Supplemental data

Case reports

Case A.1 presented with aHUS at the age of three years and had 22 subsequent episodes during her childhood and adolescence. The 23rd episode, at the age of 14 years, was accompanied by acute kidney injury, which resolved after six cycles of plasma exchange.

At the age of 18 years she was referred to our department, expecting her first child (12th gestational week). She received 200 mL of fresh frozen plasma every other week until the day of the vaginal delivery at 40th gestational week (induced due to increasing proteinuria and blood pressure) and was solely monitored afterwards for 6 months. During the pregnancy proteinuria increased to a maximum of 821 mg/g creatinine but decreased after the pregnancy to levels between 200 and 700 mg/g creatinine (reference value: <200 mg/g creatinine). Antihypertensive therapy was not required during pregnancy and serum creatinine was within the reference interval.

The same treatment regimen was followed during her second pregnancy three years later. Again, she showed no signs of complement activation or rise in serum creatinine (stable at 0.75 mg/dL) during pregnancy, but developed significant proteinuria with a maximum of 2800 mg/g creatinine.

Proteinuria persisted after her second pregnancy (2900 mg/g creatinine 19 months after the second delivery) despite serum creatinine levels within the normal range and a later performed biopsy confirmed renal TMA.

In addition to the *CFI* (p.G342E)[1] and *MCP (p.D257Vfs*42)*[2] mutations, the patient is homozygous for the rare alleles of p.R102G and p.P314L polymorphisms in *C3*. This haplotype was shown to be a susceptibility factor for dense deposit disease.[3] Carriers

of these variants were reported to have lower alternative pathway activity and in hemolysis assays p.G102 activated the alternative pathway more efficiently and had higher hemolytic activity compared with the p.R102 C3 protein as this amino acid change influences the efficiency of regulation by factor H.[4]

Case A.2 presented with end-stage renal disease due to chronic-recurrent TMA aged 24 years. The patient of African descent received hemodialysis treatment until the age of 26 when she successfully received a kidney transplant. Three years later she became pregnant with her first child. We initiated prophylactic plasma infusions (200 mL fresh frozen plasma every other week) beginning at 14th gestational week. The pregnancy was uneventful and the treatment was stopped after delivery.

Prompted by the uneventful course of her first pregnancy that was adequately treated with low doses of plasma infusions (2.5 mL/ kg body weight), she was only monitored during her second and third pregnancy without any specific aHUS treatment. After an uneventful second pregnancy, the third pregnancy was terminated in 33rd gestational week because of intrauterine death of the fetus most likely due to infection of the child. No signs of complement activation were detected in the mother and follow-up was unremarkable.

Since receiving her kidney transplant the serum creatinine levels ranged between 0.7 and 1.3 mg/dL without significant proteinuria but microalbuminuria (maximum 86 mg albumin/g creatinine).

Case A.2 harbors a *CFI* mutation (*p.I416L*), which is causing a quantitative factor I deficiency and is very likely pathogenic.[1,5-8]

Case C.1: The African patient initially presented with an episode of p-aHUS on the day of the delivery of her first child by cesarean section. She developed severe uterine bleeding and subsequent TMA, which did resolve 20 days after onset. A kidney biopsy confirmed the biochemical signs of acute TMA. She underwent 26 plasma exchanges within 41 days while receiving rituximab (2000 mg in two doses) and prednisolone therapy (starting dose 100mg, conventionally tapered). A seizure, most likely due to posterior reversible encephalopathic syndrome, was successfully terminated with lorazepam 2 mg. Following discharge from the hospital 8 weeks after delivery her C3c levels remained decreased (0.84 mg/dL) and LDH levels rose to 465 U/L (reference interval < 248 U/L), which is why she received 1 L of fresh frozen plasma once again. Twelve months later the C3c level was 0.69 mg/dL (reference interval 0.9 mg/L), but without clinical signs of TMA.

Thirteen months after the emergency cesarean section of her first child, she presented in 21st gestational week of her 2nd pregnancy and was referred to our facility. We initiated prophylactic plasma infusions (10 mL/kg body weight) every other week. The patient was closely monitored and hypertension was controlled with methyl-dopa (daily dosage between 500 mg and 1000 mg). Delivery was planned as cesarean section and 800 ml of plasma each were infused before and one day after delivery. Hemoglobin, thrombocytes, serum creatinine and C-reactive protein were daily monitored and four days after delivery the mother and her newborn were discharged. We proceeded with prophylactic plasma infusions every other week for 12 weeks. During her second pregnancy and the postpartum period she did not show any signs of complement activation, despite slightly decreased C3c concentrations below the lower limit of reference values (77.9 to 92.4 mg/dL) until 6 months after delivery.

Aged 23 years she visited our outpatient clinic 3 weeks pregnant with her third child. Similar to her second pregnancy we initiated preventive plasma infusions with 10 mL/kg body weight every other week starting at 14th gestational week. Despite H1N1 influenza at 18th gestational week, she showed no signs of TMA throughout her pregnancy. Delivery was successfully managed with the same treatment regimen as for her second pregnancy.

Again C3c levels were slightly decreased during the postpartum period (72.1 to 87.1 mg/dL; reference range: 90 to 180 mg/dL, determined in a non-pregnant population), but this time intermittent significant proteinuria developed (900 mg/g creatinine, serum creatinine levels below 1.0 mg/dL). Prophylactic plasma infusions were given until 7 months postpartum. Currently her C3c levels are still decreased below the lower threshold of the reference interval and proteinuria decreased.

The two mutations on *C3* (*p.K104E*, *p.D1457H*) are functionally not characterized, but based on *in silico* analysis both are likely to be pathogenic. In addition she harbors the *CFH-H3* haplotype (ref).

Case C.6: The 19 year-old mother of a one-year old boy was admitted to our clinic with classic clinical signs of aHUS after suffering from a urinary tract infection with *Escherichia coli* followed by vaginal *Candida* infection. The histological diagnosis was chronic-recurrent thrombotic microangiopathy (TMA), which was unsuccessfully treated with plasma exchange for 17 days. For six years she performed uncomplicated peritoneal dialysis until she successfully received a renal transplant and henceforward was treated with plasma exchange at day zero, one, two and three, and afterwards thrice weekly (in total 10 times) followed by plasma infusions (8 units of 200 mL fresh frozen plasma weekly for two months). Complete weaning of plasma infusion

was never performed in this patient, due to a decrease of C3 and C3c, indicative of a permanent activation of the alternative complement system.

Aged 29 years, after a modification of immunosuppressive therapy, she expected her second child. During the first trimester C3c levels were 92.15 mg/dL with no clinical signs of TMA. In the 12th gestational week prophylactic plasma infusions were intensified from monthly intervals to every other week (25 mL/kg body weight) to prevent a more severe activation of the complement system. In the 38th gestational week a cesarean section was performed after 8 units of 200 mL fresh frozen plasma had been given prior to the surgery. Serum creatinine (reference range: 0.4-0.9 mg/dL) ranged between 1.4 and 1.8 throughout the pregnancy, increased to 2.2 towards the date of delivery, but decreased again around 1st week postpartum. The C3c, C4 and CH50 were within reference values.

In 2015 her third pregnancy, in which maintenance plasma treatment was not intensified, was uneventful until 30th gestational week. A urinary tract infection treated with antibiotics was followed by vaginal mycosis, and labial herpes at the day of delivery. Proteinuria significantly increased around 36th gestational week to more than 2000 mg/g creatinine with a slight increase in serum creatinine from 1.6 to 2.0 mg/dL. Beginning in 32nd gestational week haptoglobin was not detectable, but LDH, C3c, and CH50 levels were within normal range. The baby was delivered at 38th gestational week by a cesarean section after infusion of 8 units of 200 mL fresh frozen plasma. One day after delivery she received her additional planned 8 units of fresh frozen plasma, but her physical status started to deteriorate. In addition to significantly increased infection parameters, hemoglobin levels decreased to 7.7 g/dL (reference range: 12- 16 g/dL) and platelet count was 75 G/L (50% drop). Hematologic alteration could be well controlled by the plasma infusions and fluid phase complement profile

was within normal limits. However, creatinine acutely increased (maximum 9.29 mg/dL) and a transplant biopsy confirmed acute TMA (**Figure 2**). Eculizumab was started on the 5th day after delivery (900 mg every week for four weeks). Kidney function slowly recovered under standard treatment regimen (up until week five 1200 mg every other week) of eculizumab, complicated by several adverse events (vaginal abscess, uterine bleeding). Seven months postpartum her serum creatinine was 3.5 mg/dL with a proteinuria of 1588 mg/g creatinine and well controlled blood pressure. Her C3c levels were still decreased to 62 mg/dL.

Notably, her 25 years old sister comprises an identical genotype (*CFH p.N516K*, homozygous *CFH-H3*), but never experienced an episode of aHUS so far and shows C3c within the reference interval. Of note, her first pregnancy was unremarkable without biochemical signs of TMA (prospectively followed at our department). In contrast, the mother of the two sisters harbors the *CFH-H3* risk haplotype only in heterozygous form.

Detailed Methods

Definitions

Laboratory signs of TMA were defined as mechanical hemolysis and hemoglobin < 10 g/dL, platelet count < 150 G/L or drop in platelets > 25% in 48 hours, LDH levels > 250 U/L, haptoglobin levels < 12 mg/dL and the presence of schistocytes in peripheral blood smear, associated with acute kidney injury, and absence of ADAMTS13 deficiency and absence of ADAMTS13 inhibitor (if activity was < 20% including ADAMTS-13 inhibitor determination).

Stages of CKD[9] and pre-eclampsia/HELLP were defined as detailed elsewhere.[10] For calculation of progression to ESRD, patients that received a kidney transplant after first presentation were counted as progressed to ESRD and all women were assumed not to have ESRD (regardless of if serum creatinine was known) at first pregnancy. Gestational age (gestational week + days) was calculated from the first day of the last normal menstrual period and date of birth. For calculation of at risk-time for a p-aHUS episode we arbitrarily added four weeks postpartum time to reflect the time at risk after delivery (by choosing a longer postpartum at risk time, rate moves towards null). A preterm birth was defined as birth before 36th gestational week. If only gestational weeks were known we added 3.5 days for calculation of time at risk. Small for gestational age (SGA) was defined as infants with a birth weight below the 10th percentile for gestational age and large for gestational age (LGA) was defined as a birth weight greater than the 90th percentile for age.[11]

Data collection was based on chart review for general demographic data including gender, ethnic origin, age of first known episode of TMA, family history, if applicable transplant status, laboratory and biopsy data, as well as specific treatment (plasma exchange, plasma infusion, or eculizumab).

The Austrian mother/child-book (Mutter-Kind-Pass) can be accessed at http://www.bmgf.gv.at/cms/home/attachments/0/4/6/CH1101/CMS1310413628758/mukipass_leitfaden.pdf.

Laboratory methods

Laboratory work-up included blood count, blood chemistry, coagulation parameters, blood smear, schistocyte counts, haptoglobin levels, and urinalysis. For complement

specific diagnostics C3c and C4, total complement activity of the classic (CH50) and alternative pathway, and ADAMTS-13 activity (if activity was < 20% including ADAMTS-13 inhibitor determination) were analyzed (www.kimcl.at).

Genetic investigations

Genomic DNA was extracted from peripheral blood according to standard procedures. Mutation screening of *CFH*, *CFI* and *MCP* was done by polymerase chain reaction amplification of coding exons and splice sites using primers previously described[6,12] in a modified protocol by the Laboratory for Molecular Diagnostics, Department of Laboratory Medicine, Medical University Vienna (www.kimcl.at). For patients, in which sequencing of *CFH* did not include the SNP -331C>T (discriminates between the *CFH-H3* and *H8* haplotypes), we used the term *CFH* H3/H8 haplotype.

Genetic analysis of *CFB*, *C3*, *DGKE* and *CFHR1-5* was carried out by the Research Laboratory in the 3rd Department of Internal Medicine, Semmelweis University in Budapest. For *CFB*, *C3* and *THBD* the whole coding regions of the genes were sequenced using the ABI 3130 xl genetic analyzer. In order to study copy-number alterations of *CFH*, *CFHR1*, *CFHR2*, *CFHR3* and *CFHR5*, multiplex ligation-dependent probe amplification (MLPA) was performed with SALSA MLPA probemixes P236-A3 and P296-A1 (MRC-Holland, Amsterdam, the Netherlands) following the manufacturer' s instructions.

Novel mutations were tested for functional effects using MutationTaster,[13] SIFT,[14] Provean,[15] and Polyphen-2.[16]

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