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CASE REPORT

An autoimmune lymphoproliferative syndrome initially diagnosed as Evans syndrome

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ABSTRACT

Autoimmune lymphoproliferative syndrome (ALPS) is a rare childhood disorder characterized by chronic non-malignant lymphoproliferation and autoimmunity. Patients with ALPS frequently exhibit episodic and intermittent, severe autoimmune-induced hemolytic anemia, thrombocytopenia or combined cytopenias. The co-occurrence of immune-mediated cytopenias, autoimmune thrombocytopenia and autoimmune hemolytic anemia is also known as Evans syndrome. This report describes a child who presented with Evans syndrome symptoms and who, after the detection of increased percentage of double negative T cell population in the peripheral blood, was diagnosed as ALPS. He received several courses of treatment including glucocorticoids, cyclosporine A (Cs A), and intravenous immunoglobulin (IVIG) without any clinical benefit and was treated with the antimalarial drug Fansidar®. With administration of Fansidar® (half tablet per week; containing 250 mg of pyrimethamine and 12.5 mg of sulfadoxine) combined with immunosuppressive drugs, clinical status and laboratory findings temporarily improved. After two months, the patient underwent laparoscopic splenectomy because of worsening of thrombocytopenia refractory to the treatment. There was a transient beneficial effect from splenectomy. We were unable to stop immunosuppressive therapy and Fansidar®; however, this combined therapy was successful in decreasing the number of hospitalizations and controlled his clinical symptoms more effectively for six months. Unfortunately, he was admitted to a regional hospital with high fever and died at the age of three.

Key Words: Autoimmune lymphoproliferative syndrome, Evans syndrome, treatment, fansidar®, splenectomy

ÖZET

Başlangıçta Evans sendromu tanısı alan bir otoimmünlenfoproliferatif sendrom olgusu

Otoimmun lenfoproliferatif sendrom (ALPS); kronik non-malign lenfoproliferasyonla seyreden ve oldukça nadir görülen otoimmün bir çocukluk yaş grubu hastalığıdır. Sıklıkla ciddi otoimmun hemolitik anemi, trombositopeni veya kombine sitopenilerin geliştiği atak ve remisyon dönemleri ile kendini gösterir. Gelişen immun sitopeni bulguları aynı zamanda Evans sendromu olarak da bilinmektedir. Biz burada Evans sendromu tanısı koyduğumuz ve altta yatan nedenleri araştırırken artmış double negatif T hücre populasyonunun gösterilmesi ile ALPS tanısı alan hastayı sunuyoruz. İmmünsupresif amaçlı uygulanan glukokortikoid, siklosporin A, intravenöz immunglobulin ile klinik yanıt alınamayan hastaya ek olarak antimalarial bir ilaç olan Fansidar® uygulandı. Fansidar®'ın (haftada yarım tablet; 250 mg pyrimethamine ve 12.5 mg sulfadoxine) diğer immünsupresif ilaçlar ile birlikte uygulanması ile klinik tabloda ve laboratuar bulgularında geçici olarak iyileşme elde edildi. İki aylık dönem sonrasında tedaviye dirençli trombositopeni nedeniyle klinik durumu kötüleşen hastaya laparoskopik splenektomi uygulandı ve kısa bir dönem iyileşme sağlanabildi. Sonrasında altı aylık dönem süresince klinik tabloyu kontrol altına alarak hastaneye yatış sayısında azalmayı sağlaması nedeni ile Fansidar® ile kombine uygulanan immünsupresif tedaviye ara verilemedi. Ancak hasta bu tedavileri almaktayken üç yaşına geldiğinde gelişen yüksek ateş nedeniyle başvurduğu hastanede yaşamını kaybetti.

Anahtar Sözcükler: Otoimmün lenfoproliferatif sendrom, Evans sendromu, tedavi, Fansidar®, splenektomi

INTRODUCTION

Autoimmune lymphoproliferative syndrome (ALPS) is a disorder of lymphocyte apoptosis that arises in early childhood and is characterized by massive lymphadenopathy (LAP), splenomegaly and autoimmune manifestations, especially immune-mediated cytopenias. A distinct feature of ALPS, and as an early clue to its nature, is the occurrence of markedly increased numbers and percentage of $CD3^{+}TCR\alpha\beta^{+}$ $CD4^{-}CD8^{-}$, and double negative T (DNT) cells in the circulation and lymphoid tissues [1].

In the majority of patients, ALPS is due to inherited mutations in genes that regulate lymphocyte survival by triggering programmed death of lymphocytes, or apoptosis [1]. According to the molecular abnormality found, ALPS patients are currently classified as ALPS type Ia or Ib when they carry a mutation of the tumor necrosis factor receptor superfamily 6 (TNFRSF6) gene encoding Fas or Fas ligand gene, respectively. Type II is due to mutation in the caspase 10 and type III designates patients in whom a genetic defect is yet to be identified [2,3]. However, apoptotic defect alone is not sufficient to produce the clinical manifestations of ALPS, because healthy relatives with defective in vitro apoptosis are frequently identified [4].

Indications for the treatment of ALPS patients depend on the type and severity of the symptoms. In many patients, the clinical status does not require any treatment and the severity of symptoms may decrease with time. However, hypersplenism, anemia and/or thrombocytopenia may require therapeutic intervention [1]. A number of treatment modalities are used in the management of severe ALPS cases. This has included the usage of glucocorticoids, intravenous immunoglobulin (IVIG), splenectomy or chemotherapeutic agents [5]. Bone marrow transplantation has been reported twice, with resulting correction of the Fas-deficient state [6,7]. Recently, successful usage of an antimalarial drug, sulfadoxine/pyrimethamine (Fansidar®), has been reported in six out of seven ALPS patients [7]. Long-term prognosis of ALPS is still uncertain, as the most of the patients have been followed for a relatively short time. The major determinants of morbidity and mortality in ALPS are the severity of autoimmune disease, postsplenectomy sepsis and lymphoma [2].

In this report, a 10-month-old boy with a severe form of ALPS, whose disease was refractory to conventional therapies and who was treated with Fansidar®, is presented.

CASE REPORT

The patient was the first child of nonconsanguineous parents. His family history was unremarkable. He was healthy until the age of six months when he was admitted to a local hospital with disseminated vesicular skin lesions, generalized LAP, hepatosplenomegaly (HSM) and tachycardia, and he was diagnosed with severe varicella-zoster virus (VZV) infection. Coombs-positive (IgG) hemolytic anemia, thrombocytopenia, elevated immunoglobulin levels and severe proteinuria were detected. Cytomegalovirus (CMV) IgM and IgG were also found to be positive. Lymph node and renal biopsy revealed reactive hyperplasia and mesangioproliferative glomerulonephritis with CMV inclusion body in tubular epithelia, respectively. High dose methylprednisolone, IVIG, and ganciclovir were given for the treatment and multiple red blood cell and platelet transfusions were administered. Since the patient did not respond to these treatments and was refractory to the transfusions, he was referred to our center for further treatment and plasmapheresis.

The patient was admitted to our unit with recurrent fever and bleeding from venipuncture sites at the age of 10 months. On physical examination, multiple LAP, marked HSM (7 cm and 4 cm at midclavicular line, respectively), and multiple ecchymoses were detected. Blood count revealed an anemia (Hb 5.7 g/dl,), white blood cell (WBC) count of 7.2×10^{9}/L with neutropenia (0.7×10^{9}/L), and thrombocytopenia (2×10^{9}/L). Reticulocyte count was slightly elevated (3.2%) and direct Coombs test was positive (IgG: 4+). Urinary analysis was normal and biochemistry tests were significant only for mildly elevated transaminases (ALT: 73 U/L, AST: 49 U/L, GGT: 51 U/L). Viral serologic studies including hepatitis A, hepatitis B, Parvovirus, Epstein-Barr, human immunodeficiency virus (HIV) and rubella were found to be negative. CMV IgM and whole blood polymerase chain reaction analysis for CMV were also negative. Bone marrow examination revealed mildly hypercellular marrow with megakaryocytes increased in number, mild dyserythropoiesis and no signs of malignancy. Antinuclear antibody and anti-DNA results were negative. The diagnosis of Evans syndrome was considered with these laboratory findings. Since Evans syndrome can be associated with other conditions, further evaluation was indicated. Immunological studies revealed hypergammaglobulinemia (IgG: 2850 mg/dl, IgA: 212 mg/dl, IgM: 173 mg/dl) with normal Anti A titer (Table 1). Lymphocyte subset analyses showed an elevated population of $CD3^{+}$ $TCR\alpha\beta^{+}CD4^{-}$ $CD8^{-}$ T

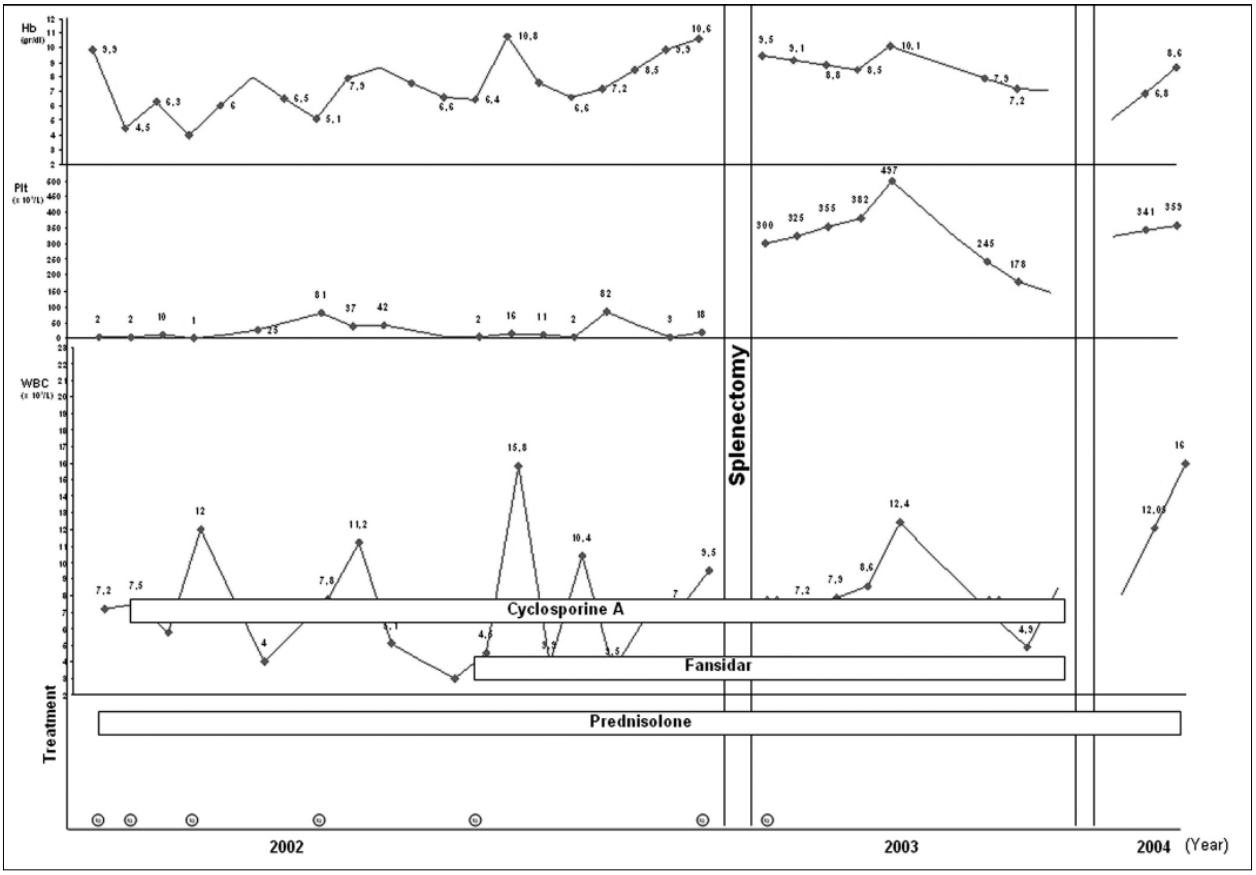

Figure. The interactions between the treatment and blood counts of patient's

cells (15% of the total lymphocyte population). Fas (CD95) expression was detected on mononuclear cells. In vitro lymphoproliferative response to phytohemagglutinin (PHA) and anti-CD3 were normal. Nitroblue tetrazolium (NBT) test was found to be normal. Chronic non-malignant lymphoproliferation and autoimmune cytopenias along with an increased proportion of DNT cells lead to the diagnosis of ALPS.

Treatment with broad-spectrum antibiotics, high-dose methylprednisolone, cyclosporine A (Cs A) and IVIG was started. However, the patient's bleeding problems (oral mucosa, gastrointestinal and urinary system) progressively worsened and he was admitted to the intensive care unit. After several weeks of treatment, his infection was better, hepatic/splenic sizes were diminished and his thrombocytopenia/anemia had slightly improved. After three months, he was discharged home on prednisolone with slow tapering, although the direct Coombs test was still strongly positive.

Despite ongoing immunosuppressive treatment, thrombocytopenia and hemolytic attacks continued. During the treatment, he was admitted to our hospital five times for infection (staphylococcal pyodermia, paronychia, pulmonary infection) and/or bleeding (oral mucosa, gastrointestinal system and urinary). Six months later, the permanent control of autoimmune cytopenias was still not achieved, and treatment with the antimalarial drug Fansidar® was started. The patient was given an oral dose of one-half tablet per week containing 250 mg of pyrimethamine and 12.5 mg of sulfadoxine. Administration of this drug led to a temporary improvement in his clinical status. Physical examination showed a decrease in HSM. Laboratory findings showed no significant change in the number of lymphocytes and neutrophils during Fansidar® treatment. At the age of two years, laparoscopic splenectomy was performed because of the worsening of thrombocytopenia refractory to IVIG, glucocorticoids, Cs A and Fansidar®. After splenectomy, the same medical therapy was continued and two months later, he was admitted again with pulmonary infection, paronychia and gastrointestinal system bleeding. Pulse methylprednisolone,

Table 1. Immunological findings of the patient

Study	*Result*	*Normal range*
Hemoglobin (g/dl)	5.7 g/dl	11.5-13.5 g/dl
WBC (x 10^9/L)	7.2x 10^9/L	5.5-15.5
MCV (fl)	75 fl	75-87
Platelet (x 10^9/L)	2 x 10^9/L	150-400
ALC (x 10^9/L)	4.8x10^9/L	3.0-9.5
ANC (x 10^9/L)	0.7x10^9/L	1.5-8.5
IgG (mg/dl)	2850	460-969
IgA (mg/dl)	212	17-69
IgM (mg/dl)	173	46-159
Isohemaglutinin (anti-A) titter	Anti A 1/32	≥ 1/8
CD3+ (%)	79.6	51-79
CD3+CD4+ (%)	35	33-55
CD3+CD8+ (%)	31	11-33
CD3+TCR$\alpha\beta$+CD4-CD8- (%)	15	< 1
CD16+56+ (%)	12.6	5-23
CD19+(%)	8	14-44
CD45RA+ (%)	79	72-93
CD45RO+ (%)	20	9-31
HLA-DR+ (%)	22	18-38
CD95+ lymphocytes (%) monocytes (%)	29 51	
Lymphoproliferative response to		
PHA (%) AntiCD3 (%)	75 70	65.8±9.2 57.5±6.2
NBT	positive	positive

granulocyte colony-stimulating factor (G-CSF) and antibacterial treatment were added to Cs A and Fansidar®. Partial clinical and laboratory improvement was detected and he was discharged from hospital. The interactions during the whole treatment and patient's blood count are shown in Figure 1. Afterwards, he was well for six months with low-dose corticosteroid (0.76 mg/kg/day prednisolone), Cs A (5.2 mg/kg/day) and Fansidar® (half tablet per week). Unfortunately, he was admitted to a regional hospital with high fever and died the same day at the age of three.

DISCUSSION

Diagnostic criteria for ALPS include a) chronic non-malignant lymphoproliferation (LAP, splenomegaly); b) elevated percentages (<1%) of DNT cells and c) defective in vitro antigen-induced lymphocyte apoptosis [1]. The identification of DNTs has been cited as a useful screening tool for centers with limited experience of lymphocyte apoptosis assays and if combined with appropriate clinical features, peripheral lymphocytosis, circulating autoantibodies and polyclonal hypergammaglobulinemia, is extremely suggestive of ALPS (8). Autoimmunity and increased occurrence of lymphoreticular malignancies are also common features.

In this report, we presented a patient with characteristic clinical and laboratory features of ALPS. These include chronic LAP and HSM, hypergammaglobulinemia, autoimmune cytopenias and expansion of DNT cells. The most common autoimmune diseases seen in ALPS are hemolytic anemia and autoimmune thrombocytopenic purpura (AITP) [2].

The cause of this disorder is unknown, but likely represents an autoimmune state secondary to immune dysregulation leading to production of multiple antibodies. Evans syndrome was originally described in 1949 and after a period it was reported in association with primary immunodeficiencies, and connective tissue and lymphoproliferative diseases [9,10]. It is obvious that there are several common clinical and laboratory features in ALPS and Evans syndrome and that these disorders may overlap [10]. The presence of Evans syndrome indicates a serious disorder of immunoregulation. In a recent report, elevated DNTs suggestive of ALPS was detected in 58% of patients diagnosed with Evans syndrome [11]. This data suggests a high prevalence of ALPS among Evans syndrome patients. The evaluation of more patients with Evans syndrome for DNT's and apoptosis defects may help the understanding of pathophysiology and diagnosis of patients who may in fact have ALPS.

There are number of gene defects which may cause ALPS. Fas, Fas ligand, and caspase 10 deficiencies are associated with several ALPS subtypes. In the remainder of patients, there is currently no defined molecular defect. Although the detectable level of Fas expression was determined in our case, this does not exclude the Fas gene defect. The apoptotic defect alone, however, is not sufficient to produce the clinical manifestations of ALPS [4] There is a complex relation-

ship between genotype, phenotype and disease penetrance. The clinical penetration and degree of inhibition of apoptosis are varied, even for the same mutation in different individuals [4, 8]. These data provides that several factors may contribute to the clinical expression of ALPS. Environmental factors, which could lead to a disturbance of homeostasis of the immune system, might also play a role in the pathogenesis. Our patient was the index case in the family. Although we did not know the genetic defect, viral infections - either VZV or CMV - seem to be the triggering factors.

Clinical management of ALPS generally is directed at autoimmune manifestations and lymphoproliferation [2]. Autoimmune cytopenias in ALPS occasionally require an intensive therapy, including a prolonged corticosteroid treatment and chemotherapeutic agents (methotrexate, cyclophosphamide, azathioprine, MMF) [2]. The patient in this report has a severe form of ALPS. Even though he received intensive immunosuppressive treatment, a long-term control of autoimmune cytopenia's wasn't achieved. When the other treatment schemes had failed to treat severe clinical signs, the anti-malarial drug Fansidar® was added to the treatment. Bosch et al. were reported Fansidar® usage in seven ALPS patients thus pyrimethamine induces apoptosis in activated lymphocytes [12]. However, Rao et al could not demonstrate any regression of adenopathy or splenomegaly and also Oren et al failed to show a positive clinical response to Fansidar therapy [13,14].

In our patient, in spite of a temporarily clinical and laboratory improvement at the beginning of Fansidar treatment, there was no clinical and hematological response after the first month of treatment. We avoided to perform splenectomy, because opportunistic infections and septicemia rate are higher and extremely fatal under the age of 2 years [13]. On the other hand, we had to perform this procedure. When we compared the clinical course before and after the splenectomy, there was a beneficial effect from the splenectomy. However, we couldn't manage to stop immunosuppressive therapy with Fansidar. Unfortunately the patient had growth retardation and developmental problems since costicosteroid was administered for a long time.

The patient was followed for 26 months. After his last discharge, he was well for six months with low-dose steroid, Cs A and Fansidar®, but he died at the age of three with an infection. In conclusion, treatment of severe forms of ALPS is challenging. The accumulating experience in diagnosis, treatment and long-term follow up of these patients will provide a better understanding of its nature and generate a guide for patient care.

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