Total IgE assessment in newborn umbilical cord blood

Mariana VIERU1,2, Anca Angela SIMIONESCU1,3, Ana Maria Alexandra STĂNESCU4, Vlad DIMA5, Florin-Dan POPESCU1,2

1 “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania
2 Department of Allergology, “Nicolae Malaxa” Clinical Hospital, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania
3 Department of Obstetrics and Gynecology, Filantropia Clinical Hospital, Bucharest, Romania
4 Department of Family Medicine, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania
5 Department of Neonatology, Filantropia Clinical Hospital, Bucharest, Romania

ABSTRACT

In this minireview we present important data on umbilical cord blood IgE antibodies in neonates and total IgE as a predictive biomarker for the development of allergen sensitization and atopic diseases later in life. Discussions regarding the methods for determining total IgE in serum or plasma from cord blood samples are focused on the main immunoassays used in different studies and clinical practice. The fluorescence enzyme immunoassay with anti-IgE covalently coupled to a capsulated cellulose polymer solid-phase is nowadays currently used to measure total IgE in human serum or plasma. The umbilical cord blood total IgE levels are quantitatively determined by using its low range assay. Specific and practical aspects regarding cord blood sampling, including specimen collection from the umbilical cord vessels and careful preparation of serum and plasma, alongside with knowledge of the principles of the immune methods used are important to avoid preanalytical and analytical errors and for obtaining accurate results of this risk biomarker for allergy.

Keywords: umbilical cord blood, total IgE, immunoassays

INTRODUCTION

The prevalence of allergic and atopic diseases has dramatically increased worldwide during the past decades with a significant healthcare and society burden [1,2]. A defective epithelial barrier hypothesis has been recently proposed to explain this increase [3], together with a biodiversity loss hypothesis that states that the contact with natural environments enriches the human microbiome, promotes immune balance and protects from allergy [4].

IgE antibodies are found in very low concentrations in newborn circulation [5]. As IgE is classically considered not to cross the transplacental barrier, other genetic and environmental factors may influence the neonatal total IgE levels [6-8]. Some gene-gene and gene-environment interactions begin in fetal stage to increase IgE production in neonates [9,10]. The regulation of IgE production may begin in utero, and this may be reflected in the levels of umbilical cord blood (UCB) IgE. Elevated values are considered to be a risk factor for subsequent development of allergic and atopic diseases [11-16], but allergen-specific IgE in UCB may not reflect intrauterine sensitization and may be a result of the maternal IgE transfer to the fetus [17]. Recently it
was shown that IgE molecules from the mother may be transferred to fetus and minimally sensitize fetal/neonatal mast cells [18].

**UMBILICAL CORD BLOOD AS BIOLOGICAL SPECIMEN FOR TOTAL IG E**

UCB is an easily accessible specimen and has been used for searching prognostic biomarkers for allergies, such as regulatory T cells, gene expression of cytokines and IgE levels. Since the seventies, UCB total IgE gained attention and was investigated as a predictive biomarker for allergic and atopic diseases [10,19-21].

Some researchers found that the UCB IgE total levels are higher in male newborns and in the case of cesarean section compare with vaginal birth [16,22-28], but others did not find such correlations or with secondhand smoke [29-30]. Although different studies revealed that elevated UCB total IgE is affected by some other factors related to maternal, placental and fetal characteristics, such findings were inconclusive and difficult to assess due to the complex influences of genetic factors [22,23,31-34].

The levels of total IgE in UCB are frequently below 0.5 kU/l [10,34], but some studies revealed that about 20-25% of newborns present raised UCB IgE levels [35,36]. Elevated UCB total IgE serum levels are considered a risk biomarker for the development of allergen sensitization and atopic manifestations in children. Some previous findings generated conflicting results. Although several trials have reported this biomarker as ineffective in predicting the development of atopic diseases in the first two years of life [37-40], most studies revealed that elevated UCB total IgE levels have a role as biomarker for the future development of allergic diseases [12,15,19,20,24,41,42]. Significant studies indicated that raised UCB total IgE levels in serum may predict early atopic symptoms [43,44], while elevated UCB levels combined with high values in before two years of age may be associated with atopic dermatitis later in childhood [39,45]. Moreover, high UCB total IgE in serum may be a predicting risk factor for developing atopic disease in older children and young adults [12,41,46].

Parents must provide informed consent for UCB sampling [24]. It is very useful to record medical information about the newborn (including sex, birth weight, gestational age, season of birth, delivery mode), mother (maternal age, prepregnancy body mass index, parity, previous pregnancy, atopic diseases, including atopic dermatitis, allergic rhinitis and asthma, and antenatal complications, including pregnancy hypertension, diabetes, infection, intrauterine growth retardation), and environmental factors (household conditions, including prenatal cat/dog exposure, home dampness and environmental tobacco smoke exposure) [10,15,22,47]. A detailed family history of atopic diseases is important to be mentioned, because the combination of elevated UCB total IgE and positive family history of allergy is strongly associated with subsequent atopic manifestations [12].

**METHODS FOR MEASUREMENT OF TOTAL IG E IN UCB SAMPLES**

Total IgE concentrations in UCB samples were initially measured with a paper radioimmunosorbent test (PRIST) with high sensitivity, serum values under 0.5 IU/ml being considered as undetectable [48]. Based on the lower analytical limit of this method and a highly degree correlated solid-phase enzyme immunoassay using monoclonal antihuman IgE designed to measure IgE between 0.2 and 50 kU/l on 0.1 ml of serum or plasma, the total IgE values obtained from umbilical vein blood samples were dichotomized into < 0.5 and ≥0.5 kU/l [49].

When comparing three different UCB sampling techniques for determining neonatal total IgE levels using a modified PRIST method, alongside with serum IgA concentration quantification by a sandwich enzyme-linked immunosorbent assay (ELISA) to assess the contamination of UCB with maternal blood, aspirated UCB from the umbilical vein or capillary collection at 4-5 days of life are preferred. If gravity-collected UCB from the umbilical vein is used (by letting the blood flow freely into a tube), maternal blood contamination should be investigated by determining IgA in all blood samples with IgE concentrations exceeding the cut-off point [50].

Methods using radioisotopes for assessing total IgE in human serum or plasma were replaced by nonisotopic immunoassays or immunonephelometry, currently in use [51].

A biotin-avidin amplified ELISA was used after the years of radioisotopic usage. UCB samples were obtained prior to delivery of the placenta. The umbilical cord was wiped to reduce the potential of maternal blood contamination during collection. The newborn plasma volume was used to allow calculation of the quantity of maternal blood needed to increase the UCB IgE for any given maternal serum IgE concentration [52].

An enzyme immunoassay with streptavidin-peroxidase-conjugate and photometrical reading for the quantitative determination of total IgE in 2 ml of UCB samples was more recently used. Umbilical IgA was measured by immunoturbidimetry in order to eliminate the possibility of mixed maternal blood [53]. Some authors suggest that IgA values higher than 32 μg/ml reflect contamination by maternal blood [54,55], while
others consider an increased UCB IgA level at above 14 μg/ml [50]. In order to ensure that the UCB samples are free from contamination by the maternal blood these levels should be less than 10 μg/ml [56], while others do not consider UCB IgA measurement mandatory due to a very low rate of such contamination [10,14].

An ELISA may be used for total IgE quantification in newborn dried blood spots (NDBS) or cord blood dried blood spots (CBDBS). For the preparation of the CBDBS, 50 μL of UCB is spotted onto a Guthrie card, then dried. The ELISA kit for total IgE contains mAb107 capture antibody, mAb182-biotin detection antibody, streptavidin-horseradish peroxidase and the IgE standard [57].

The fluorescence enzyme immunoassay (FEIA) with anti-IgE covalently coupled to a capsulated cellulose polymer solid-phase (ImmunoCAP) is nowadays currently used to measure total IgE in human serum or plasma. The UCB IgE levels are quantitatively determined by using ImmunoCAP Total IgE Low Range Assay on the Phadia instrument. Venous UCB samples are used. Elevation of UCB IgE levels are considered when greater than 0.5 kU/l [10,13,15,16]. In neonates, the cut-off values for IgE are fixed at 0.35 kU/l [23]. Nephelometry may be used to measure maternal serum total IgE in order to correlate with UCB values [58].

Arterial UCB may also be obtained from neonates for total plasma IgE assessment, and collected in EDTA-treated tubes immediately after delivery of the placenta, preferably alongside with the control blood gases performed on UCB (5 min after birth and immediately after cord clamping). Maternal blood is also collected in sterile tubes immediately prior to delivery (20-30 min before expulsion or before incision in C-section deliveries) for mother-child sample pairing used in correlation studies. The simultaneously quantification of plasma IgE with other immunoglobulins (IgA, IgM, IgG1, IgG2, IgG3, IgG4) from UCB and mothers may be performed by a multiplex immunoassay using a human antibody isotype panel, magnetic microsphere beads and a Luminex instrument platform. This immunoassay sensitivity is 0.003 ng/ml for IgE, and 0.34 ng/ml for IgA, 6.41 ng/ml for IgM, 2.11 ng/ml for IgG1, 16.07 ng/ml for IgG2, 0.08 ng/ml for IgG3, 0.56 ng/ml for IgG4 [59]. For total IgE, one kU/l is equal to 2.4 ng/ml [60,61].

Finally, it is important to underline that total IgE quantification is usually performed in serum or plasma from venous samples or capillary blood. For the serum preparation, whole blood is collected in commercially available tubes (red topped ones) and allowed to clot by leaving it undisturbed at room temperature (usually 15-30 min). Then the clot is removed by centrifugation at 1,000-2,000 x g for 10 min in a refrigerated centrifuge. For the plasma preparation, whole blood is collected into commercially available anticoagulant-treated tubes e.g., EDTA-treated (with lavender-colored tops). Cells are removed by centrifugation at 1500 x g for 10 min at 4°C. The collected plasma is centrifuged again at 2500 x g for 10 min at room temperature to deplete platelets in the plasma sample [59]. Following centrifugation, it is essential to immediately transfer the liquid component, serum or plasma, into a clean polypropylene tube using a Pasteur pipette. For UCB total IgE immunoassays, samples may be obtained from both the umbilical vein and artery. We should keep in mind that it is likely that umbilical cord “arterial” and umbilical cord “venous” samples are obtained from the same type of blood vessel in real practice [62]. Moreover, quality laboratory control is also critical with record keeping for each immunoassay, control specimen and proficiency testing.

**CONCLUSIONS**

Different immunoassays may be used to measure total IgE in serum or plasma from UCB samples in neonates. Serum or plasma preparation should be carefully performed according to protocols. UCB sampling may be performed from umbilical vein and artery, although venous blood is generally used for IgE immunoassays. UCB collected for other diagnostic purposes is usually obtained by allowing blood to drain from the cut end into a glass tube prior to delivery of the placenta, but for total IgE measurement the aspirated samples from the umbilical vein are preferred to the gravity-collected ones. Specific and practical aspects regarding UCB sample collection and preparation alongside with knowledge of the principles of the immune methods used are important to avoid preanalytical and analytical errors and for obtaining accurate results of this predictive risk biomarker for the development of allergic and atopic diseases later in life.
REFERENCES

1. Brough HA, Lanser BJ, Sindher SB, et al. Early intervention and prevention of allergic diseases. Allergy. 2021 Jul 13.
2. O’Connell EJ. The burden of atopy and asthma in children. Allergy. 2004;59 Suppl 78:7-11.
3. Akdis CA. Does the epithelial barrier hypothesis explain the increase in allergy, autoimmunity and other chronic conditions? Nat Rev Immunol. 2021.
4. Haathelta T. A biodiversity hypothesis. Allergy. 2019;74(8):1445-1456.
5. Holt PG, Jones CA. The development of the immune system during pregnancy and early life. Allergy. 2000;55(8):688-697.
6. Avrech OM, Samra Z, Lazarovich Z, et al. Efficacy of the placental barrier for immunoglobulins:correlations between maternal, paternal and fetal immunoglobulin levels. Int Arch Allergy Immunol. 1994;103(2):160-165.
7. Purizaca-Benites M. La placenta y la barrera placentera. Rev Peru Ginecol Obstet. 2008;54:270-278.
8. Rio-Age K, Azagra-Borronat I, Massot-Cladera M, et al. Association of Maternal Microbiota and Diet in Cord Blood Cytokine and Immunoglobulin Profiles. Int J Mol Sci. 2021;22(4):1778.
9. Hua L, Liu Q, Li J, et al. Gene-gene and gene-environment interactions on cord blood total IgE in Chinese Han children. Allergy Asthma Clin Immunol. 2021;17(1):69.
10. Chen M, Gu Y, Liu Q, et al. Relationship of cord blood IgE with maternal, fetal, and environmental factors in the Chinese population. Allergol Immunopathol (Madr). 2021;49(3):50-55.
11. Jacobsen HP, Herskind AM, Nielsen BW, et al. IgE in unselected like-sexed monozygotic and dizygotic twins at birth and at 6 to 9 years of age:high but dissimilar genetic influence on IgE levels. J Allergy Clin Immunol. 2001;107:659-663.
12. Pesonen M, Kallio MJ, Siimes MA, et al. Cord serum immunoglobulin E as a risk factor for allergic symptoms and sensitization in children and young adults. Pediatr Allergy Immunol. 2009;20:12-18.
13. Yang KD, Chang JC, Chuang H, et al. Gene-gene and gene-environment interactions on IgE production in prenatal stage. Allergy. 2010;65:731-739.
14. Hong X, Tsai HJ, Liu X, et al. Does genetic regulation of IgE begin in utero? Evidence from T(H)1/T(H)2 gene polymorphisms and cord blood total IgE. J Allergy Clin Immunol. 2010;126(1059-67):1067.
15. Chen CH, Lee YL, Wu MH, et al. Environmental tobacco smoke and male sex modify the influence of IL-13 genetic variants on cord blood IgE levels. Pediatr Allergy Immunol. 2012;23:456-463.
16. Chen CH, Lee YL, Wu MH, et al. Sex-moderrated interactions between IL4/IL13 pathway genes and prenatal environment on cord blood IgE levels. Clin Exp Allergy. 2019;49:1128-1138.
17. Bannelýkke K, Pipper CB, Bisgaard H. Sensitization does not develop in utero. J Allergy Clin Immunol. 2008;121(3):646-651.
18. Honda Keith Y, Kabashima K. Maternal IgE is transferred to fetuses with IgG and minimally sensitizes fetal/neonatal skin mast cells. J Allergy Clin Immunol. 2021 Jun 25;S0091-6749(21)00905-2.
19. Shah PS, Wegienga G, Hlavstad S, et al. The relationship between cord blood immunoglobulin E levels and allergy-related outcomes in young adults. Ann Allergy Asthma Immunol. 2011;106:245-251.
20. Nissen SP, Kjaer HF, Hest A, et al. Can family history and cord blood IgE predict sensitization and allergic diseases up to adulthood? Pediatr Allergy Immunol. 2015;26:42-48.
21. Chawes. Low-grade disease activity in early life precedes childhood asthma and allergy. Dan Med J. 2016;63:B5272.
22. Nabanvi M, Ghorbani R, Asadi AM, et al. Factors associated with cord blood IgE levels. Asian Pac J Allergy Immunol. 2013;31(2):157-162.
23. De Amici M, Perotti F, Marseglia GL, et al. Cord and blood levels of newborn IgE:correlation, role and influence of maternal IgE. Immunobiology. 2017;222:450-453.
24. Mohammadzadeh I, Haghshenas M, Asefi S, et al. IgE level in newborn umbilical cord and its relationship with some maternal factors. Clin Mol Allergy. 2019;17:11.
25. Petrovičová O, Bánovčin P, Babuškivcová E, et al. Factors modifying cord blood IgE levels - a pilot study. Epidemio Mikrobiol Imunol. 2016;65:226-231.
26. Scirica CV, Gold DR, Ryan L, et al. Predictors of cord blood IgE levels in children at risk for asthma and atopy. J Allergy Clin Immunol. 2007;119:81-88.
27. Croner S, Kjellman N, Eriksson B, et al. IgE screening in 1701 newborn infants and the development of atopic disease during infancy. Arch Dis Child. 1982;57(5):364-368.
28. Caracta CF. Gender differences in pulmonary disease. Mt Sinai J Med. 2003;70(4):215-224.
29. Karimi M, Mohijian M, Nodoshan H, et al. Relationship between stress during pregnancy and cord blood IgE level. J Shahid Sadoughi Univ Med Sci Yazd. 2012;20:142-151.
30. Gurbuz T, Karakol B, Onal ZE, et al. Evaluation of in utero sensitization by screening antigen-specific immunoglobulin E levels in umbilical cord blood. Postepy Dermatol Alergol. 2015;32:184-187.
31. Hernández E, Barraza-Villarreal A, Escamilla-Núñez MC, et al. Prenatal determinants of cord blood total immunoglobulin E levels in Mexican newborns. Allergy Asthma Proc. 2013;34:e27-34.
32. Meulenbroek LA, KnippeLS LM. Cord blood IgE: fetal or maternal? Clin Exp Allergy. 2015;45:1012-1014.
33. Latif-Pupovc H, Lokaj-Berisha V, Lumezi B. Relationship of cord blood immunoglobulin E and maternal immunoglobulin E with birth order and maternal history of allergy in Albanian mother/neonate pairs. Open Access Maced J Med Sci. 2017;5:751-756.
34. Fereidouni M, Nami FA, Serki E, et al. Evaluation of cord blood immunoglobulin E and its association with maternal factors in a group of Iranian newborns. J Cell Biochem. 2019;120:13658-13663.
35. Ferguson A, Dimich-Ward H, Becker A, et al. Elevated cord blood IgE is associated with recurrent wheeze and atopy at 7 yrs in a high risk cohort. Pediatr Allergy Immunol. 2009;20:710-713.
36. Liu C-A, Wang C-L, Chuang H, et al. Prediction of elevated cord blood IgE levels by maternal IgE levels, and the neonate's gender and gestational age. Chang Gung Med J. 2003;26:561-568.
37. Erikkson TH, Sigurgeirsson B, Ardal B, et al. Cord blood IgE levels are influenced by gestational age but do not predict allergic manifestations in infants. Pediatr Allergy Immunol. 1994;5:5-10.
38. Bergmann RL, Edenharter G, Bergmann KE, et al. Predictability of early atopy by cord blood-IgE and parental history. Clin Exp Allergy. 1997;27:752-760.
39. Hansen LG, Høst A, Balken S, et al. Cord blood IgE II. Prediction of atopic disease. A follow-up at the age of 18 months. Allergy. 1992;47:397-403.
40. Ledrup Carlsen KC, Carlsen KH, Nafstad P, et al. Perinatal risk factors for recurrent wheeze in early life. Pediatr Allergy Immunol. 1999;10:89-95.
41. Sadeghnajad A, Karmous W, Davis S, et al. Raised cord serum immunoglobulin E increases the risk of allergic sensitisation at ages 4 and 10 and asthma at age 10. Thorax. 2004;59:936-942.
42. KaaN A, Dimich-Ward H, Manfreda J, et al. Cord blood IgEIts determinants and prediction of development of asthma and other allergic disorders at 12 months. Ann Allergy Asthma Immunol. 2000;84(1):37-42.
43. Businco L, Marchetti F, Pellegrini G, et al. Predictive value of cord blood IgE levels in ‘at-risk’ newborn babies and influence of type of feeding. Clin Allergy. 1983;13:503-508.
44. Chandra RK, Puri S, Cheema PS. Predictive value of cord blood IgE in the development of atopic disease and role of breast-feeding in its prevention. Clin Allergy. 1985;15:517-522.
45. Jehnke H, Nordberg LA, Vach W, et al. Patterns of sensitization in infants and its relation to atopic dermatitis. Pediatr Allergy Immunol. 2006;17:591-600.
46. Croner S, Kjellman NIM. Development of atopic disease in relation to family history and cord blood IgE levels. *Pediatr Allergy Immunol*. 1990;1:14-20.

47. Susanto NH, Vicendese D, Salim A, et al. Effect of season of birth on cord blood IgE and IgE at birth: A systematic review and meta-analysis. *Environ Res*. 2017;157:198-205.

48. Michel FB, Bousquet J, Greillier P, et al. Comparison of cord blood immunoglobulin E concentrations and maternal allergy for the prediction of atopic diseases in infancy. *J Allergy Clin Immunol*. 1980;65(6):422-430.

49. Tariq SM, Arshad SH, Matthews SM, et al. Elevated cord serum IgE increases the risk of aeroallergen sensitization without increasing respiratory allergic symptoms in early childhood. *Clin Exp Allergy*. 1999;29:1042-1048.

50. Lilja G, Magnusson CG, Johansson SG, et al. Neonatal IgE levels and three different blood sampling techniques. *Allergy*. 1992;47(5):522-526.

51. Popescu FD, Vieru M. Precision medicine allergy immunoassay methods for assessing immunoglobulin E sensitization to aeroallergen molecules. *World J Methodol*. 2018;8(3):17-36.