

METHODOLOGY

GUIDELINES FOR EMERGENCY SPECIMEN COLLECTION FROM CRITICALLY ILL PATIENTS SUSPECTED OF HAVING METABOLIC DISEASES

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ABSTRACT

Many metabolic diseases of genetic origin have an acute and life-threatening presentation. Sometimes the patient progressively worsens and eventually dies before a final diagnosis is reached. For a genetic disease, the diagnosis is important not only for the patient but also for his family, as it will be essential for preventive measures such as carrier detection, genetic counseling and prenatal diagnosis. Since a definite diagnosis depends on the collection of appropriate specimens, and since in many cases time is critical, in this paper we present some guidelines for emergency specimen collection from critically ill patients suspected of having metabolic disease.

INTRODUCTION

Among the more than 4,000 Mendelian genetic traits already identified in humans (McKusick, 1989), nearly 250 are inborn errors of metabolism (IEM) (Galjaard *et al.*, 1987). The presentation of these disorders is variable, and in many cases the hypothesis of an IEM should be considered in an acutely ill newborn, infant or even in an older patient (Bickel, 1987; Burton, 1987; Greene *et al.*, 1988; Kolschutter, 1983). Although some simple tests may be of value in the first steps of

the laboratory evaluation of a patient with suspected metabolic disease (Edwards *et al.*, 1988; Green, 1989), in most cases the diagnosis will be based on sophisticated procedures that frequently involve a reference laboratory. Although many IEMs are not yet treatable, the diagnosis of a genetic disease is important for the patient's family. Preventive measures such as genetic counseling, carrier detection and prenatal diagnosis can only be offered if a definite diagnosis is reached. Sometimes the downhill progression of a metabolic disease is fast and precludes the collection of appropriate specimens for the necessary biochemical studies. With this situation in mind, a set of guidelines for the emergency collection of specimens from acutely ill patients is presented to facilitate the diagnosis of IEMs even in the cases in which the lethal outcome can not be avoided.

THE GUIDELINES

The types of specimens useful for the diagnosis of a genetic metabolic disease are presented in Table I, in decreasing order of importance, together with instructions for their collection. These guidelines include recommendations by many authors (Burton, 1987; Green, 1989; Holton, 1982; Leonard, 1985), and the specimens are divided into three groups: a) most informative specimens, which should always be collected (urine and plasma or serum); b) usually very informative specimens, which should be collected whenever possible (heparinized blood, skin biopsy and CSF); c) specimens informative for specific situations, to be collected in selected situations or when no information is available about the actual need for such material (total blood for DNA studies and tissue samples).

COMMENTS

Every time an IEM is suspected in an acutely ill patient, the clinical and laboratory staff should act jointly for the prompt collection and appropriate processing and storage of adequate specimens (preferably before the introduction of provisory therapeutic measures such as interruption of feeding, acidosis correction or exchange transfusions). One member of the team should be elected to coordinate the large number of professionals that are usually involved in the investigation, and also to contact reference laboratories whenever necessary. The team should remember that if the patient does not improve and takes a downhill course, the counseling that will be given to the family, especially in terms of the prevention of new cases, will depend on the adequacy of specimen collection from the index case.

Table I - Guidelines for emergency specimen collection from critically ill patients suspected of having genetic metabolic disease.

Most important specimens (always to be collected):

1. Urine: Collect at least 30 ml (more if possible) of a random urine specimen; if necessary, pool many small-volume specimens; do not use preservatives; store at -20°C .
2. Plasma or Serum: Obtain at least 3 ml of plasma or serum and store at -20°C ; if the patient is to be submitted to blood transfusion, be sure that the sample was collected before hand.

Very useful specimens (to be collected whenever possible):

3. Heparinized blood: Collect 8 to 10 ml of blood into a heparinized syringe and keep at $+4^{\circ}\text{C}$ (do not freeze); also be sure that the sample is collected before any blood transfusion; this sample will be essential for enzyme assays on leukocytes and erythrocytes.
4. Skin Biopsy: Collect under sterile conditions (extremely important !) and place sample in a sterile flask with medium for cell culture (this may be the standard culture medium used for karyotyping); in an emergency situation, use sterile saline instead; never use agar; samples collected up to 24 hours after death are usually viable provided they are not infected; keep at $+4^{\circ}\text{C}$.
5. Cerebrospinal Fluid (CSF): Collect 2 to 3 ml of CSF, centrifuge to discard cells and store the supernatant at -20°C .

Specimens useful in selected situations (to be collected when indicated or when the indication is not clear):

6. Blood for DNA analysis: Whenever possible, collect 20 ml of whole blood using EDTA as an anticoagulant and freeze at -20°C ; this specimen can be used, if necessary, for DNA studies.
 7. Tissue Sample: To be collected when the diagnostic suspicion should be clarified by a biochemical analysis of a specific tissue; the most useful tissue is usually liver, but skeletal muscle and heart muscle may also be informative; if possible, collect 2 or 3 fragments by percutaneous needle biopsy; samples collected up to 2 hours after death are usually viable; pack the sample in aluminum foil and freeze immediately at -20°C ; do not add formaline!
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RESUMO

Muitas doenças metabólicas têm uma apresentação aguda que coloca o paciente em risco de vida. Algumas vezes o paciente deteriora rapidamente e evolui para óbito antes que um diagnóstico definitivo seja alcançado. No caso de uma doença genética, o diagnóstico é importante não apenas para o paciente mas também para a sua família, uma vez que será essencial para as medidas preventivas (detecção de portadores, aconselhamento genético, diagnóstico pré-natal) a serem adotadas. Uma vez que o diagnóstico definitivo depende da coleta de amostras apropriadas e, sendo o tempo muitas vezes crítico, neste artigo são apresentadas algumas instruções para a coleta em situação de emergência de amostras de pacientes agudamente enfermos nos quais se suspeita de uma doença metabólica.

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