Introduction:

The dried blood spots (DBS) are used for quantification of drugs [1], metabolites [2] and proteins [3], while providing advantages over conventional serum or plasma samples, such as less invasive sample collection and lower requirements for processing and handling. DBS typically contain capillary blood (cDBS) collected from a fingertips (adults) or a heelprick (neonates). However, some studies may utilize venous blood (vDBS) collected via venipuncture instead [3]. This leads to the need for calibration of analytes levels in cDBS and vDBS. It was shown that levels of small molecules (e.g. glucose or cholesterol) are different in cDBS vs. plasma [4]. However, limited data are available on protein concentration levels. We intended to further explore protein levels, particularly levels of acute phase proteins (APPs) i.e. alpha-1-antitrypsin (AAT), alpha-1-acid glycoprotein 1 (AAG1) and alpha-1-acid glycoprotein 2 (AAG2) as well as constitutive protein serum amyloid A4 (SAA4) and immunoglobulin A1 (IgA1) in cDBS and vDBS.

Methods:

vDBS were collected from elbow vein and cDBS from fingertip of 13 healthy volunteers at University Hospital Brno after approval from IRB. Sample preparation is shown in the Figure 1. Samples were injected in 5% acetonitrile with 0.1% formic acid onto UHPLC analytical column (C18 Peptide BSH column, 1.7 μm, 2.1 mm i.d. x 100 mm). Peptides were analyzed using UHPLC/SMRM-MS in positive ion mode. All data were acquired in dynamic SMRM mode. To maintain SMRM assay selectivity 3-5 SMRM qualifier transitions were monitored per peptide and a single SMRM qualifier transition was used for quantification.

Results:

Absolute quantification of SAA4, A1AT, A1AG1, A2AG2 and IgA1 was performed in both cDBS and vDBS from 13 healthy volunteers (Table 3). Analysis of Variance (ANOVA) was performed and the result provided p-value of 0.298 indicating no significant difference between capillary and venous blood. Average number of Spearman correlation coefficient was 0.847 ± 0.137 with p-value of the correlation test 0.006 ± 0.011 (Table 2).

Conclusion:

In this study we have quantified three acute phase proteins (alpha-1-antitrypsin, alpha-1-acid glycoprotein 1 and alpha-1-acid glycoprotein 2), constitutive protein serum amyloid A4 and immunoglobulin A1 heavy constant alpha 1 (IgA1) and immuno-globulin A1 (IgA1) in cDBS and vDBS by multiplex protein assay based on UHPLC/SMRM-MS. We have found no statistically significant differences between levels of selected proteins in cDBS and vDBS and thus we suggest that the determination of protein markers of inflammation in cDBS is comparable to conventional assays in plasma or serum.

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