detection. All blood and urine samples were extracted through SPE ELUT-3 mL columns. Initially, 1.0 mL blood or urine samples were mixed with 2.0 mL phosphate buffer (pH~9.2) and 10 μL internal standards (fluorazepam) in the glass centrifuge tube. Then the mixture was agitated for 10 seconds in a horizontal shaker, centrifuged at 35.000 rpm speed for 5 minutes and transferred to a clean tube. Prepared samples (centrifugates) were poured into SPE columns and left for 10 to 20 minutes for complete absorption. Extraction by adding twice with 5 mL of ethyl acetate. The extract collected in the tube was evaporated under the stream of nitrogen at the temperature of 40°C. The dry residue was dissolved in 200 μL of methanol and then injected into the LC-MS/MS chromatographic system.

For the qualitative drug detection the system of liquid chromatography–time-of-flight mass spectrometry (LC/MS TOF) was used and for the quantitative drug detection, the liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used. The chromatographic column Zorbax SB-C18 and system were operated in a gradient mode. Mobile phase A was 5 mM ammonium formate and 0.01% formic acid in water. Mobile phase B was 0.01% formic acid in acetonitrile. The received chromatograms were processed by means of a qualitative determination program, using in the device integrated library of medicines and narcotic substances. In a biological specimen, at a concentration of carfentanyl above 8 ng/mL, it is detected by a routine data processing program. To detect lower concentrations of carfentanyl target search is used based on its ionized mass and time of release.

## 3. Results

According to the data of the SFMS during the last 2 years (period from 2015 to 2017) there have been 63 cases of death from drug poisoning 15 of which were positive for carfentanyl. All postmortem examinations were performed in SFMS.

All the decedents were adults from 24 to 67 years old, the majority of whom were men (12 cases). The mean age of female decedents was $35.0 \pm 2$, while the mean age of male ones was $36.5 \pm 12$. No statistically significant difference was found between the mean age of subjects in 2 gender groups ($P > .5$). Most of them were found dead in the district of Vilnius known for drug trafficking or in other places. One woman was found dead at home. Just a week before in a hospital, she had undergone detoxication program from heroin abuse.

During external corpse examination injection marks (IMs) were found in most of the cases in 1 or more of the following locations: above the clavicle, in the elbow pits, forearms, groins, on the dorsal surfaces of the hands. Also syringes, foil pieces were found in some of the decedents' clothes pockets. During internal examination, the signs of sudden death were found in most of the cases: venous congestion of internal organs, thin blood in the heart chambers, edematous lungs with multiple petechial hemorrhages on the surface and edema of the brain (EOB).

**Table 1**

**Autopsy findings.**

| Case No. | Age | IM | SD | PHH | PHL | DTB | IOH | EOL | EOB | ACA | LVH | IHF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 44 | + | + | + | + | + | + | + | + | − | − (1.0) | − |
| II | 35 | + | + | − | + | + | + | + | + | − | − (1.0) | − |
| III | 34 | + | + | − | + | + | + | + | + | − | − (1.0) | − |
| IV | 36 | + | + | − | + | + | + | + | + | − | − (1.4) | |
| V | 27 | + | + | − | + | + | + | + | + | − | − (0.9) | − |
| VI | 33 | + | + | − | + | + | + | + | + | Up to 50% | + (2.0) | + |
| VII | 37 | + | + | + | + | + | + | + | + | − | − (1.4) | − |
| VIII | 24 | + | + | − | − | + | + | + | + | Up to 50% | − (1.4) | − |
| IX | 25 | + | + | − | − | + | + | + | + | − | + (1.8) | − |
| X | 67 | + | + | − | − | + | + | + | + | Up to 50% | + (1.5) | + |
| XI | 45 | − | + | − | − | + | + | + | + | Up to 50% | + (2.0) | + |
| XII | 37 | + | + | + | + | + | + | + | + | − | + (1.5) | − |
| XIII | 38 | + | + | + | + | + | + | + | + | − | − (1.4) | − |
| XIV | 35 | + | + | + | + | + | + | + | + | − | − (1.2) | − |
| XV | 26 | − | + | − | − | + | + | + | + | − | − (1.3) | − |

ACA = atherosclerosis of coronary arteries, DTB = dark thin blood in the heart chambers, EOB = edema of the brain, EOL = edema of lungs, IHF = interstitial heart fibrosis, IM = injection mark, IOH = internal organs' hyperemia, LVH = left ventricular hypertrophy (left ventricular wall thickness in cm), PHH = petechial hemorrhages on the heart surface, PHL = petechial hemorrhages on the lung surface, SD = sudden death features.

There were 2 cases with multiple petechial hemorrhages on the surface of the heart in addition to the signs mentioned above. Advanced coronary artery atherosclerosis (stenosis up to 50%) was found in 4 cases, myocardial fibrosis in 3 cases, and left ventricular hypertrophy (LVH) (left ventricular wall thickness more than 1.5 cm) in 6 cases. Hepatic steatosis was found in all of the cases. There were no pathological changes in other organs (Table 1).

In 4 cases alcohol was detected in blood and urine samples. In 9 cases concentration of carfentanyl in blood varied from 0.2 to 9.3 ng/mL, and in 6 cases there was no carfentanyl detected in blood. The mean of carfentanyl concentration in blood was $3.39 \pm 3.62$ ng/mL. In 14 cases, carfentanyl was found in the urine, 10 of them with concentrations from 2.7 to 10.4 ng/mL, and 4 with only traces of the drug. The mean of carfentanyl concentration in urine was $6.07 \pm 2.49$ ng/mL. In 1 case the bladder was empty and the sample could not be taken, but traces of carfentanyl were found in internal organ (liver, kidney) samples. In all cases, carfentanyl findings were accompanied by other drugs. Morphine and/or diphenhydramine were found almost in all of the cases (14) with 8 cases positive for both of them. In 5 cases 6-monoacetylmorphine (6-MAM) (1 of the active metabolites of heroin) and codeine were found in urine. Another opioid, methadone, was found in 1 blood sample. One urine sample was positive for benzoylecgonine, methylecgonidine (metabolites of cocaine), and lorazepam. In 2 cases, samples were positive for methamphetamine. In one of them, other medications—metoprolol, chloroquine, levomepromazine—were also found. Lastly, other benzodiazepines—bromazepam and 7-aminoclonazepam (a clonazepam metabolite) were found in 3 of the cases (Table 2).

Carfentanyl sole was prevalent in 2 cases. The external and internal examination of these revealed the signs of sudden death: venous congestion of internal organs, thin blood in the heart chambers, edematous lungs with multiple petechial hemorrhages on the surface and EOB. Furthermore, there was no difference in postmortem findings between these 2 cases and the rest, which included multiple opioids. Thus, the cases presented in the report show that carfentanyl exposure causes signs and symptoms similar to other opioid toxicity.

## 4. Discussion

Carfentanyl was first synthesized in 1974 by Paul Janssen's research team. At the beginning of Paul Janssen's research in

**Table 2**

**Toxicological results.**

| Substance | Case No. / *Sample* | I | II | III | IV | V | VI | VII | VIII | IX | X | XI | XII | XIII | XIV | XV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alcohol (‰) | Blood | 0.83 | – | – | – | – | – | 0.48 | 0.42 | – | – | – | – | – | 0.88 | – |
| | Urine | 1.43 | – | – | – | – | – | 0.35 | – | – | – | – | – | – | 1.58 | – |
| Carfentanyl (ng/ml) | Blood | 5.1 | 6.0 | 9.3 | 7.6 | – | – | – | 1.0 | 0.2 | 0.3 | – | 0.7 | – | – | 0.3 |
| | Urine | 10.4 | 7.3 | 4.9 | 8.6 | 2.7 | 5.82 | 2.72 | ** | * | 7.39 | * | 6.53 | 4.35 | * | * |
| Morphine | Blood | + | – | + | – | – | + | + | – | – | – | – | – | – | – | + |
| | Urine | + | + | + | + | + | + | + | – | – | – | – | – | + | + | + |
| 6-MAM | Blood | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| | Urine | * | – | – | + | – | + | * | – | – | – | – | – | * | – | – |
| Diphenhydramine | Blood | + | + | – | – | – | + | + | + | – | + | – | – | – | – | + |
| | Urine | + | + | + | + | + | + | + | – | * | + | – | * | – | + | + |
| Codeine | Blood | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| | Urine | * | – | – | + | – | * | * | – | – | – | – | – | * | – | – |
| Benzoylecgonine | Blood | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| | Urine | – | – | – | + | – | – | – | – | – | – | – | – | – | – | – |
| Methylecgonidine | Blood | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| | Urine | – | – | – | + | – | – | – | – | – | – | – | – | – | – | – |
| Metamphetamine | Blood | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| | Urine | – | – | – | – | + | – | + | – | – | – | – | – | – | – | – |
| Metoprolol | Blood | – | – | – | – | – | – | + | – | – | – | – | – | – | – | – |
| | Urine | – | – | – | – | – | – | + | – | – | – | – | – | – | – | – |
| Chloroquine | Blood | – | – | – | – | – | – | + | – | – | – | – | – | – | – | – |
| | Urine | – | – | – | – | – | – | + | – | – | – | – | – | – | – | – |
| Levomepromazine | Blood | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| | Urine | – | – | – | – | – | – | + | – | – | – | – | – | – | – | – |
| Methadone | Blood | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| | Urine | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Lorazepam | Blood | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| | Urine | – | – | – | + | – | – | – | – | – | – | – | – | – | – | – |
| 7-ACLZ | Blood | – | – | – | – | – | – | – | + | – | – | + | – | – | – | – |
| | Urine | – | * | – | – | – | – | – | – | – | - | * | – | – | – | – |
| Nordazepam | Blood | – | – | – | – | – | – | – | + | – | – | – | – | – | – | – |
| | Urine | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Bromazepame | Blood | – | – | – | – | – | – | – | – | – | – | – | + | – | – | – |
| | Urine | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

6-MAM = 6-monoacetylmorphine, 7-ACLZ = aminoclonazepam.
* - traces.

1953, morphine was the standard and strongest analgesic for the relief of pain.[7] Now carfentanyl, a synthetic fentanyl derivate, is one of the most potent opioids known and since 1986 is used mostly in veterinary medicine, to tranquilize exotic wildlife animals in order to perform examination and procedures.[7,8]

Carfentanyl is 4-anilidopiperidine structurally similar to fentanyl.[7] In vivo carfentanyl acts primarily on the mu opioid receptors as an agonist.[9] Carfentanyl lowest $ED_{50}$ in rats is 0.00037 mg/kg, so it has clinical potency up to 10.000 times that of morphine and 100 times that of fentanyl.[10] $LD_{50}$ in rats is 3.13 mg/kg. $LD_{50}$ in humans is unknown. 1 mg of carfentanyl is approximately equivalent to 8 to 10 g of morphine.[7]

Due to its extreme potency, carfentanyl is not approved for clinical use in humans, except as a radioligand for positron-emission tomography (PET) imaging, where very low doses are required. In veterinary medicine, it successfully tranquilizes large animals. Carfentanyl is administered intramuscularly via dart injection.[11,12] The drug has a rapid onset in animals, and is metabolized by liver and excreted into bile or urine.[13]

In humans, carfentanyl is metabolized in hepatocytes as well, where N-dealkylation and monohydroxylation of the piperidine ring is the dominant pathway. Despite the fact that carfentanyl is categorized as a "high clearance" compound, it might be rapidly distributed and stored in fatty tissues that could prolong its toxicity.[14]

Carfentanyl induces similar effects to other opioids, however, due to its potency, it also induces strong side effects such as sedation, respiratory depression, rise in systemic blood pressure, temperature and decrease in heart and respiratory rates.[11,13] Naltrexone is the antidote of carfentanyl, eliminating its effects.[6,12] When treating carfentanyl overdose in animals, 100 mg of naltrexone is needed for 1 mg of carfentanyl.[12]

In humans, carfentanyl toxic exposure can occur through accidental injection and by absorption through mucous membranes (eyes, nose, mouth) or by direct absorption through damaged skin.[6,12]

In 1 documented case of human exposure to carfentanyl a 47-year-old veterinarian was accidentally splashed in the eyes and mouth with the contents of a tranquillizer dart containing 1.5 mg carfentanyl citrate and 50 mg xylazine hydrochloride. 2 minutes later he felt drowsy. He was administered with antidote naltrexone (100 mg), however, an hour later, while in hospital, he still complained of mild and transient chest discomfort, which disappeared a day later.[6]

Carfentanyl has also been reported to have applications in anti-terrorist interventions as incapacitating agent, used by Russian safety forces in the hostage rescue operation in October, 2002, in the Dubrovka theatre, Moscow. Carfentanyl and remifentanyl were found on a shirt sample and carfentanyl metabolite norcarfentanyl was found in a urine sample. It is possible that an aerosol containing a mixture of carfentanyl and remifentanyl was used.[15–18]

## 5. Conclusion

Summarizing the above, the cases presented in the report show that carfentanyl exposure causes signs and symptoms similar to other opioid toxicity. The drugs, which include carfentanyl, intoxication may even be fatal if appropriate treatment is not available. Therefore, nowadays it is very important to draw forensic medicine expert's attention to new substances in drug trade in Lithuania and other members of The Baltic States, as well.

## Author contributions

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