Suspect profiling through detection and mapping of Haemoglobin variants in blood evidence

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Overview

Blood is the most frequent type of evidence encountered at the scene of major crimes. In addition to developing methods for the operational and reliable detection of this biofluid[1,2], efforts have been also directed towards providing intelligence to narrow down the pool of suspects [3,4]. In this context, the detection of Haemoglobin variants (HbV) from bloodstains of bloodmarks yields suspect profiling intelligence and may indicate bio-geographical provenance.

Methods

Blood stains were enzymatically digested with trypsin at 20 µg/mL (containing 0.1% RapiGest) and incubated at 37 °C for 1 h. Blood marks were digested with trypsin at 25 ng/µL containing 0.1% RapiGest, using the TM3 Sprayer (*HTX Technologies LLC*) and incubated for 2 h at 50 °C. Matrix coating with α-cyano-4-hydroxycinnamic acid was performed at 10 mg/mL in 70:30 ACN:TFAaq 0.2% using the TM M3 Sprayer.

MALDI profiling data were acquired from three MALDI mass spectrometers, namely the MALDI QToF Synapt G2 HDMS (Waters Corp. Manchester, UK), MALDI TOF/TOF rapifleX and MALDI qTOF timsTOF fleX (ttfleX, Bruker Daltonik GmbH, Bremen, Germany) whereas MALDI imaging data were primarily acquired from the ttfleX.

Conclusions

The development of bottom-up proteomics MALDI MS Imaging based approaches on different high end mass spectrometers has allowed to conclude: (i) it is possible to correctly assign the type of HbV present in each of the patient blood samples investigated in a blind study; *(ii)* the highest available sensitivity is required (ttfleX) to enable the detection of all the 6 HbV investigated; *(ii)* it is possible to image all the 6 variants in blood fingermarks and reconstruct the ridge pattern; detection and imaging of these species is compatible with the prior application of CSI enhancement techniques.

Acknowledgments

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References

1. Kennedy K. et al. 2020, Sci Rep https://doi.org/10.1038/s41598-020-74253-z; **2.** Kennedy K. et al. 2021, FSI doi: 10.1016/j.forsciint.2021.110774. **3.**Heaton C, Witt M et al, 2021, Analyst 10.1039/d1an00578b; **4.** M. Witt et al, *Bruker Daltonik Application Note MSI-22*, 2021;

Results

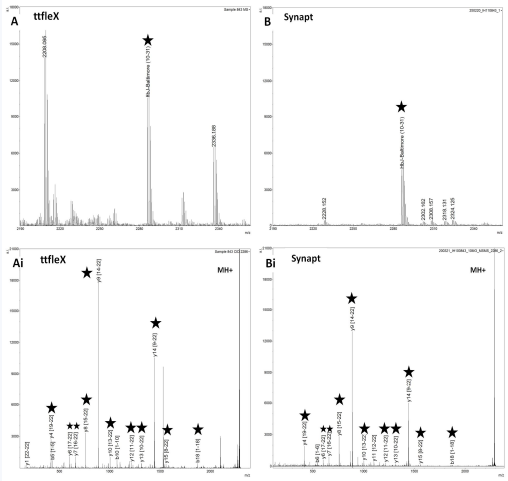

Fig 1. MALDI MS and MS/MS analysis of sample 843 on ttfleX (A, Ai)) and Synapt (B, Bi); Ai and Bi: MALDI MS/MS spectra of the ion at nominal *m/z* 2286 (HbJ-Baltimore (10-31) SAVTALWDKVNVDEVGGEALGR) confirming presence of this variant.

The ttfleX exhibited a superior sensitivity both in MS mode (A,B - star symbols indicate the proteotypic peptide (HbJ-Baltimore (10-31)), and in MS/MS mode (Ai, Bi - star symbol indicates the fragments detected in common).

ttfleX				rapifleX			Synapt			
Sample no.	Experim. ID	proteotypic peptide *m/z*	Mass Accuracy (ppm)	Experim. ID	proteotypic peptide *m/z*	Mass Accuracy (ppm)	Experim. ID	proteotypic peptide *m/z*	Mass Accuracy (ppm)	True ID
843	HbJ-Baltimore	2286.172	-0.1	HbJ-Baltimore	2286.171	-0.4	HbJ-Baltimore	2286.165	3.6	HbJ-Baltimore
844	HbC	694.425 951.559	0 3.1	HbC	694.426	1.2	HbC	951.57	8.2	HbC
845	HbC	1865.057	-3.9	HbC	694.426 951.565	10.5 2.6	HbC	694.422 951.561	-4.2 -1.1	HbC
846	HbE	1829.976 2227.217	0.3 -1.1	HbD-Iran	1313.68	-0.6	HbE	916.468 1829.956 1313.706	-4.3 -5.6 -8.6	HbE
847	HbE	1829.976	0.3	HbE	1313.708 2227.221	7.1 0.7	HbE	916.471 1829.971	-2.2 -2.4	HbE
848	HbD-Punjab	1377.716	-1.3	no variant	NA	NA	no variant	NA	NA	HbD-Punjab
849	no variant	NA	NA	no variant	NA	NA	no variant	NA	NA	HbD-Punjab
850	HbJ-Baltimore	2286.172	-3.2	HbJ-Baltimore	2286.166	-3.5	HbJ-Baltimore	2286.145	12.1	HbJ-Baltimore
851	HbD-Iran	1313.681 2227.183	-0.2 -0.1	HbD-Iran	1313.676 2227.18	-3.7 -1.1	HbD-Iran	1313.674 2227.190	-4.9 3.2	HbD-Iran

Table 1. MALDI MS of patients' blood samples containing the six variants investigated. The same set of samples was analysed on three different instruments, namely the timsTOF fleX (ttfleX), the rapifleX and the Synapt G2 HDMS. Cells are coloured in green if the correct variant was identified; cells are coloured in red if the incorrect Hb var or no Hb var was identified. Spectra were run in duplicates on the ttfleX and in triplicates on the rapifleX and on the Synapt. In the table only one replicate *m/z* per proteotypic peptide detected is reported having the lowest relative error.

The ttfleX identified all the variants but in one case namely for patient 849. No instrument identified the presence of the HbV in this case, despite the same variant was detected by the ttfleX in a different patient.

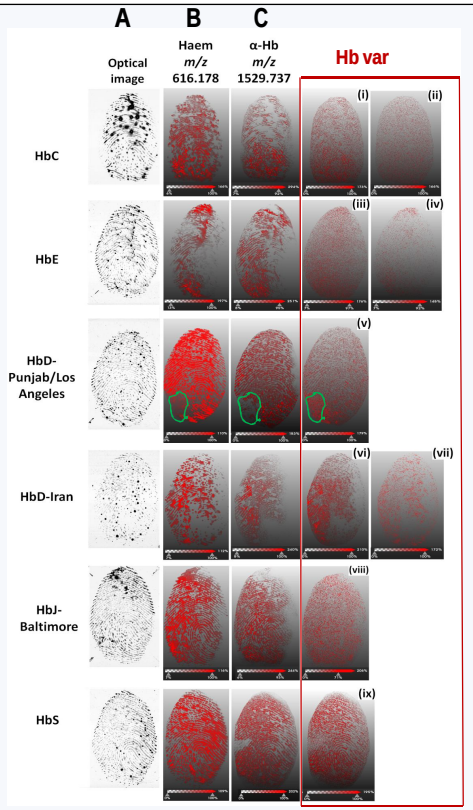

Fig 2. MALDI MS images of Haemoglobin variants (Hb var) proteotypic peptides from silicon fingertips contaminated with patients' blood (mass accuracy within ±10 ppm). **Hb var have a very low incidence and somewhat linked to biogeographical provenance. Hence their detection and visualisation in blood marks would provide intelligence on the suspect/crime circumstances and narrow down the pool of suspects[2].**

(A) Optical image of the blood mark; (B) MALDI MS images of haem; (C) MALDI MS images of an α-haemoglobin peptide; ((i)–(ix)) MALDI MS images of proteotypic Hb var peptides detected and confirmed in MALDI MS and MS/MS mode. Proteotypic peptides are shown for:

(i) HbC (2–7) *m/z* 694.420
(ii) HbC (2–9) *m/z* 951.563,
(iii) HbE (10–27) *m/z* 1829.976
(iv) HbE (10–31) *m/z* 2227.220
(v) HbD-Punjab/LA (121–133) *m/z* 1377.719
(vi) HbD-Iran (19–31) *m/z* 1313.687
(vii) HbD-Iran (10–31) *m/z* 2227.183
(viii) HbJ-Baltimore (10–31) *m/z* 2286.173
(ix) HbS (2–9) *m/z* 922.536

Ion suppression of (v) by haem and αHb peptide is particularly evident in the bottom left area of the mark (in green).

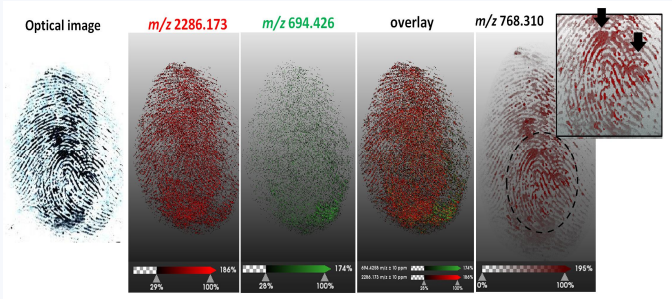

Fig 3. MALDI MS images of overlapping fingerprints contaminated with blood containing *HbJ-Baltimore (red)* and *HbC variants (green)* respectively and pre-enhanced with AB1. **It is possible to distinguish between different Hb variants in overlapping blood marks in pseudo-operational conditions whilst providing ridge detail. This could be useful to differentiate the blood of a victim from that of their assailant[2]**

HbJ-Baltimore blood fingerprint (*m/z* 2286.173) has been separated from HbC blood fingerprint (*m/z* 694.426); the ion at *m/z* 768.310 shows clearly two ulnar loops (labelled with the black arrows), indicating the presence of two fingermarks.