

Sustained hyperferritinemia in a child with macrophage activation syndrome secondary to systemic juvenile idiopathic arthritis - perforinopathy: case based review

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Received: 24th April 2017, Revised: 5th August 2017, Accepted: 23rd September 2017

SUMMARY: Çakan M, Aktay-Ayaz N, Gemici H, Annayev A, Çıtak A, Akçay A, Öztürk G. Sustained hyperferritinemia in a child with macrophage activation syndrome secondary to systemic juvenile idiopathic arthritis - perforinopathy: case based review. Turk J Pediatr 2018; 60: 598-603.

Systemic juvenile idiopathic arthritis is a subtype of juvenile idiopathic arthritis and characterized by arthritis and many systemic features like fever, rash, hepatosplenomegaly, lymphadenopathy and serositis. Macrophage activation syndrome is the most dreadful complication of systemic juvenile idiopathic arthritis and can cause mortality and morbidity if not recognized and treated early and aggressively.

Hemophagocytic lymphohistiocytosis (HLH) is characterized by diminished or absent activities of natural killer cells and cytotoxic T lymphocytes leading to cytokine storm and uncontrolled activation of T cells and macrophages. Primary (familial) HLH is a group of autosomal recessive disorders caused by mutations in the perforin and other related genes and distinctive for onset during early infancy and high rate of mortality. Secondary HLH may be caused by infectious, oncologic and rheumatologic disorders. The term Perforinopathy is used to describe cases with classical familial HLH and also for cases with familial HLH gene mutations but not following a classical familial HLH course.

Herein we report a case of chronic perforinopathy in which clinical symptoms started with systemic juvenile idiopathic arthritis and severe macrophage activation syndrome that needed plasma exchange and extracorporeal membrane oxygenation during acute period and ongoing interleukin-1 blockage for sustained hyperferritinemia.

Key words: hyperferritinemia, macrophage activation syndrome, perforin A91V, perforinopathy, systemic juvenile idiopathic arthritis.

Juvenile idiopathic arthritis (JIA) is the most common cause of chronic arthritis in children.¹ The diagnosis of JIA is made by the presence of arthritis for more than 6 weeks in a child younger than 16 years of age and in whom there is no other identified cause for arthritis.² JIA is not a homogenous disease and has several subtypes. Classification systems were developed for JIA and the last one, ILAR classification, divides JIA into 7 subtypes.¹ Systemic JIA (sJIA) comprises 5% to 15% of children with JIA. sJIA is characterized by arthritis and

systemic features such as fever, rash, serositis, lymphadenopathy and hepatosplenomegaly.² As sJIA has many accompanying systemic features, follows a course different from other JIA subtypes and lacks association with certain HLA types and autoantibodies, debate is whether it should be included in the category of JIA, or whether it should be considered as an autoinflammatory disease.¹

Hemophagocytic lymphohistiocytosis (HLH) is a term that describes a spectrum of

disorders characterized by accumulation of well-differentiated mononuclear cells with a macrophage phenotype exhibiting hemophagocytic activity.³ HLH is divided as primary (familial) HLH and secondary HLH. Secondary HLH may be caused by infectious, oncologic and rheumatologic diseases^{4,5}. Systemic JIA, Kawasaki disease and systemic lupus erythematosus are the most common causes of rheumatologic disorders that can lead to secondary HLH also known as macrophage activation syndrome (MAS).⁶ Perforinopathy is the term suggested to be used by Voskoboinik et al.⁷ to describe patients with complete or partial loss of perforin function that can lead to classical familial HLH or late onset MAS, and hematological malignancy.

Case Report

A 9-year-old boy was admitted to the hospital with the complaints of fever and arthralgia for three weeks. He was hospitalized in another center for a week because of the fever. As fever did not subside, and slowly progressing respiratory distress started, he was referred to our emergency room. He had jugular venous distension, mild dyspnea and cardiac sounds were deeply located. Chest X-ray revealed

no pneumonic infiltration but cardiomegaly. Echocardiographic examination showed 30 mm pericardial effusion. The child was transferred to pediatric intensive care unit (PICU) and pericardiocentesis, that yielded serosanguineous fluid, was performed. The child was admitted to pediatric rheumatology ward. He had diurnal fever (peaking to 39.5 °C), accompanied by evanescent macular rash, myalgia and arthralgia. Arthritis on the left knee and left ankle and 2 cm splenomegaly was noticed. He was the first child of the non-consanguineous healthy parents and had a healthy younger brother. The family stated that the child did not have any known disease and was totally healthy until 3 weeks ago.

Laboratory tests showed leukocytosis, anemia, thrombocytosis, high acute phase reactants, hypoalbuminemia, and hyperferritinemia (*ferritin: 13.670 ng/ml*) (Table I - 1st day results). Bone marrow aspiration did not show any malignant cells or hemophagocytic cells. Blood, urine and pericardial fluid cultures, bacterial and viral serology studies did not yield any possible causative agent. Especially, Epstein-Barr virus and cytomegalovirus serologic and DNA studies that were twice repeated became negative. Thorax and abdominal computerized tomography (CT) examinations did not show

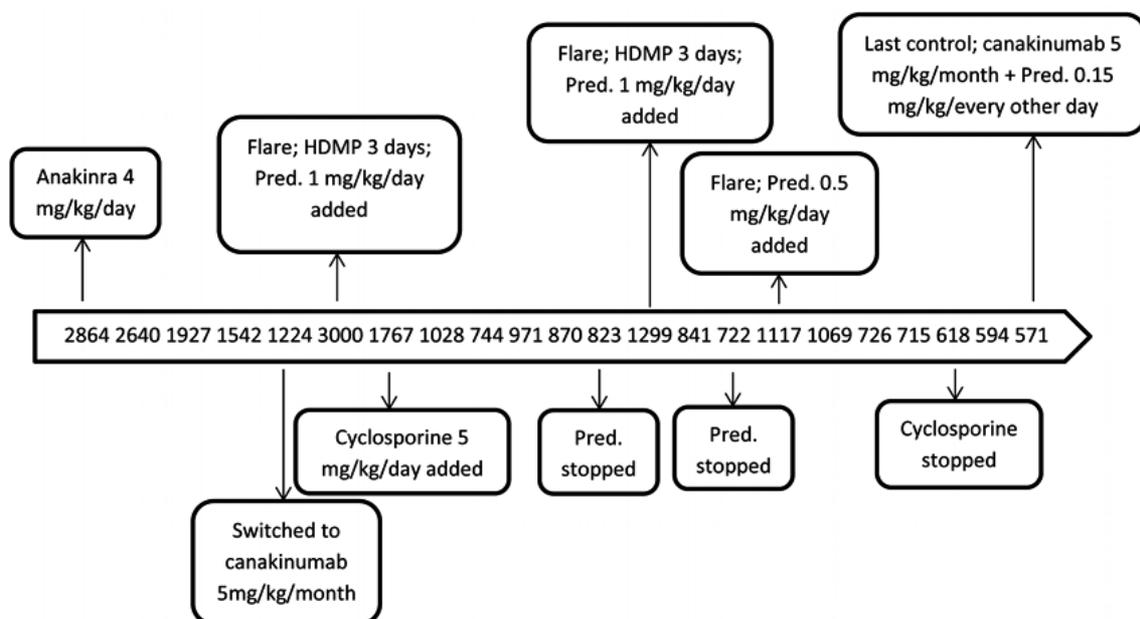


Fig. 1. Monthly checked serum ferritin levels (ng/ml) and treatment modifications made during 1.5 years of follow-up. HDMP: High-dose methylprednisolone (30 mg/kg/day); CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; Pred: Prednisolone.

any mass or lymphadenopathy. Broad spectrum antibiotics that were started in the PICU were continued. The clinical picture of the patient was compatible with sJIA and MAS. High-dose methylprednisolone (30 mg/kg/day, 3 days) and intravenous immunoglobulin (1 g/kg, 2 days) were given and continued with intravenous prednisolone (2 mg/kg/day) and oral cyclosporine (5 mg/kg/day). On the 7th day, daily fever peaks continued and laboratory tests showed extreme hyperferritinemia (*ferritin: 100.000 ng/ml*) and worsening in all parameters (Table I - 7th day results). Tuberculin skin test was negative but quantiferon assay analysis became positive on the 7th day so anti-tuberculosis treatment consisting of isoniazid, rifampicin, ethambutol and streptomycin was initiated with the working diagnosis of tuberculous pericarditis.

The clinical picture of the patient was stable but borderline hypotension developed on the 8th day and the child was sent to the PICU of another center for plasma exchange. The next day, he developed respiratory depression and inotrope resistant hypotension in the PICU. He was intubated and ejection fraction was around 20%. Hypotension was unresponsive to all medications, so he was connected to extracorporeal membrane oxygenation (ECMO). The patient spent a month in the PICU. He remained intubated for 13 days, and ECMO was applied for 9 days, plasma exchange for 15 days and continuous veno-venous hemofiltration for 15 days. Control bone marrow aspiration showed hemophagocytic cells. Anti-tuberculosis

treatment, prednisolone and cyclosporine were continued in the PICU. He was admitted back to the rheumatology ward after a month. The child still had daily fever peaks and laboratory tests showed persistence of high ferritin levels (*ferritin: 8.534 ng/ml*) and thrombocytopenia (Table I - 35th day results). We were planning to start anakinra from the first day but the child had suspicious tuberculosis infection. As fasting gastric and pericardial fluid cultures and PCR analysis did not yield any positive result for *M.tuberculosis* and thorax CT was not suggestive of a tuberculosis infection, tuberculosis treatment was discontinued and followed with isoniazid (INH) prophylaxis. Anakinra 4 mg/kg/day was initiated and finally on the 6th day of anakinra the fever resolved.

On the 2nd month he developed severe mid-abdominal pain and amylase and lipase levels were elevated. The clinical picture was thought to be cyclosporine induced pancreatitis and cyclosporine was discontinued. Amylase and lipase levels became normalised in 2 weeks. Three weeks later he developed severe local infection on the left inguinal catheter area and fever again. Broad spectrum antibiotics were restarted and as ferritin level rose up to 10.064 ng/ml anakinra dosage was increased to 8 mg/kg/day. Wound infection healed totally in 3 weeks. The child was discharged 100 days later with INH and anakinra (4 mg/kg/day). He had mild hypertransaminasemia and high ferritin levels (*ferritin: 2.864 ng/ml*) (Table I- 100th day results) during discharge and hypertransaminasemia was thought to be

Table I. Laboratory Parameters of the Patient.

	1 st day	7 th day	35 th day	100 th day
Leukocytes	20.200/mm ³	12.700/mm ³	4900/mm ³	10.500/mm ³
Hemoglobin	8.2 g/dl	12.9 g/dl (transfused)	12.1 g/dl	11.5 g/dl
Platelets	553.000/mm ³	80.000/mm ³	68.000/mm ³	354.000/mm ³
CRP	154 mg/L	19.4 mg/L	28 mg/L	0.5 mg/L
ESR	95 mm/hr	12 mm/hr	30 mm/hr	10 mm/hr
Albumin	2.2 g/dl	2.4 g/dl	3.2 g/dl	3.8 g/dl
AST	42 U/L	1209 U/L	35 U/L	114 U/L
ALT	19 U/L	582 U/L	20 U/L	139 U/L
Ferritin	13.670 ng/ml	100.000 ng/ml	8.534 ng/ml	2.864 ng/ml
Triglyceride	199 mg/dl	678 mg/dl	335 mg/dl	175 mg/dl
LDH	489 U/L	2091 U/L	223 U/L	155 U/L

CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase.

INH side effect and INH was discontinued for 2 weeks and restarted after normalization of liver function tests. Four months later anakinra was switched to canakinumab (5 mg/kg/month) due to painful daily injections.

He has been followed for 1.5 years and had three flares of sJIA during this period. In every flare he had fever for 2 or 3 days without any other symptom or sign and all flares were seen 2-3 weeks after drug modifications. Figure 1 shows ferritin levels that were checked every month and treatment modifications that were made. The main question was although he was clinically stable and all other laboratory parameters were normal why ferritin levels were still high. So we looked for familial HLH genes namely PRF1, UNC13D, and STX11. We found heterozygous A91V variant in the perforin gene. The mother and brother had wild type gene but the father had heterozygous A91V variant too. The child was consulted to pediatric hematology team for bone marrow transplantation (BMT). After consideration of benefits and harms of BMT and discussions with the family, the decision was that although he was a candidate for BMT, it should not be considered as an option at the moment as the child was doing well with the current medical treatment.

The child is currently stable on monthly canakinumab (5 mg/kg) and oral prednisolone 0.15 mg/kg/every other day. Physical and laboratory examinations are normal by all parameters except for hyperferritinemia that is fluctuating around 500 ng/ml. Informed consent was received from the family.

Discussion

A thorough review of the literature via PubMed search (2004–2017; terms: perforin A91V, systemic juvenile idiopathic arthritis, macrophage activation syndrome, hemophagocytic lymphohistiocytosis, hyperferritinemia) was performed to retrieve data regarding the perforin mutations and macrophage activation syndrome secondary to systemic juvenile idiopathic arthritis.

Hemophagocytic lymphohistiocytosis is a term that is used to describe a spectrum of disorders characterized by accumulation of well-differentiated mononuclear cells with a macrophage phenotype exhibiting

hemophagocytic activity.^{3,6} HLH is divided as primary (familial) HLH and secondary HLH. Secondary HLH may be caused by infectious, oncologic and rheumatologic etiologies and when caused by rheumatologic disorders, it is traditionally called MAS.⁴ The most common cause of rheumatologic disorder associated with MAS in children is sJIA. sJIA is a subtype of JIA and is characterized by arthritis, fever, rash, serositis, hepatosplenomegaly and lymphadenopathy.⁸ MAS is a severe and potentially lethal complication of sJIA. Clinical features are non-remitting high fever, hepatosplenomegaly, lymphadenopathy, hemorrhages and central nervous system dysfunction. On laboratory tests cytopenias, abnormal liver function tests, decreased erythrocyte sedimentation rate (due to consumption of fibrinogen), hypertriglyceridemia, hyponatremia, hypoalbuminemia, elevated levels of serum sCD25 and cCD163 levels, hemophagocytosis on tissue examinations, and most strikingly hyperferritinemia are observed.^{3,8} Our case had all clinical and laboratory features of sJIA and severe MAS that could not have been controlled with medical treatment and needed plasma exchange and ECMO.

Familial HLH (fHLH) is a group of rare autosomal recessive disorders caused by genetic defects in packaging, exocytosis or function of cytotoxic granules leading to inability of natural killer cells and cytotoxic T cells to kill targeted cells causing an overwhelming inflammatory response and excessive cytokine production resulting in sustained macrophage activation.^{3,6,9} There are 5 types of fHLH and also some immune deficiency disorders like Griscelli syndrome, Chédiak-Higashi syndrome, Hermansky-Pudlak syndrome type II, X-linked lymphoproliferative syndromes and many others may cause to fHLH.^{4,9} The clinical and laboratory features are very similar in fHLH and secondary HLH. To differentiate sJIA complicated with MAS from primary and other secondary forms of HLH, several classification/diagnostic criteria sets have been developed.⁸ The age of onset is less than 1 year of age in 70% of fHLH cases but there is no known upper age limit for the onset of the disease and the only curative method is bone marrow transplantation.⁹

Perforin is a pore-forming protein that is critical for the function of cytotoxic lymphocytes in immune responses to microorganisms and in surveillance for malignancies.^{9,10} The most common cause of fHLH is perforin deficiency (type 2 fHLH) and mutations in the perforin gene (PRF1) accounts for 20-50% of fHLH cases, while mutations in three other genes, UNC13D, STX11, and STXBP2 (type 3,4,5 fHLH, respectively) impair the delivery of perforin into the immune synapse.^{3,7,10,11} fHLH type 1 is linked to the 9q21.3 locus, comprises around 10% of fHLH cases and can be recognized by impaired natural killer cell function but not associated with the absence of perforin expression.⁹ It is clear that homozygote pathogenic mutations in fHLH genes result in fatal illness that mostly present under 2 years of age. But there are some cases that carry mutations in fHLH genes and do not fit to this category and Voskoboinik et al.⁷ proposed the term perforinopathy to consider functional perforin deficiencies as a spectrum of clinical presentations and not as a single disease entity. They suggested that the term acute perforinopathy should be used to describe cases with classical fHLH that is diagnosed under 2 years of age with bi-allelic mutations in fHLH genes leading to complete loss-of-function. Sub-acute perforinopathy term was suggested for cases with inflammatory disease that has remitting/relapsing course that presented after 2 years of age with bi-allelic mutations in fHLH genes leading to partial loss-of-function. Chronic perforinopathy term was suggested to be used in patients presenting during adolescence or adulthood with MAS, hematological malignancy, or lymphoproliferative disease and having mono-allelic mutations in the PRF1 gene. Most importantly, mono-allelic mutations in the PRF1 gene (most commonly A91V variant) were considered as a contributing factor but not as a causative factor.⁷

Several studies have shown that patients with sJIA have dysfunction of natural killer cells.¹² Vastert et al.¹⁰ have shown that at the time of MAS in sJIA patients there were even more marked decreases in perforin levels of natural killer cells along with a further decrease of lytic natural killer cell function. In the same study, 56 sJIA patients were analyzed for PRF1 gene mutations and 11 (20%) of them were found to be heterozygous for missense

mutations in PRF1 (7 of them had A91V variant). Interestingly, heterozygosity for A91V was twice as frequent in sJIA patients with a history of MAS compared with sJIA patients without a history of MAS (20 vs 9.8). The authors concluded that low perforin expression and lytic function of natural killer cells in sJIA patients were further depressed during MAS and having heterozygous mutations in the PRF1 gene could be a risk factor for MAS development in sJIA patients.¹⁰ Huasong et al.¹³ studied A91V gene analysis in 31 MAS cases and no variant was found and they concluded that ethnic and regional differences may play a role in the pathogenesis.

A91V sequence variation in PRF1 gene is considered as a neutral polymorphism by some authors. They state that carriage rate of A91V in the Caucasian population is as high as 17.5%-21%, so the incidence of HLH should be much higher than observed in population.^{14,15} In papers that considered A91V as a pathogenic mutation, all cases were older children or young adults having atypical fHLH features and the authors commented that probably this was not definitive causative factor but contributing.¹⁶⁻¹⁸ Okur et al.¹⁹ looked for the frequency of A91V sequence variant in 44 secondary HLH patients and found that 5 (11.3%) of them had A91V mutation and all of the patients had an infection. In the same study they searched for the frequency of A91V variant in 159 healthy Turkish controls and found that 7 (4.4%) of them had A91V variation. They concluded that A91V mutation is not uncommon in the general population but having this mutation increases the risk of secondary HLH in patients with an underlying condition, especially those with an underlying infection. Also, there are some studies stating that having heterozygous A91V variant in the PRF1 gene could predispose individuals to leukemia, lymphoma and melanoma.^{7,20-22}

In our clinic, we do not normally check any fHLH genes in sJIA patients with or without MAS. We wanted to look for perforin and other fHLH gene mutations for this patient because the patient's course was extremely unusual for a sJIA-MAS patient. MAS attack was very severe which needed ECMO and plasma exchange and more importantly after normalization of clinical and all other laboratory parameters ferritin

levels did not get back to normal. We also do not think that A91V variant was a causative factor for MAS but was a contributing factor in our case. The limitation of our manuscript is that we were unable to perform any functional studies and measure cytokine levels.

In conclusion, like many authors, we think that having heterozygous PRF1 gene variants - with some residual perforin activity - may cause late onset and atypical MAS attacks when encountered with a triggering factor and we recommend checking for PRF1 gene in these cases.

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