University of Groningen

Assessment of carnitine excretion and its ratio to plasma free carnitine as a biomarker for primary carnitine deficiency in newborns

Crefcoeur, Loek L.; Heiner-Fokkema, M. Rebecca; Maase, Rose E.; Visser, Gepke; de Sain-van der Velden, Monique G.M.

Link to publication in University of Groningen/UMCG research database

RESEARCH REPORT

JIMD REPORTS SSIEM   WILEY

Assessment of carnitine excretion and its ratio to plasma free carnitine as a biomarker for primary carnitine deficiency in newborns

Loek L. Crefcoeur[1,2] | M. Rebecca Heiner-Fokkema[3] | Rose E. Maase[4] |
Gepke Visser[1,2] | Monique G. M. de Sain-van der Velden[5]

[1]Department of Metabolic Diseases, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands

[2]Division of Metabolic Diseases, Amsterdam Gastroenterology and Metabolism, Emma Children's Hospital, Amsterdam UMC, Location University of Amsterdam, Amsterdam, The Netherlands

[3]Department of Laboratory Medicine, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

[4]Department Biologicals, Screening and Innovation, Dutch National Institute for Public Health and the Environment, Bilthoven, The Netherlands

[5]Section Metabolic Diagnostics, Department of Genetics, University Medical Centre Utrecht, Utrecht, The Netherlands

Correspondence
Monique G. M. de Sain-van der Velden, Section Metabolic Diagnostics, Department of Genetics, University Medical Centre Utrecht, Utrecht, The Netherlands.
Email: m.g.desain@umcutrecht.nl

Abstract

In the Netherlands, newborns are referred by the newborn screening (NBS) Program when a low free carnitine (C0) concentration (<5 μmol/l) is detected in their NBS dried blood spot. This leads to ~85% false positive referrals who all need an invasive, expensive and lengthy evaluation. We investigated whether a ratio of urine C0 / plasma C0 (Ratio$_{U:P}$) can improve the follow-up protocol for primary carnitine deficiency (PCD). A retrospective study was performed in all Dutch metabolic centres, using samples from newborns and mothers referred by NBS due to low C0 concentration. Samples were included when C0 excretion and plasma C0 concentration were sampled on the same day. Ratio$_{U:P}$ was calculated as (urine C0 [μmol/mmol creatinine])/(plasma C0 [μmol/l]). Data were available for 59 patients with genetically confirmed PCD and 68 individuals without PCD. The Ratio$_{U:P}$ in PCD patients was significantly higher (p value < 0.001) than in those without PCD, median [IQR], respectively: 3.4 [1.2–9.5], 0.4 [0.3–0.8], area under the curve (AUC) 0.837. Classified for age (up to 1 month) and without carnitine suppletion (PCD; $N = 12$, Non-PCD; $N = 40$), medians were 6.20 [4.4–8.8] and 0.37 [0.24–0.56], respectively. The AUC for Ratio$_{U:P}$ was 0.996 with a cut-off required for 100% sensitivity at 1.7 (yielding one false positive case). Ratio$_{U:P}$ accurately discriminates between positive and false positive newborn referrals for PCD by NBS. Ratio$_{U:P}$ is less effective as a discriminative tool for PCD in adults and for individuals that receive carnitine suppletion.

KEYWORDS
carnitine, FCE, newborn screening, PCD, plasma, ratio, urine

1 | INTRODUCTION

Acylcarnitines are valuable metabolites used in newborn screening (NBS), as abnormal plasma acylcarnitine profiles are indicative of several inborn errors of metabolism (IEM).[1,2] In case of carnitine depletion, theoretically, the measured concentration of other acylcarnitines may also be reduced, which in turn could give rise to false negative reporting.[3,4] C0 is therefore considered essential for reliable interpretation of the acylcarnitine profile. All newborns with a C0 concentration <5 μmol/l are referred to a paediatric metabolic specialist for evaluation.[5]

In the past 15 years, in the Netherlands ~15% of newborns referred for follow up of low C0 were diagnosed with primary carnitine deficiency (PCD; OMIM no. 212140). In the remaining 85% no underlying cause for the low C0 was identified and they were able to retain normal plasma C0 concentrations without carnitine supplementation. Furthermore, no other IEM was identified in any of these false positive referrals. Follow-up of these false-positive referrals is challenging, requiring a long period of confirmatory testing, which may include measurement of residual carnitine transporter activity in cultured fibroblasts and/or sequencing of the *SLC22A5* gene.[6] The prolonged uncertainty regarding the health of their newborn child during this extensive evaluation, can cause significant anxiety in the families concerned.[7] A faster exclusion of the diagnosis PCD may reduce harm by false-positive referral.

PCD is an autosomal recessive disorder caused by variations in the *SLC22A5* gene, encoding the Organic Cation Transporter Novel 2 (OCTN2) protein.[8,9] OCTN2 maintains intracellular C0 concentrations by transporting carnitine into cells, and reabsorbing C0 in the renal tubuli.[10–12] Defects in OCTN2 cause a decrease of plasma (and intracellular) C0 concentrations and an increased renal waste by inadequate tubular reabsorption.[10] This causes a shifted balance of plasma C0 versus C0 excretion in PCD patients, which can be determined by calculating the fractional carnitine excretion (FCE) as: ((urine C0 × plasma creatinine[Cr])/(plasma C0 × urine Cr)) × 100%. FCE has been shown to be increased in PCD patients, ranging from 3% to 190%, with proposed normal ranges of <4%. Whilst FCE may be a promising marker for early differentiation of healthy newborns and PCD-affected newborns, information on FCE is limited, as most case descriptions at the time of writing are derived from conference abstracts, with restricted descriptions of control groups, timing of sampling and circumstances at sampling (e.g., with or without carnitine supplementation).[13–17]

In the Netherlands, plasma Cr is not routinely measured in newborns referred for low C0. However, in most Dutch metabolic specialist centres, plasma C0, C0 excretion and urine Cr are measured upon referral. We investigated whether a ratio of C0 excretion to plasma C0 could be an effective diagnostic tool at the time of referral to improve and simplify the follow-up protocol for PCD.

2 | MATERIALS AND METHODS

2.1 | Study design

Urine and plasma C0 results were retrospectively collected. Inclusion criteria were: (1) C0 excretion and plasma C0 were sampled on the same day and (2) sampled individuals were referred after identification by NBS

TABLE 1 Baseline characteristics

	No PCD N = 68			PCD N = 59		
	N/Median (%)/[IQR]	Range		N/Median (%)/[IQR]	Range	p Value
Gender (male, N)	19 (27.9)			12 (20.3)		0.431
Referral						<0.001
Newborn (N)	54 (79.4)			27 (45.8)		
Maternal (N)	14 (20.6)			32 (54.2)		
Age at sampling						
Age (median, days)	13 [10–92.3]	7–13 400		9215 [53–12 319]	5–14 600	<0.001
Age <1 month (N)	40 (58.8)			15 (25.4)		<0.001
Plasma C0 concentration (median, μmol/l)	11.1 [8.6–17.7]	3.2–79		9.0 [6.7–22.0]	3.6–73.0	0.087
C0 excretion (median, μmol/mmol Cr)	5.0 [3.0–11.0]	0–1460		34.5 [9.6–127.6]	0.7–1520	<0.001
Ratio$_{U:P}$ (median)	0.4 [0.3–0.8]	0–31.6		3.4 [1.2–9.5]	0.06–31.0	<0.001
On carnitine suppletion (yes, N)	6 (8.8)			22 (37.3)		<0.001

Abbreviations: C0, free carnitine; Cr, creatinine; PCD, primary carnitine deficiency.

—WILEY

FIGURE 1 Violin plots and corresponding ROC-curves (with 95% confidence interval) for plasma C0, C0 excretion and Ratio$_{U:P}$ in (A) the complete dataset, (B) dataset classified for suppletion and (C) dataset classified for age at sampling. AUC, area under the curve; C0, carnitine; Cr, creatinine; PCD, primary carnitine deficiency; ROC, receiver operator characteristic
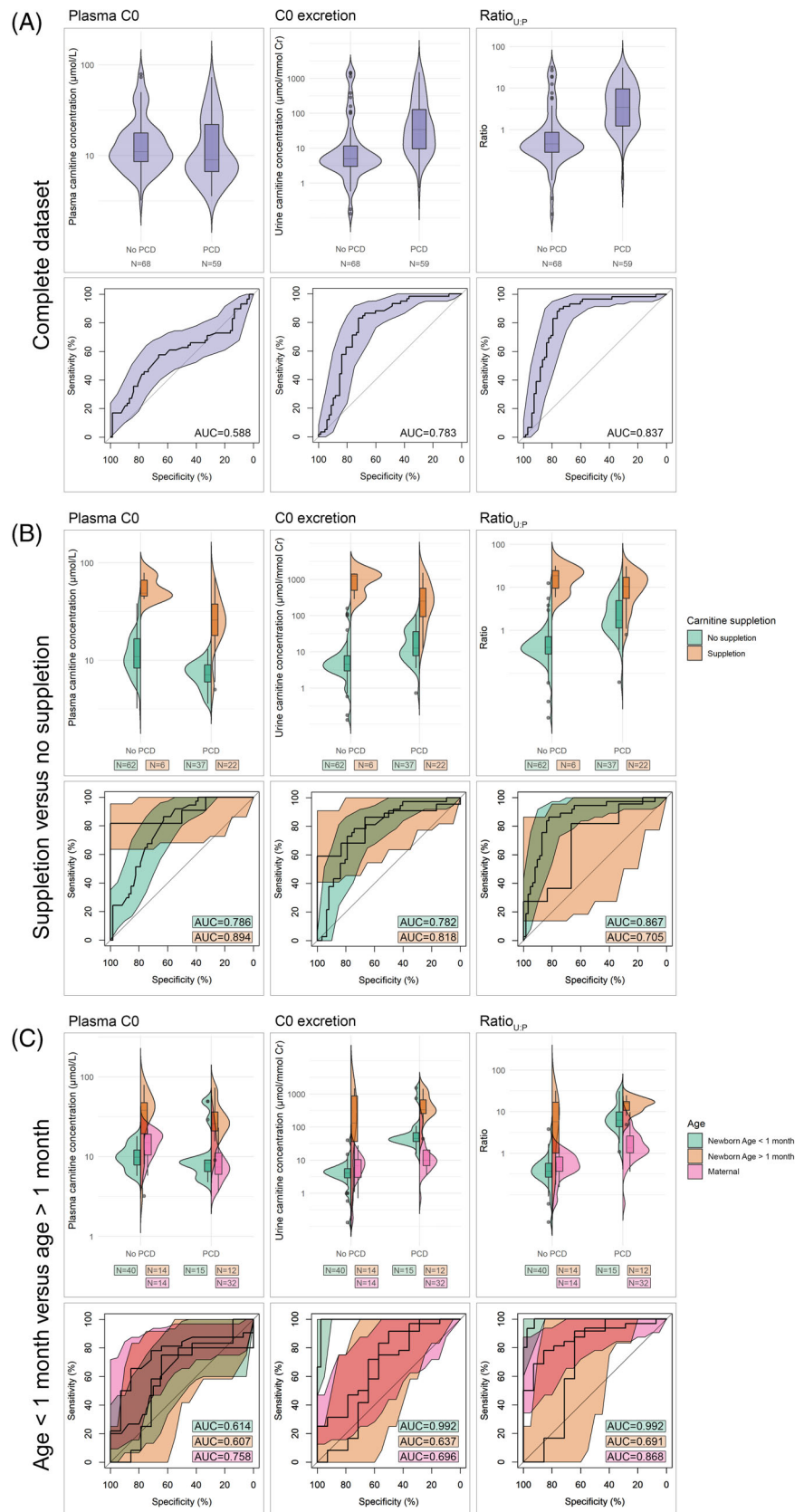
(newborns and mothers of newborns referred by NBS). The following data were collected: C0 and Cr excretion, plasma C0 concentration, age at sampling, sex, received

carnitine suppletion at the time of sampling and PCD diagnosis (confirmed genetically, presented in Table S1). Written informed consent was obtained from all
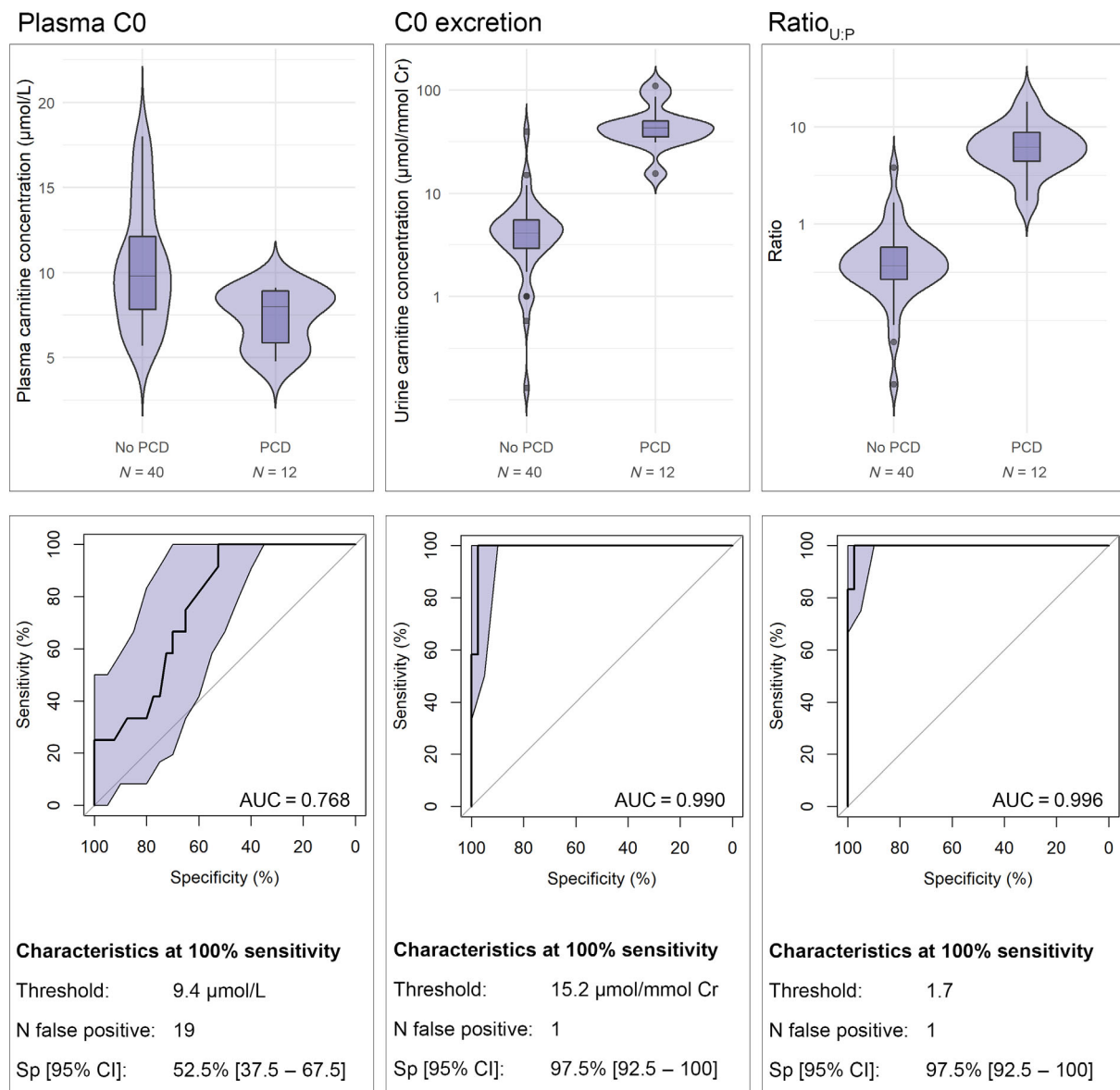
Age < 1 month and without suppletion

FIGURE 2 Violin plots and corresponding ROC-curves (with 95% CI) for plasma C0, C0 excretion and ratio$_{U:P}$ in newborns referred for low C0 in NBS, sampled before age 1 month and without carnitine suppletion at time of sampling. Provided with ROC analysis for each test are the diagnostic characteristics at the threshold for 100% sensitivity. AUC, area under the curve; C0, carnitine; CI, confidence interval; Cr, creatinine; NBS, newborn screening; PCD, primary carnitine deficiency; ROC, receiver operator characteristic; Sp, specificity

participants and/or their caregivers (METC protocol number 19-234/M). The study was conducted in accordance with the principles of the Declaration of Helsinki.

2.2 | Calculation of carnitine excretion in relation to plasma carnitine

The following equation was used to calculate the ratio of urine C0 to plasma C0 (Ratio$_{U:P}$):

$$\text{Ratio}_{U:P} = \frac{\text{Urine C0} \,(\mu\text{mol}/\text{mmol Cr})}{\text{Plasma C0} \,(\mu\text{mol}/\text{l})}$$

2.3 | Diagnostic accuracy analysis

Receiver operator characteristic (ROC) curve analysis was performed for plasma C0, C0 excretion and Ratio$_{U:P}$, with PCD as the classifier. ROC curve analysis was then repeated in data classified for carnitine suppletion (with

or without suppletion at time of sampling) and age (age at sampling up to 1 month and age at sampling above 1 month).

2.4 | Statistics

All analyses were performed using R (version 4.1.3, R Core Team, Vienna, Austria). DeLong's test was used to compare two ROC curves. The Mann–Whitney U-test was used to compare continuous data between PCD and non-PCD. The chi-squared test was used to compare two categorical data between two groups. Significance was assumed for $p < 0.05$.

3 | RESULTS

Baseline characteristics are provided in Table 1. In the non-PCD ($n = 68$) versus the PCD ($n = 59$) population, respectively, the median [interquartile range (IQR)] plasma C0 concentration was 11.1 [8.6–17.7] and 9.0 [6.7–22.0] μmol/l, median C0 excretion was 5.0 [3.0–11.0] and 34.5 [9.6–127.6] μmol/mmol Cr and median $Ratio_{U:P}$ was 0.4 [0.3–0.8] and 3.4 [1.2–9.5]. The non-PCD population contained more samples from newborns ($N = 54$ [79.4%] vs. PCD: $N = 27$ [45.8%]) and a lower age at sampling (13 days vs. PCD: 9215 days [25 years]). In the non-PCD population, six (8.8%) received carnitine suppletion at the time of sampling, versus 22 (37.3%) in the PCD population.

3.1 | Diagnostic accuracy of $Ratio_{U:P}$

Unclassified, area under the curve (AUC)'s [95%CI] for plasma C0, C0 excretion and $Ratio_{U:P}$ were: 0.588 [0.485–0.691], 0.783 [0.701–0.866] and 0.837 [0.763–0.912], respectively (Figure 1A). To further evaluate the accuracy of $Ratio_{U:P}$ in the target population (non-supplemented newborns), data were classified for carnitine suppletion (with or without suppletion; Figure 1B) as well as age (age ≤1 month and age >1 month; Figure 1C). AUC for $Ratio_{U:P}$ increased in an unsupplemented population (0.867 [0.790–0.943] vs. 0.705 [0.437–0.973]) and in a population with an age up to 1 month (0.992 [0.977–1] vs. 0.691 [0.464–0.917] for newborns sampled at age above 1 month and 0.868 [0.763–0.974] for maternal samples).

The final analysis, in an unsupplemented population with an age up to 1 month (median age at sampling: 11 days), demonstrated the following median [IQR] for non-PCD ($n = 40$) and PCD ($n = 12$), respectively: plasma C0 9.8 [7.8–12.1] and 8.0 [5.9–8.9] ($p = 0.005$), C0 excretion 4.0 [2.8–5.4] and 43.0 [35.1–50.2] ($p < 0.001$),

$Ratio_{U:P}$ 0.37 [0.24–0.56] and 6.2 [4.4–8.8] ($p < 0.001$). The AUC's [95% CI] for plasma C0, C0 excretion and $Ratio_{U:P}$ were respectively: 0.768 [0.634–0.901], 0.990 [0.968–1], 0.996 [0.986–1] (Figure 2). The corresponding thresholds for 100% sensitivity were 9.4 μmol/l, 15.2 μmol/mmol Cr, 1.7, respectively. The specificity [95% CI] at 100% sensitivity for C0 excretion and $Ratio_{U:P}$ both was 92% [92.5%–100%] (yielding one false positive).

4 | DISCUSSION

This study demonstrates that the ratio of C0 excretion to plasma C0 can be used as an early discriminative test for PCD in newborns referred by NBS. In our study population, applying a threshold of 1.7 the $Ratio_{U:P}$ yields 12 true positive cases, 1 false positive case and correctly identifies as negative 39 newborns that do not have PCD (median ratio in healthy newborns is 0.37). Using the $Ratio_{U:P}$ significantly decreases the duration of follow-up, cost and harm of false positive referrals by NBS.

PCD is implemented in several NBS programs across the world[1,18–20] and NBS for PCD is known to have a poor positive predictive value (PPV), ranging from 1.6% to 4.7%.[21,22] In part due to the high false positive rate, the benefit of screening for PCD remains in question.[22,23] The high false positive rate, in addition to the identification of many asymptomatic individuals, led to discontinuation of screening for PCD in New Zealand.[23] Detection of PCD following NBS in the Netherlands performs slightly better (PPV 14.6%), which might be due to a different NBS procedure. If C0 concentration is low in the DBS, a second NBS sample is taken and newborns are only referred when the low C0 concentration persists.[5] However, this algorithm comes at the cost of timeliness. In the Netherlands initial DBS's are obtained 72–168 h after birth and in the event a second sample is requested, there is a further delay of 2–3 days before a newborn is referred.[1,18–20] $Ratio_{U:P}$ does not improve the PPV of NBS for PCD, but it can mitigate the harm of the high amount of false positive referrals by quickly ruling out PCD.

The difference in relative carnitine excretion is less pronounced in supplemented individuals (Figure 1B). This is expected, as the plasma concentration threshold for tubular carnitine reabsorption is 40–60 μmol/l.[24] Therefore, supplemented healthy individuals could quickly increase their relative carnitine excretion, approaching values of PCD patients (with or without suppletion). Indeed, the median plasma C0 concentration of healthy supplemented individuals was 49 μmol/l (range 42.6–79), with a $Ratio_{U:P}$ of 18.8 (range 5.9–31.6; Table S2). Therefore, we recommend using the $Ratio_{U:P}$ as a test prior to initiating carnitine supplementation.

Ratio$_{U:P}$ is less effective as a discriminative test in a population above the age of 1 month (Figure 1C). It is important to note that many of the newborns in this category received carnitine suppletion when sampled (11/12 PCD and 6/14 non-PCD), and as discussed above, this may have confounded the results in this population. The other part of this population consists of mothers, sampled ~1–2 weeks after delivery ($N = 46$ out of 72). During the first trimester, glomerular filtration rate increases and slowly recovers 6–8 weeks after delivery.[25] This may cause an underestimation of Ratio$_{U:P}$ in PCD-affected mothers. Furthermore, maternal PCD cases identified through NBS likely have milder phenotypes, as many are reported to be completely asymptomatic at the time of diagnosis.[26–29] A milder defect may result in less pronounced renal C0 wasting, leading to a lower Ratio$_{U:P}$ (median Ratio$_{U:P}$ maternal PCD: 1.35, newborn PCD: 9.83; Table S3). Last, in general, with increasing age, the population becomes less homogenous, with more diverse renal functions, body masses and diets that may all differently impact carnitine homeostasis.

Thus far, only FCE has been investigated as a diagnostic parameter expressing relative carnitine excretion.[14–17] Theoretically, FCE may be superior to Ratio$_{U:P}$, as it corrects for Cr excretion by taking plasma Cr levels into account. However, for the purpose of fast exclusion of PCD, this correction is only beneficial (compared with Ratio$_{U:P}$) when Cr-excretion is increased due to elevated plasma levels of Cr—in that case, C0 excretion and Ratio$_{U:P}$ would appear low, whereas FCE would still be elevated, due to the high plasma Cr in the numerator of the equation. Causes for primarily elevated plasma Cr include increased muscle degradation (e.g., due to a myopathy) and strenuous exercise, which are extremely rare in newborns and are therefore unlikely to cause false negatives when using Ratio$_{U:P}$.[30] Other causes for Cr disturbances are renal insufficiency, where plasma Cr rises, as urine Cr decreases,[31] which would lead to a perceived increase of both FCE and Ratio$_{U:P}$ or glomerular hyperfiltration, where urine Cr is increased and plasma Cr decreases, which would lead to a perceived decrease of both FCE and Ratio$_{U:P}$.[25,32] Unfortunately, we could not compare FCE to Ratio$_{U:P}$ in our NBS population, as plasma Cr was often not available. Future research comparing FCE to Ratio$_{U:P}$ as a marker for PCD is required.

We acknowledge the limitations of using the ratio$_{U:P}$ as a discriminative tool for PCD. First, carnitine in urine is not routinely available for clinical decision makers and larger studies are necessary to validate the threshold. Finally, immaturity of renal absorption may result in decreased ability of kidneys to preserve carnitine.

Interestingly, solely C0 excretion can accurately discriminate PCD from false-positives in an unsupplemented, newborn population (with an AUC of 0.990). The added benefit of C0 excretion in NBS has been reported previously in a conference abstract by Gallant et al.,[33] demonstrating the following C0 excretion (nmol/mg Cr) (median [range]): PCD 401 [45–1030], false positives 28 [7–516]. They report increased accuracy when combining newborn plasma and urine C0 concentrations. As C0 excretion and plasma C0 are generally sampled simultaneously we would recommend using the ratio$_{U:P}$ rather than urine excretion values.

In conclusion, the ratio of urinary free carnitine over plasma free carnitine can effectively discriminate between true and false positive referrals from NBS for PCD reducing time to diagnosis and mitigating the negative effects of a false positive referral. The ratio is most effective in neonates under 1 month of age and prior to carnitine supplementation.

CONFLICT OF INTEREST

Loek Crefcoeur, Rebecca Heiner, Rose Maase, Gepke Visser and Monique de Sain declare no potential conflicts of interest.

DATA AVAILABILITY STATEMENT

Supporting data are made available on an online open data repository.

ETHICS STATEMENT

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (Medical Research Ethics Committee Utrecht; METC protocol number 19-234/M). Written informed consents of all included individuals were obtained.

ORCID

Loek L. Crefcoeur https://orcid.org/0000-0003-2057-5525

M. Rebecca Heiner-Fokkema https://orcid.org/0000-0002-3477-0770

Monique G. M. de Sain-van der Velden https://orcid.org/0000-0003-3033-4397

REFERENCES

1. Loeber JG, Platis D, Zetterstrom RH, et al. Neonatal screening in Europe revisited: an ISNS perspective on the current state and developments since 2010. *Int J neonatal Screen.* 2021; 7(1):15.
2. Therrell BL, Padilla CD, Loeber JG, et al. Current status of newborn screening worldwide: 2015. *Semin Perinatol.* 2015; 39(3):171-187.
3. Ahrens-Nicklas RC, Serdaroglu E, Muraresku C, Ficicioglu C. Cobalamin C disease missed by newborn screening in a patient with low carnitine level. *JIMD Rep.* 2015;23:71-75.
4. Lin Y, Wang W, Lin C, et al. Biochemical and molecular features of Chinese patients with glutaric acidemia type 1 detected through newborn screening. *Orphanet J Rare Dis.* 2021; 16(1):339.
5. Afkap/beslissingscriteria Neonatale screening. Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Centrum Gezondheidsbescherming (GZB). Afdeling Biologische geneesmiddelen, Screening en Innovatie (BSI). 2020. https://webshare.zenya.work/lv3tvdk3gr61tbnx/Document.aspx. Published 2022. Updated January 27, 2020. Accessed May 6, 2022.
6. Magoulas PL, El-Hattab AW. Systemic primary carnitine deficiency: an overview of clinical manifestations, diagnosis, and management. *Orphanet J Rare Dis.* 2012;7(1):68.
7. van Dijk T, Kater A, Jansen M, et al. Expanding neonatal bloodspot screening: a multi-stakeholder perspective. *Front Pediatr.* 2021;9:706394.
8. Tang NLS, Ganapathy V, Wu X, et al. Mutations of OCTN2, an organic cation/carnitine transporter lead to deficient cellular carnitine uptake in primary carnitine deficiency. *Hum Mol Genet.* 1999;8(4):655-660.
9. Nezu J-I, Tamai I, Oku A, et al. Primary systemic carnitine deficiency is caused by mutations in a gene encoding sodium ion-dependent carnitine transporter. *Nat Genet.* 1999;21(1): 91-94.
10. Longo N. Primary carnitine deficiency and newborn screening for disorders of the carnitine cycle. *Ann Nutr Metab.* 2016; 68(3):5-9.
11. Longo N, Di San A, Filippo C, Pasquali M. Disorders of carnitine transport and the carnitine cycle. *American journal of medical genetics - seminars in medical. Genetics.* 2006;142C(2):77-85.
12. Tamai I, Ohashi R, Nezu JI, et al. Molecular and functional identification of sodium ion-dependent, high affinity human carnitine transporter OCTN2. *J Biol Chem.* 1998;273(32): 20378-20382.
13. Bernardini I, Rizzo WB, Dalakas M, Bernar J, Gahl WA. Plasma and muscle free carnitine deficiency due to renal Fanconi syndrome. *J Clin Invest.* 1985;75(4):1124-1130.
14. Boonyawat B, Liammongkolkul S, Vijarnsorn C, Vatanavicharn N. Carnitine Uptake Disorder Identified by Expanded Newborn Screening in Thailand. Poster presentation presented at: Annual Symposium of the Society for the Study of Inborn Errors of Metabolism; Athens; 2018.
15. Bzduch V, Gorova R, Addova G, et al. Fractional excretion of free carnitine in urine as an important marker for carnitine uptake defect. Poster presentation presented at: Annual Symposium of the Society for the Study of Inborn Errors of Metabolism; Athens 2018.
16. Kobayashi H, Hasegawa Y, Endo M, Purevsuren J, Yamaguchi S. ESI-MS/MS study of acylcarnitine profiles in urine from patients with organic acidemias and fatty acid oxidation disorders. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2007;855(1):80-87.
17. Krywawych S, Davison J, Winter R, Heales S. Fractional renal carnitine clearance relative to creatinine clearance in patients with systemic primary l-carnitine deficiency. Poster presentation presented at: Annual Symposium of the Society for the Study of Inborn Errors of Metabolism; Rotterdam. 2019.
18. Therrell BL Jr, Padilla CD. Newborn screening in the developing countries. *Curr Opin Pediatr.* 2018;30(6):734-739.
19. Lin Y, Xu H, Zhou D, et al. Screening 3.4 million newborns for primary carnitine deficiency in Zhejiang Province. *China Clinica Chimica Acta.* 2020;507:199-204.
20. McCandless SE, Wright EJ. Mandatory newborn screening in the United States: history, current status, and existential challenges. *Birth Defects Res.* 2020;112(4):350-366.
21. Gallant NM, Leydiker K, Wilnai Y, et al. Biochemical characteristics of newborns with carnitine transporter defect identified by newborn screening in California. *Mol Genet Metab.* 2017;122(3):76-84.
22. Schiergens KA, Weiss KJ, Roschinger W, et al. Newborn screening for carnitine transporter defect in Bavaria and the long-term follow-up of the identified newborns and mothers: assessing the benefit and possible harm based on 19 (1/2) years of experience. *Mol Genet Metab Rep.* 2021;28:100776.
23. Wilson C, Knoll D, de Hora M, Kyle C, Glamuzina E, Webster D. The decision to discontinue screening for carnitine uptake disorder in New Zealand. *J Inherit Metab Dis.* 2019; 42(1):86-92.
24. Evans AM, Fornasini G. Pharmacokinetics of L-carnitine. *Clin Pharmacokinet.* 2003;42(11):941-967.
25. Cortinovis M, Perico N, Ruggenenti P, Remuzzi A, Remuzzi G. Glomerular hyperfiltration. *Nat Rev Nephrol.* 2022;18:435-451.
26. El-Hattab AW, Li FY, Shen J, et al. Maternal systemic primary carnitine deficiency uncovered by newborn screening: clinical, biochemical, and molecular aspects. *Genet Med.* 2010;12(1): 19-24.
27. Lee NC, Tang NLS, Chien YH, et al. Diagnoses of newborns and mothers with carnitine uptake defects through newborn screening. *Mol Genet Metab.* 2010;100(1):46-50.
28. Rose EC, di San Filippo CA, Ndukwe Erlingsson UC, Ardon O, Pasquali M, Longo N. Genotype-phenotype correlation in primary carnitine deficiency. *Hum Mutat.* 2012;33(1):118-123.
29. Schimmenti LA, Crombez EA, Schwahn BC, et al. Expanded newborn screening identifies maternal primary carnitine deficiency. *Mol Genet Metab.* 2007;90(4):441-445.
30. Hosten AO. BUN and creatinine. In: Walker HK, Hall WD, Hurst JW, eds. *Clinical Methods: The History, Physical, and Laboratory Examinations.* Butterworths; 1990.

31. Tynkevich E, Flamant M, Haymann JP, et al. Decrease in urinary creatinine excretion in early stage chronic kidney disease. *PLoS One.* 2014;9(11):e111949.

32. Helal I, Fick-Brosnahan GM, Reed-Gitomer B, Schrier RW. Glomerular hyperfiltration: definitions, mechanisms and clinical implications. *Nat Rev Nephrol.* 2012;8(5):293-300.

33. Gallant NM, Wilnai Y, Lee C, et al. Optimal methods for diagnosis of systemic primary carnitine deficiency suspected by newborn screening. *J Inherit Metab Dis.* 2013;36(2):S112.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

TABLE S1 Genetic variants of the PCD patients in the study cohort

TABLE S2 Characteristics of individuals with and without suppletion

TABLE S3 Characteristics of individuals identified by newborn screening (Newborn) or by newborn screening of their child (Maternal)

TABLE S4 AUC's and 100% sensitivity cut-off values for all classified groups