Antidote preparedness and toxic alcohol poisoning; cause, diagnosis and management

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SUMMARY

Background

Antidotes are an important part of the treatment of poisonings. For some poisonings, it is time-critical, and therefore necessary that the treatment is available when needed. The Norwegian recommendations for antidote stocking in hospitals are based on hospital size; small-, large- and regional hospital without the sizes being properly defined. It is not known whether hospitals comply with these recommendations.

The morbidity and mortality after poisoning with the toxic alcohols methanol and ethylene glycol are high. Ethylene glycol poisoning typically happens as individual events after intentional or unintentional ingestion. On the other hand, methanol poisoning often occurs as large outbreaks with many affected after ingestion of alcohol also containing methanol. This is especially apparent in countries with high consumption of unrecorded alcohol, such as homemade alcohol. It is unknown whether toxic amounts of methanol are formed during home distillation of alcohol from rice. The diagnosis of both these poisonings is particularly challenging due to nonspecific clinical features and limited access to the analytical methods to confirm the diagnosis. Early diagnosis can improve the prognosis if treatment is given early. Methods for rapid bedside diagnosis can contribute to this, but is still not available. Antidote together with dialysis is in most cases the standard treatment of care for both poisoning, fomepizole being the preferred antidote. Access to fomepizole is often a limitation due to the current price, and unnecessary high dosage should therefore be avoided.

This thesis aims to describe the antidote preparedness in Norwegian hospitals (paper I), further focusing on toxic alcohol poisoning in terms of cause (paper II), diagnostics (paper III and IV), and management (paper V).

Methods

To evaluate the antidote preparedness in Norwegian hospitals according to the national

recommendations, a survey was sent to all 50 Norwegian hospitals treating acute poisonings (paper I). We defined 31 hospitals as small, 15 as large and four as regional.

In paper II, we evaluated whether homemade alcohol distilled from rice could result in toxic amounts of methanol produced as a by-product. The production process was observed in 20 private households in a province in Northern Vietnam, a country known to have homemade alcohol production. The final product was then analysed for methanol.

The enzyme formate oxidase (FOX) can be used to measure the toxic metabolite formate for diagnosis of methanol poisoning. In paper III, we tested the sensitivity and specificity of a modified variant of this enzyme in aqueous solutions. Further, the specificity of point of care (POC)-model with this modified FOX-enzyme was evaluated. In paper IV, the POC-model described in paper III was used clinically in one patient with methanol poisoning.

Paper V is a prospective observational study of adult patients with suspected toxic alcohol poisoning treated with the antidote fomepizole and continuous renal replacement therapy (CRRT). Fomepizole plasma concentration was measured to evaluate whether the recommended dose gave plasma concentration above the minimum desired value of 10 µmol/L (0.8 mg/L) and to describe the pharmacokinetics of fomepizole during CRRT.

Results

Paper I

The response rate was 100 % as all hospitals responded. Only 22 % of the hospitals stocked the antidotes recommended for their hospital size. The 15 hospitals classified as large hospitals were the least compliant with the recommendations while the four regional hospitals were fully compliant.

Paper II

There were detectable levels of methanol in 85 % of the samples from private households. The highest concentration detected was 1.2 mmol/L (37 mg/L), which would require 424 litres of homemade alcohol to be drunk to achieve a toxic plasma concentration.

Paper III

The sensitivity of the modified FOX-enzyme in aqueous solution was 100 % for formate concentrations between 1-20 mmol/L (4.6-92 mg/dL), and the specificity was 97 %. When the POC-model was used bedside with samples from patients with metabolic acidosis (n=14) of various aetiology, no false positive results were obtained.

Paper IV

One patient with suspected methanol poisoning presented with visual disturbances, dyspnoea, and metabolic acidosis. One drop of whole blood was applied to the POC-model and the result showed high positive formate (> 10 mmol/L, 46 mg/dL). This was confirmed as 19 mmol/L (87 mg/dL) with gas chromatography mass spectrometry (GC-MS).

Paper V

The fomepizole concentration was above the minimum value of 10 µmol/L (0.8 mg/L) in 98 % of the plasma samples. CRRT clearance of fomepizole was 28 mL/min and the saturation coefficient/sieving coefficient was 0.85.

Conclusion

The antidote availability in Norwegian hospitals was variable as only one out of five followed the national recommendations. This, together with the fact that the size of the hospitals is not properly defined, indicate that it is time to revise these recommendations. Classification according to the urgency of availability instead of hospital size, as done in some other countries, should be considered. Homemade alcohol from uncontrolled rice distillation did contain methanol, but not in toxic amounts. This indicates that methanol poisoning after consuming homemade alcohol from rice, could be due to other reasons than the production process, for example by methanol being added post-production. A modified FOX-enzyme for detection of formate had a high sensitivity and specificity. When the POC-model was used bedside with samples from patients with metabolic acidosis, no false positives were registered. More data from clinical trials are needed before this POC-model can be used in clinical practice. We found that the new dosing regimens for fomepizole during CRRT was sufficient to obtain the minimum required plasma concentration of the antidote.

SAMMENDRAG

Bakgrunn

Antidoter er en viktig del av behandlingen ved forgiftninger. For enkelte forgiftninger er antidotbehandlingen tidskritisk, og det er derfor nødvendig at den er tilgjengelig når det er indikasjon for å starte behandling. De norske anbefalingene for lagerhold av antidoter i sykehus er organisert etter sykehusstørrelse; små, store, og region sykehus, uten at størrelsen på sykehusene er godt definert. Det er ukjent om sykehusene følger de nasjonale anbefalingene.

Morbiditet og mortalitet etter forgiftninger med de toksiske alkoholene metanol og etylenglykol er høy. Etylenglykolforgiftninger skjer vanligvis som enkelthendelser etter tilsiktet eller utilsiktet inntak. Derimot skjer metanolforgiftninger som store utbrudd med svært mange affiserte etter inntak av alkohol som også inneholder metanol. Dette forekommer spesielt i land med høyt forbruk av uregistrert alkohol, som for eksempel hjemmelaget alkohol. Det er imidlertid ikke kjent om toksiske nivåer av metanol dannes ved hjemme-destillasjon av alkohol fra ris. Det er spesielt utfordrende å diagnostisere begge disse forgiftningene på grunn av uspesifikke kliniske funn og begrenset tilgang til analytiske metoder for å bekrefte diagnosen. Tidlig diagnostikk kan forbedre prognosen dersom behandlingen blir igangsatt tidlig. Metoder for rask pasientnær diagnostikk kan bidra til dette, men er fortsatt ikke tilgjengelig. Sammen med dialyse er antidot i de fleste tilfeller standard behandling av begge forgiftninger, og fomepizol er foretrukket antidot. Tilgangen til fomepizol er ofte begrenset på grunn av høy pris, og behandling med unødvendig høy dose bør derfor unngås.

Denne avhandlingen har som mål å beskrive antidotberedskapen ved norske sykehus (artikkel I), og deretter fokusere på toksiske alkoholforgiftninger i forhold til årsak (artikkel II), diagnostikk (artikkel III og IV) og behandling (V).

Metoder

For å evaluere antidotberedskapen ved Norske sykehus mot de nasjonale anbefalingene, ble en spørreundersøkelse sendt til 50 sykehus som behandler akutte forgiftninger (artikkel I). Vi definerte 31 sykehus som små, 15 som store og fire som regionsykehus.

I artikkel II undersøkte vi om hjemmelaget alkohol destillert fra ris dannet toksiske mengder metanol som et biprodukt. Produksjonsprosessen ble observert i 20 private husholdninger i en provins i Nord Vietnam, et land kjent for omfattende produksjon av hjemmelaget alkohol. Sluttproduktet ble analysert for innhold av metanol.

Enzymet format oksidase (FOX) kan brukes til å måle den toksiske metabolitten maursyre (format) i diagnostikken av metanolforgiftninger. I artikkel III undersøkte vi sensitivitet og spesifisitet av en modifisert variant av dette enzymet i vandig løsning. Videre evaluerte vi spesifisiteten av en pasientnær/«point of care» (POC)-modell med dette modifiserte enzymet. I artikkel IV, ble POC-modellen som beskrevet i artikkel III brukt klinisk på en pasient med metanolforgiftning.

Artikkel V er en prospektiv observasjonsstudie på voksne pasienter med mistenkt toksisk alkoholforgiftning som fikk behandling med antidotet fomepizol og kontinuerlig dialyse (CRRT). Plasmakonsentrasjonen av fomepizol ble målt for å evaluere om doseringsanbefalingene ga en konsentrasjon over ønsket verdi på 10 µmol/L (0,8 mg/L), og for å beskrive farmakokinetikken av fomepizol ved samtidig CRRT.

Resultat

Artikkel I

Svarprosenten var 100 % da alle sykehusene svarte på undersøkelsen. Det var bare 22 % av sykehusene som lagret alle antidotene i henhold til anbefalingene for sin sykehusstørrelse. De 15 sykehusene som var klassifisert som store sykehus hadde dårligst etterlevelse i forhold til anbefalingene, mens alle de fire regionsykehus fulgte anbefalingene.

Artikkel II

Metanol ble detektert i 85 % av prøvene fra private husholdninger. Den høyeste konsentrasjonen som ble målt var 1,2 mmol/L (37 mg/L), som betyr at 424 liter hjemmelaget alkohol må å drikkes for å oppnå en toksisk plasmakonsentrasjon.

Artikkel III

Sensitiviteten av det modifiserte FOX-enzymet var 100 % for maursyre konsentrasjoner mellom 1-20 mmol/L (4,6-92 mg/dL), og spesifisiteten var 97 %. Når POC-modellen ble brukt med prøver fra pasienter med metabolske acidoser av forskjellige årsaker, fant man ingen falske positive prøver.

Artikkel IV

En pasient med mistenkt metanolforgiftning innkom med synsforstyrrelser, dyspné og metabolsk acidose. En dråpe fullblod ble påført POC-modellen og viste høy positiv maursyre (> 10 mmol/L, 46 mg/dL). Dette ble også bekreftet som 19 mmol/L (97,4 mg/dL) med gasskromatografi-massespektrometri (GC-MS).

Artikkel V

Plasmakonsentrasjonen av fomepizol var over ønsket verdi på 10 µmol/L (0,8 mg/L) i 98 % av plasmaprøvene. CRRT-clearance av fomepizol var 28 mL/min og metnings koeffisienten/sieving-koeffisienten var 0,85.

Konklusjon

Tilgjengeligheten av antidoter ved norske sykehus var varierende, og det var kun ett av fem sykehus som fulgte de nasjonale anbefalingene for sin sykehusstørrelse. Dette, sammen med det faktum at størrelse på sykehusene ikke er godt nok definert, indikerer at det kan være på tide å revidere disse anbefalingene. Klassifisering etter hastegrad for å gi antidot i stedet for sykehusstørrelse, hvilket gjøres i enkelte andre land allerede, bør vurderes. Hjemmelaget alkohol laget ved ukontrollert destillasjon av ris inneholdt metanol, men ikke i toksiske mengder. Dette indikerer at årsaken til metanolforgiftninger etter å ha drukket hjemmelaget alkohol fra ris kan skyldes andre årsaker enn produksjonen, for eksempel at metanol blir tilsatt etter produksjon. Et modifisert FOX enzym for deteksjon av maursyre hadde høy

sensitivitet og spesifisitet. Da POC-modellen ble brukt med prøver fra pasienter med metabolsk acidose, fant man ingen falsk positive prøver. Mer data fra kliniske studier er nødvendig før denne POC-modellen kan brukes i klinisk praksis. Vi fant at de nye doseringsanbefalingene for fomepizol og CRRT ga ønsket plasmakonsentrasjon av fomepizol.

LIST OF PUBLICATIONS

I. **A survey of the antidote preparedness in Norwegian hospitals**

Lao YE, Goffeng A, Spillum BJ, Jacobsen D, Nakstad ER, Hovda KE

European journal of hospital pharmacy. 2021:ejhpharm-2020-002544

DOI: https://doi.org/10.1136/ejhpharm-2020-002544

II. **Methanol content in homemade alcohol from a province in North Vietnam**

Lao Y, Pham BD, Le HT, Nguyen Van H, Hovda KE

Drug and alcohol review. 2019 Jul;38(5):537-542.

DOI: https://doi.org/10.1111/dar.12937

III. **An enzymatic assay with formate oxidase for point-of-care diagnosis of methanol poisoning**

Lao YE, Heyerdahl F, Jacobsen D, Hovda KE

Accepted for publication in Basic & Clinical Pharmacology & Toxicology

IV. **Formate test for bedside diagnosis of methanol poisoning**

Hovda KE, Lao YE, Gadeholt G, Jacobsen D

Basic Clin Pharmacol Toxicol. 2021;129(1):86-8

DOI: https://doi.org/10.1111/bcpt.13597

V. **Fomepizole dosing during continuous renal replacement therapy, an observational study**

Lao YE, Vartdal T, Froeyshov S, Latimer B, Kvaerner C, Mataric M, Holm P, Foreid S, Jacobsen D, McMartin K, Hovda KE

Clin Toxicol (Phila). 2021:1-7

DOI: https://doi.org/10.1080/15563650.2021.1980581

LIST OF ABBREVIATIONS AND CONVERSION FACTORS

ADH: alcohol dehydrogenase

AG: anion gap

ALDH: aldehyde dehydrogenase

CNS: central nervous system

C_p: Target toxic plasma concentration methanol

CRRT: continuous renal replacement therapy

CVVHD: continuous veno-venous haemodialysis

CVVHDF: continuous veno-venous hemodiafiltration

D: dialysate concentration

DMPS: dimercaptopropanesulphonate

DMSA: dimercaptosuccinic acid

EG: ethylene glycol

F: bioavailability

FDA: United States Food and Drug Administration

FDH: formate dehydrogenase

FOX: formate oxidase

GC: gas chromatography

GC-MS: gas chromatography mass spectrometry

ICU: intensive care unit

IHD: intermittent haemodialysis

IPCS: The international Programme on Chemical Safety

k_e: elimination constant

LMIC: low- and middle-income countries

OG: osmolal gap

P: plasma concentration

POC: point-of-care

Q_D: dialysate flow rate

Q_E: effluent flow rate

Q_{UF}: ultrafiltration flow rate

RRT: renal replacement therapy

TBC: total body clearance

$T_{1/2}$: plasma half-life

UK: United Kingdom

US: United States

Vd: volume of distribution

4-CP: 4-carboxypyrazole

4-OHMP: 4-hydroxomethylpyrazole

Conversion factors from mmol/L to mg/dL for ethanol, formate and methanol are 4.6, 4.6 and 3.2. For fomepizole the conversion factor from µmol/L to mg/L is 0.08.

1. BACKGROUND

1.1. Antidote preparedness

When antidote treatment is indicated for a poisoning, it is necessary that the treatment is available and started in time. Several studies have reported insufficient stocking of antidotes in different countries (1-10). Recommendations for antidote stocking are important tools in the emergency preparedness work to ensure good antidote availability. In Europe, there are no common guidelines, and each country must create its own. The International Programme on Chemical Safety (IPCS), United States (US) and United Kingdom (UK) have classified their recommendations according to the urgency of availability (11-13).

1.1.1. Norwegian recommendations for antidote stocking

A survey from 2002 described the antidote preparedness in Norway as unsatisfactory with lack of recommendations (14). The Norwegian Poison Information Centre published national recommendations for antidote stocking in hospitals in 2007 and classified them according to hospital size; “recommended in all hospitals”, “addition for large hospitals” and “addition for regional hospitals”. Previously, the hospitals in Norway were classified as local hospitals, central hospitals and regional hospitals. These terms are no longer in use and the recommendations as such are outdated. Today we have four regional health trusts; South-East, West, Central-Norway and North where each region consists of several local hospital trusts. There are six university hospitals in the country. Among these, South-East and West have two university hospitals each. Except for the terminology “university hospital”, there is no official definition of hospital size. The recommendations are continuously updated regarding recommended antidotes, but they are still categorized according to an outdated classification system for hospitals as described above and without the hospital size being defined. Furthermore, there are no recommended minimum quantities of each antidote (15). Since 2002, no new survey has been conducted and it is therefore unknown whether the hospitals are compliant with the national recommendations or not.

1.2. Toxic alcohols

One of the poisonings where antidote is an important part of the treatment are toxic alcohols. Due to their toxicity, toxic alcohols are chemicals not meant for oral consumption. Methanol, ethylene glycol (EG), isopropanol, diethylene glycol and propylene glycol are all defined as toxic alcohols. In this thesis, the term toxic alcohol will include methanol and EG, and the main focus will be on methanol poisoning.

A toxic alcohol poisoning occurs after ingestion of methanol or EG, which then is metabolized to toxic substances that cause the clinical features. Methanol and EG poisonings have several similarities regarding metabolism, clinical course and treatment. The overall mortality and risk of permanent sequelae is high, typically reported to be between 29-46 % and 10-18 % (16-18), if treatment is initiated late or is absent (16, 17, 19-26). Methanol poisoning causes little or no inebriation, but it is rather characterized by a latency period of 12-24 hours after ingestion followed by nausea, vomiting, chest pain, hyperventilation, metabolic acidosis and visual disturbances (16, 21, 24-26). Coma, respiratory and circulatory failure can develop without treatment (21, 24, 25). EG poisoning on the other hand initially creates an inebriating effect with a central nervous system (CNS) depression. A shorter latency period (compared to methanol) of 4-12 hours follows before the symptoms typically occurs (21-23, 26). Untreated it will typically progress to coma, convulsions and renal failure (22, 23).

1.3. History

In the literature, methanol has been used as far back as ancient times by the Egyptians. It was not until 1856 that toxicity was mentioned following occupational exposure, and the ocular effects were mentioned (27). The first cases of methanol poisonings were seen at the end of the 19th century. Before that, methanol production originated from wood distillation which gave a product unsuitable for drinking due to its bad smell and taste (28). The discovery of a new and cheap production method yielded a purer product that was drinkable, and which could be used as a substitute for the more expensive ethanol (28). Following this, several cases of blindness and death were reported in Europe and the US after ingestion of methanol (28). However, there was much disagreement as to whether the

cause of the poisoning was methanol itself or impurities in the product. In 1922, there was a large outbreak of methanol poisoning in Hamburg (28). The following year the methanol was found to be pure, and thus impurities could not have caused the poisoning (28).

The latency period was described early, but the hypothesis of the metabolite being the toxic component was not supported until 1953 in a review by Bennet (29). Conflicting results in animals was one of the main reasons why it took a long time to find the cause and mechanism of toxicity. In 1955, Roe concluded that the findings in animal studies were not consistent with what was seen in clinical practice, and that this could be caused by different metabolism of methanol in animals and humans (30). Initial studies were therefore conducted in monkeys (31, 32), which were then used as a model for methanol poisoning in humans (21). In 1975 it was concluded that the accumulation of formic acid was the cause of the metabolic acidosis (32), and this was later confirmed in humans (33, 34).

Regarding the treatment of methanol poisoning, Harrop and Benedict already discovered in 1920 that a methanol poisoned patient was acidotic, and thus gave treatment with bicarbonate (28). The importance of treating the metabolic acidosis was nevertheless not recognized until 1946 bye Roe (28). As early as the beginning of the 20th century, the discussion about the effect of treatment with ethanol was started, but this was also not recognized until the 1940s by Roe (28). Removal of methanol by haemodialysis in humans was first documented in 1961 (35). In 1969 it was shown in vitro that 4-methylpyrazole (fomepizole) inhibited the enzyme alcohol dehydrogenase (ADH) (36), the enzyme being responsible for the first step in the metabolism of methanol. The first case reports of fomepizole use in patients for EG poisoning were published in the 1980s (37, 38) and in the 1990s for methanol poisoning (39). The clinical trials for fomepizole treatment in EG and methanol poisoning were conducted between 1995-1997 (40, 41) and the United States Food and Drug Administration (FDA) then approved fomepizole for methanol poisoning (in 2000). The potential role of folate therapy in reducing the formate concentration by increasing its metabolism was documented by Noker et al. in 1980 (42).

EG was first synthesized in 1859 by a French chemist. The coolant effect was discovered in the early 20th century and has since been used in antifreeze products and cooling water in

cars. Initially it was thought to be non-toxic and it was also used as a pharmaceutical excipient (43). It was used as a substitute for ethanol because of easy access, a sweet taste and the inebriating effect. It was not until 1930 the first case of EG poisoning was reported (44). The clinical effect of haemodialysis was documented in late 1950s (45) and ethanol as treatment for EG poisoning was recommended in 1965 (46). The approval of fomepizole by FDA for this indication came in 1999.

1.4. Methanol

Methanol (CH_3OH) is a clear, colourless and volatile liquid with an odour similar to ethanol, which is easily miscible with the other alcohols (47). Methanol is an industrial alcohol that is also used as an environmentally friendly fuel. It is commonly added to consumer products, for example paint and cosmetics. Poisoning occurs by ingestion of methanol, which is then metabolized to the toxic metabolite formic acid that causes the clinical features (21, 24-26). The metabolism is by multiple steps (Figure 1). First slowly by ADH to formaldehyde (CH_2O) and then rapidly by aldehyde dehydrogenase (ALDH) to formic acid (HCOOH). With a pKa value of 3.75 it will almost completely dissociate to the corresponding anion formate at physiological pH. Formate is then metabolized to CO_2 and water. This latter reaction is folate dependent, and the limited amounts of folate in humans leads to formate accumulation (21). This causes the metabolic acidosis (33, 34) and the ocular toxic effects (48) that are the classic signs and symptoms of methanol poisoning. Formaldehyde is also toxic, but is not thought to have a role in methanol poisonings due to its short half-life (49).

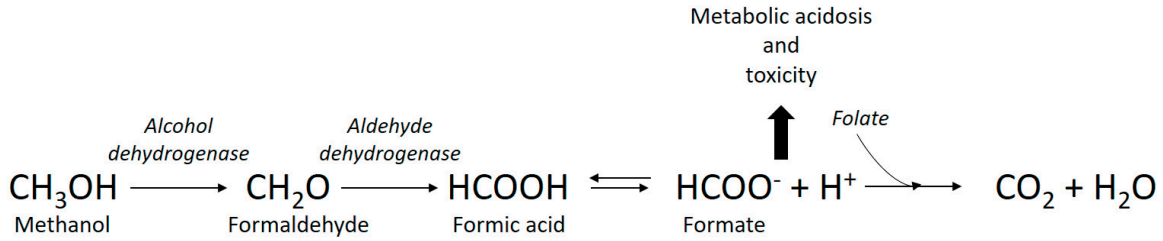

Figure 1: Metabolism of methanol

1.5. Ethylene glycol

EG ($C_2H_4(OH)_2$) is a colourless and odourless liquid. Compared to methanol it has a sweet taste and will give an inebriating effect similar to ethanol. It is found in many consumer products and is perhaps best known for its use as an antifreeze. EG poisoning occur after ingestion of EG which is then metabolized by multiple steps to toxic metabolites. The first step by ADH to glycolaldehyde, further by ALDH to glycolic acid (Figure 2) (21-23, 26). The latter (with a pKa of 3.83) will almost completely dissociate to glycolate and give a metabolic acidosis. In addition, glycolic acid will be partly converted to oxalic acid that will immediately dissociate and bind calcium to form insoluble calcium oxalate crystals responsible for the renal toxicity (23, 50).

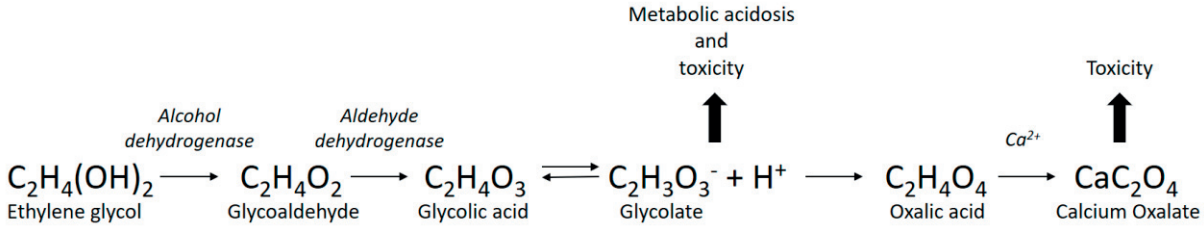

Figure 2: Metabolism of ethylene glycol

1.6. Epidemiology

Most cases of methanol poisonings occur after ingestion, but single cases of toxicity after exposure by inhalation (51, 52) and skin (53, 54) are also reported. Methanol poisonings can be sporadic single cases or a mass outbreak. Individual cases can be suicidal ingestion or unintentional ingestion in children. Mass outbreaks have been seen all over the world (16-18, 55, 56), but most frequently occur in low- and middle-income countries (LMIC) (56). During the COVID-19 pandemic, there have also been single cases and massive outbreaks of methanol poisonings due to ingestion of hand sanitizers containing methanol (57-59).

A common underlying factor for outbreaks of methanol poisoning is that they occur after ingestion of unrecorded alcohol, for example homemade or illegal alcohol. In Norway, a country with high alcohol taxes, illegal alcohol diluted with methanol led to outbreaks in 1979 and in 2002-2005 (16, 60), as well as small “outbreaks” seen from time to other. Even

in countries like the Czech Republic and Estonia where alcohol prices are lower, there have been cases of contamination with cheaper methanol leading to massive outbreaks (17, 18). The alcohol in these outbreaks contained varying amounts of methanol from 20 to 100 % (16-18). In countries where alcohol is banned, like Libya and Iran, there is typically an illegal market that has led to major outbreaks due to alcohol contaminated with methanol (56, 61). Indonesia faces methanol poisonings of an almost endemic character, whereas e.g., India, Cambodia, Vietnam, Turkey, and Bangladesh are other countries where this occurs on a seemingly frequent epidemic basis (62).

Homemade alcohol production by fermentation and distillation for small-scale production or for making traditional beverages is common in several countries. As an example, Kenya had one of several large outbreaks in 2014 (56). In addition to illegal alcohol consumption, they have the traditional homemade spirit Chang'aa, where methanol is added to give it a "kick". There is often believed that poor distillation of ethanol is the cause of methanol poisoning in cases where homemade alcohol was the source of the poisoning. The boiling point of methanol (65°C) is lower than ethanol (78°C). Without sufficient knowledge of the distillation process and equipment for temperature control, some methanol will be evaporated in the distillation process before the temperature is sufficiently high during the distillation process. The raw material in the production is also important. Methanol is produced from pectin (63), which are abundant in fruit. The fermenting microbes that produce pectin methylesterase will promote this process (64). Although the final product contains some methanol, it is not typical that the amount is toxic.

In contrast to methanol, EG poisonings are often isolated cases of self-harm/suicidal poisonings or children (or animals) accidently drinking it because of its sweet taste (22). Nevertheless, large outbreaks of intentional ingestions have also been reported (65). In general, methanol poisoning occurs in LMIC, while EG poisoning typically occurs in high-income countries or where anti-freeze is commonly used.

1.7. Diagnosis

1.7.1. Methanol

Known exposure history, clinical features, physical examinations, and laboratory investigations may lead to suspicion of methanol poisoning. In many cases, there is no known exposure, the patient is comatose, and the clinical features of methanol poisoning are often non clear or specific (21, 24, 25). The gold standard for diagnosis is the measurement of plasma methanol by gas chromatography (GC) (24), which is available in large hospitals in high-income countries. However, this is rarely available as a 24-hour service. In the absence of this or pending the result, the measurement of anion gap (AG) and osmolal gap (OG) are often used for diagnostic purposes (66, 67). Methanol will increase the osmolality, and the osmolal gap will be elevated in the early phase of the poisoning (Figure 3) (67). With increasing metabolism with molar equivalent formation of formate, the OG will decrease, and the AG increase due to accumulation of formate (67). This is a very commonly used strategy, but as an indirect diagnostic method, it has is limitations: Other alcohols will increase the OG, and if ethanol is congested, its contribution must be subtracted. Other conditions such as diabetic- and alcoholic ketoacidosis (68, 69), and to a lesser extent chronic kidney disease (70), can also increase the OG and AG. In addition, severely ill patients with multiple organ failure will release substances that can contribute to the OG (71, 72). Measurement of the toxic metabolite formate by an enzymatic/spectrophotometric method is an alternative to methanol detection (73, 74). This is still a method that requires access to trained laboratory personnel, and the availability of this in hospitals is therefore limited. In countries where outbreaks of methanol poisoning occur, there is no or very limited access to any of these analytical methods, and the diagnosis of methanol poisoning thus becomes very difficult (56, 61, 75). Early initiation of treatment is necessary to minimize morbidity and mortality and the development of a simpler method for diagnosis could therefore be lifesaving.

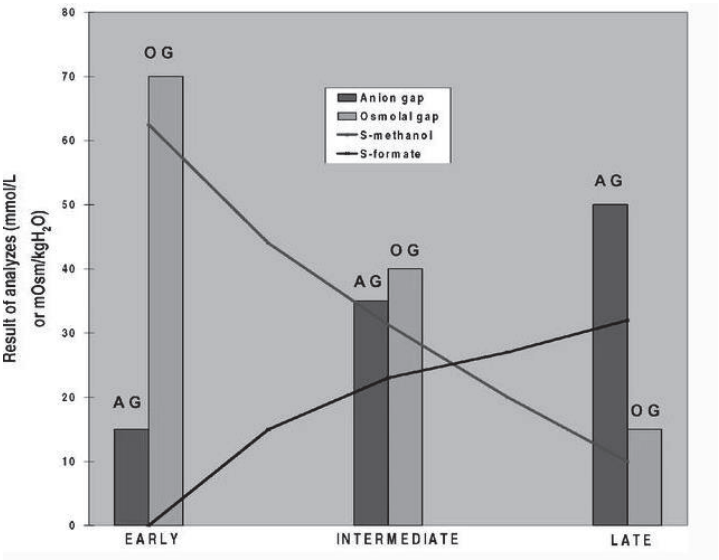

Figure 3: Change of osmolal gap (OG) and anion gap (AG) during the different stages of methanol poisoning. Figure from Hovda et al. (67), reprinted with permission.

1.7.2. New methods for diagnosis of methanol poisoning

New methods for methanol detection using enzymes or oxidizing agents have been investigated (76-78). None of the methods using only one enzyme or oxidizing agent have been promising, since it has not been possible to distinguish between methanol and ethanol if both are ingested.

A novel assay for detection of formate using formate dehydrogenase enzyme (FDH) has been developed (79). A point-of-care- (POC) model with this enzyme enables fast bedside diagnostic from a single drop of blood. Unfortunately, this enzyme is not stable in room temperature over time. Therefore, cool storage and transport is required, making it inconvenient and expensive. The enzyme formate oxidase (FOX) is on the other hand stable in room temperature, but the sensitivity and specificity of this enzyme have not yet been systematically tested.

A POC-model with a modified FOX-enzyme requiring only one drop of whole blood, plasma or serum has been developed. The presence of formate will give a visual colour that can be compared to a colour scale for semi-quantitative detection of formate: negative (corresponding to < 1mmol/L, 4.6 mg/dL), low positive (1-10 mmol/L, 4.6-46 mg/dL) and high

positive (> 10 mmol/L, 46 mg/dL). The specificity of the POC-model and its clinical applicability are not known.

1.7.3. Ethylene glycol

Detection of EG in biological fluid can be performed with an enzymatic- (80) or chromatographic method (81). Like methanol poisoning, AG and OG may be used as an indirect method for diagnosis (21-23). Presence of EG will increase the OG, which then gradually decreases when EG is metabolized. In the early phase of the poisoning, the AG is normal, while it increases as the metabolite glycolate is formed (22, 23). Microscopy of the urine for calcium oxalate crystals can also be used to support the diagnosis, but a positive finding alone is not enough to make the EG diagnosis, and crystals are not always apparent, even in the severe cases (21-23). The “lactate gap” is sometimes uses as a surrogate marker for EG poisoning: Arterial blood gas analysers that use lactate oxidase are usually not able to separate lactate from glycolate and will typically give a falsely increased result. Some lactate analysers are more specific, e.g., gas chromatography mass spectrometry (GC-MS) methods, lactate dehydrogenase methods etc. The “lactate gap” can therefore indirectly indicate the presence of the toxic metabolite glycolate (82, 83). Compared to the diagnosis of methanol poisoning, EG poisoning is somewhat less challenging due to the availability of both non-specific and specific methods in high-income countries where most EG poisonings occur.

1.8. Treatment of methanol and ethylene glycol poisoning

The treatment consists of blocking the metabolism of methanol to formate and EG to glycolate with an antidote, correction of the metabolic acidosis with intravenous sodium bicarbonate, and removing the toxic alcohol and its metabolites with haemodialysis (21, 22, 25, 26). The dialysis will also correct the acidosis. Further, the use of folic or folinic acid is used in methanol poisoning to theoretically increase the endogenous metabolism of formate to non-toxic compounds (CO_2 + H_2O) (24, 25), despite a lack of clinical trial evidence. A combination of the above is typically given to the patients, in addition to supportive treatment.

1.8.1. Antidote treatment

*Ethano*l inhibits the metabolism of the toxic alcohols by having a greater affinity for the ADH-enzyme compared to methanol and EG (22, 25, 26). Reduced production of the toxic metabolites has been demonstrated, but because of combination with other treatment, the documentation of ethanol's effect alone has been limited (55, 84-88). Due to the competition for the ADH-enzyme, the molar ethanol concentration in plasma should be at least a quarter of the molar concentration of the toxic alcohol in plasma (89). For simplicity, a concentration of 22 mmol/L (100 mg/dL) has been recommended as a target, and it should be monitored every 1-2 hours to ensure optimal effect (21, 22, 25, 26, 88).

Fomepizole (4-MP) is a competitive inhibitor of ADH (36). The minimum plasma concentration required to inhibit the metabolism of methanol and EG is 10 µmol/L (0.8 mg/L), as shown in studies on non-human primates (90). Therapeutic drug monitoring of fomepizole is not necessary. The treatment effect of fomepizole has been documented, but without control groups it has not been possible to distinguish between the effect of fomepizole and dialysis (40, 41, 91, 92). When comparing the effect of ethanol versus fomepizole, no difference in outcome has been found (18, 93). Fomepizole is still recommended as the drug of choice due to less serious side effects, particularly in pregnant women and children, and because there is no need for therapeutic drug monitoring (22, 25, 26). In the absence of access to fomepizole, ethanol is recommended orally or intravenously (22, 25, 26). This is often the case in LMIC, which unfortunately also typically do not have access to plasma ethanol analysis to monitor the treatment (61).

The recommended fomepizole dose is 15 mg/kg as a loading dose, followed by 10 mg/kg every 12 hours with dose increase to 15 mg/kg from the fifth dose onwards (26, 88, 94). The need for the dose increase is assumed to be due to fomepizole's auto-induction of its own metabolism. Renal replacement therapy (RRT) removes fomepizole (95, 96); it is therefore recommended to give the maintenance dose every 4 hours or as continuous infusions of 1 mg/kg/hour during RRT (88, 94). In the dosing recommendations from Brent (94), no distinction was made between intermittent and continuous renal replacement therapy (CRRT). Based on the theory of less drug removal during CRRT compared to intermittent

haemodialysis (IHD) and one case report, McMartin et al. have suggested that the maintenance dose should be given every 8 hours or that the continuous infusion should be reduced to 0.5 mg/kg/hour (88). Apart for one case report published as a conference abstract (97) (now included in the present material), there are no available data to support that these dosage recommendations achieve the desired plasma concentrations of fomepizole above 10 µmol/L (0.8 mg/L).

Theoretically, *folate* treatment (in the form of folic- or folinic acid) may be beneficial in methanol poisoning, but the only human data are case reports and case series (98). The good safety profile of folate indicates a low risk by giving this treatment. In particular, It should be considered in the absence of access to other treatment or in patients where folate deficiency is suspected (98).

1.8.2. Dialysis

Haemodialysis removes methanol, EG and their toxic metabolites formate and glycolate, respectively (21, 85, 99-102). For methanol poisoning, IHD is preferred over CRRT in terms of methanol and formate removal (103), as well as time to correct acidosis (104). No difference in case fatality or survival with visual/CNS sequelae has, however, been demonstrated (105). EG, unlike methanol, has a significant renal excretion (approximately 20 %), in addition to liver metabolism (22). Therefore, EG-poisoned patients with normal renal function receiving early treatment with fomepizole do not necessarily need dialysis (106, 107). In a recently published systematic review (108), monotherapy with fomepizole has now been suggested in patients with moderate acute kidney injury and/or metabolic acidosis.

2. AIM OF THE THESIS

The overall aim of this thesis consists of two parts:

1) To describe the general antidote preparedness in Norwegian hospitals.
2) To add to the knowledge of toxic alcohol poisoning in terms of the cause, diagnostics and the management.

2.1. Paper I

Antidotes are an important part of the treatment of poisonings. For some poisonings, the treatment is time-critical, and the antidote must be available at the hospital. Our experience from clinical practice is that some hospitals do not have time-critical antidotes available, and that the national recommendations are not followed. The aim of this paper was to:

1. Describe the antidote preparedness in Norwegian hospitals.
2. Examine the hospitals' compliance with the national recommendations.

2.2. Paper II

Individual cases and large outbreaks of methanol poisoning are frequent in countries with unrecorded alcohol consumption. It is necessary to understand the cause of these poisoning when drinking for example homemade alcohol, in order to take preventive measures. Therefore, the aims were as follows:

1. Investigate whether homemade alcohol distilled from rice could produce toxic amounts of methanol.
2. Compare the results with other studies of homemade alcohol.

2.3. Paper III

There is a profound lack of simple tests for the diagnosis of methanol poisoning, especially for bedside (POC) use. An enzymatic method for detection of the toxic metabolite formate with the use of a FOX-enzyme is a possible solution, and a POC-model with this modified enzyme has recently been developed. This led to the following aims:

1. Test the sensitivity and specificity of this modified FOX-enzyme.
2. Test the specificity of a POC-model using this modified FOX-enzyme.

3. Test the clinical applicability of this POC-model.

2.4. Paper IV

The POC-model described in paper III has not been used clinically in patients with methanol poisoning. The aim was to describe the use of the POC-model in a patient with suspected methanol poisoning.

2.5. Paper V

Fomepizole is removed by RRT, and the dose must therefore be increased. Recent recommendations have suggested a lower dosing of fomepizole during CRRT as compared to IHD. This is based on theoretical considerations and one single case report. Therefore, the following aims were defined:

1. Examine whether the newly proposed dose recommendations for fomepizole during CRRT achieve a plasma concentration above 10 µmol/L (0.8 mg/L), the level that inhibits toxic metabolite formation.
2. Examine the elimination kinetics of fomepizole during CRRT.

3. MATERIAL AND METHODS

3.1. Paper I

Study design and participants

This study was a survey of the antidote preparedness in Norwegian hospitals that treat patients with acute poisoning. We compared the findings with the national recommendations for stockpiling of antidotes in hospitals (15). A digital survey was sent to all 50 hospitals treating acute poisoning.

Method

The national recommendations for antidote stockpiling in hospitals are categorized by hospital size; “all hospitals”, “addition for large hospitals” and “addition for regional hospitals” (Table 1). These recommendations do not properly define the size of a hospital. To compare with the national recommendations, we divided the hospitals in three groups: “small hospital”, “large hospitals” and “regional hospitals”. We defined a regional hospital as the university hospital in each of the four health regions. In the two regions with more than one university hospital, the largest was chosen as the regional hospital and the other defined as a large hospital. Furthermore, we defined a large hospital as the one with the largest population catchment area in each local hospital thrust (109). If this was the regional hospital, the next largest was defined as large. The remaining hospitals were defined as small and should therefore adhere to the antidote recommendations for “all hospitals”. This resulted in 31 hospitals defined as small hospitals, 15 as large hospitals and four as regional hospitals.

The digital survey was sent by email in April 2016 to a pharmacist in those cases where the hospital pharmacy was responsible for the follow-up of the antidote storage. Otherwise, it was sent to a nurse or doctor with similar responsibility. Two reminders by email were sent to those who did not respond and eventually contacted by phone if no response. The survey was closed in June 2016. All hospitals were asked about the availability of 35 antidotes according to the national recommendations at that time (Table 1).

Table 1: National recommendations for antidote stockpiling in Norwegian hospitals from April 2016, which are categorized after hospital size and without a recommended minimum stock (15).

Recommended in all hospitals	**Additional recommendations for large and regional hospitals**	**Additional recommendations for regional hospitals**
Acetylcysteine	Calcium folinate injection	Dimercaptopropanesulphonate (DMPS)
Activated charcoal	Cyproheptadine	Dimercaptosuccinic acid (DMSA)
Atropine	Dantrolene	Penicillamine
Biperiden	Deferoxamine	Prussian blue
Calcium gluconate (local and intravenous)	Digoxin immune FAB (DigiFab®)	Sodium calcium edetate
Ethanol	Fomepizole	
Flumazenil	Methylthioninium chloride	
Glucagon	Obidoxime	
Hydroxocobalamin	Octreotide	
Ipecac syrup	Pyridoxine injection	
Lipid emulsion	Silibinin	
Naloxone	Sodium thiosulfate	
Physostigmine	*Vipera berus* antivenom (ViperaTab®)	
Phytomenadione		
Protamine sulfate		
Sodium sulfate		

Ethical considerations

The survey did not contain any personal information and ethical approval was not relevant for this kind of study. The participants consented to respond to the survey in advance.

3.2. Paper II

Study design and participants

This observation study was conducted in Phu Tho province in northern Vietnam, because this is a province known for its high incidence of homemade alcohol production. In addition, Phu Tho General hospital has treated cases with methanol poisoning. This is a province with both urban and rural/mountain areas and therefore two different communes were chosen to participate: one urban and one rural/mountain. Within each commune, 10 private households with known homemade alcohol production were included. This number was chosen based on convenience.

Methods

The production process of homemade alcohol takes several days. Therefore, the research group observed part of the production and the other parts only demonstrated. Some households had ongoing production in several stages, and for these households more of the process was observed. The production from rice by distillation was carried out without any kind of temperature control. From each household, a batch of one litre was collected and analysed for the content of ethanol and methanol. For comparison, 5 bottles of rice alcohol from each commune that were sold by street vendors/restaurants were also analysed for the same substances. This gave a total of 30 samples.

Calculations and statistics

The methanol toxicity of the homemade alcohol was estimated by calculating the volume necessary for a person weighting 70 kg to drink in order to reach a toxic plasma concentration. The following equations were used

$$Dose = \frac{Cp \times Vd}{F}$$

$$Volume = \frac{Dose}{Methanol\ concentration}$$

Where Cp is the target toxic plasma concentration of methanol, Vd the volume of distribution and F the bioavailability. We defined 10 mmol/L (32 mg/dL) as a toxic plasma concentration, the level where antidote treatment is recommended (88). The Vd used was

0.7 L/kg (24) and, for simplification, bioavailability was set to 1.0. If the bioavailability is lower, the corresponding volume to drink will be larger.

In order to compare the concentration of ethanol and methanol between the homemade alcohol and the bottles bought from street vendors/restaurants, a Mann-Whitney test was used.

Ethical considerations

Ethical approval was not relevant for this kind of study, but approval from the head of health service in the province was obtained.

3.3. Paper III

The FOX-enzyme can be used in a POC-model for detection of formate. This paper describes the experiments with a modified FOX-enzyme in aqueous solutions and with a POC-model containing this enzyme.

Sensitivity and specificity testing of the FOX-enzyme

In order to test the sensitivity of the modified FOX-enzyme in aqueous solutions, nine concentrations of formate between 1-20 mmol/L (4.6-92 mg/dL) were tested. Since a threshold value of 1 mmol/L (4.6 mg/dL) was chosen to separate toxic concentrations from endogenous levels, an additional test for the concentrations 0, 0.5, 1.5 and 2.0 mmol/L (0, 2.3, 6.9 and 9.2 mg/dL) was conducted. In addition, the specificity in aqueous solutions was tested against 18 different substances that could potentially interfere or be present in the target patient group: acetone, ascorbate, beta-hydroxybutyrate, diethylene glycol, DL-lactate, ethanol, ethylenediamintetraacetic acid (EDTA), ethylene glycol, fomepizole, glycerol, glycolate, isopropanol, L-pyroglutamate, methanol, methylene blue, oxalate, salicylate and urate. The sensitivity and specificity were evaluated with a spectrometer at 653 nm wavelength. The measured value after five minutes divided by the value at the start was used (ratio), and we defined a ratio ≥ 0.8 as a negative sample and a ratio ≤ 0.7 as a positive sample. The solutions were also examined visually for a colour change.

Description of POC-model

The POC-model was used as follows: One drop of whole blood, plasma or serum was applied to the test strip (Figure 4). A filter removes all red blood cells and the enzymatic reaction with the FOX-enzyme takes place on a reactive membrane. The result is read visually for a colour change that will appear in the presence of formate. For semi-quantitative detection, the test strip is compared to a colour scale consisting of three parts; negative (< 1mmol/L, 4.6 mg/dL), low positive (1-10 mmol/L, 4.6-46 mg/dL) and high positive (> 10 mmol/L, 46 mg/dL).

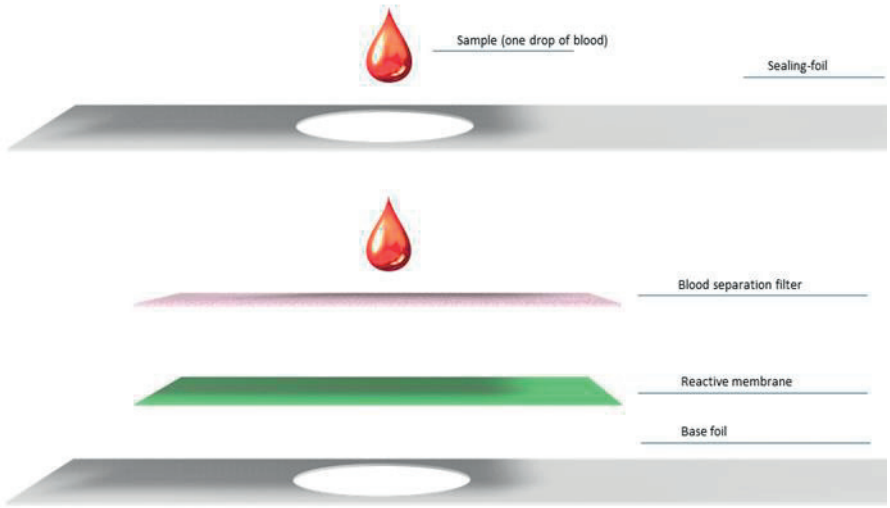

Figure 4: Principles for the point-of-care (POC)-model for detection of formate

Specificity of the POC-model

The specificity of the POC-model was evaluated bedside with a drop of blood from 14 patients with metabolic acidosis of various origin, not due to methanol poisoning. The result was not verified with GC-MS.

Clinical applicability of POC-model

This was performed by using four blood samples spiked with different concentrations of formate: two negative, one low positive (3 mmol/L, 14 mg/dL) and one high positive

(20 mmol/L, 92 mg/dL). The result was read individually by four intensive care unit (ICU) doctors and two ICU-nurses, and the true result was blinded to them. To determine the extent of agreement between several participants the inter-rater reliability was calculated with Fleiss' kappa (110).

Ethical consideration

The Data Protection Officer at Oslo University Hospital approved the study for testing of the specificity of the POC-model, as this was considered a quality (study case number 21/15801). The biological material used was destroyed immediately after analysis.

3.4. Paper IV

A patient with suspected methanol poisoning with a severe metabolic acidosis and visual disturbances presented to hospital. The POC-model described in paper III was used with a drop of whole blood from the patient to see how it performed in clinical practice. Written consent was obtained from next of kin.

3.5. Paper V

Study design and participants

This study was a prospective observational study of adult patients (>18 year) with suspected or confirmed toxic alcohol poisoning treated with fomepizole and CRRT. Patients were recruited from Oslo University Hospital, Akershus University Hospital, Baerum Hospital, Ostfold Hospital Kalnes and Levanger Hospital. The study period was from June 2019 to November 2020. Data from two pilot patients collected before the formal study period was included in the material.

Treatment

All patients received fomepizole according to dosage recommendations for fomepizole and CRRT; 15 mg/kg as a loading dose followed by a maintenance dose of 10 mg/kg every 8 hours. A continuous infusion of 0.5 mg/kg/hour as maintenance dose during CRRT was also an option. It was up to the treating physician to decide which of the dosing alternatives to prescribe. Local guidelines were followed when choosing CRRT modality and settings.

Method

Blood and dialysate samples were collected at intervals of 1-2 hours to measure the plasma concentrations of fomepizole and to calculate the elimination kinetics of fomepizole during CRRT. To ensure that a sample was taken at the time when the fomepizole concentration was at its lowest (C_0) for fixed doses of fomepizole, a sample was also collected immediately before each fomepizole dose. Sampling lasted as long as the patient received fomepizole and CRRT.

Calculations

Continuous veno-venous haemodialysis (CVVHD) and continuous veno-venous hemodiafiltration (CVVHDF) were the CRRT modalities used. The degree of drug removal by the filter is represented by the sieving coefficient for filtration and saturation coefficient for haemodialysis. The following equation was used to calculate CRRT clearance for CVVHD:

$Clearance_{CVVHD} = (D/P) \times Q_D$

Where (D/P) is the saturation coefficient, with fomepizole dialysate concentration (D), fomepizole plasma concentration (P), and dialysate flow rate (Q_D) (111). The patients who received CVVHDF used post-filter replacement fluid, and the following equation was used to calculate clearance:

$Clearance_{CVVHDF} = (D/P) \times Q_E = (D/P) \times (Q_{UF}+Q_D)$

For CVVHDF the sieving/saturation coefficient was used for (D/P), and with ultrafiltration flow rates (Q_{UF}) plus dialysate flow rate (Q_D) is the total effluent rate (Q_E) (111, 112).

To find the elimination kinetics of fomepizole during CRRT we used concentration-time graphs (zero-order) and semi-log graphs (first-order kinetics). The R^2 value was calculated to identify whether there was a linear drug decline (zero-order) or a log linear drug decline (first-order). A R^2 value of one indicates that elimination kinetics follow that order.

The half-life ($T_{1/2}$) was calculated for first-order elimination with the following equation:

$T_{1/2} = (\ln 2/k_e)$ (113)

where k_e is the elimination rate constant.

Total body clearance (TBC) was determined by the equation

$TBC = k_e \times Vd$ (113)

Where Vd is the volume of distribution

Ethical considerations

Regional Committee for Medical and Health Research Ethics (2017/981/REK South-East D) approved the study. All patients gave consent and for those who were unable to do so, a next of kin provided the consent. Approval from the Data Protection Officer at Oslo University Hospital was not necessary since this was before 2018. The study was registered in Clinicaltrials.gov (NCT04649138).

4. RESULTS

4.1. Paper I

In this study, we investigated the antidote preparedness in Norwegian hospitals. All 50 hospitals responded to the survey, and this resulted in a 100 % response rate. The most commonly used antidotes (acetylcysteine, activated charcoal, atropine, flumazenil, naloxone and phytomenadione) were available in all hospitals, and the availability of the other antidotes varied (Table 2 paper I). Only 11/50 (22 %) of the hospitals were fully compliant with the recommendations and stored all recommended antidotes for their hospital size. All four regional hospitals were fully compliant with the recommendations, six small hospitals stored all recommended antidotes, while large hospitals where the least compliant: Only one of these hospitals stored all antidotes recommended for that hospital size. In large hospitals, cyproheptadine for serotonergic syndrome was the antidote least available, whereas sodium sulphate for barium poisoning was least available at small hospitals.

4.2. Paper II

In this paper, we examined the content of methanol in homemade alcohol distilled from rice without temperature control. Eighty-five percent (17/20) of the samples from private households contained detectable levels of methanol, with a median concentration of 0.3 mmol/L (9 mg/L) (range 0.1-1.2 mmol/L, 2-37 mg/L). For the purchased rice alcohol, the corresponding numbers were 60 % and 0.4 mmol/L (12 mg/L) (range 0.1-0.8 mmol/L, 2-25 mg/L). None of the samples contained toxic levels of methanol - a theoretical ingestion of 424 litres of the sample with the highest concentration would be required to produce a toxic plasma concentration of methanol.

4.3. Paper III

In this paper, we studied a novel formate assay with a modified FOX-enzyme for diagnosis of methanol poisoning. The sensitivity of the FOX-enzyme in aqueous solutions was 100 % for all formate concentrations tested above or equal to the threshold value 1 mmol/L (range 1-

20 mmol/L, 4.6-92 mg/dL). The result was confirmed by visual detection for colour change. The specificity of the FOX-enzyme was 97 %, with only one false positive sample.

We tested the specificity of a POC-model bedside with whole blood samples from 14 patients with metabolic acidosis: six diabetic ketoacidosis, three ethylene glycol poisonings, one renal failure, one lactic acidosis and three patients without suspicion of methanol poisoning with a metabolic acidosis of unknown origin. All samples were evaluated as negative.

The clinical applicability of the POC-model was good when used by the clinical staff. Only one of the participants identified one sample wrongly; a negative sample interpreted as low positive. This gave a Fleiss' kappa value of 0.87 ($p<0.001$), 95 % CI (0.69-1.05), suggesting an almost perfect strength of agreement (114) when comparing the inter-rater reliability between the clinical staff.

4.4. Paper IV

When the POC-model was used on a patient with suspected methanol poisoning, the result showed high positive (> 10 mmol/L, 46 mg/dL formate). The result was verified by GC-MS showing 62 mmol/L (199 mg/dL) of methanol and 19 mmol/L (87 mg/dL) of formate.

4.5. Paper V

In this paper, we included 10 patients that were treated with fomepizole and CRRT. Three patients had confirmed methanol poisoning, four had confirmed EG poisoning and three had suspected toxic alcohol poisoning that were shown to be negative. This was verified or excluded with GC-MS. Fomepizole was administered as fixed doses in nine patients, and one received a continuous infusion. Seven patients received CVVHD while three patients received CVVHDF with post-filter replacement fluid. A total 120 of 123 plasma samples were above the required minimum concentration of 10 µmol/L (0.8 mg/L) (Figure 5). The through plasma concentration (C_0), measured before each new dose, was a median of 108 µmol/L (9 mg/L) (range 2-168 µmol/L, 0.2-14 mg/L). The lowest plasma concentration of fomepizole for the patient receiving continuous infusion of fomepizole was 70 µmol/L (6 mg/L).

Calculation of R^2 value for the elimination of fomepizole during CRRT gave a value of approximately 1 for both zero- and first-order elimination (Table 2 paper V). The half-life ($T_{1/2}$) for the first-order elimination was calculated to be 5.6 hours (median), range 1.3-10.5 hours. CRRT removed fomepizole with a saturation/sieving coefficient of 0.85 (median) and range of 0.46-0-96. This gave a CRRT clearance of 28 mL/min (median) and range 8-35 mL/min. Clearance of fomepizole by CRRT was 22 % (median), range 9-44 % of the total body clearance (TBC).

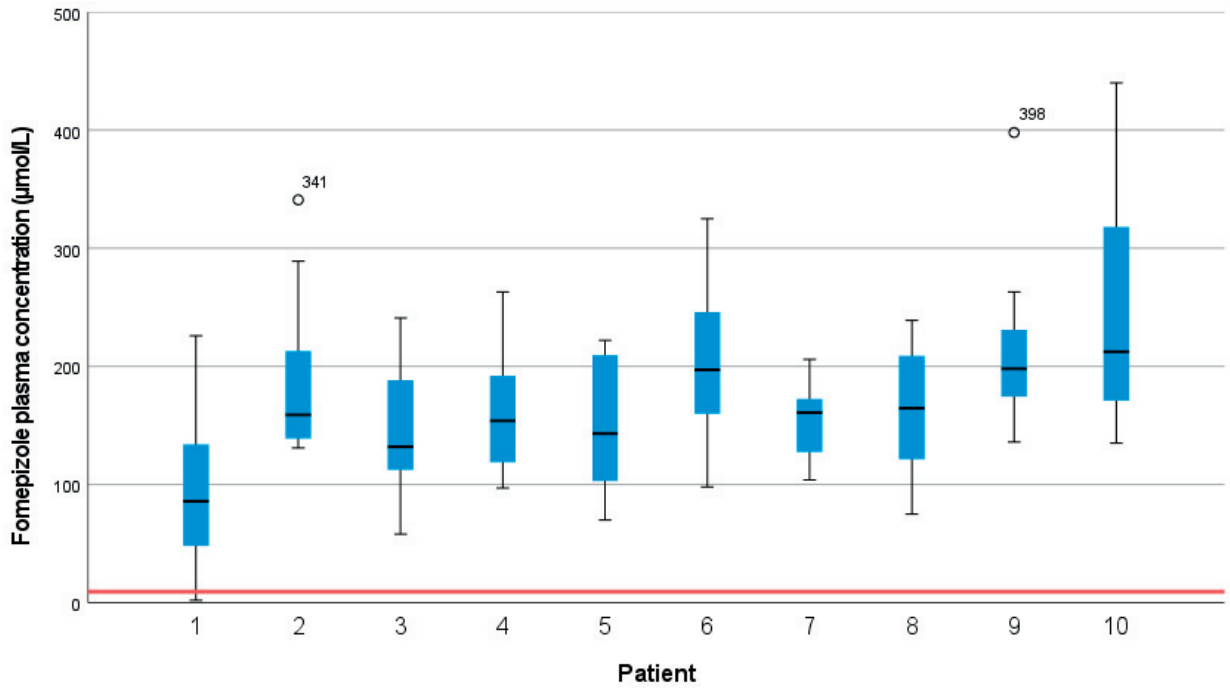

Figure 5: Plasma concentration of fomepizole measured. Patient 1-4 and 6-10 received fixed doses of fomepizole and patient 5 a continuous infusion.

5. DISCUSSION

5.1. Methodological considerations

5.1.1. Validity

The internal validity describes whether the right methods have been chosen to answer the research question. It can be negatively affected by selection bias, information bias and statistical confounding (115). The results obtained must be transferable from the study population to a general target population, i.e. generalization, and this is the external validity (115). Validity will be discussed in the following sections for each paper.

5.1.2. Paper I

The methodological limitation of this study is that it is a self-reported survey. We therefore do not know the actual amounts of antidotes available at that particular time. Three reminders were sent to those who had not responded. This was necessary to ensure a high response rate, resulting in 100 % response, which is a strength. There is a possibility that hospitals may have made improvements after receiving the questionnaire, before finally responded to the survey. Thus, it may have led to a seemingly better antidote preparedness at the time of reporting.

The national recommendations for antidote stockpiling are categorized by hospital size without this being properly defined. We therefore had to define these ourselves and tried to group the hospitals accordingly. It is a strength that we defined a regional hospital as a university hospital, as this is an official term for a given type of hospital. This is also supported by the result that showed that all four regional hospitals were fully compliant with the recommendations. We chose population catchment area to find the large hospital in each local hospital thrust, as these numbers were publicly available. This is a weakness since we also could have requested the number of hospitals beds or asked the hospital staff if they would classify their hospital as being a small or large hospital. Regarding the latter, it would have been difficult to find the right person who could answer this, and it would again likely have led to biases, with the possibility that many hospitals would then define themselves as small.

From experience, we knew that antidotes are stored in several locations in hospitals rather than in one particular place. Therefore, we added a question asking whether the antidote was located in another location at the hospital. Where “Do not know” was answered, we contacted the responder to find out if this actually could be located in another location. If “Do not know” was maintained this was classified as “no”. At the time of the study, the use of digital tools to find the location of various drugs in a hospital was a new feature, and also not available in all hospitals. We may therefore have underestimated the actual antidote availability.

5.1.3. Paper II

Study design

This study was an observational study to investigate whether uncontrolled production of homemade alcohol from rice could lead to toxic amounts of methanol as a by-product. One limitation in our study is that it was conducted in two communes in the same province in Northern Vietnam. The province and the communes chosen were known to be areas with a lot of homemade alcohol production. This limits the generalizability of homemade alcohol elsewhere and outside of Vietnam, possibly not reflecting the variation in the country. Rice was used as raw material in the alcohol production, and the results can therefore not be generalized to alcohol made from other raw material. The sample size of 20 households is also small. The number was chosen based on available resources.

There are few studies on homemade alcohol where the origin of the alcohol is known, as they are purchased from markets, distribution sites or through a network of contacts. In our study, the production sites were visited, and parts of the production process directly observed by the study personnel and this is a strength.

Selection bias

The 20 households included were not randomly selected. They were chosen by the head of the commune health centre and there is thus a risk of selection bias. For the commune, it will not be beneficial to get a result that indicates that homemade alcohol contains toxic amounts of methanol. Therefore, it is a possibility that the households were chosen with this in mind. On the other hand, whether alcohol is contaminated by methanol is difficult to

know in advance. Interview of the participants (unpublished data) revealed that they were different in terms of experience with homemade alcohol production (2-32 years of experience), and the size of the production (20-140 litres per week). We therefore believe that selection bias is less likely.

Calculations

In order to compare with other studies, we chose a body weight of 70 kg when calculating the estimated volume to drink to achieve a toxic plasma concentration of methanol. The average weight in Vietnam for men is 61.2 kg and women 54.0 kg (116). At a weight lower than 70 kg, a smaller amount of methanol is needed to reach a toxic plasma concentration and, correspondingly, a smaller volume to drink. In our study, the methanol content was negligible, and the corresponding volume required to achieve a toxic effect for a bodyweight of 61 kg and 54 kg was 369 L and 327 L, and thus not relevant for the result of our study.

The calculation of the minimum volume to drink in order to obtain a toxic plasma concentration of methanol is a theoretical consideration. For simplification, we chose a bioavailability of one (since this is unknown). If the oral absorption is lower, the corresponding volume resulting in a toxic effect will be even larger than our calculated volume. The equation used for calculation is for one single loading dose. This may apply to small volumes such as a shot (37-44 mL). In practice, drinking typically happens over time with repeated exposure. This simplification of using the loading dose calculation is thus a conservative estimate of the actual volume needed to obtain a toxic dose. Further, there will also be an ongoing metabolism of methanol as well as elimination by the lungs while blood ethanol concentration drops (117). In practice, the drinking volume required to achieve a toxic plasma concentration will be even larger than our estimated minimum volume.

5.1.4. Paper III

Paper III describes the preliminary experiments with the modified FOX-enzyme used in a novel formate assay and a POC-model for diagnosis of methanol poisoning.

The sensitivity and specificity of the FOX-enzyme were tested in aqueous solutions. This is a limitation since a future diagnostic test will be used on biological material such as blood,

plasma, or serum. However, this is a stepwise process, and data from the aqueous solution needs to be generated to allow for further testing on biological material. A strength of the sensitivity testing is that clinically relevant formate concentrations are included in the analysis (73, 74). In addition, a separate experiment was conducted to ensure that the FOX-enzyme could distinguish even very low positive concentrations from the endogenous levels.

For specificity, we also tested 18 different substances. The goal of a future POC-test is a screening tool for metabolic acidosis of unknown origin, to verify or exclude methanol poisoning as the cause. We have therefore tested against the substances we consider the most relevant in that context: substances that may give a metabolic acidosis, the other toxic alcohols and their metabolites, substances that can potentially interfere with the colour reaction, and the most common anticoagulant used in blood sample tubes. We did not test the unstable ketone body acetoacetate or the metabolite 2-hydroxyethoxacetic acid, which cause the acidosis after diethylene glycol exposure (118). The anticoagulant EDTA was the only one that was tested, since these are the anticoagulant tubes most frequently used in our emergency department. Heparin is also used as an anticoagulant in blood sample tubes and blood gas syringes. When specificity of the POC-model was investigated, we used a drop of whole blood that was applied with a blood gas syringe containing heparin. Thus, a potential effect of heparin on the specificity of the POC-model would have been detected. In these initial experiments, we also chose to test only one clinically relevant concentration of each substance. Since extreme values for all substances were not tested, the result thus cannot be generalized to single cases with extreme values.

The specificity of the POC-model was tested in real-time with patient samples. A total of 14 patients were tested and the low number of patients is a weakness. This was also a quality study, and the results were not verified with the gold standard.

The reading of the POC-model results by clinical staff was performed with six participants: four ICU-doctors and two ICU-nurses, representing a typical target group for the device. Each participant individually assessed the results, and the true result was blinded to them. This was not a full usability study. No power calculation was performed and there was a low number of participants, which is a limitation.

5.1.5. Paper IV

This paper describes one case report where we used the POC-model on samples from a patient with methanol poisoning. With only one patient, the result cannot be generalized, but it documents the clinical potential of the POC-model, and it was used in a real-life setting. A strength of this paper is the use of the POC-model in the relevant clinical context, and that the methanol and formate levels were later verified with GC-MS.

5.1.6. Paper V

Study design

This study was a prospective observation study with 10 patients treated with fomepizole and CRRT. The largest methodological weakness is the limited number of patients. However, when studying an infrequent diagnosis such as toxic alcohol poisoning, including a high number of patients is very difficult.

Two CRRT modalities were used in our study: CVVHD and CVVHDF with post-filter replacement fluid. No one received continuous veno-venous hemofiltration (CVVHF) or CVVHDF with pre-filter replacement fluid. The type of CRRT modality can affect the degree of drug removal, but this applies for large drug molecules. Drugs ≤ 2000 Daltons are considered small, and are removed regardless of CRRT modality (111). The size of fomepizole is only 82.1 Daltons, thus the choice of CRRT modality should not affect the amount of drug to be removed. However, effluent flow rate will affect drug removal and clearance for the different CRRT modalities. This will especially be important when using a higher effluent volume than the recommended 20-25 mL/kg/hour, as more fomepizole will be removed, giving a risk of underdosing and therapy failure (119). Eight of ten patients (80 %) had an effluent volume within the recommendations. While this only represents a small number, it is nonetheless an indication that the results could be generalizable for the recommended effluent volume.

One patient received a continuous infusion of fomepizole, whereas the nine others received fixed doses. The result can therefore not necessarily be generalized to patients receiving continuous infusion of fomepizole. From a theoretical perspective, the elimination kinetics should not be different compared to fixed doses. Further, the lowest concentration

measured for the patient who received a continuous infusion of fomepizole was seven times higher than the recommended minimum. From a practical perspective, it is preferable to give fixed doses of fomepizole since a continuous infusion will occupy an intravenous line, although the total maintenance dose needed for fixed doses (10 mg/kg every 8 hour) is somewhat higher than continuous infusion (1 mg/kg/hour).

We did not measure fomepizole or the metabolite 4-carboxypyrazole (4-CP) in the urine. However, renal excretion in healthy volunteers is low (< 3%) (120, 121). We have assumed that it is negligible, and that removal of fomepizole is by dialysis and metabolism in these patients. The Vd was used to calculate TBC, and previous reported values for fomepizole in healthy volunteers were 0.57 (121) and 0.66 L/kg (122). The Vd for some drugs may be changed in critically ill patients (123) and we therefore used 0.67 L/kg based on relevant unpublished patient data (later published (124)). This value is slightly higher than previously published data and may in theory give a slight overestimation of TBC. These data were later published, confirming the average Vd in methanol and ethylene glycol poisoned patients to be 0.68 and 0.66 L/kg, respectively (124).

5.2. Discussion of main findings

5.2.1. Antidote preparedness in Norwegian hospitals (paper I)

In this study we found that the availability of antidotes in Norwegian hospitals varies, just as studies from other countries have shown (3, 6-10). Frequently used antidotes such as acetylcysteine, atropine, flumazenil and naloxone were available in all hospitals, but not necessarily enough to treat a 70 kg patient. The latter can be explained by the fact that there is no minimum required stock in the Norwegian recommendations. In a study from UK they found that the same four antidotes were available in all hospitals, but not correct stock levels (10). Contrary to our recommendations, they require that the hospitals have enough antidotes available to start treatment of one adult patient and continue for 24 hours.

We found that only 22 % of the hospitals where fully compliant with the national recommendations. The poor compliance can probably be explained by the fact that the

recommendations lack definition of hospital size. It is difficult to compare our results with studies from other countries for several reason: Many studies are old and those carried out before 2000 are not relevant to the current situation. New antidotes have been approved (e.g., fomepizole) and in general there has been more focus on drug preparedness in recent times, especially in high-income countries. Some studies only cover part of the country, such as the study from Australia (3) and Spain (7), whereas other countries lack national recommendations (3, 8, 9). Countries such as Kuwait (8) and Lebanon (9) also have a completely different organization of the health care system as compared to Norway. Therefore, the studies from UK (10) and Denmark (6) are more comparable. On the other hand, the latter was conducted between 1999 and 2002 and is therefore less relevant. Denmark is also smaller than Norway in size and does not have the same challenges as regards to long distances between the hospitals. The guidelines for stocking of antidotes in the UK are also divided into three categories, but rather than hospital size, they have used timely availability; immediately available (category A), available within 1 hour (category B) and to be held within a geographical region available within 4-6 hours (category C) (12). Considering that UK guidelines are categorized by urgency of availability and not hospital size, the results are not directly comparable. When comparing category A and B antidotes, the majority are the same as recommended stocked in all hospitals, with an addition for large and regional hospitals in Norway (Table 1). The additional recommendations for only regional hospitals are all category C antidotes in UK, except for penicillamine, which was removed from the UK recommendations in 2017. The last national audit for UK in 2018-2019 found that most hospitals were not compliant with the recommendations for categories A and B when taking both availability and quantity into account (10). However, when looking solely at availability the result was better. This may indicate that the compliance in UK is somewhat better than Norway, when recommended stock levels are not included. One explanation for this result is that it may be easier for decision makers to make an assessment when considering timely availability, as this is a more specific recommendation about ensuring optimal treatment. This may also explain the poor result for small hospitals in Norway, which might have been better if the term immediately available was used. This supports the argument that our national recommendations are outdated and that they need revision. The urgency of availability is a much better approach since antidote treatment can

be time critical. The recommendations should also be evidence-based where there is literature available.

5.2.2. Methanol content in homemade alcohol (paper II)

Although 85 % of the samples from private households contained detectable levels of methanol, none of the samples were even close to containing toxic concentrations of methanol. Other studies on homemade alcohol have found varying results (125-131). In a study from Romania with homemade Tuica, a traditional Romanian alcohol made from plum, they found toxic concentrations of methanol (2684 mmol/L = 86 000 mg/L) (129). When we used our method to estimate minimal volume to drink to reach toxic plasma concentration of methanol, 0.2 L of the Tuica sample with the highest methanol concentration was required to obtain potential toxic concentrations. The corresponding volume for the Tuica sample with the lowest concentration of methanol was 26 L. The source of these samples was known since they were collected from local Tuica distilleries that were visited, but the production was not observed. Another study from Romania with homemade Tuica also found toxic concentrations of methanol (591 mmol/L = 18929 mg/L), but it is unknown where and how the samples was collected (130). In a study from Poland, the sample with the highest concentration of methanol (236 mmol/L = 7570 mg/L) could potentially be toxic (132). According to the label, this sample was also made from plum, but it is uncertain whether it was homemade or not since, it reported in the paper as being unrecorded alcohol without further specification. Fruit contains pectin that will metabolize to methanol (63). Alcohol made from fruit may therefore contain a higher concentration of methanol than alcohol made from rice. By consuming large amounts of the samples with the highest methanol concentration in the studies from Ukraine (125) and China (126), over several days, it is theoretically possible to reach toxic plasma concentrations – especially if combined with ethanol that will prolong methanol half-life. This could theoretically happen in a single incident, but it is highly unlikely that a large number of patients, as seen in the reported large methanol outbreaks (16-18), have all consumed these massive amounts. One reason why these previous studies found different results than we did is likely because their alcohol was made from fruit rather than rice as presented. Furthermore, the origin of the alcohol in these studies is often unknown since they come from markets or other distributions sites. Since it is unknown how they were made, there is a possibility that methanol may have been

added after production. This makes our study unique, since we have shown that homemade alcohol from rice made in private homes without any temperature control did not produce toxic amounts of methanol. This is important knowledge when uncovering the causes of methanol poisoning outbreaks where homemade alcohol from rice has been ingested.

5.2.3. Sensitivity and specificity of the FOX-enzyme (paper III)

In these first published data on the sensitivity and specificity of the modified FOX-enzyme, we found a high sensitivity and specificity.

The *sensitivity* data covers the formate concentration range between 1-20 mmol/L (4.6-92 mg/dL). During an outbreak in 1979 in Norway, all patient samples analysed for formate contained a concentration between 4.8-17.1 mmol/L (22.1-78.7 mg/dL), except for one that was below the detection limit of 0.4 mmol/L (1.8 mg/dL) (34). The latter was a patient with a methanol concentration of 35 mmol/L (112 mg/dl) and ethanol of 37 mmol/L (170 mg/dL), which illustrates that the metabolism of methanol was inhibited by ethanol. In a later outbreak in Norway, the formate concentration was above 10 mmol/L (46 mg/dL) in all symptomatic patients and between 0.5-8.3 mmol/L (2.3-38.2 mg/dL) in the four asymptomatic ones (73). Similarly, from the large outbreak in the Czech Republic, the median formate concentration was 13.4 mmol/L (61.7 mg/dL) and the highest 25.2 mmol/L (116.0 mg/dL) (18). The median values for symptomatic patients with visual disturbances or dyspnoea were 15.2 mmol/L (70.0 mg/dL) and 15.4 mmol/L (70.9 mg/dL) respectively (74). The results from Paper III thus showed that the modified FOX-enzyme sensitivity is high for the formate concentrations relevant in clinical practice – as also demonstrated in the present case report (paper IV).

For the additional 18 substances tested for specificity, only isopropanol gave a false positive result. The colour change to green instead of blue may indicate that this is a different kind of reaction, but this requires further studies. Previous studies with another enzyme (alcohol oxidase) for detection of methanol poisoning have failed to distinguish between methanol and ethanol (76, 78). Since ethanol is commonly co-ingested, a diagnostic method for methanol poisoning must be able to separate these two alcohols, which this novel formate assay with FOX-enzyme does. The high specificity of the FOX-enzyme also covers the most

common substances that potentially may be present in patients who are the target group for this test. This study is therefore an important contribution in the work of developing a POC-test for methanol poisoning.

5.2.4. Specificity of the POC-model (paper III)

When used bedside on 14 patients with metabolic acidosis of various aetiologies, no false positive results were obtained. The target group for a future POC-test is patients with metabolic acidosis of unknown origin to verify or exclude methanol poisoning as the cause. Our results cover a wide range of causes giving a metabolic acidosis; diabetic ketoacidosis, lactic acidosis, renal acidosis and acidosis due to ethylene glycol poisoning. Alcoholic ketoacidosis was not included, but the FOX-enzyme had high specificity for both lactate and beta-hydroxybuturate, typically responsible for the acid-base disturbances in these patients, and did not report a positive result for these two compounds.

5.2.5. Fomepizole dosing during CRRT (paper V)

We found that the plasma concentration fomepizole during CRRT was above the minimum value of 10 µmol/L (0.8 mg/L) in 98 % (120/123) of the samples. Three samples were below 10 µmol/L (0.8 mg/L), and they all came from the same patient, and it was after the fifth dose. This low concentration is due to increased elimination and the possible causes for this are: increased CRRT clearance, increased metabolism or auto-induction of its own metabolism. The patient received CVVHD, and the CRRT clearance is affected by the dialysate flow. The latter was constant during the observation time and the CRRT clearance calculated to 22 mL/min, which is also lower than the median value in the data material. In addition, the CRRT clearance was calculated for dose four and five since no dialysate samples were collected until the fourth dose. The three values that were too low in this patient can thus not be explained by increased CRRT clearance. Increased liver metabolism can often be explained by a drug interaction, but this patient did not receive any enzyme-inducing drugs. From animal studies, fomepizole is metabolised to 4-hydroxymethylpyrazole (4-OHMP) and 4-CP (133). Human data has confirmed that the primary metabolite is 4-CP, and it is believed that this metabolite is produced by enzymes of the cytochrome P450 system (121). Fomepizole also induces CYP 2E1 (134, 135); if this is the enzyme responsible for fomepizole metabolism, it can in theory initiate its own metabolism. In healthy volunteers,

metabolism increased over time, and this observation was explained by auto-induction (121). This is the reason why guidelines recommend that the maintenance dose is increased from 10 mg/kg to 15 mg/kg from the fifth dose, which was not done in our patient. The low plasma concentration in our patient may thus be explained by auto-induction. The three samples with too low concentration had no clinical significance for the patient, since fomepizole treatment was discontinued after the last dose. In general, long treatment time with fomepizole is less frequent. From a retrospective study in 436 patients given fomepizole, a median of two (IQR 1-3) maintenance doses following a loading dose were administered (136).

5.2.6. Elimination kinetics of fomepizole during CRRT (Paper V)

We found that the elimination kinetics of fomepizole during CRRT followed both zero- and first-order kinetics, which in practice may be difficult to explain. Although we may have had too few data points to make these calculations, the elimination kinetics of fomepizole without dialysis is described as zero-order in animals (90, 137, 138) and healthy volunteers (121). Elimination kinetics data from poisoned patients are limited; however in one study with five patients, it was best described by first-order kinetics (139). The observation time (up to three days) was longer than in our study. In addition, three patients also received haemo- or peritoneal dialysis and ethanol treatment – all procedures that will influence elimination towards first order (less elimination through metabolism). Fomepizole is eliminated by first order kinetics during IHD (124). A possible explanation of our findings may therefore be that the endogenous fomepizole elimination (mainly metabolism) is non-linear (zero order) – and the influence of CRRT (linear elimination, first order) makes it look like a combined zero- and first order elimination.

6. CONCLUSION & CLINICAL IMPLICATIONS

This thesis describes antidote preparedness in Norwegian hospitals and various aspects of toxic alcohol poisoning in terms of cause, diagnosis and management. The conclusions of each paper according to their aims and the clinical implications of these are as follows:

Paper I

The antidote preparedness in Norwegian hospitals varied, and only 22 % of the hospitals where fully compliant with the national recommendations. Large hospitals were least compliant, while all four regional hospitals were fully compliant. The compliance with the recommendations was not satisfactory, and clearly has a potential for improvement in order to increase antidote preparedness. We have also emphasized that it was difficult to comply with the recommendations categorized after hospital size when this was not properly defined. This indicates that there is a need to revise the recommendations, and that time of availability is likely a better indicator than hospital size.

Paper II

1. Homemade alcohol distilled from rice did not produce toxic amounts of methanol.
2. Other studies have found varying results from non-toxic to toxic amounts of methanol.

These findings are important to understand the cause of methanol poisoning when drinking homemade alcohol from rice, also in order to trace the source of the toxic alcohol during outbreaks. Preventive measures, such as public information about the risks of drinking homemade alcohol of unknown origin, should be considered.

Paper III

1. The sensitivity and specificity of the tested modified FOX-enzyme were high.
2. The specificity of the POC-model was high when tested bedside on patients with metabolic acidosis of different origin.
3. The clinical applicability of the POC-model was good and there was almost a perfect strength of agreement between the participants when comparing inter-rater reliability.

Paper IV

When the POC-model was used in clinical practice on a patient with suspected methanol poisoning, the test showed a high positive formate (> 10 mmol/L, 46 mg/dL), within a few minutes, consistent with the GC-MS result.

The results of papers III and IV are the preliminary studies in the work of developing a POC-test for detection of formate. Ultimately, this could provide completely new possibilities for diagnosing methanol poisonings where this is not possible today, as well as providing a significantly faster diagnosis where analytical methods are already available.

Paper V

1. The new (reduced) dose recommendations for fomepizole during CRRT achieved the desired plasma concentration above 10 µmol/L (0.8 mg/L)
2. The elimination of fomepizole during CRRT followed both zero- and first order kinetics – and we present an explanation for this. During the first-order elimination, the median half-life was calculated as 5.6 hours. CRRT removed fomepizole with a saturation coefficient/sieving coefficient of 0.85 (median) giving a CRRT clearance of 28 mL/min (median).

We have shown that the desired plasma concentration of fomepizole is achieved with the new (reduced) dosing recommendations during CRRT. Previous dosing recommendations for fomepizole did not differentiate between IHD and CRRT (94), and compared to these, only half the maintenance dose is now required during CRRT. In the event of an outbreak of methanol poisoning with limited access to fomepizole, more patients may therefore be treated correctly. In addition, the cost of the maintenance dose will be significantly reduced.

7. FUTURE RESEARCH

To prevent outbreaks of methanol poisoning in LMIC with homemade alcohol production, there is a great need for public information about the risk of drinking liquor of unknown origin. Even though we have shown that homemade alcohol from rice does not produce toxic amounts of methanol, people still get methanol poisoning after this. Knowledge and awareness about methanol poisoning among the public must be increased to ensure that help is sought in case of suspected poisoning. In addition, health personnel should also be aware of the possibility for such poisoning when treating patients drinking unrecorded alcohol or simply as a differential diagnosis in metabolic acidosis of unknown origin. For future research, the effect of introducing such measures should be studied.

In this thesis, we have presented the preliminary results from the innovative work of developing a POC-model for detection of formate to be used for diagnosing of methanol poisoning. This device is not available on the market yet, and further research is needed to develop the test. A full usability study must be carried out on a broad group of users in addition to ICU doctors and nurses. The sensitivity and specificity of the final product must be tested in clinical trials and the results should be verified with GC-MS. However, it is difficult to predict where a major outbreak will occur next so that equipment and ethics approval can all be in place for a study. Equipment for GC-MS verification of the diagnosis would be difficult in a LMIC. One solution to this could be to collect the blood samples from an outbreak and afterwards do the GC-MS verification in a country where this is available.

We found that the plasma concentrations of fomepizole during CRRT in toxic alcohol poisoning were well above the desired minimum concentration. Future research should evaluate whether the maintenance dose during CRRT may be even further reduced. A possible tool in this research could be to use our data to create a pharmacokinetic model for fomepizole dosing during RRT.

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9. PAPERS I-V

I

II

Drug and Alcohol REVIEW

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Methanol content in homemade alcohol from a province in North Vietnam

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Abstract

Introduction and Aims. *Methanol poisonings pose a major risk especially where illegal alcohol is consumed. The source of the methanol in the drinks are debated. We aimed to evaluate whether home distillation of alcohol made from rice was capable of producing toxic amounts of methanol.* ***Design and Methods.*** *Twenty households with homemade alcohol production in Phu Tho province in Vietnam were included in this pilot study. We followed the whole production process and an alcohol sample from each household was analysed for methanol content.* ***Results.*** *17 (85%) of the samples contained detectable levels of methanol. The median concentration was 9 mg/L (range 2–37 mg/L). To develop clinical symptoms of methanol poisoning from the sample with the highest concentration would require drinking more than 424 L.* ***Discussion and Conclusions.*** *Homemade alcohol from rice did not contain sufficient amount of methanol to cause toxicity in our study. This supports the theory of methanol being added to ethanol post production for economical purposes as the main source of mass poisonings.* [Lao Y, Pham BD, Le HT, Nguyen Van H, Hovda KE. Methanol content in homemade alcohol from a province in North Vietnam. *Drug Alcohol Rev* 2019;38:537–542]

Key words: methanol, alcohol, poisoning, Vietnam.

Introduction

Methanol poisoning after unknowingly consuming pure methanol, or more often, a mixture of ethanol and methanol is a worldwide problem with a high morbidity and mortality [1–5]. This is particularly affecting developing countries [1,5–7]. These poisonings occur more frequently in countries with a high unrecorded alcohol consumption, such as homemade alcohol or alcohol sold outside government regulations. Vietnam is one of the countries that according to the World Health Organization, has a high proportion of unrecorded alcohol consumption [8]. The source is mainly homemade liquor made from rice. There are no major outbreaks of methanol poisonings reported in Vietnam in the literature in English, but there are several reports of deadly alcohol poisonings due to methanol in the media [9–12]. The origin of the toxic amount of methanol in these poisonings is unknown.

Methanol is an organic chemical used for industrial purpose as a solvent and a fuel. It is also found in automotive products such as antifreeze and windscreen wash. This makes it cheap and easily accessible. Ethanol on the other hand is often subjected to high taxes as well as legal restrictions in some countries. Methanol is typically mixed with ethanol containing beverages for a financial gain. The boiling point of methanol is 65°C (149°F), and 78°C (173°F) for ethanol. Distillation of ethanol without temperature control can thus cause methanol to be produced along with ethanol. In addition, the fermentation with microbes can produce methanol [13].

It is a common opinion that toxic amounts of methanol originate from poor home distillation of ethanol. In order to make a strategy on how to prevent methanol poisoning it is necessary to know if homemade alcohol could produce toxic amounts of methanol. Studies done previously have shown that the methanol content in homemade alcohol is below toxic limits [14–18]. However, the samples are from markets or other distribution sites. There is no control of the origin of the alcohol product or the production itself. To

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evaluate whether home distillation of alcohol from rice could produce toxic amounts of methanol, we investigated the methanol content by following the whole production process directly from local producers in a province in North Vietnam.

The aim of this study was therefore to conduct a pilot study to evaluate whether home distillation of alcohol from rice could produce toxic amounts of methanol. In addition, we compared the results with other studies on distillation of homemade alcohol.

Methods

Observation of homemade alcohol production

This study was conducted in two communes in Phu Tho province, in North Vietnam. This is a province with many households producing homemade alcohol without a license for production or distribution. The province was chosen because of methanol poisoning cases treated at Phu Tho General Hospital. One commune was an urban area and one was a rural area/mountain area. Both communes were known to have homemade alcohol production.

Twenty households with alcohol production were included in this study, 10 from each commune. Each household was chosen by asking the head of commune health centre in each commune to find a household distilling alcohol at the time of inclusion. In each household, the person in charge of the production was interviewed and the alcohol production observed.

All households made alcohol from rice in a four-step process: First, they cleaned and cooked the rice. Thereafter they cooled the rice down and added yeast for fermentation. The alcohol was then distilled without any temperature control, and the final product was a mix of all the distilled alcohol. The final product was dispensed in plastic cans or bottles. They were stored inside the home brewers' house and mainly sold to neighbours and surrounding community. All participants used rice as main ingredient, but they used different kind of yeast. None of the home brewers had a license for production or distribution and there was no quality control of the final product.

Sample collection

After observation of the production, 1 L of the batch from each household was collected. In addition, one bottle of rice-distilled alcohol was bought randomly at five different street vendors or street eateries selling alcohol in each commune. This gave a total of 30 samples; 20 from home brewers and 10 from street vendors/street eateries. None of the street vendors or eateries had a license to sell alcohol. The origin of this alcohol was from home brewers in the village. All the samples were sent for analysis of methanol- and ethanol content at Hanoi University of Science at Vietnam National University.

Analytical procedure

The ethanol concentration was measured with a hydrometer, and gas chromatography with flame ionization detector (FID) used for detection and quantification methanol.

Turbid liquids were filtered through a 0.2 μm membrane before analysis for detecting methanol. The substances for analysis were mixed with ethanol and water, volume ratio 1:2 to develop a standard curve. The concentrations in the standard curves ranged from 0.5–500 mg/L. The methanol concentration was calculated based on the standard curve of the gas chromatograph.

Methanol concentration is often expressed as g/hL pure alcohol or g/hL 100% alcohol by volume. This makes it easier to compare different samples without considering the alcoholic concentration. We have used mg/L as a measure for methanol concentration in the samples, thus not taken the alcoholic concentration into account.

Calculation of minimum volume to drink to obtain methanol poisoning

To evaluate whether the alcohol contained toxic levels of methanol, we calculated how much a 70 kg person must drink to reach a toxic serum concentration. The volume of distribution (Vd) for methanol is 0.7 L/kg [19]. We chose the serum concentration for initiating therapy with alcohol dehydrogenase inhibitors 10 mmol/L (32 mg/dL) as a toxic threshold value [20]. This is a theoretical maximum (hence a conservative approach) since there will be an ongoing metabolism of methanol after the parallel ethanol level is reduced.

Statistics

Statistics were performed using SPSS version 25. The comparison of ethanol and methanol content between the alcohol from home brewers and alcohol bought from street vendors/eateries was done by using a Mann–Whitney test. Statistical calculations were done with a 0.05 level of significance.

Ethics

The study did not involve any human intervention and no ethical approval was sought. However, the head of health service of Phu Tho province approved that the study could be conducted. All the 20 households were asked to participate in the study and everyone consented.

Results

Table 1 shows the concentration of ethanol and methanol in samples from home brewers and street vendors/eateries. Of the home brewer samples, 17 (85%) contained detectable levels of methanol. The median concentration was 9 mg/L (range 2–37 mg/L). Compared to alcohol bought on the streets, six samples (60%) contained methanol with a median concentration of 12 mg/L (range 2–25 mg/L). None of the samples contained toxic levels of methanol. There was no significant difference ($P = 0.70$) between the concentration of methanol in the two groups.

The concentration of ethanol was significantly higher in the samples from home brewers compared to the samples bought on the street ($P = 0.04$). The median ethanol content in the samples from home brewers was 37% vol (range 29–58% vol). Corresponding data for the samples from the street were 32% vol (range 29–38% vol).

The comparison of methanol concentration in homemade alcohol from various studies is shown in Table 2.

Discussion

This study did not show any toxic amounts of methanol in homemade alcohol from rice. To develop clinical symptoms from methanol poisoning when drinking the homemade sample with the highest methanol concentration would take more than 424 L.

This pilot study is the first to observe and trace all steps in the production of homemade alcohol in a country with regular reports of toxic alcohol incidents.

Table 1. *Methanol and ethanol concentration from analysis of 30 samples*

Sample	Origin	Ethanol concentration (% vol)	Methanol concentration (mg/L)	Volume (L) to drink to obtain a serum methanol of 10 mmol/L
1	Home brewers	38	37	424
2	Home brewers	29	28	560
3	Home brewers	38	20	784
4	Home brewers	38	13	1206
5	Home brewers	29	12	1307
6	Home brewers	32	12	1307
7	Home brewers	37	11	1425
8	Home brewers	32	9	1742
9	Home brewers	39	9	1742
10	Home brewers	35	9	1742
11	Home brewers	35	7	2240
12	Home brewers	36	6	2613
13	Home brewers	43	5	3136
14	Home brewers	58	2	7840
15	Home brewers	37	2	7840
16	Home brewers	55	2	7840
17	Home brewers	34	2	7840
18	Home brewers	56	Not detected	—
19	Home brewers	39	Not detected	—
20	Home brewers	30	Not detected	—
21	Street	31	25	627
22	Street	30	21	747
23	Street	35	14	1120
24	Street	36	10	1568
25	Street	32	5	3136
26	Street	37	2	7840
27	Street	38	Not detected	—
28	Street	31	Not detected	—
29	Street	30	Not detected	—
30	Street	29	Not detected	—

Table 2. *Summary of methanol concentrations in homemade alcohol in various studies*

Country	Type of alcohol	Main ingredient	Number of samples	Production observed and samples collected in this connection	Highest measured methanol concentration (mg/L)	Median measured methanol concentration (mg/L)	Volume (L) to drink of sample with highest concentration to obtain a serum methanol of 10 mmol/L	Unit methanol concentration stated in original article	Reference
Romania	Home-distilled Tuica	Fruits, grains, industrial ethanol or unknown	35	No	86 000	2500	0.2	g/dL	[21]
Poland	Unrecorded alcohol; homemade, illegally imported and untaxed alcohol	Grain, fruits, sugar or unknown	33	No	7570	73	2	g/hL pure alcohol	[16]
Ukraine	Homemade Samohon for personal use	Sugar, fruits or unknown	31	No	2620	57	6	g/hL pure alcohol	[17]
China	Bai jiu	Grains	61	No	1979	278	8	g/hL pure alcohol	[18]
Vietnam	Homemade	Rice or unknown	4	No	750	46	21	g/hL pure alcohol	[15]
Vietnam	Home distilled	Rice	20	Observed production	37	9	424	mg/L	Present study
Guatemala	Illegal Cuxa	Sugarcane	9	No	6.5[a]	NA	2412	mg/L	[14]

[a]Average concentration, highest concentration not available.

Compared to the other studies on homemade distilled alcohol, none of them followed the production. The only study which found potentially toxic levels of methanol was done in Romania by Levy *et al.* [21]. They analysed 35 samples of Tuica alcohol, a distilled alcohol from fruit. The sample with highest methanol concentration was 86 000 mg/L. It would require only 0.2 L of this sample to possibly develop symptoms of methanol poisoning. Compared to our study the alcohol was made from fruit. Pectins are abundant in fruit and methanol is the end product of pectin metabolism [22]. Alcohol from fruit will usually contain more methanol, but not toxic amounts. Another difference is their estimated toxic amount of methanol in a 70 kg patient. The rationale for this discrepancy is their use of a Vd of 0.6 L/kg [23], vs. our Vd of 0.7 L/kg [19]. Further, they defined a lower toxic serum concentration of methanol. Their threshold is based on the old methanol serum action level for haemodialysis – 7.8 mmol/L (25 mg/dl) [23]. We have chosen the new threshold for initiating alcohol dehydrogenase inhibitors 10 mmol/L (32 mg/dl) as the lower toxic limit [20]. Using Vd of 0,6 L/kg and 7.8 mmol/L (25 mg/dl) as toxic serum methanol concentration instead, gives a volume of 0.1 L to drink to develop symptoms of methanol poisoning. The potential for toxicity is reflected by the level of the toxic metabolite (formic acid/formate). Given the molar 1:1 ratio between methanol and formate, this is the lowest theoretical level it would take to reach that threshold value of 10 mmol/L.

In the studies from Poland [16], Ukraine [17] and China [18] there is a theoretical possibility that the volume could be relevant in people consuming a constantly high volume of alcohol over a few days. However, it seems unlikely that a higher number of patients as we see in some outbreaks could consume the significantly larger amount it would take to cause death or severe sequelae (brain damage or blindness). Given the metabolism of methanol would have a gradual onset with the concomitant ethanol in the blood, the actual amount needed would be even higher than these theoretical ones. Compared to our study, none of the other studies traced the production process, and there is a potential for addition of methanol prior to sampling.

The present data does not support the theory that home distillation of alcohol from rice will produce toxic amounts of methanol to cause multiple deaths and severe sequelae in several patients. This is in accordance with most former studies on the topic [14–18]. Still, it is possible that single individuals consuming large amounts of alcohol from a selected number of poorly distilled alcohols could encounter clinical features from methanol poisoning. Our study supports the theory that these methanol poisonings in most or all situations are likely due to the addition of methanol for financial gain [4,5]. However, the main ingredient and type of yeast for fermentation may affect the content of methanol produced. This variation calls for more extensive studies both in number of samples and in variety of regions, yet still with production being observed rather than alcohol bought in the aftermath. Such studies should have a varying base of ingredients from rice to various fruits, as well as different types of yeast.

Limitations

The present study is a pilot study, and the main limitations are thus the small sample size, the limited geographical variation and all the alcohol being produced solely from rice. However, by following all the production and distribution steps, it can serve as a model for a more extensive study. In addition, the results are comparable to other less thorough, yet similar studies. A follow-up study should be performed in different regions, with different commodities and with a larger number of producers. The head of commune health centre helped identifying households that were going to start their alcohol production at the time on inclusion. Because of this lack of randomisation, there is a risk of selection bias.

Conclusion

Home distillation of alcohol from rice did not give any toxic amounts of methanol. To become even symptomatic, it would require such a large volume to drink that it is not feasible. Thus, the results from this pilot study supports the theory of methanol being added to alcohol post production. More large-scale studies are warranted to evaluate whether home distillation could possibly be a source of toxic levels of methanol in alcohol containing liquor.

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Conflict of Interest

MI had no role in the interpretation of data, conclusion and writing of the article or the decision on where

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III

An enzymatic assay with formate oxidase for point-of-care diagnosis of methanol poisoning

Running title: Point-of-care test for detection of formate

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Abstract

Gas chromatographic analysis for quantification of plasma methanol requires laboratory equipment and personnel, and it is typically unavailable in short time notice, especially in low- and middle-income countries. Detection of formate with the enzyme formate oxidase (FOX) is a promising method that can make the diagnosis of methanol poisoning simple and fast. The aims of this study were to test the sensitivity and specificity of a modified FOX enzyme, and to test the specificity of a point-of-care-model (POC) containing FOX enzyme with samples from patients with metabolic acidosis. The sensitivity and specificity of FOX enzyme in aqueous solution was evaluated with a spectrometer and by visual detection for colour change. Formate concentrations between 1-20 mmol/L were used to test sensitivity and 18 potentially interfering substances were tested for specificity. The sensitivity of the FOX enzyme was 100% and the specificity 97%. When specificity of the POC-model was tested, no false positives were detected. As such, the sensitivity and specificity of this modified FOX enzyme for detection of formate was high. The results with this enzyme confirm the potential for its use in formate analysis as a fast diagnosis of methanol poisoning.

Introduction and Background

Methanol poisoning has a high mortality and morbidity, but effective treatment exists provided early diagnosis and initiation of treatment.[1-6] The gold standard for diagnosis is detection of plasma methanol by gas chromatography (GC).[7] This is rarely available around the clock even in large university hospitals in high-income countries, and most often it is not available at all in low and middle-income countries. Increased anion gap and osmolal gap are used as surrogate markers instead, but they are unspecific.[8, 9] Further, osmolality analyses done by the proper method with freezing point depression, is also typically not available in low and middle-income countries.

Methanol itself is not toxic, but it is metabolized by alcohol dehydrogenase via formaldehyde to formic acid, the latter being responsible for the toxic effects. Patients who present late to hospital may have already metabolized most of the methanol to formic acid, and methanol may therefore be below the detection limit with corresponding high levels of formate.[10, 11] The use of an enzymatic method for measuring formate is a good alternative, but currently it is in limited use as it requires specific enzymes as formate dehydrogenase,[10] a spectrophotometer and capable technicians.[10, 12] The typically unspecific clinical features make the diagnosis of methanol poisoning challenging, often with a delayed initiation of treatment as the result. In countries where large outbreaks of methanol poisoning occur regularly, both non-specific and specific blood tests are often unavailable. Thus, there is an obvious need for a simple and specific blood test for diagnosis of methanol poisoning.

The ideal point-of-care (POC) test for methanol poisoning should give a diagnosis within minutes from a minimal amount of blood, and with a simple visual readout. It should be easy to use, and it should be stable in room temperature. As such, a formate dehydrogenase-based system works in the laboratory, but it has stability issues and would need a cold chain for transport and storage.[13] This is impractical and expensive, thus limiting the potential availability. The enzyme formate oxidase (FOX) can also be used for enzymatic detection of formate, and it is stable in room temperature. However, both the sensitivity and specificity of this enzyme in the diagnosis of methanol poisoning are unknown.

We have tested a modified FOX enzyme in a simplified POC-model. A formate concentration below 1 mmol/L was set as a threshold value for a negative test, to ensure that endogenous levels were not considered positive.[14] The aims of this study were to test the sensitivity and specificity of a modified FOX enzyme. Secondly, we wanted to test the specificity of the POC-model with patient samples. Further, we wanted to test the clinical applicability of the POC-model by having clinical staff read the result of samples spiked with various concentrations of formate.

Materials and Methods

The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies.[15]

The experiments conducted were divided in two parts: I) testing the sensitivity and specificity of a modified FOX enzyme, and II) testing specificity and clinical applicability of a POC-model containing the modified FOX enzyme.

The principle for the enzymatic method for formate detection is a two-step reaction:

1) $HCOOH + O_2 \rightarrow CO_2 + H_2O_2$ (catalysed by the FOX enzyme)

2) Leuco Dye (colourless) + $H_2O_2 \rightarrow$ Leuco Dye (colour) + H_2O (catalysed by horseradish peroxidase)

Part I: FOX enzyme

Sensitivity and specificity testing of the FOX enzyme

The sensitivity and specificity of FOX enzyme were tested in aqueous solution by a spectrometer and by visual detection for a colour change to blue. Broadcom Q-mini spectrometer reading at 653 nm wavelength was used, and the ratio between the measurements after five minutes and at the start was calculated. A ratio ≥ 0.8 was defined as a negative sample and a ratio ≤ 0.7 as a positive sample. The FOX enzyme solution used was a mixture of the modified FOX enzyme and other substances that will not be disclosed due to intellectual property rights.

For sensitivity, nine cuvettes were separately filled with a known concentration of formate diluted in NaCl 9 mg/mL before being mixed with the FOX enzyme solution. The following formate concentrations were used: 1, 2, 4, 5, 6, 8, 10, 15 and 20 mmol/L. For control, nine new cuvettes were filled with the FOX enzyme solution only. To ensure that the FOX enzyme could separate a negative sample from a positive sample that was close to the chosen threshold value of 1 mmol/L, a separate test was performed for the following formate concentrations: 0, 0.5, 1.5 and 2.0 mmol/L. The experiment was repeated eight times for 0 mmol/L and 10 times for the other three formate concentrations. This gave 29 samples with formate in the concentration range of 1-20 mmol/L for calculation of sensitivity. In addition to the negative formate samples (0 and 0.5 mmol/L, repeated eight and 10 times respectively), the specificity was tested against 18 different substances in concentrations thought to be clinically relevant (Table 1). This gave 36 samples for calculation of specificity. Eighteen cuvettes were filled with a known concentration of the substances to be tested

(Table 1) together with the FOX enzyme solution. For each cuvette, an associated control with only FOX enzyme solution was made.

Part II: POC-model

POC-model description

The POC-model is a test strip that requires one drop (40 µL) of whole blood, plasma or serum, which is applied to the front of the test strip (Figure 1). Red blood cells are removed from whole blood by a separation filter, whereas the enzymatic reaction with the FOX enzyme takes place at a reactive membrane. The presence of formate will generate a visible colour change at the back of the test strip. To evaluate the result, the test strip was compared to a defined colour scale for semi-quantitative detection of formate: negative (corresponding to < 1mmol/L), low positive (1-10 mmol/L) and high positive (> 10 mmol/L) (Figure 2).

Specificity of POC-model with patient samples

The specificity was tested bedside with one drop of whole blood from 14 patients with metabolic acidosis from various origins, where inclusion criteria was set to a base excess < -5 mmol/L (= base deficit > 5 mmol/L). The result was evaluated by visual reading after four minutes against the colour scale for semi-quantitative detection (negative, low positive or high positive) by two evaluators (one intensive care (ICU) nurse and one author YEL).

Clinical applicability of POC-model

Four ICU doctors and two ICU-nurses were randomly selected to read the results of the POC-model. None of the participants were familiar with the POC-model or how to read the result in advance. Four different blood samples were tested: Two negative samples, one sample spiked with 3 mmol/L formate (low positive) and one spiked with 20 mmol/L formate (high positive). One drop of sample was added to the test strip and repeated for each sample. After four minutes, the participant individually assessed the results of the test by visually defining whether each test was negative, low positive or high positive. This was repeated for each participant and the true result was blinded to them. Fleiss' kappa was used to evaluate the inter-rater reliability between the participants since there were more than two of them.[16] Kappa values between 0.61-0.80 is considered substantial strength of agreement and more than 0.80 is considered almost perfect strength of agreement.[17] IBM SPSS® Statistics for Windows (Armonk, NY, USA) version 27 was used for the statistical calculations.

Ethical considerations

Specificity of the POC-model with patient samples was a quality control study and approved by the Data Protection Officer at Oslo University Hospital (case number 21/15801). Experiments involving biological material was destroyed immediately after use.

Results

Part I: FOX enzyme

Sensitivity and specificity testing of the FOX enzyme

All 29 samples with formate concentrations higher or equal to 1 mmol/L were evaluated as positive with a ratio ≤ 0.7 after five minutes (Figure 3), giving a sensitivity of 100%. The average ratio was 0.4 (range 0-0.7). The results for samples with low formate concentrations near the threshold value (1 mmol/L) are presented in Figure 4. Of note, the positive samples with formate 1.5 and 2 mmol/L have a ratio ≤ 0.7 after five minutes and can be separated from the negative samples 0 and 0.5 mmol/L with a ratio ≥ 0.8.

As regards to the nine formate concentrations tested between 1-20 mmol/L, they all gave a visual colour change to blue after five minutes. The results for 2 mmol/L, 5 mmol/L and 10 mmol/L are presented in Figure 5. Note that samples with formate changed colour to blue indicating a positive sample.

Regarding specificity, only one of the 36 negative samples gave a false positive result, giving a specificity of 97%. The sample with isopropanol had a ratio of 0.5 (positive sample) and changed colour, but it immediately turned green instead of a gradual blue development.

Part II: POC-model

Specificity of POC-model with bedside patient samples

All samples from patients with metabolic acidosis of other origins than methanol poisonings were evaluated as negative: six patients had diabetic ketoacidosis, three had confirmed ethylene glycol poisoning, one had renal failure with metabolic acidosis, one had lactic acidosis, and three had metabolic acidosis of unknown origin without suspicion of methanol poisoning.

Clinical applicability of the POC-model

The high positive (20 mmol/L), low positive (3 mmol/L) and one negative sample were identified correctly by all six clinical staff participants. The second negative sample was identified correctly by 5/6 clinical staff participants, whereas one assessed it as low positive.

Comparing the inter-rater reliability between the six clinical staff participants gave a Fleiss' kappa value of 0.87 ($p<0.001$), 95% CI (0.69-1.05), suggesting an almost perfect strength of agreement.[17]

Discussion

The present study evaluated the sensitivity and specificity of this modified FOX enzyme in aqueous solution. The sensitivity for formate was high in the concentration range of 1-20 mmol/L, being most relevant in clinical practice. During a methanol outbreak in Norway, all symptomatic patients had a formate concentration above 10 mmol/L, whereas four patients without symptoms had a formate level between 0.5-8.3 mmol/L.[10] From the large outbreak in the Czech Republic with 101 hospitalized patients, the median formate concentration on admission was 10.9 mmol/L, whereas the highest was 25.2 mmol/L.[11] Formate concentrations were higher in patients with clinical features. Those with visual disturbances had a median value of 15.2 mmol/L and those with dyspnoea 15.4 mmol/L.[12] One patient presented with visual disturbances and a formate concentration of 3.7 mmol/L. This case most likely represented an analytical error, as it did not correspond to the arterial blood gas and anion gap on arrival (personal communication with main author S. Zakharov).

The specificity of the FOX enzyme in aqueous solution was high with only one false positive. Isopropanol had a ratio of 0.5 after five minutes and changed colour immediately to green when the FOX enzyme solution was added. This colour was different from the enzymatic reaction that occurred over time in the presence of formate. The fact that the colour change occurred immediately, and to a different colour may indicate that this was a different type of reaction. The substances tested for specificity represent relevant differential diagnoses in metabolic acidosis, substances potentially interfering with the colour reaction, or common additives to blood sampling tubes (Table 1).

Previous attempts with enzymatic assays for detection of methanol have shown that the specificity was low towards ethanol.[18, 19] In a study from Hack et al., they used a commercial product with alcohol oxidase for detection of ethanol in saliva, to test if methanol, isopropanol and ethylene glycol could be detected when added to plasma.[18] They found that the sensitivity for methanol was good, but it did not distinguish between concomitant ingestion of ethanol. Similarly, when Shin et al. used

alcohol oxidase in a liquid-based system to test for methanol and ethanol in saliva, it was not possible to separate between the two alcohols.[19] Such co-ingestion is very common and avoiding a false positive result is crucial. In the present study, the FOX enzyme in aqueous solution did not react with ethanol, and so did not give a false positive result. When the POC-model was tested bedside with samples from 14 patients with metabolic acidosis of various aetiologies, none gave a false positive result. The POC-model is meant to be a screening tool in metabolic acidosis of unknown origin to verify, or exclude, methanol as the cause. The present data thus support its usefulness as a diagnostic tool.

When testing the clinical applicability of the POC-model, the samples were correctly identified by the clinical staff, except for one participant who identified one negative sample as a low positive. With a low positive test (1-10 mmol/L), the patient is likely asymptomatic.[10, 12] This indicates that treatment should not be initiated. However, a control test is then indicated after 2-4 hours to evaluate whether the formate concentrations are steady, decreasing/normalizing, or increasing. In the latter case, treatment should be initiated. This will ensure that non-significant intakes of methanol do not lead to unnecessary treatment, whereas the control after a given period would indicate if the formate concentration is on the rise. If the patient develops clinical features before the control test, an additional test should be run immediately, to find out whether the features are because of methanol poisoning or not. Therefore, this one negative sample that was erroneously read as low positive would in the clinical situation have triggered a following test after 2-4 hours and would thus not have led to unnecessary treatment. In case of uncertainty as to whether the test is negative or low positive, we recommend it to be interpreted as a low positive and thus be followed by a repeated test.

The negative sample identified correctly by all clinical staff was a blood sample from a patient that previously had a false positive methanol result with the gold standard GC-mass spectrometry (GC-MS). The initial false positive methanol result was followed by a second false positive result on GC-MS. This led to initiation of antidote treatment of this severely acidotic patient. Treatment was continued until repeated reanalysis with GC-MS showed a negative result, also later confirmed by a formate assay by GC-MS. The patient in question would clearly have been a candidate for this POC-model due to the metabolic acidosis of unknown origin. As shown in our study, everyone evaluated this as negative sample and the patient would not have received the antidote treatment based on this.

In most of countries where large outbreaks of methanol poisoning occur, there is limited or no possibility of confirming or eliminating the diagnosis. Treatment facilities are also often limited both

as regard to dialysis and ICU-capacity. This is also true for the preferred antidote, fomepizole, which is typically not available due to its current cost. As such, the presented POC-model may offer new possibilities for diagnosis and treatment, but also a chance to avoid unnecessary treatment. The test is based on detection of the toxic metabolite formate rather than the parent alcohol. Without formate present, methanol poisoning will not give any clinical features.[10, 12] The high sensitivity of the FOX enzyme will detect even low concentrations of formate, and it allows detection even hours before the clinical features become apparent. Further, patients who present late after ingestion may not have any methanol left on admission due to its elimination by metabolism. In a study from Zakharov et al., two of the patients had a methanol concentration below detection limit, but detection of formate confirmed methanol poisoning.[12] On the other hand, a negative formate test does not rule out methanol poisoning. This may be seen in patients who present early or have co-ingested ethanol.[2] However, these patients would have no clinical features from the methanol poisoning. An algorithm for the clinical use of a formate analysis in diagnosing methanol poisoning has earlier been suggested.[7]

The POC-model will also offer opportunities in countries that have facilities to confirm the diagnosis. In the absence of a 24-hour service, or if the samples must be sent to another hospital for analysis, this test enables a faster diagnosis. Even in large university hospitals where specific analyses are available around the clock, the result using the POC-model may be obtained bedside within less than five minutes rather than hours. We recently published the first case report where this POC-model was used in clinical practice on a methanol poisoned patient:[20] The test showed high positive formate even before the result of the blood gas analysis returned, and antidote treatment was started immediately.

Fomepizole is the preferred antidote for treatment of methanol poisoning, but it is not available in most low- and middle-income countries due to the current price. Ethanol is therefore often used as the only available antidote.[21] The main disadvantages of using ethanol, is the difficult dosing and the need for monitoring of plasma concentration to ensure the recommended ethanol level of 22 mmol/L (100 mg/dL).[5, 6] In the absence of ethanol analyses, the current POC-model for formate detection may also be used to monitor the antidote effect. Increased formate concentrations indicate that the metabolism of methanol is not blocked, and the ethanol dosage needs to be increased.

In our study, we have shown that this modified FOX enzyme has a high sensitivity and specificity, thus being promising for use in diagnosing methanol poisoning. Further development and clinical trials are warranted.

Limitations

The sensitivity and specificity of the FOX enzyme is only tested in aqueous solutions and not whole blood, plasma, or serum. Further, when specificity was tested with the 18 mentioned substances, only one defined concentration per substance – although high - were tested. It is therefore unknown whether more extreme concentrations of the substances in question can have an effect. When specificity of the POC-model was tested bedside with samples from patients with metabolic acidosis, we only included 14 patients. In addition, none of them had alcoholic ketoacidosis, which is a very important differential diagnosis. However, neither lactate nor beta-hydroxybutyrate or ethanol gave a false positive result when FOX enzyme specificity was tested separately. The clinical applicability of the POC-model was only tested with six ICU personnel and is hence not to be considered as a full usability test, rather as a pilot study to show the feasibility of such a POC-test.

Conclusion

The present data showed a high sensitivity and specificity of a modified FOX enzyme in aqueous solutions for detecting formate. When a POC-model containing FOX enzyme was tested for specificity with bedside samples from patients with metabolic acidosis of other origin than methanol poisoning, no false positives were detected. The current method may provide opportunities for quick and easy diagnosis of methanol poisoning where this is not possible today, and it can reduce the time from sampling to obtaining the result, even where this is available around the clock.

Conflict of interest

FH, DJ and KEH are inventors and co-owners of the company Orphan Diagnostics, which has developed the enzyme and the current POC-model. There is no commercial product on the market at present.

Data availability statement

Data from the current study will be available upon request.

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Figure legends

Figure 1: Illustration of principles for point-of-care model.

Figure 2: Illustration of point-of-care model with three level scale for semi-quantitative detection of formate; negative < 1 mmol/L, low positive 1-10 mmol/L and high positive > 10 mmol/L. Note. *Adapted from* "Formate test for bedside diagnosis of methanol poisoning," by KE Hovda et al., 2021, *Basic Clin Pharmacol Toxicol*., 129, p. 87 [19].

Figure 3: Sensitivity of formate oxidase (FOX) enzyme in aqueous solutions evaluated with a spectrometer for nine formate concentrations between 1-20 mmol/L. Y-axis is the ratio between the measured value at a given time and start (T=0). A ratio ≥ 0.8 after five minutes was defined as a negative sample and a ratio ≤ 0.7 as a positive sample.

Figure 4: Sensitivity of formate oxidase (FOX) enzyme in aqueous solutions evaluated with a spectrometer for low concentrations of formate. Y-axis is the ratio between the average measured value for each concentration at a given time and start (T=0). A ratio ≥ 0.8 after five minutes was defined as a negative sample and a ratio ≤ 0.7 as a positive sample.

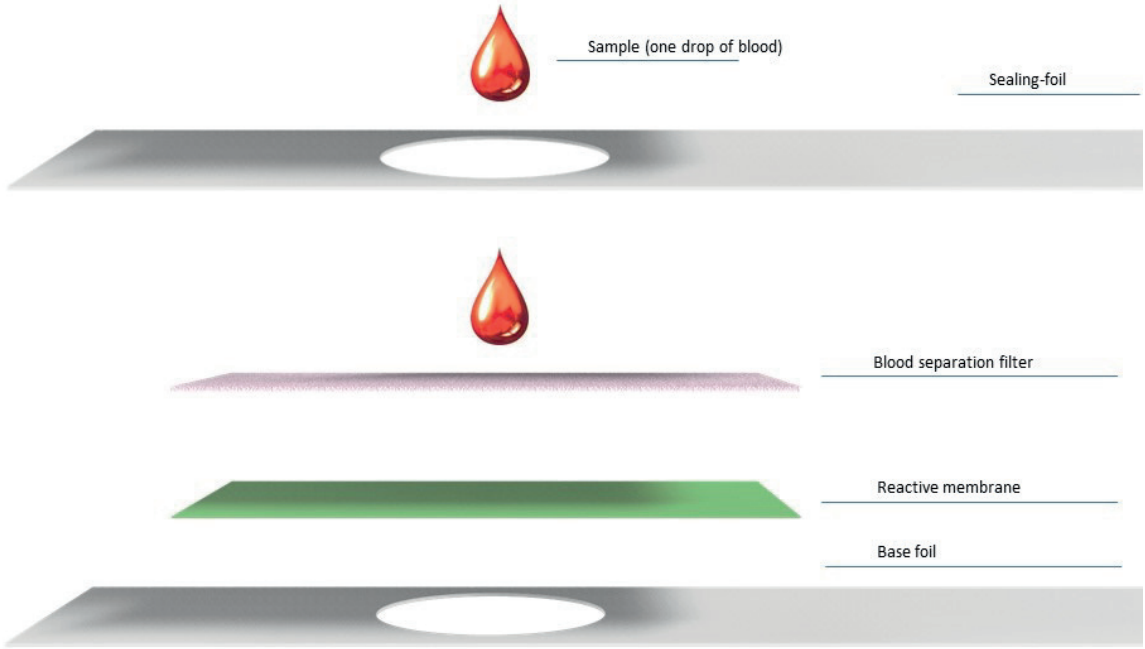
Figure 5: Illustration of the reaction between formate and formate oxidase (FOX) enzyme that gives a colour change to blue after 5 minutes. From left; formate 10 mmol/L; 5 mmol/L; 2 mmol/L and control (without formate).

Table 1: Substances tested for specificity

Substance	**Concentration tested (mmol/L)**	**Explanation**
Acetone	52	Could be present in ketoacidosis
Ascorbate	1000	Could potentially interfere with colour reaction
Beta-hydroxybutyrate	20	Major component in ketoacidosis
Diethylene glycol	10	Toxic alcohol
DL-lactate	20	Cause of lactacidosis*
Ethanol	66	Antidote
Ethylenediamin-tetraacetic acid (EDTA)	51	Anticoagulant in blood sample tubes
Ethylene glycol	51	Toxic alcohol
Fomepizole	0.5	Antidote
Glycerol	2	Could be present in metabolic acidosis
Glycolate	20	Cause of acidosis in ethylene glycol poisoning
Isopropanol	51	Toxic alcohol
L-pyroglutamate	20	Metabolite in the glutathione cycle, may cause metabolic acidosis after e.g. paracetamol use
Methanol	96	Toxic alcohol
Methylene blue	0.05	Could potentially interfere with colour reaction
Oxalate	0.02	Metabolite of ethylene glycol
Salicylate	10	Possible cause of metabolic acidosis
Urate	4	Could potentially interfere with colour reaction

*Most common cause of metabolic acidosis

Figure 1: Illustration of principles for point-of-care model.



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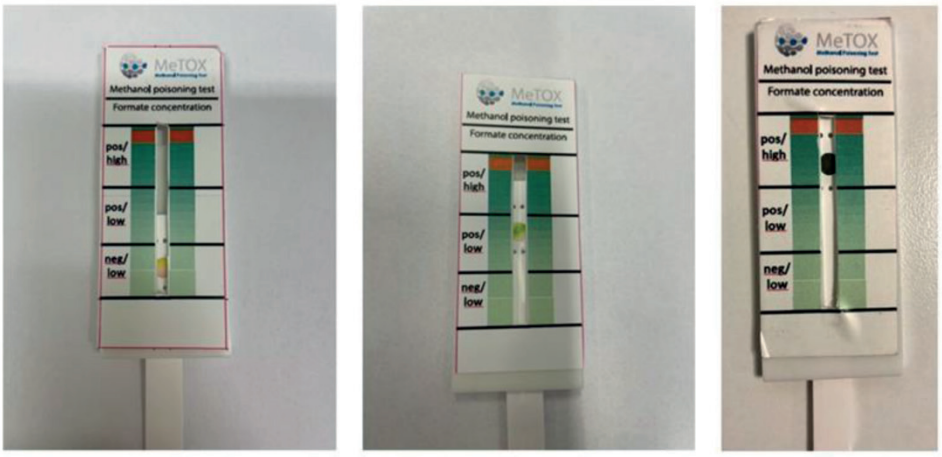

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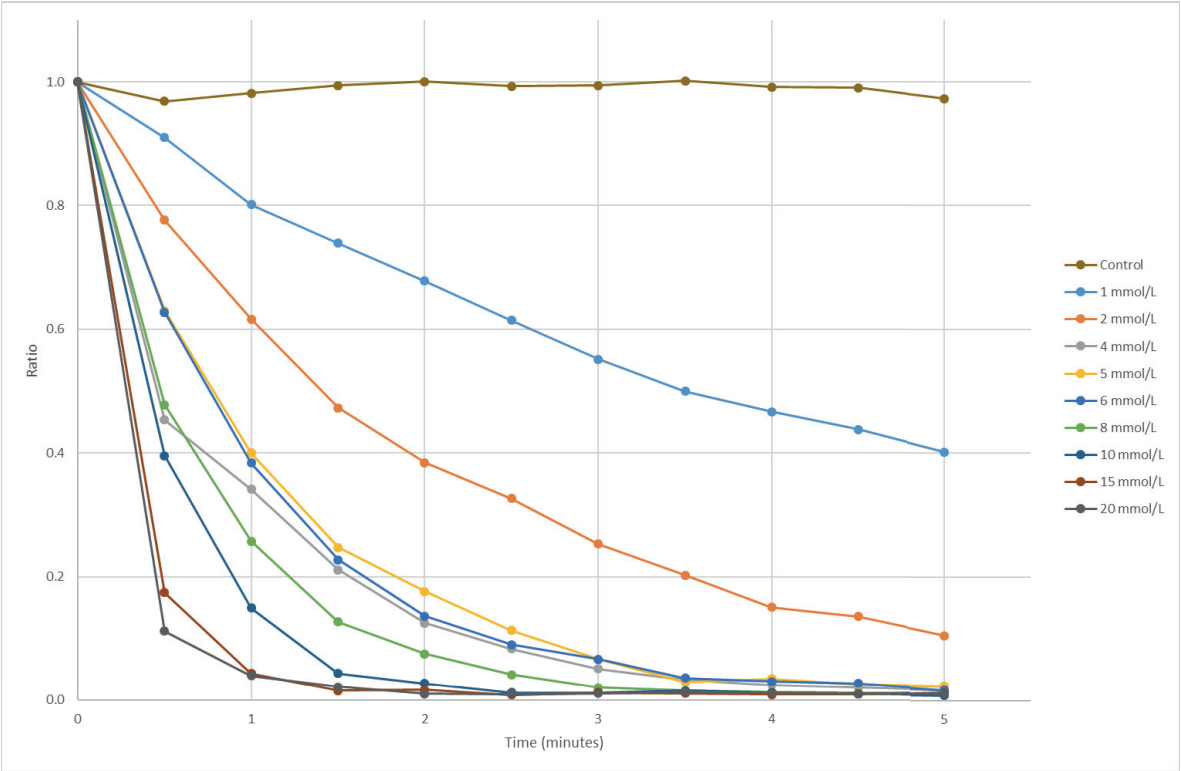

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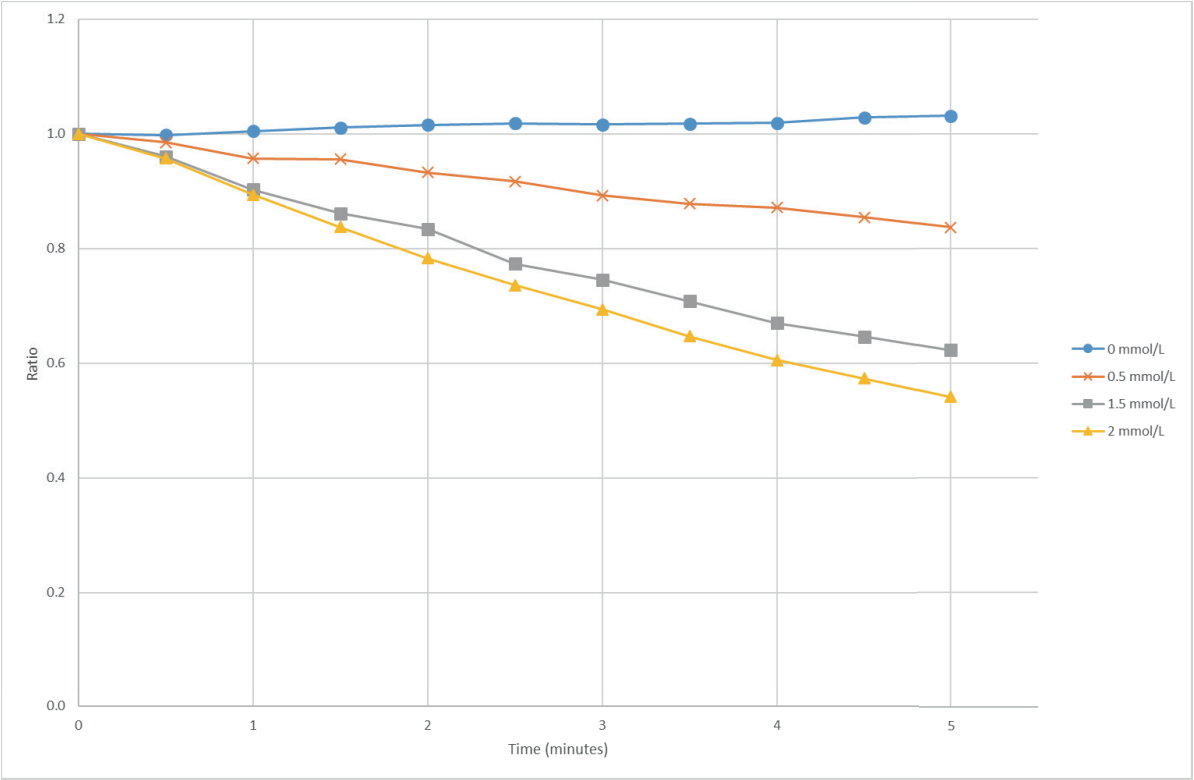

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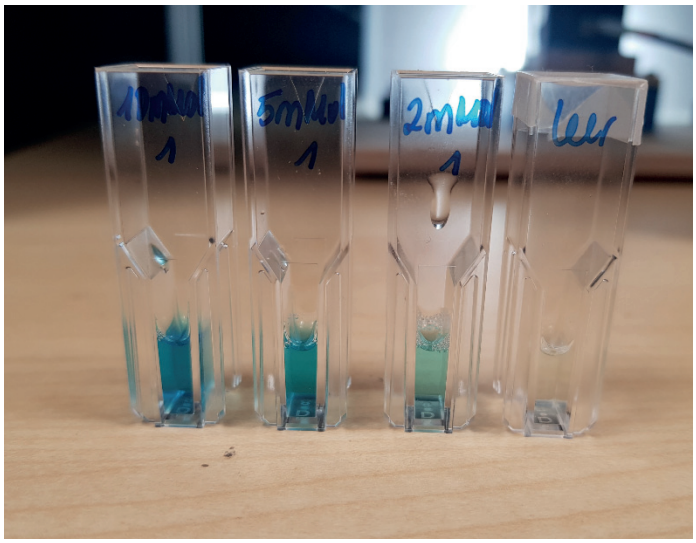

IV

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SHORT COMMUNICATION

BCPT Basic & Clinical Pharmacology & Toxicology

Formate test for bedside diagnosis of methanol poisoning

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Abstract

Methanol poisoning kills thousands of people every year and remains a diagnostic challenge, especially where the resources are scarce, but also in high-income countries worldwide. We are in the course of developing a bedside strip to detect formate – the toxic metabolite of methanol. We hereby present the first clinical methanol case where formate was detected bedside from a drop of blood: The patient, a 61-year-old male, was admitted with a suspect methanol poisoning and severe metabolic acidosis. The test strip was positive after 3 minutes. Sodium bicarbonate (500 mmol/L), fomepizole, dialysis and folinic acid were given based on the positive test. The diagnosis was some hours later confirmed by GC-MS, showing a methanol concentration of 62 mmol/L (200 mg/dL) and a formate concentration of 19 mmol/L. Implementation of this technology into routine clinical use can potentially offer an opportunity for a step change in the management of methanol poisoning.

KEYWORDS

analytical methods, clinical toxicology, toxicological agents, toxicological methods

1 | INTRODUCTION

Outbreaks of methanol poisoning from illicit alcohol are a major global public health problem, particularly affecting areas of poverty in low- and middle-income countries.[1] The diagnosis of methanol poisoning is challenging, requiring blood gas analysis and then laboratory-based chromatographic measurement of methanol concentrations. This usually takes hours if at all available in the receiving hospital. Even in the best-equipped University centres in the Western world, the delay for methanol analyses is a common and frustrating situation. We have studied the possible use of a formate analysis as a highly sensitive and specific way of diagnosing methanol poisoning[2] and developed a simple diagnostic test strip based on the similar enzymatic reaction (using formate dehydrogenase).[3] Although this test proved promising,[4] the stability of the enzyme was limited at room temperature which made it dependent on a cold chain, significantly increasing the potential application cost of the method. With that in mind, we modified a formate oxidase enzyme to ensure adequate temperature stability, activity and specificity.

Methanol itself is not toxic, but it is metabolized by alcohol dehydrogenase in the liver via formaldehyde (short half-life and no clinical relevance) to formic acid/formate. Formic acid/formate is further metabolized to non-toxic compounds. Due to lack of folate reserves in primates being necessary for the latter metabolism, formic acid/formate accumulates, explaining the toxicity in methanol poisoning.[5]

2 | MATERIALS AND METHODS

Forty microliters of testing material (whole blood, plasma or serum) are applied to one side of the strip. Any red blood cells are retained in the first layer, and the cell-free filtrate reaches the reaction layer, containing a formate-dependent

colour-generating system. Our design gives a cut-off value of 1 mmol/L separating a negative from a low positive test. We have made the test semi-quantitative at three different levels: negative (<1 mmol/L), low positive (2-10 mmol/L) and high positive (>10 mmol/L). *Negative* means no formate (or only endogenous) levels are present, indicating either no methanol present, or inhibited metabolism by concurrent ethanol in the blood: in either case, without detectable formate, the patient cannot have symptoms from methanol poisoning. *Low positive* means that formate is present, but in low-/sub-clinical levels. This is typical for the early stages or small intake of methanol. A new sample should be drawn, in for example one hour, to see if the levels are increasing, decreasing or at a steady level. *High positive* means that >10mmol/L formate is present, and the patients are typically acidotic and symptomatic. Assessment can be done by the use of a simple flowchart addressing the use of a formate analysis in a clinical setting.[6]

For specificity evaluation, we have tested against the following substances (unpublished material): D-/L-lactate, betahydroxy-butyrate, glycolate, pyroglutamate, ascorbic acid, ethanol, methanol, ethylene glycol, isopropanol, glycerol, di-ethylene glycol, acetone, semicarbazide, glycolic acid, oxalic acid, methylene blue, fomepizole and EDTA. There was no influence on the specificity testing from any of the tested substances (i.e. no false positives). This prototype has not been made commercially available for technical and aesthetic reasons, but an improved product is under development. As an example of the value of the product, we hereby present the first report of use in a patient poisoned with methanol admitted to our hospital.

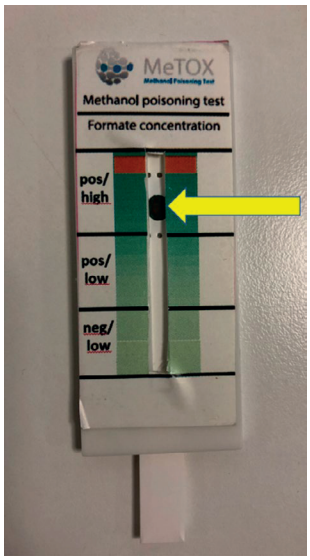

FIGURE 1 The test strip showing a high concentration (>10 mmol/L) of formate in blood after approx. 3 minutes. Pos/low range is set to 2-10 mmol/L and neg/low <1 mmol/L

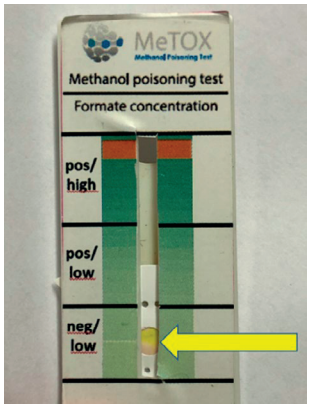

FIGURE 2 After the initial dialysis session, the test was negative and formate was no longer detectable by GC-MS

3 | RESULTS

A 61-year-old man presented to the Emergency Department with a suspected methanol poisoning with visual disturbances, dyspnoea and metabolic acidosis (pH 6.91, pCO_2 3.12kPa (26 mmHg), HCO_3 4.6 mmol/L (4.6 mEq), base deficit 28 mmol/L (28 mEq), lactate 8.5 mmol/L). A drop of blood was placed on the prototype formate test strip (Figure 1). The high formate concentration (>10 mmol/L based on the test strip) and the blood gas results strongly indicated methanol poisoning presenting late with accumulation of both formate and lactate.[5] He was treated with $NaHCO_3$, fomepizole, folinic acid, mechanical ventilation and haemodialysis. Later, his admission methanol concentration was reported at 62 mmol/L (200 mg/dL) with a formate concentration of 19 mmol/L, measured using GC-MS. A blood test strip was negative after dialysis (Figure 2), as was GC-MS for formate. He admitted drinking methanol-containing liquor two days earlier. He was discharged with typical reduced visual acuity and cerebral complications because of late presentation.

4 | DISCUSSION

This is the first report of this prototype test strip for rapid bedside diagnosis of methanol poisoning. With the presence of formate being obligatory to develop symptoms from methanol poisoning, this test can represent a new era for clinicians faced by the difficult diagnosis of methanol poisoning.

4.1 | Limitations

This manuscript is describing a single case report: whereas the use of formate itself is already established in the literature,

this specific method described needs to be further validated in larger studies.

ACKNOWLEDGEMENT

We want to thank prof. Michael Eddleston for his help and critical comments on this manuscript.

CONFLICT OF INTEREST

KEH, GG and DJ are all co-inventors of the device, and all are co-owners of the company Orphan Diagnostics (OD). OD is at present working on making a similar device commercially available with the help of governmental research- and innovation funding. There is no commercial product available at present.

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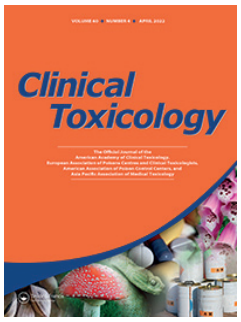

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Fomepizole dosing during continuous renal replacement therapy – an observational study

Yvonne E. Lao, Trond Vartdal, Sten Froeyshov, Brian Latimer, Christiane Kvaerner, Marija Mataric, Peter Holm, Siri Foreid, Dag Jacobsen, Kenneth McMartin & Knut Erik Hovda

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