Diagnosis of immunodeficiency caused by a purine nucleoside phosphorylase defect by using tandem mass spectrometry on dried blood spots

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Background: Purine nucleoside phosphorylase (PNP) deficiency is a rare form of autosomal recessive combined primary immunodeficiency caused by a enzyme defect leading to the accumulation of inosine, 2′-deoxy-inosine (dIno), guanosine, and 2′-deoxy-guanosine (dGuo) in all cells, especially lymphocytes. Treatments are available and curative for PNP deficiency, but their efficacy depends on the early approach. PNP–combined immunodeficiency complies with the criteria for inclusion in a newborn screening program.

Objective: This study evaluate whether mass spectrometry can identify metabolite abnormalities in dried blood spots (DBSs) from affected patients, with the final goal of individuating the disease at birth during routine newborn screening.

Methods: DBS samples from 9 patients with genetically confirmed PNP–combined immunodeficiency, 10,000 DBS samples from healthy newborns, and 240 DBSs from healthy donors of different age ranges were examined. Inosine, dIno, guanosine, and dGuo were tested by using tandem mass spectrometry (TMS). T-cell receptor excision circle (TREC) and kappa-deleting recombination excision circle (KREC) levels were evaluated by using quantitative RT-PCR only for the 2 patients (patients 8 and 9) whose neonatal DBSs were available.

Results: Mean levels of guanosine, inosine, dGuo, and dIno were 4.4, 133.3, 3.6, and 3.8 μmol/L, respectively, in affected patients. No indeterminate or false-positive results were found. In patient 8 TREC levels were borderline and KREC levels were abnormal; in patient 9 TRECs were undetectable, whereas KREC levels were normal.

Conclusion: TMS is a valid method for diagnosis of PNP deficiency on DBSs of affected patients at a negligible cost. TMS identifies newborns with PNP deficiency, whereas TREC or KREC measurement alone can fail. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *Purine nucleoside phosphorylase, severe combined immunodeficiency, newborn screening, tandem mass spectrometry, late-onset, delayed-onset, purine nucleoside phosphorylase–combined immunodeficiency, T-cell receptor excision circle, inherited disorder*

Primary immunodeficiencies are a group of severe diseases that affect the immune system. The most severe phenotype is severe combined immunodeficiency (SCID), in which both cellular and humoral immunity are affected.[1] Even though healthy at birth, infants with SCID usually die of severe infections in the first years of life unless early and effective therapy is provided.[1] Usually, the diagnosis of SCID is hypothesized because of the presence of a severe infection; at that time, permanent damage can already be present.[1] Therefore an early diagnosis in the preinfection period is desirable to save affected patients and to afford them good

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Abbreviations used
ACTB: β-Actin
ADA: Adenosine deaminase
CID: Combined immunodeficiency
DBS: Dried blood spot
dGuo: 2′-Deoxy-guanosine
dIno: 2′-Deoxy-inosine
IS: Internal standard
KREC: Kappa-deleting recombination excision circle
PNP: Purine nucleoside phosphorylase
SCID: Severe combined immunodeficiency
TMS: Tandem mass spectrometry
TREC: T cell-receptor excision circle

quality of life. Newborn screening of SCID has been proposed and is already performed in several countries.[2] The high mortality and morbidity rates of the disease, availability of curative therapies, higher effectiveness of those therapies when performed during the early presymptomatic phase, and availability of specific disease markers makes the SCIDs a group of diseases deserving of newborn screening.[3]

Purine nucleoside phosphorylase (PNP) deficiency is a rare form of autosomal recessive combined immunodeficiency (CID), accounting for approximately 4% of patients with SCID[4-6] with a wide spectrum of immunologic severity up to PNP-SCID. Nevertheless, its true incidence and prevalence remains unknown because of misdiagnosis and insufficient available laboratory testing.

PNP is a key enzyme in the purine degradation and salvage pathway that catalyses phosphorolysis of purine nucleosides and 2′-deoxy-nucleosides, such as guanosine, 2′-deoxy-guanosine (dGuo), inosine, and 2′-deoxy-inosine (dIno), to their respective purine bases and pentose-1-phosphates.[7] Loss of PNP function blocks production of xanthine, hypoxanthine, and uric acid and allows PNP substrates to undergo alternative metabolism to toxic metabolites. The intracellular accumulation of deoxyguanosine triphosphate derived from dGuo is thought to be toxic to lymphoid cells, and this or other effects of PNP deficiency result in various neurologic abnormalities in up to half of patients with the condition.[6,8]

PNP deficiency is both genetically and clinically heterogeneous: inheriting alleles with mutations that profoundly decrease or abolish enzyme activity results in CID, which has a wide clinical spectrum up to SCID; less detrimental mutations confer milder phenotypes.[8] PNP deficiency should be suspected in a patient with T lymphopenia, recurrent infections of the upper and lower respiratory tract caused by common bacterial pathogens or opportunistic organisms, neurologic abnormalities, autoimmunity, or malignancy.[1,4-6,8] Hematopoietic stem cell transplantation might be curative, at least for the immunologic aspects of the disease, but its efficacy is higher if performed early to restore lymphoid cells that express active PNP and provide both immune function and metabolic detoxification to other organs before irreversible damage has occurred.

PNP deficiency is usually diagnosed in already symptomatic patients by assaying enzyme activity in hemolysates prepared from fresh or frozen packed erythrocytes, in dried blood spots (DBSs), or in blood mononuclear cells or fibroblasts. The diagnosis can also be established or confirmed by means of analysis of PNP genotype.[8] We have recently demonstrated that tandem mass spectrometry (TMS) can easily identify abnormal metabolites in a similar disorder, SCID, because of inherited deficiency of adenosine deaminase (ADA).[9] The specificity and sensitivity of TMS diagnosis of ADA deficiency is very high and has a very low cost when used on DBSs during a routine newborn screening procedure.[9,10] Since 2010, a pilot population-based newborn screening study for SCID has been started in Tuscany, Italy, using both TMS[9,10] and molecular methods to evaluate T-cell receptor excision circles (TREC)[11] and, more recently, kappa-deleting recombination excision circles (KREC) levels.[12] TRECs and KRECs are well-known markers of primary immunodeficiency disorders used in newborn screening programs. TREC and KREC levels are evaluated by using quantitative real-time PCR on DBSs. However, both TREC and KREC quantitative analysis might fail in identification of some immunodeficiencies[9] and cannot individuate delayed or late-onset ADA deficiency.

The aim of the present work was to evaluate whether TMS can identify PNP metabolites in DBSs, with the final goal of finding a sensitive and reliable tool able to identify patients with PNP-CID at birth during routine newborn screening.

METHODS

Patients

DBS samples from 9 patients with genetically confirmed PNP deficiency were evaluated. Four of them were from Turkey, 2 were from Germany, 1 was from Argentina, 1 was from the United Kingdom, and 1 was from Sweden. DBSs were taken at the time of diagnosis in 7 of these patients (patients 1-7), although for 2 patients (patients 8 and 9), stored DBSs taken at birth during routine newborn screening were retrieved and retrospectively tested. The 2 patients (patients 8 and 9) with available DBSs taken at birth came from the United Kingdom and Sweden.

All patients had severe recurrent infections, neurologic symptoms, or both. Clinical data are summarized in Table I.

Carriers

In addition to samples from affected patients, DBSs from the mother of patient 1 and both parents from patients 3 and 6 were analyzed. All of them were single-allele carriers of the mutation and clinically healthy.

Guthrie cards from PNP-deficient patients, carriers, and control subjects

All experiments for the quantitation of PNP substrates were conducted in compliance with institutional review board guidelines (protocol no. 7949/2011). Informed consent was obtained from parents or guardians.

As controls, 10,000 DBSs from neonates (9,300 full-term and 700 premature infants weighing <1,800 g) born in the period between January 2012 and May 2013 were analyzed; 240 DBSs from healthy subjects divided into 4 age groups were also analyzed.

DBSs were obtained from spotted blood on filter paper (903; Whatman GmbH, Dassel, Germany) immediately after withdrawal of the samples to prevent further metabolic conversions.

Guthrie card collection procedure for newborn screening

DBS samples are routinely collected from all neonates born in the Tuscany and Umbria regions of Italy (about 45,000 samples per year). Collection is recommended at between 48 and 72 hours of life. Blood samples are obtained by means of heel sticks, spotted on filter paper (903, Whatman GmbH), dried, and sent daily by courier to the central newborn screening laboratory.