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# CASE REPORT

## Markedly raised haemolysis index with upper limit of normal serum potassium levels

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*Abstract*

*Introduction*: Haemolytic specimens are a frequent occurrence in clinical laboratories, and they interfere with the analysis of many tests. *Case report*: We describe here an unusual case of leptospirosis complicated by haemolytic anaemia in a 70-year-old man with established kidney failure. He presented with an abrupt onset of shortness of breath, flushing and erythematous rash after completing haemodialysis. The patient's biochemistry test samples were however rejected twice as they were grossly haemolysed. The integrated auto-verification alert system implemented in the hospital's laboratory information system alerted the staff of the possibility of *in vivo* haemolysis. *Discussion*: The auto-verification alert system effectively distinguishes between *in vitro* and *in vivo* haemolysis and as such can be utilised as a diagnostic aid in patients with suspected intravascular haemolysis.

*Keywords*: *in vivo* haemolysis, *in vitro* haemolysis, haemolytic index, serum potassium, auto-verification alert system

### INTRODUCTION

Haemolysis is a pathological process characterised by the breakdown of red blood cells (RBC) with disruption of the cell membrane resulting in the release of haemoglobin and other intracellular components to the surrounding plasma.[1] Haemolysis can occur from two sources, either *in vivo*, which is primarily due to pathological disorders such as autoimmune haemolytic anaemia or transfusion reaction or *in vitro* due to improper specimen collection, processing or transport.[1]

The distinction between these two sources of haemolysis is of vital importance as demonstrated by a case report of a patient who died of electromechanical dissociation cardiac arrest due to overlooked hyperkalaemia. In this case, the laboratory policy did not include warning clinicians about potassium results in haemolysed samples.[2] Hence, not reporting a potassium result may imply to clinicians that it cannot be measured analytically. In *in vivo* haemolysis, however, a potassium result may be of clinical use. Here we report a case of *in vivo* haemolysis, which was timely detected owing to the auto-verification (AV) alert system implemented in a tertiary government hospital's laboratory information system (LIS) in Malaysia.

### CASE REPORT

A 70-year-old man with underlying hypertension and established kidney failure (EKF) presented to the emergency department complaining of an abrupt onset of shortness of breath after completing 4 hours of haemodialysis. The patient also vomited twice and developed sudden flushing, non-pruritic erythematous rash, systolic hypertension and tachypnoea.

The patient's renal profile (RP), cardiac enzyme (CE) and liver function test (LFT) requests were rejected twice as both samples were grossly haemolysed with haemolysis indices (HI) of 3672 mg/dL and 3355 mg/dL, respectively although they were taken by proper sampling method. This caused the results to be flagged on the analyser. The markedly raised HI

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value of > 400 mg/dL further prompted the AV system not to release other routine biochemistry results. A similar outcome was observed for the third specimen with a reported HI of 3210 mg/dL.

The AV computer-based algorithm in this hospital has been modified to include an auto-reflex *in-vivo* haemolysis verification alert. *In-vivo* haemolysis verification application is an automatic computer prompt that alerts laboratory staff to the possibility of *in vivo* haemolysis. This auto-reflex *in-vivo* haemolysis verification alert has been programmed to pick up potassium values that are not critically elevated (< 6.0 mmol/L) despite gross haemolysis (HI > 400 mg/dL), hence alerting the laboratory staff to all three samples received for this patient. However, since the AV alert system was still at the early stages of implementation, the laboratory staffs were uncertain of *in viv*o haemolysis and as such rejected the first two samples. Following this incident, a standard operating procedure with a flow chart (Figure 1) was designed to illustrate the process of handling samples suspected of *in vivo* haemolysis.

Based on this third alert, the Chemical Pathologist in charge was notified and immediately ordered for the results to be released to the clinician. Since the hospital's LIS is programmed not to release biochemistry results from grossly haemolysed samples, the patient's results were manually inserted into the system with an accompanying remark, which stated "Gross haemolysis noted. Blood test results to be interpreted with caution". Based on the patient's presentation and his blood test results, a diagnosis of haemodialysis related-haemolysis was made, which explained the patient's flushed appearance and persistent grossly haemolysed samples. Laboratory results on admission showed anaemia with raised white cell count (WCC) and C-reactive protein (CRP) with deranged coagulation profile (Table 1)**.** The direct antiglobulin test (DAT) was negative.

On the third day of admission, he became jaundiced, hypotensive and tachycardic with worsening metabolic acidosis, deranged LFT and coagulopathy (Table 1). There were several

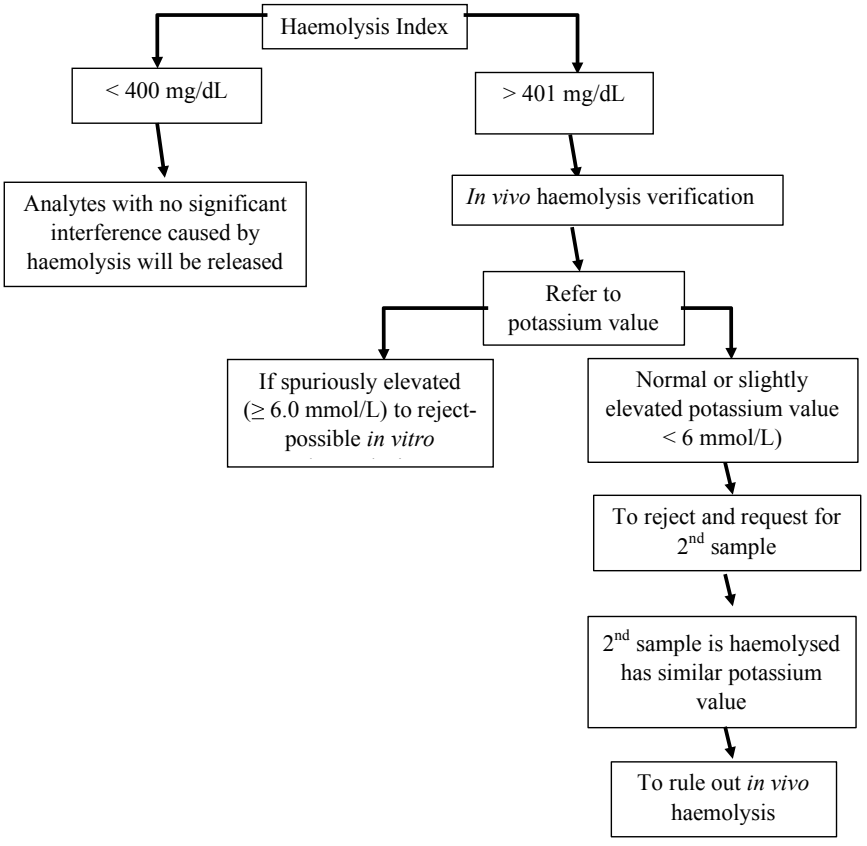


FIG. 1: Flow chart of the laboratory's approach to haemolysed sample

**TABLE 1: Patient's laboratory investigations**

| | | Admission | | | |
|---|---|---|---|---|---|
| **Laboratory Parameters** | | **(Day 1)** | **(Day 2)** | **(Day 3)** | **(Day 4)** |
| ***HAEMATOLOGY*** | | | | | |
| FBC | Hb (12.0-15.0) g/dL | 10.2 | 9.4 | 9.6 | 7.4 |
| | Platelet (150-400) $x10^9$/L | 202 | 74 | 113 | 105 |
| | WCC (4.0-10.0) $x10^9$/L | 32.9 | 24.6 | 22.2 | 17.9 |
| FBP | Mild leucoerythroblastic picture with occasional RBCs seen. No significant spherocytes, polychromasia and fragmented cells seen. Features suggestive of underlying infection/inflammation. | | | | |
| COAG | PT (12.0-14.5) sec | | 33.6 | 34.7 | 37.9 |
| | APTT (31.0-43.0) sec | | 41.0 | 42.8 | 42.8 |
| | INR (1.0) | | 3.0 | 3.0 | 3.2 |
| ***BIOCHEMISTRY*** | | | | | |
| RP | Urea (2.76-8.07) mmol/L | 11.70 | 15.80 | 26.30 | 21.90 |
| | Creatinine (62-106) $\mu$mol/L | 528 | 594 | 677 | 593 |
| | Na (136-145) mmol/L | 139 | 138 | 137 | 138 |
| | K (3.5-5.1) mmol/L | 5.5 | 5.1 | 5.4 | 5.8 |
| LFT | Total bilirubin(<21) $\mu$mol/L | 119 | 141 | 376 | 468 |
| | Direct bilirubin(<3.4) $\mu$mol/L | | 32 | 110 | 231 |
| | Indirect bilirubin(0-16) $\mu$mol/L | | 109 | 266 | 237 |
| | Total protein (66-87) g/L | 83 | 81 | 74 | 56 |
| | Albumin (35-52) g/L | 28 | 29 | 31 | 26 |
| | ALP (40-129) U/L | 77 | 62 | 63 | 94 |
| | ALT (<41) U/L | 382 | 1352 | 4266 | 4524 |
| | AST (<40) U/L | | 2153 | 8986 | 10995 |
| | Calcium total (2.15-2.50) mmol/L | 2.19 | 2.19 | | 1.94 |
| | Phosphate Inorganic (0.81-1.45) mmol/L | 2.08 | 2.21 | | 2.30 |
| | Magnesium (0.66-1.07) mmol/L | | 0.84 | | 1.04 |
| | Uric acid (202.3-416.5) $\mu$mol/L | | 251 | 253 | |
| | Ammonia (16-60) $\mu$mol/L | | | 136 | |
| | Ferritin (30-400) $\mu$g/L | | | | 392282 |
| | Lactate (0.5-2.2) mmol/L | | | 12.6 | 13.6 |
| | Amylase (28-100) U/L | | | 1898 | 1435 |
| | LDH (<250) U/L | 7368 | 11392 | 14061 | 13760 |
| CE | CK (<190) U/L | | 327 | 1587 | 10764 |
| | CK-/MB Mass (CKMB) (<6.22) $\mu$g/L | | | 8.25 | 27.20 |
| CRP | (<5) mg/L | | 53.6 | | |

| | | | | | |
|---|---|---|---|---|---|
| VBG | pH (7.35-7.43) | 7.45 | 7.37 | | |
| | $pCO_2$ (41-51) mm/Hg | 24 | 27 | | |
| | $HCO_3$ (23-29) mmol/L | 16 | 15 | | |
| ABG | pH(7.35-7.45) | | | 7.45 | 7.29 |
| | $pO_2$ (83-108) mmHg | | | 64 | 90 |
| | $pCO_2$(35-45) mmHg | | | 28 | 20 |
| | $HCO_3$ (21-26) mmol/L | | | 19 | 9 |
| | $SO_2$(94-98) % | | | 80 | 100 |
| TDM | Acetaminophen($\mu$g/mL) | | | <5 | |
| HIL | Haemolysis (mg/dL) | 3210 | 2763 | 936 | 304 |
| | Icteric (mg/dL) | 7 | 6 | 23 | 35 |
| | Lipaemic | 100 | 107 | 174 | 131 |

***MICROBIOLOGY***

| | |
|---|---|
| Blood C & S | No Growth |
| Urine C & S | No Growth |
| Leptospira IgM | Positive |
| Hepatitis B Ag | Nonreactive |
| Hepatitis C Ab | Nonreactive |
| Urine analysis | Positive for urobilingen and trace blood with a urine RBC count of 1 |

FBC: full blood count, FBP: full blood picture, WCC: white cell count, Hb: haemoglobin, RBC: red blood cell, COAG: coagulation profile,PT: prothrombin time, INR: international normalized ratio, APTT: activated partial thromboplastin time, Na: sodium, K: potassium, ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, LDH: Lactate Dehydrogenase, CE: cardiac enzyme, CK: creatinine kinase, CRP: c-reactive protein, VBG: venous blood gas, ABG: arterial blood gas, TDM: therapeutic drug monitoring

abnormal results noted during the third and fourth day of admission. The patient had significantly high levels of serum ferritin probably due to the release of ferritin from haemolysed RBC as well as an acute phase response to the infection.[3] Hyperamylasaemia in this patient with EKF is due to impaired renal clearance.[4]

Ultrasound of the abdomen showed right pleural effusion, bilateral renal parenchymal disease, normal appearance of the liver parenchyma and a contracted gallbladder with no signs of an acute process and no evidence of intra-abdominal collection. The patient's diagnosis was revised to septic shock secondary to leptospirosis with multiorgan failure when the Leptospira IgM result came back as positive. He was immediately transferred to the intensive care unit. Unfortunately, the patient succumbed to his illness on the fourth day of admission.

## DISCUSSION

It is always a challenge for laboratories to distinguish between *in vitro* and *in vivo* haemolysis. *In vitro* haemolysis can release large quantities of intracellular potassium even with mild haemolysis. On the other hand, a grossly haemolysed sample with a normal or mildly elevated potassium level should raise a suspicion of *in vivo* haemolysis. The potassium level is a true value of the analyte from the haemolysed sample and is not an artefact of methodological interference.[5] In other words, any increase in potassium level resulting from *in vivo* haemolysis is true hyperkalaemia. In practice, when haemolysis occurs *in vitro*, there will not be haemolysis of subsequent repeat samples provided a proper sampling method is used. However, in this case, as haemolysis persisted in the next two samples, *in vivo* haemolysis was considered as the most probable cause.[2] If a typical AV system with a HI value cut-off of 400 mg/dL was used solely in this case, *in vivo* haemolysis would have been missed. This is because the system would have automatically flagged the biochemistry results, interpreted the results as *in vitro* haemolysis and would have not released the results. The highlight of this case

is the modification that has been done to the AV system in this hospital, which includes auto reflex of the haemolysis alert notification. It has been programmed to pick up potassium values that are not critically elevated (< 6.0 mmol/L) despite gross haemolysis (> 400 mg/dL), thus alerting laboratory staff to the possibility of *in vivo* haemolysis.

*In vivo* haemolysis in leptospirosis results from the insertion of lipopolysaccharide, which is the main constituent of the outer cell wall of Leptospira into phospholipid bilayers of RBC membrane causing loss of phospholipids, resulting in osmotic instability of the RBC.[6] Potassium released in all causes of *in vivo* haemolysis is dispersed throughout body fluids, which would decrease the otherwise raised concentration.[5] In this case although the potassium values were slightly higher than the upper reference limit (5.1 mmol/L), they were not spuriously elevated and were not of critical value (≥ 6.0 mmol/L). A study by Zou *et al*. (2013) reported that for every increase in the HI of 100 mg/dL, there will be an estimated increase of potassium of 0.5–0.8 mmol/L.[7] In this instance since the HI was > 3000 mg/dL, the potassium value was expected to be >10 mmol/L.

Characteristic signs of *in vivo* haemolysis are an increased indirect bilirubin level and reticulocyte count, which indicates marrow compensatory response.[3] However, contrary to that, the reticulocyte count in this patient was normal. This was most likely due to infection by leptospirosis as compensatory reticulocytosis is inadequate or absent in cases of marrow involvement, infection or autoimmune reaction against the bone marrow.[3] In this patient, bilirubin and transaminases showed an increasing trend despite the reducing HI values with the indirect bilirubin being higher than direct bilirubin. This could be attributed to hepatocellular damage secondary to a leptospirosis infection. Furthermore, positive urinalysis for blood and urobilinogen on Day 1 of admission is evidence that *in vivo* haemolysis has taken place. Excessive RBC lysis releases haemoglobin into urine and with haemoglobin breakdown comes increased bilirubin production that increases the amount of urobilinogen formed and excreted in the urine.[8]

An elevated lactate dehydrogenase (LDH) level with indirect hyperbilirubinaemia supports the diagnosis of haemolytic anaemia in this clinically jaundiced patient. LDH was significantly rising despite the decreasing trend of HI. This was due to cellular necrosis and increased tissue turnover that occurs in hepatitis secondary to leptospirosis.[9] The raised serum phosphate was due to reduced renal excretion in EKF. Although serum alkaline phosphatase (ALP) is commonly raised in leptospirosis due to hepatocellular injury, in this case, ALP was within the normal range due to haemolysis.[9] Haemolysis causes an intra-erythrocyte release of magnesium ions that inhibit ALP activity in the haemolysed sample.[10] The markedly elevated CK is contributed by both haemolysis that can cause falsely elevated CK values due to the RBC enzyme adenylate kinase, which is involved in the enzymatic reaction for CK[1] and rhabdomyolysis, which may result from proteins that act as toxins in the host during leptospiral infection.[11] Increased value of CKMB is presumably due to myocarditis secondary to leptospirosis.[12]

The AV system was implemented in this tertiary hospital based on the Clinical and Laboratory Standards Institute (CLSI) guidelines on Autoverification of Clinical Laboratory Test Results, which focuses on the process for validating and implementing AV protocols.[13] Since its implementation, the AV system has greatly reduced manual review time and effort by laboratory staff. This gives staff more free time to deal with certain tests that require offline steps such as manual dilutions or to investigate questionable test results. In this case, once the analyser had flagged this sample, the AV system deemed the results inappropriate to be released and the automated haemolysis alert notification appeared. Automated HI application, which was created in the AV system is one of the filters for the programme alerting the laboratory staff of the grossly haemolysed sample. This application utilises a rule-based algorithm (Figure 1). We define gross haemolysis as a soluble Hb ≥ 400 mg/dL as estimated by the manufacturer's HI > 400 mg/dL. This HI cut-off value of 400 mg/dL was selected by our laboratory as haemolysis at this concentration was shown to cause interference in approximately 40% of the routine analytes yielding a bias >10%. This data was obtained from the manufacturer's package inserts for various analytes (Table 2).[14]

Machine learning algorithms and sophisticated statistical functions have been applied in AV to discover new approaches to identify pre-analytical and analytical errors. AV alert employs specific decision algorithms making use of criteria based on consistency checks to assess the effect of interference on the analyte.[15] This application assisted the laboratory staff in

**TABLE 2. Examples of analytes and their respective HI reported to cause interference[14]**

| No. Analyte | HI (mg/dL) |
|---|---|
| ALT | 90 |
| Albumin | 1000 |
| ALP | 200 |
| Ammonia | 200 |
| Amylase | 500 |
| AST | 40 |
| Bilirubin, Direct | 25 |
| Bilirubin, Total | 800 |
| Calcium | 1000 |
| Chloride | 1000 |
| Cholesterol, Total | 700 |
| CK | 100 |
| Creatinine | 1000 |
| GGT | 200 |
| Glucose | 1000 |
| HDL Cholesterol | 1200 |
| Lactate | 1000 |
| LDH | 15 |
| Magnesium | 800 |
| Phosphate Inorganic | 300 |
| Potassium | 90 |
| Protein, Total | 500 |
| Sodium | 1000 |
| Triglycerides | 700 |
| Urea | 1000 |
| Uric Acid | 1000 |

ALT: Alanine Aminotransferase, ALP: Alkaline Phosphatase, AST: aspartate aminotransferase, CK: creatinine kinase, GGT: Gamma Glutamyl Transferase, LDH: Lactate Dehydrogenase, HDL cholesterol: High density lipoprotein cholesterol

detecting, analysing and reporting haemolysis and alerting the clinicians of possible result interference for *in vivo* haemolysis (Table 2).[14] In this case, based on the AV alert, the staff noticed that the potassium results were not spuriously elevated despite the gross haemolysis in the first two samples and thus immediately alerted the Chemical Pathologist in charge to the possibility of *in vivo* haemolysis when the third sample revealed similar result discrepancy.

## CONCLUSION

Haemolysis is the most common cause of sample rejection by the laboratory. To ensure prompt identification of clinically important haemolytic disorders, a systematic and well-designed AV module in the management of haemolysed sample would be ideal in diagnostic laboratories.

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