PO-CON1810E

SHIMADZU
Excellence in Science

A novel detection of sulfated hemoglobin using Matrix-Assisted Laser Desorption Ionisation Time-Of-Flight (MALDI-TOF) mass spectrometry

ASMS 2018 MP 520

Suzanne Docherty[1]; Ray Iles[2]; Tom Kweku Abban[3]; Raminta Zmuidinaite[2]; Matthew E Openshaw[3]; Martin Besser[4]

[1]Norfolk and Norwich University Hospital NHS Foundation Trust, Norwich, United Kingdom;
[2]MAP Sciences, Bedford, United Kingdom;
[3]Shimadzu, Manchester, United Kingdom;
[4]Addenbrookes Cambridge University Hospital, Cambridge, United Kingdom

MAP SCIENCES

A novel detection of sulfated hemoglobin using Matrix-Assisted Laser Desorption Ionisation Time-Of-Flight (MALDI-TOF) mass spectrometry

Introduction

- Sulfhemoglobinemia is a rare, but acquirable blood disorder, which occurs from irreversible binding of sulfur compounds to heme, thereby producing sulphated hemoglobin (SulfHb).
- Clinical diagnosis is challenging - patients present similarly to those with methylated hemoglobin (MetHb).
- Laboratory diagnosis is problematic – there is overlap between the absorption spectra of SulfHb and MetHb using arterial blood gas analysers.
- Clinical case study - Sulfhaemoglobinemia was suspected in a 73-year old female with central cyanosis, a low oxygen saturation (SaO_2~75%), a normal arterial partial pressure of oxygen (pO_2~12kPa), and atypical blood appearance (Figure 1).
- As a potential alternative to complementary methods in the laboratory, we present a simple approach using MALDI-TOF mass spectrometry to identify SulfHb in whole blood.

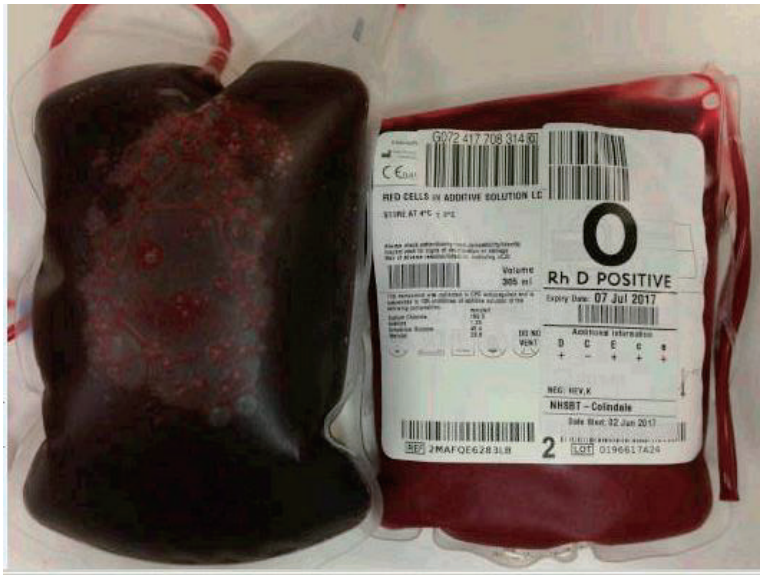

Figure 1. Photograph illustrating the color difference between the patients blood (left, with greenish tinge) and donor unit being exchanged (right, typical red color).

Methods and Materials

- MALDI-TOF MS of blood for heme adducts (500 – 700 m/z)
  - Eluted blood (from dried blood spots on Guthrie cards, and as citrated whole blood), were analysed on a MALDI-8020 benchtop linear MALDI-TOF mass spectrometer (Shimadzu, Manchester, UK), using CHCA (20mg/mL in 0.1% trifluoroacetic acid/ acetonitrile,1:2 (v/v)) in a classic sandwich method (Figure 2).
  - Control blood samples M and F, along with the patient's blood samples at presentation immediately prior to transfusion, post transfusion, and 1 month after magnesium sulfate ingestion was halted, were analysed.
- Visible spectral absorption was measured at 10nm intervals between 500 and 700nm.

A novel detection of sulfated hemoglobin using Matrix-Assisted Laser Desorption Ionisation Time-Of-Flight (MALDI-TOF) mass spectrometry

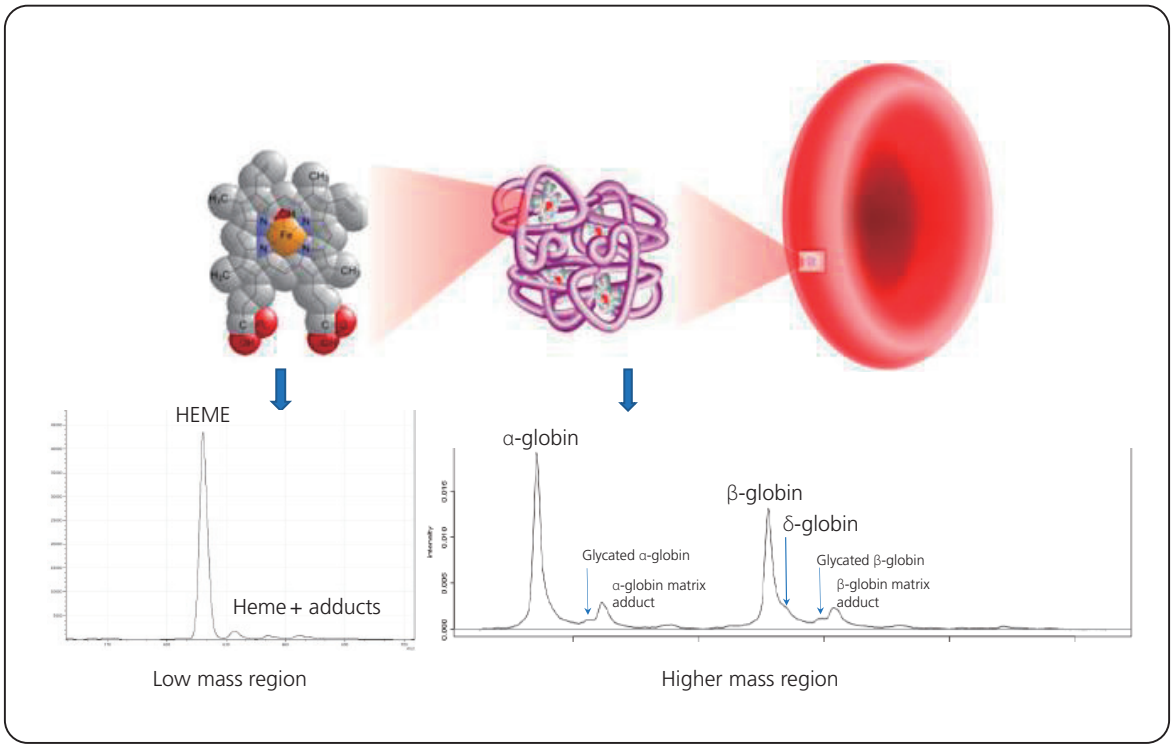

Figure 2. Illustration of lysis of RBCs, by adding whole blood to distilled water, resulting in haemoglobin release, which dissociates into free alpha & beta globin protein chains, along with free prosthetic heme. These are then resolved simply by MALDI-TOF mass spectrometry, thus allowing relative quantification of variants and adducts for clinical diagnostic purposes.

Results

- The low oxygen saturation led clinicians to query MetHb, but hospital laboratory investigations, using arterial blood gas analysers from different platforms, returned error messages for MetHb quantification, highlighting the difficulty in SulfHb identification.
- SulHb was diagnosed by MALDI-TOF MS (Figure 3) and prompted clinicians to check the drug history of the patient, who was found to have a prolonged use of an antacid (Milk of Magnesia, which contains $MgSO_4$).
- MALDI-TOF mass spectrometry analysis (Figure 3) confirmed the presence of SulfHb in the patient's blood:
  - Heme adduct peaks corresponding to hydrogen sulphide, sulphur monoxide and sulphur dioxide bound to the heme moiety, was seen in the patient's samples but not controls.
  - These heme adducts signals significantly decreased following blood unit exchange transfusions; and virtually disappeared when the source of sulfation was identified and eliminated from her pre-existing treatment regime.
- Spectrophotometry analysis (Figure 4), with a peak absorbance at 620nm wavelength, corroborated the MALDI MS diagnosis, although it was suggestive of the presence of SulfHb, rather than confirmatory.
- The trend in oxygen saturation monitoring (Figure 5) shows the outcome of the patient with treatment, and highlights the improvement after elimination of the source of sulfation.

A novel detection of sulfated hemoglobin using Matrix-Assisted Laser Desorption Ionisation Time-Of-Flight (MALDI-TOF) mass spectrometry

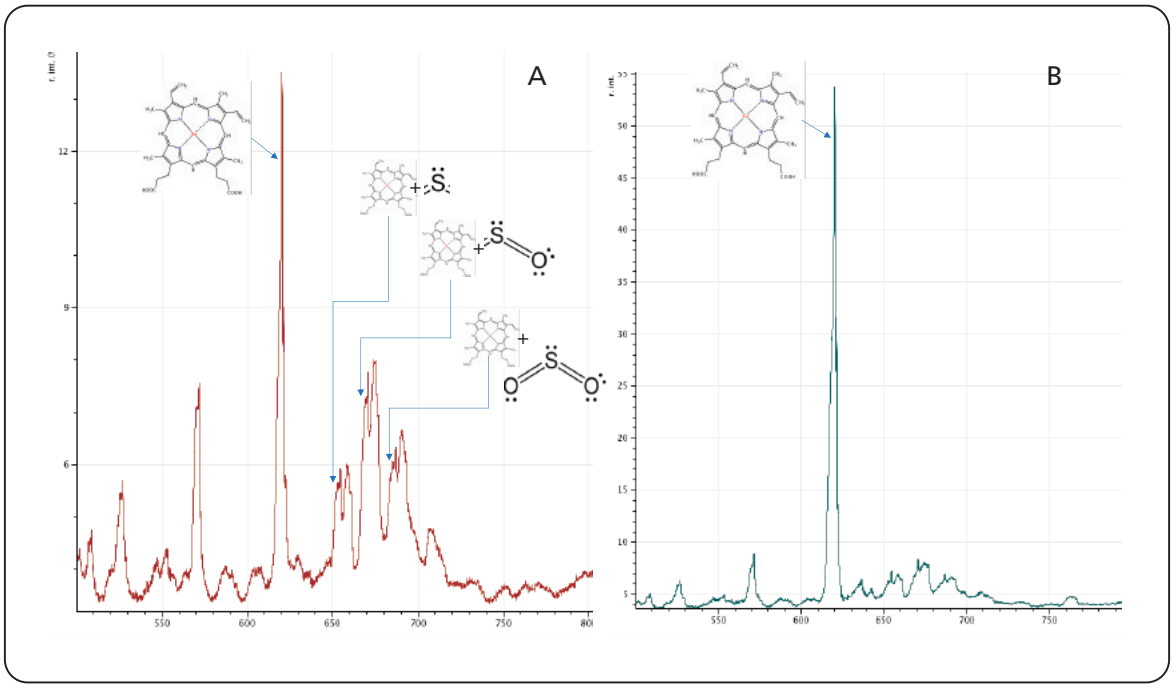

Figure 3. MALDI-TOF mass spectra of patient's lysed blood demonstrating heme and heme adducts pre (panel A) and post transfusion (panel B). Positions of Heme plus sulfur, Sulfur monoxide and Sulfur dioxide are indicated.

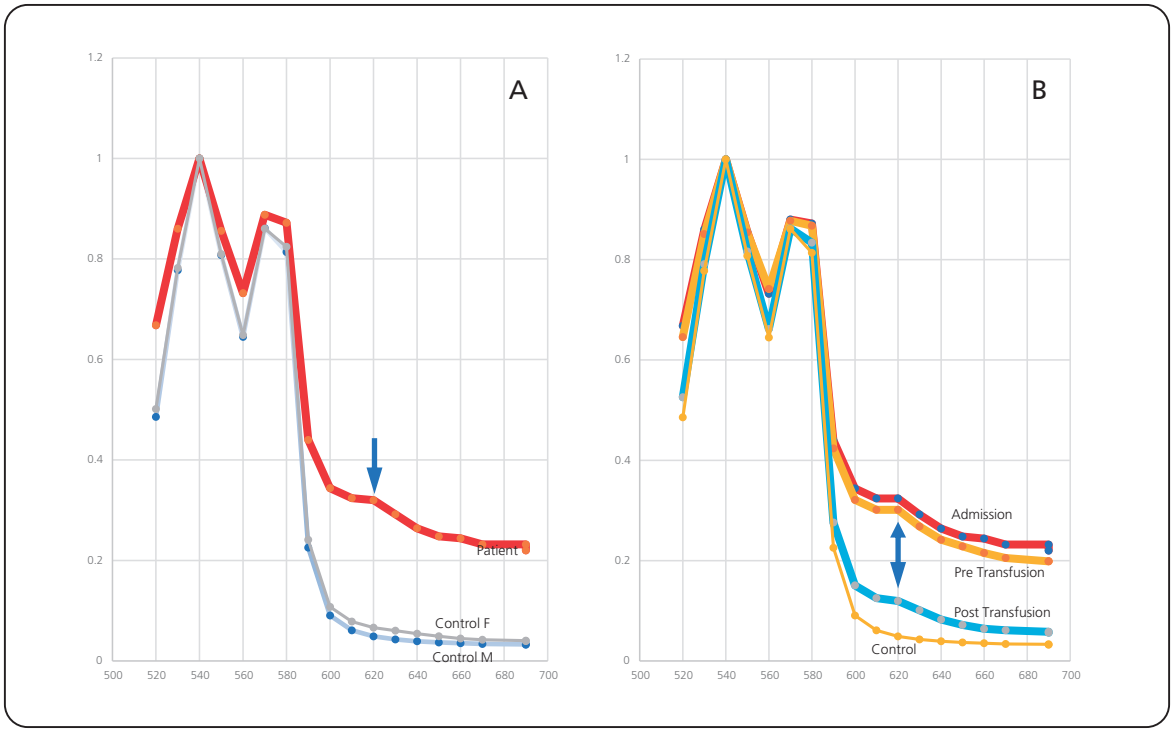

Figure 4. Absorption spectra of hemoglobin samples. Panel A: Patient and a female (F) and male (M) control samples; arrowed is an increased absorption peak at 620nm. Panel B: Patients sample on admission, pre-transfusion and post-transfusion compared to control sample; double arrowed is absorption peak at 620nm seen in all patient samples.

A novel detection of sulfated hemoglobin using Matrix-Assisted Laser Desorption Ionisation Time-Of-Flight (MALDI-TOF) mass spectrometry

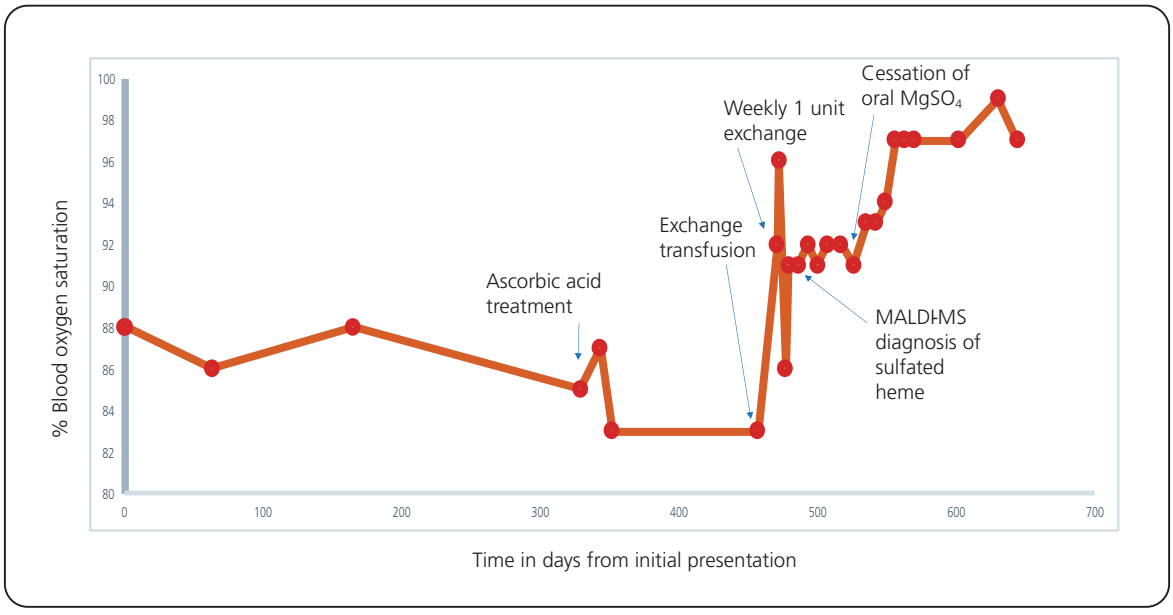

Figure 5 - Plot of patients oxygen saturation values from presentation to treatment and final cessation of ingestion of causative agent.

Conclusions

- Direct measurement of sulphur poisoning, either environmental or iatrogenic, is now possible using this rapid and cost effective test.

Corresponding author: suzanne@docherty.com

Disclaimer: The products and applications in this presentation are intended for Research Use Only (RUO). Not for use in diagnostic procedures.

First Edition: June, 2018

SHIMADZU

Shimadzu Corporation

www.shimadzu.com/an/