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Case Report

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Coombs-negative severe hemolytic anemia and possible autoimmune disease in an adult following cytomegalovirus infection

Abstract

Hemolysis is a quite uncommon complication of Cytomegalovirus (CMV) infection, which could rarely present with severe manifestations. Diagnosis is usually based on exclusion of other causes of hemolytic anemia, history of CMV infection, accompanied by positive serological tests and positive direct Coombs (DAT) Test, evidence indicative of post-infection autoimmune response. Although most cases present no symptoms, there have been reports of severe post CMV infection hemolysis requiring treatment with steroids, immunoglobulin or even splenectomy.

It is herby described the case of a 19-year-old immune competent woman, who presented with severe hemolytic anemia following primary CMV infection. The patient's Hemoglobin (Hb) level was 5.8g/dl on the day of admission. Extensive laboratory testing for causes of anemia, active infections, systemic diseases and autoimmunity showed positive Antinuclear Antibodies and positive CMV IgM and IgG Antibodies. Although direct Coombs Test were persistently negative, post infection autoimmunity was suspected and after steroid treatment was initiated, the patient achieved full recovery. In immune competent individuals suffering from post CMV infection hemolysis a stay and watch policy could be employed. However, as far as an autoimmune mechanism is suspected the patient is eligible for steroid treatment.

Keywords: hemolysis, immunoglobulin, antinuclear antibodies, autoimmune response, lymphadenopathy, arthralgia, symptomatology

Volume 3 Issue 1 - 2016

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Received: October 29, 2016 | **Published:** November 18, 2016

Abbreviations: CMV, cytomegalovirus; DAT, direct anti-globulin test; Hb, hemoglobin; RBC, red blood cells; G-6-PD, glucose-6-phosphate dehydrogenase; PNH, paroxysmal nocturnal hemoglobinuria; ANA, antinuclear antibodies

Introduction

CMV infection is quite common among young adults. Age stratified studies revile a bimodal pattern of high CMV seroprevalence both during early childhood and during early adulthood. The virus is secreted almost at all body fluids of an infected individual, so close contact to an infected person could result to viral transmission. Vertical transmission congenital infection and day care children close contact could be causative of early childhood peak. Furthermore, it is strongly supported that young adulthood peak could be linked to the commencement of individuals sexual life. Given the fact that the infection is much more prevalent among low socioeconomic societies, CMV IgG seropositivity rates vary widely among different populations studied, ranging from 60-100%.[1,2] Most immune competent adults infected present no symptoms, whereas mild to moderate symptoms, such as prolonged fever, lymphadenopathy and arthralgia may also occur, a syndrome looking a lot alike to infectious mononucleosis.[2] Hemolysis is a rather uncommon complication of primary CMV infection and is usually accompanied by thrombocytopenia. Although in the majority of the cases it is asymptomatic and shelf-detained, rarely can be severe and life threatening, even among non-immune compromised individuals.[3] It has to be underlined that in the absence of symptomatology during primary CMV infection, post infection hemolysis may be the leading cause of the patient's visit to the physician.

Diagnosis of CMV induced hemolytic anemia require at first chemistry tests to establish hemolysis as the cause of anemia and direct Coombs (Direct Anti-globulin Test (DAT)), which is indicative of the presence of immunoglobulin, or complement or both in vivo on the patient's Red Blood Cells (RBC). A positive direct Coombs (DAT), which is representative of autoimmunity as the cause of hemolysis, accompanied by history of recent CMV infection with positive IgM and IgG titers, identify the presence of a CMV induced autoimmune mechanism. As a result, apart from supportive measures further special treatment can/should be provided such as Ganciclovir, gamma globulins and/or steroid.[4–6] It is hereby reported the case of an apparently healthy young woman suffering from severe CMV induced hemolytic anemia.

Case description

A 19-year-old healthy woman was admitted to our hospital complaining of acute onset fatigue and lack of appetite. She reported no symptoms indicative of recent infection and was under no medication. She didn't report any other medical condition and she hadn't traveled abroad during the past year. On physical examination splenomegaly was noticed, whereas liver size was normal and she presented no signs of lymphadenopathy. By the time of her admission, her hemoglobin levels (Hb) were 5.8g/dl (normal range, 12.0-15.4g/dl). Although no treatment had been initiated at that time, a series of laboratory tests took place in order to evaluate the cause of anemia.

On the first day of admission the patient was diagnosed with severe hemolysis. All laboratory tests run are shown in Table 1. Total and indirect bilirubin levels and LDH levels were high and haptoglobin levels were very low. Urine analysis was positive for urobilinogen and negative for hemoglobin. Reticulocytes levels were very high by the day of admission. Peripheral blood smears showed evidence of RBC fragmentation and no spherocytosis.

Table 1 6months follow up of the patient. By the second day of admission steroids were prescribed (grey cells). By the 10th day the patient was dismissed. The patient is still followed as an outpatient

	1st day of admission	2nd day of admission- Steroid administration	3rd day of admission	8th day of admission	15th day, patient dismissed- followed as outpatient	36th day- 5 weeks- tests back to normal	7th week follow up- no recurrence	2 months- follow up- no recurrence	3 months- follow up- no recurrence	6 months- follow up- no recurrence
Ht (36- 45%)	18	22	26	26	34	38	42			43
Hb (12.0- 15.4%)	5.8	7.7	8.8	8.8	11.2	12.6	13.8			14.3
MCV (80- 99 fl)	105	102	103	101	99	98	95			89
RDW (11.5- 14.5%)	24.9	23.9	25.7	24.8	20.2	13.7	13.4			
Retinocytes (0.5- 2.0%)	15.9	15.4	17.3	4.4	4.4	2.1	2.3			
WBC (3.9- 10.2x 10^9 / l)	5.8	4.4	4.3	3.6	5.6	8	9.2			10.5
Neutrophile count (%)	67.5				78.9	82.3	76.6			84.1
PLT (150- 370 x 10^9 /l)	253	237	266	215	254	275	315			229
ESR (<12 mm/1st hour)	93									
Urea (2.7- 6.8 mmol/l)/ Creatinine (45- 84 µmol/l)/ Uric Acid (140- 340 µmol/l)/ Potasium (3.7- 5.3 mmol/l)/ Sodium (135- 142 mmol/l)/ Calcium (2.1- 2.6 mmol/l)	_/58/_/4.2/ 136/2.48				3/58/168/ 4.3/140/_	3.8/58/191 /4.0/138/_				
Glucose (3.6- 5.6 mmol/l)	5.7				6.9	7.2				
Total Bilirubin (<20 µmol/l)	39		41	25	22	15				
Direct Bilirubin (<5 µmol/l)	13		14	10	8.3	5.6				
Indirect Bilirubin	26		27	15	13.7	9.4				

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Table Continued....

	1st day of admission	2nd day of admission- Steroid administration	3rd day of admission	8th day of admission	15th day, patient dismissed- followed as outpatient	36th day- 5 weeks- tests back to normal	7th week follow up- no recurrence	2 months- follow up- no recurrence	3 months- follow up- no recurrence	6 months- follow up- no recurrence
AST (<41U/L)/ ALT (<41U/L)/ ALP (35- 110 U/L)/ γ- GT (<40 U/L)	48/ 34/ 82/ 12				18/22/ 71/15	16/24/ 58/16				
LDH (<250 U/L)	585		503	415	349	201				
CRP (<5 mg/l)	11									
CPK (<170 U/L)	46									
Total Protein (60- 80 g/l)	73									
Infectious Pathogens										
EBV- VCA IgG/ EBV- VCA IgM/ EBV- EBNA IgG		Positive/ Negative/ Positive								
Parvo IgG/ Parvo IgM		Positive/ Negative								
CMV IgG		Positive titer:12 sample/cut-off						Positive titer:97 sample/ cut-off		Positive titer:188 sample/ cut- off
CMV IgM		Positive titer: 7 sample/cut- off						Positive titer: 1.4 sample/ cut-off		Negative titer: 0.64 sample/ cut- off
CMV IgG avidity (<0.40 recent infection, >0.65 past infection)		0.356- recent infection						0.482- grey zone		
HBsAg/ Anti HCV/ HIV I-II		Negative/_/_						_/ Negative/ negative		
Rheumatological Tests										
RF		Negative								
ANA		Positive titer: 1/1280 type: nuclear dots						Positive titer: 1/1280 type: nuclear dots	Positive titer: 1/1280 type: nuclear dots	Positive titer: 1/1280 type: nuclear dots

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Anti-ds DNA/ Anti-Ro/ Anti- La/ Anti Sm/ Anti RNP/ Anti U1 sn RNP/ Anti-Scl-70		Negative						Negative		
ANCA: c-ANCA/ p-ANCA		Negative						Negative		
Haptoglobin (0.3- 2.0 g/l)		<0.1						0.41		
IgG (7.0- 16.0 g/l)/ C3 (0.9- 1.8 g/l)/ C4 (0.2- 0.45 g/l)		9.05/1.10/0.22								

Although the patient didn't report Glucose-6-Phosphate Dehydrogenase (G-6-PD) deficiency, both G-6-PD and pyruvate kinase testing was conducted showing no abnormal results. Hemoglobin electrophoresis showed no abnormal variants, so further hereditary hemolytic causes were excluded. Copper levels were in a normal range. Paroxysmal Nocturnal Hemoglobinuria (PNH) was also excluded using Flow Cytometry. By the time the patient was admitted and diagnosed with hemolysis, a broad spectrum direct Coombs test, against human IgG and complement C3d was run, which turned to be negative. High clinical suspicion of autoimmune hemolytic anemia demanded repetitive testing using mono specific antibodies against IgG, IgA, IgM and C3d which was also negative. Further testing of an eluate of the patient's RBC, didn't detect any auto antibodies.

Although the patient reported no symptoms of recent infection and had no signs of lymphadenopathy, she was diagnosed with recent CMV infection with CMV IgG positive (titer: 12 sample/cut- off) and IgM positive (titer: 7 sample/cut- off). Presence of primary CMV infection was further confirmed by CMV IgG avidity, which showed immaturity of IgG antibodies (0.356<0.400 representative of recent CMV infection). Active EBV and Parvo B19 infection was excluded as the patient had undetectable levels EBV- VCA IgM and Parvo B19 IgM Antibodies, although she showed evidence of past exposure to both pathogens (EBV: EBV-VCA IgG and EBV-EBNA IgG positive, Parvo B19 IgG positive). Further testing for Hepatitis B, Hepatitis C and HIV I/II was negative Table 1. In addition, any subclinical systemic or rheumatology disease, which could present with signs of hemolysis, had to be excluded. Clinical examination and radiology testing didn't show any signs of systemic disease such as sarcoidosis, whereas rheumatology basic laboratory tests revealed high titers of Antinuclear Antibodies (ANA) (titer 1:1280) following a nuclear dot pattern Table 1.

As a result, hemolysis was linked to recent CMV infection through an autoimmune mechanism and steroid therapy (prednisone 1mg/kg) was administered by the second day of admission. No further supportive measures were required and the patient's haematocrit and hemoglobin increased during the following days of hospitalization, accompanied by reduction of total bilirubin and LDH Table 1. Five weeks later her hemoglobin and bilirubin levels were back to normal and the patient achieved full recovery. Steroid therapy followed a weekly pattern of dose reduction. The patient presented no relapse both during and after her recovery.

During patient's recovery a serum sample was tested for CMV IgG and IgM two months later demonstrating rise of the IgG titer:97 sample/cut-off, decline of IgM titer:1.4 sample/cut-off and slight increase of CMV IgG avidity titer (0.482<0.65) still indicative of primary recent CMV infection during the previous trimester. The patient continued to be followed and 6 months later CMV IgG titer was further higher 188sample/cut-off and IgM were negative. Furthermore, during follow up ANA titer (1/1280) was persistently high, although the patient achieved full recovery.

Discussion

It has to be underlined that in immune competent individuals post CMV infection complications are quite rare. The disease usually runs presenting either no symptoms or flu like or mononucleosis like syndrome. As a result post infection complications may be the cause of the patient's presentation to the physician. Other well established complications or actual manifestations of CMV infection, which may be the result of either human's immunologic response to the pathogen or direct invasion of the virus in specific tissues include almost all systems: gastrointestinal tract: gastroenteritis, colitis, central nervous system: encephalomyelitis, encephalitis, meningoencephalitis, hematology disorders: thrombocytopenia, hemolytic anemia, disseminated intravascular coagulation, myelodysplastic changes, pancytopenia, splenic rupture and other rare manifestations such as vascular thrombosis, ocular involvement and pulmonary disease. Taking a closer look at the hematological disorders accompanying CMV infection, it is strongly supported that pancytopenia and

hematopoiesis inhibition could be due to direct cytopathic effect of the virus, whereas hemolysis is the result of an immune mediated mechanism against CMV.[2] Most cases of post CMV hemolysis are subclinical or quite mild requiring no further treatment. Given the fact that mild post CMV infection hemolysis is usually diagnosed during a patient's extended laboratory testing for other manifestation/ complication, for example thrombocytopenia, the actual incidence of this complication is not known. Among 20 patients suffering from CMV infection tested, 9 showed laboratory tests of hemolysis and none of those patients presented any symptom.[3]

However, the 19-year-old woman described in the present case was admitted with hemoglobin levels of 5.8g/dl, indicating severe anemia. In addition, she reported no medical history of recent infection, even with mild symptoms. As a result, symptomatic anemia was extensively evaluated with basic laboratory tests, which disclosed hemolytic anemia accompanied by negative direct Coombs. Further serological tests run in order to evaluate active infection proved to be negative, apart from IgG and IgM CMV Antibodies, representative of resent asymptomatic CMV infection. It has to be pointed out that are infection or reactivation of a previously acquired CMV infection, could also establish itself following this serological pattern. However, immaturity of CMV IgG Antibodies, as demonstrated by low CMV IgG avidity confirmed primary recent asymptomatic CMV infection. Exclusion of further hemolytic anemia causes supported the suspicion that hemolysis was due to CMV induced autoimmunity. There have been reported cases of post CMV infection hemolysis, where diagnosis was achieved by exclusion of other causes of hemolytic anemia and serological evidence of recent CMV infection.[1,3,6–11]

Although the patient was diagnosed with autoimmune hemolytic anemia, repetitive direct Coombs testing turned to be negative. Based on the fact that direct Coombs using poly specific IgG and C3d may at first turn negative, further testing using mono specific IgG, IgM, IgA and C3d was performed, which also turned to be negative. Furthermore, an eluate of the patient's RBC was tested, detecting no auto antibodies. Consequently, there was no laboratory result that could ground the under suspicion autoimmune mechanism responsible for post CMV infection hemolysis. Although on this setting a positive direct Coombs (DAT) is a useful tool, indicative of autoimmunity,[9] there have been published data that report the presence of post CMV infection hemolytic anemia with persistently negative direct Coombs.[1,3,11,12] A previously published case report of an 18-year-old woman with negative direct Coombs CMV induced hemolysis supported that the patient by the time of admission had increased osmotic fragility of her RBC, a laboratory finding, which detained by the time the patient recovered.[12] Another report of symptomatic CMV infection- fever up to 39.5°C for 14days, and post infection severe hemolysis has been described.[11] A week after symptom relief, the patient was admitted with jaundice and anemia, diagnosed with Coombs negative, post CMV infection hemolytic anemia.[11] However, the actual autoimmunity pathway causing CMV induced hemolysis hasn't been clarified yet.[2] It is strongly supported that cross-reactivity between the patients' erythrocyte and CMV antigens is not a causative factor for post infection hemolysis.[1]

The patient's hemolytic anemia turned to be responsive to steroids and she experienced no recurrence during recovery and follow up, which reinforces the fact that the complication was due to autoimmunity. It has to be underlined that there are no specific guidelines for post CMV induced hemolytic anemia treatment. Some reported cases required no treatment, while the patient was closely followed.[11,12] On the other hand, steroids have been successfully used in severe cases and the patients achieved full recovery.[5,3] Combination of anti viral and steroids is another therapeutic pattern used.[13] Anti-globulin has also played a significant role in post CMV infection therapeutics.[4,6]

To our knowledge there have been a few cases reported of Coombs negative, CMV induced severe hemolysis.[1,3,11,12] The incidence of this complication and its' immunity- mediated mechanism require further research. As a result, both present and previously published reports underline the need of high clinical awareness. CMV induced hemolysis should be taken into account when other more frequent causes (both acquired and hereditary) of hemolytic anemia are excluded. Post CMV infection hemolysis may present with direct Coombs negative hemolytic anemia. Steroid, anti-globulin and Ganciclovir treatment have been used for treatment of patients with severe CMV induced hemolysis.

Acknowledgements

None.

Conflict of interest

The author declares no conflict of interest.

References

1. Ludwig A, Hengel H. Epidemiological impact and disease burden of congenital cytomegalovirus infection in Europe. *Euro Surveill*. 2009;14(9):26–32.
2. Rafailidis PI, Mourtzoukou EG, Varbobitis IC, et al. Severe cytomegalovirus infection in apparently immunocompetent patients: a systematic review. *Virol J*. 2008;5(1):47.
3. Horwitz CA, Skradski K, Reece E, et al. Haemolytic anaemia in previously healthy adult patients with CMV infections: report of two cases and an evaluation of subclinical haemolysis in CMV mononucleosis. *Scand J Haematol*. 1984;33(1):35–42.
4. Coghill J, Comeau T, Shea T, et al. Acute hemolysis in a patient with cytomegalovirus pneumonitis Treated with intravenous immunoglobulin (IVIG). *Biol Blood Marrow Transplant.* 2006;12(7):786–788.
5. Eisenberg MJ, Kaplan B. Cytomegalovirus-induced thrombocytopenia in an immuno competent adult. *West J Med*. 1993;158(5):525–526.
6. Juneja SK, Phillips KA, Speed B, et al. High-dose gamma-globulin responsive haemolysis due to cytomegalovirus in an immunocompetent adult. *Br J Haematol*. 1996;95(2):433–435.
7. Houston MC, Porter LL, Jenkins DE, et al. Acute immune hemolytic anemia in adults after cytomegalovirus infection. *South Med J*. 1980;73(9):1270–1274.
8. Kaneko S, Sato M, Sasaki G, et al. Case of cytomegalovirus-associated direct anti-globulin test-negative autoimmune hemolytic anemia. *Pediatr Int*. 2013;55(6):785–788.
9. Salloum E, Lundberg WB. Hemolytic anemia with positive direct antiglobulin test secondary to spontaneous cytomegalovirus infection in healthy adults. *Acta Haematol*. 1994;92(1):39–41.
10. van Spronsen DJ, Breed WP. Cytomegalovirus-induced thrombocytopenia and haemolysis in an immuno competent adult. *Br J Haematol*. 1996;92(1):218–220.
11. Veldhuis W, Janssen M, Kortlandt W, et al. Coombs-negative severe haemolytic anaemia in an immuno competent adult following cytomegalovirus infection. *Eur J Clin Microbiol Infect Dis*. 2004;23(11):844–847.

12. Saito M, Nakamura S, Kawasaki S, et al. Transient cytomegalovirus-induced hemolysis in an immuno competent woman. *Am J Hematol*. 1999;62(2):123–124.

13. Hwang N, Kim EH, Han SY, et al. Severe cytomegalovirus colitis with hemolytic anemia mimicking travelers' diarrhea. *Int J Infect Dis*. 2015;37:104–106.

Citation: Notter J, Plack A, Wirz S, et al. Coombs-negative severe hemolytic anemia and possible autoimmune disease in an adult following cytomegalovirus infection. *Hematol Transfus Int J.* 2016;3(1):130–135. DOI: 10.15406/htij.2016.03.00057