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Sweat as a clinical sample: what is done and what should be done

“The clinical importance of sweat has traditionally been limited to the determination of chloride for the diagnosis of cystic fibrosis and incipient determination of drugs.”

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“The role of sweat in clinical analysis is poor, but this sample deserves a prominent place that can be achieved after development of appropriate research.”

Sweat is known for being a clear, hypotonic biofluid produced by eccrine and procrine glands located in the epidermis, with a slightly acidic pH (between 4.0 and 6.8), and composed mainly by water (99%), containing the so-called electrolytes (e.g., sodium, chloride and potassium), urea, pyruvate and lactate; but also proteins, peptides, amines, amino acids and metal ions in smaller concentrations, together with inhibitors, antigens, antibodies and a variety of xenobiotics such as drugs, cosmetics and ethanol [1]. The clinical importance of sweat has traditionally been limited to the determination of chloride for the diagnosis of cystic fibrosis (CF) and incipient determination of drugs [1]. The present spread of ‘omics’ disciplines, and particularly of metabolomics as the youngest of the big ‘omics,’ has open a fan of possibilities to the use of sweat as clinical sample. Except for the case of some high molecular weight proteins, which reach sweat by different intracellular storages in particular situations [2,3], most sweat components are small molecules resulting from metabolic pathways; therefore, their study pertains to the metabolomics field, the omics of small molecules typically (<1000 Da or <1500 Da).

The present advantages of sweat as clinical

sample that push a growing research are: its no invasive sampling, crucial in advanced states of some diseases and that makes unnecessary health personnel care; its simple but varied composition that can be the basis for its clinical interest for diagnostic. On the other hand, some present shortcomings are: the low secreted volume, the variable secretion among individuals and the lack of optimized analytical protocols, which are mainly necessary in metabolomics. Therefore, the main aspects to which immediate attention should be paid with the dual purpose of increasing the advantages and overcoming shortcomings should encompass all steps of the analytical process are as follows.

Sweat sampling

The diagnostic of some diseases such as CF promoted in the past the improvement of old sweat samplers such as occlusive bandages, giving place to present commercial Macroduct and Megaduct devices [4]. They alleviated shortcomings such as the difficulty to produce enough sweat for analysis, sample evaporation, need for a trained staff and errors in the results owing to the presence of pilocarpine; therefore, more precise the determination of chloride for CF diagnostic were then possible. Nevertheless, present more complex uses of sweat – mainly when involved in omics analyses – make mandatory the design of new samplers with total overcoming of present shortcomings and

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even offering new advantages. In dealing with sweat sampling for drug abuse monitoring, patches provide a long-term continuous monitoring of drug exposure [4]. The recent hydrogel micropatches for sampling and profiling skin metabolites are an example of the research in this area [5]. Also, the necessity for different devices for sweat sampling may result from more in depth studies on mapping different body zones as the irreproducibility of the present samplers (designed for sampling in arms) made imprecise the differences in sweat composition depending on the body area [6].

Normalization of the sampled volume

A pending goal in the use of sweat as clinical sample is normalization as the protocols assayed so far have not provided successful results. Thus, Appenzeller *et al.* [7] used the concentration of sodium and potassium as an internal reference to normalize the volume of sweat collected by patch samplers. The authors measured the target metabolites by capillary zone electrophoresis–diode-array detector for indirect detection at 214 nm, and concluded that K^+ cannot be used for normalization owing to the high inter-individual variability, while Na^+ displayed very low variability within groups, making it suitable for normalization. Nevertheless, the authors did not assay the normalization system in sweat from CF patients, who alter their sodium concentration. More research on this analytical aspect is mandatory that should be developed with more complex instrumentation that provides more information on different parameters to be jointly treated.

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Sample preparation

Sweat is a relatively simple sample that does not require preparation for CF diagnostic; while for other analyses it is mandatory treatment to lead the target analytes to the appropriate state for measurement, which depends on both the analytes and the analytical equipment. This is the case with drugs analysis [1] and in the so far scant applications in the genomics [8] and proteomics fields [9]. The recent use of sweat as sample to search for metabolomics biomarkers [10] or quantitative analysis of amino acids [11] has shown as unnecessary sample cleanup (using SpinColumn SPE cartridges because the low sample volume). Sample dilution demonstrated to be enough to overcome interferences and avoid derivatization reactions when LC–MS is used instead of an automatic amino acids analyzer [12]. More in depth research is necessary to propose other sample

preparation steps that can be required particularly in targeted metabolomics analysis.

Sweat analysis

The complexity of equipment for sweat analysis depends on the target analyte(s).

In dealing with the determination of a single compound, as is the case for chloride for CF diagnostic, there are a number of dedicated instruments – usually simple commercial devices – based mainly on potentiometry, but also others based on osmolarity, conductivity or coulombimetric measurements [13]. Example of the interest in improving CF diagnosis is the recent CF quantum® sweat test [14]. Mention apart among single analytes determined in sweat deserves pH. This parameter can be correlated to sodium concentration and sweat rate in order to indicate to users the proper time to hydrate during physical exercise and avoid the risk of muscle cramps; and also changes in the pH of the skin play a role in the pathogenesis of skin diseases, such as dermatitis and acne [15]. These known aspects of sweat-pH monitoring have promoted the development of a smartphone based accessory and method for the rapid colorimetric detection of pH in sweat [16], and also an autonomous, robust and wearable microfluidic platform capable of performing online analysis of pH in sweat [17]. These and similar devices can help in searching for other relationships between sweat pH and individual healthy/ill states.

Sweat is also an excellent sample for drug analysis as the whole human body produces between 300 and 700 ml/day of sweat. In the context of the absorption/distribution/metabolism/excretion cycle of drugs, a small but sufficient fraction of a drug is excreted in sweat; therefore, drug can be tested in this biofluid [1] with several advantages over blood or urine such as less opportunity for sample adulteration, longer time-detection windows in some cases and noninvasiveness as compared with blood [1]; however, analyses of the drug in sweat provided a time window detection of up to a week or more after administration [1]. Other advantage of sweat as clinical sample is that drugs with basic nature can accumulate in sweat from blood due to the difference of pH between the two matrices [1]. An aspect to be taken into account in future research is that most of the authors working on this field determine the target drug (at LOQ as low as 0.4 ng drug/sample), but not its metabolites [18]. Monitoring the precursor and metabolites in individuals under different conditions could provide information on potential different metabolic pathways closely related with the healthy/ill state of the individual.

Along with essential metals, sweat is also a known excretory route for xenometals; therefore, their analysis

with subsequent interpretation of comparative results are a xenometabolomics matter. In addition, depending on the oxidation state of the target metal, the research belongs to metabolomics subdisciplines such as ionomics or metallomics. Metals xenometabolites can be no toxic, as is the case with Ni, or toxic as Cd, Pb, Hg and As; all them can enter the organism as metals or salts and, when absorbed through the skin, they are first solubilized by sweat. Therefore, blood or urine might not reflect properly dermal absorption, which is better expressed in sweat. In addition, optimization of the potential of sweating as a therapeutic excretory mechanism merits further research, as does research into homeostasis of no common metals, and conditioning or adaptation to regular sweating by athletes to be matched with studies of excretion of trace elements.

Nevertheless, it is the search for metabolomics biomarkers of key diseases as lung cancer [19] the area of outstanding interest, as demonstrated by the changes in the excreted metabolites with different advancement of the target disease. The growing development of metabolomics data treatment and metabolomics

databases opens new possibilities to the information provided by cutting-edge analytical instrumentation, excellent tools for the small volumes characteristic of sweat samples.

Conclusion

Further research is mandatory to demonstrate and take profit from the wide potential of sweat as clinical sample. Metabolomics is the field in which the most fruitful results are foreseeable.

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