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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:
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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 Susi [1] for neonatal screening for phenylketonuria [2–5]. 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 disease [6].

Using aseptic technique, blood is withdrawn from patients using puncture by micro blood lancet.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 processing [2].

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 used [6]. 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 worldwide [5].

Coupled plasma mass spectrometry is used for determination of metals in traces, mainly essential metals including human immunodeficiency virus cuprum, zinc, molybdenium 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

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the DBS methodologies are refined further, they have the potential to be used in epidemiological studies for detection of metals in traces in newborns [9].

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 analyzed [7].

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 technique [7].

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 intoxication [4]. The analysis is possible even post-mortem [10].

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 studies [6]. In case DBS is validated for the use in pharmacokinetic studies, it can be used in monitoring 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 days [11–13].

It has also been shown that the detection and genotypisation 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 drugs [16] and monitoring of the adherence [17].

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 folylpolyglutamate 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 μL 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 feasible [22], 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-

fined 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.

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

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 Capiau 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, Capiau 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 ± 5μL 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-effective [3], 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 possible [22].

Conclusion

Using optimised sample preparation and analysis, the DBS technique has significant potential applications in preclinical 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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