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Complement profile in neonates of different gestational ages

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Abstract: Background: Blood levels of regulators of the complement system in preterm babies were reported in few studies only. The aim of this study was to set up a complement profile in premature and term babies focusing on the development of blood levels of key regulatory proteins which may allow an estimation of potential susceptibility to infection.

Methods: Complement activity (CH50), levels of mannan binding lectin (MBL), complement regulators (Factors H and I, C1 inhibitor, properdin) and C3a as marker of complement activation were assessed in three groups of healthy newborns: 1) prematures (≤ 34 weeks), 2) late prematures (>34 - <37 weeks) and 3) term neonates (≥ 37 weeks).

Results: CH50 increased with gestational age with lower titers in cord blood than in day 5 post delivery venous blood. MBL concentrations were not significantly different among groups. Quantitative and functional C1 Inhibitor were below adult normal range in prematures <34 weeks, and lower in cord blood as compared to day 5. Factor I, factor H and properdin, remained below adult values in all groups. Low C3a levels excluded that low complement titers were due to activation-induced consumption.

Conclusion: These results demonstrate the relative immaturity of the complement system and its regulation, especially in premature infants. Our findings point to an increased susceptibility to infections during the early neonatal period.

Keywords: Complement; C1inhibitor; Factor H; Factor I; MBL; Properdin; C3a; Premature Neonates

INTRODUCTION

Complement components are synthesized early in fetal life although with a relative deficiency of most of the complement proteins in comparison with adult levels [1-6]. C5, C7 and C9 appear after 5, 14 and 18 weeks of pregnancy, respectively [7]. Placenta transfer of C3 was excluded due to the detection of allotypic differences in maternal and newborns sera[8]. The first components to be detected are C3 and C4 in week 5,5 and 8 in fetal life, respectively [9, 10]. Factor B is present in the fetus at approximately 10 weeks of gestation [9].

The ontogeny of other components is not well documented but one may assume that all the components can be detected by week18 to 20 week of pregnancy [2,9]. In premature babies, total functional activity of the classical pathway and levels of the components C3, C4 and C5 are lower than those in comparison with term neonates [8, 10, 11]. Reports on complement profiles in term newborns indicate comparative low levels in comparison to adults[12,13,14,15] . Lau et al. found rising levels from 25 weeks of gestation up to 20 weeks post full term in a longitudinal study of prematures[15].

Mannan-binding lectin (MBL) is thought to play an important part in innate antimicrobial defence system of the body inducing the so called lectin pathway.of complement activation. In premature babies, where the passive transfer of protective maternal antibodies is interrupted, MBL function could be of relevance to compensate for an increased susceptibility to infectious diseases.

Complement as one of the most potent inflammatory system requires effective regulation to limit damage at the site of inflammation. Low properdin levels have been reported previously in cord blood from term newborns [16]. C1Inhibitor (C1inh) acts as an important multifunctional regulator in various kinine systems. We (MK) previously found no substantial reduction of C1inh in preterm neonates [17]. Factor H and factor I concentrations are usually below adult normal range in term neonates [2,6,16] , but only rare data are available for premature babies (<34 weeks).

The aim of this study was to establish a complement profile in premature and term babies focusing on the ontogeny of key regulatory proteins.

Patients and Methods

Serum and EDTA-plasma were obtained from cord blood and from venous blood taken at the 5th day after delivery of premature and term babies of different gestational age (Table 1), divided into aliquots and stored frozen at -70^{o}C. The study was performed upon informed consent of the parents and approval by the Ethical Committees of the Hospital Santa Marcelina, Sao Paulo, Brazil, and the Department of Pediatrics, USP Sao Paulo, Brazil. The newborns were initially admitted with suspicious infection and ethical committee approved sample collection. After careful blood analysis and cultures, in addition to the follow up, a clinical apparent infection was completely excluded. Gestational age was obtained considering maternal history and confirmed by clinical assessment of maturity according to the Capurro score [18] for term newborns and the New Ballard Score [19] for preterm newborns. Prematurity was considered for a gestational age of less than 34 weeks (group 1), late

premature babies were of the 34th- <37th week (group 2) and term neonates were ≥ 37 weeks old (group 3).

Functional activity of the classical pathway (CH50) of complement was measured in a hemolytic assay according to described procedures [20]. Serum C1inh protein levels were measured by nephelometry and functional activity of the regulator was analysed by the method of Levy and Lepow (1959) [21]. Plasma concentrations of the complement regulators, factors H and I, were assessed by rocket immunoelectrophoresis, using goat antibodies to human factor H or factor I (Miles Scientific, ICN Biochemicals, Eschwege, Germany). Purified factors H and I (Quidel, San Diego, CA, USA) were taken as standards [22]. Applying this technique, normal ranges (mean±2 S.D., n=40 adults) were found to be 360-680/μg/ml (factor H) and 60-90/μg/ml (factor I), respectively.

C3a/C3adesArg was quantified by ELISA (Progen, Heidelberg, Germany) as described by Zilow et al, 1989 [23]. From previous observations, normal values in healthy preterm neonates were considered <255ng/ml [6]

Results are expressed as median and presented as box plots with 25th and 75th percentiles including minimum and maximum levels. Kruskall-Wallis test for data comparison among the 3 groups and Mann-Whitney test for the comparison of results from cord blood and from 5th day samples within the individual neonate groups were applied for statistical analysis. Statistical significance was assumed at $p < 0.05$. In order to evaluate the relative concentration with respect to the respective parameter's normal adult range, we expressed its median concentration as percentage of minimal and maximal normal values.

Results

Hemolytic activity of the classical pathway was undetectable in cord blood of 7/13, 3/13 and 0/16 in groups 1, 2 and 3, respectively. At the 5th day after delivery, total complement activity was still not detectable in 2/10 (group 1) and 1/13 (group 2) (Figure 1). Functional activity of the classical pathway increased up to the 5th day in neonates of gestational age above 36 weeks (Table 2).

Cord blood levels of quantitative and functional C1 inhibitor (protein and function) and of factors H and I progressively increased with gestational age. Except for CH50, 5th days levels of all other parameter showed no significant variations (Table 2, Figures 1, 2, 3, 4 and 5). Preterm babies of less than 34 weeks showed statistically increasing levels of quantitative and functional C1 inhibitor and factor H from cord blood samples up to the 5th day. Premature neonates within 34 and 37 weeks of gestation had significant higher values of CH50, functional C1 inhibitor and Factor H in the 5th day samples. In term babies all parameters except for MBL increased from cord blood to the 5th day samples (Table 2, Figures 1-5). Signs of complement activation as reflected by elevated C3a values were only observed in 3 cord blood samples of group 1 and two of the group of prematures without statistically significant difference between the groups (Figure 6).

Discussion

Infections remain an important cause of morbidity and mortality in neonates and the incidence of invasive bacterial infections is largely dependent on gestational age and birth weight.

Several proteins synthetized by the liver show a rapid increase after birth, including coagulation products and complement proteins.

It is well known that complement as an essential part of the immune system has not gained its full function in newborns. This impairment of the innate immune response poses a considerable risk for severe infections in neonates, which is not compensated by a mature specific immune system [24,25,26]. Birth occurs at various stages of fetal maturity with a variable impact on infection susceptibility [24,27].

Transfer of immunoglobulin G occurs largely after 32 weeks of gestation, resulting in only low plasma levels in preterm infants. IgG levels decrease over the first weeks after delivery, leading to relative hypogammaglobulinemia. Considering these aspects, effector mechanisms of the innate immune response are certainly of great relevance to protect the newborn against a hostile environment *extra utero*.

Functional impairment of the classical pathway activity (CH50) was observed in almost all samples, with higher levels in term babies as previously described [6,11,25,28,29,30,31]. In most studies protein levels of individual components (except C7) as well as total hemolytic activity of the classical pathway (CH50) and of the alternative pathway (AP50) have been described to be significatly lower in neonates than in adults and to correlate with gestational age. [5,32]. In a recent metanalysis covering data of a great variety of complement tests, Mc Greal et al [31], found a large range for neonatal CH50 levels from 52 to 81% of adult mean

values with 58.6% as average value for normal term neonates. Preterm neonates starting at 26–27 weeks gestation had CH50 values of 32–36% of normal adult values [31]. In our study, classical pathway activity was undetectable in cord blood of 7/13 premature newborns of <34 week but could be demonstrated 5 days after delivery in most neonates in this group.

Lau et al (1995) [15] found in 168 preterm infants with a mean gestational age of 29 weeks that MBL levels rapidly rise after 25 weeks of pregnancy, later confirmed by other studies [12,13]**.** It was hypothesized that MBL might compensate for low Ig levels with respect to survival in preterm infants with hypogammaglobulinaemia and low complement levels.[15].

Kilkpatrick et al [14] demonstrated that MBL levels in umbilical cord blood samples of term neonates are similar in distribution as those in adult blood donors. He suggested the transition from a sterile to a nonsterile environment as a plausible explanation for the increased MBL values after birth. In contrast, Terai & Kobayashi [12] argued that increased MBL concentrations might be the consequence of stress in labor and delivery. No differences were found between samples taken after vaginal or surgical delivery [14]. We could not detect any significant difference in median MBL levels of premature, late premature and term neonates. It rather appears that MBL measured in cord blood reflect MBL genotype distribution.

Factors H and I occur early in fetal life, between the 12nd and 14th week of pregnancy, with 15 and 24% of adult levels increasing to 54 and 61%, respectively, in cord blood [9]. Summarizing 6 studies, McGreal et al [31] calculated a mean Factor H level in term neonates of 63.6% of adult concentrations, while Factor I levels were slightly lower with an average of

50.8%. These levels are obviously still lower than adult levels at 6 months after birth [2] but reach adult levels at one year of age [16]. We also found persistently reduced levels of factor H and I in premature babies. Levels for Factor H and Factor I were below adult normal ranges in almost all cord blood samples and increased until the 5th day post-partum, without reaching adult concentrations.

One may speculate that low concentrations of complement components leading to reduced lytic and opsonic complement activity may sufficiently be kept in balance by low levels of complement regulators. However, low factor H and I levels may lead to a dysfunctional regulation especially of the alternative pathway especially in severe inflammatory renal diseases such as aHUS and MPGN [33]. Higher levels in C3bBbP, an activation product specific to the alternative pathway, than of C1rsC1Inh (classical pathway) in infected neonates [1], may, at least in part, reflect an impaired regulation of the alternative pathway as a consequence of low factors H and I concentrations.

Properdin is significantly lower in the sera of children during their first year of life [16]. In our preterm and term infants properdin levels were very low reaching only 25% of adult mean concentrations. It is well known that in the absence of properdin serum bactericidal activity is considerably diminished. Like in deficiencies of the late components of complement- properdin deficiencies are associated with severe bacterial infections, esp. with meningococci [34].

C1 inhibitor, recognized not only as a regulator protein of the three activation pathways of complement system but also in various other kinin-generating systems, has not been extensively followed in fetal and neonatal life. Johnston et al. (1983) [11] found that C1 inhibitor in term neonates was 62% of

adult levels and that this regulator was reduced, rather than elevated, in mothers as compared to nonpregnant controls. Cat et al. (1993) [17] reported low functional titer in preterm infants (corresponding to 42–84% of adult levels). We observed in our preterm babies levels even as low as 23% of adult concentrations.

Certain maternal complement levels at birth greatly vary from those of nonpregnant women [9,11], complement concentrations in newborns could, therefore, be misinterpreted if the respective maternal values are used as the denominator for calculating neonatal level ratios. Whereas many maternal complement concentrations are increased by 26–57% relative to adults, C1q, C1r, C1inhibitor, properdin, and factor D, are decreased in maternal serum [32].

Complement activation, primarily through the action of the anaphylatoxins C3a and C5a, leads to the recruitment of innate and adaptive immune cells to the site of inflammation and microbial invasion [35]. Analysis of C3 activation products therefore appears to be useful in detecting *in utero* and neonatal infection [3,36]. Significantly higher levels of C3adesArg fragments and C3bBbP were found in term and preterm infants [36] with proven infection and may serve as useful indicator for ARDS in neonates [37]. As in our three samples with elevated C3a levels no clinical or analytical evidence of infection was given, we assume artificial complement activation due to suboptimal sample handling.

In conclusion, our results suggest that even extreme preterm neonates are able to regulate their immature complement system. Their age-related low protein levels and not the established adult plasma concentrations have to be considered if a deficiency is assumed. Although low titers of classical and

alternative pathway function may be considered as risk factors for infections, an impaired regulation may pose an additional risk for uncontrolled complement-mediated inflammatory tissue destruction, as it has been described for neonatal HMD and ARDS. The scenario of exposure to infective agents in preterm (and term) neonates is not rare considering that they are frequently exposed to venipuncture, catheters, endotracheal intubation and other procedures that break immunity barriers.

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Table 1 – Characteristics of the neonatal study group.

Table 2 –Serum concentrations of CH50, C1 inhibitor, factors H and I, properdin and MBL in neonates of different gestational age

Figure 1 – CH50 values in cord blood and 5th day after birth in newborns according to gestational age

Figure 2 – C1INH levels in cord blood and 5th day after birth in newborns according to gestational age

Figure 3 – Functional C1INH values in cord blood and 5th day after birth in newborns according to gestational age

Figure 4 – Factor H levels in cord blood and 5th day after birth in newborns according to gestational age

Figure 5 – Factor I levels in cord blood and 5th day after birth in newborns according to gestational age

Figure 6 - C3a levels in cord blood and 5th day after birth in newborns according to gestational age

Table 1 – Characteristics of the neonatal study group.

	< 34 weeks		34 - <37 weeks		≥ 37 weeks	
	Cord blood	5th day	Cord blood	5th day	Cord blood	5th day
Male:Female	4:9	3:7	5:8	4:9	9:7	10:7
Weight (g)	1292.3 (860-1690)	1338 (950-1690)	2101.5 (1720-2550)	2124.6 (1720-2550)	3153.8 (2750-3650)	3169.4 (2750-3650)

Cord blood: sample collection during delivery; 5th day: samples collection after 5 days postpartum.

Table 2 –Serum levels of CH50, C1 inhibitor, factors H and I, properdin and MBL in neonates of different gestational ages

	Gestational age	< 34 weeks		34- <37 weeks		≥ 37 weeks		P value (Mann-Whitney)			P value (Kruskal-Wallis)	
	Normal range (adults)	Cord blood (%)*	5th day (%)*	Cord blood (%)*	5th day (%)*	Cord blood (%)*	5th day (%)*	< 34w cb vs 5th day	34-<37 w cb vs 5th day	>37 w cb vs 5th day	Cord Blood (3groups)	5th day (3 groups)
CH50 (U/mL)	78-132	0 (0)	47,5 (36 -61)	40 (30.3-51.3)	62 (47-79.5)	56,5 (42.8-72.4)	70 (53-89.7)	NS	0.029	0.005	0.003	0.006
C1inh (mg/dL)	20-40	13 (21.7- 43)	22 (55 -110)	14 (35-70)	24 (60-120)	20 (50-100)	28 (70-140)	0.001	NS	0.002	0.001	NS
C1inh function (U/mL)	10-40	8,5 (21.2-85)	13 (32.5-130)	10,5 (26.2-105)	15 (37.5-150)	14 (35-140)	18 (45-180)	0.017	0.038	0.03	0.01	NS
Factor H (µg/mL)	302-822	255 (31-84.4)	350 (42.5-116)	160 (19.5-53)	269 (32.7-89)	160 (19.5-53)	395 (48-130.8)	NS	NS	0.034	0.005	NS
Factor I (µg/mL)	60-90	34 (38 -57)	38 (42.2-63.3)	36 (40-60)	42 (46.7-70)	43 (47.7-71.6)	50 (55.6-83.3)	NS	NS	0.037	0.001	NS
Properdin (µg/mL)	16-46	3.9 (8,5-24,4)	3.1 (6,7-19,4)	4.4 (9,6-27,5)	5.4 (11,7-33,8)	4.1 (8,9-25,6)	4.1 (8,9-25,6)	NS	NS	NS	NS	0.012
MBL (ng/mL)	>50	22-2196	0-2860	0-601	0-613	0-2086	0-3282	NS	NS	NS	NS	NS

(*) Values in parenthesis correspond to the percentage of the median newborn levels with reference to the normal ranges for adult healthy individuals; NS – not significant

Figure 1

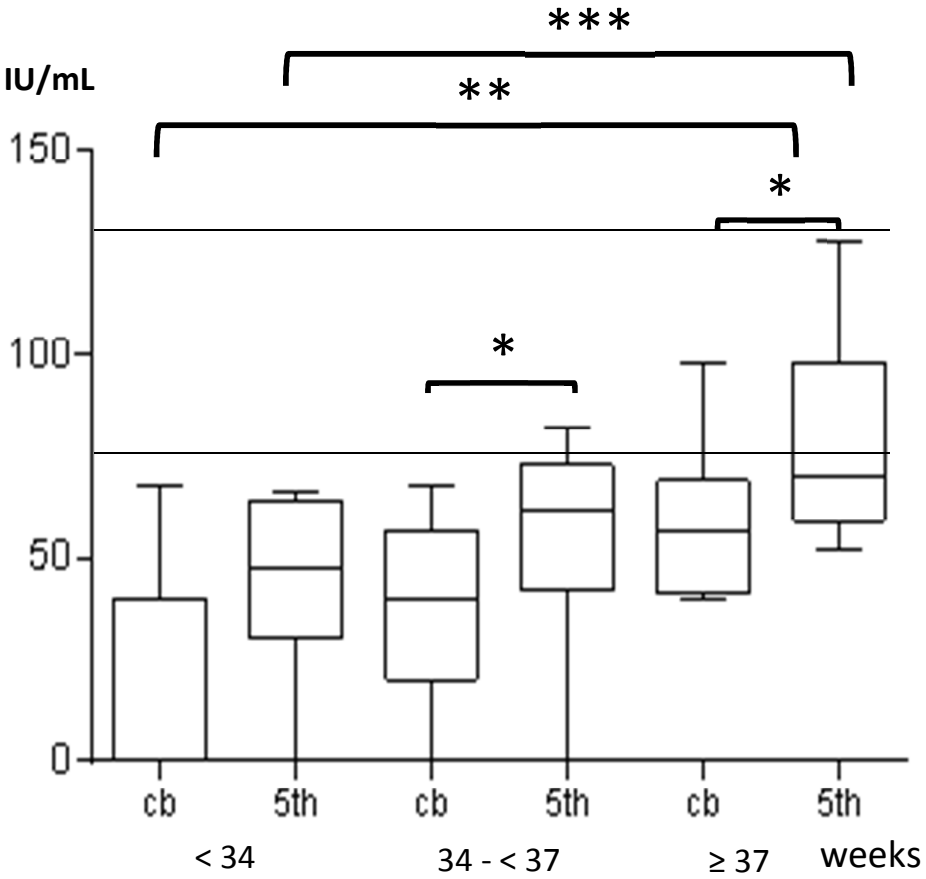

Figure 2

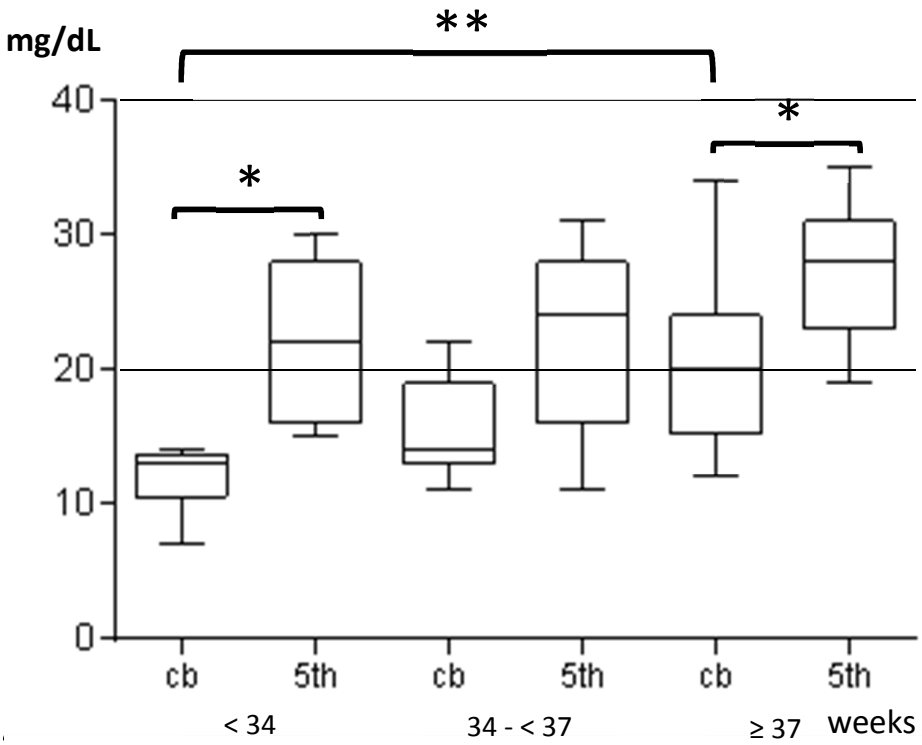

Figure 3

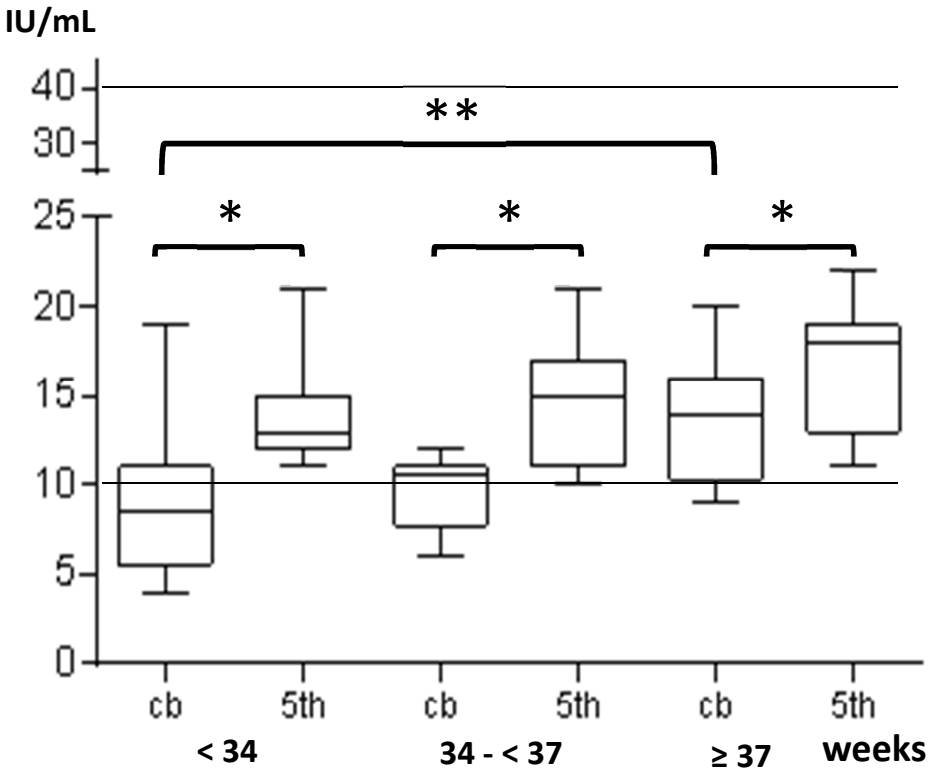

Figure 4

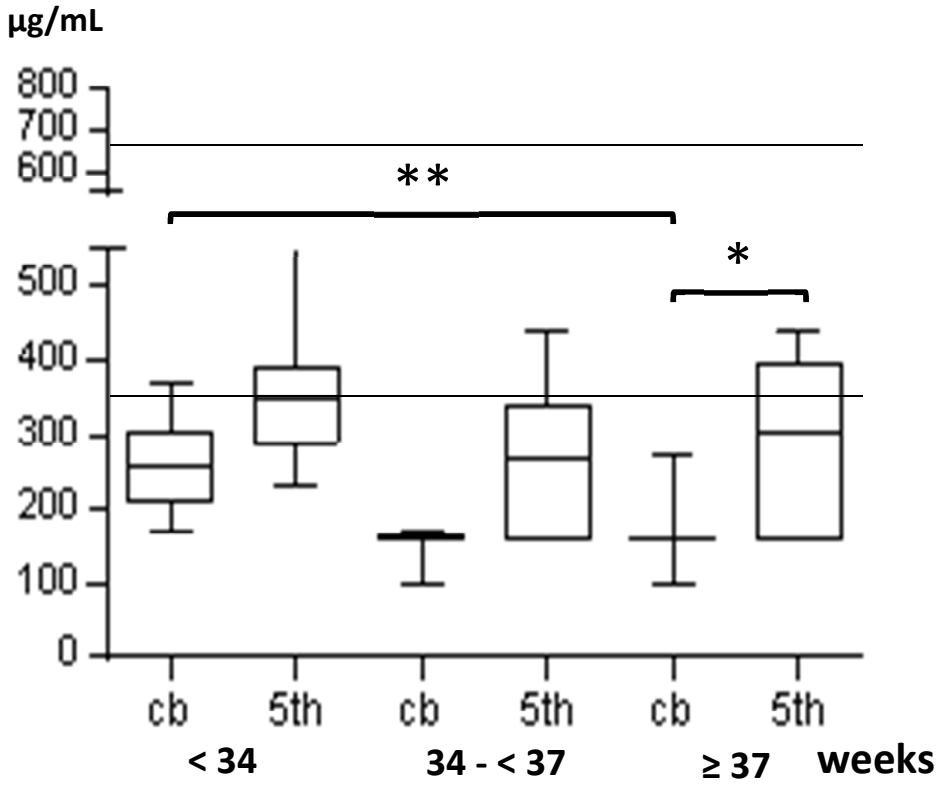
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Figure 5

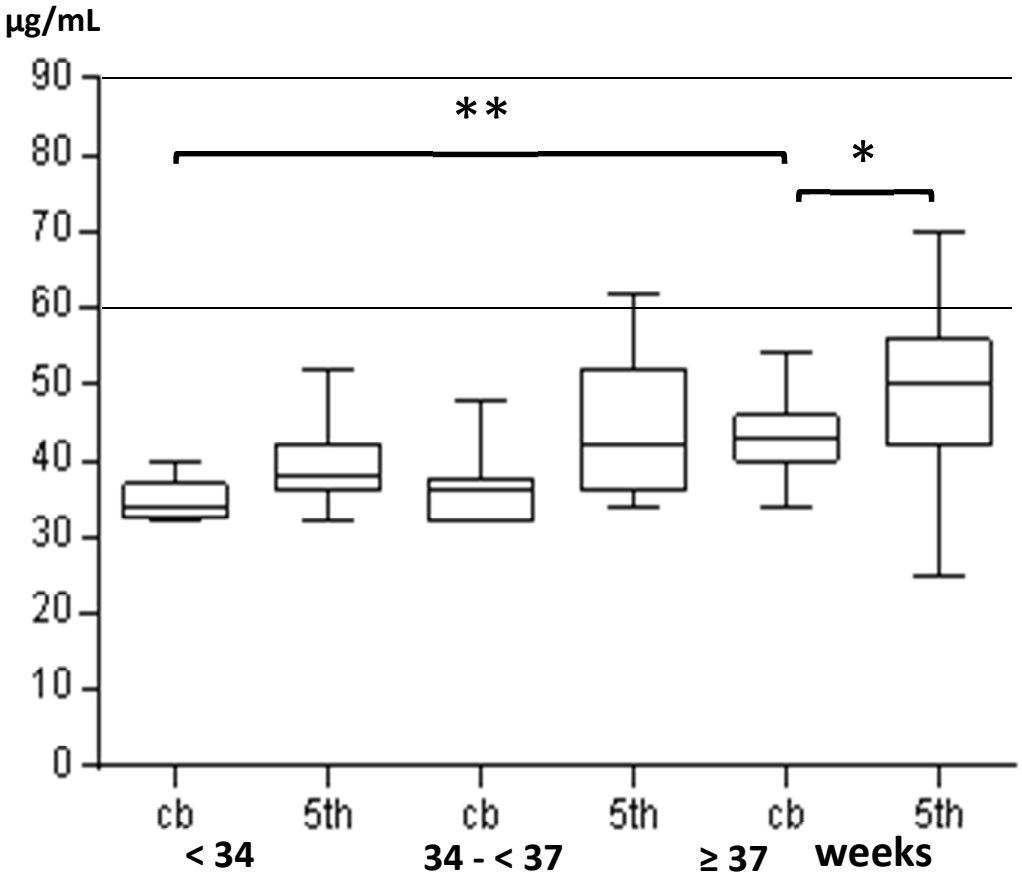
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Figure 6