Haemolysis as influence & interference factor

L. Thomas
Kirschbaumweg 8, 60489 Frankfurt Deutschland - e-mail: th-books@t-online.de

INTRODUCTION

Haemolysis is an important interference factor that must be considered when making laboratory measurements. Its influence should not be ignored. Because of the automation of many analytical processes, including increased automation of the pre-analytical phase, screening test material for haemolysis is often lacking. In particular samples collected for complete blood testing in non-laboratory settings have to be transported over longer distances because of the increasing consolidation of laboratories. As a result there is an increased risk of haemolysis during storage and/or transportation. Even if haemolysis is not visually detectable, a discharge of intracellular constituents into the plasma/serum can have occurred. If the tests are on patients with the haemolytic syndrome (in-vivo haemolysis), then differentiating it from in vitro haemolysis, mostly resulting from inappropriate specimen collection, is rarely possible. Consequently, analytical results are often false, fluctuating between either too high or too low, or giving unexpected pathological findings, for such measurements as potassium, LDH, AST, acid phosphatase, and/or neurone-specific enolase.

The objective of this report is to point out the extent of haemolytic interferences in laboratory findings, and thereby to decrease the number of inappropriate specimen collections and testing.

HAEMOLYSIS

Haemolysis is the release of intracellular components from erythrocytes, thrombocytes and leukocytes into the extracellular fluid, i.e. the plasma or serum (1). Haemolysis is visible as a red colouration of the plasma or serum after centrifugation of the sample. Reports in the literature on the concentration of free haemoglobin, which is visible as a red coloration in the plasma or serum, varies between 100 and 300 mg/L.

Haemolysis can lead to changes of a specific parameter in the test material. It is called a biological influence factor if the release of the blood-cell constituents took place in vivo. In vitro haemolysis is an interference factor if it occurs after specimen collection and changes the results of the analytical process (2).

Thrombocytolysis and granulocytolysis can also influence test results without visual haemolysis (3). On examination of the coagulation process it is evident that thrombocytolysis is responsible for the higher concentration of a number of intracellular components in serum compared to the plasma. An intravascular destruction of leukocytes can lead to increased lysozyme levels in myeloid and monocytic leukaemia's.

CAUSES OF HAEMOLYSIS

Visual pre-analytical inspection of centrifuged blood samples for haemolysis is the reason for rejection in 60% of the rejected samples. In a medical study (4), 3.3% of the specimens that were sent to the laboratory for clinical chemical investigation were haemolysed. When examining the reason, in only 3.2% of haemolysed samples was in vivo haemolysis the cause.

IN VITRO-HAEMOLYSIS (1)

Several factors causing in vitro haemolysis are encountered during the venipuncture procedure (Table 1). Further possibilities for inaccuracies are presented in Table 2.

IN VIVO-HAEMOLYSIS

In-vivo haemolysis is caused by antibodies, biochemically through medications, by toxic substances, through hereditary factors (e.g. haemoglobinopathies), through enzyme defects (acholuric jaundice) or by infections (e.g. malaria). When suspecting in vivo haemolysis the plasma

Table 1
Causes of haemolysis during venipuncture

- strong aspiration, particularly while puncturing superficial veins. Aspiration using thin needles should cause less haemolysis than use of large ones because the flow-rate, flow speed and turbulence is less, and as a result haemolysis is reported to be lower (5).
- partial obstruction of a venous or arterial catheter. As a result there is a more intense aspiration if the sample is collected with a syringe
- specimen collection with a syringe and subsequent splitting of the sample into several tubes

Riprodotto da JIFCC 2002; 13(4), versione elettronica, consultabile al sito internet di IFCC all'indirizzo www.ifcc.org. Per gentile autorizzazione del coordinatore della Communications and Publications Division della IFCC, Dr. Bernard Gouget

Table 2
Problems encountered after specimen collection

- shaking the blood too vigorously
- centrifuging the blood before completion of coagulation
- centrifugation of partially coagulated specimens from patients on anticoagulants
- positive or negative pressure in sample tubes
- blood dilution with hypotonic solution
- freeze-thawing of whole blood
- storage or transport of whole blood over several days at ambient temperatures

should be checked to exclude the possibility of additional in vitro haemolysis1 caused by the coagulation process.

HAEMOLYSIS DETECTION

Haemolysis is visible in non-icteric samples as a red coloration of serum and plasma. It is visible to the eye if the concentration of free haemoglobin is above 300 mg/L. Quantitation of free haemoglobin below this concentration is obtained immuno-nephelometrically (7) or spectrophotometrically using a bichromatic method (e.g. ACA). The upper reference limit for free haemoglobin in plasma is 20 mg/L; for serum it is 50 mg/L.

PLASMA/SERUM CHANGES THROUGH HAEMOLYSIS

Released components of blood cells, whose intracellular concentrations are more than 10x higher than their extracellular concentrations, might increase the plasma/serum concentrations of these components significantly through in vivo and in vitro haemolysis. In particular potassium, LD and AST levels become elevated. Measured components whose levels are higher in serum than in plasma come from thrombocytes, i.e. potassium, neurone-specific enolase and acid phosphatase. Indicators of haemolysis are presented in Table 3.

DIFFERENTIATION OF IN VIVO- AND IN VITRO-HAEMOLYSIS

Even if in vitro haemolysis occurs more frequent than in vivo, the latter is of greater clinical importance because of its pathological origin, and because the parameters influenced by haemolysis are relevant for diagnosis, follow-up and therapeutic monitoring of the diseases. In cases of suspected in vivo haemolysis sample rejection is considered malpractice. For this reason a differentiation (in vivo/in vitro) has to be carried out on every haemolytic sample.

In vivo released haemoglobin is bound to haptoglobin and transported into the reticuloendothelial system (RES), mostly to the spleen. Free haemoglobin can only be measured in plasma if the haptoglobin transport capacity is exceeded. Then haptoglobin cannot be measured using immunonephelometric or immunoturbidimetric methods. Haptoglobin concentrations in the reference range can be measured if there is a simultaneous acute-phase response (CRP increased) or in patients with hypersplenism. Increase in haptoglobin concentration and free haemoglobin has been reported in patients with plasmacytoma (8).

In patients having the HELLP-syndrome the concentration of haptoglobin in plasma can be normal on diagnosis. However, it will decrease within the following 10 days (10).

When suspecting a haemolytic syndrome the decrease of haptoglobin and the rise in LD, indirect bilirubin and the reticulocyte index depend on the degree of haemolysis. In extensive haemolysis, changes in LD, indirect bilirubin and haptoglobin will occur within 24 hours. However, a rise in the reticulocyte index occurs 2 days later, at the earliest. An increase in indirect bilirubin is only measurable if the haemolysis rate (usually 0,8%) rises above 5%. In intravasal haemolysis a rise in LD (which causes a change in the LD electrophoresis pattern particularly of LD1and LD2) can only be measured if the reticulocyte index is above 10%. In a subject with an LD activity of 165 U/L and an in vitro haemolysis of 800 mg the serum haemoglobin caused a 58% increase in LD activity /10/. Massive intravasal haemolysis, with a decrease in the haematocrit of more than 25% within 12 hours, can cause a hypertri-glyceridaemia. Reduced tri-glyceride metabolism is caused by diminished micro circulation and/or mobilization of free fatty acids, and their re-esterification to triglycerides (11). Criteria to distinguish in vivo and in vitro haemolysis are shown in Table 4.

Table 3
Indicators of haemolysis

- red coloration of plasma/serum
- unexpected increase in potassium, LD, AST, acid phosphatase, neurone-specific enolase levels
- decrease of haptoglobin concentra-tion
- increase indirect bilirubin concentration
- rise in reticulocyte-index

Table 4
Different types of haemolysis

In vitro-haemolysis parallel increase of haemoglobin (red coloration of plasma/serum), potassium, LD and AST respectively, but haptoglobin and reticulocyte-index remains normal,
unforeseen increase in potassium, but no red coloration of plasma/serum, LD in reference range, i.e. if whole blood is stored for several days,
In vivo-haemolysis parallel increase in haemoglobin (red coloration of plasma/serum) and LD but no parallel increase in potassium,
no red coloration of plasma/serum, but decrease in haptoglobin and potential increase in LD, indirect bilirubin and/or reticulocyte-index, respectively,
serum/plasma without red coloration, but increase in LD, potassium and acid phosphatase. In plasma no increase of these parameters (i.e. has been noticed in thrombocytosis)

HAEMOLYSIS AS AN INTERFERENCE FACTOR

Constituents that have been released from blood cells and that subsequently have been mixed with the extracellular fluid can change the plasma/serum concentration or activity of certain components for the following reasons.

Increased or decreased values because of a concentration gradient between the cells and the plasma.

Constituents released from blood cells can interfere with the chemical reactions used to analyse for plasma/serum components (e.g. the peroxidase activity of haemoglobin affects bilirubin measurement and released adenylkinase the measurement of CK).

Haemoglobin in the sample can interfere with chemical reactions because it changes the molar extinction coefficient of the substrate or reaction product to be measured.

Hemoglobin absorbs light very strongly at 415 nm (Soret wave). Haemolysis therefore increases absorption in this wavelength range and causes an apparent increase in the concentration of analytes measured in this range.

Interferences of clinical chemical tests through haemolysis have been pub-lished many times[12–14]. A list of frequently interfering parameters is presented in Table 5.

REFERENCES

1. Guder W. Haemolysis as an influence and interference factor in clinical chemistry. J Clin Chem Clin Biochem 1986; 24: 125-6.
2. Guder W. Einflußgrößen und Stör-faktorenbei klinisch-chemischen Untersuchungen. Internist 1980;21: 533-42
3. Guder W. Fonseca-Wollheim F. Heil W. Schmitt M, Töpfer G. Wisser, H.Zawta B. Die hämolytische, ikterische und iipämische probe. Empfehlungen zur Erkennung und Vermeidung klinisch relevanter Störungen. DG Klinische Chemie-Mitteilungen 1999; 30: 167-77.
4. Carraro P. Servidio P, Plebani M. Haemolyzed specimens: a reason for rejection or clinical challenge? Clin Chem 2000; 46: 306-7.
5. Moss G, Staunton C. Blood flow, needle size and hemolysis. Examining an old wives' tale, N Engl J Med1970, 282, 967.

Table 5
Interferences in parameters and methods

Parameter, method	Comments
Aspartataminotransferase (AST)	The activity of AST in erythrocytes is 40x higher than in plasma. In patients with AST activities in the reference range haemolysis with haemoglobin values of 1.5 g/L causes an elevated AST activity.
Bilirubin	False low concentrations are measured using the Jendrassik-Gróf-method, because the pseudoperoxidase activity of haemoglobin inhibits the formation of the azo dye. The inhibition can be observed if the free haemoglobin concentration in serum is higher than 0,8 g/L[12].
Creatinkinase (CK)	Released erythrocyte adenylkinase increases the enzymatically measured CK- and CK-MB activities. Adenylatkinase added to the chemical reaction mixture cannot be inhibited through AMP and diadenosinpentaphospate. Consequently there is an increase in the measurement signal.
Fe (iron)	Potentially haemoglobin is a huge source for iron. However, the additive iron effect is insignificant[14], because the iron-porphyrin binding is stronger than the iron-transferin binding and methods for the determination of iron only measure iron released from transferrin.
Total protein	The additive effect of haemoglobin on the total protein concentration is small, but significant.
Uric acid	Only high haemoglobin concentrations cause lower serum values. The uricase-catalase method (Kageyama-reaction) is more susceptible to interference than the uricase-peroxidase method.
Potassium	The concentration of potassium in red blood cells is approximately 25 x higher than in plasma. The concentration of potassium is increased, even if the in vitro haemolysis is not visible through red coloration. This can be noticed if a whole blood sample with low glucose values is stored several hours at room temperature.
Inorganic phosphate	Blood cells have a high phosphate level, but the major part is organically bound. The addition of organic phosphate esters to serum can produce a release of inorganic phosphate that can falsely increased phosphate concentrations. For this reason serum should be separated from erythrocytes within 2 hours after specimen collection.
Serum protein-electrophoresis	Haemoglobin-haptoglobin-complexes move between the α2- and ß-Globulin fractions. Free haemoglobin migrates as a diffuse reddish band in the ß-globulin fraction.
Immunoassays	Immunoassays are evaluated by diagnostic kit manufacturers for interferences to haemolysis in the same way as other clinical chemistry tests. However, the manufacturers often only add haemoglobin (mostly human methemoglobin is used) to samples. When suspecting an haemolysis interference factor of an immunoassay haemoglobin should not be confused with haemolysis. Blood cells contain components other than haemoglobin that can hamper immunoassays. Therefore, it is very important to ask the product manufacturer how haemolysis testing was performed.

6. Heins M, Hell W, Withold W. Storageof serum or whole blood samples? Effects of time and temperature on 22 serum analytes. Eur J Clin Chem Clin Biochem 1995, 33: 231-8.
7. Lammers M. Gressner AM. Immunonephelometric quantification of free haemoglobin. J Clin Chem Clin Biochem 1987, 25:363-7.
8. Lohse A. Schmitz-Reinhard B. Hyperhaptoglbinämie bei Plasmo-zytomund Hämolyse. Dtsch Med Wschr 1985, 110:1433-4.
9. Wilke G, Rath W, Schutz E, Armstrong VW, Kuhn W. Haptoglobin as a sensitive marker of haemolysis in HELLP-syndrome. Int J Gynecol Obstet 1992, 39: 29-34
10. Podiasek SJ, McPherson RA. New lactate dehydrogenase-IgM complexes. Laboratory Medicine 1989, 20:617-9
11. Druml W, Grimm G, Laggner AN, Schneeweiß B, Lenz K. Hyperlipaemia in acute haemolysis. Klein Wochenschr 1991; 69: 426-9.
12. Sonntag O. Haemolysis as an interference factor in clinical chemistry. J Clin Chem Clin Biochem 1986; 24; 127-39
13. Glick MR, Ryder KW, Jackson SA. Graphical comparisons of interferences in clinical chemistry instru-mentation. Clin Chem 1986, 32:470-5
14. Grafmeyer D, Bondon M, Machon M, Levillain R, The influence of bilirubin, haemolysis and turbidity on 20 analytical tests performed on automatic analyzers, Eur J Clin Chem Clin Biochem 1995; 33:31-52