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Case Report

Fatal acute haemolysis and methaemoglobinaemia in a man with renal failure and Alkaptonuria – Is nitisinone the solution?

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ABSTRACT

Haemolysis and methaemoglobinaemia (MetHb) are rare metabolic complications that can occur in Alkaptonuria (AKU), for which there is no curative treatment. Presented is a case of a man who had AKU, and serves as a reminder of life-threatening complications that can occur with haemolysis and MetHb. This case presents an opportunity to revisit important considerations relating to the investigation and treatment of haemolysis and MetHb with a view to raising awareness, and in doing so hopefully reducing the uniformly fatal outcome. Additionally it is proposed that treatment of haemolysis and MetHb with nitisinone is considered as a potentially lifesaving treatment as it is believed that reducing the concentration of circulating homogentisic acid will reduce oxidative stress.

1. Introduction

Alkaptonuria (AKU, OMIM 203500) is a rare disorder of the tyrosine metabolic pathway that is inherited in an autosomal recessive manner, occurring in 1 in 250,000 people [1]. It results from a defect in the *HGD* gene, which encodes homogentisate 1,2-dioxygenase (HGD, EC 1.13.11.5) [2]. The major metabolic consequence of this defect is the significant accumulation of homogentisic acid (HGA) in circulation, and excretion in urine. This increase in HGA is central to the pathophysiology of the disorder, and the deposition of a dark pigment in tissues in a process referred to as ochronosis. The clinical manifestations of this disease are typically observed from the third decade of life and often severely debilitating, but are not generally considered to result in a fatal outcome (see reference [3] for a recent review). Srsen et al. [4] concluded that life span in patients with AKU is unaffected and similar views have also led to the disease being perceived as relatively benign. This has hindered progressing understanding the condition and developing effective disease-modifying therapies. Treatments are mostly palliative and supportive in nature, including underutilised physiotherapy, pain relief and joint replacement. Recently there has been a major focus on the use of nitisinone for the treatment of AKU. This competitive inhibitor of 4-hydroxyphenylpyruvate dioxygenase (HPPD, EC 1.13.11.27) is highly efficacious in reducing HGA [5–10] and has been shown to slow the progress of ochronosis and its consequences [11].

Less commonly observed in AKU are the life threatening acute complications – haemolysis and or methaemoglobinaemia (MetHb). In all 11 cases that have been reported (Table 1) in AKU the mortality rate was 100% [12–22]. Below is a report of a recent case of haemolysis and MetHb in a man who had AKU, and serves as a reminder of life-threatening complications that can occur. This case presents an opportunity to revisit important considerations relating to the investigation and treatment of haemolysis and MetHb in the context of AKU, with a view to raising awareness, and in doing so hopefully reducing the uniformly fatal outcome. Moreover it enables discussion around the potential use of nitisinone as a potentially lifesaving treatment for these patients.

2. Case report

A 48-year old man underwent a successful uncomplicated elective knee arthroplasty. Prior to knee surgery, haemoglobin was within the normal reference interval (136 g/L). Three days later he presented to hospital with myocardial tightness to his chest, this was thought to have

Abbreviations: AKU, alkaptonuria; HGA, homogentisic acid; AKI, acute kidney injury; CKD, chronic kidney disease; MetHb, methaemoglobin; G6PD, glucose-6-phosphate dehydrogenase

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Table 1
Summary of cases of the acute haematological complications observed in patients with AKU reported in the literature. Adapted from Davison et al. [23].

Age (yrs) Gender	Primary illness	Trigger	MetHb present/max concentration (%)	Treatment	Outcome	Reference
50 Male	Arthropathy Type 2 diabetes CKD	Exacerbation of COAD Metabolic acidosis Haemolysis	Yes 34	Methylene blue	Death	[12]
66 Female	Aortic valve surgery COAD Hereditary telangiectasias Diabetes	AKI Sepsis	Yes No value reported	Unknown	Death	[13]
59 Male	CKD	Exacerbation of CKD Metabolic acidosis Haemolysis Sepsis	Yes 29.9	Haemodialysis Methylene blue Vitamin C Transfusion	Death	[14]
79 Male	Arthropathy	AKI Sepsis	Yes 27.9	Transfusion Vitamin C	Death	[15]
24 Male	CKD Hypertension Epilepsy	Exacerbation of CKD Haemolysis Metabolic acidosis	No	Haemodialysis/filtration N-Acetyl cysteine Vitamin C Transfusion	Death	[16]
50 Female	None	AKI Sepsis	Yes 26.8	Haemodialysis Methylene blue Transfusion	Death	[17]
27 Male	CKD Renal transplant Aortic insufficiency ?Erythropoeitic protoporphyria	Haemolysis Thromocytopenia Acute liver failure	No	Haemodialysis Antibiotics Transfusion	Death	[18]
72 Female	Diabetes mellitus Gastric ulcers Ischaemic heart disease Arthropathy	AKI Dehydration	Yes 43.6	Methylene blue Ascorbic acid Renal replacement therapy Transfusion	Death	[19]
63 Male	CKD Hypertension	Urosepsis Hydronephrosis Calculi Exacerbation of CKD Metabolic acidosis Haemolysis	Yes 25.1	Haemodialysis/filtration N-Acetyl cysteine Vitamin C Transfusion	Death	[20]
63 Female	1 week history of anorexia, nausea, abdominal pain	AKI Haemolysis Not known to have AKU, diagnosed at post mortem	Yes No value reported	Plasmaphoresis Exchange transfusion	Death	[21]
60 Female	ESRF Arthropathy Mitral valve replacement Anaemia	3 day history of weakness Anaemia	Yes 24.5	Exchange transfusion Methylene blue Haemodialysis Antibiotics	Death	[22]
48 Male	**Asthma** **Umbilical hernia** **Renal stones** **Knee arthroplasty**	**Pain to left side** **Nausea and vomiting** **Ascites** **Anaemia** **Jaundice**	**Yes** **> 30**	**Haemodialysis** **Vitamin C**	**Death**	**Current case**

CKD – chronic kidney disease; AKI – acute kidney injury; COAD – chronic obstructive airways disease; MetHb – methaemoglobin; ESRF – end stage renal failure. All bold text represent abnormal results - outside of the reference interval.

been brought on by stress and renal colic pain. At this time he had low haemoglobin (Table 2). Five days after this episode he presented to his local hospital with pain to his left side, nausea and vomiting. He had anaemia of unexplained origin, was jaundiced (Table 2) and had ascites. He was not on nitisinone or any other regular medications at the time of presentation and did not have a history of haemolysis. At this time he was considered to be nutritionally replete and was not on a protein restricted diet.

Past medical history included a diagnosis of AKU five years previously, documented by increased urine HGA. He also had a history of asthma and an umbilical hernia (~4 cm). His right kidney was reduced in size by 10 cm on ultrasonography, and had previous episodes of renal colic and renal stones (three months previously serum creatinine was 93 μmol/L, eGFR > 60 mL/min/1.73 m^2 using an enzymatic creatinine assay).

Eight months prior to this acute presentation the patient completed their participation in a clinical trial (Suitability Of Nitisinone in AKU 2 (SONIA-2), ClinicalTrials.gov Identifier: NCT01916382) evaluating a disease modifying treatment for his AKU and required him to take 10 mg daily nitisinone over a four-year period (urinary HGA concentrations in this patient pre- and four years post nitisinone were 48,447 and 200 μmol/24 h, respectively (personal communication Ranganath LR), reference range < 2.92 μmol/24 h [24]). Following the clinical trial funding was not available to continue treatment with nitisinone; nitisinone was not made available as part of the clinical trial upon its termination and this was conveyed to patients through a patient information sheet when they were enrolled. This was because the patients were not local and came from all around Europe and also from Jordan, making it impossible to administer nitisinone safely with the required metabolic and dietetic support and monitoring. It is presumed that HGA returned to pre-nitisinone concentrations (concentration not measured), based on our experience in the National AKU Centre in the United Kingdom, where previous high levels of HGA in serum and urine return by two weeks of stopping nitisinone, in keeping with the known

Table 2
Summary of abnormal biochemistry and haematology blood results from the acute admission to hospital.

Test	Reference interval	5 Days before admission	Day of admission	1 Day after admission
Haemoglobin	118–148 g/L	**98**	**81**	
Reticulocytes	0.50–1.50%			**6.4**
Bilirubin	< 21 μmol/L	**9**	**35**	**48**
ALT	< 35 U/L	**69**	29	
AST	< 45 U/L	**45**	**57**	
LDH	< 250 U/L		**2632**	**2498**
Haptoglobin	0.32–2.5 g/L		**< 0.32**	**0.12**
Total calcium	2.2–2.6 mmol/L		2.30	**2.15**
Phosphate	0.8–1.5 mmol/L		**1.48**	**2.06**
Sodium	133–146 mmol/L	**144**	**132**	140
Potassium	3.5–5.3 mmol/L	**4.0**	**5.9**	**6.5**
Urea	2.5–7.8 mmol/L	**12.1**	**35.6**	**26.3**
Creatinine[a]	50–130 μmol/L	**145**	121	91
eGFR (CKD-EPI)	> 60 mL/min/1.73 m^2	**45**	61	69
C-Reactive protein	< 5 mg/L		**130**	**158**
Procalcitonin	0.05–0.1 ng/mL		**3.01**	

All results in bold text are outside of the reference interval. Only abnormal results are included in this table despite other investigations being performed.
[a] Creatinine measurements performed using an enzymatic essay.

half-life of 54 h. Whilst participating in the SONIA-2 clinical trial he presented with several episodes of renal stones (1–3 stones on four occasions), and despite intermittent renal impairment (serum creatinine based on Jaffe based assay varied from 90 to 131 μmol/L between November 2014 to October 2018) secondary to the renal stones, he did not develop any haematological complications (haemoglobin concentrations ranged from 148 to 160 g/L between November 2014 to October 2018). The severity of AKU in this patient was assessed using the AKU severity score index (AKUSSI) [25] during the SONIA-2 clinical trial. His overall AKUSSI score (includes a score for joint, spine and clinical) was 104 before starting nitisinone and 138 following 4 years of treatment with nitisinone, showing the severity of his AKU did worsen over this period. Recurrent renal stones contributed significantly to the AKUSSI scores increasing during SONIA-2 in this patient (personal communication Ranganath LR).

Laboratory tests carried out at the most recent acute presentation showed profoundly low haemoglobin (Table 2) with a normal mean cell volume, consistent with normocytic anaemia. Peripheral blood smear did not reveal any abnormal cell forms (e.g. spherocytes or elliptocytes). Additional laboratory tests revealed a marked elevation in LDH and reticulocytes, with a very low haptoglobin; together confirming a diagnosis of haemolysis (Table 2). The cause of the haemolysis at this time was not known and the patient received a blood transfusion to increase his haemoglobin. A few hours after admission the patient appeared to deteriorate and additional laboratory investigations were performed; Coombs test was negative, excluding autoimmune haemolytic anaemia and arterial blood gas analysis (Table 3) revealed a profound MetHb, with decreased oxyhaemoglobin and a mild acidaemia. Over the course of the day MetHb increased and oxyhaemoglobin decreased; oxygen therapy was administered to combat hypoxia, but this did not improve his clinical picture. Additionally, the patient had raised inflammatory markers (Table 2), suggesting an infection of unknown origin. Assessment of renal function at the time showed a disproportionate increase in urea compared to creatinine (eGFR > 60 mL/min/1.73 m^2) and mild hyperkalaemia, this pre-renal picture was thought to reflect dehydration and increased haemoglobin breakdown. Insulin and dextrose were administered at this time to treat the hyperkalaemia and to rehydrate the patient.

Over the course of the admission the patient was also given the antioxidant vitamin C (1 g in 5 mL water) and haemodialysis to reduce the metabolic consequences of haemolysis and oxidative stress. This was unsuccessful and the patient deteriorated further; with MetHb and lactate increasing to > 30% and > 17 mmol/L, respectively (Table 3). Pre-terminally, the patient became markedly hyperkalaemic and hyperphosphataemic, possibly secondary to red blood cell break down and further deterioration of renal function. In an attempt to combat this the patient underwent further haemodialysis and had a repeat blood transfusion. Unfortunately the patient did not respond and went into multi-organ failure, and passed away soon after.

3. Discussion

Haemolysis and MetHb are life threatening acute complications that can occur in AKU as a consequence of increased oxidative stress. Currently there are no guidelines or best practice recommendations available for the management of these patients. Outside of AKU the investigation and management of these complications are well documented [26–28].

Sadly, this is the sixth case report of a patient presenting acutely with both haemolysis and MetHb in a patient with AKU, and like all other cases in the literature it presented a challenge both in the context of diagnosis and management. There is little to be gained from an extensive comparison between the case presented and those previously reported to have haemolysis and or MetHb. It is curious however to note that in only 5 of 11 cases both haemolysis and MetHb were reported [12–14,20,21].

HGA is central to the metabolic consequences described above and it has been purported that it is converted to 'soluble melanins', which create a pro-oxidant environment acting as a catalyst for oxidative stress in AKU [29–33]. Additionally it has been demonstrated that tissue injury can be induced by benzoquinone acetic acid (BQA), a product of the oxidation of HGA by polyphenol oxidase. The formation of BQA is likely to have a role in the formation of MetHb as it has be shown that quinone imine compounds are involved in the formation of MetHb [34]. Recently advanced oxidation protein products have also been shown to be increased in AKU, providing further evidence of

Table 3
Summary of arterial blood gas results from the acute admission to hospital.

Blood gas measurement	Reference interval	Day of admission (time in 24 h format)								1 Day after admission	
		07:21	8.23	9.53	12.05	14.05	17.48	19.59	23.22	17.54	22.42
pH	7.34–7.44	**7.32**	**7.33**	7.43	7.41	7.43	7.43	7.38	7.43	**7.17**	**< 7.0**
pCO_2	4.7–6.0 kPa	**4.0**	**3.9**	**3.7**	**3.9**	**3.7**	**3.5**	**4.0**	**3.6**	**2.9**	4.7
pO_2	11.3–14.0 kPa	**9.6**	**10.4**	**10.5**	16.7	24.4	23.7	12.5	13.6	15.2	16.1
Oxyhaemoglobin	96–99%	73.3	70.6	69.1	67.3	60.8	60.0	63.6	59.8	70.2	65.3
Carboxyhaemoglobin	< 1.5%	**4.2**	**3.7**		**3.1**	1.0	0.7	2.0	1.9	**3.7**	1.0
Methaemoglobin	< 1.5%	**19.5**	**23.6**	**25.8**	**29.6**	**> 30**	**> 30**	**> 30**	**> 30**	**> 30**	**> 30**
Oxygen saturation	96–99%	96.1	97.1	98.2	100	100	100	100	100	100	100
Lactate	0.5–2.2 mmol/L	0.6	0.5	0.6	0.9	1.6	2.1	1.9	**2.8**	**> 17.0**	**> 17.0**

All results in bold text are outside of the reference interval.

oxidative stress [35–37]. However, an increase in HGA alone is unlikely to be the cause of MetHb and or haemolysis, as there are a number of patients with renal impairment in AKU who never seem to develop MetHb and haemolysis. It is far more probable that a pre-existing disturbed redox status (genetic or acquired) could provide a pro-oxidant environment. As previously reviewed [23], there are several factors known to favour oxidative stress, including (i) acute kidney injury (AKI) and AKI on background of chronic kidney disease (CKD), (ii) vitamin and trace element deficiencies and (iii) genetic deficiencies in the red blood cell membrane (e.g. spherocytosis) or enzymes (e.g. glucose-6-phosphate dehydrogenase (G6PD)). In the case presented the patient had a long history of renal stones, but no history of severe AKI and or CKD. Common to all previous cases is that all patients had AKI, in some cases this was a new presentation and in others it occurred on a background of CKD. An additional consideration in the patient presented is the impact that the surgery (performed five days before presentation) may have had on renal function. Whilst surgery was performed without complication, it is well known that surgery causes increased catabolic stress and may be worsened by blood loss and or dehydration, thus impairing renal function. The impact of this is likely to be magnified in a patient with AKU as the kidney plays a critical role in both the elimination and secretion of HGA from the body [38].

Six of 11 patients also presented with sepsis [13,14,16,17,20,22] and the case presented herein also had an infection of unknown origin, which may have aggravated their renal function further due to an increased catabolism, leading to an increased flux down the tyrosine metabolic pathway resulting in increased HGA production, further contributing to oxidative stress.

In all 11 cases (Table 1), and the case presented here, HGA was not measured at the time of the acute presentation, but is presumed to have been increased as kidney function was impaired and patients were not on HGA-lowering treatment; the actual measurement of HGA in this syndrome has never been documented.

Together the accumulation of uraemic toxins and HGA are likely to have increased oxidative stress. It is noteworthy that the measurement of creatinine using enzymatic assays is unreliable and can lead to an under estimation in creatinine results and therefore an overestimation of estimated glomerular filtration rate [39]. It is the authors' recommendation that creatinine based measurements for the assessment of renal function, in AKU generally and more so when much higher concentrations are suspected following renal failure, should be done using a Jaffe based colorimetric assay as they were when the patient participated in the SONIA-2 clinical trial.

Common to all cases including this one is that vitamin and trace element levels were not evaluated, nor were red cell enzyme defects (e.g. G6PD) assessed. If such trace element deficiencies were to be uncovered, it could also lend itself to therapeutic intervention. It is much harder to anticipate the presence of genetic abnormalities of red cell predisposing to haemolysis, but these are an important consideration and should be borne in mind.

Parenteral iron, repeated blood transfusions and haemodialysis are also thought to contribute to oxidative stress and together with the factors mentioned above may overwhelm the endogenous anti-oxidant capacity of the body leading to haemolysis and MetHb. In the case presented the patient received two blood transfusions and haemodialysis. Both modes of treatment have been utilised in the majority of cases reported in AKU patients with haemolysis and or MetHb without success, despite their potentially beneficial effects in treating hyperkalaemia and anaemia, respectively.

The only anti-oxidant therapy trialled in the case presented was vitamin C. While vitamin C is an important substrate required for anti-oxidant defence mechanisms (i.e. reduction of thioredoxin and glutathione) it is only a small part of a somewhat complex anti-oxidant system. Moreover caution has to be taken in using vitamin C as it can induce haemolysis in patients with G6PD deficiency [40]. Other potential anti-oxidant treatment options not utilised in this patient include methylene blue and *n*-acetyl-cysteine. The rationale for using methylene blue is that it reduces iron in haemoglobin back to its oxygen carrying state Fe^{2+} thus improving the delivery of oxygen and reducing oxidative stress. N-Acetyl-cysteine has also be trialled previously cases reported [15,20] in an attempt to reduce glutathione and reduce oxidative stress, but both have been used without success in previous reported cases.

It is clear from this case and those that have been previously reported that there is limited awareness of the life threatening nature of acute haemolysis and or MetHb in AKU, and that all standard treatments are not effective enough to save an AKU patients life. So what are we missing? The biggest metabolic perturbation observed in AKU is the accumulation of HGA and thus if the additional 'insult' for the haematological complications in AKU is the formation of pro-oxidants as a consequence of HGA accumulating, then the inhibition of its formation should be a potential lifesaving treatment. As alluded to earlier nitisinone is a highly efficacious inhibitor of HGA through its action on HPPD and is known to work rapidly (i.e. 60% decrease in circulating HGA within 48 h on 2 mg dose (Ranganath LR, unpublished data)). In this case (and in all other cases reported) nitisinone was not trialled, and had been stopped eight months prior to admission as the patient was on it as part of a clinical trial. It is difficult to prove in this patient that stopping nitisinone after having a 10 mg daily dose caused a progressive increase in oxidative stress and contributed to these complications. Nitisinone was not used in this case because of the limited availability and its unproven efficacy in the setting of acute haemolysis and MetHb, but in our opinion, this should not be a barrier to its use. Nitisinone is widely available in most parts of the World easily as it is used as life-saving therapy in hereditary tyrosinaemia 1 (OMIM 276700), where it is the current standard of care. It should be possible to work with the local paediatric community to obtain nitisinone on a compassionate emergent basis in such situations. The key is to consider this potentially lifesaving treatment in the context of unexplained anaemia, deterioration in renal function and suboptimal antioxidant defence mechanisms. Only by using nitisinone in this scenario in AKU can we prove that nitisinone is the answer to what is otherwise a uniformly fatal outcome.

3.1. Learning points

- A drop in haemoglobin/unexplained anaemia in AKU should lead to methaemoglobin measurement; this biochemical assay is widely available, rapid and reliable thus facilitating a rapid diagnosis.
- A Jaffe colorimetric assay for the measurement of creatinine should be used to assess renal function (eGFR) in AKU on an annual basis and not an enzymatic assay, to avoid complacency.
- Nitisinone should be ordered urgently through the local paediatric community and if necessary by contacting the manufacturers (Swedish Orphan Biovitrum) to administer to the patient as a potentially lifesaving treatment.
- Patients with AKU should have antioxidant status assessed, including the measurement of the trace elements copper, zinc and selenium and be screened for deficiency in G6PD deficiency as this is risk factor for haemolysis.

Compliance with ethics guidelines

All procedures reported in this manuscript were in accordance with the ethical standards of the local Hospital ethics committee and with the Helsinki Declaration of 1975, as revised in 2000. Ethical approval was obtained from the family of the patient reported in this case report.

Author contributions

Davison AS wrote the first draft of this manuscript. Davison AS, Luangrath E, Selvi E, Ranganath LR reviewed and revised the final

manuscript.

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Declaration of Competing Interest

Davison AS, Luangrath E, Selvi E, Ranganath LR have no conflict of interest.

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