

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

Christoph Bührer¹, Ömer Erdevi^{2,10}, Anton van Kaam³, Angelika Berger⁴, Evelyn Lechner⁵, Benjamin Bar-Oz⁶, Karel Allegaert^{7,10}, Tom Stiris⁸, İstemi Han Çelik^{2,10} and Janet Berrington⁹; on behalf of the REDEXAM study group¹¹

BACKGROUND: To validate the 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 eight European and Middle East countries.

METHODS: Prospective observational study in 967 preterm infants <30 weeks' gestational age assessing the capacity of urinary NTproBNP on days of life (DOLs) 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 with 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% confidence interval 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.

CONCLUSION: 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.

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 manner 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), whereas the pain and stress response to ophthalmoscopy is poorly blunted by oral sucrose (11).

Although a considerable proportion of very preterm infants develop some degree of ROP, only a small fraction progresses 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 used on noninvasive samples by local laboratories.

In response to increased preload 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, N-terminal B-type natriuretic peptide (NTproBNP, amino acids 1–76). Measuring NTproBNP concentrations is widely being used for the 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

¹Department of Neonatology, Charité Universitätsmedizin Berlin, Berlin, Germany; ²Department of Neonatology, Zekai Tahir Burak Maternity Hospital, Ankara, Turkey; ³Department of Neonatology, Emma Children's Hospital, Academic Medical Center Amsterdam, Amsterdam, The Netherlands; ⁴Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Vienna, Austria; ⁵Department of Neonatology, Kepler University Medical Center, Linz, Austria; ⁶Department of Neonatology, Hadassah Medical Center, Jerusalem, Israel; ⁷Department of Development and Regeneration, KU Leuven, Leuven, Belgium; ⁸Department of Neonatal Intensive Care, Oslo University Hospital, Oslo, Norway; ⁹Newcastle Neonatal Service, Royal Victoria Infirmary, Newcastle upon Tyne, UK; ¹⁰Current affiliation: Department of Neonatology, Ankara University School of Medicine, Ankara, Turkey (Ö.E.); Intensive Care and Department of Pediatric Surgery, Erasmus Medical Center, Sophia Children's Hospital, Rotterdam, The Netherlands (K.A.); Etlik Zübeyde Hanım Women's Diseases Education and Research Hospital, Ankara, Turkey (İ.H.Ç.). Correspondence: Christoph Bührer (christoph.buehrer@charite.de)

¹¹Further members of the REDEXAM study group are listed above References.

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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 with 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, REDucing Eye EXAMinations in preterm infants) in neonatal intensive units in eight European and Middle East countries.

METHODS

Study Setting

Neonatal intensive care units are located 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 who 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 -80°C 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 analyzer, Roche Diagnostics International, Rotkreuz, Switzerland) as described previously (17), whereas 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 by birth weight.

ROP Outcomes

ROP-screening examinations using serial binocular indirect ophthalmoscopy were carried out by experienced local ophthalmologists according to local and national guidelines and were 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 analyzed using SPSS 23.0 (SPSS, Chicago, IL). As NTproBNP results and other variables lacked a normal distribution, data are presented as median and interquartile range. 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 two groups) or the Kruskal-Wallis test (more than two groups, followed by *post hoc* analysis employing the Mann-Whitney *U*-test). For paired samples, the Wilcoxon test was used. 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, $n = 118$; the 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 (interquartile range) gestational age was $27^3/7$ ($26^1/7 - 28^6/7$) weeks and birth weight was 945 (780–1,165) g. The actual age at which urine was collected for DOL14 samples ($n = 904$) was 14.35 ± 1.37 days (mean \pm SD) and 28.33 ± 1.68 days for DOL28 samples ($n = 839$). In all, 36 infants died before discharge. Among the survivors, 550 infants were without 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 with 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 (815–1,180) g, $P < 0.001$), and lower proportional weight gain between birth and DOL28 (31.4 (19.0–41.9)% vs. 36.1 (24.8–48.1)%, $P = 0.007$) but not between birth and DOL14 ($P = 0.080$). The sex ratio, multiple vs. singleton pregnancy, and maternal age did not differ between survivors with or without ROP treatment ($P > 0.1$). Patient characteristics by country are given in [Table 1](#).

Urinary NTproBNP Concentrations and UNBCR

Baseline data. Urinary NTproBNP concentrations declined from median 992 (330–4,082) pg/ml on DOL14 to 470 (170–1,465) 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) $\times 10^{-4}$, $P = 0.018$) 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), $P = 0.195$).

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%)
The 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%)

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 with survivors without ROP intervention (n = 837; **Table 2**).

Survivors with no/little ROP (stage 0 or 1) had lower urinary NTproBNP concentrations and UNBCRs 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 threefold in infants with ROP stage 2 and approximately fivefold in infants with ROP stage 3, as compared with the levels in 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 ROC curves (**Table 4**). ROC areas of NTproBNP or UNBCR to predict survival without ROP treatment were larger on DOL14 than on DOL28, whereas 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 significantly different from chance in Norway (0.432).

The three variables that differed significantly between infants who did and those who did not survive without ROP treatment and that were known on DOL14 (gestational age, birth weight, and 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 on DOL28 (gestational age, birth weight,

Table 2. Urinary NTproBNP concentrations and UNBCR in survivors without ROP requiring treatment, survivors who had severe ROP requiring treatment (laser or intravitreal injection), and infants who died

	Survivors, no ROP treatment (n = 837)	Survivors, ROP treatment (n = 94)	Died (n = 36)	P
<i>NTproBNP (pg/ml)</i>				
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
<i>UNBCR (×10⁻⁴)</i>				
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

DOL, day of life; NTproBNP, N-terminal B-type natriuretic peptide; ROC, receiver operating characteristics; ROP, retinopathy of prematurity; UNBCR, urinary NTproBNP/creatinine ratio.

Numbers represent median and interquartile range.

NTproBNP DOL14, NTproBNP DOL28, and proportional weight gain DOL28). All factors were retained in the final equations. Linear coefficients (β) 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 on DOL14 were 300 (208–636) vs. 656 (341–2,753) vs. 1,539 (464–3,627) in survivors without ROP treatment, survivors with ROP

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

	No ROP	ROP 1	ROP 2	ROP 3	P
<i>NTproBNP (pg/ml)</i>					
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
<i>UNBCR (×10⁻⁴)</i>					
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

DOL, day of living; NTproBNP, N-terminal B-type natriuretic peptide; ROP, retinopathy of prematurity; UNBCR, urinary NTproBNP/creatinine ratio.

Table 4. Areas under ROC curves to predict survival without ROP treatment

Variable	Area	95% CI	P
<i>NTproBNP</i>			
DOL14	0.731	0.686–0.777	<0.001
DOL28	0.683	0.622–0.745	<0.001
<i>UNBCR</i>			
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

CI, confidence interval; DOL, day of life; NTproBNP, N-terminal B-type natriuretic peptide; ROC, receiver operating characteristics; ROP, retinopathy of prematurity; UNBCR, urinary NTproBNP/creatinine ratio.

Score DOL14: $300 - 0.156 \times \text{gestational age (weeks)} - 0.118 \times \text{birth weight (g)} + 0.146 \times \text{NTproBNP DOL14 (pg/ml)}$.

Score DOL28: $300 - 0.118 \times \text{gestational age (weeks)} - 0.131 \times \text{birth weight (g)} + 0.067 \times \text{NTproBNP DOL14 (pg/ml)} + 0.180 \times \text{NTproBNP DOL28 (pg/ml)} - 0.110 \times \text{proportional weight gain birth to DOL 28 (fraction)}$.

treatment, and infants who died, respectively ($P < 0.001$), and scores on DOL28 were 212 (315–666) vs. 851 (353–3,930) vs. 1,247 (482–2,511), respectively ($P < 0.001$).

No infant with a score of 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.

Although 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 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 insulin-like growth factor 1 levels, we hypothesize that circulatory compromise and increased BNP release during the first month of life have 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), whereas 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 used 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 standardization of ROP outcomes by central review of ophthalmoscopic 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 analyzed 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. Although overall predictive power of NTproBNP measurements was lower than that 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 generalizability with proportional weight gain (20,21) or risk factor-based models (22,24). In contrast to the expectations underlying this multinational endeavor, 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, whereas low levels are 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.

REDEXAM STUDY GROUP

Sinan Abu-Leil¹², Benjamin Bausenhardt¹³, Christoph Czernik¹⁴, Uğur Dilmen^{15,21}, Nicholas Embleton¹⁶, Zivanit Ergaz-Shaltiel¹², Lara

Garabedian¹⁷, Irene Lok¹⁸, Tone Nordvik¹⁹, Wes Onland¹⁸, Øivind Ringen¹⁹, Thomas Skeath¹⁶, Oliver Wagner²⁰

¹²Hadassah Medical Center, Jerusalem, Israel; ¹³Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Vienna, Austria; ¹⁴Charité Universitätsmedizin Berlin, Berlin, Germany; ¹⁵Zekai Tahir Burak Maternity Hospital, Ankara, Turkey; ¹⁶Royal Victoria Infirmary, Newcastle upon Tyne, UK; ¹⁷Department of Development and Regeneration, Catholic University of Leuven, Leuven, Belgium; ¹⁸Emma Children's Hospital, Academic Medical Center Amsterdam, Amsterdam, The Netherlands; ¹⁹Oslo University Hospital, Oslo, Norway; ²⁰Kepler University Medical Center, Linz, Austria.

²¹Deceased.

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