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Evolution of tyrosinemia type 1 disease in patients treated with nitisinone in Spain

María Luz Couce, MD, PhD[a,*], Paula Sánchez-Pintos, MD, PhD[a], Luís Aldámiz-Echevarría, MD, PhD[b], Isidro Vitoria, MD, PhD[c], Victor Navas, MD, PhD[d], Elena Martín-Hernández, MD[e], Camila García-Volpe, MD[f], Guillem Pintos, MD, PhD[g], Luis Peña-Quintana, MD, PhD[h], Tomás Hernández, MD[i], David Gil, MD, PhD[j], Félix Sánchez-Valverde, MD, PhD[k], María Bueno, MD, PhD[l], Iria Roca, SC[a], Encarna López-Ruzafa, MD[m], Carmen Díaz-Fernández[n]

Abstract

Treatment with nitisinone (NTBC) has brought about a drastic improvement in the treatment and prognosis of hereditary tyrosinemia type I (HT1). We conducted a retrospective observational multicentric study in Spanish HT1 patients treated with NTBC to assess clinical and biochemical long-term evolution.

We evaluated 52 patients, 7 adults and 45 children, treated with NTBC considering: age at diagnosis, diagnosis by clinical symptoms, or by newborn screening (NBS); phenotype (acute/subacute/chronic), mutational analysis; symptoms at diagnosis and clinical course; biochemical markers; doses of NTBC; treatment adherence; anthropometric evolution; and neurocognitive outcome.

The average follow-up period was 6.1 ± 4.9 and 10.6 ± 5.4 years in patients with early and late diagnosis respectively. All patients received NTBC from diagnosis with an average dose of 0.82 mg/kg/d. All NBS-patients (n = 8) were asymptomatic at diagnosis except 1 case with acute liver failure, and all remain free of liver and renal disease in follow-up. Liver and renal affectation was markedly more frequent at diagnosis in patients with late diagnosis ($P < .001$ and .03, respectively), with ulterior positive hepatic and renal course in 86.4% and 93.2% of no-NBS patients, although 1 patient with good metabolic control developed hepatocarcinoma.

Despite a satisfactory global nutritional evolution, 46.1% of patients showed overweight/obesity. Interestingly lower body mass index was observed in patients with good dietary adherence (20.40 ± 4.43 vs 24.30 ± 6.10; $P = .08$) and those with good pharmacological adherence (21.19 ± 4.68 vs 28.58 ± 213.79).

intellectual quotient was ≥85 in all NBS- and 68.75% of late diagnosis cases evaluated, 15% of which need pedagogical support, and 6.8% (3/44) showed school failure.

Among the 12 variants identified in fumarylacetoacetate hydrolase gene, 1 of them novel (H63D), the most prevalent in Spanish population is c.554–1 G>T.

After NTBC treatment a reduction in tyrosine and alpha-fetoprotein levels was observed in all the study groups, significant for alpha-fetoprotein in no NBS-group ($P = .03$), especially in subacute/chronic forms ($P = .018$).

This series confirms that NTBC treatment had clearly improved the prognosis and quality of life of HT1 patients, but it also shows frequent cognitive dysfunctions and learning difficulties in medium-term follow-up, and, in a novel way, a high percentage of overweight/obesity.

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[a] Unit of Diagnosis and Treatment of Congenital Metabolic Diseases, S. Neonatology, Department of Pediatrics, Clínico Universitario de Santiago de Compostela, CIBERER, Health Research Institute of Santiago de Compostela (IDIS), [b] Unit of Metabolism, Department of Pediatrics, Hospital de Cruces, Group of Metabolism, Biocruces Health Research Institute, CIBERER, [c] Unit of Metabolopathies Hospital Universitario La Fe, [d] Pediatric Gastroenterology and Nutrition Unit Hospital Carlos Haya, Málaga, [e] Unit of Mitochondrial and Congenital Metabolic Diseases, Hospital 12 de Octubre, [f] Pediatric Gastroenterology, Hepatology and Nutrition Unit, H. San Joan de Deu, Barcelona, [g] Minority disease Unit, Hospital Vall d'Hebron, [h] Gastroenterology and Nutrition Unit Complejo Hospitalario Universitario Insular-Materno Infantil, CIBEROBN, Las Palmas de Gran Canaria University, Las Palmas, [i] Pediatric Service, Hospital General de Albacete, [j] Pediatric Gastroenterology, Hepatology and Nutrition Unit Hospital Virgen da Arrixaca, Murcia, [k] Gastroenterology and Nutrition Unit, Hospital Virgen del Camino, Pamplona, [l] Metabolic Congenital Diseases Unit, Hospital Virgen del Rocío, Sevilla, [m] Gastroenterology Unit, H. de Torrecárdenas, Almería, [n] Unit of Hepatology and Infantile Hepatic Transplantation, Hospital Universitario La Paz, Madrid, Spain.

[*] Correspondence: María Luz Couce, Hospital Clinico Universitario de Santiago de Compostela, Santiago de Compostela, A Coruña, Spain (e-mail: maria.luz.couce.pico@sergas.es).

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Abbreviations: AFP = alpha-fetoprotein, BMI = body mass index, FAH = fumarylacetoacetate hydrolase, HCC = hepatocellular carcinoma, HT1 = hereditary tyrosinemia type 1, IQ = intellectual quotient, NBS = newborn screening, NTBC = nitisinone, SA = succinylacetone, Tyr = tyrosine.

Keywords: nephrocalcinosis, phenotype, severe liver dysfunction, tubulopathy, tyrosine

1. Introduction

Hereditary tyrosinemia type 1 (HT1, OMIM 276700) is a severe autosomal recessive disorder due to deficiency of fumarylacetoacetate hydrolase (FAH, EC 3.7.1.2), the last enzyme in tyrosine (Tyr) degradation pathway. Due to this defect, toxic metabolites including succinylacetone (SA), maleylacetoacetate, and fumarylacetoacetate, are formed. These products cause severe liver dysfunction, renal tubulopathy, porphyria like syndrome, cardiomyopathy, and hepatocellular carcinoma (HCC) early in life.[1,2] Acute (presenting before 6 months of age), subacute (presenting between 6 and 12 months) and chronic (presenting after 1 year) forms of the disease have been described.[1] The age at onset of symptoms broadly correlates with severity.[3] The HT1 frequency worldwide is about 1 in 100,000 individuals, but a higher incidence is observed in some regions such as Turkey, Quebec; and India.[4] The *FAH* gene has been cloned and mapped in human chromosome 15q, and 100 *FAH* mutations have been reported so far.[4]

Neonatal mass screening is conducted in many countries around the world and is essential to guarantee early diagnosis and treatment during the neonatal period. Tyr as a screening parameter is not appropriate due to the high rate of false-positive, related to transient neonatal tyrosinemia, and false-negative results; SA is the screening parameter of choice.[5,6]

Until the early 1990s, the only strategies available to manage the symptoms of HT1 were protein-restricted diets (usually low in phenylalanine, methionine, and Tyr) and liver transplantation. In 1992, an alternative treatment, (2-(2-nitro-trifluoromethylbenzoyl) 1, 3-cyclohexanedione, also known as nitisinone or NTBC, was introduced.[7] This treatment inhibits the upstream enzyme 4-hydroxyphenylpyruvate dioxygenase, thereby preventing the formation of the toxic metabolites fumarylacetoacetate and succinylacetoacetate (Fig. 1). NTBC treatment strongly increases plasma Tyr concentrations, so this therapy should be complementary to diet restricted in Tyr and its precursor phenylalanine (Phe) and should not be administered alone.[8,9] Liver dysfunction is now controlled in >90% of patients, and extrahepatic manifestations have been abolished or greatly improved with combined treatment.[10] It is essential an early start of NTBC therapy to avoid sequelae; ideally in the neonatal period.[1,11,12] Eye pain, eye itching/conjunctivitis, corneal crystals, and

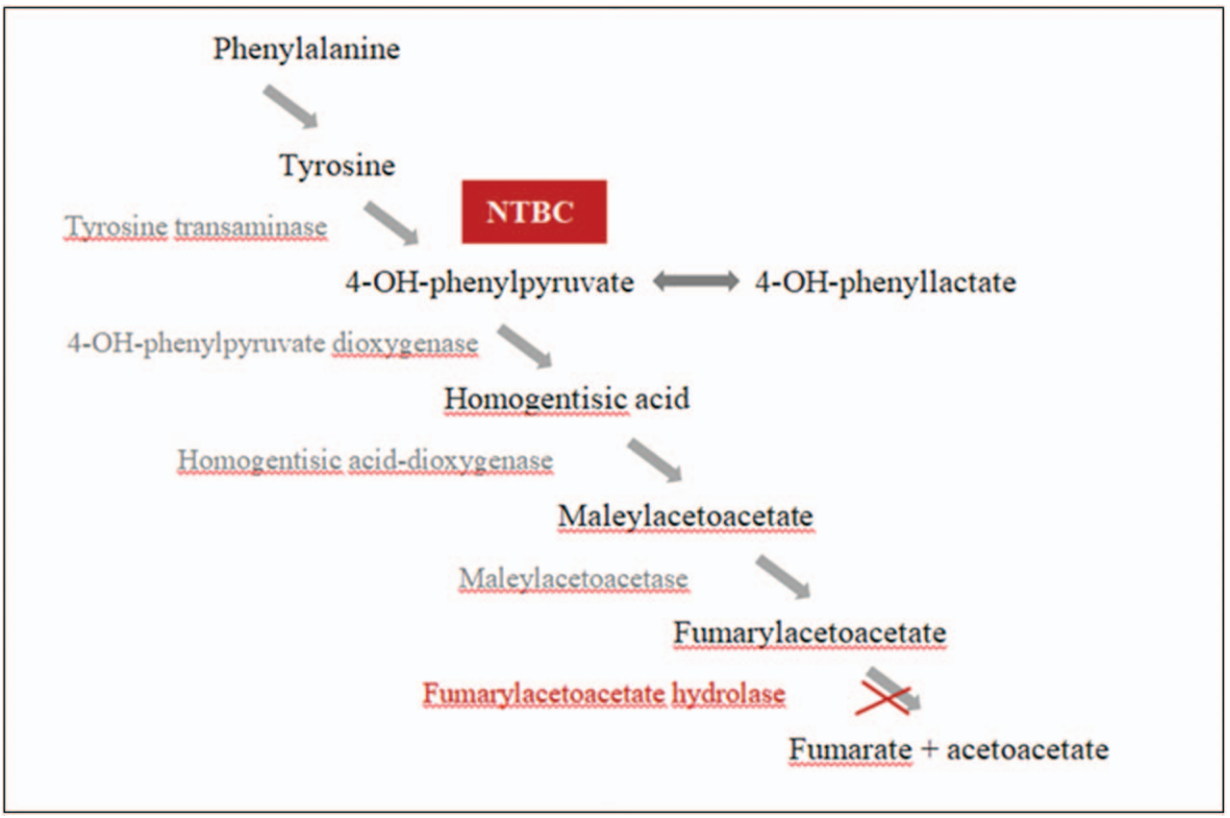

Figure 1. Metabolic pathway of tyrosine. Fumarylacetoacetate hydrolase is the deficient enzyme in tyrosinemia type 1. NTBC inhibits the upstream enzyme 4-hydroxyphenylpyruvate dioxygenase preventing the formation of the toxic metabolites fumarylacetoacetate and succinylacetoacetate. NTBC=nitisinone.

thrombocytopenia are the most common side effects reported[6] and are mostly related to high Tyr levels and reversible when Tyr levels decrease by strict dietary adherence. The risk of HCC has markedly diminished after the introduction of NTBC, although its occurrence has been also described in some HT1 subjects under NTBC therapy.[1,13,14] Neurocognitive deficits could also represent a problem in long-term management.[2,15,16]

Few studies until now had assessed in wide series the evolution of HT1 patients treated with NTBC.[6,11] The aim of this multicentric study is to evaluate Spanish patients with HT1 in treatment with nitisinone, including novels aspects, not previously studied, as nutritional assessment and anthropometric parameters.

2. Methods

2.1. Study design

This is a retrospective observational multicentric study developed in Spanish HT1 patients treated with NTBC. The study protocol was approved by the local research ethics committee of each Center. All HT1 patients who received treatment with NTBC were considered eligible and were recruited after informed consent had been obtained.

2.2. Population

Patients were recollected through a noninterventional post-authorization safety study to evaluate long-term safety of NTBC (Orfadin) treatment in standard clinical care. Data were covered by the physicians responsible for these patients from 14 regions in Spain. The contact was made through the Scientific Society of Inborn Errors of Metabolism of Spain.

At diagnosis, the following parameters were evaluated: age and year of diagnosis, sex, type of diagnosis (by clinical symptoms or by newborn screening [NBS]), mutational analysis of the *FAH* gene, phenotype (acute, subacute, or chronic attending to the onset of symptoms: <6 months; 6–24 months or >24 months, respectively), presence of clinical symptoms and/or biochemical markers and/or imaging studies suggesting diagnosis (liver, renal, haematological, cognitive, ophthalmologic status, and others). Treatment was held according to Spanish Guidelines,[17] during follow-up patients received not only NTBC treatment but also a dietary restricted in Tyr and Phe, according to age and tolerance. Clinical course was subsequently monitored. Follow-up included start date of NTBC treatment and follow-up time; dose of NTBC per kg administered; adherence to diet and pharmacological treatment (good, regular, poor, and very poor); measurement of SA, Phe, Tyr, alpha-fetoprotein (AFP) and NTBC blood concentrations, SA in urine; assessment of anthropometric parameters (weight, height, body mass index [BMI]); evolution of clinical symptoms (liver, renal, haematological, cognitive, ophthalmologic status, and others); development or not of HCC; behavioral assessment (deficit or not of attention and hyperactivity syndrome); schooling (normal or with support); intellectual quotient (IQ) testing.

2.3. Methods

2.3.1. Anthropometric methods. Recumbent length was measured with a measuring board and weight with a manual baby scale until the age of 24 months. Thereafter, standing height was measured with a wall-mounted stadiometer and body weight, to the nearest 100 g, with digital scales. Patients were weighted barefoot and after overnight fasting. Weight and height percentiles and *z*-scores were calculated using the online nutritional assessment tool of the Spanish Society of Gastroenterology, Hepatology, and Nutrition (www.gastroinf.es).

The nutritional status was performed by calculating the BMI as BMI = weight (kg)/height^2 (m^2). Subjects were classified according to BMI by using the WHO Child Growth Standards (underweight: BMI percentile <15; normal: BMI percentile 15–85, overweight: BMI percentile 85–95, obese: BMI percentile >95).[18,19]

2.3.2. Analytical methods. NBS for HT1 by levels of Tyr (cut-off level: 200 μmol/L) and SA as the second marker or only SA on filter paper blood spots using tandem mass spectrometry was done in the regions of Spain where expanded NBS was first performed (Galicia, Cataluña, Madrid, and Murcia).

Quantitative analysis of amino acids (Phe, Tyr) in plasma samples was carried out by ion-exchange chromatography (Biochrom 30 autoanalyzer) after deproteinization of the sample (plasma or urine) with 5-sulfosalicylic acid. SA was detected in dried blood spot by a kit of Chromsystems (Teknokroma, Barcelona, Spain) by tandem mass spectrometry. AFP and some other routine biochemical parameters were analyzed in the respective laboratories associated with each center, but because the reference ranges of these parameters are standardized, we assumed that the slight variability among them is of no clinical significance. NTBC concentrations were evaluated by using tandem-mass spectrometry.[20]

Reference range values for biochemical parameters were: SA (<0.1 μmol/L in blood and <1 mmol/mol creatinine in urine), Phe (35–120 μmol/L), Tyr (<400 μmol/L), AFP (<10 μg/L, in the first year of life less than 77 μg/L in girls and 22 μg/L in boys), NTBC (30–60 μmol/L).

- Abdominal ultrasound and/or liver magnetic resonance imaging, and ophthalmologic examination were also done in each reference center according to the chronology indicated in the Spanish follow-up protocol.
- Molecular testing: DNA was isolated and sequenced by standard procedures for blood samples of all patients and their parents. Molecular analysis of *FAH* was performed using standard procedures. Primers were designed to overlap the coding sequences and their flanking regions (sequences available on request). Polymerase chain reaction products were purified by ExoSap (usb) enzyme and sequenced using a Big Dye Terminator Cycler Sequencing Ready reaction kit and the manufacturer's protocol (Applied Biosystems, Waltham, Massachusetts). The sequencing reactions were performed in an ABI 3130XL Genetic Analyser.

2.3.3. Cognitive function. Cognitive function was assessed by psychomotor development index (PDI) or IQ using the Wechsler intelligence scale R for school-age children, the McCarthy Scales of Children's Abilities for preschool-age children, and the Brunet–Lèzine scale in infants. The overall index score of PDI or IQ is considered in the normal range when it is above 85.

2.4. Statistical analysis

Data were analyzed using the R statistical package v3.4.0 [R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna,

Austria. URL https://www.r-project.org/, last accessed: 12/07/2018]. Sample normality was assessed using both the Kolmogorov–Smirnov and the Shapiro–Wilk tests. In cases where one of the variables was quantitative and the other qualitative, we applied the Student *t* test if the quantitative variable was normally distributed, and the Wilcoxon signed-rank test otherwise. Fisher exact test or Chi-squared tests were used when both variables were qualitative. To assess correlations between quantitative variables, we used the Pearson correlation for normally distributed data and the Spearman rho otherwise. The resulting *P*-values were adjusted using the Bonferroni correction and only values lower than .05 were considered significant.

3. Results

3.1. Diagnosis

We evaluated 52 Spanish patients, 7 adults and 45 children (57.7% male; mean age: 11 years and 8 months, range: 2 months–24 years) with HT1. The characteristics of the study population are detailed in Table 1. All patients receive treatment with NTBC from diagnosis. Eight of them (p1–p8) were early detected by NBS, all being asymptomatic except for 1 that already had liver failure data at diagnosis. In 5 patients NBS was performed using Tyr in blood spot as a primary marker and SA as a secondary marker, and in the remaining 3 patients SA was the primary marker. The average age for diagnosis was 12.7 days (range 7–21).

A total of 44 (84.6%) of our patients were diagnosed at a later stage due to by the appearance of clinical symptoms (no-NBS group) (median age at diagnosis: 241 days, range: 22–1428) with noteworthy delay in diagnosis respect with NBS cases ($P < .001$). Acute presentation was the most frequent form (24/44; 54.5%), followed by subacute presentation in 31.8% (14/44) and chronic presentation in 13.6% (6/44). Liver affectation was markedly more frequent at diagnosis in cases with late diagnosis (43/44) compared to cases detected by NBS (1/8) ($P < .001$). In acute forms, acute liver failure stands out as the most frequent hepatic manifestation (75% – 18/24) followed by hepatomegaly (25% – 6/24) and hepatic nodules (20.8% – 5/24). In those patients with subacute/chronic form the incidence of hepatomegaly, in 3 patients together with splenomegaly, and hepatic nodules is greater (90% and 35%, respectively). In a patient with a chronic form the debut was due to a hepatoblastoma.

In no-NBS group kidney involvement at diagnosis was present in 20/44 (45.4%), also more frequent than in NBS-group ($P = .03$), including renal tubular dysfunction (17/44), nephromegaly (7/44), nephrocalcinosis (7/44), renal dysfunction (1/44), and Fanconi syndrome (1/44, patient 14), with higher frequency in acute forms of tubulopathy and nephrocalcinosis and in subacute/chronic forms of nefromegaly. Hypophosphatemic rickets was observed in 4 acute and 1 subacute patients, and 3 patients had neurological crises at diagnosis. Other manifestations at diagnosis in no-NBS group were vomiting (18%), hypoglycemia (15.9%), sepsis (9%), and growth failure (6.8%).

As reflected in Table 2, Tyr, AFP, and SA levels at diagnosis were slightly lower in NBS-group than in those with late diagnosis (Tyr: 416.7 ± 153.5 vs 469.95 ± 231 μmol/L, $P = .79$; AFP: 4.3 vs 5.9 ng/mL, $P = .84$; and SA: 0.42 vs 0.5 μmol/L, $P = .53$). Both AFP and Tyr levels show a lineal correlation with the time of evolution of the disease at diagnosis with higher levels in chronic forms of tyrosinemia ($P = 0.048$ for AFP). No relevant differences were observed in Phe levels between the 2 groups.

3.2. Follow-up

The average follow-up period was 6.1 ± 4.9 years in patients with early diagnosis and 10.6 ± 5.4 years in those with late diagnosis. All patients received NTBC from diagnosis with an evolving average dose of 0.82 mg/kg/d (range: 0.44–1.34) according to NTBC levels. A good diet adherence was observed in 87% of NBS-patients and 65% of patients with late diagnosis, while only 1 patient showed poor adherence to pharmacological treatment. General clinical outcome was positive in most patients; only 6 patients, all of them not diagnosed by NBS, developed hepatic manifestations, in addition to the patient with hepatoblastoma at diagnosis: hepatic nodules (3; cases number 32, 39, and 50), hepatic hyperechogenicity (case number 40), hepatic cirrhosis (case 50), and hepatic steatosis (case 45). Only 1 case of new hepatic malignancy was observed in our series: a female patient with a subacute form (case number 42), good dietetic and therapeutic adherence, and Tyr and NTBC levels within range, who developed a hepatocarcinoma at the age of 13 years and needed liver transplantation. All NBS patients continued free of liver and renal disease.

In no-NBS group renal evolution was satisfactory after treatment in 41/44 patients, 1 patient developed renal dysfunction secondary to chemotherapy treatment of hepatoblastoma and 2 maintained nephrocalcinosis. Hematological and ophthalmological evolution was good except in 1 patient, with very poor diet and treatment adherence, who developed ocular manifestations. Six patients, 5 of them with acute form and 1 with subacute diagnosis, presented seizures: photosensitive epilepsy (1/6), myoclonic epilepsy (2/6), absence crisis (1/6), and 2 with an isolated seizure.

It highlights that anthropometric evolution is our series is satisfactory as almost half of the HT1 patients maintained a BMI adequate to their age and sex (47.06%). Only 3 patients, all of them of late diagnosis, showed underweight in their follow-up, nevertheless the percentage of obesity and overweight in our study population is high, and interestingly, no significant differences were observed in this percentage between NBS and no-NBS groups. No patient has shown development of diabetes or hyperglycemia so far.

We evaluate the correlation between BMI with several variables: Tyr levels, duration of pharmacologic or dietetic treatment, and adherence to both treatments, and we found a significant correlation between BMI and NTBC treatment (*P*-value: $9.62e^{-05}$; rho = 0.5987515). BMI was both lower in patients with good dietary adherence (20.40 ± 4.43 vs 24.30 ± 6.10; $P = .08$) and in those with good adherence to NTBC treatment (21.19 ± 4.68 vs 28.58 ± 213.79).

Learning difficulties were not observed in NBS-patients but in late-diagnosis group pedagogical support or curricular adaptation was necessary in 15% of patients, and 6.8% (3/44) showed school failure, 1 case with concomitant diagnosis of Angelman syndrome and 2 with poor diet and pharmacological adherence. Thirty-eight patients had cognitive evaluation. IQ was ≥85 in all NBS- and 68.75% of late diagnosis cases evaluated; in this second group, 3 patients with acute-onset and 1 with chronic presentation, showed IQ ≤70. A negative correlation between the duration of NTBC treatment and IQ was not verified in our series by a logistic regression model. Seventeen percent of HT1

Table 1

Characteristics of tyrosinemia 1 patients at diagnosis.

Patient number	Sex	Age at diagnosis	Diagnosis type	Phenotype	SA	TYR	AF	Genetic study	Clinical symptoms at diagnosis					
									Cognitive status	Hepatic symptoms/ image test	Renal symptoms/ image test	Hypophosphatemic rickets	Neurological crises	Other symptoms
1	F	19 d	NBS	AS	0.57	317	1.1	c.554–1G>T (IVS6–1G>T)/c.1062+5G>A (IVS12+5G>A)	Good	N	N	N	N	–
2	F	7 d	NBS	AS	0.40	243	2.6	c.554–1G>T (IVS6–1G>T)/ c.1062+5G>A (IVS12+5G>A)	Good	N	N	N	N	–
3	M	9 d	NBS	AS	0.4	430	6.4	c.554–1 G>T (IVS6–1 G>T) in homozygosis-	Good	N	N	N	N	Laryngomalacia
4	M	15 d	NBS	AS	0.4	396		c.554–1G>T (IVS6–1 G>T) in homozygosis	Good	N	N	N	N	–
5	M	21 d	NBS	AS	0.32	371		–	Good	N	N	N	N	–
6	F	10 d	NBS	AS	0.5	471		–	Good	N	N	N	N	–
7	M	7 d	NBS	AS	0.4	602		–	Good	N	N	N	N	–
8	M	14 d	NBS	Acute	0.1	458		c.554–1 G>T (IVS6–1 G>T) in homozygosis	Good	AHF	N	N	N	–
9	F	1 mo 3 d	CS	Acute	0.46	510	2	c.1062+5G>A (IVS 12+5 G-A) in homozygosis	Good	AHF	RTD/Nc	Y	N	–
10	M	4 mo 7 d	CS	Acute	0.43	266	3.6	c.554 1G>T (IVS6- 1G>T) in homozygosis	Poor	AHF	RTD/Nc/Nm	Y	Y	
11	M	1 mo 24 d	CS	Acute	un	255	6.4	c.707–1G>A (IVS8–1G >A) in homozygosis	Regular	AHF	RTD/Nm	Y	N	Sepsis/GI bleeding
12	F	3 mo 3 d	CS	Acute	0.4	463	1.3	c.5541G>T (IVS6–1G>T)/ c.233G>A (p. Trp78X)	Good	Hepatomegaly	N	N	N	Sepsis
13	F	4 mo 23 d	CS	Acute	0.3	421	5.1	c.782C>T (p.Pro261Leu) in homozygosis	Good	Hepatomegaly/Hepatic nodules	RTD	N	N	Epistaxis
14	M	5 mo	CS	Acute				–	Good	N	RTD/FS	Y	N	–
15	M	22 d	CS	Acute				c.554–1 G>T (IVS6–1 G>T) in homozygosis	Good	Hepatomegaly Hepatic nodules	N	N	N	Sepsis
16	F	4 mo 20 d	CS	Acute				c.554–1 G>T (IVS6–1 G>T) in homozygosis	Good	Hepatomegaly/ Hepatic nodules	RTD/Nc	N	N	–
17	M	4 mo 24 d	CS	Acute				–	Good	AHF	N	N	N	–
18	M	27 d	CS	Acute				–	Good	AHF	RTD/Nc	N	N	–
19	F	4 mo 26 d	CS	Acute				c.554–1 G>T (IVS6–1 G>T) in homozygosis	Good	Hepatomegaly/Hepatic nodules	N	N	N	–
20	F	22 d	CS	Acute				c.554–1 G>T (IVS6–1 G>T) in homozygosis	Good	Hepatomegaly	N	N	N	–
21	M	3 mo 12 d	CS	Acute	0.65	641	7.2	c.554 1G>T (IVS6–1 G>T) /n.d.	Regular	AHF	N	N	N	Hypogliyemia
22	F	5 mo 26 d	CS	Acute	0.6	233	2.1	c.913G>C (p.Gly305Arg)/c.1062+5G>A (IVS12+5G>A)	Regular	AHF	N	N	N	Hypoglucemia
23	M	1 mo 15 d	CS	Acute	0.7	482	8	c.1062+5G>A (IVS12+5G>A) in homozygosis	Regular	AHF	N	N	N	Hypoglucemia
24	M	3 mo 12 d	CS	Acute	0.6	480	3.9	c.1062+5G>A (IVS12+5G>A) in homozygosis	Regular	AHF	RTD/Nm	N	N	Hypoglucemia
25	M	1 mo 18 d	CS	Acute		193	1.4	–	Poor	AHF	RTD	N	N	Vomiting/GI bleeding
26	F	1 mo 8 d	CS	Acute		99	19	c.1062+5G>A (IVS12+5G>A)/c.982C>T (p.GLn328X)	Regular	AHF	RTD/Nc	N	Y	Vomiting
27	M	3 mo 28 d	CS	Acute		570	5.3	c.554–1 G>T (IVS6–1 G>T) in homozygosis	Good	AHF	N	N	N	Vomiting Hypoglucemia
28	M	4 mo 2 d	CS	Acute		186	2.3	c.554–1 G>T (IVS6–1 G>T) in homozygosis	Good	AHF	N	N	N	Vomiting
29	M	2 mo 13 d	CS	Acute				–	Good	AHF	N	N	N	–
30	F	2 mo 25 d	CS	Acute				–	Good	AHF	RTD	N	N	–

(*continued*)

Table 1

(continued).

Patient number	Sex	Age at diagnosis	Diagnosis type	Phenotype	SA	TYR	AF	Genetic study	Clinical symptoms at diagnosis					
									Cognitive status	Hepatic symptoms/ image test	Renal symptoms/ image test	Hypophosphatemic rickets	Neurological crises	Other symptoms
31	M	2 mo 7 d	CS	Acute				–	Good	AHF	N	N	N	–
32	M	19 d	CS	Acute				–	Regular	AHF Hepatic nodules	N	N	N	–
33	M	6 mo 2 d	CS	Subacute		494	2.2	c.554 1G>T (IVS6–1 G>T) in homozygosis	Regular	AHF	N	Y	N	Vomiting Growth ret.
34	F	8 mo	CS	Subacute	un	452	1.3	c.554–1G>T ((IVS6–1 G>T)/ c.1027G>T (p.Gly 343Trp)	Regular	Hepatomegaly Cirrhosis	RTD	N	N	Vomiting
35	M	8 mo 25 d	CS	Subacute	0.14	530	3.78		Good	Hepatomegaly. Coagulopathy	RTD/Nm	N	N	Vomiting
36	M	11 mo 3 d	CS	Subacute	0.14	572	2.52	c.554–1 G>T (IVS6–1 G>T) in homozygosis	Regular	Hepatomegaly Coagulopathy Hepatic nodules	RTD	N	N	Sepsis GI bleeding
37	F	7 mo 4 d	CS	Subacute	0.2	375	1.28	c.982C>T (p.Gln328X) in homozygosis		Hepatosplenomegaly	N	N	N	N
38	M	1 yr 20 d	CS	Subacute				c.554–1 G>T (IVS6–1G>T)/ c.974C>T/p.Thr325Met	Good	Hepatomegaly Hepatic nodules	Nm	N	N	Growth ret. Hypoglucemia
39	M	9 mo 27 d	CS	Subacute	0.8	414	N	c.1062 + 5G>A (IVS12 + 5 G>A)/ c.554–1 G>T (IVS6–1 G>T)	Good	Hepatomegaly	Nm	N	N	N
40	F	8 mo 16 d	CS	Subacute	0.6	313	N	c. 398 A>T (p.His133Leu) in homozygosis	Good	Hepatomegaly Coagulopathy	RTD/Nc	N	Y	Growth ret. Hypoglucemia
41	F	8 mo 9 d	CS	Subacute				c.554–1 G>T (IVS6–1 G>T) in homozygosis	Regular	Hepatomegaly Hepatic nodules	N	N	N	–
42	F	11 mo 26 d	CS	Subacute		370	13.6	c.554–1 G>T (IVS6–1 G>T) in homozygosis	Good	Hepatomegaly Coagulopathy	N	N	N	N
43	F	7 mo 3 d	CS	Subacute				–	Good	Hepatomegaly Hepatic nodules	N	N	N	N
44	M	10 mo 28 d	CS	Subacute				–	Good	Hepatomegaly	N	N	N	N
45	M	1 yr 1 mo	CS	Subacute				–	Good	Hepatosplenomegaly	RTD	N	N	N
46	M	1 yr 5 mo	CS	Subacute				–	Good	Hepatoblastoma	N	N	N	N
47	F	2 yr 3 mo	CS	Chronic	0.35	377	5.7	c.782C>T (p.Pro261Leu) in homozygosis	Poor	Hepatomegaly/cirrhosis	RD	N	N	N
48	F	2 yr 5 mo	CS	Chronic	0.3	352	Normal	c.554–1G>T (IVS6–1 G>T)/ c.982C>T (p.Gln328X)	Good	Hepatomegaly Hepatic nodules	N	N	N	N
49	M	2 yr 1 mo	CS	Chronic	0.6	268	Normal	–	Good	Hepatosplenomegaly/ Hepatic nodules	N	N	N	N
50	F	3 yr 11 mo	CS	Chronic	0.5	719	3.7	c.554–1 G>T (IVS6–1 G>T) in homozygosis	Good	Hepatomegaly Hepatic nodules	RDT/Nm/Nc	N	N	N
51	M	2 yr 26 d	CS	Chronic		830	10.2	H63D in homozygosis	Good	Hepatomegaly	N	N	N	Vomiting
52	F	23 mo	CS	Chronic		193	4.4	c.554–1 G>T (IVS6–1 G>T) in homozygosis	Good	Hepatomegaly Hepatic nodules	N	N	N	N

AHF = acute liver failure, AS = asymptomatic, CS = clinical symptoms, d = day, F = female, FS = Fanconi syndrome, GI bleeding = gastrointestinal bleeding, Growth ret = growth retardation, M = male, mo = month, N = no, NBS = newborn screening, Nc = nephrocalcinosis, Nd = no detected, Nm = nephromegaly, P = patient, RD = renal disease, RTD = renal tubular dysfunction, un = undetectable, yr = year, Y = yes.

Table 2

Clinical, analytical, and anthropometric evolution of tyrosinemia type 1 Spanish patients.

n (%)	All sample (n = 52)	Newborn diagnosis (NBS) (n = 8)	Clinical diagnosis			P^1	P^2
			All (n = 44)	Acute (n = 24)	Subacute or chronic (n = 20)		
Age at diagnosis, d	206 (7–1428)	13 (7–25)	241 (22–1428)	104 (22–370)	406 (33–1428)	.0001	.0004
Clinical manifestations							
Poor status at diagnosis							
Hepatic	44 (84.6%)	1 (12.5%)	43 (97.7%)	23 (95.8%)	20 (100%)	<.001	.36
Renal	20 (38.4%)	0	20 (45.4%)	11 (45.8%)	9 (45%)	.03	.53
Hematological	16 (30.7%)	0	16 (36.3%)	8 (33.3%)	8 (40%)	.01	.42
Ophthalmic	1 (1.9%)	0	1 (2.2%)	1 (4.2%)	0	.70	.32
Neurological	3 (5.7%)	0	3 (6.8%)	2 (8.3)	1 (5%)	.73	.55
Poor status follow-up							
Hepatic	6 (11.5%)	0	6 (15%)	1 (4.2%)	5 (25%)	.06	.05
Renal	3 (5.7%)	0	3 (6.7%)	2 (8.3%)	1 (5%)	.70	.55
Hematological	1 (1.9%)	0	1 (1.9%)	0	1 (5%)	.34	.28
Ophthalmic	1 (1.9%)	0	1 (1.9%)	0	1 (5%)	.40	.26
Neurological	6 (11.5%)	0	6 (15%)	0	1 (5%)		
Analytical parameters							
At diagnosis:							
Succinilacetone, μmol/L	0.50 (0.37–0.70)	0.42 (0.37–0.80)	0.50 (0.30–0.75)	0.50 (0.40–0.75)	0.45 (0.30–0.91)	.79	.71
Alpha-fetoprotein, μg/L	5.33 (3.10–8.60)	4.30 (3.25–7.65)	5.9 (3.05–9.5)	5.07 (2.30–6.95)	6.00 (3.85–13.6)	.84	.05
Phenylalanine, μmol/L	44.57 ± 15.02	42.46 ± 16.53	46.90 ± 14.33	46.90 ± 14.33	42.62 ± 15.68	.69	.35
Tyrosine, μmol/L	461.9 ± 220.4	416.7 ± 153.5	469.95 ± 231	453.08 ± 234.6	490.2 ± 230.9	.53	.60
Follow-up:							
Succinilacetone, μmol/L	0.43 (0.18–19)	0.40 (0.3–0.57)	0.60 (0.30–0.65)	0.60 (0.50–0.67)	0.42 (0.18–0.65)	–	.71
Alpha-fetoprotein, μg/L	4.64 (3.1–8.6)	2.6 (1.6–8.6)	2.36 (1.13–4.35)	3.05 (1.9–7.4)	1.90 (0.55–3.76)	.91	.02
Tyrosine, μmol/L	408.4 ± 161.1	393.1 ± 121.5	427.29 ± 151.9	413.1 ± 183.1	437.2 ± 135.7	.41	.99
Treatment							
NTBC levels, μmol/L	36.51 (8–80)	38.06 (8–30)	39.81 (12–80)	41.95 (12–80)	37.25 (12–80)	.76	.10
Diet compliance							
Good	36 (69.23%)	7 (87.5%)		14 (58.33%)	15 (75%)	.09	.13
Regular/poor	16 (30.76%)	1 (12.5%)		10 (41.6%)	5 (25%)		
Anthropometric evolution							
BMI							
Normal	24 (47.06%)	4 (57.14%)		9 (37.5%)	11 (55%)	.980	.770
Underweight	3 (5.88%)	0		3 (12.5%)	0		
Overweight + obese	24 (46.15%)	3 (42.86%)		12 (50%)	9 (45%)		
Cognitive status							
PDI or IQ							
Normal	38 (73.08%)	8 (100%)	30 (68.08%)	15 (62.5%)	15 (75%)	.009	.51
Poor	14 (26.92%)	0	14 (31.82%)	9 (37.5%)	5 (25%)		

Data expressed as mean, except for succinilacetone; alpha-fetoprotein; and NTBC levels, express as median and range in parentheses.
BMI = body mass index, IQ = intellectual quotient, NBS = newborn diagnosis, NTBC = nitisinone, PDI = psychomotor development index.
P^1 = newborn diagnosis versus clinical diagnosis, P^2 = acute versus subacute + chronic presentation.

patients showed attention hyperactivity and attention deficit and no neurological crises were observed in the study cohort during the follow-up period.

After treatment with NTBC a reduction in Tyr and AFP levels was observed in all the study groups, significant for AFP in the group with clinical diagnosis ($P = .03$) and specially in the subacute/chronic forms ($P = .018$) (Fig. 2).

Genetic study was carried out in 36 patients and 12 different variants in *FAH* gene were detected. All variants were previously described,[26–31] except a novel variant (H63D) identified in

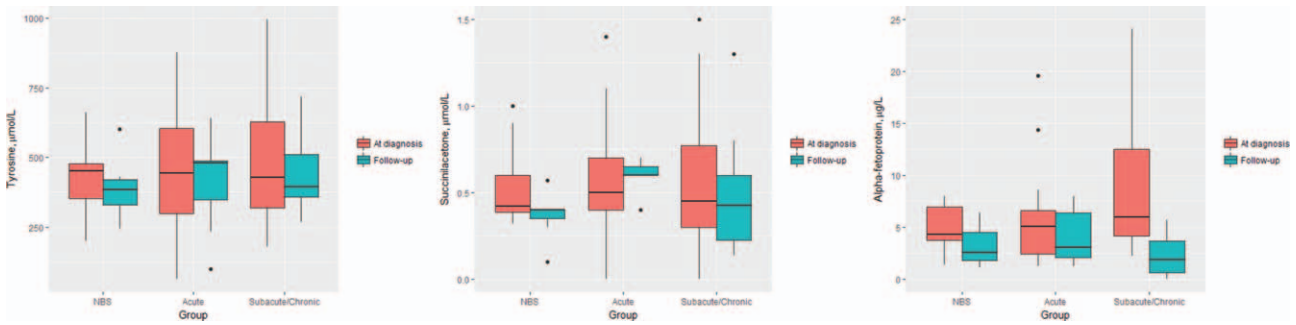

Figure 2. Comparison between tyrosine, succinylacetone and alpha-fetoprotein levels in the NBS, acute and subacute/chronic patients at diagnosis and in the follow-up, after treatment with NTBC. NBS = neonatal screening, NTBC = nitisinone.

Table 3

Molecular study of the Spanish HT1 patients.

Variant	Number of cases (n = 36)	Number of alleles (n = 71)	Percentage	
			Heterozygosis	Homozygosis
c.554–1G>T	23 (63.89%)	39 (54.9%)	7 (30.43%)	16 (69.57%)
c.1062 + 5G>A	10 (27.78%)	14 (19.7%)	6 (60%)	4 (40%)
c.982C>T	3 (8.33%)	4 (5.6%)	2 (66.67%)	1 (33.33%)
c.782C>T	2 (5.56%)	4 (5.6%)	0 (0%)	2 (100%)
c.398A>T	1 (2.78%)	2 (2.8%)	0 (0%)	1 (100%)
938delC	1 (2.78%)	1 (1.4%)	1 (100%)	0 (0%)
c.1027G>T	1 (2.78%)	1 (1.4%)	1 (100%)	0 (0%)
c.233G>A	1 (2.78%)	1 (1.4%)	1 (100%)	0 (0%)
c.974C>T	1 (2.78%)	1 (1.4%)	1 (100%)	0 (0%)
c.836A>G	1 (2.78%)	1 (1.4%)	1 (100%)	0 (0%)
c.913G>C	1 (2.78%)	1 (1.4%)	1 (100%)	0 (0%)
H63D	1 (2.78%)	2 (2.8%)	0	1 (100%)

Percentages were calculated with respect to the 36 patients in whom the genetic study was carried out.
HT1 = hereditary tyrosinemia type I.

homozygosis in a male patient with a chronic form of HT1. The most prevalent Spanish mutation is c.554–1 G>T (IVS6–1 G>T) (63.89% – 23/36), 16 patients showed this mutation in homozygosis (16/23 – 69.57%) and 7 in heterozygosis (7/23 – 30.43%) (Table 3). In the group of patients homozygous for c.554–1 G>T, 93.8% had hepatic and 50% renal involvement at diagnosis. The hepatic evolution of HT1 patients with the aforementioned variant, considered together in both homo and heterozygosis, is slightly worse when compared to patients with other mutations (poor hepatic status in the follow-up: 12.5% vs 8.33%, respectively, $P = .95$). Moreover, the patient who developed hepatocarcinoma is homozygous for this mutation. Thus, the mutation c.554–1 G>T, despite the absence of statistical significance with patients harboring other mutations, probably due to the low sample size in mutational comparison groups, seems to condition a worse hepatic prognosis even in spite of the treatment. The comparison between the follow-up according to the genotype is shown in Table 4.

4. Discussion

NTBC has brought about a drastic improvement in the treatment and prognosis of HT1 as all the clinical multiorganic complications associated to toxic metabolites accumulation in this pathology have been shown to be preventable with NTBC therapy.[6,11,21] Evidence supported that earlier treatment with NTBC and dietary protein restriction within the first 2 months of life showed a lower rate of complications (hepatic and renal dysfunction)[12]; shorter hospital admissions and a reduced need for liver transplantation.[22] In accordance with previous series of HT1,[6,11,21,23] our revision confirms a clear clinical benefit with the combination of NTBC and dietary treatment. From the 43 patients with hepatic affectation at diagnosis (1 NBS-patient, 23 with acute onset and 19 with subacute-chronic presentation) only 6 maintain hepatic damage in the follow-up. In The Québec NTBC study no patient with early treatment (before 1 month of age) had developed to date HCC; in our series, with a long-follow up period (up to 15 years in some patients), but shorter than the surveillance of the referred study (up to 20 years), a new hepatic malignancy was observed in the follow-up in a patient diagnosed at about 1 year of age who maintained a good metabolic control.

As signaled by Geppert et al systematic review,[22] any of our patients developed renal dysfunction after NTBC treatment and, moreover, renal outcome of those HT1 patients with renal pretreatment involvement was meanly satisfactory.

A good metabolic control, judged by normalization of plasma SA and Tyr levels, was achieved quickly in treated patients. In our series, the greatest effect in reducing AFP levels was observed in no-NBS group, especially in those patients with subacute/chronic forms (Fig. 1).

Table 4

Comparison between hepatic and renal evolution according to the genotype.

Hepatic evolution							
All patients (n = 52)		Patients with molecular study (n = 36)		Patients homozygous for c.554-1 G>T (n = 16)		Patients with other variant (n = 12)		*P*
Good status	46 (88.46%)	Good status	32 (88.89%)	Good status	14 (87.5%)	Good status	11 (91.67%)	.95
Poor status	6 (11.54%)	Poor status	4 (11.11%)	Poor status	2 (12.5%)	Poor status	1 (8.33%)	
Renal evolution								
All patients (n = 52)		Patients with molecular study (n = 36)		Patients homozygous for c.554-1 G>T (n = 16)		Patients with other variant (n = 12)		*p*
Good status	49 (94.23%)	Good status	34 (94.44%)	Good status	16 (100%)	Good status	11 (91.67%)	.85
Poor status	3 (5.77%)	Poor status	2 (5.56%)	Poor status	0 (0%)	Poor status	1 (8.33%)	

The comparison between patients with c.554-1 G>T in homozygosis versus those with other variant was made by the Fisher test with Benjamini–Hochberg correction.

Actually, despite this good metabolic response, a main concern in HT1 treatment is the high frequency of neurocognitive impairment of HT1 patients showing a lower IQ, impaired executive functioning, motor abilities, and social cognition and schooling problems.[2,15,16] The exact pathophysiological mechanisms underlying these impairments were not well elucidated.[24] Despite that no significant correlation between Tyr or Phe plasma levels and IQ at school age was found in a recent study,[25] a relation with chronic hypertyrosinemia has been suggested and some clinical reports seemed to support this hypothesis.[23,26]

Our series showed a clearly better neurocognitive outcome in NBS-patients with early treatment. Learning difficulties were only observed in 23% of late-diagnosis patients The finding of frequent attention hyperactivity and attention deficit (17%) is consistent with the results reported in other studies[27] and less of 10% of HT1 patients presented seizures, percentage similar to the observed in an European cross-sectional study.[6]

It is remarkable in our series that QI in the follow-up remains within a normal range in all the NBS-patients evaluated and in 73% of those with clinical diagnosis and only 4 patients from the 38 with QI assessment showed QI <70. This results contrast with those reported previously with a high number of patients with intellectual and psychomotor delay.[15,21,25]

Although the observation of a negative correlation between the duration of treatment with NTBC and IQ,[2] a recent animal model showed that this slower learning and cognitive impairment is caused by HT1 and not by NTBC treatment itself.[28] Our results endorse it, as no significant relation was demonstrated by a logistic regression model between the duration of NTBC treatment and IQ. Neither correlation was objectified between IQ and Phe/Tyr quotient (Spearman rho: 0.11; *P*-value: .57).

Although the low number of patients with poor dietetic-therapeutic adherence prevents an adequate statistical comparison, the mean BMI is clearly lower in those patients with good treatment compliance, which does not suggest that overweight/obesity is related to the use of special formulas free of Tyr and phenylalanine, which contrasts with studies carried out on other inborn errors of amino acid metabolism as phenylketonuria.[30]

The most prevalent mutation in our cohort, c.554–1 G>T (IVS6–1 G>T), is a splice mutation with higher prevalence in Southern Europe and in Mediterranean region, previously reported in other studies of Spanish HT1 patients,[10,29,31] and ranks second in prevalence in European HT1 patients.[4] The second mutation in frequency was c.1062+5 G>A [IVS 12+5 (G->A)], also a splice mutation previously described in our country.[10,31,32] Both mutations together accounts for 74.6% of the *FAH* alleles investigated (c.554–1 G>T: 39 alleles and c.1062 +5 G>A: 14 alleles). The rest of mutations are rare in our population: c.982C>T,[30] c.836A>G,[31] c.233G>A,[31] c.974C>T[10] and 1 of them (c.782C>T) was identified for the first time in Spanish HT1 patients. This mutation, c.782C>T, was found in 2 Caucasians patients from Madrid is a missense mutation found in 100% of Ashkenazi-Jewish patients examined in Israel[33] and also described in Saudi Arabia and Egypt.[34]

The main limitations of our study are the small number of NBS patients included, reflecting that HT1 screening is not included in the neonatal screening of most regions of our country which results in a limitation of the statistical power of some comparisons as well as the fact that the mutational study was not performed in the entire population studied.

This series confirms that NTBC treatment had clearly improved the vital prognosis and quality of life of HT1 patients, but it also reveals cognitive manifestations and learning difficulties a medium term in a considerable number of patients, as well as, in a novel way, a high percentage of overweight/obesity in HT1 patients.

Author contributions

Conceptualization: María-Luz Couce, Luis Aldámiz-Echevarría, Isidro Vitoria, Luis Peña-Quintana.
Data curation: Victor Navas, Elena Martín-Hernández, Camila García-Volpe, Guillem Pintos, Luis Peña-Quintana, Tomás Hernández, David Gil-Ortega, Félix Sánchez-Valverde, María Bueno, Encarna López-Ruzafa.
Formal analysis: María-Luz Couce, Elena Martín-Hernández, Luis Peña-Quintana, Iria Roca.
Funding acquisition: Carmen Diaz.
Methodology: María-Luz Couce, Isidro Vitoria, Victor Navas, Elena Martín-Hernández, Camila García-Volpe, Guillem Pintos, Tomás Hernández, David Gil-Ortega, Félix Sánchez-Valverde, María Bueno, Encarna López-Ruzafa.
Software: Iria Roca.
Supervision: María-Luz Couce, Luis Aldámiz-Echevarría, Isidro Vitoria, Iria Roca, Carmen Diaz.
Validation: María-Luz Couce, María Bueno.
Writing – original draft: María-Luz Couce, Paula Sánchez-Pintos.

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