UZ LEUVEN | LABORATORIUMGENEESKUNDE

CAT
Critically Appraised Topic

Opstellen van een diagnostisch algoritme voor laboratoriumdiagnostiek bij high anion gap en osmolar gap acidose

English title:
Laboratory diagnostics for high anion gap metabolic acidosis and osmolar gap acidosis: designing a diagnostic process flowchart

Author: Matthias Weemaes
Supervisoren: Prof. Dr. P. Vermeersch, apr. Biol. Nele van den Eede
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CLINICAL BOTTOM LINE

Belangrijkste weerhouden bevindingen/conclusies. Iemand die niet veel tijd heeft, moet hier de correcte weergave van de besluiten vinden.

The diagnosis of D-lactic acidosis, 5-oxoprolinuria, ethylene glycol and methanol, rare causes of high anion gap acidosis, is often missed or delayed. Workup of high anion gap acidoses and osmolar gaps is often incomplete or clinicians do not know how to interpret the result. To bridge this gap we designed a diagnostic process flowchart for high anion gap metabolic acidosis which clinicians can use for diagnostic purposes and which can be implemented into an order entry system as a form of clinical decision/diagnosis support.

CLINICAL/DIAGNOSTIC SCENARIO

Hoe/waarom is men tot de vraagstelling gekomen? Wat is eventueel de huidige praktijk en waarom wordt die nu in vraag gesteld?

High anion gap metabolic acidosis can have different etiologies. Acronyms, like GOLDMARK, can aid in the differential diagnostic process. However, diagnostic workup is often incomplete and this can lead to delayed or missed diagnoses.
This critically appraised topic wants to address these issues by firstly constructing a diagnostic process flowchart that clinicians can use for the workup of 'high anion gap metabolic acidosis' and secondly by assessing several case histories and investigating whether a comprehensive and streamlined flowchart can possibly address gaps in the diagnostic process.

High anion gap metabole acidose kent verschillende oorzaken. Acronymen, zoals GOLDMARK, kunnen helpen in het differentieel diagnostisch proces. Echter, vaak gebeurt de diagnostische workup onvolledig en kan dit leiden tot een laattijdige of verkeerde diagnose.
Deze CAT heeft als een tweeledig doel: enerzijds wensen wij een diagnostische flowchart op te stellen die de clinici van intern of externe centra, indien zij dit wensen, kunnen gebruiken als leidraad voor het onderzoeken van de etiologie van een high anion gap metabole acidose bij patiënten. Anderzijds wensen wij, aan de hand van casuïstiek, te onderzoeken hoe vaak een diagnostisch workup voor high anion gap metabole acidose adequaat gebeurt.

QUESTION(S)

1) *Constructing a diagnostic process flowchart for 'high anion gap metabolic acidosis' (HAGMA).*
2) *Reviewing pathophysiology of D-lactate acidosis, 5-oxoprolinuria, methanol and ethylene glycol intoxication.*
3) *How big is the problem?*
4) *General considerations for work-up of HAGMA*

1) Constructing a diagnostic process flowchart for 'high anion gap metabolic acidosis' (HAGMA).

Traditionally, the diagnosis for high anion gap metabolic acidosis (HAGMA) is made through the use of one or more acronyms. These acronyms, in which every letter signifies a cause of HAGMA, serve as mnemonic aids in differentiating HAGMA etiologies. All of these acronyms (GOLDMARK, KUSMALE, MUD PILES, etc.) contain somewhat different letters, depending on the (current) frequency of occurrence of the different HAGMA-etiologies and, although each of those have their own added value, the most recent is also the most useful in applying, namely GOLDMARK.[1] An exploded view of the GOLDMARK acronym shows us the following etiologies for HAGMA: **G** (Glycols), **O** (5-oxoprolinuria), **L** (L-lactate), **D** (D-lactate), **M** (Methanol), **A** (Aspirin), **R** (renal failure) and **K** (keto-acidosis). Despite the fact that KUSMALE and MUD PILES can add **A/P** ((par)aldehyde) and **I** (iron/isoniazide) to the diagnostic workflow, these etiologies are currently considered to be extremely rare and thus not included in the most recent acronym GOLDMARK. [1] Also, newly 'discovered' etiologies (D-lactate, 5-oxoproline, propyleneglycol) are included in the newer GOLDMARK acronym.[1] A comparison between acronyms is listed in table 1. Acronym letters with similar meaning were joined on the same line in table 1.

GOLDMARK	KUSMALE	MUD PILES
Glycols	**E**thylene glycol	**E**thylene glycol
Oxoproline		
L-lactate	**L**actate	**L**actate
D-lactate		
Methanol	**M**ethanol	**M**ethanol
Aspirin	**S**alicylate	**S**alicylate
Renal failure	**U**remia	**U**remia
Ketoacidosis	**K**etoacidosis	**D**iabetes
	Aldehyde	**P**araldehyde
		Iron/Isoniazide

Table 1: comparison between high anion gap metabolic acidosis acronyms, with GOLDMARK as default. Acronym letters with similar meaning are on the same line.

Despite availability of comprehensive and easy-to-use mnemonics we have the impression that too often diagnoses are made on the basis of incomplete laboratory results or that sometimes a clear diagnosis is not made at all. This is especially true for the more rare but relevant causes included in the GOLDMARK (D-lactate, 5-oxoproline).

To address these issues and to improve the diagnostic process around HAGMA we consulted the emergency department and constructed a diagnostic process flowchart (figure 1), based on the GOLDMARK and other, older, acronyms. Starting from the thesis done by B. Decru[2], discussing all possible causes of metabolic acidosis through literature review, this flowchart was constructed in such a way that the clinician should be able to intuitively go through the whole diagnostic process without potentially missing relevant etiologies. Also, by using this flowchart, trainees can train their clinical and differential diagnostic thinking around this framework and perpetuate this process for future HAGMA diagnostic challenges. Finally, this flowchart could potentially be used for demand management with electronic order entry tools.

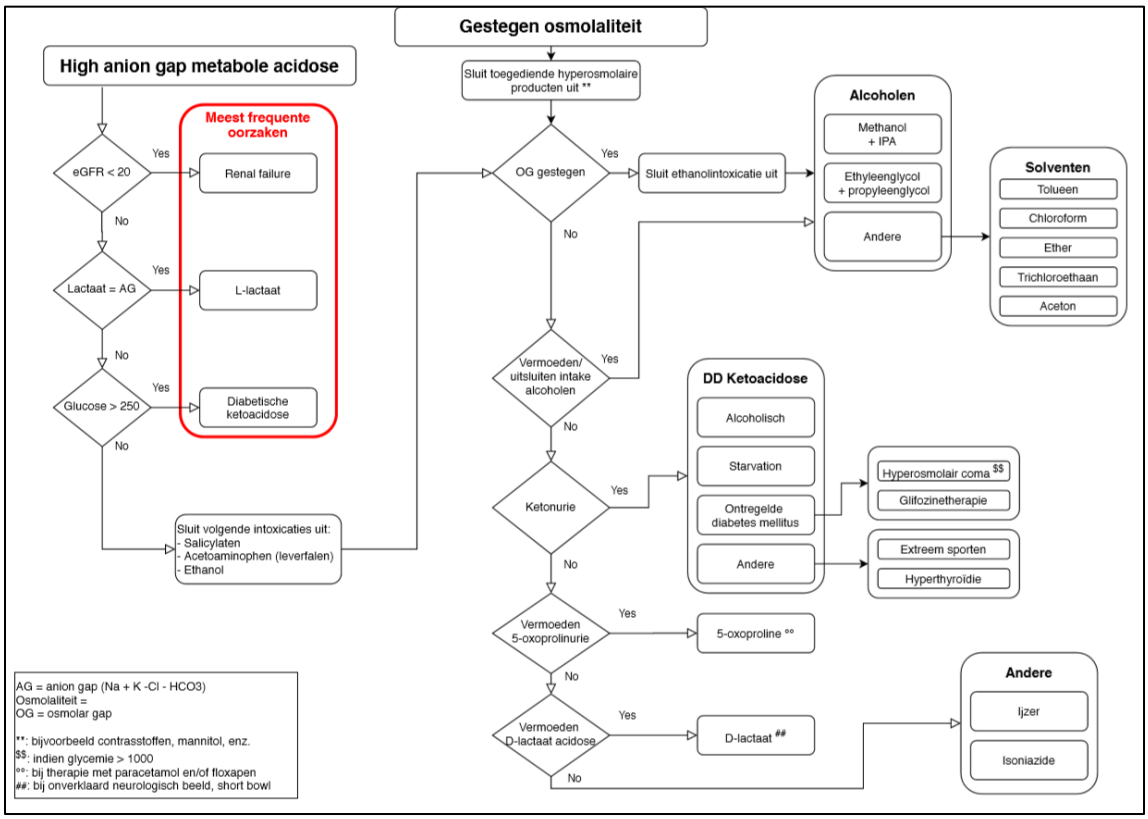

Figure 1: diagnostic process flowchart for 'high anion gap metabolic acidosis'
The full-resolution image of the flowchart is also added in the attachments at the end of this document.

2) Reviewing case histories of D-lactate acidosis, 5-oxoprolinuria, methanol and ethylene glycol intoxication.

a. D-lactate acidosis

Pathophysiology and diagnosis

D-lactate is the stereoisomer of L-lactate and although the lactic acid pool (1-2 mmol/L) normally consists exclusively of L-lactate, traces of D-lactate might be formed by the glyoxylate pathway from methylglyoxal.[3-6] Humans can also take up D-lactate via the gastrointestinal tract from fermented foods (e.g. yoghurt) or produced by colonic bacterial flora from undigested simple carbohydrates.[5-7]

Under normal circumstances, most simple carbohydrates are reabsorbed in the small intestine and only small amounts of L- and D-lactate are produced in the colon. In patients with short bowel syndrome, however, larger amounts of simple carbohydrates can reach the colon after a carbohydrate rich meal, which can result in significant D-lactate production (via bacterial D-lactate dehydrogenase or LD-lactate racemase) and subsequent uptake via the gastrointestinal tract.[5,7,8] Patients with bacterial overgrowth of the small intestine are also at risk of increased D-lactate uptake.

D-lactate acidosis typically presents with atypical neurological symptoms and a high anion gap metabolic acidosis. Short bowel is a significant risk factor for developing D-lactate acidosis. Especially, when a high anion gap acidosis remains unexplained and risk factors for developing D-lactate acidosis apply, D-lactate acidosis should be excluded.

Diagnosis of D-lactate acidosis is challenging. Routine chemistry methods (core lab and POCT) only measure L-lactate and D-lactate should be measured in a blood sample that matches the anion gap increase. The fact that D-lactic acidosis is typically not included in the initial differential diagnoses makes establishing an D-lactic acidosis diagnosis often difficult.[4,9] In some cases, it may take several D-lactic acidosis episodes to establish the diagnosis.[10]

As the enzymes used in routine chemistry and point-of-care testing are selective for L-lactate and do not measure D-lactate, other techniques should be used. Gas chromatography of a (urine) sample can detect lactate but does not differentiate between L- or D-lactate isomers. D-lactate should be measured timely with an assay containing a D-lactate selective enzyme. At the laboratory of UZ Leuven, we apply an in-house developed assay containing a D-lactate specific

lactate dehydrogenase (D-LD, from Lactobacillus leichmannii) and measure kinetically the conversion of NAD^+ to NADH at 340 nm.[11]

b. 5-oxoprolinuria

Pathophysiology and diagnosis

Two rare inborn errors of metabolism, either a defect in 5-glutathionsynthetase or 5-oxoprolinase, can cause 5-oxoprolinuria. More frequent, however, 5-oxoprolinuria is caused by a non-inherited disruption of the gamma-glutamyl cycle (figure 2). Several molecules can disturb the gamma-glutamyl cycle, especially acetaminophen which is the main cause of 5-oxoprolinuria, but also flucloxacillin and vigabatrin.[12-14]

Metabolization of acetaminophen occurs through conjugation with a glucuronide or sulfate or through oxidation by the cytochrome P450 enzyme pathway. This oxidation pathway produces the metabolite (N-acetyl-p-benzoquinone imine or NAPQI) which is consequently conjugated with glutathion and further metabolized to produce both cysteine and mercapturic acid conjugates.[15] Acute intoxication or chronic use of acetaminophen causes depletion of glutathione, which in turn releases the inhibition of gamma-glutamyl-cysteine synthetase, causing an increase in gamma-glutamylcysteine and 5-oxoproline.[16]

Furthermore, flucloxacillin and vigabatrine inhibit 5-oxoprolinase enzyme activity and can consequently cause an excess increase in 5-oxoprolin.[17]

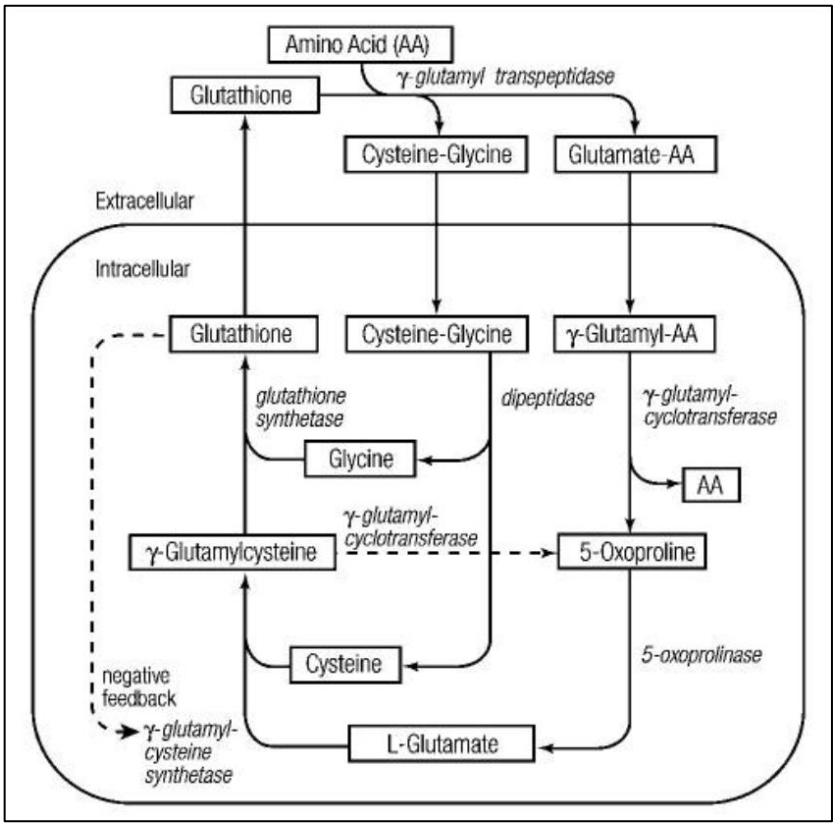

Figure 2: metabolic pathways of gamma-glutamyl cycle

Risk factors for developing 5-oxoproline HAGMA include severe sepsis, malnutrition, liver failure, chronic alcoholism and acetaminophen.

Diagnosis of 5-oxoprolinuria often proves difficult because, like D-lactic acidosis, it is an often overlooked cause of HAGMA. Oxoprolinuria is, however, not uncommon nowadays as many elderly people undergo surgery (e.g. orthopedic surgery) and are often prescribed acetaminophen for post-operative pain. The typical patient to consider for the diagnosis of 5-oxoprolinuria is a female, elderly patient receiving acetaminophen (and flucloxacilline) postoperatively developing a HAGMA. In such and related cases, always consider excluding 5-oxprolinuria.

Chromatographic analysis of 5-oxoproline in urine will objectify the diagnosis.

c. Methanol

Pathophysiology and diagnosis

Methanol is used in several industrial products, ranging from model airplane fuel to perfumes and wind shield washer. Throughout history several sporadic epidemics of mass methanol poisoning (e.g. bootleg whiskey) have been recorded, mostly due to tainted beverages, following improper/illegal distillation of alcohol for consuming purposes.[18]

Alcohols are rapidly absorbed after oral ingestion. Following ingestion, methanol is metabolized by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) into formaldehyde and formic acid, the latter causing toxicity through inhibition of mitochondrial processes (e.g. opticus nerve) and causing tissue hypoxia and accumulation of lactic acid through disturbing the redox-state in the body. Methanol undergoes minimal renal clearing and has an average half-life of 30-54 hours, in patients receiving concurrent ethanol infusion treatment.[19]

In addition to a (hetero)anamnesis suggestive of methanol ingestion, a raised anion gap which cannot be accounted for by the more common causes of HAGMA should trigger the exclusion of toxic alcohol ingestion. Determining the osmolar gap, defined as measured blood osmolality minus calculated osmolality, is primordial when assessing a patient suspected of methanol ingestion. Because the osmolality is only influenced by the osmotically active methanol and the anion gap and blood pH is only influenced by its metabolite (formic acid) it is imperative that assessment of a possible methanol (also true for other alcohols) intoxication should include both a calculated anion gap and a calculated osmolar gap. Generally speaking, a HAGMA in a methanol intoxication points to formic acid. On the other hand, an osmolar gap points towards presence of methanol in the patient's blood. To better illustrate these kinetics we refer to figure 3. Upon ingestion of methanol, which is osmotically active, only the blood osmolar gap will show an increase but as methanol is gradually metabolized and formic acid is produced, the osmolar gap decreases and the anion gap increases. Eventually, when methanol is completely metabolized and only formic acid remains, only a high anion gap will be present. Also, because ethanol is also competing for metabolization by ADH, the HAGMA can take longer to develop. However, if the patient has recently ingested methanol (or other toxic alcohol), an osmolar gap will be present.[18] Moreover, because ethanol is often co-ingested with other toxic alcohols, it can be informative to calculate an ethanol-corrected osmolar gap to indicate indicate its presence.[18]

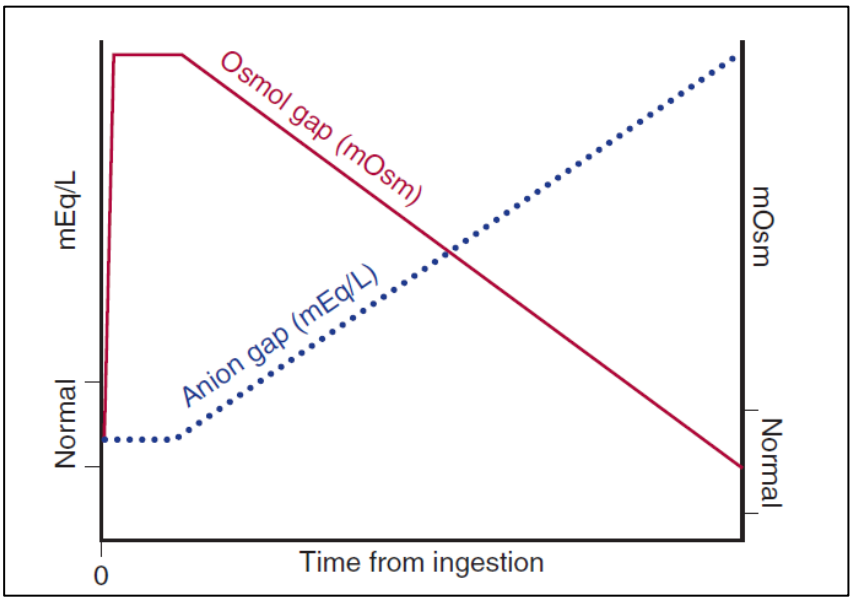

Figure 3: Reciprocal relationship of anion gap and osmolar gap over time (hours). Note that patients presenting early may have a normal anion gap while patients who present late may have a normal osmolar gap. (Goldfrank's Toxicological Emergencies 2011)

Inebriation, a common finding among those intoxicated with ethanol, is associated with all alcohols and severity is linked with the molecular weight of the molecule; the higher the molecular weight, the higher the probability of inebriation (isopropanolol = ethylene glycol > ethanol > methanol). Other clinical manifestations, due to inhibition of mitochondria and tissue hypoxia mentioned earlier, include visual complaints, ranging from visual impairment to total blindness, renal failure, sometimes associated with myoglobulinuria, central nervous system depression, abdominal pain and pancreatitis. Also, because lactate production can occur with methanol

intoxication as well with other causes of hypoxia, it is important to differentiate between the two.

Remark: Although isopropanolol is not included in the scope of this paper it is worth mentioning it because of the fact that it is so ubiquitously present in products we daily use. When ingested, it is metabolized to aceton (osmotically active) but because no organic acid compound is formed a HAGMA does not occur.[18] Moreover, ketosis without HAGMA can be diagnostic for isopropanolol poisoning.[18] Given its frequent use, sometimes in combination with methanol, we recommend to systematically measure isopropanol when methanol is requested. At UZ Leuven, both alcohols can be combined in a single analysis, using GC-FID.

d. Ethylene glycol

Pathophysiology and diagnosis

Similarly to methanol, ethylene glycol is used in industrial products. Today it is primarily used as an engine coolant antifreeze in car radiators.[18] Its sweet taste is a contributing factor to unintentional ingestions. Metabolization of ethylene glycol occurs via the same two enzymes responsible for the metabolization of methanol (ADH and ALDH) with formation of glycolic acid. However, lactate dehydrogenase (LDH), additionally forms glyoxylic acid from glycolic acid. One of the end-products is oxalic acid which forms a complex with calcium and builds up at the glomeruli after crystallization, explaining its nephrotoxicity. Besides metabolization, ethylene glycol is also cleared by the kidneys and has half-life of 11-18 hours. [18]
Diagnosing an ethylene glycol intoxication requires measuring both the anion gap and osmolar gap, similar to when assessing a possible methanol or other toxic alcohol intoxications. The same inverse relation (figure 2) is observed with ethylene glycol. Calcium oxalate can be observed in urine samples of patients.
Besides renal failure and inebriation, ethylene glycol can, in cases of severe poisoning, cause hypocalcemia due to presence of oxalic acid but also cerebral edema and cranial nerve dysfunction.

3) How big is the problem?

First of all, the rare etiologies of high anion gap acidosis (D-lactate, 5-oxoproline, methanol and ethylene glycol) are of course rare. Incidence numbers are lacking for D-lactic acidosis and 5-oxoprolinuria. The US government reported 6599 single exposure cases of ethylene glycol and 1975 cases of methanol in 2018.[20] In the UK, referrals for toxic alcohol ingestion amounted to 85 cases of which ethylene glycol was the agent most commonly involved.[21] To have an estimate of the total number of laboratory test orders, we queried our laboratory information system for all D-lactate (enzymatic assay, plasma), 5-oxoproline (GC-MS, urine) , methanol (GC-FID, whole blood) and ethylene glycol (GC-MS, whole blood) intoxications from the 1st of January 2015 until the 31st of December 2019. During this 5-year period the laboratory of UZ Leuven analyzed 99 samples for D-lactate, 33 samples for 5-oxoproline, 155 samples for methanol and 60 samples for ethylene glycol intoxication.
The positivity rates of those samples, based on the upper limit of the reference interval[22], were 61.6% (61/99) for D-lactate, 81.8% (27/33) for 5-oxoproline, 5.8% (9/155) for methanol and 18.3% (11/60) for ethylene glycol. To limit the number of cases and thus making review of case histories feasible, we made a selection of all the positive samples by using an arbitrary cut-off of 0.5 mmol/L for D-lactate and 100 mmol/mol creatinine for 5-oxoproline.

Additionally, exclusion criteria were: measurements sent to us from external laboratories (because it was not possible to review those cases), multiple measurements in a single patient (e.g. to monitor effect of treatment), documented inborn errors of metabolism (e.g. for 5-oxoprolinuria) and no documented HAGMA (no elevated AG and/or acidosis) or, for the methanol and ethylene glycol cases no documented osmolar gap. Finally, we excluded 2 cases, in which traces of methanol were found in the blood. In these cases we concluded that the traces of methanol found were likely due to chronic alcohol abuse.[23] After exclusion, we identified the following possible cases: 3 cases of D-lactate aciduria, 5 cases of 5-oxoprolinuria, 1 case of methanol intoxication and 1 case of ethylene glycol poisoning (table 2).

	Gender	Age	Result	Time (hours) from HAGMA diagnosis until requesting result in previous column
D-lactate	V	35	0.63 mmol/L (second episode: 21.02 mmol/L)	84
	V	43	0.65 mmol/L	10
	V	60	0.64 mmol/L	216
5-oxoproline	V	68	41960 mmol/mol creatinin	39
	V	49	32795 mmol/mol creatinin	6
	V	55	11493 mmol/mol creatinin	35
	V	70	3506 mmol/mol creatinin	3
	V	68	942 mmol/mol creatinin	3
Methanol	M	24	0.19 g/L	0
Ethylene glycol	V	43	4.3 g/L	0

Table 2: Reviewed cases

These cases are a mix of confirmed cases and possible cases, based on the electronic health record (EHR). The goal of reviewing these cases, possible and confirmed, was to assess completeness of the diagnostic process and to assess whether the flowchart mentioned above can improve the diagnostic process by using it as a framework. For every case we established the baseline moment (date and time) at which point a HAGMA could be determined by a calculated high AG and a decreased bicarbonate or negative base excess. From there we assessed all the step-by-step measurements included in the flowchart: eGFR, lactate, glucose, salicylate, acetaminophen, osmolality (and thus also osmolar gap), ethanol, methanol, ethylene glycol, ketones in urine, 5-oxoproline in urine and D-lactic acid. We checked which of the flowchart tests were demanded per case (demanded yes or no) and noted how long it took from the baseline HAGMA measurement to request these additional tests. Generally we noticed that the lactate, osmolality and ketone in urine measurements were sometimes requested with notable delay from the baseline HAGMA establishment. The average (and max time) between HAGMA diagnosis and requesting the additional test were 14,4 hours (max = 64 hours) for lactate, 34.8 hours (max = 144 hours) for osmolality and 35.1 hours (max = 216 hours) for ketones in urine, as shown in table 3.

Checkpoint	Measured within x hours? (Avg)	Measured within x hours? (Max)
eGFR <20	0.0	0.0
lactaat = AG	14.4	64.0
glucose >250	4.7	32.0
paracetamol	4.8	14.5
salicylaten	0.0	0.0
osmolaliteit	34.8	144.0
ethanol	4.0	12.0
methanol	0.0	0.0
EG	0.0	0.0
ketonen in urine	35.1	216.0
5-oxo	39.9	168.0
D-lactaat	79.0	216.0

Table 3: average and maximum time between HAGMA or OG diagnosis and request of additional tests. Those tests with the largest delay in requesting are marked in red.

For the suspected diagnoses of D-lactic acid and 5-oxoprolinuria we found that adequate diagnostics are generally delayed; as these are often not thought of as possible differential diagnoses, this finding is not illogical. For a D-lactic acidosis we saw that, for a particular case, the definitive diagnosis took several admissions spread over a 2 year period. The unexplained neurological symptoms were consecutively

attributed to Wernicke's encephalopathy and renal failure. Due to partially unknown kinetics of D-lactic acid in the human body it can be difficult to prove that the HAGMA is due to D-lactic acid. Generally speaking, a sample for D-lactic acid measurement and estimation of the anion gap should be taken as soon as a patient presents itself with neurological symptoms. Delaying sampling might result in false negative results or results not matching the high AG.[10]

For methanol and ethylene glycol we noticed that the diagnosis was almost immediately included in the diagnostic work-out in the emergency department and toxicological dosage of methanol and/or ethylene glycol were immediately requested. In diagnosing and treating methanol, ethylene glycol and other toxic alcohol intoxications, it is useful to make a quick assessment of the patient's clinical condition by measuring AG and osmolar gap.[24,25] As such, fast startup of antidote and dialysis can be ensured if necessary. Later on, adjuvantia (e.g. folate in methanol cases, thiamin and pyridoxine in ethylene glycol cases) can also be administered.
Quickly determining the osmolar gap can indicate the amount of toxic alcohol (e.g. methanol or ethylene glycol) in the blood, until they are metabolized. In case of suspicion of ethylene glycol poisoning, the presence of a lactate gap between POCT and the core lab (due to cross-reaction of glyoxylic acid and glycolic acid on some blood gas analysers[26], but not on routine analysers (depending on the enzyme used in the assay), should elicit immediate action if found abnormal.

4) General considerations for work-up of HAGMA

- Remeasure electrolytes (sodium, potassium, chloride, bicarbonate) + AG
- Measure glucose, lactate, creatinine and urea
- Measure ketones
- Measure salicylates, acetaminophen and ethanol
- Measure osmolality and calculate osmolar gap
- If the parameters mentioned above don't indicate intoxication with higher methanol/ethylene glycol but anamnesis is suggestive of ingestion, request methanol or ethylene glycol.
- Unexplained HAGMA's should always warrant further investigation and should prompt thinking of 5-oxoproline of D-lactic acid as possible differential diagnoses.

Conclusion

Despite of the limited number of cases that were reviewed we did observe a trend of incomplete, delayed and missed diagnoses concerning the more rare spectrum of HAGMA causes. We speculate that, by using a flowchart, future diagnostics for HAGMA can be streamlined and improved upon. The causes of HAGMA mentioned above are rare and often forgotten (especially D-lactic acidosis and 5-oxoprolinuria) but are not insignificant. First of all, thinking about the more rare causes of HAGMA is important, especially when a HAGMA remains unexplained and is attributed to renal failure or lactic acidosis which sometimes serve as 'passe-partout' even though, on their own, they cannot explain the HAGMA completely. Secondly, adequate diagnostics with right laboratory tests at the right time are necessary.[27]

COMMENTS

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TO DO/ACTIONS

1) Prospectively validate and fine-tune the HAGMA flowchart
2) Implement the flowchart in UZ Leuven

ATTACHMENTS

Attachment 1
HAGMA flowchart

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Attachment 1