

Anti-neutrophil Cytoplasmic Antibody Positivity in Five Children with Systemic Lupus Erythematosus - What is the Importance of this Finding?

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SUMMARY Juvenile systemic lupus erythematosus (JSLE) is a systemic autoimmune chronic disease that can affect any part of the body. It is characterized by the formation of antibodies against nuclear antigens. Vasculitis may be found in SLE, but it scarcely complies with anti-neutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (AAV) criteria. We report five cases of severe JSLE associated with AAV diagnosed between 1991 and 2013 in three university-based tertiary care centers. The patients (3 girls and 2 boys, aged 12 to 17) presented with a severe clinical picture and the following features: cytopenia (n=5), autoimmune hepatitis (n=3), lupus nephritis (n=1), pancreatitis (n=1), secondary antiphospholipid syndrome (n=2), impending respiratory failure (n=2), and gastrointestinal bleeding (n=1).

All patients were proteinase 3 (PR3) ANCA positive, while two of them were myeloperoxidase (MPO) and PR3 ANCAs positive at the same time. They were treated with corticosteroids and immunosuppressive drugs. Remission of the disease was achieved in three patients. The course of the disease was worsening in two patients and we included rituximab (anti-CD20) in therapy. All of our patients presented as the most severe SLE patients, who must be diagnosed as soon as possible and treated very intensively. Since the comorbidity of JSLE and AAV occurs very rarely in children, presentation of such patients, their clinical pictures, treatment, and the course of the diseases are experiences that can be of great help.

KEYWORDS: systemic lupus erythematosus; ANCAs; ANCA-associated vasculitis

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multi-system autoimmune connective tissue disorder with various clinical presentations that can imitate many diseases (1,2). Fifteen to twenty percent of SLE cases

occur before the age of 16. When presenting in childhood, SLE is often more severe and usually includes the vital organs (3). It is characterized by the presence of multiple autoantibodies, including serum antibod-

ies against nuclear components (ANA), circulating immune complexes, and activation of the complement system. Anti-neutrophil cytoplasmic antibodies (ANCA) can also be detected. It is a group of autoantibodies directed against various neutrophil and monocyte granule and lysosome constituents. Clinically significant are ANCAs directed to proteinase 3 or myeloperoxidase, whereas the importance of other ANCAs is unknown (4). ANCAs are strongly associated with small vessel vasculitis (AAV), which includes Wegener's granulomatosis, microscopic polyangiitis (MPA), and Churg-Strauss syndrome (CSS) (5). According to studies, approximately 15-20% of adults (6) and up to 69% of children with SLE have a positive ANCA finding, but without signs of vasculitis (7). It is estimated that in 4% of SLE patients clinical signs of vasculitis may be found (8). Only five patients diagnosed with SLE and AAV have been described in the literature. To the best of our knowledge, there is only one publication regarding children who suffer from both JSLE and AAV. Since the coexistence of JSLE and AAV occurs very rarely in children, we report five cases of severe JSLE associated with clinical and laboratory signs of AAV.

PATIENTS AND METHODS

We reviewed the medical records of all patients aged 1-18 years who were diagnosed with JSLE (according to the revised ACR criteria) (9,10) and ANCA-associated vasculitis (according to ACR/EULAR criteria) (11,12,13) during the period of 1991-2013 at the Pediatrics Departments at three university tertiary care hospitals (Zagreb, Split, Rijeka). Sera of all children were tested for the presence of ANA using indirect immunofluorescence (IIF) on Hep-2 cells (Euroimmun, Lübeck, Germany). Sera positive for IIF were then tested by antigen-specific enzyme-linked immunosorbent assay – ELISA (Euroimmun, Lübeck, Germany). ANCA tests were determined by IIF using in-house preparations of ethanol – fixed human purified neutrophils as cellular substrate. MPO- and PR3-ANCA were performed using ELISA (Euroimmun, Lübeck, Germany). Positive C-ANCA, P-ANCA serum, and negative control were included in every ANCA determination. Before we started gathering the data, parental consent and ethics committee approvals were obtained.

CASE PRESENTATIONS

Case 1

A 12-year-old girl, whose disease started four weeks prior to hospitalization, presented with myal-

gia, polyarthralgia, vasculitic palm rash, right palm swelling, hepatosplenomegaly, and persistent low-grade fever (Table 1). The third day after admission she became tachydyspnoic and orthopnoic with respiratory insufficiency and pale-green skin. In the laboratory findings there were severe anemia, leucopenia, and markedly increased transaminases, highly elevated erythrocyte sedimentation rate (ESR), C – reactive protein (CRP), and polyclonal gammopathy (Table 2). ANAs were also significantly positive (dsDNA, histone, SS-A, SS-B, U1-RNP) with highly elevated ANCA (PR3 ANCA) and positive anticardiolipin antibodies (Table 2). MSCT of the abdomen showed an enlarged spleen, liver, and retroperitoneal lymphadenopathy. We started treatment with pulse corticosteroids (three consecutive days), pulse cyclophosphamide (6x), and azathioprine, after which the clinical picture and laboratory findings became normalized with the exception of slight thrombocytopenia. She has been in complete remission for five years (Tables 2 and 3).

Case 2

This case involved a 14-year-old girl who was diagnosed with polyarticular juvenile idiopathic arthritis (JIA) in 2008 and treated with corticosteroids and methotrexate at a local hospital. Her first hospitalization at the University Hospital, Department of Pediatric Rheumatology and Immunology came three years later because of her deteriorating condition, when she presented with general distress, severe headaches, nausea, and arthralgias. A physical examination showed radiocarpal, metacarpophalangeal, and talocrural edema, swan-neck deformities of the fingers and prominent vasculitic rash on the palms (Table 1). The laboratory work-up revealed elevated ESR, highly elevated transaminases, anemia, thrombocytopenia, hypergammaglobulinaemia, low complement with positive ANAs, dsDNA, and histone antibodies, positive PR3 ANCAs, and AGLM (Table 1 and 2). A liver biopsy showed chronic active hepatitis and MR of brain CNS vasculitis. We started treatment with corticosteroid pulse therapy (three consecutive days) followed by corticosteroids orally and pulse cyclophosphamide therapy (6x). Since there were no clinical or laboratory result improvements, rituximab was added into the therapy. The course of her disease was later complicated by thrombophlebitis and suspected deep vein thrombosis of the lower leg. Clinically, this girl is much better now, but according to laboratory findings, the disease is still somewhat active (Tables 2 and 3).

Table 1. Clinical characteristics of 5 patients with juvenile SLE and ANCA-associated vasculitis

	Patient 1 13.5 years/ F	Patient 2 15.5 years/ F	Patient 3 15 years/ F	Patient 4 15 years /M	Patient 5 17 years/ M
Diagnosis	SLE, ANCA-associated vasculitis, autoimmune hepatitis, secondary APLS	SLE, ANCA-associated vasculitis, JIA, autoimmune hepatitis	SLE, ANCA-associated vasculitis, autoimmune hepatitis acute pancreatitis	SLE, lupus nephritis V, ANCA-associated vasculitis, secondary APLS	SLE, ANCA- associated vasculitis autoimmune hepatitis
Clinical signs of SLE	Myalgia, polyarthralgia, hepatosplenomegaly, retroperitoneal lymphadenopathy	Polyarthritis	Retroperitoneal lymphadenopathy, severe abdominal pain, pancreatitis	Malar rash on face, cervical lymphadenopathy, polyarthralgia, abdominal pain	Abdominal pain
Clinical signs of ANCA- associated vasculitis	Impending respiratory failure, vasculitic rash at the palms	Vasculitic rash at the palms, severe headaches, CNS vasculitis	Vasculitic rash on arms and legs, impending respiratory failure	Livedo reticularis of legs, facial vasculitic rash, necrotic changes on palms, severe headache, asthma, membranous nephritis class V	Gastrointestinal bleeding, nasal inflammation
Constitutional symptoms	Subfebrile, loss of appetite, adynamic, pale-green skin, general distress	Loss of appetite and weight, nausea, general distress	Febrile, general distress	Fever, fatigue, headache, general distress	Fatigue, loss of appetite

ANCA: anti-neutrophil cytoplasmic antibodies; CNS: central nervous system; SLE: systemic lupus erythematosus.

Case 3

A 14-year-old girl was hospitalized for severe abdominal pain in the upper right quadrant with a fever and general distress. She was diagnosed with acute pancreatitis (elevation of amylases) and a hepatic lesion (elevation of transaminases and bilirubin). A liver biopsy specimen indicated signs of highly active chronic hepatitis, and an abdominal multi-slice computer tomography (MSCT) showed enlarged lymph nodes retroperitoneally. Since lymphoma was suspected, an explorative laparotomy was performed. Histology of the removed lymph nodes excluded malignancy, confirming reactive inflammatory changes. After a laparotomy was done the general condition of patient was aggravated by respiratory failure and a vasculitic rash. Laboratory findings showed highly elevated ESR and CRP, anemia, and hypergammaglobulinaemia, with positive anti-dsDNA, histone, U1RNP antibodies, and ANCA (Table 2). The patient was treated with corticosteroids orally followed by pulse cyclophosphamide therapy (6x). She responded well and the dose of corticosteroids was slowly reduced. Azathioprine and hydroxychloroquine were entered into therapy. Today, she is in complete clinical and laboratory remission at a follow-up of almost four years (Table 2).

Case 4

A 13-year-old boy was hospitalized three weeks after the onset of his disease that presented with fever, severe headaches, abdominal pain, polyarthralgia, and a prominent malar rash on the face, a rash on the legs consistent with livedo reticularis, cervical lymphadenopathy, and fatigue. He had suffered from asthma for several years. Laboratory findings showed anemia, leucopenia, thrombocytopenia, and lower levels of complement with positive ANA (dsDNA, histone, SS-A, SS-B, U1-RNP, Smith), ANCA-PR3, and anticardiolipin antibodies (Table 2). An Magnetic resonance imaging (MRI) of the brain was performed because of suspected CNS vasculitis, but the result was normal. Corticosteroids and hydroxychloroquine were entered into therapy. After two weeks of such treatment, proteinuria was observed (total proteins were 1.73 g in daily urine). A renal biopsy according to the ISN/RPS 2004 classification showed membranous lupus nephritis (class V). Pulse doses of cyclophosphamide (6x) were introduced into therapy. After the last pulse of cyclophosphamide, proteinuria was again observed (2.5g in daily urine) and we started with mycophenolate mofetil, but with no success. Unfortunately, he developed nephrotic syndrome.

Table 2. Immunological findings of five patients with juvenile SLE and ANCA-associated vasculitis

Immunological findings	Patient 1		Patient 2		Patient 3		Patient 4		Patient 5	
	Onset of disease	Last follow up (after 5 y)	Onset of disease	Last follow up (after 3 y)	Onset of disease	Last follow up (after 4 y)	Onset of disease	Last follow up (after 2.5 y)	Onset of disease	Last follow up (after 3,5 y)
ANA	1:10240	speckled	1:1600	Homogenous	1:3200	Homogenous	1:3200	neg	1:1600	1:800
Anti ds-DNA (IU/ml)	173	neg	349	neg	268	neg	585	nd	603	164
Anti-histone antibodies (U/ml)	293	neg	290	105	327	nd	351	nd	360	124
ANCA	1:20480	neg	1:160	neg	1:1280	1:40	1:10240	neg	1:5120	neg
MPO (RU/ml) (n.v. < 20)	neg	neg	14	7	139	nd	6	neg	148	nd
PR3 (RU/ml) (n.v. < 20)	86	neg	25	5	25	nd	96	neg	42	nd
IgG (g/l) (n.v. 7.0-16.0)	48.22	8,87	22.01	10.95	73.58	21.87	17.07	3.87	38.28	9.92
IgA (g/l) (n.v. 0.7-4.0)	<0.05	0.09	0.72	0.46	3.39	2.49	2.68	1.65	2.74	1.74
IgM (g/l) (n.v. 0.4-2.3)	2.61	0.66	1.89	0.38	1.51	2.26	1.21	0.22	0.82	0,81
IgE (kIU/L) (n.v. < 100)	2532 kIU/L	> 2000	nd	nd	393.3	nd	980	190	82.9	29.8
A/G inversion	0.48	1.91	0.91	1.53	0.36	1.02	1.02	1.66	0.7	1.6

ANCA: anti-neutrophil cytoplasmic antibodies; APLS: antiphospholipid syndrome; CNS: central nervous system; SLE: systemic lupus erythematosus; y=years.

Therefore we decided to include rituximab. With that treatment proteinuria has been well controlled for 2.5 years.

Case 5

A 17-year-old boy was hospitalized because of evaluation of liver damage that was detected by accident during the work-up for respiratory infection (purulent nasal discharge). Seven months prior to hospitalization, he noticed blood in the stool several times. Three weeks prior to admission, he was fatigued and had a loss of appetite. During hospitalization, he suffered severe abdominal pain with vomiting and gastrointestinal bleeding (Table 1). Laboratory work-up showed elevated ESR and transaminases, hypergammaglobulinaemia, positive ANA (dsDNA, histone), ANCAs (both PR3 and MPO), and AGLM with lower levels of complement (Table 2). A liver biopsy disclosed autoimmune hepatitis of high activity with necrosis. He was treated with corticosteroids and azathioprine. After 3.5 years of follow-up he is clinically well, but antibodies are still positive (Table 2).

DISCUSSION

We report on five patients with a confirmed diagnosis of juvenile SLE and AAV and discuss the disease characteristics, treatment modalities, course of the disease and available criteria. Descriptions of such patients in the literature are scarce, especially in children. Searching the literature, we have found only one description of such a patient during childhood (14). Although the time between the manifestations of these two diseases could be more than 10 years (15), all our cases had clinical and laboratory features of both diseases already present during the initial diagnostic assessment. Their overall clinical presentation appeared heterogeneous. A similar observation has been noted in a study of SLE-AAV overlap syndrome in adults (15). Two patients had impending respiratory failure; one had renal involvement, one CNS vasculitis, and in one the gastrointestinal system was involved. Most of the patients described in the literature (15), including our cases, had very pronounced respiratory manifestations, which leads us to the conclusion that the respiratory system may be at a very high risk of being affected in this comorbidity. On the other

Table 3. Laboratory findings of five patients with juvenile SLE and AAV

Laboratory findings	Patient 1		Patient 2		Patient 3		Patient 4		Patient 5	
	Onset of disease	Last follow up (after 5 y)	Onset of disease	Last follow up (after 3 y)	Onset of disease	Last follow up (after 4 y)	Onset of disease	Last follow up (after 2.5 y)	Onset of disease	Last follow up (after 3,5 y)
ESR (mm/h)	93	29	26	6	124	21	55	17	30	21
CRP (mg/L)	25.5	<0.3	2.5	0.5	21.2	2.0	0.4	1.0	0.2	0.1
Hgb (g/L)	95	122	112	115	98	123	97	139	143	141
Hct (L/L)	0.28	0.42	0.343	0.35	0.30	0.38	0.28	0.397	0.426	0.41
WBC $\times 10^9/L$	3.0	3.65	5.7	3.3	10.2	4.6	3.5	7.63	7.30	7.7
PLT $\times 10^9/L$	274	162	137	115	477	274	121	313	326	203
AST (U/L)	663	25	1194	29	482	37	59	19	473	18
ALT (U/L)	352	21	602	22	437	27	46	27	1009	17
GGT (U/L)	96	16	63	18	228	63	18	26	312	21

AST: aspartate aminotransferase; ALT: alanin aminotransferase; GGT: gama glutamil transferase; Hgb: hemoglobin; Hct: hematocrit; PLT: platelet; WBC: white blood cells

hand, low renal involvement in our patients was not in accordance with the published literature (14,15). It seems that in our pediatric patients the kidneys were less affected than in adults with this syndrome. The only reported case of a child with SLE-Wegener granulomatosis association that we found also had significant renal involvement. The gastrointestinal system in one of our patients was affected, and it is important to note that the Japanese Research Group of Intractable Vasculitis, Ministry of Health, Labor, and Welfare (MHLW) included gastrointestinal bleeding among the diagnostic criteria for a precise diagnosis of AAV (16). What was similar in most of our cases (four out of five patients) was the presence of a vasculitic rash. Vasculitic rash is a characteristic and common skin finding of small-sized vessel vasculitis and has therefore been recognized as a significant sign: we found that it is also included in the abovementioned AAV diagnostic criteria used in Japan. Even though vasculitic rash and gastrointestinal bleeding are not incorporated into the ACR/EULAR criteria, in view of our experience, we suggest that these entities can be used as surrogate or additional descriptors in cases of suspected small-vessel vasculitis, especially in childhood.

Considering the fact that most of our patients had another autoimmune or inflammatory disease (JIA, autoimmune hepatitis, secondary ALPS, pancreatitis) it seems that in patients with this association the occurrence of certain autoimmune or inflammatory disorders might be increased. Several papers report the possible mechanisms involved in the pathogenesis of these disorders within the separate SLE or AAV diagnoses. Those mechanisms include genetic

predisposition, vasculitis, immune complex deposition, vascular intimal thickening and inflammation, thromboembolic events, generalized sororities, use of immunosuppressive therapy, etc. (17,18). The presence of some of these autoimmune diseases in such patients is partly in line with the literature since cases with this association and ALPS have already been described (15). We have not found descriptions about patients with comorbidity of AAV, SLE, and autoimmune hepatitis (AIH), as we did in most of our cases.

Regarding laboratory findings, the literature states that all such patients displayed cytopenia, which is in line with our findings (15). Three of our patients had thrombocytopenia, three leukopenia, and three anemia. It is also interesting that three of our patients had extremely high serum immunoglobulin E (IgE) levels (Table 2), but we were not able to find similar reports. One of those cases had asthma, but others did not have any evident reason that could lead to an elevation of IgE. It might be very interesting to test IgE in severe SLE patients with concomitant vasculitis to see if there is any connection. Laboratory tests also revealed high titer of ANA, anti-dsDNA and anti-histone, and highly positive ANCA in all patients. It is important to consider the possibility of false positive ANCA due to the high titer of ANA, leading to a false diagnosis of the comorbidity of SLE and AAV. The literature reports that ANCA positivity, when tested by IIF in SLE patients, is higher than when tested by ELISA, because of the presence of non-PR3, non-MPO ANCA and the difficulty in differentiating p-ANCA from ANA by IIF (19). According to the International Consensus Statement on Testing and Reporting of ANCA from 1999, serum samples containing ANCA

by IIF should be tested in ELISAs for PR3-ANCA and MPO-ANCA. Moreover, all serum samples should be tested in ELISAs for PR3-ANCA and MPO-ANCA, as we did in all our cases (20).

We should also mention that some authors noticed a positive correlation between hypergammaglobulinemia and c-ANCA titers. They hypothesized that polyclonal B cell proliferation may lead to the presence of c-ANCA antibodies (21) and false positive results. Hypergammaglobulinemia was found in all our patients but we cannot say that there is positive correlation between the level of hypergammaglobulinaemia and, in our case, the level of p-ANCA titres.

There are contradictory data in the literature regarding positive correlations between ANCA antibodies and specific symptoms. While some authors state that there is a positive correlation between ANCA and a specific symptom (such as serositis, livedo reticularis, venous thrombosis, and arthritis) and with disease activity of SLE (22-24), other authors have found that there is no association either between ANCA and specific organ involvement or between the level of ANCA and disease activity (25,26). It is important to point out that most of our patients presented with severe initial symptoms and with highly positive ANCA during that period. According to our results, the level of ANCA could be a marker of disease activity.

Disease treatment was similar to the treatment recommended in available literature (14,15). Corticosteroids together with immunosuppressives (pulses of cyclophosphamide) were used as an induction therapy. In two patients who did not respond well we administered rituximab (anti-CD20), which has shown satisfactory results so far.

Since there were very few patients described with this overlap syndrome and they were followed for quite a short period, we can say that the course of disease is actually unknown. Two patients died (one of them was child), and the others are in remission (14,15). We followed our patients for almost five years. Three of them are in remission and other two have satisfactory control of the disease.

CONCLUSION

Even though there is very small amount of information in the literature, it seems that the comorbidity of juvenile SLE and ANCA-associated vasculitis is not as rare as we expected, and that doctors should be aware of it. All of our patients presented as the most severe patients with both SLE and AAV clinical and biological features, who must be diagnosed as soon as possible and treated very intensively. In this way, it is definitely possible to prolong survival, improve

the quality of life, develop novel therapies, and determine the best approach for providing care to this complex group of patients.

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