How I treat autoimmune lymphoproliferative syndrome (ALPS)

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Abstract:

Autoimmune Lymphoproliferative Syndrome (ALPS) represents a failure of apoptotic mechanisms to maintain lymphocyte homeostasis, permitting accumulation of lymphoid mass and persistence of autoreactive cells that often manifest in childhood with chronic nonmalignant lymphadenopathy, hepatosplenomegaly, and recurring multilineage cytopenias. Cytopenias in these patients can be due to splenic sequestration as well as autoimmune complications manifesting as autoimmune hemolytic anemia (AIHA), immune-mediated thrombocytopenia (ITP), and autoimmune neutropenia (AIN). More than 300 families with hereditary ALPS have now been described; nearly 500 patients from these families have been studied and followed worldwide over the last 20 years by our colleagues and our selves. Some of these patients with FAS mutations affecting the intracellular portion of the FAS protein also have an increased risk of B-cell lymphoma. Here is an outline of the best approaches to diagnosis, follow up and management of ALPS, its associated cytopenias and other complications due to infiltrative lymphoproliferation and autoimmunity.
Introduction:
Role of Fas signaling in immune cell homeostasis and pathophysiology of ALPS

Clinical and Laboratory Features:
Hypersplenism, Lymphadenopathy, Autoimmune Cytopenias and Biomarkers

Genetics
Caused by heterozygous mutation of FAS and other apoptosis pathway genes transmitted in an autosomal dominant fashion with variable penetrance

Diagnosis and Classification of ALPS
Required diagnostic criteria include the presence of chronic lymphadenopathy and/or splenomegaly associated with increased circulating CD4-/CD8-double-negative (DN) T lymphocytes

Risk of lymphoma transformation in ALPS
FAS is a tumor suppressor gene conferring a risk of lymphoma in ALPS patients

Principles of management at presentation:
Genetic counseling and lymphoma surveillance using CT and FDG-PET scans
Management of lymphoproliferation and hypersplenism

Splenectomy and care of its aftermath
Majority of them relapse with multilineage cytopenias following splenectomy, while nearly a third of them have opportunistic post splenectomy infections (OPSI)

Treatment of ALPS associated refractory cytopenias and hypersplenism:
Successful use of mycophenolate mofetil and sirolimus as steroid sparing measures

Role of HSCT in ALPS

Implications of ALPS and Future Directions
Novel and nontoxic lympholytic therapies are necessary to control the lymphoproliferative process in children with ALPS, an otherwise rare disorder.
Introduction

Autoimmunity results from failure of self-tolerance, which can be further divided into central and peripheral tolerance. Central tolerance is fostered by apoptosis through elimination of autoreactive lymphocytes in generative lymphoid organs (the bone marrow and thymus); while mechanisms of peripheral tolerance include anergy, deletion by apoptosis and suppression by regulatory T cells to avoid autoimmunity and tissue damage. Apoptosis, the intrinsic program of cell death, is triggered by receptor-ligand interactions at the cell surface (the extrinsic pathway) or by the activation of mitochondrial proteins (the intrinsic pathway), leading to processing and activation of caspases and their downstream targets. Lymphocyte apoptosis mediated by the cell surface receptor FAS plays a pivotal role in lymphocyte homeostasis. FAS (also termed CD95/AP01) is a member of the tumor necrosis factor receptor superfamily (TNFRSF) of proteins that directly trigger apoptosis to maintain lymphocyte homeostasis, peripheral immune tolerance and prevent autoimmunity. It is a membrane bound molecule that is highly expressed not only on activated B and T lymphocytes, but also present in other cells such as hepatocytes. FAS is present on the cell surface as a pre-formed trimer. Its binding with FAS ligand leads to conformational changes on the intracellular portion of FAS protein triggering rapid recruitment of the death domain (DD) of the adaptor protein FADD (Fas-associated death domain) to the homologous DD in the cytoplasmic tail of FAS. This is followed by the recruitment of pro-caspases 8 or 10 through the interaction of their death-effector domains with the amino termini of
FADD. The resulting Fas/FADD/caspase complex is termed the death-inducing signaling complex (DISC) that incites a further cascade of caspase activation culminating in the death of cells (Figure 1) \(^5,6\).

Role of FAS in maintaining lymphocyte homeostasis and peripheral immune tolerance to prevent autoimmunity was first elucidated by studies in Fas-deficient MRL/lpr \(^/-\) mice. MRL mice homozygous for Fas mutations develop hypergammaglobulinemia, glomerulonephritis, massive lymphadenopathy and expansion of an otherwise rare and unique population of T cell receptor (TCR) \(\alpha\beta^+\) cells that lack expression of both CD4 and CD8, and hence known as the double-negative (DN) T cells, hereafter referred to as the DNT cells \(^7\). Despite being a hallmark of the disease, the role of the DNT cells in ALPS is not completely understood, partly due to the constraints of working with these cells, as they remain difficult to grow in vitro.

The understanding of the role of FAS in the immune system was further refined by recent experiments demonstrating that FAS is not key to the control of the acute immune responses in cells, i.e. following exposure to viral antigens; but more importantly it is a critical regulator of immune homeostasis during chronic infections\(^9-10\).

Taken together these studies provided insights into the pathophysiology of a similar syndrome being observed in humans that has subsequently been called the autoimmune lymphoproliferative syndrome (ALPS) \(^11-14\).

**Clinical and Laboratory Features:**
The first clinical manifestation of ALPS is often chronic lymphadenopathy and/or splenomegaly in an otherwise healthy child, often recognized by the pediatrician in a well baby clinic. Symptomatic multilineage cytopenias that are also chronic and refractory are typically most severe in early childhood, paralleling the age of expansion of the lymphocyte repertoire in children, and these tend to improve in adolescents and young adults. A large proportion of ALPS patients may present initially with episodes of fatigue, pallor and icterus associated with hemolytic anemia, spontaneous bruises and mucocutaneous hemorrhages due to thrombocytopenia; or bacterial infections associated with neutropenia. The potential for developing multiple autoimmune as well as infiltrative lymphoproliferative disorders involving different organ systems such as uveitis, hepatitis, glomerulonephritis, infiltrative pulmonary lesions and encephalitis and myelitis (manifesting as aseptic meningitis) in some patients with ALPS is being recognized upon their long term follow up over many years (Table 1)\textsuperscript{15-19}.

The most common laboratory findings include the presence of cytopenias secondary to autoimmune destruction or splenic sequestration and polyclonal hyperggammaglobulinemia. Anemia is nearly universal owing to a combination of factors including hypersplenism, autoimmunity, and iron deficiency. Eosinophilia and monocytosis are also frequent findings in patients with ALPS, although the exact pathophysiologic mechanism for these is uncertain\textsuperscript{20}. Multiple autoantibodies, frequently including those against red blood cells presenting as a positive Coomb's direct antiglobulin test (DAT) have been demonstrated, even in the absence of overt autoimmune disease\textsuperscript{21,22}. One instance of acquired factor VIII inhibitor antibody has
been reported in a ALPS patient\textsuperscript{23}. As discussed in more detail below, serum IL-10, soluble FAS Ligand (sFASL) and vitamin B12 are elevated in ALPS patients with FAS mutations, and are useful biomarkers. Peripheral blood immunophenotyping reveals elevation of the characteristic DNT cells, pathognomonic of ALPS.

\textbf{Genetics:}

ALPS is one of the first well-characterized human genetic diseases of apoptosis. Autosomal dominant transmission of heterozygous germline FAS mutations account for the majority of ALPS cases while somatic mutations in FAS limited to subsets of lymphocytes account for the second largest group of patients in our cohort\textsuperscript{24,25}. Nonetheless, pedigrees of families carrying an ALPS associated germline mutation reveal some family members who carry the genetic defect but have mild or absent phenotypic expression of the disease\textsuperscript{26}. This suggests that other factors, including modifier genes and haploinsufficiency may be involved in determining phenotypic expression\textsuperscript{27,28}. Interestingly, it has recently been demonstrated that the development of acquired \textit{FAS} mutations in DNT cells may be a determinant factor for the appearance of clinical symptoms in some patients with milder germline \textit{FAS} mutations\textsuperscript{27}. This kind of variable penetrance and expressivity is also seen in the animal models, as mice with homozygous \textit{Fas} mutations in the MRL background develop full-blown disease while in other genetic backgrounds it may be clinically silent or more subdued.

Some of these patients with germline FAS mutations affecting the intracellular portion of the FAS protein also have a significantly increased risk of
developing Hodgkin and non-Hodgkin lymphoma, underscoring the critical role played by cell surface receptor-mediated apoptosis in eliminating redundant proliferating lymphocytes with autoreactive and oncogenic potential. A handful of patients have germline deleterious mutations in genes encoding FASL or CASP10.

**Diagnosis and Classification of ALPS:**

Presentation of children with generalized adenopathy, splenomegaly and autoimmune multilineage cytopenias represents a diagnostic challenge because their clinical and laboratory features overlap with those of other childhood hematological disorders including lymphoma, hemophagocytic lymphohistiocytosis (HLH), hereditary spherocytosis, Evans Syndrome and Rosai-Dorfman Disease. Some immunological disorders associated with autoimmune phenomena, like common variable immunodeficiency (CVID) and Wiskott-Aldrich syndrome must be distinguished from ALPS. In 1999, investigators at the National Institutes of Health (NIH) suggested criteria to establish the diagnosis of ALPS. Since then, with approximately 500 ALPS patients studied worldwide, significant advances in our understanding of the disease led to the revisions of the diagnostic criteria and classification scheme following the first international ALPS workshop held at NIH in 2009.

Diagnosis of ALPS is currently based on presence of two Required and six Additional criteria. Required criteria include the presence of chronic lymphadenopathy and/or splenomegaly and elevated circulating TCRαβ+DNT cells.
Additional criteria are further divided into Primary and Secondary category (Table 2). An abnormal lymphocyte apoptosis assay and the presence of pathogenic mutations in genes of the FAS pathway are the primary additional criteria. Secondary additional criteria consist of elevated circulating biomarkers, characteristic histopathology and family history compatible with ALPS. For a definitive ALPS diagnosis a patient has to meet both Required criteria and either of the two Primary Additional Criterion (Table 2). A probable ALPS diagnosis can be entertained if both the Required criteria and any one of the Secondary Additional criterion 3, 4, 5, or 6 are present. Patients with probable ALPS should be treated and monitored in the same way as patients with a definitive diagnosis, but treating physicians are advised to pursue a genetic or apoptosis assay based diagnostic work up whenever possible.

Besides the presence of chronic (>6 months), non-malignant, non-infectious lymphadenopathy, presence of elevated circulating TCRαβ+-DNT cells (the second required criteria) is the hallmark of this disease. Identifying the characteristic TCRαβ+ expression on the DNT cells is critical as increased TCRγδ+-DNT cells is often a common reactive feature secondary to many disorders. For a diagnosis of ALPS a minimum of 1.5% of total lymphocytes (or 2.5% of T lymphocytes) should be TCRαβ+-DNT cells, in the setting of normal or elevated lymphocyte counts. The presence of lymphopenia invalidates this criterion, as its impact on the relative distribution of TCRαβ+-DNT cells is currently unknown. In contrast, elevation of TCRαβ+DNT cells above 3% of the total lymphocytes (or >5% of T lymphocytes) is seldom seen in any conditions other than ALPS \(37,38\).
One of the Primary Additional criteria is an abnormal lymphocyte apoptosis assay. However, it is no longer considered essential for the diagnosis of ALPS, as patients with both somatic FAS mutations and germline FASL mutations can present with normal in vitro FAS-induced apoptosis assays. However, the presence of a reproducible apoptotic defect in patients who fulfill other Required criteria is diagnostic of ALPS. This assay is only offered by very few specialized centers, making its routine clinical use impractical. For this reason, the presence of germline or somatic deleterious mutations in FAS, FASL or CASP10 is considered diagnostic of ALPS-FAS, ALPS-FASL and ALPS-CASP10, respectively. Patients who fulfill the criteria for diagnosis of ALPS with indeterminate genetic cause are classified as ALPS-U denoting an as yet undefined category.

Gene sequencing for ALPS is currently offered by selected commercial laboratories; however, as polymorphisms in FAS are not uncommon, a diagnostic mutation should be based on prior identification of the mutation linked to a diagnosis of ALPS or a proven functional consequence of the change in association with a new mutation. Existing databases of pathogenic FAS mutations are publicly available and can be used for diagnostic help (NCBI NIH ALPS website http://www3.niaid.nih.gov/topics/ALPS/).

Patients with ALPS like syndromes caused by germline mutations in CASP8 and somatic mutations in NRAS and KRAS are currently classified separately as ALPS related apoptosis disorders (Table 3). The latter group of patients with somatic NRAS and KRAS mutations present with autoimmune phenomena, massive splenomegaly, modest lymphadenopathy and normal or only marginally elevated
TCRαβ+ DNT cells. Their lymph node histopathology is also not typical of ALPS-FAS as it lacks the characteristic paracortical expansion populated with TCRαβ+-DNT cells (see below). Additionally, these patients show abnormalities of the myeloid compartment, with chronic persistent monocytosis, mimicking juvenile myelomonocytic leukemia (JMML) in otherwise asymptomatic younger patients. Hence, these patients are now classified as RAS-associated autoimmune leukoproliferative disorder (RALD).

Presence of elevated TCRαβ+-DNT cells and high serum or plasma levels of either interleukin-10 (IL-10), IL-18, soluble FAS ligand (sFASL) or vitamin B12 can accurately predict the presence of germline or somatic FAS mutations and can be used for ALPS diagnosis. These biomarkers can predict a mutation in FAS with a post-test probability ranging from 85 to 97%, depending on the biomarker used and the number of TCRαβ+-DNT cells. The use of any of these biomarkers as diagnostic criteria greatly facilitates the diagnosis in settings that lack access to advanced genetic sequence analysis or functional testing of cell biology (Figure 2).

Autoimmune cytopenias and hypergammaglobulinemia in patients with lymphoproliferation associated with elevated TCRαβ+DNT cells indicates high likelihood of ALPS. Characteristic lymph node histopathological findings are also helpful towards a diagnosis of ALPS-FAS. These include paracortical expansion due to infiltration by polyclonal TCRαβ+-DNT cells accompanied by follicular hyperplasia and polyclonal plasmacytosis. Marked TCRαβ+-DNT cell infiltration in some cases can lead to architectural effacement of lymph nodes and infiltrative changes in bone marrow and spleen, leading in some instances to an erroneous
diagnosis of peripheral T-cell lymphoma. The diagnostic workup for ALPS following a lymph node biopsy should include flow cytometry, immunohistochemical evaluations, or molecular studies that rule out clonal B and T cell population. Finally a positive family history for non-malignant and non-infectious lymphadenopathy and/or splenomegaly with or without autoimmune cytopenias is helpful since many ALPS patients have family members with similar clinical histories.

**Risk of Lymphoma Transformation in ALPS:**

Physiological apoptosis is critical in tumor surveillance as FAS, a putative tumor suppressor is silenced in many tumors. In one report, 20% of B-cell lymphomas derived from (post) germinal center B cells carried somatic mutations in the exon 9 of the FAS gene coding for the intracellular death domain of the protein. Somatic CASP10 gene mutations were noted in 14.5% of non-Hodgkins lymphoma (NHL) in another study. The risk of an ALPS patient developing Hodgkin's lymphoma (HL) is estimated at 50 times that of the general population and the risk of NHL is increased 14 fold in them. The ALPS associated lymphoma cohort at the NIH Clinical Center currently consists of twenty patients (15 males and 5 females) from 15 families. Their median age at lymphoma diagnosis was 17 years (range 5 years to 50 years). Eleven patients had HL, 9 patients had B cell NHL. Eighteen out of 20 patients had germline heterozygous mutations of the FAS gene affecting the intracellular portion of the protein, two patients have had no mutation.
identified. Most ALPS patients with lymphoma respond to conventional multi-agent chemotherapy and radiation. Four patients, 3 with HL, and 1 with NHL are deceased due to progressive disease and one of them developed histiocytic sarcoma following Hodgkin lymphoma\(^5\). This underscores the importance of surveillance for lymphoma in families with ALPS patients. Conversely, ALPS should also be suspected as a possible diagnosis in patients with a history of previous lymphoma presenting with non-malignant lymphadenopathy and increased DNT cells in peripheral blood and lymph node immunohistochemistry during follow up.

Associated personal or family history of childhood onset autoimmune cytopenias, lymphadenopathy and splenomegaly should be sought in all such patients to rule out ALPS.

**Principles of management at presentation:**

**Genetic counseling and lymphoma surveillance**

Genetic counseling is an integral part of the evaluation of a family with ALPS patients in our clinic. Often there is more than one affected individual in a family. Assessment of relatives of ALPS probands with mutations in genes encoding FAS or CASP10 usually identifies a parent, sibling or a more distant relative with identical heterozygous mutations inherited in an autosomal dominant fashion. Many such subjects share clinical features of ALPS with family members incumbent upon the variable penetrance of a given disease causing gene alteration\(^4\). Elevation in DNT cell numbers, serum vitamin B12 and IL-10 levels are typically not present in the absence of the clinical stigmata of ALPS. Patient and family members are educated in
our clinic to seek timely help for any systemic symptoms, flare ups of cytopenias, or unexpected focal fluctuations in lymph node and spleen size.

Chronic generalized adenopathy in ALPS patients can fluctuate over time in its size by up to 20-30% and create some concern of evolving lymphoma if one or more regional group of nodes enlarges unusually. Hence these patients need close clinical observation and have been followed with serial CT and PET scans every 2-3 years in our clinic. Some of them may have to undergo biopsy whenever there is clinical suspicion for lymphoma based on systemic symptoms and focal exacerbation of adenopathy. Non-invasive modalities of assessment are desirable in ALPS to determine if a biopsy is warranted and which of the many enlarged nodes will likely yield informative tissue. Positron emission tomography (PET) using [18F] fluoro- 2-deoxy-D-glucose (FDG) uptake, as a measure of cellular glucose metabolism, has become a standard in the staging and follow up evaluation of cancers, including lymphoma. We are exploring the value of whole body FDG-PET scan to determine whether qualitative or quantitative FDG localization can help differentiate ALPS patients with benign, albeit prominent, adenopathy from those with ALPS-associated lymphomas. Though the biodistribution of FDG in patients with ALPS is abnormal, it may still be possible to discriminate between ALPS associated adenopathy and ALPS associated lymphoma in an individual (Figures 3 and 4) based on the clinical circumstances.

There is also a role for FDG-PET scans in post-chemotherapy follow up to rule out lymphoma relapse in the background of regenerating ALPS related adenopathy as FDG-PET can be a valuable diagnostic tool to help choose a lymph
node for biopsy based on degree of FDG avidity in ALPS patients with suspected lymphoma. ALPS associated adenopathy recurs and must be distinguished from relapsing lymphoma in these patients\textsuperscript{53,54}.

Management of lymphoproliferation and hypersplenism:
Although massive, often visible, adenopathy in growing children can incite considerable anxiety and these children can be socially ostracized, treatment is not specifically indicated to shrink lymph nodes for cosmetic reasons alone. Ongoing surveillance in these patients should include careful attention to changes in lymph node size or appearance of new focal or generalized lymphadenopathy and worsening splenomegaly. The degree of generalized lymphadenopathy is documented longitudinally in a consistent fashion in our clinic. Following grading guidelines with a lymph node matrix covering all groups of nodes (Cervical, Axillary, Inguinal etc.) are used in our clinic for longitudinal follow up of these patients over many years: Grade 1, few shotty nodes; Grade 2, multiple 1-2cm nodes; Grade 3, multiple nodes, some >2 cm; Grade 4, extensive visible adenopathy\textsuperscript{55}. This grading is performed using physical examination and periodic (biannual or as necessary) CT scan evaluations of neck, chest, abdomen and pelvis (Figure 3). Clinically, splenomegaly is documented by consistently measuring its palpable extent below costal margin in the mid clavicular line; now a days a more accurate serial volumetric assessments of splenomegaly can also be made from 3D reconstructions of CT scans (Figure 4). Neither corticosteroids nor immunosuppressive drugs like
azathioprine, cyclosporin or mycophenolate mofitil reliably shrink the spleen or
lymph nodes in patients with ALPS.

Spleen-guards fabricated out of thermo-plastic material by our Rehabilitation
Medicine Department have been used with apparent success in protecting active
children with large spleens from splenic rupture. This has allowed them to
participate in usual school activities including sports programs with abundant
cautions. Preclinical studies using MRL/lpr-/- mice have been undertaken to explore
the role of different classes of drugs as lympholytic agents including Notch signal
modulators, HDAC inhibitors and Arsenic Trioxide. Using the
immunosuppressive medication, Rapamycin (Sirolimus), both adenopathy and the
spleen size can be reduced in MRL/lpr-/- mice and ALPS patients. However, in
the latter, indication for therapy should remain relief from refractory cytopenias
due to significant hypersplenism (see below).

**Splenectomy and care of its aftermath:**

Approximately, one half of more than 250 ALPS patients being followed in our clinic
have had a splenectomy in order to manage their chronic and refractory cytopenias.
Many of them underwent the procedure prior to their diagnosis of ALPS could be
suspected. Asplenic ALPS patients require vigilance for septicemia due to
pneumococcal bacteremia, which can be fatal. Eight asplenic ALPS patients have
had fatal opportunistic infections and several have presented with pneumococcal sepsis following splenectomy. In a smaller substudy of 70 ALPS-FAS patients with adequate long-term follow up, 34 individuals (49%) from 25 families underwent splenectomy at a median age of 10 years (range 1-53 years). Their average length of post-splenectomy follow up was 13 years (range 3 months to 38 years) accounting for a total follow up of 454 person years. The documented reasons for splenectomy included splenomegaly with cytopenias (79%), splenomegaly without cytopenias (15%) and cytopenias without splenomegaly (6%). This study also showed that ALPS-FAS patients have a high frequency of recurrence of cytopenias (56%) and sepsis (29%) following splenectomy; as 19 out of 34 patients developed or relapsed with multilineage cytopenias of grade 2 or higher requiring further treatment interventions (Figure 5). Eleven patients developed sepsis 8 months to 36 years post-splenectomy resulting in 2 fatalities. Many of them developed multiple septic episodes; one patient presented with 6 episodes of documented pneumococcal sepsis.

ALPS patients may be more vulnerable to sepsis as they lack circulating CD27+ memory B lymphocyte populations. It may account for their inability to mount or sustain protective levels of antibodies directed against pneumococcal polysaccharide antigens following vaccination. Based on our experience, all asplenic ALPS patients should preferably remain on long-term antibiotic prophylaxis against pneumococcal sepsis using penicillin V or fluoroquinolones like Levofoxacin. In addition to advising our asplenic patients to wear Medic alert bracelets; we also educate them and their parents and guardians about the importance of seeking
medical care promptly for a significant febrile illness requiring IV antibiotics until bacterial sepsis is ruled out. Our recommendations for asplenic ALPS patients include lifelong daily antibiotic prophylaxis as well as periodic surveillance and reimmunization against pneumococci using a combination of both 13-valent conjugate (Prevnar-13) and 23-valent polysaccharide (Pneumovax) vaccines every 4-5 years.

Recently there has been an increased awareness of some associated morbidity and mortality including OPSI, upon long-term follow up of patients after splenectomy for other indications as well. Thus, splenectomy should be avoided unless it is the only remaining measure to control chronic, refractory, life-threatening cytopenias in ALPS patients. Even under those circumstances feasibility and efficacy of a partial splenectomy or splenic embolization should be explored.

**Treatment of ALPS associated refractory cytopenias:**

The initial management of patients with ALPS-related autoimmune cytopenias (AIHA, ITP, AIN) is similar to sporadic immune cytopenias in other patient populations. Most recently updated immune thrombocytopenia treatment guidelines and recommendations from the American Society of Hematology (2011) can be broadly applicable to ALPS patients with chronic and persistent thrombocytopenia. While long-term and careful follow-up of patients with chronic multilineage cytopenias after any treatment is necessary to determine relative risks and benefits, in our practice following caveats do apply specifically to ALPS patients (Figure 6):
1. Immune-suppression with corticosteroids: We use high-dose pulse therapy with intravenous (IV) methylprednisolone (5-10mg/kg) followed by low-dose oral prednisone (1-2mg/kg) maintenance therapy that can often be successfully tapered over several weeks (8-12 weeks) as ALPS patients often have chronic and refractory disease. IV Methyl prednisolone at doses as high as 30mg/kg/day X 1-3 days may have to be used in some patients with profoundly refractory cytopenias (e.g. Hemoglobin <5gm requiring intensive care for hypoxia). However one has to be aware of the usual steroid related short and long-term morbidities as Cushingoid body habitus, hypertension, cataracts, hyperglycemia, osteopenia and avascular necrosis of the hip have been noted in some of our patients. This has prompted us to look for steroid sparing measures (see below).

2. Intravenous immunoglobulin G (IVIG) (1-2gm/kg) given concomitantly with pulse dose methylprednisolone may benefit some patients with severe AIHA by abrogating antibody-mediated red cell destruction and allowing packed red blood cell (PRBC) transfusion support for severe anemia. We avoid using WinRho for isolated thrombocytopenia in ALPS patients, as many of them are DAT positive and likely to develop hemolysis.

3. ALPS patients with isolated chronic neutropenia and associated infections can also benefit from twice or thrice weekly, low-dose (1-2 microgram/kg) G-CSF (granulocyte colony stimulating factor) given subcutaneously.

4. Standard dose Rituximab (375mg/M2 /Week X 4) for treatment of refractory, chronic cytopenias in children has been used by others as well as in 12 ALPS patients in our cohort. In seven out of nine patients with ALPS and
thrombocytopenia, rituximab therapy led to median response duration of 21 months (range 14–36 months). In contrast, none of the three children treated with rituximab for AIHA responded. Noted toxicities included profound and prolonged hypogammaglobulinemia in three patients requiring replacement IVIG, total absence of antibody response to polysaccharide vaccines lasting up to 4 years after rituximab infusions in one patient and prolonged neutropenia in one other patient. These toxicities constitute an additional infection risk burden, especially in asplenic individuals, and may warrant avoidance of rituximab in ALPS patients until other immunosuppressive medication options are exhausted.

5. Use of Mycophenolate mofetil (MMF) in 13 children with ALPS, given twice daily orally at a dose of 600 mg/M2/dose for chronic cytopenias, was initially described by us in 2005. This preliminary experience suggested that MMF has spared chronic steroid usage in patients with ALPS-associated cytopenias. ALPS patients, especially those with massive splenomegaly and hypersplenism, can often be refractory to standard dose short-term corticosteroid regimens, IVIG and PRBC transfusions and may require other measures. We have used MMF in 61 ALPS patients over the past 11 years as ongoing long-term steroid sparing immune-suppression for chronic and refractory cytopenias as well as other autoimmune manifestations such as uveitis, glomerulonephritis and pulmonary lesions (Figure 4). Their median age is 10 years (range 6 months-43 years) with a median follow up of 3 years (range 3 months-11 years). Fifty-six patients responded to MMF as defined by maintenance of adequate blood counts and reduction in its dosage or cessation of other immunosuppressive agents; however five of them required other
therapies later on as their cytopenias became more refractory and one of them died due to relapse with a refractory, overwhelming AIHA following a period of response to MMF for 5 years. Though 16 patients had undergone splenectomy prior to initiating MMF; in some patients, MMF has allowed splenectomy to be avoided or postponed until the very young children had aged and would better tolerate surgical asplenia. However it is critical to use MMF only in the context of a steroid sparing measure and not as a first line upfront therapy to treat significant cytopenias.

We usually suggest addition of MMF to the therapy regimen as and when the patients are being treated with corticosteroids for a flare up of their cytopenia (as reflected by a complete blood count with hemoglobin <8gms, Absolute Neutrophil Count <500 cells or Platelets <50,000) while they are being slowly tapered off their corticosteroids over 8-12 week period. MMF should be given concomitantly for at least 2 weeks to reach effective therapeutic drug levels in plasma while the patient is on a tapering schedule of corticosteroids (Figure 6). No evidence of increased infection risk or other significant toxicities has been noted in any of our ALPS patients on chronic MMF therapy over the last 11 years.

6. Rapamycin (Sirolimus), an m-TOR inhibitor has recently been used successfully by Teachey et al as well as others in ALPS patients. Most ALPS patients on rapamycin show a good response and many achieve a normal blood count for the first time in their life since infancy. A second advantage is that rapamycin reduces lymphoproliferation; as the enlarged lymph nodes and spleen often shrink significantly with reduction of the signature DNT cell numbers. However these patients, just like the patients on MMF, need to be maintained on
sirolimus for the long term and monitored for its toxicities diligently. Many ALPS patients requiring sirolimus present with significant lymphoproliferative burden evidenced by massive splenomegaly and adenopathy. Their cytopenias should be refractory to upfront corticosteroid therapy and/or steroid sparing measures including MMF. After the initial loading dose of 3mg/M2, patients should take sirolimus orally once daily at 2.5 mg/M2/day (up to a maximum daily dose of 4mg), to achieve a target 24-hour trough drug level of 5-15 ng/mL. Levels of sirolimus should be drawn at least twice per week until steady state is reached, then weekly or monthly thereafter. Children less than 15 years of age may metabolize sirolimus more briskly than adults and may need twice daily dosing to achieve targeted trough levels. However ensuring adequate kidney and liver function while monitoring for toxic side effects of sirolimus including T cell immunosuppression, hypercholesterolemia and stomatitis etc is imperative through interval clinical evaluations, measurement of blood drug levels and dose adjustment.

Before commencing medications like MMF or Sirolimus, their risk benefit ratio should be taken into consideration for an individual ALPS patient, as they often may have to be exposed to long-term ongoing immunosuppression to remain free from their refractory cytopenias. There have been reports of successful clinical use of other agents including hydroxychloroquine, dapsone, azathioprine and 6-mercaptopurine for relief of chronic cytopenias in children and adults and they should also be considered as a viable option in some patients with ALPS74,75.
Role of Hematopoietic Stem Cell Transplantation (HSCT) in ALPS:

Both short and long prognosis of most ALPS patients appears to be good. In our entire cohort consisting of 257 individuals, only 13 ALPS patients have died, 8 due to postsplenectomy sepsis, 1 due to overwhelming hemolytic anemia and toxicities of its treatment and 4 due to progressive malignancy. The chronic cytopenias seen in many improve with age and many of them continue responding to conventional short-term immunosuppressive treatment. Thus, there is no need to entertain allogenic HSCT in the vast majority of patients with ALPS. However, HSCT has been reportedly successful in selected ALPS patients with lymphoma, polyarteritis nodosa or very severe phenotype due to homozygous FAS mutation and refractory cytopenias. A few other ALPS patients have been transplanted with variable results, most failures being due to progression of their malignancy, ineffective engraftment due to nonmyeloablative conditioning and opportunistic infections. Of course, a sibling who carries the same apoptosis pathway mutation as the proband is not an appropriate marrow donor, even if asymptomatic.

Additionally, mortality due to matched unrelated donor (MUD) derived HSCT is too high (~35%) to justify this procedure for the vast majority of patients with a chronic disease like ALPS in which a near normal lifespan can otherwise be expected. Though most ALPS patients get better as they grow older, some of them do require chronic long-term immunosuppression, most often for multilineage cytopenias and other end organ damage due to lymphoproliferation or its therapy. If a patient’s ALPS associated lymphoproliferative disorder is likely to require life-long potent
immunosuppression that in itself is likely to lead to more morbidity to end organs like liver, kidney and lungs. HSCT may have a role in selected ALPS patients with a more severe phenotype as they may benefit from a life long respite from ALPS, its complications and their therapies\textsuperscript{47,79}.

**Implications of ALPS and Future Directions:**

The major determinants of morbidity and mortality in ALPS depend on the severity of the autoimmune disease; hypersplenism and asplenia related sepsis, as well as development of lymphoma (Table 4). Patients with mutations abrogating function of the intracellular domain of the FAS protein are at risk of developing lymphomas and they need diligent life-long follow up. It is necessary to understand the natural history of this otherwise rare disorder to prognosticate which of these patients might benefit from early interventions like use of mycophenolate mofetil, sirolimus or stem cell transplantation while avoiding a splenectomy and make risk adaptive recommendations. This decision has to be based on their underlying disease phenotype and assessment of the risk benefit ratio of any proposed treatment regimen. Role of thrombopoietin mimetic agents and histone deacetylase inhibitors in managing ALPS associated recalcitrant cytopenias and hypersplenism need further exploration. Novel and nontoxic lympholytic therapies are necessary to control the lymphoproliferative process in children with ALPS who have chronic lymphadenopathy and splenomegaly. They also require long term monitoring and collaborative follow-up by multiple sub-specialists that are familiar with ALPS.
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Authorship:

Contributions: V.K.R. participated in clinical care and wrote the manuscript; J.B.O provided laboratory diagnostic work up, reviewed and edited the manuscript.

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References:


Table 1:

Clinical Features of ALPS

<table>
<thead>
<tr>
<th>Feature</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphadenopathy</td>
<td>96%</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>95%</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>72%</td>
</tr>
<tr>
<td>Splenectomy</td>
<td>49%</td>
</tr>
<tr>
<td>AIHA</td>
<td>29%</td>
</tr>
<tr>
<td>ITP</td>
<td>23%</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>19%</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>1%</td>
</tr>
<tr>
<td>Liver Dysfunction</td>
<td>5%</td>
</tr>
<tr>
<td>Infiltrative Lung Lesions</td>
<td>4%</td>
</tr>
<tr>
<td>Eye lesions</td>
<td>0.7%</td>
</tr>
</tbody>
</table>
Table 2: Revised Diagnostic Criteria for ALPS Based on First International ALPS Work Shop 2009.36

Required Criteria

1. Chronic (>6 months), non-malignant, non-infectious lymphadenopathy and/or splenomegaly.
2. Elevated CD3⁺ TCRαβ⁺CD4⁺CD8⁺ DNT cells (>1.5% of total lymphocytes or >2.5% of CD3⁺ lymphocytes) in the setting of normal or elevated lymphocyte counts

Additional Criteria

Primary

1. Defective lymphocyte apoptosis in two separate assays
2. Somatic or germline pathogenic mutation in FAS, FASLG or CASP10;

Secondary

3. Elevated plasma sFASL levels (>200 pg/ml), plasma IL-10 levels (>20 pg/ml), serum or plasma Vitamin B12 levels (>1500 ng/L) OR plasma IL-18 levels above 500 pg/ml;
4. Typical immuno-histological findings as reviewed by a hematopathologist
5. Autoimmune cytopenias (hemolytic anemia, thrombocytopenia or neutropenia) with elevated IgG levels (polyclonal hypergammaglobulinemia);
6. Family history of a non-malignant/non-infectious lymphoproliferation with or without autoimmunity.

Definitive diagnosis: Both required criteria plus one primary accessory criterion.
Probable diagnosis: Both required criteria plus one secondary accessory criterion.
Table 3: ALPS Classification and distribution of different categories of patients seen and evaluated at NIH Clinical Center as part of our current cohort.

<table>
<thead>
<tr>
<th>ALPS Classification</th>
<th>Chronic LPD/ splenomegaly</th>
<th>Elevated alpha/beta DNT’s</th>
<th>Apoptosis defect</th>
<th>% ALPS Cases (N = 257)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALPS-FAS (Germline mutation)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>72% (185)</td>
</tr>
<tr>
<td>ALPS-sFAS (Somatic mutation)</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>0.5% (14)</td>
</tr>
<tr>
<td>ALPS-FASLG</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>&lt;1% (2)</td>
</tr>
<tr>
<td>ALPS-CASP10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>&lt;1% (4)</td>
</tr>
<tr>
<td>ALPS-U</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>20% (52)</td>
</tr>
</tbody>
</table>

**ALPS Related Apoptosis Disorders**

| CASPASE 8 Deficiency State | + | +/- | +/- | 2 |
| RALD (Somatic NRAS and KRAS mutations) | + | +/- | +/- | 6 |

RALD: RAS associated autoimmune leukoproliferative disorder.
Table 4:

Salient Aspects of Management and Prognosis of ALPS

- Diagnosis and genetic counseling at presentation
- Significant number of ALPS patients do not need any intervention for asymptomatic lymphadenopathy and splenomegaly that often seems to get better with age.
- Use of spleen guards made of thermo-plastic material for protecting enlarged spleens from trauma
- Avoid splenectomy.
- For unavoidable surgical asplenia: Use 13-valent conjugate and 23-valent polysaccharide vaccines against pneumococcal sepsis
- Med alert bracelet, fever alert and long term antibiotic prophylaxis
- Autoimmune Cytopenias: short-term steroids and IVIG
- Steroid-sparing measures: mycophenolate mofetil and sirolimus
- Vigilance for lymphoma: Role of periodic CT and FDG-PET scans.
- Deaths (13/357 patients) in our cohort:
  - Death due to sepsis with asplenia (9), malignancies (4)
Figure Legends:

**Figure 1:** Schematic diagram of current understanding of extrinsic and intrinsic apoptosis pathways. The prototypical receptor of the extrinsic pathway is FAS. It recruits the adaptor FADD and the procaspases 8 and 10 upon ligation. The caspases are then cleaved in order to further activate other downstream caspases, leading to cell death. The intrinsic pathway is controlled by proteins of the BCL2 family, and triggered by stimuli such as DNA damage and growth factor withdrawal. These stimuli ultimately lead to activation of caspase 9 and downstream effector caspases. There is a crosstalk between the two pathways in some cell types.

**Figure 2:** Signature Biomarkers of ALPS-FAS. Biomarkers are very useful to predict the presence of FAS mutations in patients with features of ALPS\(^3\). The increasing (A) or decreasing (B) post-test probabilities of having a FAS mutation in patients with different combinations of biomarkers are shown.

**Figure 3:** CT and FDG-PET scans featuring ALPS-FAS associated lymphadenopathy and splenomegaly. Patient 230 is a 10 year old female, with asymptomatic adenopathy and splenomegaly. Patient 232 is a 22 year old male, with asymptomatic and visible cervical and axillary lymphadenopathy and modest splenomegaly. No intervention was indicated in both patients. Note increased uptake in the spleen as a reflection of lymphoproliferation compared to liver in both patients.

**Figure 4:** More illustrative examples of FDG-PET and CT scan appearances of some ALPS-FAS patients showing splenomegaly and lymphadenopathy: Patient 004 (25 year old male who presented with fever, mouth ulcers and neutropenia; suspected lymphoma was ruled out following these scans and biopsy of cervical lymph node,
patient underwent splenectomy for persistent cytopenias); patient 072 (12 year old asymptomtatic male with cervical and axillary adenopathy); patient 161 (9 year old male treated with MMF for chronic cytopenias for the last 8 years, 3D CT reconstruction is showing enlarged spleen with volume measured at 1972 cm$^3$) and patient 317 (22 year old male with splenomegaly, CT scan is showing a spleen spanning 27 cm). Bottom panel is showing the Chest CT scan appearance in a 19 year old male (ALPS patient 109) with otherwise asymptomatic nodular lymphocytic pulmonary infiltrates. This patient has also been on therapy for his chronic cytopenias with MMF for the last 7 years.

**Figure 5:** Causes and consequences of splenectomy in a subset of 34 ALPS-FAS patients who have undergone long term follow up in our clinic. Note that more than half of them have relapsed with multilineage cytopenias following splenectomy requiring further therapeutic interventions, while a third of them have had septic episodes.

**Figure 6:** Management suggestions for ALPS associated chronic refractory cytopenias. This schematic diagram is included only as a suggested guideline for managing children with ALPS associated autoimmune multilineage cytopenias. Use of G-CSF may be warranted for severe neutropenia associated with systemic infections. Similarly use of other chemotherapeutic and immunosuppressive agents besides mycophenolate mofetil (MMF) and sirolimus (Rapamycin) (e.g. hydroxychloroquine, methotrexate, mercaptopurine, vincristine, azathioprine, cyclosporine,) can also be considered as a steroid sparing measure; or used while
avoiding or postponing surgical splenectomy at the discretion of the treating clinicians based on the circumstances of a specific patient.
Figures

Apoptosis Pathways

Extrinsic

FAS Ligand
FAS
FADD
Procaspses 8/10
Active caspases

Intrinsic

Growth factor withdrawal
Chemotherapy
DNA damage: x-ray

BCL2 family

Effector Caspases (3/7)

APOPTOSIS

Figure 1
Figure 2
Figures 3 and 4
Causes and consequences of splenectomy in ALPS (N=34)

Figure 5
Management Suggestions for ALPS Associated Chronic Refractory Cytopenia

**Initial treatment**
- oral prednisone (1-2mg/kg/day) for 1 week, then taper slowly over 8-12 weeks.
- +/- IVIG

---

**No Response:**
- IV methylprednisolone (5-10mg/kg/day) X 3 days +/- IVIG
dose based on clinical severity of cytopenia
Followed by oral prednisone (2mg/kg/day) tapered slowly over 8-12 weeks

---

**Initial Response with breakthrough cytopenia during corticosteroid tapering**
- (Hg <8, ANC <500, Platelets <50,000):
- Increase dose of oral prednisone to 1-2mg/kg/day and commence slow taper again over 8-12 weeks
- Add oral mycophenolate mofetil (MMF)
  - 1200mg/M2/day divided twice daily
  - Plan to continue MMF long term

---

**Response to MMF with breakthrough cytopenia**
- Consider short term pulse dose of corticosteroid
  - (oral prednisone 1-2mg/kg/day or higher equivalent dose up to 5mg/kg/day based on clinical severity of cytopenia)
- Continue MMF

---

**Lack of sustained response to MMF**
- Consider rituximab or other chemotherapeutic and immunosuppressive agents

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**No Response:**
- Consider rituximab

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**No Response: Assess for Splenectomy**

**If hypersplenism is a major contributor to cytopenia**

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Figure 6
How I treat autoimmune lymphoproliferative syndrome

V. Koneti Rao and João Bosco Oliveira