Identifying autoimmune lymphoproliferative syndrome (ALPS) in children with Evans syndrome: a multi-institutional study

Authors: Alix E. Seif,1 Catherine S. Manno,2 Cecilia Sheen,1 Stephan A. Grupp,1 and David T. Teachey1

Affiliations: 1Divisions of Hematology and Oncology, Department of Pediatrics, Children’s Hospital of Philadelphia, Philadelphia, PA, 19104; 2Department of Pediatrics, New York University School of Medicine

Running Title: Identifying ALPS in Evans syndrome patients

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Corresponding Author: David T. Teachey, MD. Divisions of Hematology and Oncology, Children’s Hospital of Philadelphia, 3008 CTRB, 3501 Civic Center Boulevard, Philadelphia, PA 19104. Email: teacheyd@email.chop.edu
Abstract

Autoimmune Lymphoproliferative Syndrome (ALPS) is a disorder of abnormal lymphocyte survival caused by dysregulation of the Fas apoptotic pathway. Clinical manifestations of ALPS include autoimmune cytopenias, organomegaly, and lymphadenopathy. These findings overlap with Evans Syndrome (ES), defined by presence of at least two autoimmune cytopenias. We hypothesized a subset of patients with ES have ALPS and tested 45 children at 22 institutions, measuring peripheral blood double-negative T cells (DNTs) and Fas-mediated apoptosis. ALPS was diagnosed in 47% of patients tested. Markedly elevated DNTs (≥5%) were a strong predictor of ALPS (positive predictive value 94%), while no patients with DNTs <2.5% had ALPS on apoptosis testing. Severity of cytopenias and elevated immunoglobulin levels also predicted ALPS. This is the largest published series describing children with ES and documents a high rate of ALPS among pediatric ES patients. These data suggest that children with ES should be screened for ALPS with DNTs.
Introduction

Autoimmune lymphoproliferative syndrome (ALPS) is a disorder of disrupted lymphocyte homeostasis caused by defective Fas-mediated apoptosis. Clinical manifestations include autoimmune cytopenias, organomegaly, lymphadenopathy, and increased risk of malignancy. Similar clinical features are observed in Evans Syndrome (ES), a hematologic disorder defined by autoimmune destruction of at least two hematologic cell types after exclusion of other diagnoses. We hypothesized a subset of patients diagnosed with ES may have ALPS and previously demonstrated in a small single institutional study that a significant percentage of patients diagnosed with ES have ALPS. To confirm our hypothesis, we conducted a multi-institutional observational study with primary aims to determine the prevalence of ALPS among patients diagnosed with ES and to evaluate DNTs (double-negative T cells; phenotype: CD3+, CD4-, CD8-, TCR-αβ+) as a screening tool for ALPS in patients with ES.

Materials and Methods

We recruited patients diagnosed with ES or multiple unexplained autoimmune cytopenias at 22 institutions. Inclusion and exclusion criteria are depicted in Table 1B. Referring clinicians sent samples for centralized testing and conducted a standardized physical and laboratory assessment of lymphadenopathy, organomegaly, complete blood counts, hematologic autoantibodies, anti-nuclear antibodies (ANA), serum immunoglobulin G (IgG), and anti-phospholipid antibodies (APLA). DNTs were measured in our clinical immunology laboratory, where the assay is performed routinely. We performed in vitro assessment of Fas-mediated apoptosis, the functional test used to confirm ALPS diagnosis. Methods for both of these assays are published and validated. All studies were performed using a Children’s Hospital of Philadelphia IRB-approved protocol with informed consent in accordance with the Declaration of Helsinki. Statistical analyses were performed using Prism 4 for Windows (GraphPad Software).
Results and Discussion

A total of 73 patients were referred for evaluation. Of these, 11 did not meet diagnostic criteria for ES, and an additional 17 were excluded for incomplete clinical and/or laboratory data, leaving 45 patients for analysis (Supplemental figure 1A). Mean age at diagnosis was 7.1 years, with females comprising 24% of the cohort (n=12). Gender, age, and ethnicity were not associated with ALPS diagnosis (Supplemental Figure 1B). No patients were related, and six patients had a first-degree relative with a history of autoimmune disease (3 with ITP, 1 with AIHA, 1 with CVID, and 1 with celiac disease).

Twenty-one of 45 patients (47%) had elevated DNTs and defective Fas-mediated apoptosis consistent with a diagnosis of ALPS (Figure 1A). Clinical factors predictive of defective Fas-mediated apoptosis were (Figure 1B): severity of cytopenias, defined as requiring treatment with immunosuppressive medications at least twice a year (OR 5.6; 95% CI 1.43-21.9, P =.015), presence of lymphadenopathy (OR 4; 95% CI 1.15-13.9, P = .038), and elevated IgG (OR 13.7; 95% CI 1.4-134, P = .016). While clinically evident lymphoproliferation was a predictor of ALPS, 4/21 patients were found to have significant autoimmunity, elevated DNTs, and defective Fas-mediated apoptosis without clinically identifiable lymphoproliferation. Whether these patients have a forme fruste of ALPS or an ALPS-like syndrome is up for debate. Specific cytopenias and hematologic antibodies were not predictive of ALPS, nor were ANA, APLA or hepatomegaly.

DNTs, an atypical T cell population increased in ALPS patients, were assessed in all patients (Figure 1A). The upper limit of normal for peripheral blood DNTs at our laboratory is 2.5%, established by testing an extended panel of normal controls. In this cohort, 16/17 patients with DNTs ≥5% had defective Fas-mediated apoptosis. The patient with a normal apoptosis assay and elevated DNTs was subsequently found to have a FAS mutation, and his negative apoptosis assay may represent a false negative. All 13
patients with DNTs <2.5% had normal apoptosis assays. In our series, normal DNTs had 100% sensitivity and 100% negative predictive value for ALPS; whereas, DNTs ≥5% had 96% specificity and 94% positive predictive value for a defective apoptosis assay. Of the patients with moderately elevated DNTs (2.5-4.9%), nearly half (5/14) had defective apoptosis.

Approximately 70% of patients with ALPS have identifiable genetic mutations in FAS, FASL, or CASP10. Seventeen of the 21 patients who met the laboratory diagnostic criteria for ALPS underwent genetic testing, and 9/17 (53%) had an identifiable mutation. The lower rate of detectable mutations in this study may reflect an underlying biologic difference in children who present with autoimmunity as a primary symptom rather than lymphoproliferation or the low prevalence of the disease and small numbers evaluated in prior studies.

An identified genetic mutation is a supportive criterion for ALPS and not part of the currently accepted standard definition. Polymorphisms in FAS are common, and a mutation must be proven functional to be useful for diagnosis. Accordingly, our IRB only approved genetic testing on the patients with abnormal apoptosis. Our study classified ALPS by the accepted consensus definition first proposed by the NIH in the 1990s. A number of groups, including ours have tried to identify new assays that predict ALPS (e.g., soluble Fas ligand, vitamin B12, and IL-10), but these have only been successful in identifying ALPS type 1A patients (those with mutations in FAS) and are not strong predictors of other ALPS types. Currently, the only means of diagnosing ALPS type III patients is with the apoptosis assay. This group encompasses 20-30% of ALPS patients.

Patients with somatic mutation variant ALPS (1s) and Fas ligand variant (1b) have normal apoptosis assays. Fas ligand deficiency has been reported in 2 cases of ALPS. ALPS due to somatic DNT cells carrying a Fas mutation is thought to occur in 2-5% of patients and should be ruled out in patients with elevated DNT cells and appropriate clinical phenotype. Based on the rarity of these sub-types of ALPS,
only 1 or 2 patients classified as “non-ALPS” in our series may have had ALPS. This small degree of possible misclassification would not change the findings of the study.

ALPS is presumed to be a rare condition with only a few hundred reported cases. Finding a high rate of ALPS in patients previously identified as having ES suggests ALPS may be more prevalent than previously thought. Our detection of true prevalence of ALPS in patients diagnosed with ES may be skewed by referral bias. However, a clear diagnosis is crucial, as understanding the biology of these different entities may help with identifying targeted therapies, genetic counseling, and avoiding potentially harmful treatments in the future. In particular, treatment for ALPS may vary from that of ES, as clinical trials have identified mycophenolate mofetil and sirolimus to be effective medications for children with ALPS; whereas, data for targeted therapies for ES are limited by its nature as a diagnosis of exclusion.13-14 Patients with ALPS who undergo splenectomy have an increased risk of developing post-splenectomy sepsis despite appropriate vaccination and antibiotic prophylaxis.1 If possible, splenectomy should be avoided in patients with ALPS. Trials have suggested rituximab may be relatively contraindicated in ALPS patients as ALPS patients may develop long-term immunodeficiency after treatment.15 As ES is a diagnosis of exclusion, children with idiopathic autoimmune cytopenias should be tested for ALPS, especially prior to undergoing potentially harmful treatments such as splenectomy or rituximab. These results demonstrate that childhood ES may be a distinct entity from ES in adults, as the high prevalence of ALPS in children contrasts with a recent study of ES in adults that found no ALPS cases; notably, the study was not designed to detect prevalence of ALPS and only tested the few patients with atypical lymphoproliferation.16

In summary, we found a significant percentage of pediatric patients diagnosed with Evans syndrome have ALPS, that severity in number and presentation of cytopenias predicts the diagnosis, and that some children without the classic lymphoproliferative signs are also at risk. Given the important differences in management between ALPS and ES, we recommend that all children with ES undergo ALPS screening with DNTs as part of their diagnostic evaluation.
Acknowledgments

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Authorship contributions

A.E.S, C.S.M., S.A.G., and D.T.T designed the research and drafted the manuscript. A.E.S, D.T.T., and C.S. performed research, analyzed and interpreted data and performed statistical analysis. All authors were involved in critical revision of the manuscript.

Conflicts of Interest

C.S.M. is a consultant for Bayer Healthcare, Baxter Healthcare, and Grifols US. No author has competing financial interests to declare.
References


Table 1

A. Current Diagnostic Criteria for ALPS\textsuperscript{1,7,17}

1. Chronic non-malignant lymphoproliferation
2. Elevated peripheral blood DNTs
3. Defective in vitro Fas-mediated apoptosis*

Supportive but not diagnostic:
- Genetic mutation (FAS,FASL, CASP10, NRAS)
- Autoimmunity
- Family history
- Histopathology

**Must meet all 3 criteria for diagnosis**

*Except ALPS 1b and 1m

B. Inclusion and Exclusion Criteria

Inclusion Criteria
1. Age 1-21 years
2. Diagnosis of Evans syndrome by treating physician
3. Documented autoimmune cytopenias
   a. Either 2 or 3 cell types (RBCs, WBC, platelets), or
   b. Autoimmune thrombocytopenia and strongly positive DAT

Exclusion Criteria
1. Autoimmune disease
   a. Systemic lupus erythematosus
   b. Juvenile idiopathic arthritis
2. Malignancy
3. Immunosuppression following solid organ transplant
4. Concurrent immunomodulating medications except for low dose corticosteroids
   (≤0.5mg/kg/day) *

*Low dose corticosteroids have been shown not to affect results\textsuperscript{18}
Figure 1: Clinical and laboratory features of children with ES. Forty-five patients with ES were evaluated for ALPS by in vitro Fas-mediated apoptosis assay and DNTs. Patients with defective Fas-mediated apoptosis (consistent with ALPS) are depicted with black columns and patients with normal apoptosis assays (not consistent with ALPS) are depicted with grey columns (Figure 1A). The ordinate depicts DNTs (%), and the grey shaded bar delineates DNTs between 2.5% (upper limit of normal) and 5% (marked elevation). All patients with DNTs below 2.5% had normal apoptosis testing. All patients with DNTs ≥5% except for patient 69 had defective Fas-mediated apoptosis. Of note, this child was found to have an identifiable genetic mutation in FAS and may represent a false negative on apoptosis testing. Severe autoimmune cytopenias (requiring immunosuppressive treatment at least twice a year), lymphadenopathy, and IgG level were predictive of ALPS (Figure 1B), while pancytopenia trended towards predicting ALPS in ES. Of note, 4/21 patients ultimately found to have ALPS had no clinical evidence of lymphoproliferation. †Limited clinical data were available for ANA (performed in 20/21 children with ALPS and 24/24 without ALPS), APLA (15/21 with ALPS and 14/24 without ALPS) and IgG (15/21 with ALPS and 13/24 without ALPS). §Trend towards statistical significance. *P-value reaches significance. ALPS+ — patients with defective in vitro apoptosis, ALPS− — patients with normal in vitro apoptosis, OR — odds ratio, 95% CI — 95% confidence interval, PPV — positive predictive value. P-values calculated using Fisher’s exact test.
Figure 1

A

[Graph showing ALPS assay results with sensitivity and specificity calculations]

B

<table>
<thead>
<tr>
<th>Lab finding, n (%)</th>
<th>ALPS+ N=21</th>
<th>ALPS- N=24</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
<th>PPV (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia</td>
<td>19 (90)</td>
<td>18 (75)</td>
<td>3.17</td>
<td>0.56–17.8</td>
<td>.25</td>
<td>51.4</td>
<td>34–68</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>20 (95)</td>
<td>23 (96)</td>
<td>0.87</td>
<td>0.05–14.8</td>
<td>1</td>
<td>46.5</td>
<td>31–62</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>19 (90)</td>
<td>19 (79)</td>
<td>2.5</td>
<td>0.43–14.5</td>
<td>.42</td>
<td>50</td>
<td>33–67</td>
</tr>
<tr>
<td>Pancytopenia</td>
<td>17 (81)</td>
<td>13 (54)</td>
<td>4.36</td>
<td>0.99–19.1</td>
<td>.054*</td>
<td>56.7</td>
<td>37–75</td>
</tr>
<tr>
<td>Severe cytopenia</td>
<td>16 (76)</td>
<td>10 (42)</td>
<td>5.6</td>
<td>1.43–21.9</td>
<td>.015*</td>
<td>61.5</td>
<td>41–80</td>
</tr>
<tr>
<td>ANA</td>
<td>2 (10)</td>
<td>1 (4)</td>
<td>2.56</td>
<td>0.21–3.5</td>
<td>.58</td>
<td>66.7</td>
<td>9–99</td>
</tr>
<tr>
<td>APLA</td>
<td>2 (13)</td>
<td>3 (21)</td>
<td>0.56</td>
<td>0.08–4</td>
<td>.65</td>
<td>40</td>
<td>5–85</td>
</tr>
<tr>
<td>Elevated IgG</td>
<td>8 (53)</td>
<td>1 (8)</td>
<td>13.71</td>
<td>1.4–133.9</td>
<td>.016*</td>
<td>88.9</td>
<td>52–100</td>
</tr>
</tbody>
</table>

| Clinical finding, n (%) | | | |
|-------------------------|-----------------|-----------------|
| Adenopathy              | 14 (67)         | 8 (33)          |
| Splenomegaly            | 14 (67)         | 10 (42)         |
| Hepatomegaly            | 3 (14)          | 3 (13)          |
| Any lymphoproliferation | 17 (81)         | 13 (54)         |
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