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Dried blood spot: utilising dry blood for pharmacokinetic investigations – an old method with great future for therapeutic drug monitoring

Osušena krvna mrlja: upotreba osušene krvi za farmakokinetička istraživanja – stara metoda sa velikom budućnošću za terapijsko praćenje lekova

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Ključne reči:

krv, osušena mrlja, testiranje; fragmentografija mase; farmakokinetika; terapijska ekvivalentnost; metaboličke bolesti; neonatologija; hiv.

Introduction

Dried blood spot (DBS) is a biological sampling of the full blood. Although it was initially described back in 1910s, this technique started to be explored during 1960s by Guthrie and Susi¹ for neonatal screening for phenylketonuria²⁻⁵. Since then, DBS has been used in screening for metabolic disorders, neonatal human immunodeficiency virus (HIV) infections and therapeutic drug monitoring (TDM). In 2009, DBS was implemented in development program in the US, for pediatric anti-HIV campaign (MK-8931) due to its minimal invasiveness and potential use as the sole matrix for phase 3 studies for Alzheimer's disease⁶.

Using aseptic technique, blood is withdrawn from patients using puncture by micro blood lancet. The first sample has to be discarded because of potential contamination with tissue fluid. The following sample is then transferred to previously marked area on a specially designed filter paper and dried for defined time at room temperature^{6,7}. Drying is done on non-adsorbing area and presents an important step because humidity increases a risk of microbiological contamination of the sample. After drying, paper is stored in a waterproof plastic bag, ideally with desiccant and humidity indicator, and the sample is ready for further processing².

Various methods for dried blood spot sample processing and analysis

Various techniques have been used for DBS sample processing and analysis. Sample dilution, filtration and centrifugation are commonly used⁶. For sample analysis, immunoassays are used and they normally require long incubation, gas chromatography and sample derivatization. Modern techniques such as desorption electrospray ionization and direct analysis in real time combined with mass spectrometry have shown promising results, but sensitivity of analysis is not as good, and may compromise findings. Loss of sensitivity may be attributed to the absence of chromatographic extraction and efficiently detectable functional groups. New technologies, based on liquid chromatography with mass spectrometry (LC-MS) have better sensitivity, selectivity and speed. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) showed best results and its use is increasing worldwide⁵.

Coupled plasma mass spectrometry is used for determination of metals in traces, mainly essential metals including human immunodeficiency virus cuprum, zinc, molybdenum and selenium (Cu, Zn, Mo and Se, respectively), and non-essential metals including arsenic, cadmium, mercury and lead (As, Cd, Hg and Pb, respectively) in DBS sample. Thus, as

the DBS methodologies are refined further, they have the potential to be used in epidemiological studies for detection of metals in traces in newborns⁹.

Potential uses of dried blood spot

The DBS applications are becoming more common in clinical trials, especially epidemiological ones. A study by Norwegian Breast Cancer Screening Program (NBSCP) included 4,597 women to whom questionnaires and kits for DBS and saliva were delivered and results showed to be promising. The aim of the study was to determine concentration of vitamin D and carotenoids (lutein, zeaxanthin, β -cryptoxanthin, α -caroten, β -caroten and lycopene) and to compare results with results obtained using different analytical methods. Seventy-one percent of 4,597 women returned their DBS samples, and 93% of those analyses showed similar results. High-performance liquid chromatography (HPLC) with the UV detection and liquid chromatography with mass spectrometry were used for vitamin D and carotenoids detection. Total number of 381 samples, chosen according to selected criteria (age, energy intake and body mass index) were analyzed⁷.

In order to optimise accuracy of the DBS results, the value adjustment may be required. This will accommodate the difference between full blood and serum and plasma levels of specific biomarkers or drugs. One way to obtain plasma concentrations is by multiplying the DBS values by two. This took into consideration that hematocrit value in adult women is approximately 50%. When using the value adjustment, one study of vitamin D and carotenoids concentrations in plasma showed that values in plasma were similar to results obtained in DBS. Accordingly, the DBS technique can be used efficiently in large epidemiological studies. Considering that patients perform sampling by themselves and then send the samples via mail, a huge reduction of costs represents an important advantage which could, after additional studies and validations, be encouraging factor in wider use of this technique⁷.

Study on 10 patients admitted to the Urgent Care Center of the Hospital del Mar in Barcelona, due to acute intoxication with psychoactive substances, had DBS sampling and analysis of 23 psychoactive substances and their metabolites by using ultra-high performance liquid chromatograph with mass spectrometry (UHPLC-MS). Analysis was completely validated. Results indicated a possibility of DBS sampling use in noninvasive monitoring for the presence of psychoactive substances or intoxication⁴. The analysis is possible even post-mortem¹⁰.

The possibility of the DBS use in pharmacokinetic studies is interesting. As most clinical studies in phase I include plasma sampling, because of the advantages of DBS, its potential use should be further studied. Certain studies are ongoing and with proper communication with regulatory authorities the aim is to improve and validate methods which would enable the use of DBS in clinical pharmacokinetics/pharmacodynamics studies⁶. In case DBS is validated for the use in pharmacokinetic studies, it can be used in moni-

toring of tacrolimus in renal transplant recipients on triple immunosuppressive therapy, since the data showed that monitoring of tacrolimus blood dose is necessary in the early post-transplant days¹¹⁻¹³.

It has also been shown that the detection and genotyping of hepatitis C virus is possible on the basis of the DBS samples^{14, 15}.

Therapeutic monitoring of antiepileptic drugs contributes to individualization and optimization of the treatment. This is important due to intra- and inter-individual variabilities in concentrations which consequently affect frequency and severity of adverse events. Development and validation of specific protocols for quantification of certain antiepileptic drugs are needed for the routine implementation of DBS in TDM of antiepileptic drugs¹⁶ and monitoring of the adherence¹⁷.

Methotrexate (MTX) is an antirheumatic drug often used in therapy of juvenile idiopathic arthritis (JIA) and juvenile dermatomyositis (JDM) in children. Inter-individual concentrations vary and thus affect profile of adverse events. Monitoring of adherence is very important, but also difficult because 95% of the MTX dose is metabolized within 24 hours after administration. This is possible by monitoring methotrexate polyglutamates (MTXPG)¹⁸. MTXPG are synthesized intracellularly by γ -linked sequential addition of glutamic acid residues to MTX mediated by enzyme folyl-polyglutamate synthase and can be found in erythrocytes long after MTX was cleared from plasma. The LC-MS method for the analysis of MTXPG from the DBS sample was described and validated, and it showed linearity and precision. It enables the detection of lower MTX concentrations, which is important because MTX doses in JIA and JDM treatment are significantly lower than in cancer treatment. Along with LC-MS/MS, its use is possible when there are very small volumes (12 μ L of the full blood)¹⁹. A validated LC-MS/MS method was developed for the determination of MTX and MTXPG in Caco-2 cells exposed to MTX. This method showed to be more rapid and more sensitive compared with previously reported assays for MTX and MTXPG in other matrices^{20, 21}.

The insufficiencies of dried blood spot use

DBS has certain insufficiencies, which need detailed defining for better understanding, improvement and wider use of this technique. They are: due to small volumes of the sample, repeating the analysis is usually not feasible²², some drugs are not stable at the room temperature, light and humidity influence of hematocrit^{3, 23, 24}.

While the repeatability of the small sample analysis can be solved by using more sophisticated analytical technique and stability can be improved by upgrading the storage containers for the DBS sample, the influence of the hematocrit attracted especial attention and it is the most investigated obstacle to the use of DBS. There are two main reasons why hematocrit influences analysis after the DBS sampling: blood viscosity, which is in proportion with hematocrit value, affects volume of the blood on the filter paper of precisely de-

finer diameter, ratio of blood cells count and plasma in the sample affects relative concentration of the analyte.

Considering that DBS covers analysis of the full blood, hematocrit value will consequently affect concentration of analyte in regard to plasma. By using the equation shown below (1.0), concentration of the analyte in plasma can be predicted.

$$C_{plasma} = \frac{C_{blood}}{(1 - Hct) + Hct * f_u * \rho} \quad (1.0)$$

In case f_u is constant, the equation (1.0) can be presented in the following form :

$$C_{plasma} = \frac{C_{blood}}{1 - Hct} * f_{\rho} \quad (1.1)$$

where C_{plasma} is the concentration of the analyte in plasma, C_{blood} is the concentration in full blood, Hct is hematocrit value, f_u is unbound fraction in plasma, ρ is erythrocyte-to-plasma concentration ratio and f_{ρ} is the fraction in plasma.

In 2013, the authors Capiou et al.²⁵ showed linear correlation between potassium concentration and hematocrit in a range of 0.19–0.63, with acceptable accuracy and precision. By measuring concentration of potassium in the DBS sample, hematocrit can be easily determined, and by using above described equations, concentration of the analyte in regard to plasma can be easily calculated. The main lack of this method is the use of part of the DBS sample for analysis of potassium concentration which also requires separate preparation of the sample. To overcome this issue, Capiou et al.²⁵ showed that measuring hematocrit in the DBS sample can be done via noncontact diffuse reflectance spectroscopy. In this case, the whole quantity of the sample is preserved and a possibility of an error is decreased because the preparation of the sample is not needed.

Advantages of dried blood spot over venipuncture liquid blood sample storage containers as blood sample collection and storage method

Due to numerous advantages, this sampling technique became interesting and its potentials drew investigators attention. The advantages of DBS over venipuncture are: minimally invasive technique, less painful than venipuncture, smaller volumes of blood are sampled ($15 \pm 5 \mu\text{L}$ per one marked area of the filter paper) and therefore it is more convenient for the use in newborns and small children^{3,10,22}, cost-effective³, patients can perform self-sampling at home after proper instruction and send it to a laboratory via mail^{7,26}, suitable transport and storage during the transport of the DBS samples of the patients infected with HIV or HCV, a possibility of contamination is minimal and this kind of samples can be sent even via mail^{2-4,10}, good stability of analyte in sample^{3,10,27}, use of DBS in preclinical trials supports ethical approach to animals because sampling from tail vein is possible²².

Conclusion

Using optimised sample preparation and analysis, the DBS technique has significant potential applications in pre-clinical and clinical trials, therapeutic and toxicological drug and poison monitoring as well as large epidemiological trials. DBS represents a cost-effect model of drug analysis and can provide much needed pharmacokinetic results in an efficient and robust manner.

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REFERENCES

1. Guthrie R, Susi A. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. *Pediatrics* 1963; 32: 338–43.
2. Lehmann S, Delaby C, Vialaret J, Ducos J, Hirtz C. Current and future use of "dried blood spot" analyses in clinical chemistry. *Clin Chem Lab Med* 2013; 51(10): 1897–909.
3. Liao HW, Lin SW, Chen GY, Kuo CH. Estimation and Correction of the Blood Volume Variations of Dried Blood Spots Using a Postcolumn Infused-Internal Standard Strategy with LC-Electrospray Ionization-MS. *Anal Chem* 2016; 88(12): 6457–64.
4. Kyriakou C, Marchei E, Scaravelli G, Garcia-Algar O, Supervia A, Graziano S. Identification and quantification of psychoactive drugs in whole blood using dried blood spot (DBS) by ultra-performance liquid chromatography tandem mass spectrometry. *J Pharm Biomed Anal* 2016; 128: 53–60.
5. Rao RN. Emerging liquid chromatography-mass spectrometry technologies improving dried blood spot analysis. *Expert Rev Proteomics* 2014; 11(4): 425–30.
6. Kothare PA, Bateman KP, Dockendorf M, Stone J, Xu Y, Woolf E, et al. An Integrated Strategy for Implementation of Dried Blood Spots in Clinical Development Programs. *AAPS J* 2016; 18(2): 519–27.
7. Sakbi AK, Bastani NE, Ellingjord-Dale M, Gundersen TE, Blomhoff R, Ursin G. Feasibility of self-sampled dried blood spot and saliva samples sent by mail in a population-based study. *BMC Cancer* 2015; 15: 265.
8. Wilhelm AJ, den Burger JC, Swart EL. Therapeutic drug monitoring by dried blood spot: progress to date and future directions. *Clin Pharmacokinet* 2014; 53(11): 961–73.
9. Vacchina V, Huin V, Hulo S, Cuny D, Broly F, Renom G, et al. Use of dried blood spots and inductively coupled plasma mass spectrometry for multi-element determination in blood. *J Trace Elem Med Biol* 2014; 28(3): 255–9.
10. Odoardi S, Anzillotti L, Strano-Rossi S. Simplifying sample pre-treatment: application of dried blood spot (DBS) method to blood samples, including postmortem, for UHPLC-MS/MS analysis of drugs of abuse. *Forensic Sci Int* 2014; 243: 61–7.

11. Velickovic-Radovanovic RM, Paunovic G, Mikov M, Djordjevic V, Stojanovic M, Catic-Djordjevic A, et al. Clinical pharmacokinetics of tacrolimus after the first oral administration in renal transplant recipients on triple immunosuppressive therapy. *Basic Clin Pharmacol Toxicol* 2010; 106(6): 505–10.
12. Velickovic-Radovanovic R, Mikov M, Catic-Djordjevic A, Stefanovic N, Mitic B, Paunovic G, et al. Gender-dependent predictable pharmacokinetic method for tacrolimus exposure monitoring in kidney transplant patients. *Eur J Drug Metab Pharmacokin* 2015; 40(1): 95–102.
13. Rančić N, Dragojević-Simić V, Vavić N, Kovačević A, Šegrt Z, Drašković-Pavlović B, et al. Tacrolimus concentration/dose ratio as a therapeutic drug monitoring strategy: the influence of gender and comedication. *Vojnosanit Pregl* 2015; 72(9): 813–22.
14. Marques BL, do Espírito-Santo MP, Marques VA, Miguel JC, da Silva EF, Villela-Nogueira CA, et al. Evaluation of dried blood spot samples for hepatitis C virus detection and quantification. *J Clin Virol* 2016; 82: 139–44.
15. Greenman J, Roberts T, Cohn J, Messac L. Dried blood spot in the genotyping, quantification and storage of HCV RNA: a systematic literature review. *J Viral Hepat* 2015; 22(4): 353–61.
16. Milosbeska D, Grabnar I, Vovk T. Dried blood spots for monitoring and individualization of antiepileptic drug treatment. *Eur J Pharm Sci* 2015; 75: 25–39.
17. Shah NM, Hanwa AF, Millership JS, Collier PS, Ho P, Tan ML, et al. Adherence to antiepileptic medicines in children: a multiple-methods assessment involving dried blood spot sampling. *Epilepsia* 2013; 54(6): 1020–7.
18. Hanwa AF, AlBanab A, Rooney M, Wedderburn LR, Beresford MW, McElroy JC. Methotrexate polyglutamates as a potential marker of adherence to long-term therapy in children with juvenile idiopathic arthritis and juvenile dermatomyositis: an observational, cross-sectional study. *Arthritis Res Ther* 2015; 17: 295.
19. Hanwa AF, AlBanab A, Rooney M, Wedderburn LR, Beresford MW, McElroy JC. A novel dried blood spot-LCMS method for the quantification of methotrexate polyglutamates as a potential marker for methotrexate use in children. *PLoS One* 2014; 9(2): e89908.
20. Chen G, Fawcett JP, Mikov M, Tucker IG. Simultaneous determination of methotrexate and its polyglutamate metabolites in Caco-2 cells by liquid chromatography-tandem mass spectrometry. *J Pharm Biomed Anal* 2009; 50(2): 262–6.
21. Chen G, Fawcett JP, Mikov M, Tucker IG. Monoketocholate can decrease transcellular permeation of methotrexate across Caco-2 cell monolayers and reduce its intestinal absorption in rat. *J Pharm Pharmacol* 2009; 61(7): 953–9.
22. Enderle Y, Foerster K, Burbenne J. Clinical feasibility of dried blood spots: Analytics, validation, and applications. *J Pharm Biomed Anal* 2016; 130: 231–43.
23. Briscoe CJ, Hage DS. Factors affecting the stability of drugs and drug metabolites in biological matrices. *Bioanalysis* 2009; 1(1): 205–20.
24. Antunes MV, Charão MF, Linden R. Dried blood spots analysis with mass spectrometry: Potentials and pitfalls in therapeutic drug monitoring. *Clin Biochem* 2016; 49(13–14): 1035–46.
25. Capiau S, Wilk LS, Aalders MC, Stove CP. A Novel, Nondestructive, Dried Blood Spot-Based Hematocrit Prediction Method Using Noncontact Diffuse Reflectance Spectroscopy. *Anal Chem* 2016; 88(12): 6538–46.
26. Tanna S, Lawson G. Dried blood spot analysis to assess medication adherence and to inform personalization of treatment. *Bioanalysis* 2014; 6(21): 2825–38.
27. Wagner M, Tonoli D, Varesio E, Hopfgartner G. The use of mass spectrometry to analyze dried blood spots. *Mass Spectrom Rev* 2016; 35(3): 361–438.

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