Post-mortem toxicology: A pilot study to evaluate the use of a Bayesian network to assess the likelihood of fatality

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Highlights

- Systematic toxicological analysis should be routinely in death investigation.
- Alternative approaches to using existing toxicological data should be considered.
- Bayesian networks could be used in cases where drugs/drug combination are observed.
- Influence of post-mortem redistribution requires further investigation.

Abstract

The challenge of interpreting post-mortem drug concentrations is well documented and relies on appropriate sample collection, knowledge of case circumstances as well as reference to published tables of data, whilst taking into account the known issues of post-mortem drug redistribution and tolerance. Existing published data has evolved from simple data tables to those now including sample origin and single to poly drug use, but additional information tends to be specific to those reported in individual case studies. We have developed a Bayesian network framework to assign a likelihood of fatality based on the contribution of drug concentrations whilst taking into account the pathological findings. This expert system has been tested against casework within the coronial jurisdiction of Sunderland, UK. We demonstrate in this pilot study that the Bayesian network can be used to proffer a degree of confidence in how deaths may be reported in cases when drugs are implicated. It has also highlighted the potential for deaths to be reported according to the pathological states at post-mortem when drugs have a significant contribution that may have an impact on mortality statistics. The Bayesian network could be used as complementary approach to assist in the interpretation of post-mortem drug concentrations.

Keywords

Post-mortem toxicology; interpretation; Bayesian network; death certification

Introduction

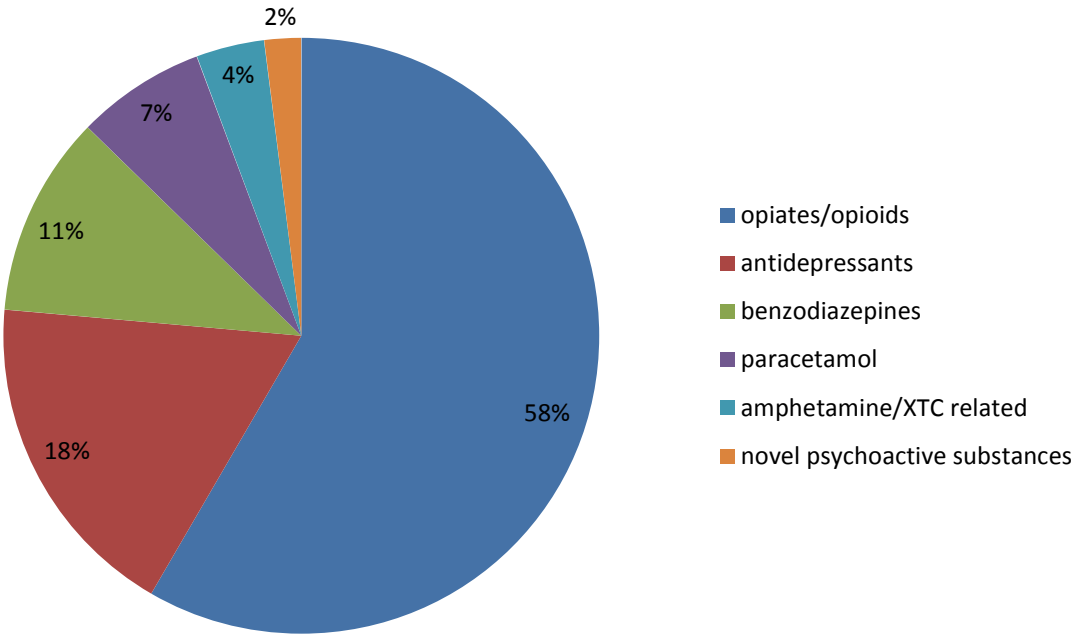
The role of post-mortem toxicology is important with respect to establishing the contribution of drug(s) to the cause of death. The issue of which drug is primarily responsible remains a confounding factor given that most cases involve multiple drug use. Compilations of the usual therapeutic, toxic and fatal drug concentrations have been published[1,2] and it seems that these are the standard 'go to' sources to provide a meaningful interpretation of the drug concentrations found in individual cases. Since the early 1990's, the understanding of post-mortem toxicology has evolved significantly with recognition of the phenomenon of post-mortem drug redistribution[3], site to site variability of drug concentrations[4], influence of tolerance, free:total drug concentration ratios[5,6,7], gender bias[8] as well as, more recently, the influence of genetic polymorphisms[9,10,11]. It has now been established and has become common practice, that peripheral blood should be obtained from a femoral vessel[4,12], yet it remains that the extent of redistribution artefacts is an unknown quantity. Whilst markers for the extent of redistribution have been evaluated[13] it still remains a challenging factor in the interpretation of post-mortem toxicology. Similarly the published reference data has evolved from that reported in serum samples to include whole blood[14] as well as distinguishing from data derived from a single drug to that in combination with alcohol and/or other drugs. Further publications have addressed other physical attributes such as age and body mass index[15,16] as well as those reported in specific case studies, usually as a consequence of a fatality due to the emergence of a new drug (whether it be a designer or new pharmaceutical drug) or unusual cases[17].

Drug contribution in forensic and coronial casework interpretation is further compounded by underlying pathological conditions such as chronic obstructive pulmonary disease, liver disease (alcohol and non-alcohol related), pneumonia or coronary artery atheroma. The contribution of drugs in these cases will naturally be taken into account, yet the published tables of toxicological data that are consulted may not indicate the presence/absence of natural disease. Of further note is the disparity on how a death is recorded in cases in different coronial jurisdictions[18]. A review of coronial services suggested a failure to identify deaths where drugs were deemed to have contributed[19], yet it does not address how to resolve the issue associated with drug related deaths where drug testing is not always routinely carried out[20,21]. The Shipman Inquiry in 2003 proposed changes to the process of death certification but also noted that greater use of toxicological analyses should be adopted in the death investigation process[22].

It is of note that 506,740 deaths were registered in England and Wales in 2013, of which 227,984 deaths were reported to coroners, reflecting a less than 1% increase (263 deaths) from 2012[23]. Of these coroners cases, 94,455 post-mortem examinations were instructed, a decrease of 359 from 2012. Furthermore, only 13,285 (14.0%) included toxicological analysis[23], but nevertheless it does see an increase from 13.3% of cases in 2012[24]. This data appears to indicate that toxicological analysis was performed on 5.8% of the cases reported to the coroner but only 2.6% of all deaths registered in England and Wales in 2013 and as such suggests that toxicological examination has not seen a significant increase in routine implementation in death investigation since the Shipman Inquiry in 2003.

According to the Office for National Statistics[24], there were only 2597 drug poisoning deaths, of which 1496 were classified as drug misuse deaths. Of the total drug poisoning deaths, 65.6% were male with an age demographic of 30-39 having the highest mortality rate[24]. The demographic of specific drug type mentioned on death certificates indicates that the opiates (heroin, morphine, codeine, dihydrocodeine) and opioids (methadone, tramadol) drug groups were by far the most prevalent, followed by the antidepressants (tricyclic, selective serotonin reuptake inhibitors and others) and benzodiazepines (of which diazepam was most prevalent in this category) See Figure 1.

Figure 1 Proportion of drug related deaths in 2012 where a named drug appeared on the death certificate. Modified from Office for National Statistics[24]



There have of course been reforms of the coroners system in England and Wales; notably that in 2006 to improve the service for a more effective investigation into deaths[25] with legislation leading to implementation of structural and procedural changes in 2009, alongside the appointment of a Chief Coroner and the concept of a coroner's investigation into death where an inquest may or may not be required. These reforms appear to suggest an impact on the trends of cause of death reported in mortality statistics[23]. Whilst these reforms have taken place, there also remains a difference in practice by hospital, clinical and forensic pathologists in the cause of death coding (part 1a) as defined using the International Classification of Diseases, 10th revision. The wording that appears on death certificates ranges from those having named specific drug combinations (but no priority given to the recorded list of drugs e.g. combination effects of morphine, codeine and alcohol); drug overdose (with no reference to specific drugs); natural disease (when drugs are present but have been deemed not to have played a vital role in the terminal outcome). Approximately 10% of the deaths reported in drug poisoning deaths had a generalised form of words on the

death certificate (e.g. drug overdose or multiple drug toxicity[24]. It is also interesting to note that it has been previously reported that in cases where no natural disease was found at post-mortem, the cause of death can be attributed to a specific combination of drugs, yet in the presence of disease with the same combination of drugs, the cause of death was attributed to the disease rather than both having a contributory factor[26]. As a consequence much useful toxicological data in unnatural or indeed natural deaths remains unavailable for interpretation for post-mortem toxicology or even public health awareness (contra-indications/adverse drug combinations).

Bayesian Statistics

The basic concept in Bayesian statistics is that of conditional probability; whenever a statement of probability (P) of an event A is given it is given under the condition of other known factors. This can be exemplified by the statement: "given the event B, the probability of the event A is x".

The notation for this is $P(A|B) = x$

Bayes theorem is defined as:

$$P(A|B) = \frac{P(B|A)P(A)}{P(B)}$$

This defines the relationship between the probabilities of A and B and the conditional probabilities of A given B and B given A.

Where;

P(A) is the prior probability i.e. the initial degree of belief in A

P(A|B) is the posterior probability i.e. the degree of belief accounting for B

This method is employed in a number of applications, where 'reasoning under uncertainty' is required e.g. medical diagnoses, stock market analysis and risk analysis, to name a few[27]. The advantage of using the Bayesian framework in such circumstances is that it can encompass both aleatory data (e.g. frequency data derived from direct experimental observation) and epistemic data (e.g. an assigned probability for an event, based upon published literature or personal experience).

Over the past two decades, a probabilistic approach has been introduced and developed as a framework for the interpretation and evaluation of forensic evidence as it has proved very useful in dealing with the evaluation of findings in the light of two competing propositions or hypotheses. Its use has been seen to be gathering momentum over the past few years[28-31] in for example DNA profiling[32], individualisation[33], bioforensics[34] and forensic entomology[35]. This approach has also been applied to the forensic autopsy[36] which, whilst limited to prediction of cause of death from war victims, does illustrate the potential for an expert system to be used as a viable probabilistic tool for cases if appropriate information pertaining to the case was added to the system.

In a forensic context, where the probabilities of two competing propositions (events) need to be considered (e.g. p(Hp) = the toxicology results account for death and p(Hd) = the underlying pathology accounts for death) through conditioning by the findings from an examination (E), and contextual information (I), Bayes theorem can be rearranged where the prior and posterior probabilities for each proposition are ratios, commonly referred to as 'odds' and the quotient of the probability of the evidence given the proposition becomes the likelihood ratio;

$$\frac{p(Hp|I,E)}{p(Hd|I,E)} = \frac{p(E|Hp,I)}{p(E|Hd,I)} \times \frac{p(Hp|I)}{p(Hd|I)}$$

Posterior odds = Likelihood Ratio x Prior Odds

Prior (contextual) information can be accounted for by conditioning the probabilities on this throughout the equation. In most cases the prior odds probability assignments are evenly distributed (i.e. p(Hp) and p(Hd) = 0.5) and therefore can effectively be ignored in the equation. In such a situation, the likelihood ratio (LR) effectively becomes equivalent to the posterior odds as a measure of how much the evidence favours one proposition over another, i.e. how our belief in the prior odds is updated by the evidence (posterior odds).

Where the LR >1 the belief in p(Hp) is increased; where LR < 1 the belief in p(Hd) is increased. In simple terms, the likelihood ratio can be expressed as;

$$LR = \frac{\text{Probability of the findings if a particular hypothesis is true p(Hp)}}{\text{Probability of the findings if an alternative hypothesis is true p(Hd)}}$$

The Bayesian approach therefore allows us, in the face of new information or evidence, to update a probability which describes our personal state of belief regarding an event which is conditioned by relevant information.

The aim of this pilot study was to establish a relational pathological-toxicological database from which a probabilistic expert system could be developed using a Bayesian network; attempting to take into account the physical attributes of the individual, the pathological findings at post-mortem and the toxicological analytical results to assist in the interpretation of a drug related/contributed death. This expert system could then be tested against 'live' casework to compare the likelihood of fatality against that actually reported.

Methods

Ethical review

The research study was approved by the University of Northumbria at Newcastle research ethics committee. Permission to use anonymous data from cases within the coronial jurisdiction of Sunderland, England was granted by the HM Coroner for the City of Sunderland.

Case selection

This was a pilot study involving data collected from cases reported to the Sunderland coroner during the period 2011-2013. Death certification in the United Kingdom is divided into two parts. Part 1 is used for the disease or condition that directly caused the death (part 1a) and any underlying cause which ultimately lead to the death (part 1b). Part 2 documents any significant disease or condition which has contributed to but not directly caused the death. Deaths from all causes (natural disease, external injuries and drug related) were included giving a total of 325 deaths during this period of time. Those cases where toxicological analysis had been undertaken were identified for inclusion in the database (n=58). Case files were examined and comprehensive pathological, including co-morbidities (chronic obstructive pulmonary disease, alcoholic liver disease, hepatitis, coronary artery atheroma or pneumonia), demographics of age, gender, BMI and toxicological case information were identified.

Relational database design

A simple relational database was constructed with the 58 cases mentioned above using Microsoft Access® to allow easy data assimilation and interrogation for the cases identified. Data included case details (age, gender, ethnicity, weight, height), pathological findings (presence of natural disease, previous medical history, reported cause of death), toxicological findings (sample origin, analyte, metabolite, parent:metabolite ratio, free:conjugated ratio) and reference tables of data (such as the therapeutic and fatal drug concentrations compiled for TIAFT by Uges[1]).

Bayesian network design

The design, construction, use and application of Bayesian networks are described in considerable detail by Jensen[37] and Jensen and Nielson[38]. In this study, there are a number of complex interdependent factors which condition upon the calculation of a likelihood ratio, making it extremely difficult to perform such a computation by hand, therefore a commercially available Bayesian network software package, (Hugin Researcher™) was employed. This software uses a graphical user interface to allow a visual construction of a particular model/ architecture under examination and is marketed as a decision making tool. It is described by the manufacturer, Hugin Expert A/S of Aalborg, Denmark, as a "compact model representation for reasoning under uncertainty"[27].

The graphical structure of Bayesian networks allows the description and modelling of possible inter-dependent relationships between different components of the problem under investigation. It consists of a series of nodes each representing a domain variable. Where one variable (child node) is dependent on another (parent node) the nodes can be connected together representing a conditional relationship between the two. The uncertainties present are represented through conditional probabilities which can be aleatory or epistemic in nature and these form the basis for the cause / relationship interactions between the various components (see Figure 2). The underlying Bayesian algorithms of such systems use these conditional probabilities to calculate the probability of different events or hypothesis given a

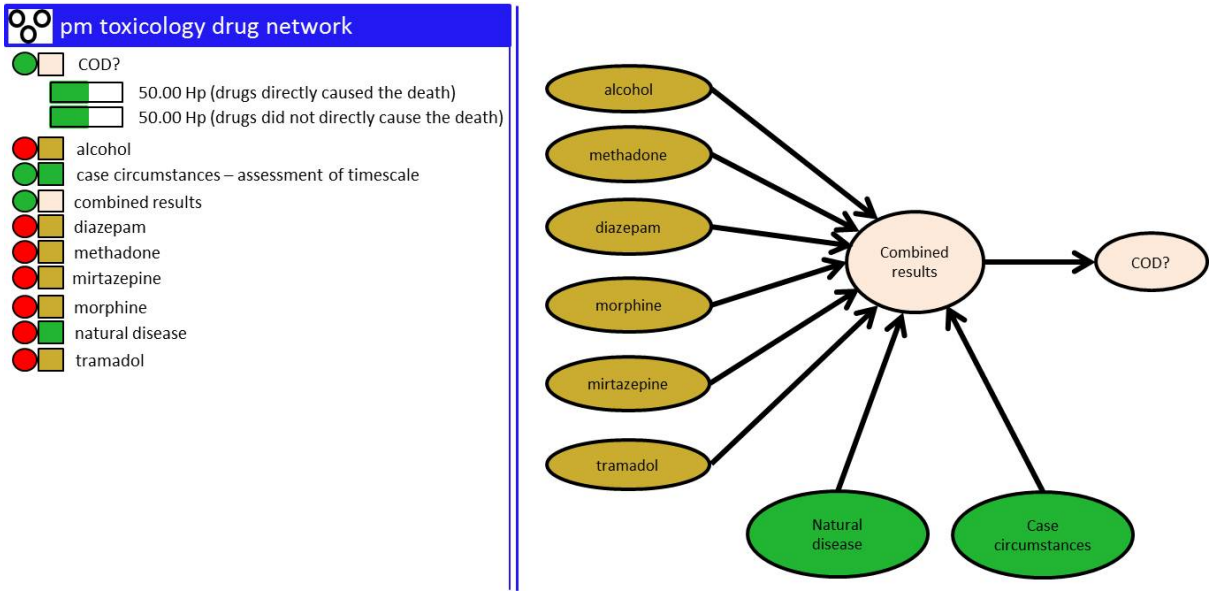
series of specific observations e.g. differential diagnosis based upon the results of clinical observations. Detailed descriptions of the available algorithms used in Hugin Expert are available[27]. These systems are tools that are designed to assist in conclusions drawn from these observations and not designed to replace the skills and knowledge of the practitioner.

For the purposes of this study, the authors felt it more appropriate and helpful to use the calculated values of p(Hp) and p(Hd) generated by the output of the Bayesian network rather than their ratio (the likelihood ratio), where;

Hypothesis Hp and associated probability p(Hp) asserts that the drug (or drug combination) is responsible for the death

Hypothesis Hd and associated probability p(Hd) asserts that the drug (or drug combination) is not responsible for the death (e.g. causal link to pathological disease).

Figure 2 Construction of the Bayesian network using Hugin Researcher™ based on drug concentration, presence of natural disease and whether the death occurred in an acute phase or representation of a period of survival



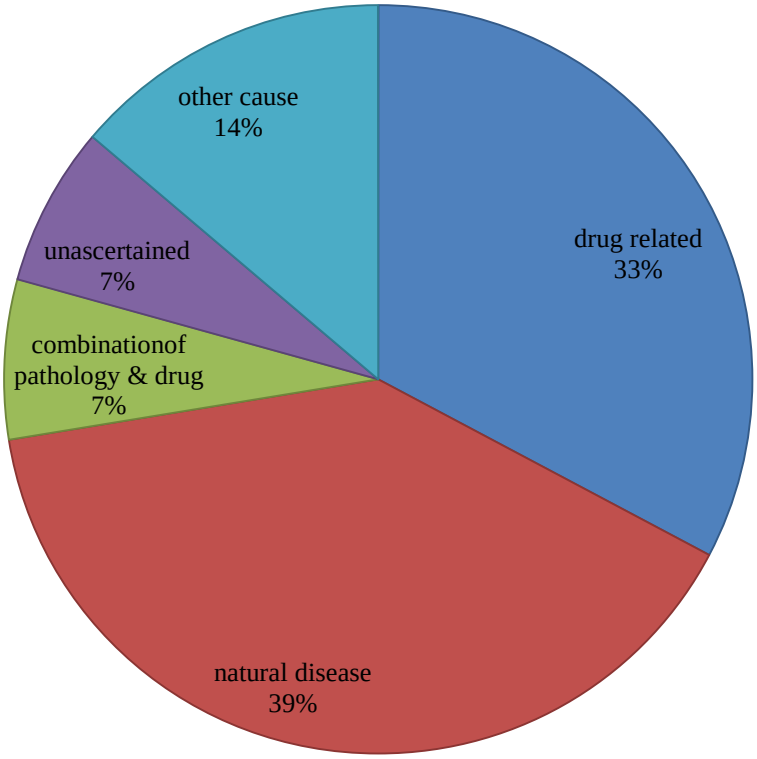
The Bayesian network used in this study is an example of a multi-parent, converging channel network, where nothing is known regarding the 'child' node except what may be inferred from the mutually independent knowledge from the 'parent' nodes. This form of architecture has previously been described by Jensen[37]. As a consequence, this architecture results in a large (convergent) 'child' node since the conditional probability must be computed against every possible combination of the states of each of the' parent nodes'. Like any Bayesian network, the architecture can (and will) be further refined if a more extensive study is carried out.

Results

Of the 58 cases analysed, named drug or drug combinations were assigned by the pathologist as the sole cause of death in 19 cases (33 %), 4 cases (7 %) reported natural disease (as part

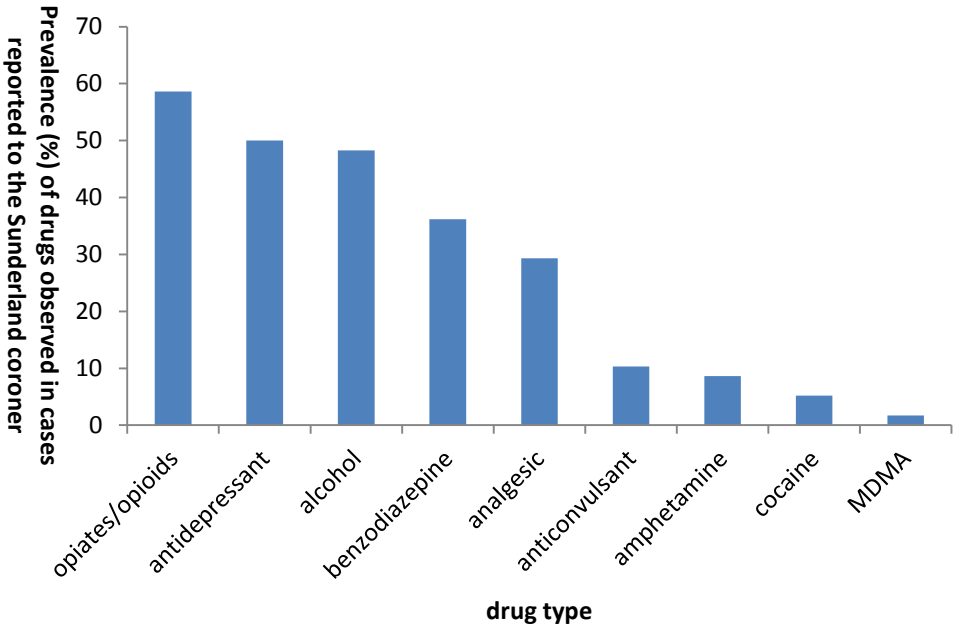
1a) with contributory effect of drug combination (as part 1b). 23 cases (40 %) reported cause of death as pathological disease, with the remaining cases as unascertained or other cause (Figure 3). The gender demographic in the drug poisoning cases was 81 % male, 19 % female and the average age at death 39.8 years male, 44.8 years female.

Figure 3 Percentage of cases by type of death as reported on death certificate



The prevalence of drug type found in all cases (figure 4) expressed as percentage indicates the opiates and opioids are the predominant drug types found in all cases (59 %) followed by antidepressants at 50 %. Opiates/opioids were found in 84 % of the drug poisoning cases. The opiate/opioid category included morphine 50%, methadone 20 % (6 % concomitant use of morphine and methadone), heroin (indicated through identification of 6 mono-acetyl morphine, 5 %), codeine 23 %, dihydrocodeine 15 %, buprenorphine 6 % and fentanyl 3 %. The antidepressants included tricylic antidepressants 31 % (amitriptyline, venlafaxine and dothiepin), selective serotonin reuptake inhibitors 48 % (citalopram, fluoxetine, sertraline and duloxetine, and other 34 % (mirtazapine as a tetracyclic antidepressant and trazodone). The anticonvulsants included gabapentin, pregabalin and carbamazepine). The pattern of drug prevalence found in these cases does not indicate a local regional variation from that reported by the Office for National Statistics[24].

Figure 4 Prevalance of drug type observed in cases reported to Sunderland Coroner 2011-2013 where toxicological examination has taken place



Of the most prevalent drug group (opiates/opioids) observed in the dataset, the concentrations of the specific drug are given in Table 1. These concentrations are similar to that which has been previously reported in the literature. For example, morphine (free) reported to have a concentration range of 0.1 mg/L→>1 mg/L, median concentration between 0.2-0.3 mg/L with morphine (total) concentrations ranging to over 2 mg/L with a median concentration between 0.3-0.6 mg/L[39].

Table 1 Opiate/opioid drug concentrations reported in cases referred to Sunderland coroner where toxicological examination was required. *metabolite of tramadol has not been reported in 3 of the 10 cases referred to coroner

Analyte	Mean (mg/L)	Range (mg/L)	Median (mg/L)	Number of cases
Methadone	0.89	0.03-1.88	0.985	8
Morphine (free)	0.67	0.03-3.85	0.165	15
Morphine (total)	1.23	0.03-6.1	0.515	15
Tramadol	1.67	0.02-5.9	1.18	10
Nortramadol	0.34	0.1-0.59	0.29	7*

However, this small dataset of opiates/opioids related deaths does indicate a higher male proportion (81 %) compared to that reported for the male population nationally (66%), but has exhibited the same age demographic. According to the Office for National Statistics[24] the North East of England was the second highest region in England to exhibit

deaths related to drug misuse which may account for the higher propensity of opiate use in the drug poisoning cases observed in this dataset (84 % compared to 58 % nationally).

Using the relational database, cases were identified on the basis of whether the death was recorded as drug related, pathological related or a combination of both for subsequent use and evaluation using the Bayesian network. Of the 58 cases where toxicological examination had been included, 5 cases were identified to cover these three classification categories of death certification. The data from these cases were used for populating the probability nodes of the Bayesian network model.

Cases examined using the Bayesian network

Case report 1

A 42 year old man (height 173 cm, weight 73 kg, BMI 24.47) was found slumped over at friend's home holding a syringe. He had a known medical history including diabetes mellitus, pancreatitis and depression. He had previously been known to misuse heroin and had previously been on a methadone maintenance therapy. He had recently been suspected of using heroin once more. Emergency services were called and life pronounced extinct. A medico legal autopsy was commissioned and was carried out 4 days after death. External examination revealed multiple track marks on the medial aspect of the left forearm with the presence of multiple recent needle puncture marks. The heart weighed 361 g and exhibited moderate to severe atheroma in the left anterior descending artery. Both lungs were markedly congested (left lung weight 892 g, right lung 1002 g). Samples of blood (femoral blood vessels) and urine were collected for systematic toxicological analysis. Analysis of the femoral blood indicated the presence of the following substances:

total morphine 0.526 mg/L; free morphine 0.366 mg/L (free:total ratio 0.7); codeine 0.04 mg/L;
diazepam 0.4 mg/L; nordazepam 0.836 mg/L (parent:metabolite ratio 0.49);
mirtazapine 0.372 mg/L;

6-monoacetylmorphine was not reported in the blood sample however it was noted that urine tested positive for opiates (qualitatively documented as morphine, codeine and 6-monoacetylmorphine).

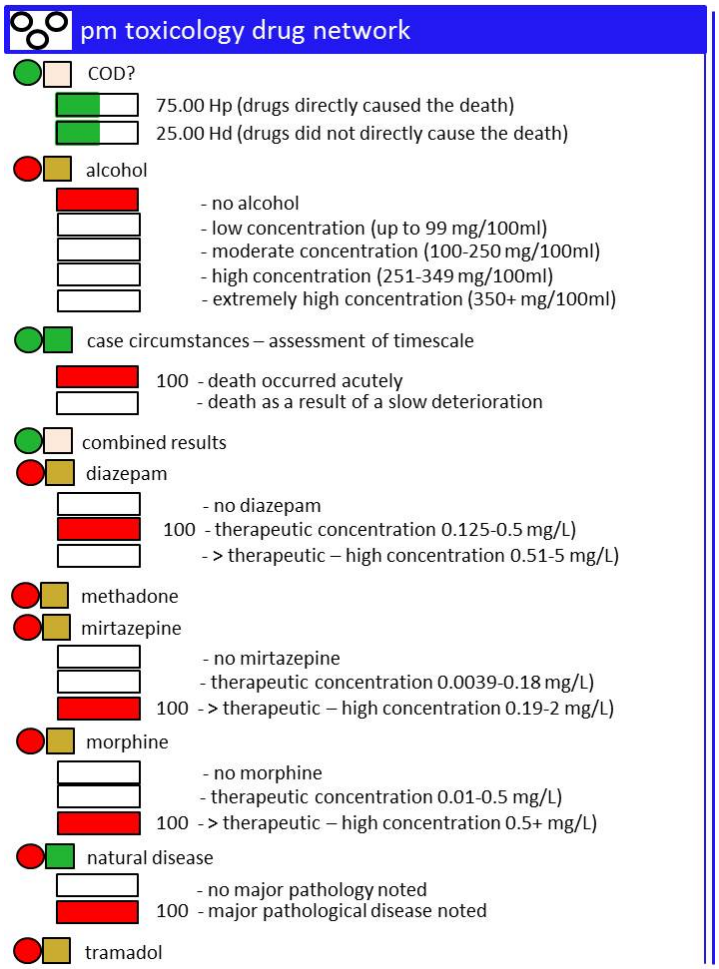
The track marks indicated recurrent long term use of these veins to inject substances. The low concentration of codeine was reported in the toxicology report to be a consequence of morphine metabolism. Some older studies indicate morphine metabolism yields codeine but as a minor metabolite and not in an appreciable concentration[40] but it is now generally accepted that there is little, if any, evidence to support this as a metabolic route. It is more likely that the presence of a low concentration of codeine such as in this case would have arisen as a consequence of an impurity (acetylcodeine) in the illicit heroin that was ingested[41].

Natural disease was noted in the form of ischaemic heart disease, with moderate to severe atheroma of one of the three main coronary arteries but this was considered to be

incidental in the light of the toxicological results. The cause of death was thus reported to be the effects of a combination of heroin, diazepam and mirtazapine. Whilst 6-monoacetylmorphine was not reported in the blood sample the inference of a heroin related death is commensurate with the qualitative analytical finding in the urine. This illustrates the importance of both case context and the medical history of the deceased, nevertheless caution should still be exerted in the interpretation of toxicological results especially in context when a direct causal role of named drug is implicated.

Having assigned probability values for each of the different states in each node table using Hugin Researcher™ based on the experience and expectations of the pathologist and toxicologist, the associated toxicological and pathological information can be selected from the expanded node list according to the post-mortem examination (see figure 5). As such, the design of the Bayesian network is limited to only offer a probability that either a pathological or toxicological cause of death is more/less likely rather than a probability of the contribution of both. It can be seen that from the Bayesian network that a toxicological cause of death was more likely than a pathological cause based on the calculated probability for p(Hp) = 0.75 and for p(Hd) = 0.25. This is in agreement with the reported cause of death.

Figure 5 Bayesian network output for case 1 indicating a probability estimate of 0.75 that drugs had a direct causal role in the death.



Case report 2

A 27 year old man (height 173 cm, weight 62 kg, BMI 20.78) with a known medical history including alcoholism and substance misuse was found unresponsive at a friend's home. Emergency services were called and on arrival rigor mortis was noted to be present in the jaw. Life was pronounced extinct and a medico legal autopsy commissioned. He had been seen earlier in the day to consume large quantity of alcohol. The post-mortem was performed 3 days following death and revealed only congestion and oedema in the lungs (left lung weight 504 g and right lung 577 g). The liver weighed 2067 g having a smooth capsular surface and a pale and fatty cut surface. It showed evidence of alcoholic liver disease with histology revealing widespread fatty change with parenchymal acute inflammation associated with damaged hepatocytes. The pancreas showed signs of fibrosis. Samples of blood (femoral blood vessels) and urine were collected for systematic toxicological analysis. Analysis of the femoral blood indicated the presence of the following substances:

alcohol 468 mg/100mL;
diazepam 0.064 mg/L; nordazepam 0.12 mg/L.

Whilst there is evidence of alcoholic liver disease and fibrosis of the pancreas it was not sufficient to account for the death. The concentration of alcohol is such that respiratory depression, coma and death would have been potentiated by the presence of diazepam even at low concentration. The cause of death in the absence of any other natural disease was reported to be the effects of a combination of alcohol and diazepam.

As illustrated by the example in case report 1, by entering the associated toxicological and pathological information into Hugin Researcher™ for this case (case report 2), the Bayesian network indicated that a toxicological cause of death was more likely than a pathological cause based on a calculated probability for $p(Hp) = 0.7$ and for $p(Hd) = 0.3$. Again (as in case 1), this is in agreement with the reported cause of death.

Case report 3

A 25 year old man (height 178 cm, weight 117 kg, BMI 37.01) was found unresponsive in bed at his home address. He was known to have a drug habit since the age of 15 and is believed to have consumed a number of Zomorph® (morphine) tablets (three strips 8-24 tablets, 10 or 30 mgs unspecified). A medico legal autopsy was commissioned and the post-mortem carried out 3 days after death. The heart weighed 521 g with no signs of natural disease. The lungs were congested and oedema present, with copious amount of frothy fluid present on cut surfaces. Histological examination indicated widespread bronchopneumonia. The liver weighed 2275 g with a pale appearance and showing focal fatty change. Blood (femoral blood vessels), urine and gastric contents were collected for systematic toxicological analysis. Analysis of the femoral blood indicated the presence of the following substances:

alcohol 119 mg/100mL;
total morphine 1.6 mg/L; free morphine 0.17 mg/L (free:total ratio 0.11);

diazepam 0.28 mg/L; nordazepam 0.66 mg/L (parent:metabolite ratio 0.42); oxazepam 0.016 mg/L;
tramadol 0.021 mg/L;
mirtazapine 0.014 mg/L;
amphetamine <0.025 mg/L;
11-nor-Δ^9-tetrahydrocannabinol 0.001 mg/L; 11-nor-Δ^9-tetrahydro-9-cannabinolic acid 0.006 mg/L.

The post-mortem examination indicated left ventricular failure resulting in pulmonary oedema. The respiratory depressant effects of morphine in combination with alcohol and benzodiazepines would have likely resulted in an inability to clear secretions from the respiratory airways and thus cause bronchopneumonia which would further exacerbate respiration difficulties. The cause of death was thus reported to be 1a. Bronchopneumonia and the effects of morphine and alcohol, 2 The effects of benzodiazepine, tramadol and mirtazapine.

By entering the associated toxicological and pathological information into Hugin Researcher™, the Bayesian network produced a probability estimate for p(Hp) = 0.38 that drugs directly caused death with a probability estimate for p(Hd) = 0.62. In this case, the Bayesian network suggests that a pathological cause of death is more likely than a toxicological cause in agreement with that reported on the death certificate. However, this case also illustrates the limitation of the network in that it does not offer a probability on the combined contribution of pathology and toxicology which in this case was deemed appropriate to include on the death certificate.

Case report 4

A 22 year old female (height 168 cm, weight 58 kg, BMI 20.64) with a known medical history including depression, anxiety and impulsive thoughts of self-harm when using alcohol was found collapsed at a friend's house. Emergency services were called and she was conveyed to hospital where life was pronounced extinct. A medico legal autopsy was commissioned and commenced 3 days following death. External examination indicated needle puncture marks to the neck, right antecubital fossa, on the back of both hands and in the left anterior tibial region and in right groin. The heart weighed 283 g, with no natural disease noted. The lungs showed congestion and oedema (left lung weight 667 g, right lung 767 g). The liver weighed 1289 g with a smooth capsular surface and unremarkable cut surface. Samples of a bottled liquid were recovered from the property and on subsequent examination found to be morphine but a low concentration (0.48 mg/ml) compared to Oramorph® oral solution (typically 2 mg/ml). Samples of blood (femoral blood vessels) and urine were collected for systematic toxicological analysis. Analysis of the femoral blood indicated the presence of the following substances:

alcohol 77 mg/100mL;
total morphine 6.1 mg/L, free morphine 3.85 mg/L (free:total ratio 0.63);
diazepam 0.412 mg/L, nordazepam 0.734 mg/L (parent:metabolite ratio 0.56), temazepam 0.038 mg/L.

The post-mortem examination showed no evidence of natural disease. Toxicology revealed a high concentration of morphine, with no 6-monacetylmorphine present in either blood or urine. In combination with the bottles of liquid morphine found it is suggestive of oral ingestion of morphine, however given the concentration of the liquid morphine it is likely that another source of morphine has also been ingested. The cause of death was thus recorded as the effects of a combination of morphine, alcohol and diazepam.

For case reports 1, 3 and 4, fatalities have been reported at morphine concentrations of 0.05-4 mg/L[1] and typically the likelihood of fatality is increased in the presence of alcohol and benzodiazepines, tolerance notwithstanding. For case 4, the Bayesian network produced a probability estimate for p(Hp) = 0.8, indicating that drugs were more likely than pathology to cause this death. This is in agreement with the reported cause of death.

Case report 5

A 39 year old male (height 175 cm, weight 88 kg, BMI 28.65) with a medical history including alcohol misuse, intravenous drug abuse and chronic pancreatitis collapsed and was conveyed to hospital with signs of tachycardia and jaundice. His medication included diazepam (2 mg) and tramadol (100 mg) with a previous prescription a year earlier for methadone (20ml daily, 1 mg/ml solution) although the latter was not re-issued. On admission to hospital a blood sample was obtained at 13:51 hrs and again at 14:09 hrs. He had a GCS of 11/15, temperature of 38.2^{o}C and rapidly deteriorated with raised troponin T and decreasing blood pressure. He suffered a cardiac arrest and was unable to be resuscitated. Life was pronounced extinct at 21:15 hrs and a post-mortem examination commissioned and carried out 3 days after death.

External examination indicated irregular scarring over the front of the arms, with needle puncture marks present in the right antecubital fossae on the back of the right forearm. The heart weighed 472 g with evidence of a hypertrophic left ventricle and a subendocardial haemorrhage affecting the aortic outflow tract. Congestion and oedema were present in the lungs (left lung weight 740 g, right lung 825 g). The liver weighed 1035 g with a markedly nodular capsular surface which was confirmed by the cut surface. Microscopically, the liver showed evidence of cirrhosis, little fat and some chronic inflammation in the expanded portal tracts in association with bile ductular proliferation.

Toxicological analysis of the blood samples taken in the ante-mortem period indicated the presence of the following substances:

Blood sample taken at 13:51 hrs (only the following drugs were reported in the toxicology report)

methadone 0.044 mg/L;
tramadol 1.49 mg/L, nortramadol 0.121 mg/L.

Blood sample taken at 14:09 hrs (only the following drugs were reported in the toxicology report)

diazepam 0.051 mg/L, nordazepam 0.155 mg/L

Samples of blood (femoral blood vessels) and urine were collected at post-mortem for systematic toxicological analysis. Analysis of the femoral blood indicated the presence of the following substances:

methadone 1.302 mg/L;
tramadol 5.895 mg/L, nortramadol 0.547 mg/L;
diazepam 0.053 mg/L, nordazepam 0.095 mg/L.

There was evidence of established cirrhosis of the liver with complications arising from this in the form of jaundice and probable oesophageal varices. There was long standing damage to the heart. The toxicological analysis of blood samples taken in life show a number of substances present at what was described as 'therapeutic' concentrations therefore given the pathological findings the cause of death was reported as 1a decompensated alcoholic cirrhosis of the liver. Given tramadol therapeutic drug concentrations are reported in the range of 0.1 to 0.8 mg/L[1, 40] it does raise the question over the reliability of the toxicological interpretation given to advise the pathologist when assigning a cause of death.

If the death certification was based on the ante-mortem drug concentrations then the Bayesian network produced a probability estimate for p(Hp) = 0.56. Since this means that p(Hp) and p(Hd) are virtually equal, the network is indicating uncertainty over the pathological and toxicological observations in this case, yet the only conclusion that can be drawn is that a toxicological cause of death is more likely than a pathological cause.

Using the post-mortem data the Bayesian network produced a probability estimate for p(Hp) = 0.91, indicating a toxicological cause of death is 'much' more likely than a pathological cause as opposed to that which has been reported.

Discussion

The decision of referral of a death to the coroner has been suggested by Mclean et al[18] to vary across different coronial jurisdictions in England and Wales. The current reforms to the coronial system and death certification recommend a reduction in the number of post-mortem examinations[25]. There is a distinct possibility that deaths where drugs have played a significant role in the death could be omitted. In the present pilot study, of the cases reported within the Sunderland Coronial jurisdiction, drugs that have been deemed as being toxicologically significant have been named on the death certificate in agreement with the recommendations of Davis[42]. It does remain though that in the current medical certification as to the cause of death there is no way to denote the priority given to each drug, or combination of drugs that have been detected. This may have an impact for public health in preventing any further deaths with specific drug combinations.

By using the Bayesian network it has been shown that in 4 of the 5 cases, the system agrees with the reported death certification and as such confers an increased level of confidence to the death certification process. As Drummer[43] points out the presence of natural disease in drug death does not always prove co-morbidity, but is important to recognise the contribution of natural disease in these cases. Whilst it is recognised that this is a small population, initial analysis using Hugin Researcher™, suggests it could be used to indicate which mechanism ie toxicology or pathology is more likely to have been observed. The primary limitation of this Bayesian network is that it does not 'learn' this combination effect and as such should not be used as a standalone diagnostic tool, especially as the probabilities indicated by the network are not absolute. As such the network could be adopted as a 'virtual' tool to assist the pathologist in their endeavour to reach an appropriate conclusion. In the other case analysed (case 5), whilst there is a natural cause of death reported, the significance of the toxicological results has not been reported in the cause of death. This has been previously reported in cases where the death has been assigned to an underlying pathological condition, notably cardiovascular disease, yet the toxicological examination reveals significant drug concentrations[21] and this case does seem to support this notion. It is proposed that the use of a Bayesian network may reduce the incidence of such cases and improve the confidence in the death certification process, whilst improving the robustness of mortality statistics. This in turn would likely have an impact for public health by potentially preventing any further deaths by specific or inappropriate drug combinations[26].

Case 5 is of note in that it unusually has both ante-mortem and post-mortem toxicology results. As previously reported drugs with a high volume of distribution (>3 L/kg)[44] have the potential for redistribution in the post-mortem period[45] with higher levels of drug associated in central vessels than in peripheral vessels. Drummer[39] indicates that whilst morphine is water soluble with a low volume of distribution (Vd 3-5 L/kg)[40], the synthetic opioid methadone (Vd ~4 L/kg)[40] is lipid soluble and is likely to exhibit an increase in concentration in the post-mortem period. Case 5 is no exception with methadone and tramadol (Vd ~3 L/kg)[40] exhibiting the redistribution artefact with diazepam (Vd 0.5-2.5 L/kg)[40] remaining stable in the same period. This case reinforces the significance of this phenomenon and its implications for post-mortem interpretation of drug concentration.

There is a significant increase of the drug concentrations found between the ante-mortem and post-mortem sampling in this case (3 days). Methadone exhibits a 29 fold increase from 0.044 mg/L to 1.302 mg/L (femoral vessel), tramadol exhibits a 3.9 fold increase from 1.49 to 5.895 mg/L which is as expected with the volumes of distribution of these drugs. Diazepam on the other hand with a lower volume of distribution remained largely unaffected with concentrations of 0.051 to 0.053mg/L consistent to that which has previously been reported[45,46]. Nevertheless, whilst the phenomenon of redistribution is well known, the degree and extent of the redistribution artefact remains undetermined in casework. Preliminary work in the late 1990s suggested that five amino acids (methionine, leucine, valine, glycine and serine) showed strong positive correlation to drug concentrations of the tricyclic antidepressant amitriptyline (known to undergo extensive redistribution due to its high volume of distribution) analysed in post-mortem pulmonary blood samples. The

results suggested that the amino acids, in particular methionine, may be a suitable marker for redistribution artefact for pulmonary drug shifts[13]. As of yet, a marker for this phenomenon has not been elucidated. Clearly, it would be of use to revisit whether there are appropriate markers to assist in post-mortem toxicological interpretation.

The Bayesian network presented here is recognised as limited however it will be expanded with further cases and a more elegant architecture to reduce the size of the convergent child node. It will further be extended to include the contribution of body mass index, gender, age and tolerance, the latter of which is of course subjective, but is recognised to play a significant role in the interpretation of the opiates/opioids[39]. One of the key functions in Hugin Researcher™ is the ability to generate simulated cases based on the conditional probabilities that have been assigned and with an increased population size, the system will become more robust in the probability estimates of whether a pathological or toxicological cause of death is more likely. Given that each drug has its own conditional probability on the outcome of the case the simulated effect of one drug or combination of drugs can then be assessed. As a concept, this preliminary data does appear to offer an additional mechanism to support the improvement of post-mortem toxicological interpretation.

Conclusion

As a pilot study, the potential of an expert system applied to post-mortem toxicology has been demonstrated. It has shown good agreement between cases reported where drugs were assigned as a cause of death but in those cases where both pathology and toxicology in combination have played a direct causal role as reported on the death certificate, the network can only offer a probability estimate as to whether either the pathology or toxicology were more likely to cause death, rather than a combination of the two. As such, it has illustrated the difficulty in how a case should be reported where there is a clear pathological cause of death but where drugs are also present at toxicological significant concentrations. It would be of use to establish a larger population of data to determine whether the individual contribution of a drug in multiple drug related fatalities can be assigned, notably in heroin or methadone related cases involving alcohol and benzodiazepines. It would also be of use at a national level to establish a clear demographic of the magnitude of drug related fatalities that are reported to the coroner and contrary to the recent recommendation of a reduction in post-mortem examinations provide support for more toxicological examinations to be routinely undertaken as recommended in the Luce report[19].

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