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Serratia marcescens as a causative factor of haemolytic uraemic syndrome/thrombotic thrombocytopenic purpura in patients with pre-existing glomerulonephritis

Serratia marcescens jako przyczyna zespołu hemolityczno--mocznicowego/zakrzepowej plamicy małopłytkowej u pacjentów chorujących na kłębuszkowe zapalenia nerek

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Abstract

The most common infective agents precipitating the haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) are *E. coli* and *Shigella species*. We present two cases of patients with pre-existing glomerulonephritis and HUS/TTP associated with a *Serratia marcescens* infection (**Adv. Clin. Exp. Med. 2003, 12, 2, 167–171**).

Key words: haemolytic uraemic syndrome, thrombotic thrombocytopenic purpura, thrombotic microangiopathy, glomerulonephritis, *Serratia marcescens.*

Streszczenie

E. coli i *Shigella species* są najczęstszymi czynnikami infekcyjnymi wywołującymi zespół hemolityczno-mocznicowy (HUS) i zakrzepową plamicę małopłytkową (TTP). W pracy przedstawiono dwa przypadki chorych na kłębuszkowe zapalenia nerek, u których wystąpienie HUS/TTP jest związane z zakażeniem *Serratia marcescens* (**Adv. Clin. Exp. Med. 2003, 12, 2, 167–171**).

Słowa kluczowe: zespół hemolityczno-mocznicowy, zakrzepowa plamica małopłytkowa, mikroangiopatia zakrzepowa, kłębuszkowe zapalenia nerek, *Serratia marcescens*.

Haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) are two syndromes, which are now increasingly referred as thrombotic microangiopathy (HUS/TTP, TMA) [1]. Both are characterized by microangiopathic haemolytic anaemia, thrombocytopenia, functional impairment of various organs: in HUS kidneys, in TTP also others. Clinical signs depend on the diverse distribution of the microvascular lesions and the consequent organ dysfunction. Aetiology of HUS/TTP can be divided in two groups: 1) infectious causes (*E. coli, Shigella*, neuraminidase producing bacteria, HIV and other viruses), 2) non-infectious causes (idiopathic, familial-genetic, drugs, tumours, pregnancy, systemic diseases, transplantation, malignant hypertension superimposed on glomerulonephritis) [2–6]. Infectious causes of HUS are typical for children and are associated with a bloody diarrhoeal prodrome caused by an *E. coli,* most commonly O157: H7, which produces shigella-like toxins I or II (SLT I or II). Apart from *E. coli,* other important SLT producing organism associated with HUS is *Shigella dysenteriae* serotype I (especially in the Indian subcontinent) [1–3]. In TTP, non-infectious causes are more frequent and other organs, except

kidneys, are frequently involved. Patients are usually older [1–3]. Beside the aetiology, whatever organ is involved in TMA, the pathology is the same, that is thrombosis of microcirculation, which is a consequence of thrombocytes adhesion and aggregation at sites of endothelial injury [1].

Case presentation

Case No. 1

Forty-two-year male was admitted due to nephrotic syndrome with normal renal excretory function. His previous history was unremarkable. On admission neurological status was normal, he was markedly oedematous, blood pressure was 150/80 mm Hg with retinopathy grade 0/I. Diuresis was 2500 mL. Laboratory findings disclosed proteinuria 20 g/24 h, haemoglobin (HGB) 15.1 g/dL, red blood cell count (RBC) 5.03 T/L, platelet count (PLT) 166 G/L, serum creatinine (Scr) 1.2 mg/dL, serum urea 23.5 mg/dL, fibrinogen 922 mg/dL, INR 0.67, total serum protein 4.6 g/L, serum albumin 2.1 g/L, total cholesterol 440 mg/dL. Other results were: immunoglobulin's G (IgG) level 220 mg/dL, complement C3c 124 mg/dL, C4 32,2 mg/dL, erythrocyte sedimentation rate (ESR) 21 mm/h and C--reactive protein negative. Carcino-embryonic antigen (CEA), alpha-fetoprotein (AFP), prostate specific antigen (PSA), indirect and direct Coomb's tests and HBs antigen were negative. Antibodies against HCV and HIV were negative. Antibodies against EBV, HSV, and CMV were negative in the IgM class and within normal range in the IgG class. Chest X-ray and abdomen ultrasonography disclosed normal images.

The renal biopsy was performed and revealed membranous glomerulonephritis (MGN). Light microscopy – (hematoxylin/eosin (HE) and periodic acid Schiff (PAS) staining showed 9 glomeruli with a uniform, marked thickening of glomerular walls and slight increase in the cell number, as well as, a moderate expansion of mesangial matrix. Singles foci of fibrosis were found in the interstitium. The periodic acid silver methanamine (PAM) staining revealed spike formation on the outer site of the glomerular basement membrane. Immunofluorescence showed granular deposits of IgG along the capillary walls. Trace amounts of IgA and IgM were found in mesangial areas.

Steroid therapy was instituted: methylprednisolone (MP) 3 × 500 mg intravenously on alternate days, prolonged by oral prednisone 1mg/kg of body weight. During the second week of steroid therapy he developed pneumonia. *Serratia marcescens* was cultured from the sputum. Subsequently acute renal failure (diuresis <1000 mL/24 h, Scr 3.4 mg/dL), anaemia (HGB 8 mg/dL with the presence of schistiocytes in the blood smear), thrombocytopenia (PLT 35 G/L) and low serum fibrinogen (200 mg/dL) were observed. Clotting time was normal, haptoglobin was undetectable. Stool examination was negative for blood and shigella-like toxins (SLT). Neurological status was normal.

Fresh frozen plasma (FFP) therapy and antibiotic against *Serratia* (piperacillin + tazobactam) were administrated. During the next five days the above symptoms subsided. Diuresis increased to 3500 mL/24 h, Scr fell down to 2.0 mg/dL, HGB increased to 10 g/dL, and PLT to 108 G/L. Steroid therapy at the above dose was continued for 2 months, afterwards, tapered slowly to 2.5 mg on alternate days at the end of one year. At the last visit in the outpatient clinic (April 2002) his proteinuria was 3 g/24 h, Scr 1.2 mg/dL, total cholesterol 202 mg/dL, HGB 15 g/dL and PLT 200 G/L.

Case No. 2

Forty-six-year female was admitted due to renal involvement in the course of systemic lupus erythematosus (SLE). SLE was diagnosed four years ago. On admission temperature was 36.6°C, legs were markedly oedematous, blood pressure was 180/90 mm Hg and retinopathy grade I/II was stated. Neurological status was normal. Laboratory findings disclosed proteinuria 2 g/24 h, HGB 10.4 g/dL, RBC 3.37 T/L, PLT 207 G/L, Scr 1.6 mg/dL, and creatinine clearance 30 mL/min (diuresis 1000 mL/24 h). Other parameters were: serum urea 98 mg/dL, fibrinogen 494 mg/dL, INR 0.81, total bilirubin 0.2 mg/dL, total serum protein 4.8 g/dL, serum albumin 2.1 mg/dL, ESR 43 mm/h, indirect and direct Coomb's tests negative. Further results were: immunoglobulin's G level 905 mg/dL, complement C3c – 33.9 mg/dL, C4 – 4.67 mg/dL, antinuclear antibodies (ANA) titre 1/320, serologic test for syphilis (USR) negative, anticardiolipin antibodies slightly positive, clotting time normal. Antibodies against HCV and HIV were negative. Antibodies against HSV were negative in the IgM class, but highly positive in the IgG class. There was no IgM for CMV and EBV detectable with normal levels of antibodies against these viruses in the IgG class. Chest X-ray and abdomen ultrasonography were normal.

The renal biopsy revealed membrano-proliferative glomerulonephritis, lupus class IV.

Light microscopy (HE and PAS staining) showed 20 glomeruli, 9 totally sclerotic, others with exuberant damages. Marked lobulization, mesangial cell proliferation with mesangial interposition, areas of necrosis and hyalinosis were pre-

sent in 11 glomeruli. In four glomeruli adhesion with Bowman's capsule were visible. In addition to these findings, diffuse interstitial inflammation with neutrophilic infiltration and fibrosis were stated. Immunofluorescence microscopy exhibited diffuse, granular deposits of IgG, IgA, IgM, C3, C1q, C4 and fibrinogen.

MP at the dose of 2 × 250 mg was administrated intravenously on alternate days. Since increases in the blood pressure (up to 240/140 mm Hg) were noted after the second pulse of MP, the dose was reduced to 125 mg on alternate days (2×) and prolonged by 0.5 mg/kg of body weight/24 h orally thereafter. Stabilization of the blood pressure around 150/90 mm Hg was achieved. During the second week of MP administration, staphylococcal pneumonia and enterococcal urinary tract infection developed. Teicoplanin was administrated. Five days later *Serratia marcescens* was cultured from the urine. Simultaneously, symptoms of TTP occurred: left arm paralysis, aphasia, worsening in renal function (Scr 3.1 mg/dL) with an increase in the activity of lactate dehydrogenase (LDH) up to 1126 U/L and an increase in total bilirubin level from 0.2 to 0.8 mg/dL. Also, a dramatic drop in haematological parameters (HGB 7.4 mg/dL, PLT 47 G/L with the presence of schistiocytes in blood smear) and low level of fibrinogen 214 mg/dL were observed. Clotting time was normal. Haptoglobin was undetectable. No SLT in excrement were found. Administration of FFP, intravenous IgG (Sandoglobulin, 5 × 12 g on alternate days) and ceftazydym resulted in the recovery from TTP within ten days, despite staphylococcal pneumonia persisting. Laboratory findings disclosed: diuresis 2500 mL/24 h, Scr 2.2 mg/dL, total bilirubin 0.4 mg/dL, HGB 8.8 mg/dL, PLT 120 G/dL, LDH 700 U/L, normal blood smear and no neurological abnormalities. She was discharged on oral steroids and antihypertensive medication. Despite this therapy and low titres of ANA (1/40), lupus nephropathy progressed and half-year later patient required regular haemodialysis therapy (serum urea 214 mg/dL, serum creatinine 5.0 mg/dL, and creatinine clearance 9 mL/min). She was successfully transplanted after one year of dialysis therapy. Up to six months after engrafment, despite cyclosporin A (CSA) administration no symptoms of HUS/TTP have occurred.

Discussion

The relationship between glomerulonephritis (GN) and secondary TMA is relatively rare. The most often reported are cases with simultaneous occurrence of HUS and acute post-infectious GN, caused by beta-haemolytic streptococci infections [6, 7]. Among other described cases were patients who developed HUS/TTP in association with membranoproliferative GN, focal segmental glomerulosclerosis, crescentic GN, and mesangial proliferative GN in the course of IgA nephropathy [8]. Concerning idiopathic membranous GN there is only one case of HUS occurring in a 20-year-old reported. Besides, single cases of HUS/TTP that occurred in nephrotic individuals have been described [4, 6, 8]. The pathogenesis of TMA in the setting of primary glomerular disease is unclear. Our first patient had history of MGN, but this solely does not seem to justify the occurrence of TMA. The most probably glomerular cells injured in the course of idiopathic MGN are podocytes but no endothelial cells [9]. Since in our patient with MGN HUS/TTP occurred after *Serratia* infection and resolved after antibiotic administration, this bacterium was supposed to contribute to the development of TMA.

Serratia marcescens, Escherichia, Shigella, Salmonella, Yersinia, Alcaligenes, Proteus, Klebsiella, Campylobacter, Citrobacter, Enterobacter, Hafnia, Aeromonas and other bacteria are the Gramm-negative bacilli from a wide enterobacteriace family. They gather and exchange self-transferable plasmids, which mediate resistance to antibiotics. It is also known that many bacteriophages can transduce DNA within this bacterial family [3, 10]. It is possible that SLT (or very similar protein) belongs to *Serratia's* toxins [11]. This putative toxin was supposed to precipitate the TMA syndrome in our patient.

The coexistence of systemic diseases and HUS/TTP is also rare. TMA is presumed to occur in 2–9.5% of adults with SLE [5, 12]. The differential diagnosis between SLE with autoimmune haemolytic anaemia and HUS/TTP is particularly difficult [13]. The situation becomes more complicated when patient with SLE has antiphospholipid antibodies, which can precipitate antiphospholipid syndrome (APL) [12, 14, 15]. Clinical symptoms of catastrophic APL are very similar to HUS/TTP. Table 1 presents the differentiation between haematological parameters observed in TTP, SLE and APL. How APL antibodies lead to thromboembolic events is still unknown. Probably the procoagulant effect of APL autoantibodies is linked to the inhibition of the natural anticoagulants [12]. Also others autoantibodies can lead to the development of TMA: anti-neutrophil cytoplasmic antibodies (ANCA), anti-factor VIII antibodies, anti-platelet antibodies, anti-protein C and S antibodies, anti-endothelial cell antibodies and anti-CD36 antibodies [1, 14].

The expensive and complicated differential diagnostic tests are important for long-term strategy

Table 1. Comparison of the haematologic characteristics of TTP, SLE, and primary APL antibodies syndrome. After: Porta C. et al.: Hematologica 1999, 84, 260–269 [14]

Tabela 1. Porównanie wartości hematologicznych charakterystycznych dla TTP, SLE i pierwotnego zespołu antyfosfolipidowego APL; według Porta C. et al. Haematologica 1999, 84, 260–269 [14]

Haematologic characteristic	TTP	SLE	Primary APL syndrome
Thrombocytopenia	consumptive	immune-mediated	immune-mediated
Microangiopathic haemolytic anaemia	present	absent	absent
Schistiocytes	present	rare	absent
APL antibodies	absent	variable	present at high titer

of treatment. Plasma exchange (infusion), the most effective method of treatment, should be promptly initiated in TMA patients [1, 12]. Moreover, autoimmunological causes require high doses of steroids, heparin and anti-vitamin K [1, 14]. In relapsing cases, it is necessary to exclude genetic predisposition [1, 16]. In our patient with SLE, APL antibodies were slightly positive. However, since the clotting time was within normal range, disseminated intravascular coagulation could be excluded. Besides, schistiocytes were present in the blood. Autoantibodies as a trigger of TMA did not seem to be the cause. Patient was treated with high doses of steroids and decreased ANA titer (from 1/320 to 1/40) was observed as a result of this treatment. The negative family history and no relapses of TMA during the observation period argue against a familial-genetic form of this syndrome, although this possibility is obviously not finally excluded. Similarly, no drug intoxication precipitating the secondary form of HUS/TTP could be suspected. Severe or accelerated hypertension should also be taken into account, especially in SLE [7]. Both patients had moderate hypertension, but there was no time relationship between increases in the blood pressure and symptoms of TMA.

In summary, the tight time-relationship between the occurrence of TMA and *Serratia marcescens* infection, as well as the disappearance of this syndrome as a result of specific anti-bacteria therapy point to the *Serratia marcescens* as a causative factor of TMA in our patients.

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