Legal Medicine 17 (2015) 172–176

Contents lists available at ScienceDirect

Legal Medicine

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

Efficacy of drug screening in forensic autopsy: Retrospective investigation of routine toxicological findings

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ARTICLE INFO

Article history:
Received 1 September 2014
Received in revised form 24 December 2014
Accepted 5 January 2015
Available online 16 January 2015

Keywords:
Forensic autopsy
Toxicological screening
Drug abuse
Illicit drug
Cause of death
Manner of death

ABSTRACT

Toxicological analysis is indispensable in forensic autopsy laboratories, but often depends on the limitations of individual institutions. The present study reviewed routine drug screening data of forensic autopsy cases ($n = 2996$) during an 18.5-year period (January 1996–June 2014) at our institute to examine the efficacy of the procedures and findings in autopsy diagnosis and interpretation. Drug screening was performed using on-site immunoassay screening devices and gas chromatography/mass spectrometry (GC/MS) in all cases, followed by re-examination using GC/MS and liquid chromatography/tandem mass spectrometry (LC/MS/MS) at a cooperating institute in specific cases in the last 4 years. GC/MS detected drugs in 486 cases (16.2%), including amphetamines ($n = 160$), major tranquilizers ($n = 72$), minor tranquilizers ($n = 294$), antidepressants ($n = 21$), cold remedies ($n = 77$), and other drugs ($n = 19$). Among these cases, fatal intoxication ($n = 123$) involved amphetamines ($n = 73$), major tranquilizers ($n = 37$), minor tranquilizers ($n = 86$), antidepressants ($n = 3$), and cold remedies ($n = 9$); most cases involved self-administration, alleged suicide and accidental overdose, while homicide was not included. These drugs were also identified in other manners of death, including homicide ($n = 40/372$), suicide ($n = 34/226$), accidental falls ($n = 27/129$), and natural death ($n = 72/514$). In these cases, on-site immunoassay screening of drugs of abuse showed negative findings in 2440 cases (81.4% in all cases), while GC/MS detected other drugs in 218 cases (7.3% in all cases), including several antipsychotic drugs, acetaminophen and salicylic acid. Further analysis using LC/MS/MS detected low concentrations of benzodiazepines in 32 cases, and also anti-diabetic and hypertensive drugs in a case of fatal abuse. These observations indicate the efficacy of systematic routine toxicological analysis to investigate not only the cause of death but also the background of fatalities in forensic autopsy. The provision of extensive drug screening is needed for forensic and social risk management, considering the marked diversity of medical and illicit drugs.

1. Introduction

Toxicological analyses are indispensable in forensic autopsy, not only for diagnosis of the cause of death, but also for detecting illicit drugs and investigating the background of unnatural deaths, including homicide, suicide and accidental casualties, as well as unexpected sudden deaths. The global standard of drug analysis includes systematic screening, identification and quantification, using instrumental analyses including immunoassay, gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS) [1,2]. However, the facility greatly depends on the limitations of individual institutions in Japan; only a limited number of institutes of forensic/legal medicine are equipped with up-to-date instruments. Meanwhile, on-site drug-of-abuse immunoassay screening devices are widely used in situations where an instrumental illicit drug immunoassay system is not available because of very strict legal regulations [3].

Considering the cost performance under such domestic circumstances, also involving limited financial resources and staff, the

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http://dx.doi.org/10.1016/j.legalmed.2015.01.001
1344-6223/

toxicological section at our institute has performed routine analyses using on-site immunoassay screening and GC/MS in all forensic autopsy cases since 1996, followed by reexamination using GC/MS and liquid chromatography/tandem mass spectrometry (LC/MS/MS) at a cooperative institute (outsourcing) in specific cases since 2010. The aim of the present study was retrospective investigation of routine drug screening data during an 18.5-year period at our institute to examine the efficacy of the procedures and findings in forensic autopsy diagnosis and interpretation.

2. Materials and methods

2.1. Autopsy database

All forensic autopsy cases (n = 2996) at our institute, which covers the southern half of Osaka City and surrounding areas, during the past 18.5 years (January 1996–June 2014) were retrospectively reviewed, excluding those where adequate specimens were not available due to advanced decomposition or skeletonization, and those with positive toxicological findings were collected. These data analyses as well as sample collections and the analyses described below were performed within the framework of our routine medicolegal casework following the autopsy guidelines (2009) and ethics guidelines (1997 and 2003) of the Japanese Society of Legal Medicine, approved by the institutional ethics committee.

Drug screening was performed by on-site drug-of-abuse immunoassay screening and GC/MS in all cases since 1996, followed by reexamination using GC/MS and liquid chromatography/tandem mass spectrometry (LC/MS/MS) in specific cases (n = 32), by M.T. at the Foundation for Promotion of Material Science and Technology of Japan (MST) since 2010. Reexamination was performed in cases of discrepancy between preliminary on-site immunoassay and GC/MS screening or negative screening results despite possible drug abuse when appropriate specimens were available.

2.2. Analytical procedures

2.2.1. Autopsy materials

Besides blood, peripheral blood, pericardial fluid (PCF), cerebrospinal fluid (CSF), bone marrow aspirate (BMA), urine, bile and stomach contents were routinely collected at autopsy for toxicology and analyzed in parallel. Pleural and peritoneal effusions were used when other specimens were not available. These specimens were stored at −20 °C until analysis after preliminary immunoassay screening.

2.2.2. On-site immunoassay drug screening devices

The Triage Panel for Drug of Abuse (DOA) (manufactured by Biosite Inc., San Diego CA, USA, purchased from Sysmex Inc., Kobe, Japan; n = 1280) and Monitect-9 (manufactured by Branan Medical Corporation, Irvine, CA, USA, purchased from Veritas, Tokyo, Japan; n = 560) were used since 2007 and 2009, respectively (Table 1). Triage DOA is an Ascend Multi Immunoassay (AMIA) system to detect phencyclidine (PCP), benzodiazepines (BZO), cocaine metabolites (COC), amphetamines (AMP), cannabinoid (THC), opiates (OPI), barbiturates (BAR) and tricyclic antidepressants (TCA) with a small quantity (140 μL) of urine [4,5]. Monitect-9 is an immunochromatography system to detect phencyclidine (PCP), benzodiazepines (BZO), cocaine metabolites (COC), amphetamines (AMP), methamphetamine (MET), cannabinoid (THC), opiates (OPI), barbiturates (BAR) and tricyclic antidepressants (TCA), requiring a larger quantity (750 μL) [6]. PCF, vitreous humor and/or pleural/peritoneal effusions were used as alternative specimens when urine was not available.

Table 1
Cut-off values in on-site immunoassay drug screening.

Drugs		Triage-DOA (ng/mL)	Monitect-9 (ng/mL)
PCP	Phencyclidine	25	25
BZO	Benzodiazepines	300	300
COC	Cocaine metabolites	300	300
MET	Methamphetamine	–	1000
AMP	Amphetamines	1000	1000
THC	Cannabinoid	50	50
OPI	Opiates	300	300
BAR	Barbiturates	300	300
TCA	Tricyclic antidepressants	1000	1000

2.2.3. Instrumental conditions

Gas chromatography/mass spectrometry: A 0.5 ml aliquot of sample was used. Automated GC/MS following solid/liquid phase extraction [7] was performed using a Shimadzu GC/MS System Model QP 5000 (column, DB-1, 30 m × 0.25 mm i.d., film 0.25 μm; column temperature, 60–230 °C; injector temperature, 200 °C; carrier gas, He at a flow rate of 40 cm/s; interface temperature, 230 °C) from January 1996 to August 2009, and Agilent Technologies GC/MS System Model 5975c MSD (column, DB-5MS, 30 m × 0.25 mm i.d., film 0.25 μm; column temperature, 100–325 °C; injector temperature, 280 °C; turbocharged carrier gas, He at a flow rate of 48 cm/s; interface temperature, 300 °C) from September 2009 to December 2013. Quantitative analytical precision was less than 10% for all drugs in each specimen.

In terms of the GC/MS system for reexamination at MST, an Agilent Technologies 7890A GC System (Column, Agilent HP-5MS, 30 m × 0.25 mm i.d., 0.25 μm) and Agilent Technologies 5975C inert MSD were used. The conditions were as follows: the initial temperature of 60 °C was maintained for 2 min, the temperature was then programmed to increase to 300 °C at a rate of 20 °C/min, and this temperature was then maintained for 5 min. The injection port and transfer line temperatures were 250 and 280 °C, respectively. The carrier gas was He and the constant pressure mode was used [8].

Liquid chromatography/tandem mass spectrometry: A 0.5 mL aliquot of sample was used. The apparatus used at MST was an automated LC/MS/MS, after which following solid/liquid phase extraction was performed using a Shimadzu Prominence system UFLC (column, 2.1 m × 150 mm i.d., L-column2 ODS; sample; column temperature, 40.0 °C; flow rate, 0.2 mL/min; injection vol.: 50 μL; mobile phase, A: 10 mM ammonium formate + 5% methanol, B: 10 mM ammonium formate + 95% methanol), and an AB Sciex Instrument Mass Spectrometer 4000QTRAP (interface: TurboV source with ESI). A triple quadrupole mass spectrometer equipped with an ESI source was used for mass analysis and detection. Acquisition mode was performed in multiple reaction monitoring (MRM) (ESI+; IonSpray Voltage (IS): 5500 V).

3. Results

3.1. Immunoassay drug screening

Either one or both of the Triage-DOA and Monitect-9 test devices showed positive findings in 556 cases (18.6%). There was discrepancy between the results of these devices for AMP (n = 49), THC (n = 28), BAR (n = 10) and TCA (n = 28), which were positive on the Triage-DOA but negative on the Monitect-9, especially in cases where putrefaction was apparent or when alternative specimens were used; however, the Monitect-9 test was positive for BZO, which was negative on the Triage-DOA, in 61 cases.