

## Exceptional Case

# Fatal oxidative haemolysis and methaemoglobinaemia in a patient with alkaptonuria and acute kidney injury

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### Abstract

Alkaptonuria (AKU) is a rare inherited disorder of tyrosine metabolism, which leads to an accumulation of homogentisic acid (HGA) and is associated with a progressive arthropathy. Fatal complications are unusual and usually result from cardiac disease or progressive renal impairment; rapidly fatal haematological complications are exceptionally rare and described in only a handful of case reports. This case involves a 63-year-old male with AKU and modest chronic kidney disease who developed rapidly fatal haemolysis and methaemoglobinuria following an episode of acute kidney injury triggered by an obstructing ureteric calculus and urosepsis. The patient succumbed despite aggressive antioxidant therapy with ascorbic acid and n-acetyl cysteine. A rapid build-up of HGA due to reduced renal clearance, triggering oxidative haemolysis and methaemoglobinuria is proposed as the mechanism. Alternative strategies to consider when conventional antioxidants fail are discussed including the potent inhibitor of HGA production, nitisonone.

**Keywords:** acute kidney injury; alkaptonuria; homogentisic acid; methaemoglobinaemia; oxidative haemolysis

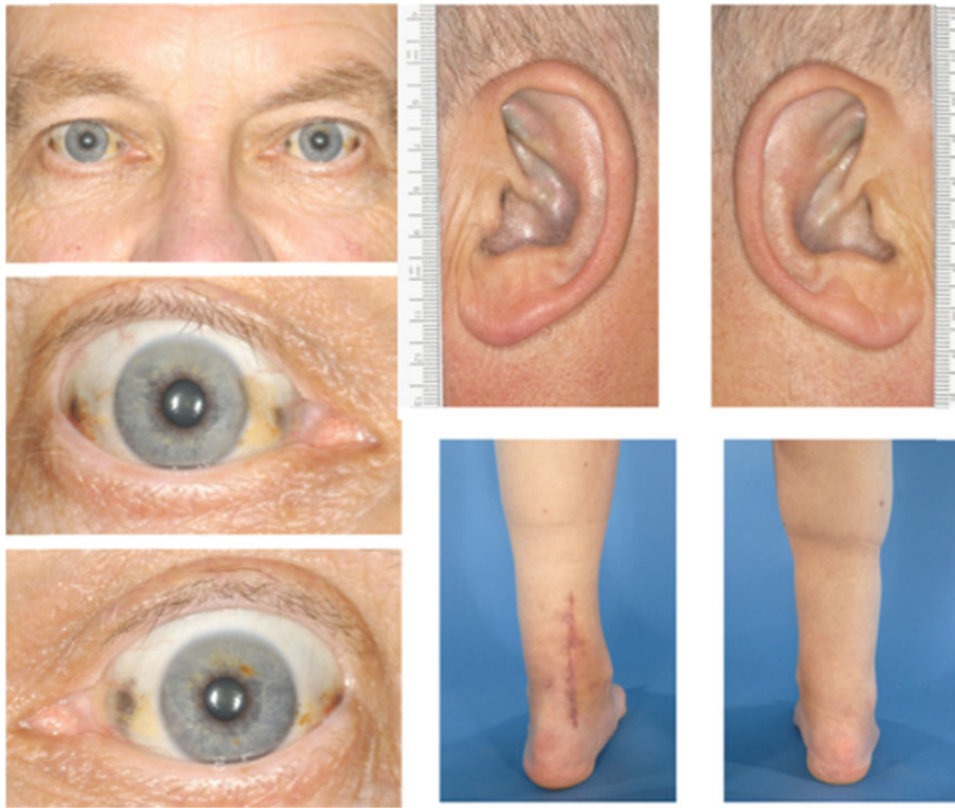
## Introduction

Alkaptonuria (AKU) is an autosomal recessive disorder of the tyrosine degradation pathway with deficient activity of homogentisate 1,2-dioxygenase leading to accumulation of homogentisic acid (HGA) [1]. It is characterized by oxidized HGA-forming pigmented benzoquinone polymers similar to melanin, which deposit in connective tissue giving the classical discoloration of ear cartilage and sclera [1]. A progressive ochronotic arthritis results and involvement of cardiac valves and coronary calcification can have serious consequences [1]. Efficient renal clearance of HGA by both glomerular filtration and tubular secretion exposes the renal parenchyma and collecting system to high concentrations of HGA predisposing to nephrolithiasis. High levels of urinary HGA are diagnostic of AKU and explain the typical discoloration seen on urinary alkalization. Haematological complications of AKU are rare but can be dramatic with case reports of fatal haemolytic anaemia associated with renal impairment [2].

## Case report

A 63-year-old man presented to his local hospital with severe urosepsis and acute kidney injury. He had been

diagnosed with AKU at the age of 40 on the basis of elevated urinary HGA, after 10 years of unexplained back pain, short stature and loss of height. He exhibited typical pigmentation of the pinnae and sclera (Figure 1). He embarked on an extensive orthopaedic career with multiple joint replacements and tendon surgery, subsequently developing hypertension and progressive chronic kidney disease with a baseline creatinine of 190 µmol/L. He had undergone elective shoulder resurfacing and radial head excision 2 weeks earlier, when his creatinine had risen to 299 µmol/L. He was admitted with fever, renal angle pain and a progression in the acute kidney injury with a creatinine of 727 µmol/L, urea 33.9 mmol/L. Additional investigations revealed a CRP of 163 mg/L, significant anaemia (Hb 74 g/L), leucocytosis (WCC  $14.6 \times 10^9/L$ ) and a serum bicarbonate of 13 mmol/L with an anion gap of 22 mmol/L (Table 1). Immediate management included crystalloid resuscitation and intravenous antibiotics (tazobactam/piperacillin). Nephrotoxic medication was suspended and anticipating the possible need for haemodialysis he was transferred to the regional renal unit and transfused 2 units of packed red cells. Computer tomography of the kidneys, ureters and bladder revealed a left hydronephrosis with an obstructing calculus at the vesicoureteric junction and a further calculus within the kidney. Tamsulosin was added to aid calculus expulsion and after 24 h, creatinine had



**Fig. 1.** Typical features of ochronosis with pigmentation of the pinnae and sclera; previous achilles tendon surgery.

**Table 1.** Laboratory profile during the initial and final 48 h

	Admission	Day 1	Day 7	Day 8 (periarrest)
Hb g/L (140–180)	74	72	69	55
HCT L/L (0.42–0.54)	0.21	0.22	0.19	0.13
MCV fL (83–98)	86	88	91	93
Retics $\times 10^9/L$ (25–85)			646.1	307.6
Ferritin $\mu g/L$ (20–300)	773.8			
B12 ng/L (200–700)	216			
Folate $\mu g/L$ (3–20)	4.9			
Haptoglobin g/L (0.2–2.0)			0.0	
O <sub>2</sub> Hb % (95–99)			78.8	
COHB % (0.5–2.5)			2.4	
HHb % (1.0–5.0)			1.9	
MetHb % (0.4–1.5)			16.8	25.1
pH (7.35–7.34)			7.354	7.322
pCO <sub>2</sub> kPa (4.67–6.00)			2.33	2.25
pO <sub>2</sub> kPa (10.67–13.33)			12.79	12.00
[FiO <sub>2</sub> ]			[4 L nasal cannula]	[15 L rebreathe mask]
Lactate (0.4–2.2)			1.2	2.2
Gluc (3.3–6.1)			8.7	
Base Excess mmol/L			–14.5	–16.3
HCO <sub>3</sub> –mmol/L (22–30)	13	12	16	
Anion Gap mmol/L		22	23	
Na mmol/L (133–146)	134	139	144	
K mmol/L (3.5–5.3)	4.1	4.4	4.3	
Cl mmol/L (95–108)		105	105	
Ur mmol/L (2.5–7.8)	33.9	34.6	38	
Cr $\mu mol/L$ (50–120)	727	286	113	
Bili $\mu mol/L$ (0–20)	14	9	7	
ALT $\mu/L$ (8–55)	21	22	24	
LDH $\mu/L$ (10–250)		263	2152	
aCa mmol/L (2.2–2.6)	1.77	1.60	2.02	
Mag mmol/L (0.7–1.0)		(0.28 day 5)	0.73	
CRP mg/L (0–4)	163	166	84	

fallen rapidly to 286  $\mu\text{mol/L}$ . No positive microbiology was identified on blood or urine cultures however during the subsequent 6 days of antibiotics this recovery of renal function continued with creatinine falling to 113  $\mu\text{mol/L}$ . Urea remained persistently elevated at  $\sim 33$  mmol/L and there was a constant metabolic acidosis despite bicarbonate therapy with an anion gap of  $\sim 20$  mmol/L and a normal lactate. Hb fell from a post-transfusion peak of 92 g/L to  $\sim 60$  g/L and LDH rose progressively from 263 to 1343  $\mu\text{L}$ . The anaemia was normochromic, normocytic with normal B12, folate, ferritin and no GI bleeding evident. Hypomagnesaemia and hypocalcaemia were corrected.

At Day 7 post-admission a striking generalized increase in black skin discoloration was noted. Peripheral pulse oximetry dropped rapidly to 70–80% on room air with only minor symptoms; the arterial  $\text{pO}_2$  on nasal cannula oxygen was 14.7 kPa. The discrepancy between  $\text{SpO}_2$  and arterial  $\text{pO}_2$  raised the question of haemoglobinopathy and fractional Hb measurement confirmed significant methaemoglobinaemia of 16.8%. Hb had fallen to 69 g/L with a normal bilirubin and markedly elevated LDH at 2152  $\mu\text{L}$ . Direct antiglobulin test was negative; and haptoglobin non-detectable. Peripheral blood film appearances were consistent with acute, severe oxidative haemolysis with blister cells, ghost/hemi-ghost cells, polychromasia and nucleated red cells. Supravital staining with brilliant cresyl blue demonstrated Heinz bodies (Figure 2). Fresh separated serum

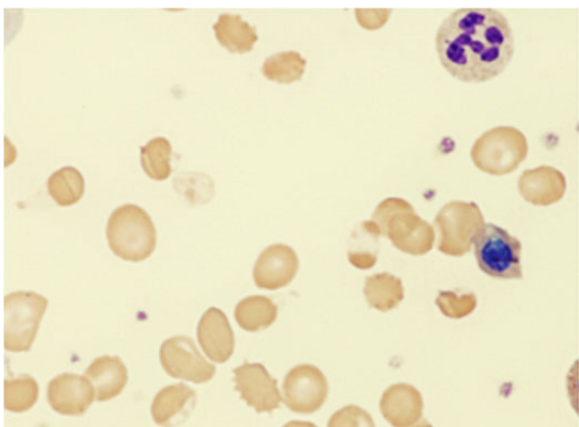


Fig. 2. Blood film consistent with oxidative haemolysis.



Fig. 3. Pigmented serum.

samples were noted to be highly pigmented (Figure 3). Intravenous N-acetylcysteine and oral ascorbic acid were commenced and tazobactam/piperacillin discontinued. Despite this, the patient deteriorated rapidly with a bradycardia leading to a pulseless electrical activity circulatory arrest not rescued by prolonged resuscitation. An arterial blood gas immediately prior to cardiorespiratory arrest revealed a worsening of the methaemoglobinaemia to 25.1%, further drop in Hb to 44 g/L and persistence of a normal lactate, high anion gap acidosis.

At autopsy, a uniform grey discoloration of the skin was noted. There was widespread ochronosis of cartilage, connective tissues and aortic atheromatous plaques (coronary arteries with mild plaque disease). There was also evidence of ochronosis of the renal parenchyma and pigmented calculi were found in the left kidney, bladder and prostatic parenchyma. There was no significant cardiac valvular or brain pathology.

## Discussion

AKU is well recognized as a condition with significant morbidity related to a progressive spondylo-arthropathy and although cardiac involvement can pose significant risks, the lifespan is not generally thought to be significantly reduced [3]. Rapidly fatal non-cardiac complications of AKU are not well recognized. One previously published case describes fatal haemolysis complicating acute kidney injury in AKU [2]; the addition of methaemoglobinaemia makes this case even more unusual, joining a handful of reports including a recently published case from Japan [4]. The cascade of events despite recovery of excretory renal function is a novel aspect of the case.

There is little doubt that AKU predisposes to renal calculi, usually in later stages of the disease, however tubular and interstitial fibrosis and tubular atrophy have been reported [5] along with ochronotic pigment deposition in the renal parenchyma [2]. The acute kidney injury in this case was almost certainly triggered by the obstructing left ureteric calculus leading to a transient hydronephrosis and urinary sepsis; however, ochronotic changes were seen within the renal parenchyma at autopsy and an interstitial component to the chronic kidney disease is possible. HGA excretion is exclusively renal by both glomerular filtration and tubular secretion and it is postulated that reduced HGA clearance led to an acute rise in HGA [6]. Quantification of plasma HGA was not locally available, but the normal lactate, high anion gap acidosis without ketonaemia points to HGA accumulation as the likeliest explanation. Rather unexpectedly, a high anion gap persisted despite recovery of renal function. Acute tubular necrosis with retarded recovery of tubular function affecting tubular secretion of HGA could explain this and would correlate with the hypomagnesaemia and hypocalcaemia.

There is *in vitro* evidence that HGA undergoing oxidation to form benzoquinone polymers acts as a trigger for haemolysis and that the oxidative process can be reversed by adding antioxidants such as ascorbic acid and glutathione [7]. Chronic kidney disease with uraemia is associated with a reduced antioxidant capacity [8]. Other causes of haemolysis were considered in this case and although the tazobactam/piperacillin was discontinued, there was no evidence of an immune-mediated haemolytic process typical of the IgG complex mechanism, which might have implicated this drug [9]. The unusual aspect of this case

involves the co-existent complication of methaemoglobinemia, formed by oxidation of the Fe<sup>2+</sup> moiety of haemoglobin to Fe<sup>3+</sup> thereby relinquishing all haem oxygen-carrying capability. A gradual increase in skin discoloration due to HGA accumulation was dramatically augmented at Day 7, very likely due to the development of methaemoglobinemia. Several drug triggers have been reported as causes of methaemoglobinemia [10], none of which were evident in this case, and it is possible that the oxidative stress provided by HGA was responsible. With methaemoglobin rising to account for 25% of a Hb which had fallen to 55 g/L as a result of the haemolytic process, ultimately there was a failure of tissue oxygen delivery leading inexorably to death.

In stable AKU, antioxidant therapy with ascorbic acid and dietary protein restriction to limit tyrosine and phenylalanine intake have been previously described, however neither strategy has been proven to alter the HGA profile or clinical outcome [11, 12]. In acute haemolytic crises associated with AKU, N-acetyl cysteine, ascorbic acid and continuous venovenous haemofiltration have not been shown to arrest haemolysis [2]. It is not known whether renal replacement therapy of any modality would effectively remove HGA or arrest the oxidative process. Specific treatment for methaemoglobinemia including exchange transfusion is recommended when the affected fraction of Hb reaches 20%; however, this guidance is difficult to interpret in the context of a falling Hb with ongoing haemolysis where a lower percentage of methaemoglobinemia is likely to have a more profound effect. Methylene blue has been used without obvious benefit [4]; its pro-oxidant capacity may exacerbate haemolysis in this setting and nephrotoxicity is an additional concern. Nitisonone is a reversible inhibitor of 4-hydroxyphenylpyruvate dioxygenase and rapidly reduces serum HGA [11, 13, 14]. Nitisonone has previously been used in hereditary tyrosinaemia type 1 and in a recently published randomized trial in AKU it decreased serum HGA levels to below detection in 60% of cases and by 95% over a 3-year period [15]. Nitisonone has not been used in the context of a haematological crisis induced by HGA toxicity in AKU and was not available for our patient, but given its good tolerability and potent ability to modify the aberrant metabolic pathway, it should be considered in future cases.

**Conflict of interest statement.** None declared. This case report has not been published elsewhere in whole or part including abstract form.

## References

1. Phornphutkul C, Introne WJ, Perry MB et al. Natural history of Alkaptonuria. *N Engl J Med* 2002; 347: 2111–2121
2. Heng AE, Courbebaisse M, Kemeny JL et al. Hemolysis in a patient with Alkaptonuria and chronic renal failure. *Am J Kidney Dis* 2010; 56: e1–e4
3. Srsen S, Vondracek J, Srsnova K et al. Analysis of the life span of alkaptonuric patients. [Slovak] *Casopis Lekaru Ceskych* 1985; 124: 1288–1291
4. Isa Y, Nihei S, Irifukuhama Y et al. A rare case of acquired methemoglobinemia associated with alkaptonuria. *Intern Med* 2014; 53: 1797–1800
5. Kazancioglu R, Taylan I, Aksak F et al. Alkaptonuria and renal failure: a case report. *J Nephrol* 2004; 17: 441–445
6. Introne WJ, Phornphutkul C, Bernardini I et al. Exacerbation of the ochronosis of alkaptonuria due to renal insufficiency and improvement after renal transplantation. *Mol Genet Metab* 2002; 77: 136–142
7. Hegedus ZL, Nayak U. Homogentisic acid and structurally related compounds as intermediates in plasma soluble melanin formation and soft tissue toxicities. *Arch Int Physiol Biochim Biophys* 1994; 102: 175–181
8. Kuroda M, Asaka S, Tofuku Y et al. Serum antioxidant activity in uremic patients. *Nephron* 1985; 41: 293–298
9. Broadberry RE, Farren TW, Bevin SV et al. Tazobactam-induced haemolytic anaemia, possibly caused by non-immunological adsorption of IgG onto patient's red cells. *Transfus Med* 2004; 14: 53–57
10. Coleman MD, Coleman NA. Drug induced methemoglobinemia. Treatment issues. *Drug Saf* 1996; 14: 394–405
11. Ranganath LR, Jarvis JC, Gallagher JA. Recent advances in management of alkaptonuria. *J Clin Pathol* 2013; 66: 367–373
12. Wolff JA, Barshop B, Nyhan WL et al. Effects of ascorbic acid in alkaptonuria: alterations in benzoquinone acetic acid and an ontogenic effect in infancy. *Pediatr Res* 1989; 26: 140–144
13. Suwannarat P, O'Brien K, Perry MB et al. Use of nitisonone in patients with alkaptonuria. *Metabolism* 2005; 54: 719–728
14. Preston AJ, Keenan CM, Sutherland H et al. Ochronotic osteoarthropathy in a mouse model of alkaptonuria, and its inhibition by nitisonone. *Ann Rheum Dis* 2014; 73: 284–289
15. Introne WJ, Perry MB, Troendle J et al. A 3-year randomized therapeutic trial of nitisonone in alkaptonuria. *Mol Genet Metab* 2011; 103: 307–314

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