Ref: Ro J Med Pract. 2021;16(Suppl4)
DOI: 10.37897/RJMP.2021.S4.4

Total IgE assessment in newborn umbilical cord blood

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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

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Article History:
Received: 21 July 2021
Accepted: 15 August 2021

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 IGE

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 IGE 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 × g for 10 min at 4°C. The collected plasma is centrifuged again at 2500 × 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.

Conflict of interest: none declared
Financial support: none declared

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