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Retinopathy of prematurity screening at ≥30 weeks: urinary NTpro-BNP performance

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Running Title

Urinary NTproBNP ROP & infants >30 weeks

Abstract

Aim

Urinary N-terminal B-type natriuretic peptide NTproBNP levels are associated with the development of retinopathy of prematurity (ROP) in infants <30 weeks gestation. The incidence of ROP in more mature infants who meet other ROP screening criteria is very low. We therefore aimed to test whether urinary NTproBNP predicted ROP development in these infants.

Methods

Prospective observational study in 151 UK infants ≥30+0 weeks gestation but also <32 weeks gestation and/or <1501g, to test the hypothesis that urinary NTproBNP levels on day of life (DOL) 14 and 28 were able to predict ROP development.

Results

Urinary NTproBNP concentrations on day 14 and day 28 of life did not differ between infants with and without ROP (medians 144mcg/ml vs 128mcg/ml respectively, p=0.86 on DOL 14 and medians 117mcg/ml vs 94mcg/ml respectively, p=0.64 on DOL28).

Conclusion

The association previously shown for infants <30 completed weeks between urinary NTproBNP and development of ROP was not seen in more mature infants. Urinary NTproBNP does not appear helpful in rationalizing direct ophthalmoscopic screening for

ROP in more mature infants, and may suggest a difference in pathophysiology of ROP in this population.

Key Notes

1) Published data for infants <30 completed weeks gestation suggests that urinary NTproBNP levels might contribute to identifying infants at risk from retinopathy of prematurity (ROP)
2) We showed that this is not true for more mature infants who still fulfil UK screening criteria
3) This difference may imply different pathophysiology of ROP development in more mature infants

Abbreviations

NTproBNP: Urinary N-terminal B-type natriuretic peptide

REDEXAM: REDucing Eye EXAMinations in preterm infants

ROP: retinopathy of prematurity

UK: United Kingdom

Introduction

Visual impairment as a result of retinopathy of prematurity (ROP) is potentially preventable by screening (1), but at present screening is largely dependent on direct ophthalmoscopy, which requires the availability of ophthalmologists willing and able to perform this. Although telemedicine and digital photography have improved access in some areas, costs, training expertise and other practicalities restrict universal screening (2-4). This and the physical discomfort and instability caused to the infant (5), and distressing nature of the examination to the family, mean that non-invasive markers of ROP development have been sought, and algorithms generated to attempt to maximize the performance of features associated with ROP development to accurately predict this (6,7). To date, no 'ideal' biomarkers of ROP have been identified and sufficiently tested, but urinary B-type natriuretic peptide (BNP) shows promise (8). Proteolytic cleavage of a precursor protein yields biologically active BNP and an inert N-terminal fragment, NTproBNP (amino acids 1-76). Measuring NTproBNP concentrations is routine in cardiac failure in children and neonates (9,10) and often available in routine health provision laboratories relatively cheaply. Concentrations of NTproBNP in blood parallel those in the urine, making non-invasive measurement possible (11). In a single-center pilot study, we showed that urinary NTproBNP/creatinine ratios (UNBCR) in the first month were significantly elevated in preterm infants who developed severe ROP, compared to controls (8). To further assess the predictive power of urinary NTproBNP concentrations and UNBCR during the first month of life to allow early identification of infants at high or low risk of severe ROP, we conducted a prospective observational study (REDEXAM, REDucing Eye EXAMinations in preterm infants) in neonatal intensive units in 8 European and Middle East countries (12).

This second study showed a strong association between urinary NTproBNP measurements on day 14 and 28 of life and subsequent ROP development, in a cohort of 967 infants of ≤29+6 weeks gestation (12). This is potentially important as this cheap, readily available test could help reduce or target direct ophthalmoscopy reducing either the number of individuals ever needing to be screened, or the number of screens undertaken in an individual, or both.

Gerull *et al* (13) recently confirmed the low incidence of retinopathy of prematurity(ROP) in Switzerland in more mature infants than those included in our second study - those between 30+0 and 31+6 weeks gestation. There was a very low incidence of any ROP (1.7% (56/3222)) and severe or treated ROP (0.16% (5/3222)) and these authors called for a review of screening criteria. Given the relatively large numbers of more mature infants compared to very immature infants, and the low rate of ROP in these more mature infants, the utility of urinary NTproBNP to predict ROP development in more mature infants who still fulfil screening criteria, is important. We therefore tested the hypothesis that urinary NTproBNP in the first month of life, in infants ≥30+0 weeks fulfilling UK ROP screening criteria by virtue of gestation (<32 completed weeks) birth weight (<1501g) or both, would predict ROP development. To do this we applied the methodology of our previous study (14) to infants with gestations ≥30+0 weeks, born only in the UK.

Methods

Study setting

7 neonatal intensive care units in the United Kingdom participated between November 2013 and May 2015. Ethical approval was obtained from Newcastle and North Tyneside 2 ethics committee.

Patients and measurements

Infants with a gestational age ≥30+0 completed weeks gestation and/or <32+0 or <1501g, alive at 10 days were eligible. Written informed parental consent was obtained. Urine was collected on DOL (day of life)14 and DOL28 (or as close as possible) by each center by their usual method (cotton wool balls or pads in the nappy, bags or clean catches by staff or parents at routine nappy changes) and stored at -80ºC until analysis. Urinary NTproBNP concentrations were determined at one laboratory on one site (Newcastle Upon Tyne, UK) in batches by standard hospital laboratory automated commercial chemiluminescent sandwich immunoassays (Roche Modula P E170 run on a cobas e 411 analyser) as described previously (8,15). Urines with a level lower than the limits of detection of the assay were attributed the lower level (50 mcg/l). We also recorded infant weight on DOL14 and DOL28. Proportional weight gain was calculated by dividing the gain of the infant's weight since birth on DOL14 or DOL28, respectively, by birth weight.

ROP outcomes

ROP screening examinations were unaffected by study participation, undertaken by serial binocular indirect ophthalmoscopy and carried out by experienced local ophthalmologists according to local and national guidelines unaware of urinary NTproBNP concentrations

(14). ROP was staged according to the International Classification of Retinopathy of Prematurity (16) and allocated treatment according to the Early Treatment for Retinopathy of Prematurity criteria (17).

Statistical analysis

NTproBNP results for infants with known ROP outcome and at least one urinary NTproBNP concentration measured were analysed using SPSS 23.0 (SPSS Inc, Chicago, IL). As NTproBNP results and other variables lacked a normal distribution, data are presented as median and range. We assessed the strength of association between continuous variables by Spearman rank order coefficients. Differences in the distribution of continuous variables were assessed by the Mann-Whitney U test.

Results

A total of 151 infants, 77 (51%) male, of median gestation 31.0 (range 30.0 – 37.0) weeks and median birth weight 1420g (range 640-2430g) participated, including 105 singletons, 39 twins (some of whom had non-surviving siblings), one triplet set and one quad set.

Five infants died before ROP screening was complete. Of surviving infants, 11 (7.5%) had any ROP: two (1.4%) stage 1, eight (5.5%) stage 2 and one (0.7%) stage 3 (zone 3 only). No infant was ever treated for ROP, and all ROP resolved.

We found that ROP of any grade was restricted to infants ≤ 32+0 weeks gestational age (n=123). None of the more mature infants who entered the study had any signs of ROP. In contrast to the cohort <30+0 weeks, there were no significant differences between infants with ROP, as opposed to infants without ROP for proportional weight gain or urinary

NTproBNP concentrations on day 14 and day 28 of life (Table 1) . Urinary NTproBNP concentrations were poorly associated with birthweight for this cohort (figure 1).

Discussion

Successfully identifying a non-invasive biomarker for development of ROP would have significant financial, family and infant benefits. Urinary NTproBNP appeared promising in pilot work (8), and in a larger population of relatively immature infants (<30+0 weeks gestation (12). However in the present study done in more mature infants who are still judged at risk of ROP by current screening criteria, a relationship between urinary NTproBNP and the development of ROP has not been demonstrated.

Although this was a relatively small study of 151 infants, this cohort is larger than that in the original pilot study (n=136) where a correlation was seen between ROP development and urinary NTproBNP, and a correlation was previously seen in the less mature UK infants (12). It is still possible that a larger study would show a relationship, but any study will suffer from the impact of assessing any test for ROP in a very low incidence population.

Little is known about the role of BNP in development of ROP. The lack of correlation between urinary NTproBNP levels and ROP in more mature infants could indicate different pathophysiology of either ROP development or BNP release in comparison to less mature infants. Equally, in the more immature population urinary NTproBNP may simply act as a proxy measure of 'sickness' related to immaturity which subsequently relates to risk of ROP development. In the more mature population, where ROP development is rare, 'sickness' may then influence BNP levels but not ROP development, causing the relationship to be lost.

We did not collect more extensive clinical data to allow this hypothesis to be tested further in this study, but future studies could explore this possibility, as well as being larger.

We conclude that in this UK cohort urinary NTproBNP based levels for risk factors for ROP established for more immature infants fail to identify infants of 30+0 weeks or more who might benefit from, or not require, screening ophthalmoscopy. Differences in other morbidities or potentially different pathophysiology of ROP development in more mature infants may explain this loss of correlation.

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Disclosure statement

There are no conflicts of interests to be disclose

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Figure 1

Relationship between NTproBNP and birth weight

Table 1

Outcome	Gestation (decimal) Median (range)	Birthweight (g) Median (range)	NTproBNP DOL14 (mcg/ml) Median (range)(n)	NTproBNP DOL 28 (mcg/ml) Median (range)(n)	Proportional weight gain Day 28 (g) Median (range)(n)
Live (n = 146)	31.0 (30-37)	1425 (810-2430)	134 (49-6748) (138)	94 (49-35000) (111)	0.312 (-10 – 0.71) (133)
Dead (n = 5)	31 (30.3-31.1)	1390 (640-1520)	140 (49-1626) (5)	94 (56-35000) (3)	0.32 (0-0.35) (3)
No ROP (n = 135)	31.1 (30-37)	1435 (820-2430)	128 (49-6748) (127)	94 (49-35000) (101)	0.32 (-10 – 0.71) (122)
ROP any grade (n = 11)	30.5 (30-32)	1250 (810 – 1605)	144 (49-1135) (11)	117 (49-582) (10)	0.34 (-10.-0.5) (11)
Group comparisons (p)					
Live vs dead	0.40	0.31	0.44	0.60	0.50
No ROP vs any ROP	**0.007**	0.06	0.86	0.64	0.50

Comparison of infants surviving and developing ROP by gestation, birth weight, NTproBNP measures on day 14 and 28, and proportional weight gain.

Figure 1

Relationship between NTproBNP and birth weight