N-terminal B-type natriuretic peptide urinary concentrations and retinopathy of prematurity

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Cite this article as: Christoph Bührer, Ömer Erdeve, Anton van Kaam, Angelika Berger, Evelyn Lechner, Benjamin Bar-Oz, Karel Allegaert, Tom Stiris, Istemi Han Çelik, Janet Berrington, on behalf of the REDEXAM study group, Sinan Abu-Leil, Benjamin Bausenhardt, Christoph Czernik, Uğur Dilmen, Nicholas Embleton, Zivanit Ergaz-Shaltiel, Lara Garabedian, Irene Lok, Tone Nordvik, Wes Onland, Øivind Ringen, Thomas Skeath, Oliver Wagner, N-terminal B-type natriuretic peptide urinary concentrations and retinopathy of prematurity, *Pediatric Research* accepted article preview 24 July 2017; doi: 10.1038/pr.2017.179.

Received 2 May 2017; accepted 26 June 2017; Accepted article preview online 24 July 2017

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Running title NTproBNP and retinopathy of prematurity

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Statement of financial support
The study has received financial support from the BLISS innovation grant (London, UK) and intramural funding

Disclosure statement
There are no conflicts of interests to be disclosed.

Category: Clinical observational study

ABSTRACT

Background and aims: To validate findings of a single-center pilot study showing elevated urinary N-terminal B-type natriuretic peptide (NTproBNP) concentrations in preterm infants subsequently developing severe retinopathy of prematurity (ROP) in a multicenter setting across 8 European and Middle East countries.

Methods: Prospective observational study in 967 preterm infants < 30 weeks gestational age assessing the capacity of urinary NTproBNP on day of life (DOL) 14 and 28 to predict ROP requiring treatment.

Results Urinary NTproBNP concentrations were markedly elevated in infants who developed ROP requiring treatment (n=94), compared to survivors without ROP treatment (n=837), at both time points [Median (interquartile range) DOL14: 8,950 (1,925-23,783) vs 3,083 (1,193-17,393) vs 816 (290-3,078) pg/ml, p<0.001] and DOL28 [2,203 (611-4,063) vs 1,671 (254-11,340) vs 408 (162-1,126) pg/ml, p<0.001]. C-statistic of NTproBNP for treated ROP or death was 0.731 (95% CI 0.654-0.774) for DOL14, and 0.683 (0.622-0.745) for DOL28 (p<0.001). Threshold scores were calculated potentially enabling around 20% of infants with low NTproBNP scores never to be screened with ophthalmoscopy.

Conclusions There is a strong association between early urinary NTproBNP and subsequent ROP development which can be used to further refine subgroups of patients with high or low risk of severe ROP.

INTRODUCTION

Retinopathy of prematurity (ROP) is responsible for a large proportion of potentially preventable childhood blindness worldwide, with approximately two-thirds of preterm infants displaying ROP-mediated visual impairment being born in middle-income countries (1). This proliferative disorder of blood vessels in the immature retina develops gradually after birth. Therefore, a window exists to screen, detect and treat in a timely fashion where indicated. Screening by indirect ophthalmoscopy is supposed to start at a corrected gestational age of 31 weeks and a chronological age of > 4 weeks. Numbers of ophthalmologists who are able and willing to perform ROP screening are often limited by liability concerns, reimbursement issues, and lack of training opportunities both in high- and middle-income countries (2-4). Besides being an expensive and limited resource, ophthalmoscopy is distressing to families to observe and potentially destabilizing infants. Adverse events related to the procedure or eye drops used include life-threatening apnea, bradycardia, hypoxia, tachycardia, emesis, and retinal hemorrhages (5-10) while the pain and stress response to ophthalmoscopy is poorly blunted by oral sucrose (11).

While a considerable proportion of very preterm infants develop some degree of ROP, only a small fraction progress to severe ROP requiring treatment (laser surgery or intravitreal pharmacological therapy). Thus, biomarkers that define infants at high or low risk of ROP requiring intervention would have wide implications for many infants, families and health economies. Such biomarkers would ideally have high sensitivity and specificity and should be done on non-invasive samples by local laboratories.

In response to increased pre- or afterload, cardiac myocytes release B-type natriuretic peptide (BNP) into the circulation. BNP acts on the heart (accelerated myocardial relaxation), the vasculature (vasodilation), and the kidneys (natriuresis). It is generated by proteolytic cleavage of a precursor protein, yielding the biologically active BNP and an inert N-terminal fragment, NTproBNP (amino acids 1-76). Measuring NTproBNP concentrations is widely

being used for assessment of cardiac failure in adults, children, and neonates (12-14). NTproBNP concentrations in blood parallel those determined in urine (15). In preterm infants, elevated NTproBNP concentrations measured in blood or urine have been shown to herald incipient bronchopulmonary dysplasia and are indicative of a hemodynamically significant patent ductus arteriosus (16).

In a single-center pilot study, we found that urinary NTproBNP/creatinine ratios (UNBCR) during the first month were significantly elevated in preterm infants who developed severe ROP, compared to controls (17). 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, **RED**ucing **E**ye E**XAM**inations in preterm infants) in neonatal intensive units in 8 European and Middle East countries.

METHODS

Study setting

Neonatal intensive care units in the United Kingdom, the Netherlands, Belgium, Norway, Germany, Austria, Turkey and Israel, after approval of the ethical committees of each site.

Patients and measurements

Preterm infants with a gestational age below 30 completed weeks alive at 10 days were eligible (recruitment periods varied by site). After written informed parental consent, spot urine samples were collected on DOL (day of life)14 and DOL28 (or as close as possible). Urine was collected by each center by their usual method, and included using 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 each site 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 (17), while urinary creatinine concentrations were determined enzymatically or by the Jaffé method. Further data recorded were maternal age and the infant's 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 by serial binocular indirect ophthalmoscopy were carried out by experienced local ophthalmologists according to local and national guidelines and not influenced in any way by this study. The ophthalmologists staged ROP according to the International Classification of Retinopathy of Prematurity (18) and allocated treatment according to the Early Treatment for Retinopathy of Prematurity criteria (19). They were unaware of urinary NTproBNP concentrations.

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 interquartile range (IQR). Dichotomous values are presented as numbers (percentages). 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 (for 2 groups) or the Kruskal Wallis test (more than 2 groups, followed by post hoc analysis employing the Mann-Whitney U test). For paired samples, the Wilcoxon test was employed. Continuous variables found to be significantly associated with a dichotomous variable by univariate analysis were entered into multivariate

linear regression analysis with backward elimination for p>0.1. Predictive values were estimated by the areas under receiver operating characteristic (ROC) curves (C-statistic).

RESULTS

Patient Characteristics

A total of 1,000 infants were recruited between January 2012 and July 2015 (exact recruitment periods varied by site). After exclusion of 33 infants (gestational age ≥ 30 weeks, *n*=13; failed urine collection both DOL14 and DOL28, *n*=20), 967 infants were included in the final analysis (UK, *n*=265; Germany, *n*=163; Turkey, *n*=118; Netherlands, *n*=106; Austria, *n*=171; Israel, *n*=71; Belgium, *n*=49; Norway, *n*=24). The cohort consisted of 517 boys (53.5 %) and 319 multiples (33.0 %; 280 twins, 35 triplets and 4 quadruplets). Median (IQR) gestational age was 27 $^{3}/_{7}$ (26 $^{1}/_{7}$ - 28 $^{6}/_{7}$) weeks, birth weight 945 (780-1165) g. The actual age urine was collected for DOL14 samples (*n*=904) was 14.35±1.37 d (mean±SD), and 28.33±1.68 d for DOL28 samples (*n*=839). 36 infants died before discharge. In survivors, 550 infants were without retinopathy of prematurity (ROP), 133 with ROP stage 1, 150 with ROP stage 2, and 98 with ROP stage 3. Treatment for ROP was performed in 94 infants (laser ablative surgery, *n*=76; intravitreal bevacizumab injections, *n*=18). Survivors with ROP treatment, compared to survivors without ROP treatment, had lower gestational age (25 $^{4}/_{7}$ (24 $^{5}/_{7}$-26 $^{6}/_{7}$) weeks *vs* 27 $^{5}/_{7}$ (26 $^{2}/_{7}$ – 29 $^{0}/_{7}$) weeks, p<0.001), lower birth weight (755 (640-905) g *vs* 980 (815-1180) g, p<0.001), and lower proportional weight gain between birth and DOL 28 (31.4 (19.0-41.9)% *vs* 36.1 (24.8-48.1)%, p=0.007) but not between birth and DOL 14 (p=0.080). The sex ratio, multiple versus singleton pregnancy, and maternal age did not differ between survivors with or without ROP treatment (p>0.1). Patients' characteristics by country are given in table 1.

Urinary NTproBNP concentrations and urinary NTproBNP/creatinine ratios (UNBCR)

Baseline data

Urinary NTproBNP concentrations declined from median 992 (330-4082) pg/mL on DOL14 to 470 (170-1465) pg/mL on DOL28 ($p<0.001$). The same pattern was observed for UNBCR (DOL14: 92 (30-413), DOL28: 39 (15-137) pg/mL, $p<0.001$).

Associations with patient characteristics

Urinary NTproBNP concentrations were inversely related to birth weight (DOL14: $R_s = -0.475$, DOL28: $R_s = -0.428$, $p<0.001$) and gestational age (DOL14: $R_s = -0.502$, DOL28: $R_s = -0.447$, $p<0.001$). UNBCR showed similar relationships with birth weight (DOL14: $R_s = -0.489$, DOL28: $R_s= -0.439$, $p<0.001$) and gestational age (DOL14: $R_s= -0.501$, DOL28: $R_s= -0.455$, $p<0.001$).

Urinary NTproBNP concentrations and UNBCR were slightly higher in boys than in girls on DOL14 (NTproBNP: 1,146 (387-4,436) *vs* 801 (272-3,527) pg/ml, p=0.010; UNBCR: 103 (36-441) *vs* 79 (24-386) $*$ 10^{-4}, p=0.018) but not on DOL28 of life (NTproBNP: 468 (197-1,575) *vs* 438 (151-1,329) pg/ml, p=0.142; UNBCR: 41(17-122) *vs* 37 (12-137), p=0.195).

Associations with survival, ROP stages, and ROP treatment

Urinary NTproBNP concentrations and UNBCR were markedly elevated in infants who subsequently died (*n*=36) and in survivors with severe ROP requiring intervention (laser or intravitreal therapy) (*n*=94), as compared to survivors without ROP intervention (*n*=837) (table 2).

Survivors with no/little ROP (stage 0 or 1) had lower urinary NTproBNP concentrations and urinary NTproBNP/creatinine ratios than infants with ROP stage 2 or ROP stage 3 (table 3). There was no significant difference between infants with no ROP and infants with ROP stage 1. In contrast, median NTproBNP levels were increased almost

3fold in infants with ROP stage 2, and approximately 5fold infants with ROP stage 3, as compared to infants without ROP.

Prediction of survival without treated ROP

The capacity of NTproBNP and UNBCR to predict survival without ROP treatment was assessed by the areas under receiver operating characteristics (ROC) curves (table 4). ROC areas of NTproBNP or UNBCR to predict survival without ROP treatment were larger on DOL14 than on DOL28, while those of UNBCR were only slightly larger than plain NTproBNP at both time points. For urinary NTproBNP DOL14, the areas under the ROC curve to predict survival without ROP treatment were very high in participating units in Turkey (0.872), Austria (0.818) and Germany (0.817), high in Belgium (0.780), Israel (0.767) and the Netherlands (0.736) but lower in the UK (0.685) and not significant different from chance in Norway (0.432).

The three variables that differed significantly between infants who did and who did not survive without ROP treatment and that were known DOL14 (gestational age, birth weight, NTproBNP DOL14) were entered into multivariate linear regression analyses aimed at survival without ROP treatment as dependent variable and using backward elimination if $p>0.1$. This was repeated for the five variables known DOL28 (gestational age, birth weight, NTproBNP DOL14, NTproBNP DOL28, proportional weight gain DOL28). All factors were retained in the final equations. Linear coefficients (ß) were then applied as weighted values to create scores predictive of survival without ROP treatment. These scores were calculated using the formula DOL14 [300 - 0.156*gestational age(weeks) - 0.118*birth weight(g) + 0.146*NTproBNP DOL14(pg/ml)] and DOL28 [300 - 0.118*gestational age(weeks) - 0.131*birth weight(g) + 0.067*NTproBNP DOL14(pg/ml) + 0.180* NTproBNP DOL28(pg/ml) - 0.110*proportional weight gain birth to DOL28 (fraction)]. Scores DOL14 were 300 (208-636) *vs.* 656 (341-2753) *vs.* 1539 (464-3627) in survivors without ROP

treatment, survivors with ROP treatment, and infants who died, respectively ($p < 0.001$), and scores DOL28 were 212 (315-666) vs. 851 (353-3930) vs. 1247 (482-2511), respectively ($p < 0.001$).

No infant with a score DOL14 < 197, and no infant with a score DOL28 < 189, developed ROP requiring treatment (thresholds for 100% specificity and 100% positive predictive value for survival without ROP requiring treatment). In this cohort 20.6 % of infants were below the DOL14 threshold and 15.3% below the DOL28 threshold ROP, translating into sensitivities of 20.6%/15.3%, and negative predictive values of 16.4%/14.3%, respectively.

DISCUSSION

This multinational study confirms that urinary NTproBNP concentrations during the first month of life are markedly elevated in preterm infants <30 weeks gestation who many weeks later develop severe ROP requiring intervention. An even larger elevation of urinary NTproBNP concentrations was observed in infants who subsequently died, linking circulatory stress in the first month of life to mortality and morbidity of very preterm infants.

While immaturity and oxygen exposure are pivotal for the development of ROP, the risk of severe ROP is further increased by poor postnatal growth. This link is thought to be mediated by low levels of insulin-like growth factor 1 (IGF-1) and serves as a base for ROP prediction models such as WINROP (20) or CHOPROP (21). Furthermore, severe ROP is more common in infants with conditions that increase myocardial pressure or volume load, such as sepsis (22, 23), blood transfusions (23, 24), pulmonary hypertension treated with inhaled nitric oxide (25), patent ductus arteriosus (23), or inotrope use (22, 26-29). Such conditions lead to BNP release and have been associated in several groups of patients with

increased NTproBNP (30-33). Thus, in addition to slow weight gain and low IGF-1 levels, we hypothesize that circulatory compromise and increased BNP release during the first month of life play a role in ROP development.

The potential physiological role of BNP itself in the development of ROP is not yet understood. Relative hypoxia triggers BNP secretion from cultured human retinal pigment epithelium (34), while retinal vessels express receptors for BNP (35). BNP, however, has so far not been investigated in animal models of oxygen-induced proliferative retinopathy. In adults without overt cardiovascular compromise, higher levels of NTproBNP were found to be associated with microvascular damage in the retina (36, 37) and other body parts (36, 38). Thus, it remains up to future experimental investigations to explore and characterize the role of cardiovascular compromise and natriuretic peptides in the pathophysiology of ROP.

Previous investigations have employed UNBCR rather than plain urinary NTproBNP concentrations to estimate the hemodynamic significance of a patent ductus arteriosus and to assess the risk of ROP or bronchopulmonary dysplasia. The power to predict survival without ROP intervention of UNBCR was only marginally better than that of plain NTproBNP, probably reflecting steady state kidney function at the age of 2-4 weeks in the infants studied. Diagnostic patterns of UNBCR and plain NTproBNP were highly similar, limiting the merits of additionally determining creatinine concentrations in urine samples. Thus, there is no need to correct for creatinine when using NTproBNP as a screening aid for ROP detection, increasing its potential in low income settings.

This study has several limitations, including the lack of standardisation of ROP outcomes by central review of ophthalmoloscopic findings. Local thresholds for ROP treatment have been shown to differ markedly by country (39) and may have affected the performance of NTproBNP. A fraction of infants with stage 3 ROP in this study were actually not treated. However, the approach taken was highly pragmatic, and we have analysed by treatment received, a clearly defined end-point. This issue has also large multicenter

prospective randomized controlled trials including those investigating the effect of different oxygen target saturations on morbidity and mortality of very preterm infants. Participating neonatal intensive care units also had different strategies of fluid management, inotropes, transfusion thresholds, and treatment of a patent ductus arteriosus, which may also affect the relative performance of NTproBNP. In our study, NTproBNP concentrations were determined at prespecified time points, not adjusted for potential peaks associated with particular events such as sepsis or surgery. While overall predictive power of NTproBNP measurements was lower than in the pilot study (17), the results also differed markedly by country. Urinary NTproBNP DOL14 below 372 pg/ml (a threshold met by more than 26% of all infants studied) was able to identify all infants who did not require ROP treatment in Turkey, Austria, Germany, Belgium, Israel and the Netherlands, but not in the UK and Norway.

NTproBNP and NTproBNP-based scores share the problem of limited generalisability with proportional weight gain (20, 21) or risk factor-based models (22, 24). In contrast to the expectations underlying this multinational endeavour, the results of this study do not allow for a change in current clinical practice in infants <30 weeks. However, elevated NTproBNP measured during the first month of life may be helpful to identify infants at greatly increased risk for severe ROP, allowing for early targeted intervention in future trials. NTproBNP may also be promising in identifying more mature infants with increased risk of ROP, who currently may be screened only at the request of the attending neonatologist.

CONCLUSIONS

Elevated urinary NTproBNP concentrations during the first month of life are strongly associated with subsequent development of severe ROP, while low levels associated with low risk of severe ROP. The overlapping ranges of NTproBNP concentrations of infants with and without severe ROP, however, preclude to harness these substantial differences into an algorithm to reduce eye examination in very preterm infants. The roles of cardiovascular compromise and natriuretic peptides in the pathophysiology of ROP remain to be determined.

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Table 1 Patients' characteristics by country (median/interquartile range or n/%, respectively)

Country	**n**	**Gestational age** (weeks)	**Birth weight** (g)	**Died** (n, %)	**ROP intervention** (n,%)
Austria	171	27 $^{4}/_{7}$ 26 $^{0}/_{7}$ - 28 $^{6}/_{7}$	920 690 – 1,146	6 (3.5%)	4 (2.3%)
Belgium	49	27 $^{3}/_{7}$ 26 $^{0}/_{7}$ - 28 $^{6}/_{7}$	980 863 – 1,120	2 (4.1%)	11 (22.4%)
Germany	163	26 $^{6}/_{7}$ 25 $^{5}/_{7}$ - 28 $^{3}/_{7}$	916 720 – 1,060	5 (3.1%)	12 (7.4%)
Israel	71	27 $^{4}/_{7}$ 26 $^{4}/_{7}$ - 29 $^{0}/_{7}$	980 870 – 1,120	0 (0.0%)	10 (14.1%)
Netherlands	106	27 $^{0}/_{7}$ 25 $^{5}/_{7}$ - 28 $^{1}/_{7}$	888 748 – 1,021	12 (11.3%)	7 (6.6%)
Norway	24	27 $^{2}/_{7}$ 25 $^{5}/_{7}$ - 28 $^{6}/_{7}$	921 785 – 1,229	0 (0.0%)	2 (8.3%)
Turkey	118	28 $^{6}/_{7}$ 27 $^{3}/_{7}$ - 29 $^{4}/_{7}$	1,135 959 – 1,290	5 (4.2%)	14 (11.9%)
United Kingdom	265	27 $^{2}/_{7}$ 26 $^{0}/_{7}$ - 28 $^{6}/_{7}$	920 772 – 1,164	6 (2.3%)	34 (12.8%)

Table 2 Urinary NTproBNP concentrations and urinary NTproBNP / creatinine ratios (UNBCR) in survivors without ROP requiring treatment, survivors who had severe ROP requiring treatment (laser or intravitreal injection), and infants who died. Numbers represent median and interquartile range.

	Survivors, no ROP treatment (n=837)	**Survivors, ROP treatment** (n=94)	**Died** (n=36)	p
NTproBNP (pg/ml)				
DOL14	**816** 290-3,078	**3,083** 1,193-17,393	**8,950** 1,925-23,783	<0.001
DOL28	**408** 162-1,126	**1,671** 254-11,340	**2,203** 611-4,063	<0.001
UNBCR ($*\ 10^{-4}$)				
DOL14	**76** 26-297	**494** 106-2,064	**971** 142-2,359	<0.001
DOL28	**34** 14-99	**147** 26-1,147	**218** 94-797	<0.001

Table 3 Urinary NTproBNP concentrations and NTproBNP/creatinine ratios (UNBCR) in preterm infants with various stages of ROP (median, interquartile range).

	No ROP	ROP 1	ROP 2	ROP 3	p
NTproBNP (pg/ml)					
DOL14	**634** 248-2,187	**796** 325-2,537	**1,929** 700-8,502	**3,614** 1,109-15,619	<0.001
DOL28	**340** 144-746	**417** 168-1,566	**856** 271-2,545	**1,956** 502-11,884	<0.001
UNBCR ($*\ 10^{-4}$)					
DOL14	**60** 22-191	**79** 29-283	**176** 59-1,000	**514** 108-2,045	<0.001
DOL28	**30** 11-69	**35** 16-122	**82** 24-272	**208** 34-1,446	<0.001

Table 4 Areas under receiver operating characteristics (ROC) curves to predict survival without ROP treatment.

Variable		Area	95% CI	p
NTproBNP				
	DOL14	0.731	0.686 - 0.777	<0.001
	DOL28	0.683	0.622 - 0.745	<0.001
UNBCR				
	DOL14	0.762	0.720 - 0.805	<0.001
	DOL28	0.701	0.641 - 0.762	<0.001
Birth weight		0.743	0.699 - 0.787	<0.001
Gestational age		0.792	0.682- 0.777	<0.001
Proportional weight gain from birth to DOL28		0.570	0.509- 0.631	0.016
Score DOL14		0.745	0.703-0.787	<0.001
Score DOL28		0.739	0.687- 0.791	<0.001

Score DOL14: 300 - 0.156*gestational age(weeks) - 0.118*birth weight (g) + 0.146*NTproBNP DOL14(pg/ml)

Score DOL28: 300 - 0.118*gestational age(weeks) - 0.131*birth weight(g) + 0.067*NTproBNP DOL14(pg/ml) + 0.180* NTproBNP DOL28(pg/ml) - 0.110*proportional weight gain birth to DOL 28 (fraction)