

## Exceptional Case

# Plasma nitrate/nitrite removal by peritoneal dialysis might predispose infants with low blood pressure to cerebral ischaemia

Mattias Carlström<sup>1</sup>, Katarina Wide<sup>2</sup>, Mikael Lundvall<sup>2</sup>, Carmen Cananau<sup>3</sup>, Anders Svensson<sup>3</sup>, Jon O. Lundberg<sup>1</sup>, Peter Bárány<sup>4</sup> and Rafael T. Krmar<sup>2</sup>

<sup>1</sup>Department of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden, <sup>2</sup>Karolinska Institute, Department for Clinical Science, Intervention and Technology, Division of Pediatrics, Karolinska University Hospital, Huddinge, Stockholm, Sweden, <sup>3</sup>Department of Radiology, Karolinska University Hospital, Huddinge, Stockholm, Sweden and <sup>4</sup>Department of Renal Medicine, Karolinska University Hospital, Huddinge, Stockholm, Sweden

Correspondence to: Rafael T. Krmar; E-mail: rafael.krmar@ki.se

### Abstract

The underlying pathogenic mechanisms of neurological complications in infants undergoing peritoneal dialysis (PD) are poorly understood. We report on four male infants treated with PD who developed symptomatic cerebral ischaemia. Blood pressure (BP) levels were low both before the event and at presentation. In two patients, we observed that the removal of nitrate and nitrite by PD could have impaired the nitrate/nitrite–nitrite oxide (NO) pathway, a system that generates NO independently of NO synthase. Our observation suggests that low BP and reduced NO bioavailability puts infants treated with PD at risk for impaired cerebral blood flow and consequently for brain ischaemia.

**Keywords:** blood pressure; cerebral ischaemia; infant; nitric oxide; peritoneal dialysis

## Introduction

Registry studies suggest that the overall survival of children in whom chronic dialysis was initiated during the neonatal period is similar to those who commenced at a later stage [1]. However, neurological complications remain a therapeutic challenge in this group of patients and significantly increase the risk of death [1]. Currently, published data lack information on the type of neurological complications presented in infants undergoing chronic peritoneal dialysis (PD) or on the underlying pathogenic mechanisms [1].

We describe four infants treated with chronic PD since the neonatal period, displaying consistently low blood pressure (BP) values and who suffered symptomatic cerebral ischaemia. A recent publication demonstrated that haemodialysis significantly lowers levels of stable nitric oxide (NO) metabolites nitrate and nitrite, which may increase the risk of cardiovascular events [2]. Therefore, we investigated whether PD may have interfered with NO-generating systems, i.e. the classical L-arginine-dependent NO synthase (NOS) or the nitrate–nitrite–NO pathway [3].

## Case reports

This retrospective observational cohort study of infants who commenced chronic PD during the neonatal period (age

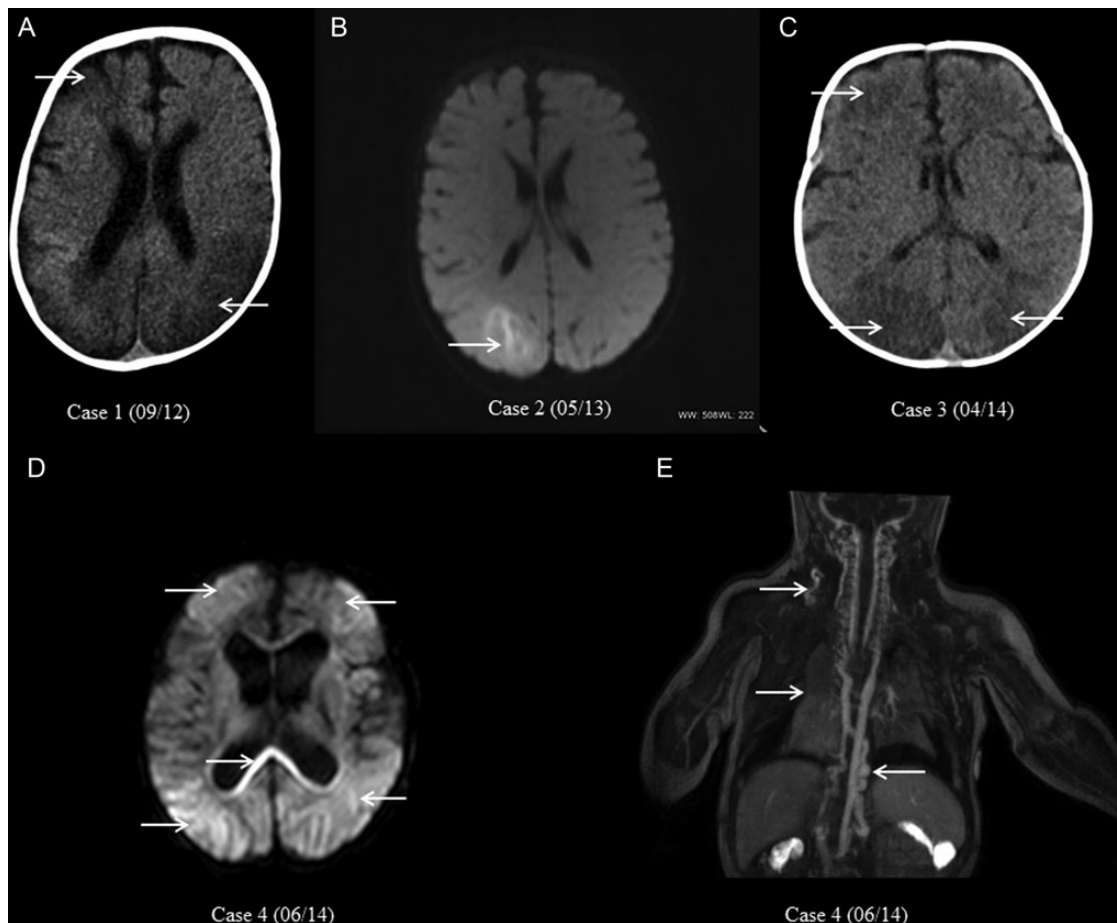
≤1 month) at our department since 2005 was approved by the hospital ethics board (Protocol number 2014/1631-31). Informed consent was waived in all but two patients who underwent additional blood and dialysate testing. Overall, 6 out of 13 consecutive patients treated with PD had neurological complications. One patient developed cerebral atrophy of unknown cause, another patient suffered cerebral hypoxic lesions due to a severe pulmonary disease and the other four patients experienced symptomatic cerebral ischaemia. In the latter patient group, the primary renal diagnosis was congenital abnormalities of the kidney and urinary tract in three patients (Case 1, 2 and 3) and autosomal recessive polycystic kidney disease in the other patient (Case 4). Peritoneal dialysis was performed on an in-patients basis. All of the patients were anuric. Dialysis dose was individualized and regarded clinically adequate if it allowed the patient to achieve a proper nutrition, to correct acid–base and electrolytic imbalances and to improve plasma creatinine and urea levels. In all patients, nutrition was provided enterally using either nasogastric tubes (Case 1, Case 3 and Case 4) or gastrostomy (Case 2).

The ischaemic event was documented at the age of 5 months (Case 1), 4.5 months (Case 2), 2.5 months (Case 3) and 2 months (Case 4), respectively. Neurological symptoms at presentation were ocular flutter (Case 1), deviation of gaze (Case 2) and seizures (Case 1, 3 and 4). At the time, no patient had an intercurrent illness. The duration of daily PD session was 14 h in Case 1 (26 mL/kg fill

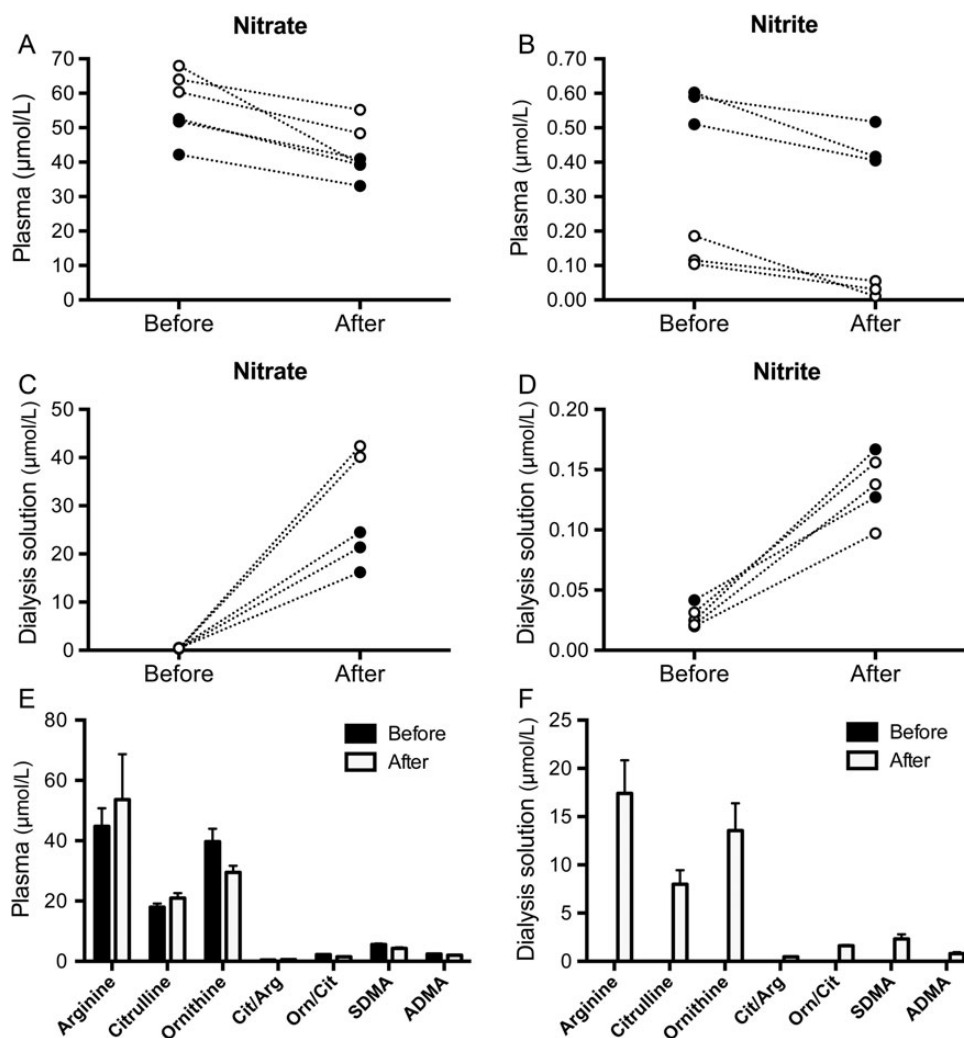
volume and 30-min dwell time), 20 h in Case 2 (17 mL/kg fill volume and 20- to 45-min dwell time), 16 h in Case 3 (26 mL/kg fill volume and 20- to 40-min dwell time) and 16 h in Case 4 (20 mL/kg fill volume and 20-min dwell time). In all patients, Physioneal 40<sup>®</sup> glucose 2.27% w/v (Baxter) was the standard PD solution used. Manual PD, i.e. the fluid exchanges were performed manually with the active participation of a nurse, was the PD procedure used in Case 2, Case 3 and Case 4, whereas Case 1 underwent automated PD (Baxter HomeChoice<sup>®</sup>). **Figure 1** shows representative neuroimages of either computed tomography scanning or magnetic resonance imaging performed after symptom onset. At presentation, none of these patients was on antihypertensive therapy. Since low systolic BP (SBP) has been associated with increased mortality in paediatric victims of traumatic brain injury, presumably due to cerebral hypoperfusion [4], we calculated the median of all documented oscillometric SBP values recorded in each patient during the 10 days previous to the first occurrence of neurological symptoms. The number of SBP measurements per patient was 24 (Case 1), 28 (Case 2), 146 (Case 3) and 22 (Case 4). Median (range) SBP values were consistently far under the 50th distribution adjusted age- and sex-related percentile in all the patients: Case 1, 53 (40–118) mmHg; Case 2, 72.5 (52–102) mmHg; Case 3, 69 (26–119) mmHg; Case 4, 56 (40–126) mmHg [5].

Two patients (Cases 1 and 4) died after 6 and 1 month following the diagnosis of brain injury. At the time of writing, the other patients (Cases 2 and 3), aged 2 and 1 year, respectively, are still on PD and none have any measurable neurologic deficit.

In Cases 3 and 4, we investigated whether PD may have impaired NO bioavailability. Plasma samples were obtained on three separate PD sessions, both at the beginning and end of each daily PD session. As previously described, a dedicated HPLC system was used to measure nitrate and nitrite content, and mass spectrometry method for analyzing amino acids linked to NOS function [6]. In both patients, reductions in plasma nitrate and nitrite levels were consistently observed at the end of the PD sessions (**Figure 2A** and **B**), whereas **Figure 2C** and **D** illustrate the increase of these inorganic anions in the total spent dialysate volume, i.e. dialysate plus ultrafiltrate per PD session. Plasma levels of arginine and citrulline remained unchanged, whereas ornithine levels decreased at the end of the PD sessions (**Figure 2E**). Plasma citrulline-to-arginine ratios, a surrogate of endothelial NOS activity, were similar at the beginning ( $0.43 \pm 0.03$ ) and at the end of the PD sessions ( $0.57 \pm 0.09$ ). On the other hand, plasma ornithine-to-citrulline ratios were reduced at the end of the PD sessions ( $2.22 \pm 0.19$  versus  $1.48 \pm 0.15$ ), which could indicate a lower arginase activity. We also investigated plasma levels of the asymmetric dimethylarginine (ADMA) and



**Fig. 1.** Native computed tomography (Cases 1 and 3) and diffusion-weighted (DW) magnetic resonance imaging (Cases 2 and 4) of the head showing ischaemic lesions (arrows). DW magnetic resonance imaging of the chest and the abdomen performed in Case 4 (**E**) showing extensive vascular thrombosis and collateral circulation (arrows). In parentheses (pictures **A**, **B**, **C**, **D**, and **E**) are depicted patient's date at symptomatic cerebral ischaemic event (month/year).



**Fig. 2.** Analysis of nitrate and nitrite content in plasma, both at the beginning (before) and end (after) of each daily PD session (**A** and **B**, respectively) and in total spent dialysate volume (dialysis solution) per daily PD session (**C** and **D**, respectively). Measurements were conducted on three separate daily PD sessions both in Case 3 (closed circles) and in Case 4 (open circles). Measurement of amino acids, relevant for NOS function, was determined in a similar way and presented as mean  $\pm$  SEM for plasma (**E**) and total spent dialysate volume (**F**).

symmetric dimethylarginine (SDMA), which are known endogenous inhibitors of NOS-dependent NO generation [7]. We observed a reduction of both plasma ADMA and SDMA levels at the end of the PD sessions (Figure 2E).

## Discussion

In the face of fluctuations in BP, the cerebral vessels possess the ability to maintain an adequate perfusion pressure. If the autoregulatory property of the cerebral vessels is intact, a decrease in BP leads to dilatation of the cerebral resistance vessels to preserve an adequate cerebral blood flow, while an increase in BP results in the opposite effect [8]. This autoregulatory property is modulated by the endothelium through the release of various vasorelaxing (e.g. NO) or vasoconstrictor (e.g. thromboxane A<sub>2</sub> and endothelin) agents [9]. Collectively, our results suggest no major effect of PD on NOS function, but rather emphasize that removal of nitrate and nitrite by PD could have limited the nitrate-nitrite-NO pathway. We speculate that in our patients, sustained low SBP, in the context of reduced NO

bioavailability, might have predisposed to episodes of silent cerebral hypoperfusion until becoming symptomatic.

Several lines of evidence indicate that NO deficiency is associated with thrombotic disorders [10]. In Case 4, with the extensive vascular thrombosis, a routine workup ruled out identifiable causes of hypercoagulable states (data not shown). To what extent PD-mediated removal of plasma nitrate and nitrite, as observed in this patient, may have contributed to thrombus formation remains to be elucidated.

In conclusion, our observations suggest that infants undergoing PD may benefit from treating low SBP levels and from preventing a decrease in the bioavailability of NO [2]. The latter could readily be achieved by supplementing the PD solutions and/or enteral feeding with physiological levels of inorganic nitrate and nitrite.

*Conflict of interest statement.* None declared.

## References

1. van Stralen KJ, Borzych-Duzalka D, Hataya H *et al.* Survival and clinical outcomes of children starting renal replacement therapy in the neonatal period. *Kidney Int* 2014; 86: 168-174

2. Bryan NS, Torregrossa AC, Mian AI et al. Acute effects of hemodialysis on nitrite and nitrate: potential cardiovascular implications in dialysis patients. *Free Radic Biol Med* 2013; 58: 46–51
3. Lundberg JO, Carlstrom M, Larsen FJ et al. Roles of dietary inorganic nitrate in cardiovascular health and disease. *Cardiovasc Res* 2011; 89: 525–532
4. MacLeod J, Lynn M, McKenney MG et al. Predictors of mortality in trauma patients. *Am Surg* 2004; 70: 805–810
5. Dionne JM, Abitbol CL, Flynn JT. Hypertension in infancy: diagnosis, management and outcome. *Pediatr Nephrol* 2012; 27: 17–32
6. Carlstrom M, Persson AE, Larsson E et al. Dietary nitrate attenuates oxidative stress, prevents cardiac and renal injuries, and reduces blood pressure in salt-induced hypertension. *Cardiovasc Res* 2011; 89: 574–585
7. Zoccali C, Bode-Boger S, Mallamaci F et al. Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. *Lancet* 2001; 358: 2113–2117
8. Paulson OB, Strandgaard S, Edvinsson L. Cerebral autoregulation. *Cerebrovasc Brain Metab Rev* 1990; 2: 161–192
9. Toda N, Ayajiki K, Okamura T. Cerebral blood flow regulation by nitric oxide: recent advances. *Pharmacol Rev* 2009; 61: 62–97
10. Loscalzo J. Nitric oxide insufficiency, platelet activation, and arterial thrombosis. *Circ Res* 2001; 88: 756–762

Received for publication: 27.11.14; Accepted in revised form: 23.1.15