Newborn screening for galactosemia: a 30-year single center experience

Francesco Porta, Severo Pagliardini, Veronica Pagliardini, Alberto Ponzone, Marco Spada
Torino, Italy

Original article

Background: **Galactosemia due to complete or near-complete galactose-1-phosphate uridyltransferase (GALT) deficiency was the first disorder added to the pioneering newborn screening panel besides phenylketonuria. In the last 50 years, many criticisms have been focused on the opportunity of its inclusion. Consequently, long-term single center experiences with this issue are generally lacking.**

Methods: **We reviewed the outcome of newborn screening for hypergalactosemia performed at our department since 1982 and the correspondent long-term clinical outcome.**

Results: **Among 1 123 909 newborns screened for hypergalactosemia, 33 showed abnormal results confirmed at second tier test. Thirteen patients were affected with classic galactosemia, 8 partial GALT deficiency, 3 severe galactokinase deficiency, 7 transient galactosemia, one congenital porto-systemic shunt, and one glucose transporter 2 deficiency. Acute neonatal liver failure in the late first week of life (5.8±1.1 days) unavoidably complicated the clinical course of classic galactosemia, unless in three second-born siblings treated on the basis of presumptive diagnosis immediately after newborn screening sample collection on day 3. Despite early treatment and long-term steadily normal peripheral blood galactose, 77% of patients with severe GALT deficiency present mild to severe intellectual disabilities. All patients with partial GALT deficiency showed normal intellectual development on a regular diet, as well as patients with galactokinase deficiency under treatment.**

Conclusions: **Availability of screening results within the fifth day after birth would allow the prevention of acute decompensation in classic galactosemia. A systematic diagnostic work-up in all positive newborns is essential to unravel the etiology of hypergalactosemia.**

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Key words: **galactose;**
galactosemia;
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Introduction

Following the inception and diffusion of newborn screening for phenylketonuria in the early sixties, classic galactosemia was firstly added to the panel.[1] Since then, many countries are screening for this disease, despite the criticisms about the opportunity of this procedure, encompassing poor screening performance and treatment ineffectiveness on long-term outcome.[2,3] In the modern thinking, however, the concept of "screening for diseases" has been overwhelmed by that of "screening for analytes", as exemplified by the wide potential of screening for hypergalactosemia. Actually, a single abnormal analyte, i.e., high blood total galactose, may address the suspect on several differential diagnoses, including inherited and congenital conditions.[4] All defects of the Leloir pathway, indeed, can be picked up by screening for hypergalactosemia, including classic galactosemia due to complete or near-complete galactose-1-phosphate uridyltransferase (GALT) deficiency, the Duarte variant due to partial GALT deficiency, uridine diphosphate galactose 4-epimerase (GALE) deficiency, and galactokinase (GK) deficiency. Glucose transporter 2 (GLUT2) deficiency, a defect of glucose transport, is an additional inherited cause leading to abnormal screening result. Moreover, congenital porto-systemic shunt (PSS) due to anatomical malformations or functional patency of physiological vessels can occasionally turn positive newborn screening as well. Actually, additional selective diagnostic procedures are essential for unravelling the precise cause of any neonatal hypergalactosemia.

Author Affiliations: Department of Pediatrics, University of Torino, Torino, Italy (Porta F, Pagliardini S, Pagliardini V, Ponzone A, Spada M)

Corresponding Author: Francesco Porta, MD, Department of Pediatrics, University of Torino, Piazza Polonia 94, 10126 Torino, Italy (Tel: +39-011-6637777; Fax: +39-011-3135382; Email: porta.franc@gmail.com)

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Our department is the Regional Reference Center for Newborn Screening of Piemonte and Valle d'Aosta and the Regional Reference Center for diagnosis and treatment of inborn errors of metabolism, performing neonatal mass screening for hypergalactosemia since 1982. Here we report our 30-year experience in this issue.

Methods

We reviewed the outcome of newborn screening for hypergalactosemia performed at our department from January 1982 to December 2012 and the correspondent long-term clinical outcome. Newborn screening was incepted using a qualitative fluorometric assay,[5] and since 2000 the first tier test has been performed by quantitative fluorescent galactose oxidase method (Neonatal total galactose assay, Perkin Elmer) to determine total galactose. Dried blood spots (DBS) on filter paper have been generally collected in the third day of life. The cut-off for total galactose was set at 10 mg/dL on the basis of assessments on 11 000 healthy newborns (99th percentile).

Newborns screened positive were recalled for second tier test with re-determination of total galactose, and in case of confirmed hypergalactosemia, addressed to GALT activity assessment on dbs and clinical evaluation.[6] Newborns with normal GALT activity underwent peripheral assessments of GALE and GK activities. Ophthalmologic evaluation and abdominal ultrasound were performed in all recalled newborns.

All detected patients were treated and followed up at our department up to now. In particular, galactose dietary restriction has been incepted in all patients with confirmed hypergalactosemia at the recall, and subsequently modulated on the basis of the definitive underlying diagnosis. Besides clinical follow-up, patients with defects of the Leloir pathway were subjected to longitudinal biochemical monitoring by regular assessments of blood galactose concentrations.

Results

Newborn screening outcome

Among 1 123 909 newborns screened for hypergalactosemia, 8991 were recalled, and 33 showed abnormal results confirmed at second tier test. Newborn screening DBS were collected at day 3.4±1.2. High galactose concentration was confirmed at re-determination performed at day 12.2±3.2 after delivery. The biochemical, instrumental, and clinical diagnostic work-up allowed the identification of 13 patients with classic galactosemia (GALT activity 2.1±2.8% of normal), 8 patients with partial GALT deficiency (GALT activity 27.0±10.1% of normal), 3 patients with severe GK deficiency (GK activity 2.1±1.6% of normal), and 7 patients with transient galactosemia (idiopathic). Additionally, one patient with congenital PSS due to an arterio-venous malformation and one patient with GLUT2 deficiency were diagnosed. Diagnostic clues and incidences of detected disorders underlying persistent hypergalactosemia are shown in Table.

Patients with classic galactosemia showed higher total galactose concentrations (first sample) with respect to both patients with partial GALT deficiency (P<0.001) and GK deficiency (P=0.042) (Fig. 1). At second tier test, galactose concentration in severe GALT deficiency was higher than in partial GALT deficiency (102.4±22.1 mg/dL *vs.* 29.3±15.6 mg/dL, P=0.005), but not different from GK deficiency (102.4±22.1 mg/dL *vs.* 87.5±8.2 mg/dL, P=0.288).

Seven patients had transient galactosemia, with abnormal galactose concentration confirmed at second tier test (29.3±15.6 mg/dL), but steadily normal galactose on a free diet after a short period of galactose restriction. All these patients showed normal GALT, GK, and GALE activity, doppler ultrasound, and ophthalmologic evaluation.

Clinical onset

Ten of the 13 patients with severe GALT deficiency were re-admitted to hospital before the availability of

Table. Diagnostic clues and incidences of disorders leading to persistent hypergalactosemia detected through 30 years of newborn screening

Detected patients	Disease	Diagnostic clue	Incidence
13	Classic galactosemia	GALT activity: 0-6%	1:86 000
8	Partial galactosemia	GALT activity: 17%-49%	1:160 000
3	GK deficiency	GK activity: 0-5%	1:370 000
1	GLUT2 deficiency	Molecular analysis *GLUT2* gene	-
1	PSS	Doppler ultrasound	-

GALT: galactose-1-phosphate uridyltransferase; GK: galactokinase; GLUT2: glucose transporter 2; PSS: porto-systemic shunt.

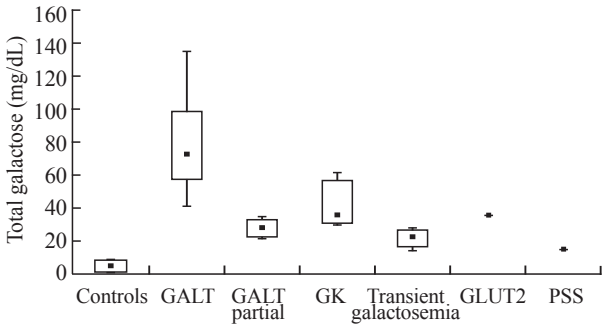

Fig. 1. Outcome of first tier test of newborn screening for galactosemia in different disorders detected during 30 years at our department. GALT: galactose-1-phosphate uridyltransferase; GK: galactokinase; GLUT2: glucose transporter 2; PSS: porto-systemic shunt.

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newborn screening results. Clinical symptoms were evident in the late first week of life (5.8±1.1 days) and quite uniform, with jaundice, lethargy, vomiting, and liver failure. In all patients, diagnosis was formulated at our center on the basis of clinical picture, further corroborated by availability of first tier screening results. Treatment with galactose free diet was effective in restoring the clinical picture over the next few days. Early acute decompensation was avoided in three siblings of affected children with severe GALT deficiency, put on galactose restriction immediately after dbs collection on the basis of presumptive diagnosis of classic galactosemia.

Among the three patients with GK deficiency, one presented isolated nuclear cataract at 12 days of life, completely cleared after galactose restriction. All other patients detected at newborn screening (GALT deficiency, transient galactosemia, GLUT2 deficiency, and PSS) were put, initially, on dietary galactose restriction with normalization of peripheral galactose concentration within the first month, and were clinically silent in the perinatal period.

Long-term follow-up

Despite early treatment and long-term lactose dietary restriction with steadily normal peripheral blood galactose (below 3 mg/dL in all patients), 10 of the 13 patients with severe GALT deficiency (77%, mean age: 15.2±5.6 years) presented mild to severe intellectual disabilities, needing special learning programs since elementary school. Three patients (23%, mean age: 16.7±11.6 years) had normal intellectual performance, following standard school programs. Ovarian insufficiency was observed in all four females with severe GALT deficiency.

The 8 patients with partial GALT deficiency (mean age: 9.2±6.5 years) showed adequate intellectual development and school performance, with normal blood galactose concentrations (1.9±0.4 mg/dL). This picture (clinical and biochemical) was shared by the three GK deficient patients on long-term dietary galactose restriction (mean age: 3.8±4.5 years, blood galactose: 1.6±1.2 mg/dL).

The patient with GLUT2 deficiency showed growth retardation since 15 months.[7] Symptomatic treatment with phosphate and bicarbonate allowed marked growth improvement. At present, the patient was 11 years old, with height on 3rd percentile and normal intellectual development. On a free mediterranean diet with fractionated meals, no symptomatic hypoglycemia was registered.

PSS in one affected patient was corrected surgically, with restoration of galactose homeostasis, uneventful clinical follow-up, and normal intellectual development.

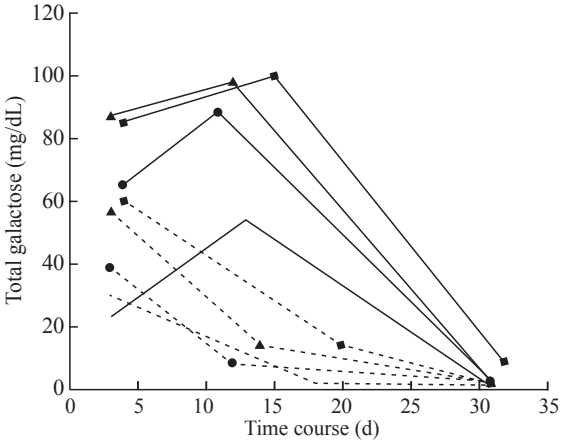

Fig. 2. Time course of peripheral galactose in four pairs of siblings with classic (marked lines) or partial (unmarked lines) galactosemia. The natural galactose accumulation observed in the first-borns (continuous lines) was prevented by early galactose restriction in the second-borns (dotted lines).

Clinical and biochemical comparison in siblings

Four pairs of affected siblings were diagnosed with classic galactosemia (2 brothers pairs, 1 sisters pair) and partial GALT deficiency (2 brothers). Gender concordance within couples was 100%. In all cases, the second born neonate was treated with galactose restriction immediately after newborn screening sampling, on the basis of presumptive diagnosis of galactosemia, classic or partial.

This procedure prevented galactose accumulation in all the second-borns, allowing earlier normalization of peripheral galactose concentration with respect to the first-borns (Fig. 2). Clinically, this has meant an uncomplicated perinatal course (mild jaundice only) in the 3 second-borns with severe GALT deficiency, in contrast with the life-threatening picture of their older siblings. On the contrary, long-term clinical course in severe GALT deficiency was not influenced by pre-symptomatic treatment. Two sisters experienced ovarian insufficiency and intellectual disabilities, two brothers had normal intellectual development, and two brothers showed normal (first-born) and impaired (second-born) intellectual outcome.

The perinatal and long-term clinical course in the two brothers with partial GALT deficiency was equally uneventful, irrespective of initiation of temporary galactose restriction.

Discussion

In the last years, several concerns have been raised about the opportunity of screening for classic galactosemia, following the concept that newborn screening for inborn errors of metabolism aims at detection of pre-

symptomatic individuals who can be successfully treated. Actually, this disorder is generally characterized by early onset life-threatening liver failure which is amenable to short-term effective treatment, whereas the long-term neurological and hormonal complications remain unpreventable.[8,9] Despite the disappointing long-term outcome, however, many countries, including the USA and developed countries, currently include classic galactosemia in newborn screening programs, also in line with the growing interest toward screening for disorders without effective treatment among prospective parents.[10] Most importantly, neonatal screening for hypergalactosemia may allow the detection of severe GALT deficiency, and other treatable conditions, as evidenced by our 30-year experience in this matter. Actually, newborn screening was the chance for the early diagnosis of 5 distinct conditions harboured by 26 newborns, namely severe and partial GALT deficiency, GK deficiency, GLUT2 deficiency, and PSS. No cases of GALE deficiency were diagnosed.

Severe GALT deficiency was the most prevalent disease, unavoidably presenting with perinatal liver failure effectively reversed by early galactose restriction. Neonatal acute decompensation was prevented in 3 cases by pre-symptomatic treatment on the basis of anamnestic diagnosis, suggesting that earlier availability of screening results could be crucial to avoid the neonatal life-threatening picture. Actually, on the basis of our experience, accessibility of newborn screening results within the fifth day of life would have potentially allowed the prevention of acute decompensations, as first tier outcome itself can potentially discern between severe and partial GALT deficiency. However, when DBS collection is anticipated at day 2, availability of newborn screening result can follow clinical presentation in severe cases.[11] Notwithstanding that earlier sampling may increase the false negative rate.

Long-term galactose restriction was ineffective on later complications of severe GALT deficiency (in spite of normal peripheral galactose concentration), such as intellectual disability or mental retardation (in almost 80% of cases) and ovarian failure (in all affected females), in line with current knowledge on this disorder.[3] Moreover, occurrence of these complications was irrespective of the perinatal clinical course, as long-term clinical outcome was concordant in 75% of affected siblings. Interestingly, we observed a substantially different long-term neurological outcome in 2 affected brothers, consistent with the hypothesized effect of epigenetic consequences on the clinical phenotype of classic galactosemia.[12]

In our experience, partial GALT deficiency is not associated with either short- or long-term complications. This finding is in agreement with the current knowledge about this issue.[4] After the first year of life, all patients were on regular diet with optimal metabolic control, confirming the recent observation that clinical and developmental outcome of this condition is good regardless of any diet changes.[13] Long-term speech and language developmental issues, however, have also been reported in patients with partial GALT deficiency,[14] suggesting that newborn screening may be helpful for the comprehension of later effects, if any, of specific biochemical phenotypes incidentally detected.

The early detection of GK deficiency warranted prompt galactose restriction with the prevention or regression of cataract in all affected patients. In our experience, newborn screening for hypergalactosemia was crucial to modify the natural ophthalmologic course of this disease. Although cataract formation in GK deficient patients have been reported despite dietary treatment and normal blood galactose,[15] the clinical course of all treated patients at our department was uneventful until to date. Strict dietary treatment and ophthalmologic follow-up of the patients are still in course.

Besides defects of the Leroir pathway, in our experience, two additional causes of neonatal hypergalactosemia were detected by newborn screening, PSS and GLUT2 deficiency. Congenital porto-systemic shunt has been reported as a potential cause of positive newborn screening for hypergalactosemia. In our experience, however, this represents the exception to the rule. Actually, in front of one detected case at newborn screening, we previously described three affected males with normal blood galactose in newborn period and even later in childhood.[16] Our findings are in agreement with a recent report on 10 patients with PSS, describing hypergalactosemia only in 30% of cases.[17] In our experience, remittent hyperammonemia with postprandial peaks was the hallmark of this vascular anomaly. This allows the speculation that idiopathic transient hyperammonemia of the newborn could be due to temporary patency of the ductus venosus of arantius, which normally closes shortly after birth. This could apply, indeed, also for transient hypergalactosemia without biochemical explanation.

GLUT2 deficiency is a rare inherited disorder of carbohydrate metabolism, with proximal renal tubular dysfunction and hepatic glycogen storage with hepatomegaly and hypoglycemia.[18] Since the substrate specificity of GLUT2 also includes galactose, decreased monosaccharide uptake by the liver results in hypergalactosemia, with potentially abnormal newborn screening results. Although no specific therapy is currently available for this disorder, symptomatic

treatment can stabilize glucose homeostasis, compensate for renal loss of various solutes, and improve growth, as observed in our cases.

In conclusion, our experience in newborn screening for hypergalactosemia shows that this procedure may detect a number of rare conditions potentially overlooked or misdiagnosed. The introduction of a systematic diagnostic work-up in all hypergalactosemic newborns is essential to this purpose, allowing treatment anticipation and modification of the natural course of some detectable conditions.

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Competing interest: The authors have no conflicts of interest to declare.

Contributors: PF ideated the study, analyzed the data, and wrote the paper. PS, PV, PA, SM collected and analyzed the data, and revised the paper.

References

1 Guthrie R. Screening for "inborn errors of metabolism" in the newborn infant-a multiple test program. Birth Defects Orig Artic Ser 1968;IV:92-98.
2 Schweitzer S. Newborn mass screening for galactosemia. Eur J Pediatr 1995;154:S37-S39.
3 Ridel KR, Leslie ND, Gilbert DL. An updated review of the long-term neurological effects of galactosemia. Pediatr Neurol 2005;33:153-161.
4 Fernandes J, Saudubray JM, van den Berghe G, Walter JH. Inborn metabolic diseases: diagnosis and treatment, 4th ed. Heidelberg: Springer, 2006.
5 Misuma H, Wada H, Kawakami M, Ninomiya H, Shohmori T. Galactose and galactose-1-phosphate spot test for galactosemia screening. Clin Chim Acta 1981;111:27-32.
6 Vaccaro Torracca AM. Galactosemia: qualitative and quantitative identification of galactose-1-phosphate. Istisan 1979;91:19.
7 Peduto A, Spada M, Alluto A, La Dolcetta M, Ponzone A, Santer R. A novel mutation in the *GLUT2* gene in a patient with Fanconi-Bickel syndrome detected by neonatal screening for galactosaemia. J Inherit Metab Dis 2004;27:279-280.
8 Fridovich-Keil JL, Gubbels CS, Spencer JB, Sanders RD, Land JA, Rubio-Gozalbo E. Ovarian function in girls and women with GALT-deficiency galactosemia. J Inherit Metab Dis 2011;34:357-366.
9 Potter NL, Nievergelt Y, Shriberg LD. Motor and speech disorders in classic galactosemia. JIMD Rep 2013;11:31-41.
10 Plass AM, van El CG, Pieters T, Cornel MC. Neonatal screening for treatable and untreatable disorders: prospective parents' opinions. Pediatrics 2010;125:e99-e106.
11 Berry GT. Galactosemia: when is it a newborn screening emergency? Mol Genet Metab 2012;106:7-11.
12 Coman DJ, Murray DW, Byrne JC, Rudd PM, Bagaglia PM, Doran PD, et al. Galactosemia, a single gene disorder with epigenetic consequences. Pediatr Res 2010;67:286-292.
13 Ficicioglu C, Thomas N, Yager C, Gallagher PR, Hussa C, Mattie A, et al. Duarte (DG) galactosemia: a pilot study of biochemical and neurodevelopmental assessment in children detected by newborn screening. Mol Genet Metab 2008;95:206-212.
14 Powell KK, Van Naarden Braun K, Singh RH, Shapira SK, Olney RS, Yeargin-Allsopp M. Long-term speech and language developmental issues among children with Duarte galactosemia. Genet Med 2009;11:874-879.
15 Janzen N, Illsinger S, Meyer U, Shin YS, Sander J, Lücke T, et al. Early cataract formation due to galactokinase deficiency: impact of newborn screening. Arch Med Res 2011;42:608-612.
16 Ferrero GB, Porta F, Biamino E, Mussa A, Garelli E, Chiappe F, et al. Remittent hyperammonemia in congenital portosystemic shunt. Eur J Pediatr 2010;169:369-372.
17 Kim MJ, Ko JS, Seo JK, Yang HR, Chang JY, Kim GB, et al. Clinical features of congenital portosystemic shunt in children. Eur J Pediatr 2012;171:395-400.
18 Santer R, Steinmann B, Schaub J. Fanconi-Bickel syndrome--a congenital defect of facilitative glucose transport. Curr Mol Med 2002;2:213-227.

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