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ORIGINAL ARTICLE

Dynamics of Selected Serum Immunological Markers During Caesarean Section

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Abstract

OBJECTIVES: The presented study aimed to describe the dynamics of the serum levels of the complement components C3, C4, and C1 inhibitor in women immediately before and after giving birth by caesarean section (CS).
DESIGN AND SETTING: 57 pregnant women undergoing caesarean section were included in this prospective observational study. Blood samples were taken 30 minutes before CS and 30 minutes after the delivery. C3, C4, and C1 inhibitor levels were analysed and the functional C1 inhibitor test performed. Angiotensin-converting enzyme concentrations before delivery were also determined.
RESULTS: Before delivery, C3 value was elevated above the reference limits for the healthy adult population in 39% of patients. Following birth, C3 median value dropped from 1.4 to 1.2 g/L. C1 inhibitor concentrations were also reduced – the median value of the C1 inhibitor before the birth was 222 mg/L, dropping to 198 mg/L after delivery. Even before the CS, C1 inhibitor concentrations were below reference range in 40% of patients, which increased to 56% after delivery; its activity however did not significantly change. In two patients with perioperative uterine hypotonia, notable complement activation was detected. ACE levels were below the normative values for adult population in 25% of patients.
CONCLUSION: Concentrations of all analysed components significantly decreased after delivery, which was not associated with blood loss or amount of intravenous liquids. This highlights the necessity of proper reporting of the time point of blood sampling in any studies or case reports detailing the immunological condition of patients in the peripartal period.

Abbreviations:

ACE	- angiotensin-converting enzyme
AFE	- amniotic fluid embolism
C1INH	- C1 inhibitor
DIC	- disseminated intravascular coagulation
IUGR	- intrauterine growth restriction

INTRODUCTION

The complement system is one of the pillars of immunity. Pregnancy is a complement amplifying condition. During pregnancy, the levels of its principal components grow (He *et al.* 2020) and the regulation protein levels decline. The ancient complement serine-protease cascade controls T cell development in order to maintain fetal tolerance. Bi-direction crosstalk between coagulation and complement is also important for a successful pregnancy (Regal, Burwick, and Fleming 2017).

Unwanted activation of the complement cascade can, however, occur in severe obstetric complications, such as life-threatening bleeding in uterine hypotonia, pre-eclampsia complications, HELLP syndrome or the anaphylactoid reaction caused by amniotic fluid embolism (AFE). The amniotic fluid embolism means the entrance of material from the fetal compartment (prostaglandins, complement activators, clotting factors) into the maternal circulation. The present options of timely diagnosis of events with the immunological background are insufficient. Where AFE is concerned, it is a diagnosis of exclusion, based only on the clinical picture of the pregnant woman/ mother within 30 minutes from the placenta delivery (Pacheco *et al.* 2016; Lynch *et al.* 2017; Kocsis and Gal 2014). The uniform diagnostic criteria proposed by Clark *et al.* (2016) include the acute hypotension to acute heart failure, accompanied by acute hypoxia to respiratory failure, normal body temperature and fibrinolysis-dominant DIC. Case reports describing various degrees of therapeutic success can be found in literature, including lipid resuscitation therapy (Lynch *et al.* 2017), extracorporeal membrane oxygenation (Huang *et al.* 2017), or administration of C1 inhibitor (Tamura *et al.* 2014a; Todo *et al.* 2015; Akasaka *et al.* 2018). In these case reports, however, it is often debatable whether or not those were cases of AFE as there is much discussion among the expert public on this issue, in particular between American authors from The Society for Maternal-Fetal Medicine (Pacheco *et al.* 2016) and Japanese authors who use much less stringent criteria (symptoms up to 12 h after delivery, DIC and severe bleeding over 1500 ml are considered sufficient criteria for AFE (Oda *et al.* 2017).

The presented study aimed to map the peripartal levels of selected complement components to set the physiological (base) levels for further research of diagnostic methods of AFE and similar acute events. Of the complement cascade components, we focused on the C3 and C4 components representing the most widely available examinations, routine in many laboratories. Of regulation proteins, we investigated C1 inhibitor in view of its prognostic as well as therapeutic potential. This acute-phase protein of the serpins family effectively controls the activation of both the classical and lectin pathways of the complement cascade, inhibits fibrinolysis as well as bradykinin. It is consumed during irreversible inhibition and can possibly act as a prognostic factor of AFE (a marked difference in C1 inhibitor activity between fatal and non-fatal AFE cases has been demonstrated (Tamura *et al.* 2014b). The C1 inhibitor concentrate is frequently used for the treatment of hereditary angioedema, more rarely in treatment of septic patients (Igonin *et al.* 2012), its successful use in obstetric complications has been also reported (Akasaka *et al.* 2018). When C1 inhibitor is depleted from the organism or its activity is low, bradykinin can cause systemic hypotension, distributive shock and participate in uterine hypotonia through increased vascular permeability (Busardo *et al.* 2015). Bradykinin is in turn degraded by the angiotensin-converting enzyme (ACE) and as estrogens in pregnancy may inhibit ACE, we also investigated the ACE levels at the end of pregnancy.

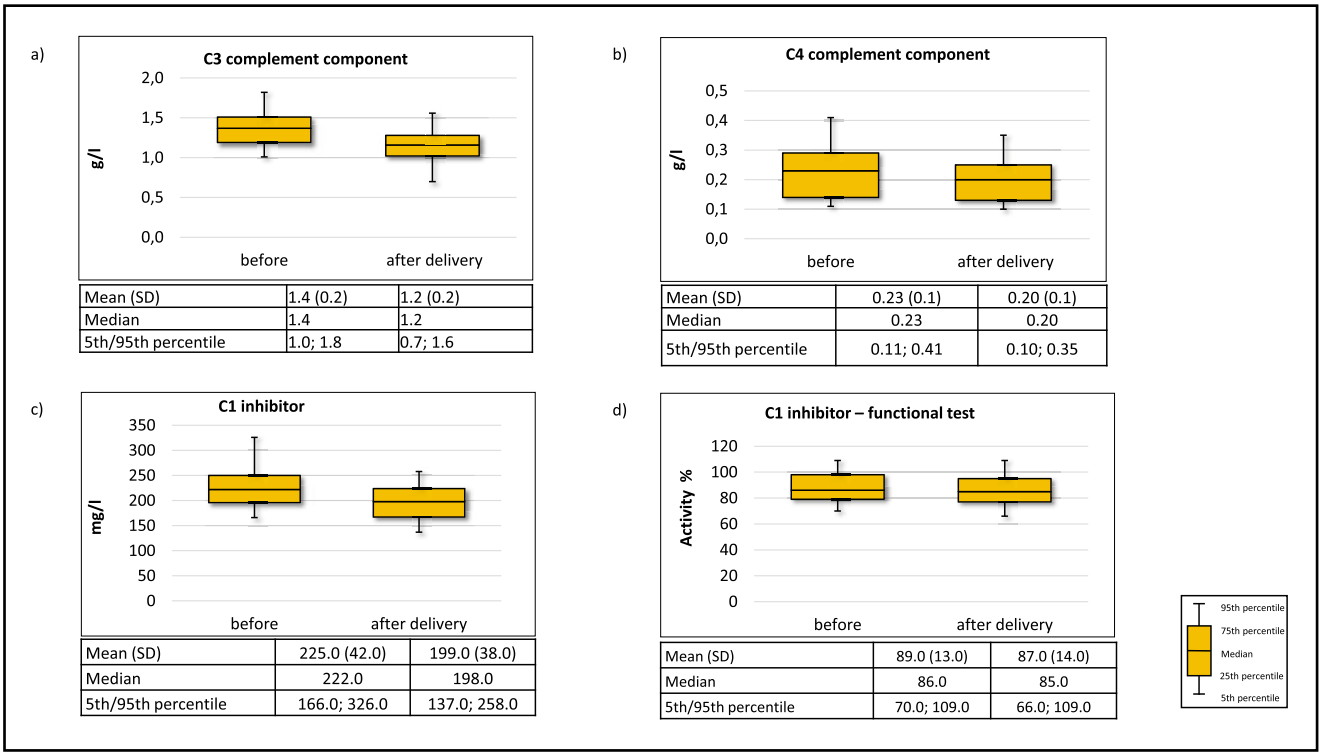
Normal reference ranges of complement components valid for the general adult population are usually used for pregnant women as well. On the perinatology.com website (www.perinatology.com, 2010), however, orientation values for C3 and C4 complement components in the individual trimesters can be found. A study comparing the complement components in the third trimester in women with physiological pregnancy and women with pre-eclampsia was published (Derzsy *et al.* 2010). A more detailed description of the increase of complement components during pregnancy has recently been published by He *et al.* (2020), whose study group, however, only included women with fully physiological pregnancy. The values in women with complicated pregnancy remain unknown.

The particular aims of this study were to a) find out if the reference levels of the selected components of the complement cascade for adult population differ from those observed peripartally in patients undergoing caesarian section and b) to provide comparison between women with physiological pregnancy and those with the complicated pregnancy. As we are looking for markers in the diagnosis of emergency conditions and as such complications of delivery and/or any significant worsening of the patient's condition usually lead to a rapid delivery through caesarean section, we focused on this type of delivery only.

MATERIAL AND METHODS

Patients and sample collection

The research was designed as a prospective observational study and was approved by the Ethics Committee of the University Hospital Brno on 4.4.2018 and registered at www.clinicaltrials.gov under No. NCT03664999. The



Mean (SD)	1.4 (0.2)	1.2 (0.2)
Median	1.4	1.2
5th/95th percentile	1.0; 1.8	0.7; 1.6

Mean (SD)	0.23 (0.1)	0.20 (0.1)
Median	0.23	0.20
5th/95th percentile	0.11; 0.41	0.10; 0.35

Mean (SD)	225.0 (42.0)	199.0 (38.0)
Median	222.0	198.0
5th/95th percentile	166.0; 326.0	137.0; 258.0

Mean (SD)	89.0 (13.0)	87.0 (14.0)
Median	86.0	85.0
5th/95th percentile	70.0; 109.0	66.0; 109.0

Fig. 1. Complement components before and after delivery in the entire group:
a), c) C3 and C1 inhibitor levels significantly decreased after delivery; b), d) C4 concentration and C1 activity did not change significantly

project was performed at the Department of Obstetrics and Gynaecology of the University Hospital Brno and Faculty of Medicine of the Masaryk University in Brno. This department serves as a regional centre for premature deliveries and pregnancy pathologies. The recruitment of patients took place from September 2018 till June 2019. The study has been reported according to STROBE statement.

Inclusion criteria: A pregnant patient undergoing delivery by caesarean section (both planned and/or emergency) expressing a consent with participation in the study and signing an informed consent form. In situations where delay would put the mother or the infant in risk, the participation in the study was not offered.

Exclusion criteria: a personal history of hepatitis or other hepatocyte damage.

60 patients were recruited into the study; three patients were excluded as two did not meet inclusion criteria and in one patient, incomplete data were recorded (Table 1). The patients were divided into two groups: a) patients without serious comorbidities with a physiological course of the pregnancy (the caesarean section was performed e.g. due to the foetus position, patient condition after the previous caesarean section, interlocked twins) and b) patients with a pathological course of pregnancy or serious comorbidities (see their detailed list in the Results). 4.9 ml blood sample was taken from each patient 30 minutes before commencing the surgery and stored in the S-Monovette Sarstedt test tube with coagulation activator and gel; where emergency CS was performed, the period was in some cases shorter but the sample was always taken before the first incision. The second sample was taken 30 minutes after delivery. This time interval was chosen because it corresponds to the diagnostic criteria for developing the anaphylactoid reaction. Data on the course of the pregnancy as well as complications during the delivery were recorded together with data on blood loss and amount of intravenous fluids. The criteria for diagnosing hypotonia were as follows: Blood loss over 500 ml necessitating the application of second-line uterotonics (such as Prostin M15 intramyometrally or Duratocin intravenously).

Immunological analysis

The blood serum was separated by centrifugation and frozen at -20 oC within an hour of sampling to prevent confounding results. Subsequently, the levels of humoral nonspecific immunity components C3, C4, as well as the concentrations and activities of the C1 inhibitor were determined at the Institute of Clinical Immunology of the St. Anne's University Hospital in Brno. C3 and C4 components were determined by immunoturbidimetric assay using Beckman Coulter reagents (Beckman Coulter, USA), C1 inhibitor concentrations using Berichrom C1 inhibitor kit (Siemens Healthineers, Germany) and its activities were established using MicroVue C1 Inhibitor Plus EIA kit (Quidel, California, USA). All analyses were performed

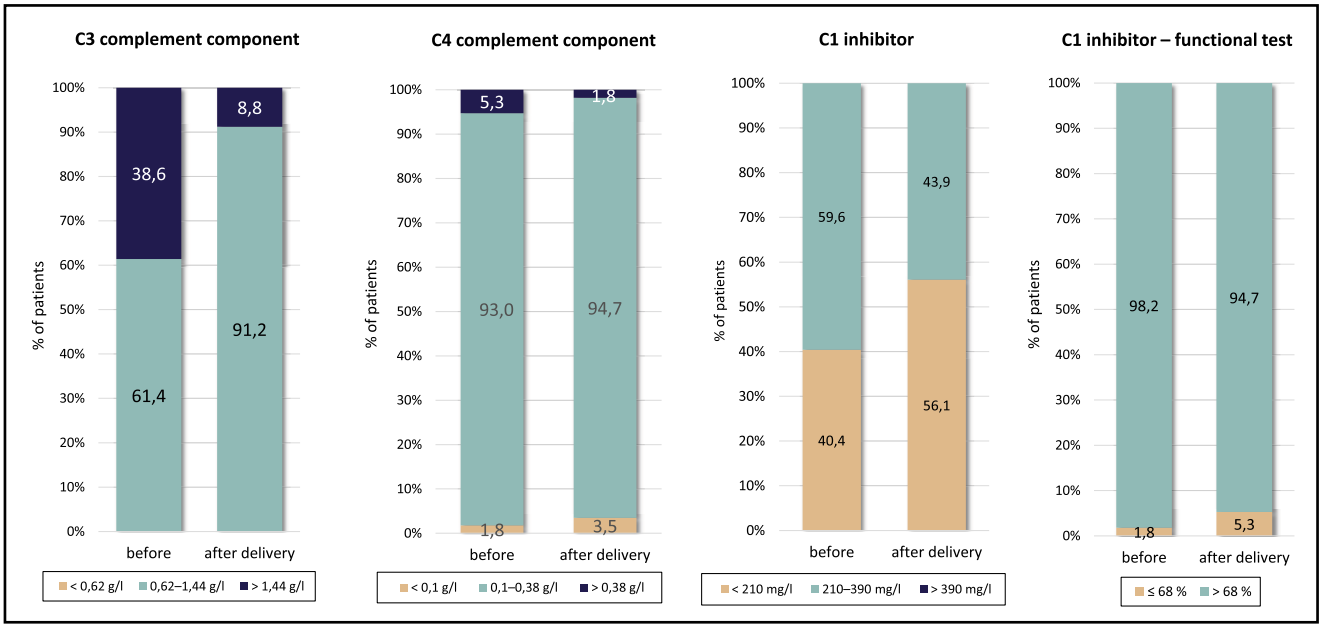


Fig. 2. Proportion of patients according to reference range for each marker (light blue normal reference range)

following the manufacturer's instructions. Samples were analysed always within 1 month of taking. A sample for determining ACE level was taken always together with the first blood sample and later analysed photometrically using Cobas 8000 analyser.

Statistical evaluation

Continuous patient characteristics were described through mean with standard deviation (SD) and median with 5th and 95th percentiles. If appropriate, continuous variables were categorized according to widely used cut-offs. Categorical parameters were summarised using absolute and relative frequencies. Relative frequencies were calculated as a percentage from the number of patients in the relevant subgroup. For one-sample or two-sample testing, respective Wilcoxon tests were used. Multiple means comparison was performed using Kruskal-Wallis test; relative frequencies in multiple groups were compared with Fisher exact test. medians of two different samples with Mann-Whitney test. $\alpha = 0.05$ was used as a required level of statistical significance. Statistical analyses were performed in IBM SPSS, Statistics (version 25.0) and R software.

RESULTS

57 patients were included into the analysis (basic patient characteristics are shown in Table 2). In 41 patients, elective CS was performed (Category 4) (RCOG, 2010), Category 3 CS was performed in 15 and Category 2 CS in 1 patient. 25 patients were in the group with physiological pregnancy and 32 patients in the group with pathological pregnancy (11x preeclampsia, of which 7 were early-onset and 4x late-onset preeclampsia, 5x gestational diabetes mellitus, 11x placental insufficiency leading to intrauterine growth restriction, 4x placenta praevia, see Table 3). Other complications included 1x HELLP, 1x placental abruption, foetal hydrops, chorioamnionitis; serious mother comorbidities included 2x ulcerative colitis, of which one necessitated parenteral nutrition during pregnancy, 1x Crohn disease necessitating ileostomy immediately following the caesarean section. Some patients had multiple pathologies. Perioperative uterine hypotonia occurred in 5 cases (3x in placenta praevia, 1x in CS due to stalled labour and 1x in a patient with gestational diabetes). In 16 patients, the CS was performed prematurely (between the 26th and 36th week of pregnancy).

In all analysed parameters, a statistical evaluation of a relationship between changes in the concentrations/activities of complement components and blood loss (as well as intravenously administered fluids) was performed. In none of the evaluated parameters, however, such relationship was found to be significant.

Regarding the **C1 inhibitor**, 60% of patients fell within the reference range for the general adult population before the delivery (reference range 210-390 mg/L), but in 40% of patients, the value was below the reference range even before the delivery. The median value of C1 inhibitor before delivery was 222 mg/L. After the delivery, the C1 inhibitor concentration further decreased to a median value of 198 mg/L and the values were below the reference ranges in 56% of patients (Fig. 1). The greatest declines, by 45% (from initial 222 mg/L to 122 mg/L) and 37% (from 220 mg/L to 137 mg/L), respectively, were observed in two patients, both of whom had perioperative uterine hypotonia requiring intervention. No significant difference in C1 inhibitor concentrations was detected between

the patient groups with physiological and pathological pregnancy (median 230 mg/L vs 215.5 mg/L prior to the delivery, decreasing to a median of 198 mg/L in both groups after the delivery). A more detailed analysis revealed that in patients with preeclampsia, the concentrations of this regulation protein were lower, namely a median of 196 mg/L before and 174 mg/L after the delivery. Where the delivery was before the 36th week, the median value of the C1 inhibitor before delivery was 226 mg/L.

In our study, we found only minuscule deviations from normal values of physiological **C1 inhibitor activity** (which is >68 %) – one patient had a negligibly reduced C1 inhibitor activity before the delivery, three after the delivery. In none of the patients, however, the C1 inhibitor activity dropped below 65%. None of the 11 patients with preeclampsia had even borderline C1 inhibitor activity (range 74% - 94%).

Before the delivery, 61% of patients showed normal **C3 component** values (reference range 0.62 – 1.44 g/L) while in 39%, the value was elevated (Fig.2). The C3 levels were always elevated in patients with autoimmune diseases (1.52 – 2.11 g/L). After the delivery, the median value of C3 concentration dropped from 1.4 g/L to 1.2 g/L. Similar to C1 inhibitor, Mann-Whitney test did not detect any significant difference between the mothers with physiological and pathological pregnancies – median C3 concentrations were 1.31 g/L vs 1.37 g/L before delivery and 1.16 g/L vs 1.15 g/L after the delivery, respectively. In women with premature delivery (before the 36th week of pregnancy), the median C3 concentration was 1.49 g/L.

Abnormally high values of **C4 complement component** (with the reference range for healthy population being 0.10–0.38 g/L) was detected in 5% of patients before the delivery; after the delivery, the values remained more or less unchanged (only a negligible insignificant decrease from median value 0.23 g/L before delivery to 0.20 g/L after delivery was recorded). The median concentration of the C4 component was significantly lower in patients with preeclampsia (0.13 g/L) than in healthy patients (0.24 g/L), p=0.034.

The levels of all markers are statistically significantly lower after the delivery than before it, as verified by the one-sample Wilcoxon test. As mentioned above, statistical testing (Kruskal-Wallis test, Fisher exact test) proved that this decline was not associated with blood loss or amount of intravenous liquids.

The **ACE** activity was within the standard range for healthy adult population in 75.5% of patients while in 24.5% of patients, a lower level was observed. The median ACE activity was 28 U/L. Of the 14 women with reduced ACE activity, 6 were women with physiological pregnancy; of 8 patients with pregnancy pathologies and reduced ACE activity, intrauterine growth restriction was present in 4 cases. The lowest ACE value (8.3 U/L) was found in a patient in the 33rd week of pregnancy with foetal hydrops.

DISCUSSION

Data on the concentrations/activities of complement components in women in labour, if available at all, usually only concern normal vaginal delivery. This can be one of the reasons why we have, in our patient group, recorded greater changes than other published studies. Benson *et al.* (2001) described a decrease of the C3 complement component by 8% to 1.17 g/L. In our patient group, the C3 concentration decreased by 21% to 1.10 g/L. In the C4 component, the situation was similar – instead of the reported decrease by 5% to 0.29 g/L, we recorded a 13% decline to 0.20 g/L.

In premature deliveries (i.e., complicated pregnancies forcing the physicians to perform an earlier CS), we observed higher C3 values (mean 1.4 g/L) than reported by Ying-dong He in the same stage of pregnancy in healthy pregnant women (mean 1.1 g/L). Similarly, in women with physiological pregnancy delivering after 36th week, our results revealed a higher mean concentration of the C3 component (1.3 g/L), which is in accordance the value of 1.2 g/L reported by the Chinese authors.

Kestlerova *et al.* (2012) studied the immunological markers in patients with preeclampsia at a time similar to that of taking the first sample in our patient group. The concentration of C4 component in patients with preeclampsia was significantly lower than in healthy adults, which corresponds well with our findings. A similar median level of C4 (0.28 g/L) in healthy pregnant women approximately 3 weeks before the delivery was also described by Derzsy *et al.* (2010). Those findings, however, differ from those reported by the aforementioned Chinese authors, namely 0.567 g/L (He *et al.* 2020). The difference is most likely due to the different methods; while for C3 detection, the Chinese authors used the same kit by Beckman Coulter as our laboratory, they used kits by BeiJia company for determining C4 levels.

In our study group, AFE (defined as sudden onset of hypotension, respiratory compromise and DIC) did not occur in any of the patients. From the perspective of immunology, the patients could have been therefore considered a uniform group. Hence, the expectation was that an unexplained drop in C3, C4 and C1INH serum levels should not occur in any of the patients. This expectation was however not met. Nishio *et al.* (2002) considers the postpartum C3 drop to 0.74 g/L (reported in a fatal AFE case) to be a sign of complement cascade activation (or, in other words, a sign of increased consumption of complement components following anaphylactoid reaction). In our patient group, the lowest postpartum C3 component concentrations were 0.63 g/L and 0.69 g/L (drop from original 1.21 g/L and 1.19 g/L, respectively). We agree with the idea that the drop of the C3 concentration was due to the complement activation – these two patients were the same in whom the strongest decline in C1 inhibitor

concentration was detected and both had uterine hypotonia. It is true that in one of those patients (a patient with placenta praevia), this decline might have been due to a substantial blood loss occurring due to the uterine hypotonia. The patient with the highest consumption of complement components (C1 inhibitor decrease by 45%, C3 by 48%) had no placental disorder, blood loss of 500 ml and intravenous fluids of 600 ml; the only complication of pregnancy was gestational diabetes. We hypothesise that the causes of uterine hypotonia may include complement activation caused by a small amount of amniotic fluid entering the mother's bloodstream and activating the complement cascade. The amount would be small enough to fail to trigger the full triad of the AFE symptoms but rather the clinical picture called "abnormal haemorrhagic type of AFE with uterine atonia and DIC" by Japanese authors (Todo *et al.* 2015). If this hypothesis is true, the different definitions by Japanese and American scientists would probably only denote different degrees of severity of a problem with an identical cause. It is indeed desirable to look for a consensus in diagnostic criteria, which would facilitate the comparability of treatment results and research of this rare but serious problem in general. The therapeutic use of the C1 inhibitor concentrate could then hopefully be a promising method for treatment of progressing hypotonia not reacting to standard uterotonic treatment or even for reversal of the lethal AFE. C1 inhibitor concentrate of 1000 units is equivalent to 8 units of fresh frozen plasma (Akasaka *et al.* 2018).

The low number of patients with individual pathologies can be perceived as a limitation of this study preventing us from drawing any conclusions where most of those pathologies are concerned. It was, however, not a primary aim of this study to set any exact background levels for patients with serious pregnancy pathologies. As many of the complications are relatively rare, acquiring a sample large enough for acquiring any statistically significant values for all pathologies would take an excessively long period and significantly increase costs of the study above the allocated means. Where rarer pathologies are concerned, we therefore provide rather a first insight into the topic.

Including women delivering before 36th week of pregnancy can also be perceived as a limitation as the levels of complement components change throughout the pregnancy period. Nevertheless, we decided to include them as many of the pathologies frequently necessitate premature delivery and we would be otherwise not able to include them into the set at all.

SUMMARY

In this paper, we report normal levels of C1 inhibitor and its activity, C3 and C4 complement component in women 30 minutes before and after delivery by caesarean section. Both patients with physiological pregnancy and various pregnancy disorders (most notably, preeclampsia) were included in the study group. Even before delivery, C3 serum levels were significantly elevated and C1 inhibitor concentration significantly lower than the normal range valid for the healthy adult population. Concentrations of all analysed components significantly decreased after delivery, which was not associated with blood loss or amount of intravenous liquids. This highlights the necessity of proper reporting of the time point of blood sampling in any studies or case reports detailing the immunological condition of patients in the peripartal period. The biggest drop in C1 inhibitor and C3 component was detected in two patients in whom uterine hypotonia occurred, indicating a possible activation of the complement cascade, possibly due to a negligible amount of amniotic fluid (insufficient for the development of a full-blown AFE) entering the mother's bloodstream. We intercede for more frequent complement components examination in practice, so we could make progress in these unclear situations and offer to patient earlier diagnostics and treatment according to simple guidelines. We suppose considering the therapeutic use of the C1 inhibitor concentrate in case of peripartal life threatening bleeding.

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