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Arginine to ornithine ratio as a diagnostic marker in patients with positive newborn screening for hyperargininemia

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ABSTRACT

Arginase deficiency is a rare inborn error of metabolism that interrupts the final step of the urea cycle. Untreated individuals often present with episodic hyperammonemia, developmental delay, cognitive impairment, and spasticity in early childhood. The newborn screening (NBS) algorithms for arginase deficiency vary between individual states in the US but often include hyperargininemia and elevated arginine to ornithine (Arg/Orn) ratio. Here, we report 14 arginase deficiency cases, including two patients with positive NBS for hyperargininemia in whom the diagnosis of arginase deficiency was delayed owing to normal or near normal plasma arginine levels on follow-up testing. To improve the detection capability for arginase deficiency, we evaluated plasma Arg/Orn ratio as a secondary diagnostic marker in positive NBS cases for hyperargininemia. We found that plasma Arg/Orn ratio combined with plasma arginine was a better marker than plasma arginine alone to differentiate patients with arginase deficiency from unaffected newborns. In fact, elevated plasma arginine in combination with an Arg/Orn ratio of $\geq$1.4 identified all 14 arginase deficiency cases. In addition, we examined the impact of age on plasma arginine and ornithine levels. Plasma arginine increased 0.94 μmol/L/day while ornithine was essentially unchanged in the first 31 days of life, which resulted in a similar increasing trend for the Arg/Orn ratio (0.01/day). This study demonstrated that plasma Arg/Orn ratio as a secondary diagnostic marker improved the detection capability for arginase deficiency in newborns with hyperargininemia, which will allow timely detection of arginase deficiency and hence initiation of treatment before developing symptoms.

Abbreviations: NBS, newborn screening; Arg, arginine; Orn, ornithine; Arg/Orn, arginine to ornithine ratio; DOL, day of life; DBS, dry bloodspot; ROC, receiver operating characteristic.

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1. Introduction

Arginase, sometimes referred to as arginase-1 (E.C·C 207800), is the final enzyme in the urea cycle, a six-enzyme, two-transporter pathway responsible for the detoxification of ammonia in the body and its conversion to urea [1]. The inherited deficiency of arginase has been shown to cause a unique syndrome, the hallmarks of which are high plasma arginine (Arg) levels, low to absent (<1% of normal) arginase activity in red blood cells, progressive spasticity, and slowing and eventual loss of cognitive milestones [2]. Current standard treatment for arginase deficiency includes lowering plasma arginine levels by dietary protein limitation, essential amino acid supplementation, and nitrogen scavengers which may have a favorable outcome both in preventing the progression of symptoms and partial reversal of some symptoms [2,3]. Moreover, enzyme replacement therapy and various DNA and RNA therapies are in clinical or preclinical development [4]. Thus, it is essential to identify patients presymptomatically and treat cases as early as possible. Most importantly, cases should not be missed. The advent of expanded newborn screening (NBS) has enabled the early diagnosis of arginase deficiency in many patients with the condition in countries with this program. Screening has allowed patients to be identified prior to the development of symptoms [5]. In the US, the U.S. Recommended Uniform Screening Panel included hyperargininemia as a secondary target for newborn screening. The primary marker for screening is the arginine level.

Arginine is a conditionally essential amino acid that plays an important quantitative and qualitative role in a number of biological pathways. It is a precursor for nitric oxide, polyamines, creatine and possibly glutamate and proline, especially in the postnatal period. The plasma arginine (Arg) level is influenced by dietary intake, endogenous synthesis both within and independent of the urea cycle, and protein turnover [6]. Given that plasma Arg level could be normal in newborns with arginase deficiency, it is necessary to utilize a secondary marker to improve test sensitivity for arginase deficiency. We have demonstrated that newborn screening can effectively and efficiently identify newborns with arginase deficiency. The use of the ratio of arginine to ornithine (Arg/Orn) or to the product of phenylalanine×leucine as secondary markers will ascertain virtually all affected individuals, with an acceptable false positive rate [5]. The challenge now is to be equally effective with clinical confirmation and treatment.

Here, we report two patients who were positive for arginase deficiency on newborn screening but who received delayed diagnoses because follow-up testing indicated normal or near normal plasma arginine levels. By incorporating the plasma Arg/Orn ratio as a secondary diagnostic marker, these two patients would have received diagnoses earlier, and the symptoms of arginase deficiency in one patient could have been prevented or lessened. An algorithm was developed using the data from 12 other patients who received a diagnosis of arginase deficiency during the newborn period and the unselected newborn population. We also showed that plasma arginine (Arg) and ornithine (Orn) levels, and the Arg/Orn ratio were relatively stable over the first month of life, obviating the need to stratify the control data.

2. Methods

2.1. Study design

In this study, we aimed to evaluate the plasma Arg/Orn ratio as a secondary diagnostic marker in newborns with positive NBS for hyperargininemia. A study request email was sent to Metab-1, an electronic mailing list for professionals in the field of inborn errors of metabolism. Cases with a confirmed diagnosis of arginase deficiency by molecular analysis, arginase activity assay in red blood cells or both were collected from respondents. The plasma Arg, Orn, and Arg/Orn ratio from these cases were analyzed and compared to those in newborns without arginase deficiency in the Quest Diagnostics database. In addition, we evaluated the correlation between age in days and plasma amino acid levels for Arg, Orn, and the Arg/Orn ratio in the unselected newborn population. The institutional review board at UCLA reviewed the study protocol and granted permission for this study.

2.2. Data collection

Patient data and demographics were collected from care providers with all protected health information removed. The results of NBS and plasma Arg, Orn, and Arg/Orn ratio levels from 14 individuals with arginase deficiency were collected for this study. The plasma Arg and Orn levels from individuals without a confirmed diagnosis of arginase deficiency between 0 and 31 days of life were acquired from the Quest Diagnostics (San Juan Capistrano, CA USA) Biochemical Genetics Laboratory database, which included 6587 samples over 6 years spanning January 2013 to December 2019.

2.3. Data analysis

The plasma Arg, Orn, and Arg/Orn levels from the 14 cases with arginase deficiency were compared to the distributions from newborns without arginase deficiency. The 97.5th percentile of this population was determined and McNemar's test was used to compare the number of cases below the 97.5th percentile between plasma arginine and Arg/Orn ratio [7]. The area under the receiver operating characteristic curves (AUC) formed using Arg and Arg/Orn ratio to discriminate between those with and without arginase deficiency were also compared by Delong's test to compare two correlated ROC curves [8]. The relationships between age and plasma Arg, Orn, and Arg/orn ratio were analyzed by linear regression. The p value <0.05 was considered statistically significant. Analysis was performed using R software version 3.4.3 [9].

3. Results

3.1. Delayed diagnosis of arginase deficiency owing to normal plasma arginine level

Patient 1 was a 4-year-old male who was born at full term following an uncomplicated pregnancy. An NBS sample collected at 17 h of age was positive for hyperargininemia (101 μmol/L, cutoff <50 μmol/L) and elevated Arg/Orn ratio (10.1, cutoff <1.4). Confirmatory tests including plasma amino acids, ammonia, and comprehensive metabolic panel were sent on day of life (DOL) 5. The plasma Arg level was within the normal range (134 μmol/L, normal range 14–135 μmol/L). He had a normal history and examination at the follow-up clinic visit. The patient was discharged with a false-positive newborn screening result for hyperargininemia. After a period of normal development, the patient developed bilateral lower extremity spasticity at 18 months of age. Plasma Arg was markedly elevated at 587 μmol/L (normal range 30–147 μmol/L). Results of an RBC arginase enzyme activity assay confirmed arginase deficiency with undetectable enzyme activity. Since the diagnosis, the patient has been on protein restriction with nonessential amino acid-free formula and has shown improvement in development and spasticity.

Patient 2 was a 4-year-old male who was born at 38 weeks following an uncomplicated pregnancy. NBS performed at 25 h of life showed elevated arginine (68 μmol/L, cut-off <50 μmol/L) and Arg/Orn ratio 4.4 (cut-off <1.4). Plasma amino acid performed on DOL5 showed mildly elevated arginine (150 μmol/L, normal range 14–135 μmol/L) with normal ammonia level. Repeated plasma amino acid levels were mildly elevated for arginine. The child continued to have normal growth and development. Subsequently, RBC arginase enzyme activity assay and plasma amino acid were performed simultaneously when the patient was 7-month-old. Plasma arginine level was normal (113 μmol/L, normal range 12–133 μmol/L) while RBC arginase enzyme activity was

undetectable, confirming arginase deficiency. The patient was followed in a clinic monthly. Plasma amino acids were also monitored monthly, and treatment was initiated following an arginine level of 402 μmol/L (normal range 30–147 μmol/L) at 9 months of age. With protein restriction and glycerol phenylbutyrate (Ravicti®) treatment, he continues to have a normal neurologic exam, though is noted for speech delay.

3.2. Arginine to ornithine ratio as a secondary marker for arginase deficiency

Given that plasma Arg levels can be normal in patients with arginase deficiency during the neonatal period, it is clearly necessary to add a secondary marker to improve the diagnostic sensitivity in NBS cases positive for hyperargininemia and arginase deficiency. The Arg/Orn ratio and other ratios have been popular second-tier discriminators used in NBS to reduce the number of false positive cases. Therefore, we collected the plasma Arg, Orn, and Arg/Orn ratio data on their initial NBS follow-up from 14 arginase deficiency patients identified by screening in the newborn period (Table 1). We found that all cases of arginase deficiency had an Arg/Orn ratio equal to or greater than 1.7 (range 1.7–16.0). Interestingly, the two cases with delayed diagnoses have the lowest plasma arginine levels as well as the lowest Arg/Orn ratios of the group. To compare the validity of using the plasma Arg level and the Arg/Orn ratio to discriminate true positives from false positive cases, we evaluated the distribution of Arg and Arg/Orn ratio in neonates with and without arginase deficiency. Data from the unselected newborn population revealed plasma Arg levels ranging from 10 to 191 μmol/L (2.5–97.5%tile), Orn levels from 27 to 312 μmol/L (2.5–97.5% tile), and Arg/Orn ratios from 0.1–1.6 (2.5–97.5%tile). Four of 14 (29%) of patients with arginase deficiency had an initial plasma Arg level (range 134–192 μmol/L) below or slightly above the 97.5th percentile (191 μmol/L) in unselected newborn population, while all 4 patients had an Arg/Orn ratio below the 97.5th percentile (ratio 1.6), which was a statistically significant difference ($p = 0.046$, Fig. 1). In addition, discrimination of newborns with arginase deficiency and unselected newborn population by ROC curve analysis was better with Arg/Orn ratio than with arginine alone (AUC = 0.998 vs. 0.980, respectively; p-value = 0.005).

3.3. Trend of plasma arginine and ornithine levels in neonatal period

A previous study suggests that plasma Arg levels change with age in children [10], which could have an impact on the diagnosis of arginase deficiency. To examine the age-related change in plasma Arg levels, we analyzed plasma Arg levels of 6587 unselected newborn population (0–31 days) obtained from Quest Diagnostics database (Fig. 2). We found that plasma Arg levels increased with age, averaging 0.94 μmol/L per day in the first 31 days of life. Plasma Orn levels, in contrast, were essentially unchanged during the neonatal period (−0.02 μmol/L per day). As a result, the Arg/Orn ratio showed a similar trend of increasing with age (0.01 per day) as the Arg alone.

4. Discussion

Arginase deficiency is a rare urea cycle disorder with an estimated minimal incidence of 1 in 1.1 million newborns in the United States [5]. It is a treatable disorder for which newborn screening and early diagnosis are now recognized as a high priority. In our previous study published in 2017 [5], we demonstrated a NBS algorithm for arginase deficiency, in which Arg in combination with Arg/Orn ratio can identify all affected individuals with a relatively low false-positive rate. To date, hyperargininemia in combination with a secondary discriminator such as Arg/Orn ratio is the most commonly used algorithm that has identified virtually all arginase deficiency newborns in screened patients [5]. However, our data showed the plasma arginine levels in newborns with arginase deficiency have significant overlap with that from the newborns without arginase deficiency. This is an important challenge in the diagnosis of arginase deficiency following a positive NBS and has led to the delayed diagnosis in the two patients reported in this study. The delayed diagnosis resulted in developmental delay, cognitive impairment, and spasticity in one patient, which could have been prevented or lessened by dietary modification if the NBS findings had been confirmed in the neonatal period.

The Arg/Orn ratio has been widely used as a second-tier discriminator in NBS for hyperargininemia and has been very successful in discriminating the affected from the unaffected in NBS. The NBS cutoff for Arg/Orn ratio ranges from 0.45 to 1.5 between the states in the US [5]. Interestingly, the two cases with delayed diagnosis of arginase deficiency in our study have the lowest plasma arginine levels (134 and 150 μmol/L) as well as the lowest plasma Arg/Orn ratios (2.2 and 1.7) on the initial NBS follow-up when compared to other arginase deficiency cases (2.9 to 16.0). At least for the small set of case study, the findings demonstrated that the Arg/Orn ratio could provide additional discriminating power to distinguish mild Arginase deficiency cases from normal newborns.

All 14 cases of arginase deficiency would be identified if an Arg/Orn ratio ≥ 1.7 was used as a secondary diagnostic marker. Since the number of newborns with arginase deficiency included in this study is limited, it is possible to have a case with an Arg/Orn ratio lower than 1.7, although it is unlikely to be much lower based on our experience in NBS. We suggest continued use of the NBS algorithm for arginase deficiency outlined in the paper by Therrell et al. [5]. With this approach, screen-positive patients will have a high probability of being true positive for arginase deficiency. In the follow-up confirmatory test, we recommend

Table 1
Plasma and NBS Arg, Orn, and Arg/Orn ratio in patients with arginase deficiency.

	Plasma					NBS		
Patient	Arginine (μmol/L)	Ornithine (μmol/L)	Arg/Orn (μmol/L)	Range (μmol/L)	DOL	Arginine (μmol/L)	Ornithine (μmol/L)	Arg/Orn (μmol/L)
1[a]	134	60	2.2	14-135	5	101	10	10.1
2[a]	150	86	1.7	14-135	5	68	16	4.3
3	263	91	2.9	14–135	15	188	46	4.1
4	192	60	3.2	14–135	16	138	26	5.3
5	268	67	4	14–135	12	351	N/A	N/A
6	179	43	4.2	6–140	2	100	29	3.4
7	233	51	4.6	N/A	N/A	261	N/A	N/A
8	299	63	4.7	6–140	5	177	22	8
9	204	42	4.9	15–160	6	248	17	15
10	282	51	5.5	N/A	N/A	233	N/A	N/A
11	881	110	8	N/A	3	377	16	22.9
12	259	32	8	N/A	6	137	9	16.1
13	528	56	9.4	20–148	7	242	N/A	N/A
14	930	58	16	14–135	7	218	N/A	N/A

[a] Cases with delayed diagnosis of arginase deficiency.

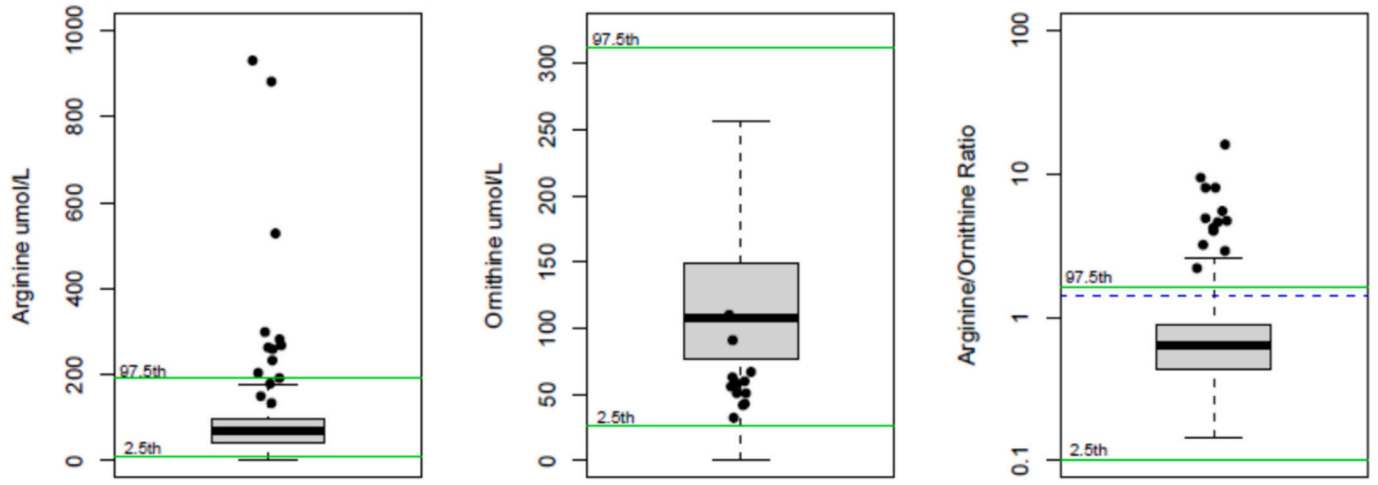

Fig. 1. The distribution of Arg, Orn, and Arg/Orn ratio in newborns.
The blue dotted line represents an Arg/Orn ratio of 1.4. The distribution of unselected newborns is represented by the box and whiskers while the points represent the cases with arginine deficiency.

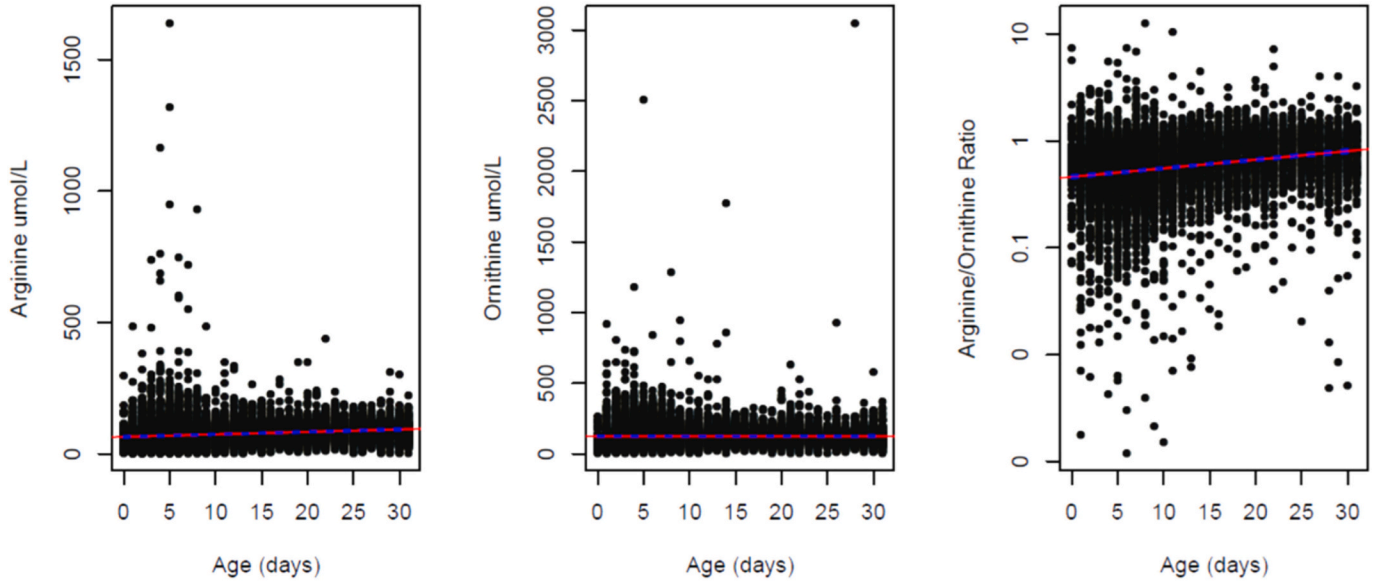

Fig. 2. The distribution of Arg, Orn, and Arg/Orn ratio during the neonatal period.
The colored line represents the trend of respective amino acids over time.

using the plasma Arg/Orn ratio $\geq$ 1.4 as the cutoff to confirm the diagnosis of arginase deficiency, which is approximately the 96th percentile among unselected population (Fig. 3). A ratio of $\geq$1.4 is also the cutoff for the DBS Arg/Orn ratio used in the California NBS program [5]. However, no referred patient should be discharged from care without a normal RBC arginase activity level or absence of *ARG1* gene mutation, because of the potential overlap with normal values and the high prior probability from the NBS algorithm. Conversely, an Arg/Orn ratio above 1.4 alone is not a valid criterion for suspecting arginase deficiency in patients who had a negative NBS or a normal plasma arginine level.

In our efforts to determine the appropriate approach to ensure accurate confirmation of arginase deficiency following a positive screening, we also established the normal values of Arg and Orn in the newborn period in a large dataset of more than 6000 newborns. The analysis demonstrated that the arginine and Arg/Orn values change slightly during the first 31 days of life whereas no significant change of ornithine values occurs, suggesting an age-related adjustment of reference range is not necessary for the neonatal period.

Author contributions

Y.H., R.S., A.F., D.W., S.C., F.L.L., P.T., D·S. were involved in conception, study design, data collection, interpretation, and manuscript preparation. C.L., I.S., R.S., J.N., S.S.B., K.J. were involved in data collection and manuscript review. C.M.R. provided statistical analysis. Y.H. and P.T. take responsibility for the collection of data, the analyses, interpretation, and publication. All authors have given approval for publication of this manuscript.

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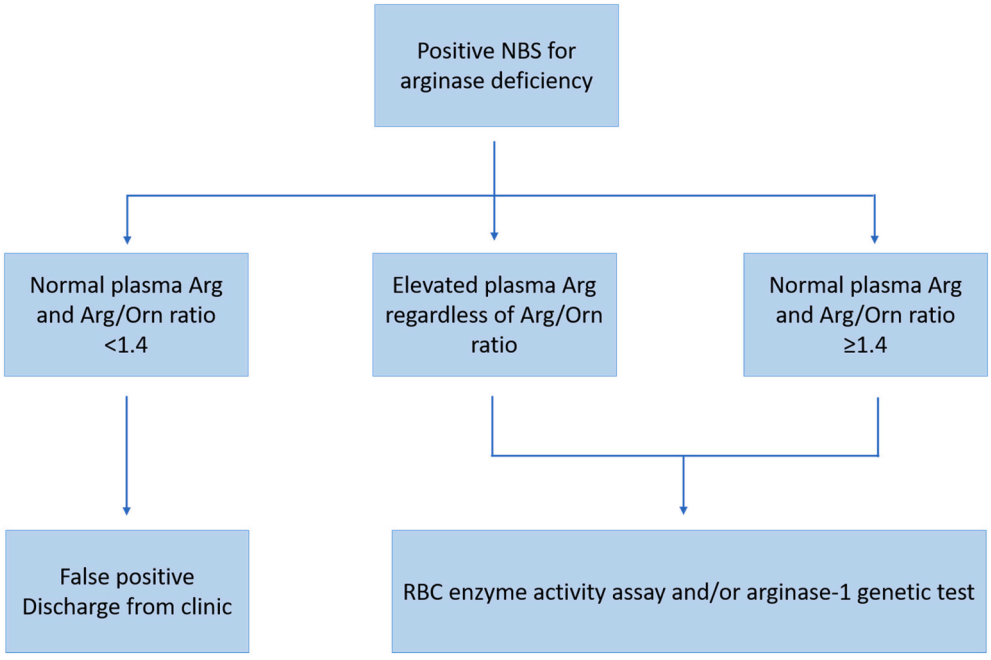

Fig. 3. Proposed algorithm for arginase deficiency workup following a positive NBS.

Ethics statement

This study involved retrospective analysis of existing patient data that were collected without patient identifiers. This study was reviewed by the institutional review board at UCLA with permission to proceed granted.

Declaration of Competing Interest

P.T, F.L.L, D.S, R.S, and C.M.R are employee of Quest Diagnostics. Other authors declare no potential conflicts of interests. None of the authors have financial gain or loss from the results of this study.

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