

Plasma levels of osteopontin from birth to adulthood

Nasenien Nourkami-Tutdibi¹  | Norbert Graf² | Rita Beier³ | Michael Zemlin¹ | Erol Tutdibi¹ 

¹Hospital for General Pediatrics and Neonatology, Saarland University Medical Center, Homburg/Saar, Germany

²Hospital for Pediatric Oncology and Hematology, Saarland University Medical Center, Homburg/Saar, Germany

³Hospital for Pediatric Oncology and Hematology, Hannover Medical School, Hannover, Germany

Correspondence

Nasenien Nourkami-Tutdibi, Hospital for General Pediatrics and Neonatology, Saarland University Medical Center, Building 9, 66421 Homburg/Saar, Germany.
Email: nasenien.nourkami@uks.eu

Abstract

Aim: Osteopontin (OPN) has been investigated as a biomarker for cancer and nonmalignant diseases during the last decades. Data about OPN as a potential biomarker in childhood diseases are still sparse, and reference values are not available in children. We aimed to establish reference values for children from birth to young adulthood and evaluate whether there are age-, gender-, and weight-specific differences.

Method: Umbilical cord blood and blood plasma samples of 117 children were collected in the Children's Hospital of Saarland University in Homburg/Saar. OPN levels were measured by ELISA, and statistical analysis was performed using SPSS software.

Results: Neonates, infants, toddlers, young children, adolescents, and adults were divided into the following six age groups: newborns (birth), infancy and toddlers (0-24 months), early childhood (3-6 years), middle childhood (7-11 years), adolescence (12-18 years), and adults (> 18 years). Highest blood OPN levels were found in the group of 0-1 years of age. OPN blood levels declined significantly with age (Spearman $r = -0.874$; $P < 0.001$).

Conclusion: Our work is the first prospective and systematic study analyzing OPN cord blood and blood plasma levels in children of all ages. It is the first study yielding reference values for different age groups from birth to young adulthood. Our data give insight on how OPN in umbilical cord blood and OPN in blood plasma are physiologically influenced during childhood development and growth with high OPN levels after birth and a constant age-related decline until the age of 14, when OPN levels reach similar values to those measured in adults.

KEYWORDS

biomarker, blood plasma, cord blood, oncogene, osteopontin, pediatric reference values

1 | INTRODUCTION

For the establishment of diagnostic or prognostic biomarkers, reference ranges need to be established. New proteins gain more attention as biomarkers in monitoring different cancer types.^{1,2} Osteopontin (OPN), a multifunctional glycosialoprotein, is involved in several physiological processes such as bone formation, angiogenesis, apoptosis, inflammation, biomineralization, cell viability, and immune response.^{3,4}

OPN acts on organisms by playing a key role in secretion levels of interleukin-10, interleukin-12, interleukin-3, interferon- γ , integrin α v β 3, nuclear factor kappa, macrophage, and T cells, regulating the osteoclast function and affecting CD44 receptors.^{5,6}

First described 1979 by Senger et al.,⁷ numerous studies were published with OPN being investigated in blood and tissue of patients with cancer and noncancer diseases.^{8,9} OPN is often described as an oncogene or cancer metastasis gene having a high potential as an

Abbreviations: BALB, bone alkaline phosphatase; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked Immunosorbent Assay; OPN, osteopontin; PDA, patent ductus arteriosus; PINP, procollagen type I; s-CTX, serum C-telopeptide of type I collagen.

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independent prognostic blood biomarker in various types of cancers.^{10,11} Studies comparing OPN in children with and without cancer are very sparse.¹²⁻¹⁷ As OPN is also a Th2 inflammation-related protein, some studies analyzed the expression of OPN and correlation of OPN to other cytokines in pediatric patients with allergic rhinitis.¹⁸⁻²⁰ A recently conducted study measured OPN levels in pediatric patients with traumatic brain injuries. Here OPN in blood plasma might have a prognostic and predictive value on pediatric patients with traumatic brain injuries.²¹ However, the potential of OPN as a biomarker in pediatric cancer patients is well accepted.²²

A systematic study of reference values for OPN from the newborn through the adult age is still lacking. Besides gender and age, factors during childhood development are well known to influence blood parameters.²³ Biomarkers and laboratory values in children are subject to age-specific variability.²⁴

The availability of age-specific reference values is indispensable for a valid interpretation of the results of laboratory tests in children, since many biomarkers vary during the physiological development of a healthy child.²⁵⁻²⁷ Most of the challenges encountered when establishing pediatric reference values are related to a growing and developing immune system that influences concentrations of various analytes routinely measured in clinical diagnostic laboratory.²⁸⁻³⁰ Differences in physical size, organ maturity, blood fluid compartments, immune and hormone responsiveness, nutrition, and metabolism are likely to affect normal analyte concentrations, such as biomarkers, in children.^{31,32} Besides this, pediatricians try to limit blood tests to a minimum due to the smaller body blood volume and the often-traumatic experience for children. Hence, clinical investigations for reference values in a pediatric setting are sparse and often done when blood for diagnostic workup has to be taken anyhow. Establishing pediatric reference values for new or existing biomarkers is challenging and requires a more complex approach as there might be significant differences within various age groups over childhood development.³³

The aim of this study was to establish reference values for OPN in umbilical cord blood of term neonates and blood plasma of infants, children, adolescents, and adults. In addition, we wanted to evaluate whether gender or body mass index (BMI; calculated as weight (kg)/[height (m)]²) influences OPN blood plasma levels.

2 | METHODS

2.1 | Sample collection and laboratory methods

This trial followed the tenets of the Declaration of Helsinki and was approved by the local ethics committee (Ärztchamber des Saarlandes, Germany, No.: 159/06). All probes were collected at the Children's Hospital of Saarland University Medical Center, Homburg/Saar, Germany. In newborns the cord blood samples were drawn from the umbilical cord vein immediately after birth. For ethical reasons, as blood tests are often a traumatic experience for children, blood samples were only collected in hospitalized children during routine and necessary blood tests (Supporting Information Table S1 lists the

distribution of study participants according to diagnostic subgroups). Inclusion criteria were the need for a venipuncture or intravenous drip placement for diagnostic procedures or treatment. A total of 181 blood samples were collected and informed consent was obtained upfront from parents for each blood sampling. Overall 37 newborns, 125 children, and 19 adults were included in the study. All blood probes were taken into 1.3 mL K2-ethylenediaminetetraacetic acid (EDTA) tubes (Sarstaedt, Nümbrecht, Germany) and stored at 8°C. Within 30 minutes after drawing, samples were centrifuged at 1000 × g for 10 minutes, and removed plasma was aliquoted and stored at -80°C until further use. The concentration of soluble OPN in plasma was determined by the enzyme-linked immunosorbent assay method (ELISA). We used the Human Osteopontin ELISA kit (product code 27158, Immuno-Biological Laboratories Co. Ltd, Gumma, Japan), which has, according to our measurements, an inter-assay coefficient of variation of 10.6% and an intra-assay coefficient of variation of 3.2%. To reduce analytical variation, all samples were measured in duplicates, and results scattering more than 10% were reanalyzed.

2.2 | Study participants and data collection

This is a cross-sectional study of neonates, children, adolescents, and adults. Pediatric participants were consecutively enrolled while attending at the Children's Hospital of Saarland University, Homburg. All neonates included in the study were healthy and term newborns (gestational age ≥ 37 completed weeks) with normal postnatal adaptation delivered at the Department of Obstetrics and Gynecology, University of Saarland. Only newborns without any signs of maternal and neonatal infection were included. Our adults were voluntary study participants and healthy employees at the Children's Hospital of Saarland. To set up a representative reference collective of pediatric patients, our probes have been sampled from children who had to have a blood test for diagnostic workup.

Blood samples from in-house patients were collected after all signs of infection were absent for at least 48 hours (C-reactive protein < 10 mg/dL, no rash or fever and clinical well-being). Here, blood was sampled within last diagnostic workup upfront to same-day discharge. Baseline characteristics such as age, gender, body weight, and height were taken from the patient's chart as well as all information regarding principal and additional diagnosis, treatment plan, and supplementary laboratory data.

A priori exclusion criteria of patients were defined on the basis of diseases and conditions possibly influencing the synthesis or regulation of OPN. These were cancer, metabolic, muscle, bone, renal, cardiac, autoimmune, and allergic diseases. Prematurity, severe acute infection, organ failure, and genetic syndromes have also been excluded from the reference group. The following diagnoses were accepted to be part of the reference group: state after cured infection, benign and self-limiting abdominal pain, migraine, cerebral contusion, coagulation defect, history of alcohol intoxication, benign epilepsy, cognitive or speech developmental delay, minor surgical interventions, diagnostic procedures (e.g., short stature workup, magnetic resonance imaging, or endoscopy) and laboratory test results within the

reference limits. In total, 117 individuals were included to final analyses based on the above criteria (see Supporting Information Table S1).

To cover the different periods of human growth and development, the reference group was divided into six subgroups: newborns (birth), infancy and toddlers (0-24 months), early childhood (3-6 years), middle childhood (7-11 years), adolescence (12-18 years), and adults (> 18 years).

2.3 | Statistical analysis

Statistical analysis was performed using SPSS software, IBM SPSS Statistics Version 20 for Windows (IBM Corporation, Armonk, NY). All tests were two-sided. Differences with $P < 0.05$ were considered significant. Descriptive statistics for continuous variables were expressed as the mean with standard deviation or medians with minimum and maximum, and for categorical variables as absolute frequencies with percentages using original untransformed values. Values of OPN were analyzed for their normal distribution by the Kolmogorov-Smirnov test and normal Q-Q plots. Natural logarithmic transformation of data was used for statistical analysis when needed. Differences among more than two independent groups were analyzed by nonparametric Kruskal-Wallis H test. Differences of OPN levels between two independent groups were assessed by nonparametric Mann-Whitney U test. In order to assess the relationship between OPN plasma levels and age in the reference group, Pearson correlation coefficient was determined. The association between height, weight, BMI, body surface area, and OPN plasma concentrations were tested by partial correlation while controlling for the effect of age. After verification of normal distribution and exclusion of outliers by applying the Tukey method, we derived OPN reference intervals by nonparametric methods and data were presented as median with 2.5th to 97.5th percentile ranges. Smoothed reference curves of OPN levels were constructed by the Lambda-Mu-Sigma (LMS) method of Cole and Green, using the LMS Chartmaker Light version 2.43 (Medical Research Council, UK).³⁴

3 | RESULTS

3.1 | Characteristics of participants

Blood samples of 117 patients were included (53.4% male and 46.6% female). Table 1 summarizes the distribution by age group and gender. Except for adults, gender was equally distributed over all age groups ($P < 0.08$). The participants' median age was 6.6 (0.0-50.9) years and was not normally distributed ($P < 0.001$). There was no significant age difference between male versus female participants (children: 4.1 (0-18.9) years, $P < 0.68$ and adults: 30 (21.1-50.9) years, $P < 0.23$).

In total, 36 healthy newborns were included in the study. All newborns had normal Apgar scores at five minutes and normal umbilical artery pH (perinatal characteristics of newborns are presented in Supporting Information Table S2). The OPN values were normally distributed ($P < 0.20$). Mean OPN blood plasma level in cord blood was 2300 ± 552 ng/mL. No significant association was seen between OPN

TABLE 1 Distribution and age of participants by age group and gender

Participants	Age group	Male	Female	Total
		n (%)	n (%)	
Newborns	Birth	23 (64%)	13 (36%)	36
		38 (37-41) ^b	38 (37-41) ^b	0.845
Children	0-2 years	2 (29%)	5 (71%)	7
		0.9 (0.1-1.6) ^c	2.4 (0.1-2.8) ^c	0.381
	3-6 years	12 (67%)	6 (33%)	18
		5.0 (3.4-6.9) ^c	5.3 (3.7-6.7) ^c	0.999
	7-11 years	15 (79%)	4 (21%)	19
10.0 (7.6-11.7) ^c		10.1 (7.9-11.3) ^c	0.999	
12-18 years	9 (50%)	9 (50%)	18	
	14.3 (12.5-17.5) ^c	16.8 (12.0-18.9) ^c	0.052	
Adults	>18 years	2 (11%)	17 (89%)	19
		40.1 (35.0-45.2) ^c	29.2 (21.1-50.9) ^c	0.351
Total		63 (53.4%)	54 (46.6%)	117

Data are given as absolute frequencies with percentages, n (%).

^aTested by Mann-Whitney test, significant for $P < 0.05$.

^bGestational age in completed weeks as median with minimum and maximum.

^cAge is given in years as median with minimum and maximum.

values and gender or any other perinatal parameters (mode of delivery, gestational age, Apgar score at five minutes, umbilical artery blood pH, birth weight, and fetal growth).

To determine possible differences in plasma OPN level, diagnostic subgroups within the included children group ($n = 63$) were compared by nonparametric Kruskal-Wallis analysis of variance. Diagnostic subgroups were not statistically different ($P < 0.47$) and were pooled together as total reference group.

3.2 | OPN blood plasma levels

OPN plasma concentrations did not show any association with weight, height, BMI, and body surface area when tested by partial correlation while controlling for the effect of age. Overall, we found an age-dependent variation of OPN blood plasma levels and could demonstrate that OPN in blood decreased significantly with growing age irrespective of gender (Spearman $r = -0.874$; $P < 0.001$). Before the calculation of reference values for OPN, the applied Tukey method identified two outliers: Patient 1 (OPN level 1429 ng/mL) was a 10.2-year-old child with neurological signs and symptoms after cerebral contusion. Patient 2 (OPN level 160 ng/mL) was a 12.0-year-old female with known von Willebrand factor syndrome and congenital cholesteatoma, who underwent mastoidectomy. Figure 1 shows the distribution of OPN blood levels according to age groups. OPN plasma level differed significantly between all six age groups (P from < 0.001 to 0.002) except for toddlers versus early childhood, middle versus early childhood and adolescence versus middle childhood.

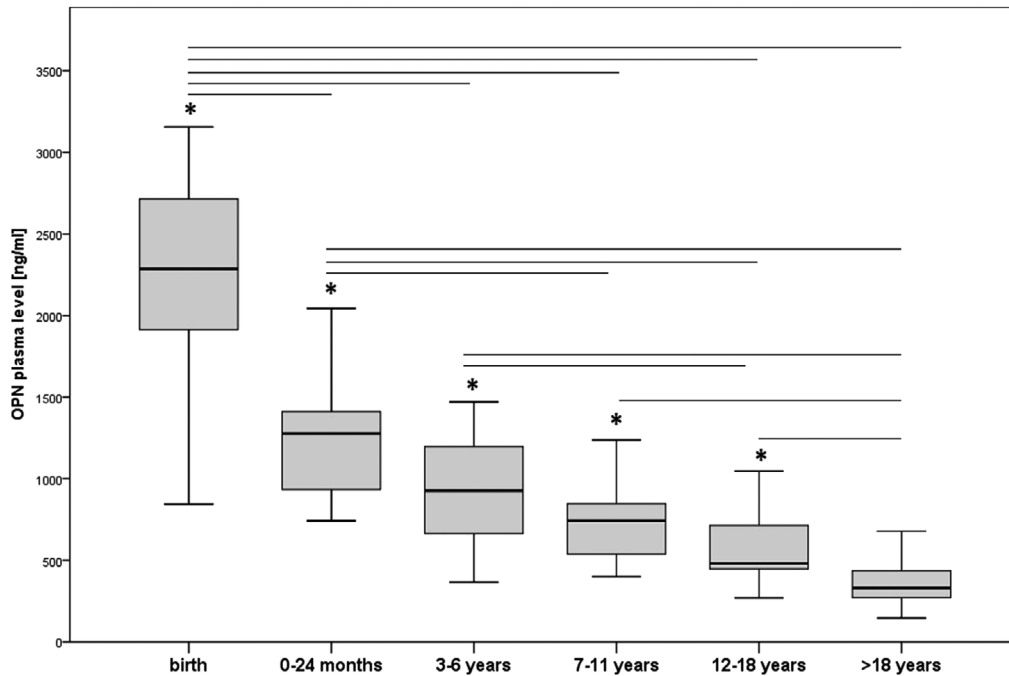


FIGURE 1 Distribution of OPN plasma levels according to age groups. Box plots represent interquartile range, whiskers the range, and horizontal lines represent the median; *Significant at $P < 0.0033$ (0.05/15) tested by Mann-Whitney U test

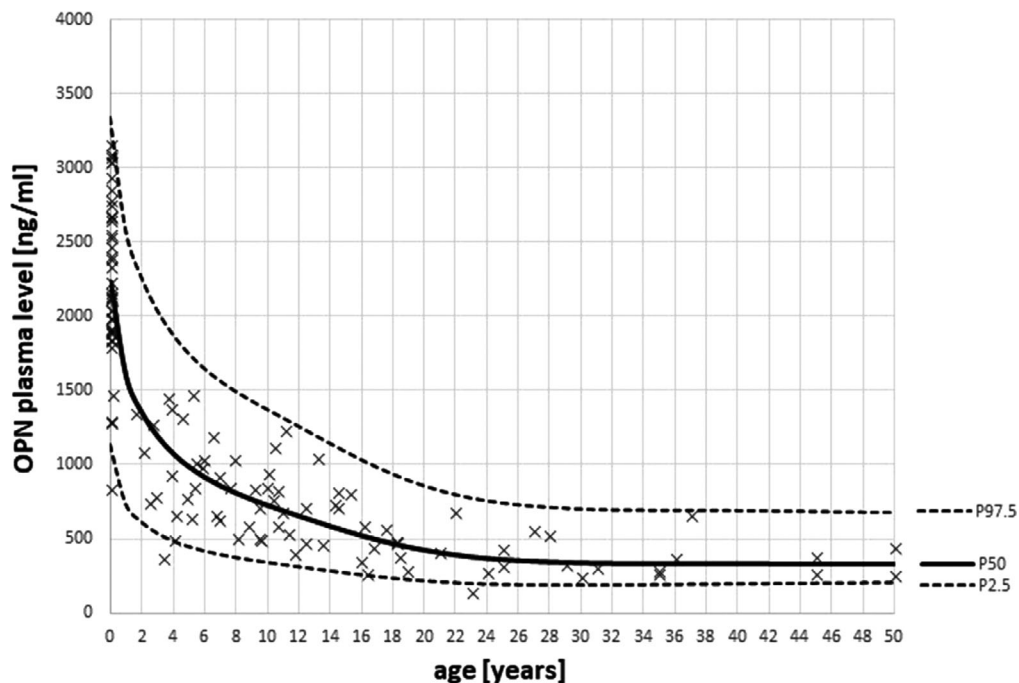


FIGURE 2 Reference ranges for OPN level in children and adults

As no difference was found between plasma OPN levels of male and female participants of all age groups, both genders were combined for the calculation of percentiles. LMS analysis revealed smoothed reference curves of OPN levels as shown in Figure 2. OPN values progressively decreased with age, particularly during infancy and toddlerhood. The percentile curve showed a great variance at birth with narrowing reference ranges toward adulthood.

4 | DISCUSSION

Here we report reference values for OPN measured in the umbilical cord blood of term neonates and blood plasma of infants, children, adolescents, and adults. This is the first study prospectively establishing reference values for OPN in a pediatric setting from birth to 20 years of age. Our data show that OPN is independent of gender

and BMI groups. We were able to demonstrate that OPN values in blood plasma are age dependent. One major finding of this trial is that OPN levels reach a maximum in umbilical cord blood and during the neonatal period of healthy term babies. In addition, our data show a significant and constant decline of OPN blood plasma levels over all age groups beyond the age of one year. We could demonstrate that OPN blood plasma levels decrease with age until they reach similar values to those measured in adults after the age of 14 years.^{35,36}

The neonatal/postnatal peak of OPN expression may be due to an increased bone turnover.^{37,38} Joung et al. showed that OPN in cord blood is significantly higher than in adults.³⁹ As they did not investigate children of other age groups, they were not able to discriminate between the rise and decline of OPN after newborn period, but they were able to show that there is an inverse correlation between OPN and gestational age. In the preterm cohort, they were also able to demonstrate that patent ductus arteriosus was independently associated with higher OPN values.⁴⁰ This is not surprising, as recently published data once again indicated OPN and its potential role in cardiac disease.^{41,42}

Other markers for bone turnover such as osteocalcin and procollagen type I show a similar peak of OPN within the first year of life.⁴³ In contrast, markers such as serum C-telopeptide of type I collagen and bone alkaline phosphatase show peak values during and a constant decline after puberty.⁴⁴ Although OPN plays an important role in bone turnover, an increase according to pubertal stage could not be seen within our data.

Age-related declines of biomarkers are a typical phenomenon during childhood, as demonstrated by Caselli et al. for adiponectin and brain natriuretic peptide.⁴⁵ In addition, many other studies establishing reference values in a pediatric setting observed interesting patterns for several analytes, proteins, or blood components in newborns, including high levels during the neonatal period.²⁶ These circumstances might be due to adaptational processes in the early neonatal period. For many years, the CALIPER program of the University of Toronto, Sick Kids Hospital, investigates the influence and interpretation of laboratory values in children. The CALIPER program is a national research initiative addressing pediatric reference values²⁸⁻³⁰

Studies analyzing OPN in a pediatric setting of nonmalignant diseases matching healthy controls are very sparse. Only a few studies have been published in the past.^{14,15} Two studies measured OPN in healthy cohorts for baseline assessment reflecting one age group without covering children of other age groups.^{39,46} Our study is the first to cover all age groups, from birth to young adults. OPN in serum yields lower values than those of plasma samples, as OPN is cleaved by thrombin after blood coagulation. Serum and plasma OPN though is highly correlated to each other,⁴⁷ according to Lanteri et al., plasma values are 3.8- to 4.8-fold higher than serum.⁴⁸

Al-Ayadhi et al. measured a mean serum OPN level of 122.3 ± 39.2 ng/mL in 42 healthy subjects with a mean age of 7.7 ± 2.5 years.¹⁶ When multiplying these values with a factor of 3.8 or 4.8, a mean

OPN value of 463.7 ng/mL or 587.0 ng/mL would be the concentration in plasma, respectively. These values are in good correlation with our mean values evaluated for children between 1 and 14 years of age. On the contrary, significantly lower OPN values were found in healthy controls within the two studies conducted by Honsawek et al. Numbers of controls were low in both studies, though with 10 and 13 children, respectively, and a mean age of 7.4 ± 3.9 and 8.3 ± 1.1 years.^{49,50} Akalema et al. analyzed OPN serum levels in children with asthma disease and 42 healthy controls with a mean age of 3.0 ± 1.9 years, measuring far lower OPN values in the cohort of healthy controls than those reported in our cohort.⁵¹ These might be due to the use of different OPN assays as well as the fact that OPN was measured in serum and not in blood plasma. The data may be biased, even though when adjusting values from sera to blood plasma as recommended by Lanteri et al.

There are a few studies measuring OPN levels in pediatric patients with type I diabetes mellitus (T1DM). Saki et al. analyzed serum OPN levels in a large cohort of patients with T1DM including 87 healthy sex- and age-matched controls. The mean age in healthy controls was 12.6 ± 5.6 years, and mean OPN levels were 13.8 ng/mL,⁵² correlating with OPN values in the blood plasma of 52.5 or 66.3 ng/mL, respectively, when multiplying with 3.8 or 4.8. Values are by far lower than those measured in healthy children of the corresponding age group in our study. Here, the use of different assays might be one cause; different ethnicity of study cohorts has to be kept in mind as well. Another study analyzed data in T1DM patients and matched healthy controls in sera that were 10-fold higher and more comparable with our results.⁵³

Talat et al. also measured serum OPN levels in T1DM patients including 60 healthy age-matched controls. The mean age in their control group was 12.0 ± 2.2 years, and mean OPN levels were 89 ± 29 ng/mL.⁵⁴ These values correlate with plasma OPN levels of 338.2 ± 110.2 ng/mL and 427.2 ± 140 ng/mL, respectively, and are in good accord with our OPN values of the same age group. Abo El-Asrar et al. measured serum OPN levels in patients with T1DM and matched healthy controls, which are also in good accord with our data. The mean age of the control group ($n = 40$) was 12.1 ± 2.9 years with mean OPN values of 90 (50-120) ng/mL. According to Lanteri et al., the OPN values in plasma should then be 342 (190-456) or 432 (240-576) ng/mL, respectively. These values are comparable with our values in healthy children, although El-Asrar et al. did not divide the cohort of controls with a range of 6-17 years into smaller age groups.⁵⁵

A large study conducted by Schreiber et al. analyzed OPN levels in tissue and serum samples of lean and obese children. The authors were able to demonstrate that OPN is a BMI independently related marker of early endothelial dysfunction on children.⁵⁶ The median OPN levels in lean, healthy children were 135 ng/mL. According to Lanteri et al., these values correspond to 513 or 648 ng/mL, respectively (mean age 12.9 ± 2.9 years), and are in good accord with our data. Schreiber et al., though, demonstrated an elevation of OPN during puberty, which is not supported by our data.

Table 2 provides an overview of OPN levels measured in umbilical cord blood, serum, and blood plasma of research studies conducted

TABLE 2 Research studies on OPN in a pediatric setting

Year	Author	Disease research topic	Sample type	Healthy controls (n)	Age (years)	OPN value (ng/mL)	Study collective (n)	Age (years)	OPN value (ng/mL)
2018	Chew ⁴⁶	Healthy subjects	Plasma	33	14.7 (13.4-16.0)	54.5 (19.8-88.4)	n.a.	n.a.	n.a.
2018	Abo El-Asar ⁵⁵	Type I diabetes mellitus (T1DM) ± microvascular complications	Serum	40	12.1 ± 2.9	90 (50-120)	87	11.5 ± 3.3	T1DM ⁺ : 120 (80-140) T1DM ⁻ : 65 (45-90)
2017	Saki ⁵²	Type I diabetes mellitus	Serum	87	12.6 ± 5.3	13.8 ± 6.3	87	12.0 ± 4.4	14.2 ± 7.4
2016	Talat ⁵⁴	Type I diabetes mellitus	Serum	60	12 ± 2.2	89 ± 29	60	11.8 ± 2.2	130.7 ± 30.4
2016	Schreier ⁵⁶	Lean healthy children vs obese children	Serum	65	12.9 ± 2.9	135 (75-175) ^a	87	11.8 ± 2.0	125 (75-150) ^a
2014	Oh ¹⁷	Langerhans cell histiocytosis ± Multisystem disease (MS) Single-system disease (SS)	Serum	12	<3 years	74 (42-100)	8	<3 years	MS ⁺ : 240 (138-456) MS ⁻ : 93 (62-214) SS: 74 (56-94)
				15	>3 years	42 (29-54)	42	>3 years	MS ⁻ : 56 (23-78) SS: 59 (31-79)
2014	Joung ⁴⁰	Term vs preterm neonates	Umbilical cord blood	147	≥37 weeks	372 ± 109	114	35-36 weeks	467 ± 149
2013	Karamizade ⁵³	Type I diabetes mellitus	Serum	86	m: 8.2 ± 3.9 f: 9.1 ± 3.2	40.9 ± 23.8 (m + f)	87	m: 8.2 ± 3.9 f: 9.1 ± 3.2	49.0 ± 22.3 (m + f)
2013	Akelma ⁵¹	Asthma	Serum	42	3.0 ± 1.9	6.0 (2.3-8.2)	51	3.7 ± 2.0	6.9 (2.4-14.5)
2012	Taranta-Janusz ¹⁴	Solitary functioning kidney	Plasma	21	11.5	4.3 (1.0-16.0)	51	11.5	6.8 (1.8-41.4)
2011	Al-Ayadhi ¹⁶	Autism	Serum	42	7.8 ± 2.5	122.3 ± 39.2	42	7.8 ± 2.5	197.1 ± 48.8
2011	Honsawek ⁵⁰	Biliary atresia	Plasma	13	8.3 ± 1.1	27.5 ± 6.4	59	8.2 ± 0.4	146.9 ± 19.1
2010	Honsawek ⁴⁹	Liver fibrosis and biliary atresia	Plasma	10	7.4 ± 3.9	15.1 ± 15.0	30	7.2 ± 3.4	47.0 ± 56.4

Values are given as mean ± standard deviation or median (25th-75th percentiles).

^aValues are derived from boxplot; m = male and f = female; n.a. = not applicable.

in a pediatric setting of different types of diseases within the last decade. Most of the studies listed below do have small cohorts of healthy controls. Some do have higher numbers of healthy controls, but none analyzed OPN values in a pediatric setting, including all age groups. Studies that analyzed OPN values in cerebrospinal fluid, urine, tear, and/or tissue samples are not listed below. Only studies with healthy controls and data about patient's age are presented in Table 2.

Finally, we also measured OPN levels in 19 healthy adult volunteers. The mean OPN level was 330 ng/mL, which shows good correlation with published literature.^{35,36}

Our study has several limitations. First, we did not use blood samples from healthy nonhospitalized children, as blood sampling is most often associated with a traumatic experience for children. This is a current and constant problem faced when trying to establish reference values for pediatric patients. Pediatric values have always been most challenging.

For that purpose, many laboratories use an entire hospitalized population and remove outliers to establish reference values.⁵⁷ Therefore, pediatric cohorts and reference values often vary from laboratory to laboratory.⁵⁸

5 | CONCLUSION

Our study is the first systematic analysis, setting up reference values for OPN over all age groups within a pediatric setting with cord blood of term neonates and blood plasma in infants, toddler, young children, adolescents, and adults. We could clearly demonstrate that OPN blood plasma levels are significantly age dependent and not related to any other variables such as gender, weight, or BMI. We need further studies with a larger number of patients to verify our data. In addition, to clarify the role of OPN in the blood plasma of children with different cancer types, further investigations are needed.

AUTHOR CONTRIBUTION

Nasenien Nourkami-Tutdibi, Erol Tutdibi, Norbert Graf, and Rita Beier designed the research. Nasenien Nourkami-Tutdibi and Erol Tutdibi coordinated data collection, performed the OPN analyses and analyzed the data. Nasenien Nourkami-Tutdibi, Erol Tutdibi, Norbert Graf, Rita Beier, and Michael Zemlin interpreted the results. Nasenien Nourkami-Tutdibi wrote the manuscript. All authors reviewed the manuscript.

CONFLICTS OF INTEREST

All the authors have no conflict of interest to declare.

DATA SHARING STATEMENT

For original data, please contact Nasenien Nourkami-Tutdibi (nasenien.nourkami@uks.eu).

ORCID

Nasenien Nourkami-Tutdibi 

<https://orcid.org/0000-0003-4240-1868>

Erol Tutdibi  <https://orcid.org/0000-0002-1866-4492>

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SUPPORTING INFORMATION

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

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