KLINIČKE I SEROLOŠKE ODLIKE JEDANAESTOMESEČNOG ODOJČETA SA PAROKSIZMALNOM HEMOGLOBINURIJOM NA HLADNOĆU

PRIKAZ SLUČAJA

CASE REPORT

CLINICAL AND SEROLOGICAL FEATURES IN AN 11-MONTH-OLD INFANT WITH PAROXYSMAL COLD HEMOGLOBINURIA

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SAŽETAK

Uvod: Paroksizmalna hemoglobinurija na hladnoću (PHH) je neuobičajen oblik autoimune hemolizne anemije. Specifičan etiološki agens nije utvrđen. Patogeneza bolesti se objašnjava prisustvom „bifaznog hemolizina" ili IgG autoantitela slabog afiniteta koje se u najvećem broju slučajeva vezuje za eritrocitni antigen P, vezujući komponente komplementa do potpune aktivacije kaskade i hemolize eritrocita. Bolest se tipično manifestuje kod dece nakon akutne infekcije, najčešće gornjih respiratornih puteva ili gastroenteritisa.

Prikaz slučaja: Prikazujemo 11-mesečno žensko odojče koje je primljeno u Odeljenje intenzivne nege Instituta zbog povraćanja, anemije, žute prebojenosti kože i sluzokože, tamno crvenog urina i opšte klonulosti. Dva dana pre prijema imala je sekreciju iz nosa. U inicijalnim laboratorijskim rezultatima registrovana je anemija sa hemoglobinom 63 g/L, hematokritom 17, 3%, retikulocitima 1,76% uz leukocitozu, trombocitozu, povišene vrednosti C reaktivnog proteina 75,7 mg/L, laktat dehidrogenaze 5365 ij/L, ukupnog/indirektnog bilirubina 67,9/64,5μmol/L, i snižen haptoglobin. U razmazu periferne krvi nije utvrđeno prisustvo šizocita. Polispecifičan direktan antiglobulinski test (DAT) bio je pozitivan 3+, a monospecifičan DAT C3d 1+. Odojče je lečeno intravenskim imunoglobulinima, transfuzijom jedne doze eritrocita, parenteralnom, dvojnom antimikrobnom terapijom i jednokratnom primenom kortikosteroida uz intravensku hidraciju i korekciju elektrolitnih poremećaja. Desetog dana hospitalizacije, uzeti su uzorci krvi za Donath-Landsteiner-ov (DL) test kojim je dokazano prisustvo „bifaznog hemolizina" i potvrđena dijagnoza PHH.

Zaključak: Kliničko prepoznavanje PHH, tranzitorna priroda „bifaznog hemolizina", i mogućnost za izvođenje DL testa su faktori od kojih zavisi pravovremena potvrda dijagnoze ovog retkog oblika AIHA.

Ključne reči: autoimuna hemolizna anemija, anti-P antitelo, Donath-Landsteiner-ov test

ABSTRACT

Introduction: Paroxysmal cold hemoglobinuria (PCH) is an uncommon form of autoimmune hemolytic anemia (AIHA). A specific etiological factor has not been determined yet. The pathogenesis of the disease is explained by the presence of "biphasic hemolysin" or low-affinity IgG autoantibody, which in most cases binds to the erythrocyte P antigen, binding components of complement until the complete activation of the cascade and hemolysis of erythrocytes. The disease typically presents in children following an acute infection, usually involving the upper respiratory tract or gastroenteritis.

Case report: We present an 11-month-old female infant who was admitted to the Intensive Care Unit of the Institute due to vomiting, anemia, yellow discoloration of the skin and mucous membranes, dark-red-colored urine, and general weakness. Two days prior to admission, she had a nasal discharge. Initial blood tests revealed a hemoglobin level of 63 g/L, hematocrit at 17.3%, reticulocyte count of 1.76%, leukocytosis, thrombocytosis, elevated C-reactive protein at 75.7 mg/L, lactate dehydrogenase at 5365 IU/L, total/indirect bilirubin at 67.9/64.5 μmol/L, and decreased haptoglobin. No schizocytosis was evident in peripheral blood. Polyspecific direct antiglobulin test (DAT) was positive 3+ and monospecific DAT was C3d 1+. The infant was treated with intravenous immunoglobulins, a single unit of red blood cells transfusion, parenteral dual antimicrobial therapy, and a single dose of corticosteroids accompanied by intravenous hydration and correction of electrolyte disturbances. On the tenth day upon admission, blood samples were taken for the Donath-Landsteiner (DL) test, which showed the presence of "biphasic hemolysin" and confirmed the diagnosis of PCH.

Conclusion: Clinical recognition of PCH, the transient nature of "biphasic hemolysin" and the possibility of performing the DL test depend on the timely confirmation diagnosis of this rare form of AIHA.

Keywords: autoimmune hemolytic anemia, anti-P antibody, Donath-Landsteiner test

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Primljeno • *Received*: May 14, 2024; **Revidirano • *Revised*:** June 7, 2024; **Prihvaćeno • *Accepted*:** June 11, 2024; ***Online first*:** June 25, 2024

DOI: 10.5937/smclk5-51033

UVOD

Paroksizmalna hemoglobinurija na hladnoću (PHH) je retka i često zanemarena forma autoimune hemolizne anemije (AIHA) koja se manifestuje intravaskularnom hemolizom i hemoglobinurijom. Specifičan etiološki agens nije utvrđen. Patogeneza bolesti se objašnjava prisustvom „bifaznog hemolizina", poliklonskog IgG autoantitela slabog afiniteta koje se u perifernoj cirkulaciji vezuje isključivo za eritrocite na temperaturama nižim u odnosu na telesnu temperaturu. U ovom procesu dolazi i do vezivanja komponenti komplementa. Iako u centralnoj cirkulaciji antitelo disocira, komplement ostaje vezan uz potpunu aktivaciju kaskade i sledstvenu hemolizu eritrocita na temperaturama oko 37°C. Ključni element u patofiziologiji bolesti je ponavljano vezivanje i disocijacija antitela u normalnoj cirkulaciji. U prošlosti je PHH bila hronična manifestacija kasnog sifilisa ili kongenitalnog sifilisa. Austrijski lekari, Julijus Donat i Karl Lendštajner su 1904. godine *in vitro* dokazali bifaznu prirodu autoantitela, a test kojim su to ustanovili koristimo i do današnjih dana. Zbog toga se u literaturi ravnopravno koriste alternativni nazivi Donath-Landsteiner antitelo i Donath-Landsteiner hemolizna anemija [1-3].

Epidemiologija i klinička slika PHH značajno se izmenila tokom poslednjih 60 – 80 godina. U današnje vreme bolest se ispoljava pretežno u dečjem uzrastu kao akutna intravaskularna hemoliza povezana sa infektivnim činiocima: virusnim infekcijama (adenovirus, influenza A, citomegalovirus, Epstein-Barr virus, virus morbila, virus mumpsa) ili bakterijskim infekcijama (*Hemophilus influenzae*, *Mycoplasma pneumoniae*, *Escherichia coli*). U kliničkoj praksi su potvrđeni pojedinačni slučajevi PHH nakon primene vakcina mRNK protiv Kovida 19, sedmovalentne konjugovane vakcine protiv pneumokoka i vakcine protiv morbila. Paroksizmalna hemoglobinurija na hladnoću može retko biti povezana sa limfoproliferativnim i autoimunim bolestima. Kod malog broja obolelih hemoliza je nepoznatog uzroka (primarni oblik) [4-7].

Tipično, bolest se manifestuje kod dece nakon akutne infekcije, najčešće gornjih respiratornih puteva, ili gastroenteritisa. Prosečan vremenski interval od pojave infekcije do ispoljavanja PHH je dve nedelje (dva dana do pet nedelja). Bolest počinje iznenada, sa hemoglobinurijom kod više od dve trećine bolesnika koja je praćena groznicom, žuticom i/ili ikterusom sklera kod više od četvrtine bolesnika. Urin je karakteristične tamno crvene do braon boje. Anemija je često teškog stepena. Direktan antiglobulinski test (DAT) je obično pozitivan samo za komplement [8].

Dijagnoza PHH se potvrđuje Donath-Landsteiner (DL) testom. Otkrivanje patognomoničnog DL antite-

INTRODUCTION

Paroxysmal cold hemoglobinuria (PCH) represents a rare and frequently underrecognized type of autoimmune hemolytic anemia (AIHA), characterized by intravascular hemolysis and hemoglobinuria. A specific etiological factor has not been determined yet. The pathogenesis of the disease is attributed to the presence of "biphasic hemolysin," a low-affinity polyclonal IgG autoantibody. This autoantibody binds exclusively to erythrocytes in the peripheral circulation at temperatures lower than body temperature. In this process, the binding of complement components occurs. Although the antibody dissociates in the central circulation, the complement remains bound, leading to the complete activation of the cascade and subsequent hemolysis of erythrocytes at temperatures around 37°C. A key element in the pathophysiology of the disease is the repeated binding and dissociation of antibodies in normal circulation. In the past, PCH was a chronic manifestation of tertiary syphilis or congenital syphilis. In 1904, Austrian doctors Julius Donath and Karl Landsteiner demonstrated the biphasic nature of autoantibodies in vitro and developed a test that is still used. For this reason, the alternative names Donath-Landsteiner antibody and Donath-Landsteiner hemolytic anemia are used with equal frequency [1-3].

Over the past 60-80 years, the epidemiology and clinical picture have changed significantly. Nowadays, the disease primarily manifests in children as acute intravascular hemolysis associated with infectious agents including viral infections (adenovirus, influenza A, cytomegalovirus, Epstein-Barr virus, measles virus, mumps virus) and bacterial infections (*Hemophilus influenzae, Mycoplasma pneumoniae, Escherichia coli*). In clinical practice, individual cases of PCH have been confirmed following the administration of mRNA vaccine against COVID-19, heptavalent pneumococcal conjugate vaccine, and measles vaccine. Paroxysmal cold hemoglobinuria can rarely be associated with lymphoproliferative and autoimmune diseases. In a small number of patients, hemolysis occurs without a known cause (primary form) [4-7].

The disease typically presents in children following an acute infection, usually involving the upper respiratory tract or gastroenteritis. The average time interval from the onset of infection to the manifestation of PCH is two weeks (two days to five weeks). The disease has a sudden onset, with hemoglobinuria present in more than two-thirds of patients, followed by fever, jaundice, and /or conjunctival icterus in more than a quarter of patients. Urine has the characteristic dark red, even brown color. Severe anemia is often present. The direct antiglobulin test (DAT) usually shows positivity only for complement [8].

la je laboratorijski izazov. Zahteva specifičnu pripremu uzoraka, inkubacije na različitim temperaturama i dobro edukovano osoblje [9].

Paroksizmalna hemoglobinurija na hladnoću je tranzitorna bolest. Uobičajeno se povlači spontano u roku od nekoliko dana do nekoliko nedelja.

Cilj rada je prikaz bolesnika kod koga je DL testom potvrđena retka forma AIHA, PHH. U Republici Srbiji, DL test je za sada moguće uraditi na Odeljenju za transfuziju krvi našeg Instituta.

PRIKAZ SLUČAJA

Žensko odojče uzrasta 11 meseci prevedeno je iz regionalnog zdravstvenog centra (RZC) zbog povraćanja, žute prebojenosti kože, sluzokože i opšte klonulosti. Nije bila febrilna. Dva dana pre prijema imala je sekreciju iz nosa, zbog čega je majka davala Aerius sirup. Rezultati laboratorijskih analiza učinjenih u RZC bili su sledeći: leukociti 23,7 x 10^9 /L, hemoglobin 74 g/L, eritrociti 2,7 x 10^{12} /L, CRP 70 mg/l, bilirubin 126 µmol/L. Pregledana je od strane hirurga koji je isključio akutnu hiruršku bolest.

Odojče telesne mase 9,670 kg i telesne dužine 75 cm primljeno je u Odeljenje intenzivne nege (OIN) Instituta. Na prijemu u OIN je poremećenog stanja svesti, tahikardična sa srčanom frekvencom oko 160/min, uz

The diagnosis of PCH is confirmed by Donath-Landsteiner (DL) test. The detection of pathognomonic DL antibodies poses a laboratory challenge. It requires a specific sample preparation, incubation at different temperatures, and well-educated personnel [9].

Paroxysmal cold hemoglobinuria is a transient disease. It typically resolves spontaneously within a span of a few days to a few weeks.

The aim of this paper is to present a patient in whom a rare form of AIHA, PCH, was confirmed by the DL test. In the Republic of Serbia DL test can currently be done at the Blood Transfusion Department of the Institute for Mother and Child Health Care of Serbia.

CASE PRESENTATION

An 11-month-old female infant was transferred from a regional health center (RHC) due to vomiting, yellow discoloration of the skin and mucous membranes, and overall lethargy. She did not have a fever. Two days prior to admission, she had nasal discharge, and her mother gave her Aerius syrup. The results of the laboratory analyses done at the RHC were as follows: leucocytes 23,7 x 10^9 /L, hemoglobin 74 g/L, erythrocytes 2,7 x 10^{12} /L, CRP 70 mg/l, bilirubin 126 µmol/L. She was examined by a surgeon, who ruled out any acute surgical condition.

Tabela 2. Višestruka regresiona analiza

Table 2. Multiple regression analysis

Rezultati laboratorijskog ispitivanja / *Laboratory test results*	***2 dana pre prijema / 2 days prior to admission***	***na dan prijema / The day of admission***	***11. dan (dan otpusta) / 11th day (day of discharge)***	***23. dan od otpusta / 23rd day upon discharge***
Hemoglobin (109,0 – 138,0 g/L) / (109.0 – 138.0 g/L)	74.0	63.0	91.0	119
Hematocrit (0,32 – 0,404 L/L) / (0.32 – 0.404 L/L)	/	0.173	0.290	0.379
Erythrocytes (4,0 – 5,0 x10^{12}/L) / (4.0 – 5.0 x10^{12}/L)	2.7	2.45	3.44	3.82
Reticulocytes (0,5 – 1,5%) / (0.5 – 1.5%)	/	1.76	7.78	1.9
Leucocytes (6 – 16 x 10^9/L) / (6 – 16 x 10^9/L)	23.7	18.7	21.2	11.6
Platelets (150,0 – 450,0 x10^9/L) / (150,0 – 450,0 x10^9/L)	/	556	557	499
CRP* (mg/L)	70	75.7	/	/
Total bilirubin (6.8 – 20.5 µmol/L)	67.9			
Direct bilirubin (1.71 – 6.84 µmol/L)	3.4			
Lactate dehydrogenase (<1100 µmol/L)	5365			
Haptoglobin (0.71 – 2.39 g/L)	0.519			
Hemoglobinuria	Positive			

žutu prebojenost kože i sluznica. Vrat bez limfadenopatije. Ždrelo blago hiperemično, nos prohodan, bez sekreta. Po postavljanju urinarnog katetera dobijen je tamno crveno prebojen urin uz proteinuriju, hematuriju i prisustvo uratnih cilindara. Na ultrazvučnom pregledu abdomena i na radiografiji abdomena i urotrakta nisu uočene patološke promene. U krvnoj slici je registrovana anemija, retikulocitoza uz leukocitozu, trombocitozu, povišen CRP, laktat dehidrogenaza, ukupan/indirektan bilirubin i snižen haptoglobin (Tabela 1).

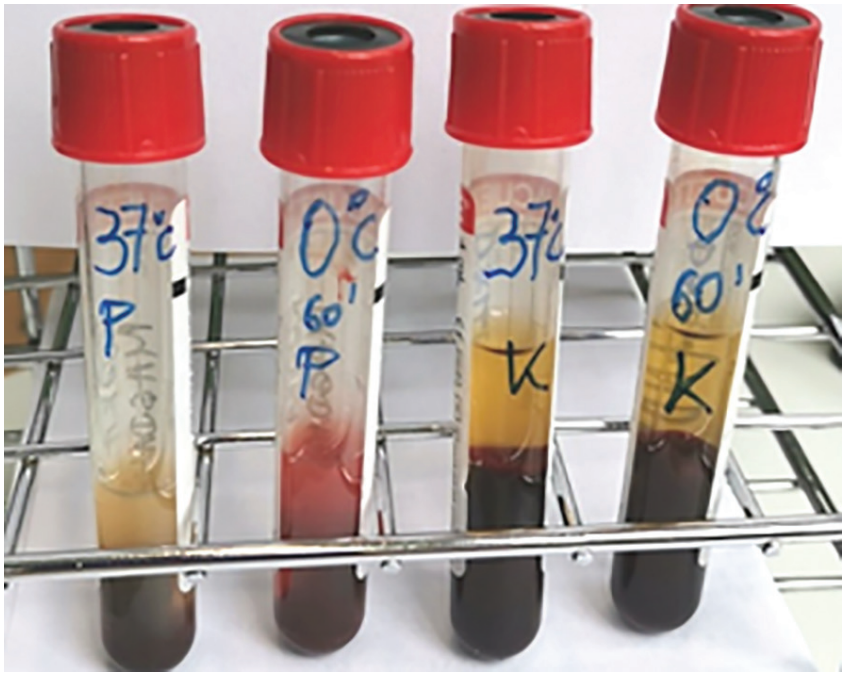
U razmazu periferne krvi nisu uočene patološke forme eritrocita (šizociti). Imunohematološkim ispitivanjem određena je: krvna grupa O RhD+, polispecifičan DAT: 3+ i monospecifičan DAT: C3d 1+, IgG–.

Lečenje je započeto primenom intravenskih imunoglobulina. Ordinirana je parenteralna dvojna antimikrobna terapija (cefotaksim, metronidazol), jednokratno je primenjena kortikosteroidna terapija uz inhibitor protonske pumpe (pantoprazol), intravensku hidraciju i korekciju elektrolitnih poremećaja. Za lečenje anemije data je transfuzija jedne doze kompatibilnih eritrocita. Diferencijalno dijagnostički zbog sumnje na hemolizno uremijski sindrom (HUS) ispitivanje je dopunjeno imunološkim testiranjem (ANCA, ANA, anti-dsDNA, C3, C4). Ostavljen je uzorak krvi radi eventualnog slanja u inostrani centar radi dijagnostike atipičnog HUS-a. Devojčica je u daljem toku stabilnog i boljeg opšteg kli-

An infant weighing 9.670 kg and measuring 75 cm in length was admitted to the Intensive Care Unit (ICU) of the Institute. On admission to the ICU, she was found to be in a disoriented state, tachycardic with a heart rate of approximately 160 bpm, and with yellow discoloration of the skin and mucous membranes. The pharynx was slightly hyperemic, with a clear nose and no secretions present. After insertion of a urinary catheter, the obtained urine was dark red in color, with the presence of proteinuria, hematuria, and urate cylinders. Ultrasound examination of the abdomen and radiography of the abdomen and urinary tract did not reveal any pathological changes. The blood count revealed anemia, reticulocytosis and leukocytosis, thrombocytosis, elevated levels of CRP, lactate dehydrogenase and total/indirect bilirubin, and diminished haptoglobin (Table 1).

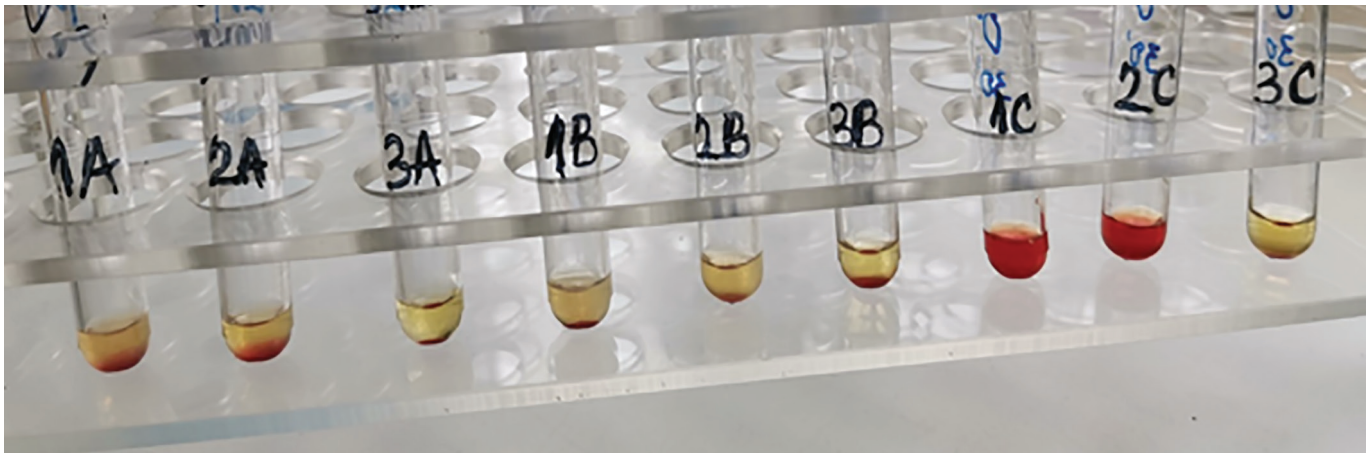
No pathological forms of erythrocytes (schizocytes) were evident in peripheral blood. The immunohematological examination revealed the following results: blood type 0, RhD positive, polyspecific DAT 3+, and monospecific DAT C3d 1+, IgG negative.

The patient was treated with intravenous immunoglobulins. Parenteral dual antimicrobial therapy (cefotaxime, metronidazole) was prescribed, followed by one dose of corticosteroid therapy and a proton pump inhibitor (pantoprazole), intravenous hydration,



Slika 1. Rezultat direktnog Donath-Landsteiner testa
P – uzorci bolesnika; K – uzorci zdrave osobe (kontrola)
Hemoliza eritrocita u uzorku bolesnika koji je inkubiran na 0°C, a zatim na 37°C. Nema hemolize u kontrolnoj epruveti. Nema hemolize u uzorcima bolesnika i zdrave osobe (kontrola) koje su inkubirane na 37°C.

Figure 1. The result of the direct Donath-Landsteiner test
P – patient samples; K – healthy person samples (control)
Hemolysis of patient erythrocytes when patient sample was incubated at 0 °C, followed by the incubation at 37°C. No hemolysis was observed in control tube.
No hemolysis was seen when patient sample and control sample were incubated at 37°C.



Slika 2. Rezultat indirektnog Donath-Landsteiner testa
Prisutna je hemoliza alogenih eritrocita kada se serum bolesnika, sa ili bez dodatog komplementa iz seruma zdrave osobe inkubira na 0 °C, a zatim na 37°C (1C i 2C). Nema hemolize u epruvetama gde je serum bolesnika ili mešavina seruma bolesnika i zdrave osobe koje su inkubirane na 0°C (1A i 2A) ili na 37°C (1B i 2B). Nema hemolize u kontrolnim epruvetama koje sadrže serum zdrave osobe (3A, 3B i 3C)

Figure 2. The result of the indirect Donath-Landsteiner test
Hemolysis of donor erythrocytes was observed when patient serum or a mixture of patient serum and normal serum (tubes 1C and 2C) were incubated at 0 °C followed by incubation at 37°C. No hemolysis was seen when patient serum or mixed serum were incubated at 0°C (tubes 1A and 2A) or 37°C (tubes 1B and 2B). No hemolysis was observed in control tubes containing normal serum only (tubes 3A,3B, 3C)

ničkog stanja, urednih vitalnih funkcija, uz iščezavanje žute prebojenosti kože.

Tokom evaluacije bolesti posumnjalo se na PHH. Desetog dana hospitalizacije uzeti su uzorci krvi za direktan i indirektan Donath-Landsteiner test. Dobijeni su pozitivni rezultati na prisustvo DL autoantitela, odnosno registrovna je hemoliza eritrocita u uzorcima bolesnika koji su sa ili bez dodavanja egzogenog komplementa inkubirani najpre na 0°C a potom na 37°C (Slika1 i Slika 2).

Odojče je 11. dana od prijema u bolnicu otpušteno kući dobrog opšteg stanja, urednih vitalnih parametara i fizikalnog nalaza uz savet za redovnu kontrolu pedijatra.

DISKUSIJA

Prikazano 11-mesečno odojče sa kliničkim i laboratorijskim znacima hemolize eritrocita predstavlja složen dijagnostički izazov.

Autoimuna hemolizna anemija je redak entitet u pedijatrijskoj populaciji sa incidencijom koja je procenjena na 0,81 slučaj na 100 000 za mlađe od 18 godina [10]. Iako se početno lečenje teške anemije sastoji od hemodinamske stabilizacije i supstitucione terapije transfuzijom kompatibilnih eritrocita, naknadno lečenje i prognoza se razlikuju u zavisnosti od etiologije. Akutna ili teška epizoda AIHA, kakva je predstavljena u ovom slučaju, može biti životno ugrožavajuća, ali konsenzus smernica za kliničko lečenje ovih pacijenata je ograničen niskom incidencijom, a nekad i nemogućnošću da se prepoznaju atipični slučajevi bolesti. U ovom slučaju, postojali su klinički i laboratorijski dokazi za

and correction of electrolyte disturbances. One unit of compatible erythrocytes was transfused to treat anemia. Differential diagnostic examination for suspected hemolytic uremic syndrome (HUS) was supplemented with immunological testing (ANCA, ANA, anti-dsDNA, C3, C4). A blood sample was collected and prepared for potential transfer to a center abroad for diagnosing atypical HUS. Later on, the girl was in a stable and improving general condition, with normal vital signs, and decreasing yellow discoloration of the skin.

During the evaluation of the disease, PCH was suspected. On the tenth day upon admission, blood samples were taken for the direct and indirect Donath-Landsteiner (DL) test. Positive results were obtained for the presence of DL autoantibodies, i.e., hemolysis of erythrocytes was observed in patient samples incubated both with and without the addition of exogenous complement, first at 0°C and then at 37°C (Image 1 and Image 2).

On the eleventh day upon admission, the infant was discharged in a stable condition, with normal vital signs and physical examination findings. She was sent home with instructions for regular check-ups with her pediatrician.

DISCUSSION

The 11-month-old infant, exhibiting clinical and laboratory signs of erythrocyte hemolysis, presents a complex diagnostic challenge.

Autoimmune hemolytic anemia is rare in the pediatric population and its estimated incidence is 0.81 cases per 100,000 in those under 18 years of age [10]. While the initial treatment of severe anemia involves hemodynamic stabilization and transfusion of compat-

intravaskularnu hemolizu uz pozitivnost DAT-a samo za komponentu komplementa C3d. Evaluacija bolesti s obzirom na tipičnu kliničku sliku išla je i u pravcu PHH.

Klinički lekari treba da imaju na umu da sumnja na PHH ne bi trebalo da zavisi od prisustva aktivne infekcije, a infekcija ne mora uvek da prethodi razvoju PHH [8,11]. Intravaskularna hemoliza i rapidno progresivna anemija mogu izazvati malaksalost, bledilo, bol u stomaku, mučninu i povraćanje [8].

Sistematski pregled Jakobsa i saradnika, obuhvatio je 125 publikacija u kojima je prikazano ukupno 230 bolesnika sa dijagnozom PHH koja je potvrđena DL testom. Distribucija učestalosti prema polu je: muški/ženski pol, 124/106 (odnos 1,17:1). Medijana uzrasta je 5 godina (8 meseci – 91 godina) sa interkvartilnim opsegom 2,6 - 51 godine. Najniža prosečna vrednost hemoglobina utvrđena je u starosnoj grupi od 10 do 19 godina (48 g/l; opseg, 23 – 106 g/l), a zatim u grupi od 0 do 9 godina (53 g/l; opseg, 20 – 129 g/l) i grupi od 20 do 29 godina (54 g/l; opseg, 27 – 161 g/l) [8].

Specifičnost bifaznog autoantitela dokazana je kod 31% odnosno 71/230 bolesnika. Kod 59/71 (83,1%) potvrđen je IgG anti-P. Druga retko prisutna antieritrocitna antitela su: IgG anti-I, IgM anti-I, anti-PP1P^{k} (Tj^{a}), IgG anti-i, IgM anti-P, IgA anti-P, anti-Pr-like i IgM antitelo neutvrđene specifičnosti [8].

Pitanje porekla autoantitela u PHH nije razjašnjeno. Prema jednom modelu, antigen P je izmenjen infektivnim agensom ili ovaj agens izaziva unakrsnu reaktivnost. Infektivni agens može indukovati promene površinskog globozida (antigena P) na membrani eritrocita, čime se povećava njegova imunogenost. Druga hipoteza sugeriše da glikosfingolipid postaje imunogen kada se veže za virusne antigene. Treća hipoteza je povećana proizvodnja autoantitela zbog povećane aktivacije limfocita ili imunološke disregulacije uzrokovane virusom. Sva 3 mehanizma mogu biti prisutna i u svakom pojedinačnom slučaju PHH [12-14].

Direktan antiglobulinski test je zlatni standard za AIHA. U PHH obično je pozitivan samo za komplement, ali može biti negativan i za C3d i za IgG.

Dijagnoza PHH se potvrđuje DL testom. Budući da se ovaj test retko izvodi, testiranje je moguće sprovesti samo u referentnim laboratorijama ili u pojedinim bolničkim bankama krvi [11,15]. Test se smatra pozitivnim kada serum bolesnika, sa ili bez dodatog komplementa iz seruma zdrave osobe, ispoljava hemolizu u epruvetama koje su prethodno inkubirane na 0°C, a zatim na 37°C, i s druge strane odsustvo hemolize eritrocita u bilo kojoj epruveti inkubiranoj odvojeno, na 0°C i na 37°C. Epruvete koje sadrže serum zdrave osobe služe kao negativna kontrola i u njihovom sadržaju ne treba da se ispolji hemoliza [9,11].

ible red blood cells, subsequent treatment and prognosis vary depending on the etiology. An acute or severe episode of AIHA, as seen in this case, can be life-threatening. However, consensus guidelines for managing these patients are limited due to the low incidence of the disease and the challenges in recognizing atypical cases. In this case, there was clinical and laboratory evidence for intravascular hemolysis with DAT positivity only for the complement component C3d. The evaluation of the disease, considering the typical clinical presentation, also suggested PCH.

Clinicians should keep in mind that suspicion of PCH should not depend on the presence of an active infection, and that infection does not always have to precede the development of PCH [8,11]. Intravascular hemolysis and rapidly progressing anemia may cause symptoms such as general weakness, pallor, abdominal pain, nausea, and vomiting [8].

The systematic review by Jacobs et al. included 125 publications where a total of 230 patients whose PCH diagnosis had been confirmed by DL test were presented. The gender-based frequency distribution was as follows: male/female, 124/106 (ratio 1.17:1). The median age is 5 years (ranging from 8 months to 91 years), with an interquartile range of 2.6 to 51 years. The lowest average value of hemoglobin was found in the age group 10-19 years (48 g/l; range 23-106 g/l), followed by the age group 0-9 years (53 g/l; range 20-129 g/l), and the age group 20-29 years (54 g/l; range 27 -161 g/l) [8].

The specificity of the biphasic autoantibody was confirmed in 31% (71/230), patients. IgG anti-P was confirmed in 59/71 (83,1%) patients. Other rarely present anti-erythrocyte antibodies include IgG anti-I, IgM anti-I, anti-PP1Pk (Tja), IgG anti-i, IgM anti-P, IgA anti-P, anti-Pr-like antibodies, and IgM antibody with undetermined specificity [8].

The origin of autoantibodies in PCH has not been clarified. According to one model, the P antigen is altered by an infectious agent, or this agent leads to cross-reactivity. An infectious agent can induce changes in the surface globoside (P antigen) on the erythrocyte membrane, which increases its immunogenicity. Another hypothesis posits that glycosphingolipids become immunogenic upon binding to viral antigens. A third hypothesis proposes that increased lymphocyte activation or immune dysregulation caused by a virus leads to heightened production of autoantibodies. All three mechanisms can be present in each individual case of PCH [12-14].

Direct antiglobulin test is a golden standard for AIHA. In PCH there is usually positivity only for complement, but it may be negative for both C3d and IgG.

The diagnosis of PCH is confirmed by DL test. Since this test is rarely performed, it can be done only in ref-

Bifazno autoantitelo je tranzitorno i detektabilno samo tokom faze akutne hemolize, zbog čega u suspektnim slučajevima DL test treba sprovesti što je ranije moguće [11]. Negativan rezultat DL testa ne isključuje dijagnozu PHH u odgovarajućem kliničkom kontekstu. Najčešći uzrok lažno negativnih rezultata je nizak i nedetektabilan titar DL antitela. Drugi mogući uzroci lažno negativnih rezultata su autoadsorpcija DL antitela u slučaju kada se odvajanje seruma ne vrši na 37°C ili neutralizacija anti-P antitela pomoću globozida u svežem serumu koji se dodaje kao izvor komplementa. Dodavanje egzogenog komplementa je važno, jer se pretpostavlja da je oboleli iscrpeo svoj komplement zbog hemoliznog procesa. Pored lažno negativnih, mogući su i lažno pozitivni rezultati, posebno kod osoba sa monofaznim IgM autoantitelima sa širokom termičkom amplitudom [16].

Paroksizmalna hemoglobinurija na hladnoću je tranzitorna bolest. Zahteva adekvatnu negu bolesnika uz kontrolu telesne temperature, odmor, hidrataciju, izbegavanje izlaganja hladnoći. Ako je anemija teška, neophodno je suportivno lečenje transfuzijom eritrocita. Bolesnika treba utopliti, a grejače krvi za transfuziju eritrocita koristiti oprezno budući da nema dovoljno dokaza koji bi podržali ovu praksu. Tipizirani eritrociti fenotipa p odnosno P-null su retki i nisu dostupni za hitnu transfuziju. Ipak, većina pacijenata kojima je potrebna transfuzija postiže zadovoljavajući posttransfuzioni hemoglobin uprkos pretpostavljenoj ili dokumentovanoj P inkompatibilnosti [11]. Antigen P je jedan od ukupno tri antigena iz krvno-grupnog sistema GLOB. Ovaj sistem označen je brojem 028 prema klasifikaciji Radne grupe za imunogenetiku eritrocita i terminologiju krvnih grupa Međunarodnog udruženja za transfuziju krvi. Gen koji kodira sintezu antigena P, *B3GALNT1* kloniran je 1998. godine. Smešten je na dugom kraku hromozoma 3 (3q26.1) [17]. Osobe koje ne eksprimiraju antigen P su vrlo retke. Procenjena učestalost fenotipa p je 5,8/1 000 000 stanovnika Evrope. Veća učestalost od 141/1 000 000, registrovana je u okrugu Vesterboten u severnoj Švedskoj. Takođe, fenotip p je češći u populaciji Japana i među Amišima [18].

Na osnovu prikaza tri bolesnika, Harada i Sonoda su zapazili da bolest ima sezonski karakter, proleće ili jesen, i ukazali na mogućnost da se dnevne varijacije temperature vazduha mogu dovesti u vezu sa temperaturno zavisnim DL antitelom. Naše iskustvo sa DL testom koji smo uveli kao standardnu operativnu proceduru od 2017. godine je slično. Kod prvog bolesnika, 23-mesečne devojčice hemolizna kriza u vezi sa PHH dokazana je DL testom u novembru i kod 11-mesečnog ženskog odojčeta u martu [15,19].

erence laboratories or in certain hospital blood banks [11,15]. The test is deemed positive if the patient's serum, either with or without added complement from a healthy individual's serum, shows hemolysis in tubes that were pre-incubated at 0°C and subsequently at 37°C. Conversely, the test is negative if there is no erythrocyte hemolysis in any tube incubated separately at 0°C and 37°C. The test tubes containing serum from a healthy person act as a negative control, and their contents should not exhibit hemolysis [9,11].

The biphasic autoantibody is transient and detectable only during the phase of acute hemolysis, which is why, in suspected cases, the DL test should be performed as early as possible [11]. A negative DL test result does not exclude PCH in an appropriate clinical context. The most common reason for false-negative results is a low and undetectable DL antibody titer. Other potential causes of false-negative results include autoadsorption of DL antibodies when serum separation is not carried out at 37°C or neutralization of anti-P antibodies by globoside in fresh serum, which is added as a complement source. Adding an exogenous complement is important, because it is assumed that the patient has exhausted their complement due to the hemolytic process. Apart from false negatives, false positives are also possible, particularly in individuals with monophasic IgM autoantibodies with a broad thermal range [16].

Paroxysmal cold hemoglobinuria is a transient disease. It requires adequate care of the patient that involves managing body temperature, ensuring adequate rest, hydration, and avoiding exposure to cold. In case of severe anemia, supportive treatment with erythrocyte transfusion is required. The patient should be warmed, but the use of blood warmers for RBC transfusion should be approached cautiously, as there is insufficient evidence to fully support this practice. Erythrocytes with the p or P-null phenotype are uncommon and not readily available for emergency transfusions. Nevertheless, most patients requiring transfusion achieve satisfactory posttransfusion hemoglobin despite presumed or documented P incompatibility [11]. P antigen is one of three antigens from the GLOB blood group system. This system is designated number 028 according to the classification of the Working Party for Red Cell Immunogenetics and Blood Group Terminology of the International Society of Blood Transfusion. The gene that encodes the synthesis of the antigen P, *B3GALNT1*, was cloned in 1998. It is located on the long arm of chromosome 3 (3q26.1) [17]. Individuals lacking the P antigen expression are very uncommon. The estimated frequency of phenotype p is 5.8/1,000,000 European inhabitants. A higher frequency of 141/1,000,000

Empirijsko lečenje AIHA često započinje kortikosteroidima. Njihovo davanje se može obustaviti kada se potvrdi dijagnoza PHH jer ne postoje dokazi koji podržavaju njihovu korist [11]. Refraktorna, rekurentna i hronična forma PHH uspešno je lečena intenzivnijim imunosupresivnim terapijama kao što su intravenski imunoglobulini, azatioprin ili rituksimab**.** Ekulizumab, humanizovano anti-C5 monoklonsko antitelo primenjen je do sada kod tri bolesnika sa PHH [20-23].

Kliničko prepoznavanje PHH, tranzitorna priroda DL antitela i mogućnost za izvođenje DL testa su faktori od kojih zavisi pravovremena potvrda dijagnoze ovog retkog oblika AIHA.

SPISAK SKRAĆENICA

ANA – antinuklearna antitela
ANCA – antineutrofilna citoplazmatska antitela
Anti-dsDNA – antitela na dvočlanu DNK
AIHA – autoimuna hemolizna anemija
CRP – C-reaktivni protein
DAT – direktan antiglobulinski test
DL – Donath-Landsteiner
GLOB – naziv krvno-grupnog sistema
HUS – hemolizno uremijski sindrom
IgG – imunoglobulin G
IgM – imunoglobulin M
OIN – Odeljenje intenzivne nege
PHH – paroksizmalna hemoglobinurija na hladnoću
RZC – regionalni zdravstveni centar

Sukob interesa: Nije prijavljen.

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was registered in Västerbotten county in the North of Sweden. Additionally, the p phenotype is more prevalent among the Japanese population and within the Amish community [18].

Based on the presentation of three patients, Harada and Sonoda noted a seasonal pattern in the disease, occurring predominantly in spring or autumn. They suggested that daily fluctuations in air temperature might be linked to the temperature-dependent DL antibody. We have similar experience with the DL test that we introduced as a standard operating procedure in 2017. In the first patient, a 23-month-old girl, hemolytic crisis related to PCH was confirmed by DL test in November, and in an 11-month-old female infant it was confirmed in March [15,19].

Empirical treatment for AIHA typically starts with corticosteroids. Their administration can be stopped when the diagnosis of PCH is confirmed as there is no evidence to support the benefit of their use [11]. The refractory, recurrent, and chronic forms of PCH have shown successful responses to more intensive immunosuppressive therapies, including intravenous immunoglobulins, azathioprine, or rituximab**.** Eculizumab, a humanized anti-C5 monoclonal antibody, has been utilized in three patients with PCH so far [20-23].

The clinical recognition of PCH, the transient nature of DL antibodies, and the feasibility of conducting the DL test all depend on promptly confirming the diagnosis of this rare form of AIHA.

ABBREVIATIONS

ANA – antinuclear antibody
ANCA – antineutrophil cytoplasmic antibody
Anti-dsDNA – anti-double-stranded DNA
AIHA – autoimmune hemolytic anemia
CRP – C-reactive protein
DAT – direct antiglobulin test
DL – Donath-Landsteiner
GLOB – the name of a blood group system
HUS – hemolytic uremic syndrome
IgG – immunoglobulin G
IgM – immunoglobulin M
ICU – Intensive Care Unit
PCH – paroxysmal cold hemoglobinuria
RHC – regional health center

Conflict of interest: None declared.

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