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► **To cite this version:**

Natacha Sloboda, Arnaud Wiedemann, Marc Merten, Amerh Alqahtani, Elise Jeannesson, et al.. Efficacy of low dose nitisinone in the management of alkaptonuria. *Molecular Genetics and Metabolism*, 2019, 127 (3), pp.184-190. 10.1016/j.ymgme.2019.06.006 . hal-03487728

HAL Id: hal-03487728

<https://hal.science/hal-03487728>

Submitted on 20 Jul 2022

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Efficacy of low dose nitisinone in the management of alkaptonuria

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Keywords: Alkaptonuria, Nitisinone, Pregnancy

Abstract

Aim: To study the efficacy of low dosage of nitisinone in alkaptonuria

Background: Alkaptonuria (AKU) is a rare genetic disease which induces deposition of homogentisic acid (HGA) in connective inducing premature arthritis, lithiasis, cardiac valve disease, fractures, muscle and tendon ruptures and osteopenia. Recent studies showed that nitisinone decreases HGA and is a beneficial therapy in AKU. This treatment induces an increase in tyrosine levels which can induces adverse effects as keratopathy.

Methods: We described the evolution HGA excretion and tyrosine evolution in 3 AKU patients treated by very low dosage of nitisinone with regards to their daily protein intakes. We also described the first pregnancy in an AKU patient treated by nitisinone.

Results: We found mild clinical signs of alkaptonuria on vertebra MRI in two young adults and homogentisate deposition in teeth of a 5 years old girl. Very low dose of nitisinone (10% of present recommended dose: 0.2 mg/day) allowed to decrease homogentisic acid by more than 90 % without increasing tyrosine levels above 500 $\mu\text{mol/}$ in these three patients.

Interpretations: The analysis of the follow-up data shows that, in our three patients, a low-dosage of nitisinone is sufficient to decrease urinary HGA without increasing plasma tyrosine levels above the threshold of 500 $\mu\text{mol/L}$.

Introduction

Alkaptonuria (AKU) is a rare, debilitating autosomal recessive disorder affecting tyrosine metabolism due to homogentisate 1,2-dioxygenase deficiency. AKU leads to accumulation of homogentisic acid (HGA) and its metabolites in tissues causing ochronosis, with darkening of cartilaginous tissues, arthritis and joint destruction [1]. Oxidative conversion of HGA leads to production of a melanin-like polymer in a process termed ochronosis. The binding of ochronotic pigments to connective tissues leads to multisystem disorder dominated by premature severe spondylo-arthropathy and cardiac valvular disease [2].

Before 2011, there was no specific treatment for AKU, and most of the possible care were palliative, largely based on analgesia and arthroplasty. Trials with vitamin C acting as an antioxidant (which decreases benzoquinone acetic acid [3], but not the rate of HGA) have turned unconvincing [4]. A low protein diet has been proposed and may be beneficial, but compliance is often limited [4]. Recently, a therapeutic strategy based on the inhibition of 4-OH phenylpyruvate dioxygenase by nitisinone (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione, or NTBC) has been proposed and has shown its biological and more recently clinical efficacy [5]. NTBC is indicated in the treatment of hereditary type 1 tyrosinemia [6]. NTBC is a potent inhibitor of 4-hydroxyphenylpyruvate dioxygenase and inhibits by the way the synthesis of HGA in case of alkaptonuria. This drug has been used off-license in the UK (National center for AKU) for patients with AKU, at a dosage of 2 mg per day, resulting in a striking decrease in the urinary and serum HGA concentrations [7]. The main side effect of this drug is to increase plasma tyrosine which should be treated by a specific diet if the level higher than expected (500 $\mu\text{mol/L}$ is the accepted level in patients treated for type I tyrosinemia) [8]. Other adverse events have been described in alkaptonuria and in tyrosinemia type I treated patients, i.e. skin rash and corneal keratopathy, probably due to increased circulating tyrosine (Tyr) levels [9, 10]. Recently, Ranagath et al report a urinary HGA decrease ranging from 80 to 90 % in the patient group treated by nitisinone (2 mg per day). They experienced 3 proven cases of corneal tyrosine keratopathy and then, they proposed a protein restriction protocol: 0.9 g/kg body weight for tyrosine values between 501 and 700 $\mu\text{mol/l}$, to 0.8 g/kg body weight for values between 701 and 900 $\mu\text{mol/l}$, and additional phenylalanine/tyrosine free meal exchanges are used for levels >900 $\mu\text{mol/l}$ [5]. Many topics remain unclear in the management of AKU by nitisinone (at what age the treatment should be consider, what is the minimal dosage of nitisinone for metabolic efficiency and how is the safety profile of nitisinone use during pregnancy in AKU). To avoid a too high increase of plasma tyrosine, we began to treat our AKU patients with a very low dose of nitisinone at 10% of the present recommended dose: 0.2 mg/day). In this paper, we report our experience of nitisinone treatment in 3 patients (two adults and a 5-years old girl) with this very low dosage of nitisinone. Moreover, the female adult patient became pregnant

while she was treated and we report the first description of nitisinone treatment during pregnancy in AKU.

Methods

Patients

The three patients (aged between 5 and 34 years) are followed for alkaptonuria at the Nancy Reference Center for Metabolic Diseases. Diagnosis was made in childhood at the appearance of black urine. We obtained a written consent from our 2 adult patients and from the parents of the third patient. A special consent was signed by the second patient for the use of nitisinone during her pregnancy.

Treatment

Patients were treated with a low dose of NTBC (Orfadin) at a dosage of 0.2 mg/day, once daily. Nitisinone 0.2mg capsule were prepared by the pharmacy of the university hospital according to the following protocol. 2 mg nitisinone capsules (n=20) are opened to obtain 40 mg of nitisinone and mannitol was added as an excipient. A homogeneous powder is done by trituration to obtain 200 capsules of 0.2 mg each. Dosage adaptations were made for two patients. NTBC was increased to 0.5 mg/day at the end of the pregnancy of patient 2 and a progressive incrementation (depending on the levels of HGA and Tyr) was performed in the child (patient 3) to reach 0.2 mg/day. The dosage of 0.2 mg/day was reached 2 years after the start of treatment.

Monitoring and follow-up

The biological monitoring was performed by urinary HGA ($\mu\text{mol}/\text{mmol}$ creatinine) and tyrosinemia ($\mu\text{mol}/\text{L}$) which were measured at the beginning and during the treatment (on average 4 times/year the first year, then twice a year the second year, then annually). The efficacy of treatment was established by a more than 90 % decrease of urinary HGA[7] while the plasma tyrosine should not exceed 500 $\mu\text{mol}/\text{l}$ as it is recommended in the treatment of tyrosinemia type I[8].

The follow-up includes a clinical examination and a nutritional monitoring at each clinic (before and during the treatment period). The nutritional follow-up included an annual biological assessment (blood count, total protein, albumin, ASAT, ALAT, homocysteine, cobalamin, selenium, zinc, calcium, phosphorus, alkaline phosphatase, 25-OH vitamin D and PTH).

At each follow-up consultation, a consultation with a specialized dietician was scheduled with a quantitative evaluation of the protein intake. The nutritional recommendations given to the patient were fitting with the most recent recommendations of the European Food Safety Authority (EFSA): proteins dietary references values between 0.66 and 0.83 g/kg/day for an adult[11].

Clinical follow-up was performed (four times/year the first year, then two times/year the second year and one time/year from the third year, with a complete clinical examination (cardiorespiratory system, abdominal palpation, neurological examination, growth). The consultation also included a monitoring of abnormal urine staining and ochronosis-type complications in adults (spine, pelvis and knee MRI, and cardiac assessment).

Analyses of homogentisic acid and tyrosine.

GC-MS analyses of homogentisic acid (HGA, expressed in mmol/mol creatine) in acidified urines samples were performed using a TRACE 1300 gas chromatograph coupled to a Polaris ion-trap mass spectrometer (Thermo Scientific) operated in electron ionization mode. The m/z 341 ion of the silylated TMS derivative was used for quantification using an external standard method. To avoid the interference between creatinine and homogentisic acid analysis[12], creatinine analysis was performed by mass spectrometry[13]. Plasma tyrosine ($\mu\text{mol/L}$) analyses were carried out using a Jeol AminoTac 500 (Tokyo, Japan) cation-exchange amino acid analyzer with post-column ninhydrin detection. All serum and urine quantitation analyses were performed by the Department of Biochemistry and Molecular Biology of the University Hospital of Nancy.

Patients and results.

The description of the three patients and the main biological results are summarized in table 1.

Patient 1 is a 26 years-old man (BMI 23.5 kg/m²) without medical history beside AKU which was diagnosed at 5 months of age (urinary HGA: 1480 mmol/mol creatinine, maximum level at 5 years: 4000 mmol/mol creatinine). Psychomotor development and growth were normal. Urinary HGA at the time of diagnosis was increased to 1480 $\mu\text{mol/mmol}$ creatinine. He was breastfed until the age of one year, then a normal-protein diet was set up. Antioxidant treatment with vitamin C was started at age 5 (500 mg/day). A low protein diet (0.5 g/kg/day) was prescribed at age 18 and was well followed thereafter. At this age, the biological screening showed mild cobalamin deficiency and a mild increase in alkaline phosphatase which induced cobalamin (1 mg monthly) and calcium and vitamin D supplementations. Lumbar bone density at this time was normal (z-score: -0.2). Clinically, he described transient knee pain at 12 years-old. He also experienced transient shoulders pain of moderate intensity after the beginning of nitisinone treatment. The regular follow-up included spine MRI (L5-S1 disc disease [Figure 1])[14] and cardiac ultrasounds every 2 years (without abnormalities to date). Treatment with Orfadin started at 21 years. The treatment result in a dramatic decrease of HGA in urines (with a nearly complete normalization (from 901 to 3 mmol/mol creatinine) associated to a mild

increase of tyrosine which exceeded only once the threshold of 500 $\mu\text{mol/L}$ (mean \pm SD: 416 \pm 142 $\mu\text{mol/L}$ [Figure 2]).

Patient 2 is a 33 years old woman (BMI 19.5 kg/m^2). She was diagnosed with AKU at 5 years (urinary dosage of HGA: 7790 mmol/mol creatinine, maximum level: 8676 mmol/mol creatinine). She has been on a mild-protein diet (1.1 g/kg/day) since her diagnosis and was put on vitamin C (500 mg/day) until she was 7 years old, then vitamin C supplementation was stopped by the family. Clinically, she reports knee and back pain since the age of 26 years old. A MRI of the spine performed at 26 years old finds generalized discs disease related to mild consequences of AKU (Figure 3)[14]. The coxo-femoral or knee MRI and the cardiac ultrasound were normal (February 2015). She has been treated by NTBC (0.2 mg/day), since February 2013 for knee pain and to avoid later complications. The treatment results in a mean decrease of more than 80% (maximum decrease: 94 %) of initial HGA level. Tyrosine level was maintained below 500 $\mu\text{mol/L}$ (393 \pm 90 $\mu\text{mol/L}$) [8] with a mildly protein restricted diet (protein intake: 1.1 g/kg/day) without use amino acids substitutes or low protein foods.

This patient became pregnant in April 2016 while she was treated by nitisinone (0.2 mg/day). She was consulting at 4 week of gestation and was informed about the lack of information in the literature about NTBC treatment and pregnancy in AKU. The decision to continue treatment was made after multidisciplinary meeting between gynecologists and metabolism specialist with the patient consent. Considering the toxicity of high phenylalanine level in maternal phenylketonuria, and in order to reduce any potential risk for the fetus [15], we aimed at keeping a target concentration of Tyr <400 $\mu\text{mol/L}$ during all pregnancy [8] (which we succeeded, with Tyr levels of 364 \pm 104 $\mu\text{mol/L}$). As urinary HGA excretion increased from 1067 to 1456 mmol/mol creatinine (Figure 4) during the third trimester of pregnancy, NTBC dosage was increased at 0.5 mg/day without significant increase in blood Tyr levels (370 \pm 55 $\mu\text{mol/L}$ during the third trimester). Pregnancy monitoring was done once a month by a dietician and a metabolism specialist. Daily protein intake was monitored to be at about 50 g protein / day (1.1 g/kg/d). This monitoring revealed micronutrient deficiencies (Zinc and Cobalamin), which were supplemented. Pregnancy monitoring was performed, as planned in France, with three ultrasounds (US) imaging at 12, 22 and 32 weeks of amenorrhea and no abnormality was detected in the fetus, in particular neither heart disease, nor cerebral malformation. She delivered in January 2017, and gave birth to a healthy baby boy (birth weight: 3.2 kg , height: 49 cm , head circumference: 35 cm). The child has been examined at one, 6 and 18 months of age and has a strictly normal growth and psychomotor development. His urine exhibits a normal color without HGA excretion.

Patient 3 is the second child of Turkish related parents. Pregnancy and delivery were unremarkable. She was seen for the first time at the age of 35 months in front of persistent black urine since the first

year of life. Clinically, she only presented a black coloration of the teeth lesions due to a baby bottle syndrome, probably in relation with homogentisate deposition (Figure 5). Psychomotor development and growth were normal. Urinary organic acids (COA) showed a huge increase in HGA (27624 $\mu\text{mol}/\text{mmol}$ creatinine) confirming the diagnosis of alkaptonuria. Mutation analysis showed that this child is homozygous for the c.595T>C mutation. In the context of early management of the disease, we felt that delaying joint and cardiac involvement in AKU patients, which can be particularly severe or disabling, was an essential point for the future quality of life of our patients, so the parents agreed to start the NTBC treatment. They were informed about potential side effects (increased Tyr levels, possible leukopenia/granulocytopenia, and potential developmental and cognitive disorders – not proved- NTBC European report)

Considering the weight of this patient and considering that we treated adults with 0.2 mg per day, we decided to begin nitisinone treatment at an initial dose of 0.05 mg/day was initiated at 36 months. We increased the dosage at 0.1 mg/day as the remaining HGA in urines was still high (5118 mmol/mol creatinine). This dosage induced a 97% HGA decrease (compared to the initial level). The dosage of NTBC was increased at 0.2 mg/day due to a secondary increase in urinary excretion of HGA probably related to an increase in protein intake (1.7 to 2.3 g/kg/day). The plasma tyrosine level was monitored and never exceeded 500 $\mu\text{mol}/\text{L}$, even at a dosage of 0.2 mg/day (mean tyrosine during the treatment: 305 +/- 111 $\mu\text{mol}/\text{L}$ [Figure 6]). A complete neurological examination was performed at each consultation and was always normal. The child has a normal development and a normal school level.

Discussion

We describe the efficacy of very low dosage nitisinone treatment in three AKU patients. The two first patients treated by nitisinone were two adults (51 and 59 years) and were treated on a very short period of time (less than one month). The first patient was treated initially with 0.35 mg bid which induced a 69% decrease of HGA from 2.9 g/day to 0.9 g/day. The dosage was then increased to 1.4 mg bid which induced a 95% decrease in HGA (0.13g/day) compared to the initial level. The second patient was treated with 0.35 mg bid which induced a 70% decrease in HGA (1.7 vs 6.4 g/day)[4]. These treatments were stopped because of the increase of tyrosine plasma levels. After these first reports, Suwannarath et al published an open-label, single-center study of 9 alkaptonuria patients (5 women, 4 men; 35-69 years of age) treated over the course of 3 to 4 months. Each patient received nitisinone in incremental doses, 0.35 mg bid followed by 1.05 mg bid and remained on this dosage and on a regular diet for 3 months. The treatment reduced HGA by 95% but the tyrosine levels increased to 755 +/- 167 $\mu\text{mol}/\text{L}$. These levels decreased to 603 +/- 114 $\mu\text{mol}/\text{L}$ when the patients took a protein-restricted diet [16]. Consecutively to this study, the dosage of 2 mg per day was used in the 3-year

randomized trial [17] and in the most recent paper showing a decrease rate of progression of alkaptonuria [5]. In this last report, Ranganath et al described three proven cases of corneal tyrosine keratopathy, two of them being symptomatic. These keratopathies were related to the increased tyrosine levels which can exceed 900 $\mu\text{mol/L}$ [5]. Consecutively, they recommended to decrease the protein intake in the patients with too high tyrosine levels, and even to implement a phenylalanine/tyrosine free amino acids mixture (used in the treatment of tyrosinemia type I) in patients who have tyrosine levels above 900 $\mu\text{mol/L}$ even if the compliance to a low protein diet by the patients remains a challenge for the treatment efficacy [18].

In our patients, the diagnoses were confirmed between 3 and 5 years of age. Excretion of HGA appears to be independent of age and is highly variable from patient to patient. As in many other metabolic disorders, there is a continuum between mild and severe forms of AKU. This continuum is clearly shown in our patients, as patient 1 (an adult) presents a mild AKU with a moderate increase of HGA in urines (1000 mmol/mol creatinine), while patient 3 (a child) exhibits a very high excretion of HGA in urines (27400 mmol/mol creatinine). HGA values were not related to age, as HGA rarely exceeded 1500 mmol/mol creatinine during infancy in patient 1 (who was treated by vitamin C).

The efficacy of treatment in patient 1 suggests that a dosage of 0.2 mg per day is sufficient to normalize HGA levels in mild patients without excessive increase of tyrosine levels. However, we wish to emphasize that this patient is very compliant with the diet (EFSA recommendations, 0.6 to 0.8 g protein/kg/day for an adult), which could explain the low HGA levels. Such adherence to the diet is a challenge for patients, but it is a key factor to reach the goals of AKU treatment with nitisinone.

In our two other patients, the very low dosage of nitisinone allowed a more than 93 % decrease of HGA in urines, but it does not mean that this will be sufficient to avoid any clinical signs later on. The published studies have been done in older patients, in whom the joints disease was already present. About 50% of patients have joint pain after 30 years, 50% undergo arthroplasty before age 55 and 50% have coronary artery calcification after age 59 [5]. Ascorbic acid, low protein diet and physiotherapy have been tried but do not alter significantly the underlying metabolic defect [4]. As AKU is a progressive storage disease, the rule used in lysosomal storage disease: “treat early to avoid further clinical signs” should be apply to AKU. Our patient 3, who have a very high level of HGA excretion already showed (as seen in the teeth) that the deposition of HGA began in infancy. We decided to treat her at 3 years to avoid the development of the disease during infancy and adolescence. The presence of disk diseases before 30 years (Figures 1 and 3) in our two adult patients (even patient 1 who exhibits a moderate excretion of HGA) is also an argument for treating early these patients. We believe that early treatment with very low dose NTBC can significantly decrease ochronosis deposits and

significantly delay (and even avoid) the age of onset of joint and systemic symptoms. The severity of the disease cannot be used to determine who should be treated early as this severity depends of multiple factors i.e. genotype, protein intake and perhaps of some other still unknown factors. The MRI of the spine our 2 adult patients showed disks disease which are more likely to be due to AKU. Hence, starting NTBC treatment during childhood or adolescence, before the onset of articular diseases, seems critical to strongly reduce the probability of spine disorders in young adults. A longitudinal study would be of interest to better assess the efficacy of an early NTBC treatment on the reduction of AKU-induced articular diseases. Serum amyloid A, chitotriosidase activity and protein thiolation index have been recently proposed as AKU biomarkers[19] and could be used to determine the evolution of the disease under nitisinone treatment.

The protein intake control must be part of the management of AKU. Although it was not described as sufficient to treat AKU [4], it is a crucial part of the management of patients treated by nitisinone as it influences the level of HGA excretion. Moreover, it will also influence tyrosine levels, which is also susceptible to raise in the case where nitisinone dosage has to be increased. Therefore, we think it is important to closely follow-up the AKU patients with a routine nutritional monitoring by a dietician, and with blood parameters, if necessary.

In patients 2 and 3, the increase of protein intake (increase of global foods intake during the third trimester of pregnancy in patient 2, and relaxation of the diet in patient 3) induced an increase in HGA excretion. However, this did not significantly modify the tyrosine levels in these two patients. These data need to be confirmed in more patients. Nevertheless, the increase of HGA excretion with higher protein intake is a critical point in the management of AKU, and compliance to the diet is probably critical to maintain the efficacy of nitisinone treatment. These elements are fully integrated in the follow-up of other metabolic disorders, such as phenylketonuria or tyrosinemia type 1 [20, 21].

The remaining question is the level of HGA which could allow to avoid clinical symptoms of AKU. In our view, expressing the data of nitisinone efficacy in percentage of decrease is not sufficient, as even if this percentage of decrease is very high (table 1), the quantitative level of HGA remains variable with time, and remains dependent on the protein intake. In patient 3, despite a decrease of more than 95 %, HGA never decreased below 2000 mmol/mol creatinine, which is higher than the maximum HGA level of our patient 1 without treatment. The plan for this patient is to improve the low protein diet and to increase the dosage of nitisinone, if HGA excretion remains above 1000 mmol/mol creatinine. This therapeutic endpoint is arbitrary, and we need long term studies to determine the level of HGA excretion which will be sufficient to avoid the appearance of further clinical signs of AKU. As it will take a decade to obtain these data, we need a long-term registry to determine the best treatment protocol

by nitisinone in AKU. These recommendations should take into account the individual responsiveness to nitisinone, and determine the level of urinary HGA for an optimal outcome. As an example, in phenylketonuria, thanks to the long-term experience, the treatment recommendations are much stricter today than before[20]. In AKU, we are just at the beginning of the nitisinone treatment story, and we need to accumulate data to find the best treatment protocol for each patient.

We did not experience any side effects in our patients treated by very low dosage of nitisinone. Tyrosine levels rarely exceeded 500 $\mu\text{mol/L}$, and this parameter sounds to be a key factor in the patient care. Indeed, it is important to maintain low levels of tyrosine, especially during childhood with developing organs. European tyrosinemia type 1 guidelines recommend keeping plasma tyrosine concentrations in the 200 – 400 $\mu\text{mol/l}$ range, until the age of 12 years old. After this age, it is common to tolerate a modest increase in tyrosine concentrations (<500 $\mu\text{mol/l}$ is considered acceptable) [8]. Indeed, eye complications are rare with Tyrosine concentrations below 800 $\mu\text{mol/l}$ [8], but there are also concerns about the cognitive outcome if tyrosine levels exceed these levels during infancy [8, 21]. Noteworthy, tyrosine levels seem more related to the nitisinone dosage than to the protein intake, as described previously [4, 5, 17].

Altogether, these elements suggest that nitisinone should be prescribed in AKU, mainly depending on HGA levels and on protein intake, and must be individually adapted to the follow-up of the metabolic parameters.

On a safety point of view, NTBC has been used in tyrosinemia type I for more than 20 years, and has been a turnabout in patients' care, as the disease outcome changed from fatal in childhood to an extended lifespan[22]. In the Orfadin datasheet of FDA[23]+u, it is notice that the most adverse reactions were thrombocytopenia, leukopenia, porphyria and ocular/visual complaints associated with elevated tyrosine levels. None of these adverse events have been observed in our 3 patients. In tyrosinemia type I, the treatment is usually started in the first month of life at a dose of 1 mg/kg/day. This is far from the dosage used in our cases which corresponds to 2% of the normal dosage for tyrosinemia type 1 for the patient 3 (a child), and to less than 0.4% of the normal dosage for tyrosinemia type 1 for the adult patients. Although adverse neurological effects have been described upon using NTBC but they were more related to high tyrosine levels (> 500 $\mu\text{mol/L}$) than to nitisinone itself[21]. This was our main reason to carefully monitor and maintain tyrosine levels below 500 $\mu\text{mol/L}$ in our patients.

Dealing with the pregnancy of patients with inborn errors of metabolism is a challenge in current clinical practice, even though AKU has never been reported to be teratogenic. Only 2 pregnancies have been reported in patients with tyrosinemia type I treated by nitisinone (1 and 0.5mg/kg/day) without

deleterious effect on the offspring [24, 25]. Even though the possible teratogenicity of NTBC was reported in animals during pregnancy [6,7], these observations have been done at a much higher dosage (5 mg/kg/day) which is 2.5 fold the maximum recommended human dose of what is used to treat type I Tyrosinemia (Summary of product characteristics Orfadin; <http://www.ema.europa.eu>). Our patient 2 was already treated by nitisinone (0.2 mg/day) before her pregnancy. As we could not know the impact of stopping the NTBC treatment, begun before pregnancy, we proposed to continue the treatment. The discontinuation of NTBC could have caused a rebound in HGA levels in our patient, and we could not extrapolate the maternal clinical consequences, in particular at the joints and cardiac level. Few data are available on discontinuation of NTBC in case of tyrosinemia[26] (appearance of neurologic crises), and none in the case of AKU.

Considering the good outcome of the two previous reports and that she received a very low dosage of nitisinone, the patient gave her consent to continue the treatment and she gave birth to a normal baby boy. We observed an increased HGA levels in our patient during the third trimester which is most probably related to the increase in protein intake which happens during the last months of pregnancy [27]. Teratogenic effect of high tyrosine levels had been published in tyrosinemia type II [15]. This is why we did a monthly metabolic follow-up and the tyrosine level never exceeded 500 $\mu\text{mol/L}$ (364 \pm 104 $\mu\text{mol/L}$) during the whole pregnancy[8]. The child is actually aged of 2 years. Concomitantly with the study of Vanclooster[14], the psychomotor development of the child is fully within the norms and provides an example of the absence of teratogenic effects of NTBC at this dosage.

Last point, as our patients took a low protein-diet, a yearly nutritional follow-up is required to avoid any micronutrient deficiency. Because of this systematic follow-up, we diagnosed a mild cobalamin deficiency in patient 1 which was then supplemented. This nutritional follow-up must be done systematically to avoid any micronutrients deficiency as it is recommended in PKU for example [20].

Conclusion

We successfully treated few young AKU patients with very-low dose of NTBC (10% of the present recommended dose) while the tyrosine levels remained below 500 $\mu\text{mol/L}$, and without any complication. We propose that nitisinone treatment should be started with this dosage, and then be adapted to every patient in function of his individual evolution. Moreover, these case reports suggested that the protein intake is also a critical parameter to achieve a good metabolic control without the risk of clinical complication (mainly keratopathy), due to excessive level of plasma tyrosine. Finally, we also described the first pregnancy in an AKU patient treated by nitisinone, with a constant efficacy on the metabolic control and a normal outcome for the offspring.

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Figure 1: Spine MRI of patient 1



Legend:

MR examination in a 27 years old man with alcaptonuria. (a, b) Sagittal T1-w.i and T2-w.i with fat saturation showing the integrity of the cervical spine. (c, d) Sagittal T1-w.i and T2-w.i with fat saturation showing a disc degeneration at the T11-T12 level characterized by a collapsed disc space and the loss of the normal appearance of the disc (arrow).

Figure 2: Evolution of urinary HGA, plasma tyrosine and protein intake in patient 1

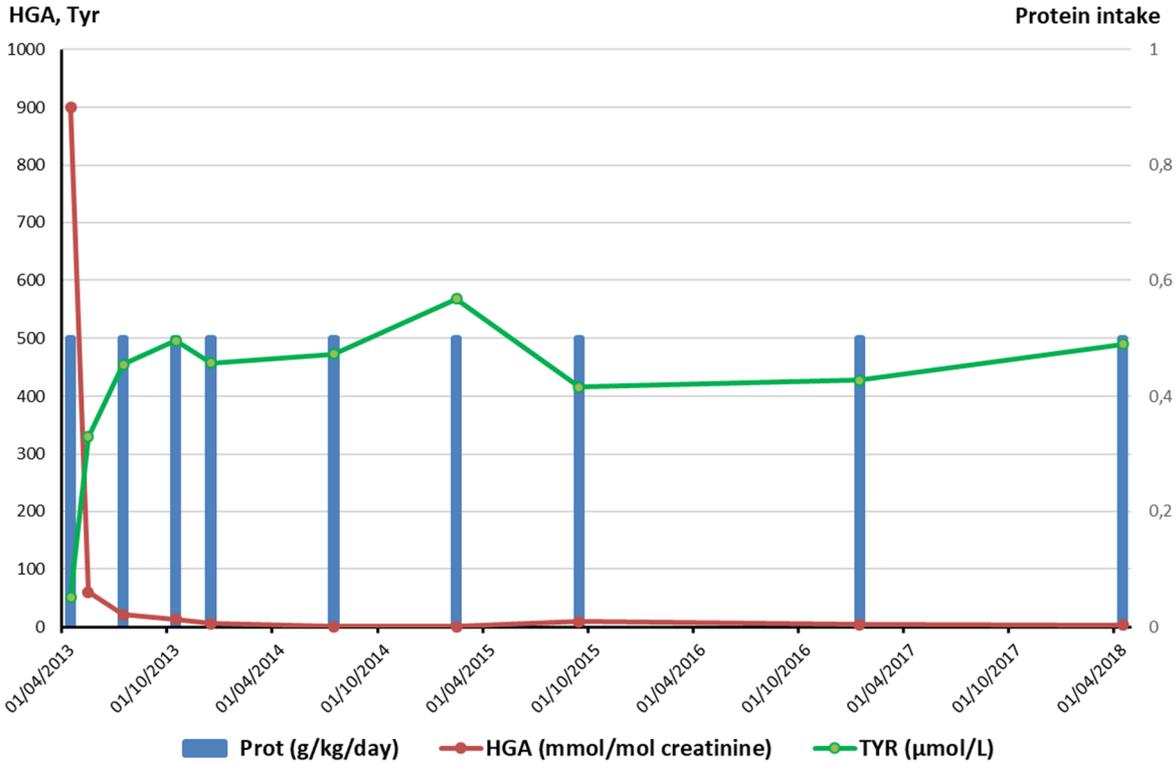


Figure 3: Spine MRI of patient 2



Legend: MR examination in a 34 years old female patient with alcaptonuria. (a, b) Sagittal T2-w.i with fat saturation showing multiple discs alteration with bulging discs at the thoracic level (arrows) and a L5-S1 disc degeneration.

Figure 5: Black inclusion in dental caries related to baby bottle syndrome [patient 3]



Figure 6: Evolution of urinary HGA, plasma tyrosine and protein intake in patient 3

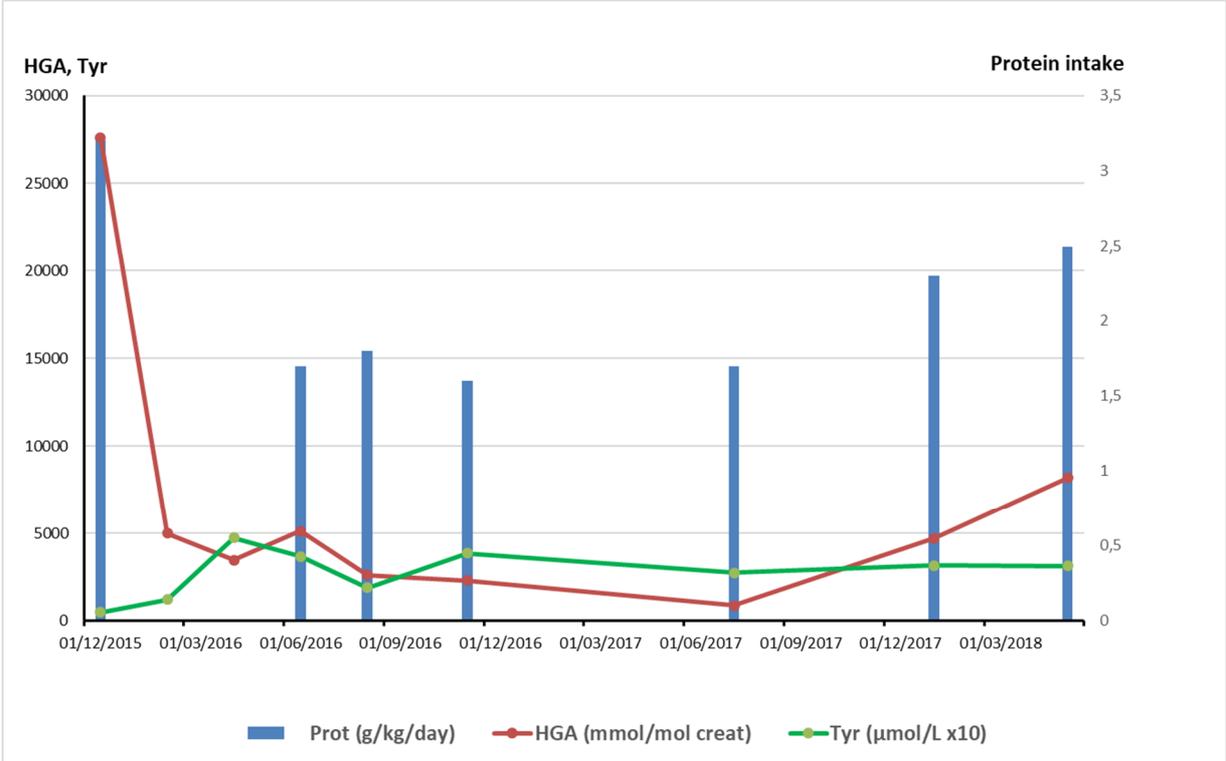


Table 1

Characteristics of the three patients

	Gender	Age at beginning of treatment (y)	Follow -up (y)	Maximum HGA level*	Minimum HGA level*	Maximum decrease (%)	Tyrosine** mean+/-SD	Maximum Tyr level**	Protein intake (g/kg/day)
Patient 1	M	21	5	1211	3	99,75	416 +/- 142	568	0.5
Patient 2	F	28	5	6472	397	93,87	374 +/- 157	585	1.1
Patient 3	F	3	3	27624	882	96,81	305 +/- 112	385	1.6 - 2.5

* HGA is expressed in mmol/mol creatinine, ** Tyrosine is expressed in $\mu\text{mol/L}$