AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME: NOVEL INSIGHTS INTO PATHOPHYSIOLOGY AND TREATMENT

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Promotor: Prof. Dr. Geneviève Laureys
Co-promotor: Dr. Victoria Bordon Cueto de Braem

Dissertation presented in the 2nd Master year in the programme of

Master of Medicine in Medicine
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Prof. Dr. Geneviève Laureys
ABSTRACT

Background  Autoimmune lymphoproliferative syndrome (ALPS) is an inherited disorder of abnormal lymphocyte survival due to defective Fas-mediated apoptosis. This causes autoreactive “double negative TCR αβ⁺ CD3⁺ CD4⁻ CD8⁻ T cells” (DNTs) to accumulate in the circulation and in lymphoid tissues. The majority of patients with ALPS carry an underlying genetic mutation, located in genes associated with the Fas apoptotic pathway. ALPS usually arises in early childhood. Clinically patients present with chronic lymphadenopathies, hepatosplenomegaly and/or autoimmune cytophenias. Patients also have an increased risk of malignancy.

Over the last decades significant progress has been made in understanding the pathophysiology of ALPS and in developing new diagnostic criteria and therapeutic agents.

Objective  ALPS is a relatively rare disorder. Worldwide, estimated cases of ALPS exceed 500, but that number has not reliably been confirmed. However, ALPS could be more common than originally thought because of low awareness in clinical practice or misdiagnosis. Diagnosis of ALPS can be a challenge partially because of its variable phenotype and laboratory findings which are unspecific and can fluctuate over time. Furthermore, recent studies have shown that some therapeutic agents commonly used for other refractory non-ALPS autoimmune cytophenias are associated with significant morbidity and mortality in ALPS. Therefore, making the correct diagnosis is important.

The aim of this study was to describe the variable phenotype in a group of patients diagnosed with ALPS in order to raise clinical awareness. Moreover, the study aims to contribute to making the correct diagnosis and choosing the most appropriate treatment option.

Additionally, the effect of treatment with Fansidar (25 mg pyrimethamine/ 500 mg sulphadoxine) on clinical outcome and laboratory results in the patient cohort is described retrospectively. In 1998, Van der Werff et al. first reported normalisation of clinical and laboratory features upon the administration of Fansidar. Since then, Fansidar has been consistently used as a first-line treatment for lymphoproliferative and autoimmune manifestations associated with ALPS in some Belgian centres.

Materials and methods  The study population consisted of 12 patients diagnosed with ALPS and followed at UZ Ghent and QFCUH in Brussels. Informed consent was obtained for all patients or their legal guardians.
UZ Ghent’s Ethics Committee approval was obtained for the retrospective data collection in medical records ranging from 1998 until present. Data on patients’ clinical, immunological and laboratory features, relevant for the diagnosis and characterisation of ALPS was collected. Furthermore, patients’ clinical outcome upon treatment was analyzed. Existing literature in patients with ALPS was reviewed. These results were compared retrospectively with our patient cohort for similarities and differences.

**Results** All patients included in the study (n=12) displayed chronic non-malignant non-infectious lymphoproliferation, autoimmune manifestations and elevated TCR αβ⁺ DNT levels (≥ 1.5% of total lymphocytes or ≥ 2.5% of CD3⁺ lymphocytes) at initial presentation. No patient has developed malignancy to date. Family history was positive in 8 out of 12 patients.

IL-10, IL-18, sFasL and vitamin B12 are biomarkers for ALPS which were recently incorporated in the diagnostic criteria. These biomarkers were inconsistently assessed in the study population described herein. Eleven out of 12 patients were treated with Fansidar as a first-line treatment or in the course of the disease. Treatment with Fansidar led to a complete or incomplete remission of autoimmune cytopenias in one-third of all patients (3/11). Upon treatment with Fansidar, TCR αβ⁺ DNT levels improved completely or significantly in 5 out of 11 patients.

**Conclusion** Clinical awareness is needed in any child with a combination of unexplained lymphoproliferation and/or autoimmune manifestations and positive family history of autoimmune cytopenias with or without non-malignant non-infectious lymphoproliferation dating back to infancy. A clinical practice algorithm is proposed providing the subsequent laboratory tests to diagnose and confirm ALPS. It is recommended that in the future IL-10, IL-18, sFasL and vitamin B12 are included in the standard diagnostic work-up for ALPS. Larger prospective cohorts or RCTs are needed to determine whether the effects seen upon treatment with Fansidar are statistically significant. These studies should preferably include data on sFasL, IL-10, IL-18 and/or vitamin B12. Since Fansidar has been effective in the treatment of autoimmune cytopenias in some patients and is not associated with severe toxicities, it could be attempted as a first-line treatment in patients with ALPS. Furthermore, close clinical follow-up, genetic counselling for the recurrence risk in descendants and patient education of constitutional symptoms for the life-long risk of malignancy are important in patients with ALPS.
SAMENVATTING

Achtergrond Auto-immuun lymfoproliferatief syndroom (ALPS) is een erfelijke aandoening met een abnormale overleving van lymfocyten als gevolg van een defect in Fas-gemedieerde apoptose. Hierdoor accumuleren “dubbel negatieve TCR αβ⁺ CD3⁺ CD4⁻ CD8⁻ T cellen” (DNTs) in de perifere circulatie en in de lymfoïde weefsels. Het merendeel van de patiënten met ALPS heeft een onderliggende genetische mutatie, gelegen in genen die coderen voor Fas-gemedieerde apoptose.


In de laatste decennia is er significante vooruitgang geboekt in inzichten in de pathofysiologie van ALPS en in het ontwikkelen van nieuwe diagnostische criteria en behandelingsmogelijkheden.

Doelstelling ALPS is een relatief zeldzame aandoening. Wereldwijd wordt het aantal patiënten op meer dan 500 geschat, maar dit aantal is nooit betrouwbaar bevestigd. ALPS zou echter meer prevalent kunnen zijn dan oorspronkelijk gedacht, door een gebrek aan kennis in de klinische praktijk of een gemiste diagnose. ALPS heeft een variabel klinisch fenotype en laboratoriumparameters die niet specifiek zijn en kunnen veranderen na verloop van tijd. Diagnose kan dus moeilijk zijn. Daarnaast hebben recente studies aangetoond dat sommige geneesmiddelen die frequent gebruikt worden in de behandeling van refractaire auto-immune cytopenieën bij andere ziektebeelden, geassocieerd zijn met een significante morbiditeit en mortaliteit in ALPS. Daarom is het belangrijk om de correcte diagnose te stellen.

Het doel van deze studie is het beschrijven van het variabel fenotype in een groep patiënten met ALPS om te zorgen voor meer klinische bewustmaking. Daarnaast tracht de studie bij te dragen tot het stellen van de juiste diagnose en het kiezen van de meest geschikte behandelingsoptie.
Bijkomend wordt het effect van behandeling met Fansidar (25 mg pyrimethamine/ 500 mg sulphadoxine) op de klinische outcome en laboratoriumresultaten van de patiëntencohorte retrospectief beschreven. Van der Werff et al. beschreven als eerste in 1998 de normalisatie van klinische en laboratorium parameters na behandeling met Fansidar. Fansidar werd sindsdien systematisch gebruikt in sommige Belgische centra als eerstelijnsbehandeling voor lymfoproliferatieve en auto-immune manifestaties in ALPS.


Resultaten Alle patiënten (n=12) in deze studiepopulatie vertoonden chronische non-maligne, non-infectieuze lymfeproliferatie, auto-immune manifestaties en een verhoogd aantal TCR αβ⁺ DNTs (≥ 1.5% van het totale aantal lymfocyten of ≥ 2.5% van het aantal CD3⁺ lymfocyten) bij de eerste presentatie. Maligniteiten werden in geen enkele patiënt vastgesteld. Familiale anamnese was positief in 8 van de 12 patiënten.

IL-10, IL-18, sFasL en vitamine B12 zijn biomarkers voor ALPS die recent opgenomen zijn in de diagnostische criteria. Deze biomarkers zijn niet consistent bepaald in de studiepopulatie die hierin beschreven is.

Fansidar werd toegediend als eerstelijnsbehandeling of in de loop van het ziekteproces in 11 van de 12 patiënten. Behandeling met Fansidar leidde tot een complete of incomplete regressie van auto-immune cytopenieën in een derde van alle patiënten (3/11). TCR αβ⁺ DNT waarden normaliseerden of verbeterden significant in 5 van de 11 patiënten na behandeling met Fansidar.
**Conclusie**  Klinische alertheid is nodig in elk kind met een combinatie van onverklaarbare lymfeproliferatie en/of auto-immune manifestaties en een positieve familiale anamnese van auto-immune cytopenieën met of zonder non-maligne non-infectieuze lymfeproliferatie teruggaand tot de kindertijd. Een algoritme voor klinische praktijk wordt voorgesteld waarin de bijhorende laboratorium testen voor de diagnose en bevestiging van ALPS beschreven worden. In de toekomst is het aanbevolen dat IL-10, IL-18, sFasL en vitamine B12 opgenomen worden in het standaard diagnostisch algoritme van ALPS. Grotere prospectieve cohorten of RCTs zijn nodig om te bepalen of de effecten van behandeling met Fansidar statistisch significant zijn. Deze studies bevatten bij voorkeur gegevens over sFasL, IL-10, IL-18 en/of vitamine B12. Aangezien behandeling met Fansidar effectief is gebleken in de behandeling van auto-immune cytopenieën in patiënten met ALPS en geen ernstige bijwerkingen beschreven zijn, kan geprobeerd worden Fansidar als eerstelijnsbehandeling in patiënten met ALPS te gebruiken. Tot slot is nauwgezette klinische opvolging, genetisch advies voor het herhalingsrisico in nakomelingen en patiënteneducatie over constitutionele symptomen gezien het levenslange risico op maligniteiten belangrijk in patiënten met ALPS.
INTRODUCTION

1. Background

Autoimmune lymphoproliferative syndrome (ALPS) is an inherited autoimmune disorder that usually arises in early childhood. It is a disorder of defective Fas-mediated T-lymphocyte apoptosis, causing autoreactive “double negative TCR αβ⁺ CD3⁺ CD4⁻ CD8⁻ T cells” (DNTs) to accumulate in the circulation and in lymphoid tissues. In healthy individuals, these TCR αβ⁺ DNTs usually constitute of less than 1% of total lymphocytes. The clinical features are consistent with normal lymphocyte production but abnormal survival and accumulation of lymphocytes. This results in a chronic, nonmalignant lymphadenopathy, hepatosplenomegaly and autoimmune manifestations. ALPS is currently defined as chronic, nonmalignant lymphoproliferation in patients with an elevated percentage of TCR αβ+ DNTs (Table 2).

ALPS usually first manifests in childhood but clinical symptoms and lymphadenopathy in particular disappear with age (1). However, elevated levels of TCR αβ⁺ DNTs and hypergammaglobulinemia persist and autoimmune cytopenias follow a relapsing course (2). The major determinants of morbidity and mortality in ALPS are the severe autoimmune disease, postsplenectomy sepsis and malignancy (3). Patients have a life-long risk of developing autoimmune disease and malignancies and require life-long follow-up (3).

The syndrome was first described by Canale and Smith in 1967 as a symptom-complex simulating malignant lymphoma (4). It is sometimes referred to as the Canale-Smith syndrome. Several patients displaying lymphadenopathy, splenomegaly and autoimmune cytopenia were described. The same features were also found in several relatives, supporting the hypothesis that the disorder was caused by a gene mutation.

The essential role of Fas in apoptosis was clarified by studies in MRL lpr/gld mice. In 1992 Watanebe-Fukunga et al. found that these mice failed to express a lymphocyte surface antigen, Fas (also called CD95 or Apo1) (5). This antigen is necessary for triggering apoptosis on lymphocytes, as reported by Trauth et al. in 1989 (6). Genetic studies have shown that the MRL/lpr phenotype results from homozygous mutations in the Fas gene (5). The MRL/gld phenotype is caused by mutations in the gene encoding Fas ligand (7). Clinically the mice present with elevated levels of peripheral TCR αβ⁺ DNTs, lymphoproliferation, hypergammaglobulinemia, glomerulonephritis and autoantibody production (5).
Various authors later described several patients as the human equivalent of these murine models with clinical features, elevated TCR αβ⁺ DNTs, defective apoptosis and specific Fas mutations (8, 9). This clinical syndrome was subsequently termed the autoimmune lymphoproliferative syndrome (ALPS).

In healthy individuals, Fas-induced apoptosis of activated peripheral T cells maintains T cell homeostasis by limiting lymphocyte accumulation during the termination phase of an immune response (3). Moreover apoptosis is critical to minimize lymphocyte autoimmune reactions against self-antigens and maintain peripheral immune tolerance (10).

Apoptosis is triggered by the interaction between the Fas receptor, a cell surface antigen and Fas ligand (FasL) (Figure 1). Upon activation and clonal expansion, T and B cells increase Fas expression and T cells increase expression of FasL (11).

The interaction between the extracellular domain of Fas and FasL causes a trimerization of Fas. This leads to the interaction the Fas-death domain (DD), which is a portion of the intracellular domain of Fas with the Fas-associated death domain protein (FADD). FADD then binds a cysteine protease caspase-8 (or sometimes caspase-10) (12). Together they form the death-inducing signalling complex (DISC). This initiates a downstream cleaving and activation of proteins, leading to proteolysis, DNA degradation and apoptosis.

There are three, partly interconnected pathways through which the caspase cascade can lead to apoptosis. The extrinsic or death receptor pathway is directly activated by a death receptor, such as Fas and leads to caspase-8 receptor-ligand interactions, which requires aggregation of the DISC.

The intrinsic or mitochondrial pathway is activated by several non-receptor mediated stress stimuli (e.g. radiation, toxins, hypoxia). Subsequent mitochondrial-initiated release of cytochrome C leads to activation of caspase-9 (13). Both pathways converge into the same effector caspases, such as caspase-3. Evidence exists that molecules in one pathway can influence the other and that the two pathways are linked (14). Moreover, The perforin/granzym pathway is an additional pathway, which can activate caspase-10 through Granzyme B or trigger an apoptose-independent pathway through Granzyme A (14). Interestingly, activation of the intrinsic pathway can be amplified by the cleavage of Bid by caspase-8 and further activation of T-Bid by Granzyme B.
Figure 1: Schematic representation of apoptotic signalling pathways. The intrinsic pathway is triggered by several stress stimuli. Subsequent mitochondrial-initiated release of cytochrome C leads to activation of caspase-9. The extrinsic pathway is triggered by the interaction of FasL and Fas and leads to the activation of caspase-8. The perforin/granzyme pathway is an additional pathway, which can activate caspase-10 through Granzyme B or trigger an apoptosis-independent pathway through Granzyme A. Activation of the intrinsic pathway can be amplified by the cleavage of Bid by caspase-8 and further activation of T-Bid by Granzyme B.

Each pathway activates its own initiator caspase (8, 9 and 10), which in turn will activate the effector caspase-3 and trigger apoptosis.

2. Pathophysiology

2.1. Genetics

The majority of patients with ALPS carry an identifiable genetic mutation, located in genes associated with the Fas apoptotic pathway. Based on these findings 5 different ALPS types have been identified (Table 3). Patients with ALPS-FAS have germline mutations in the Tumor Necrosis Factor Receptor Superfamily member 6 (TNFRSF6) gene, encoding for Fas. This gene is located on chromosome 10. The TNFRSF6 gene consists of 9 exons (15). Exons 1-5 encode the extracellular domain, responsible for binding FasL. Exon 6 encodes the transmembrane domain of Fas. Exons 7-9 encode the intracellular portion, which includes the Fas-death domain (DD), encoded by exon 9. Over 80% of patients with ALPS have mutations in the intracellular portion and the DD in particular (16).
Mutations can be either homozygous or heterozygous, presenting with a variable clinical penetrance. Patients with heterozygous TNFRSF6 mutations represent the majority of patients with ALPS. Homozygous TNFRSF6 mutations are far less frequent, but the phenotype in these patients is more severe, with an early-onset (9).

Patients with ALPS-sFAS carry somatic TNFRSF6 mutations in the TCR αβ+ DNTs and represent the second most common genetic mutations found in patients with ALPS (17). Patients with ALPS-FASLG and FAS-CASP10 carry germline mutations in genes encoding Fas ligand and caspase 10 respectively. A relatively large proportion of patients (20-30%) have an unknown genetic defect and are classified as ALPS-U (18).

**Penetrance**

The relationship between genotype, phenotype and penetrance is complex. A high degree of variability in clinical phenotype is seen in patients with ALPS and their relatives. The expression greatly depends on the genetic background. In patients carrying a heterozygous TNFRSF6 gene mutation, 70% develop clinical features (2). The specific location of mutations on the TNFRSF6 gene is associated with differences in severity and penetrance (19). Mutations affecting the intracellular Fas-death domain show a higher penetrance of ALPS clinical phenotype. Significant ALPS-related morbidity (splenectomy, autoimmune disease that requires treatment, lymphoma) is more frequently seen in relatives with intracellular mutations (19).

Recent findings suggest different molecular disease mechanisms of mutations in the intra- and extracellular domain (20). Heterozygous, missense mutations in the intracellular portion and the Fas-death domain exhibit dominant-negative interference of the signalling pathway (9, 19). The mutated Fas allele inhibits the function of wild-type allele causing an absence of Fas function, inability to bind FADD and form the DISC. Because Fas and FasL form homotrimers, the combination of a mutant and a non-affected allele results in only one out of eight configurations in a normal Fas trimer.

Mutations in the extracellular portion are thought to impair apoptosis through haploinsufficiency (20, 21). Haploinsufficiency was found to prevent the surface expression of Fas below a threshold that is needed for formation of the DISC and subsequently trigger effective apoptosis (21).
Another group of missense and frame-shift mutations retain normal Fas surface expression but are unable to bind FasL. Apoptosis is impaired to a lesser extent because the wild-type allele will allow for expression of normal Fas on the cell surface and is not inhibited by the mutated allele (20). This could account for the lower disease severity and penetrance seen in families with extracellular mutations (19, 22). Furthermore these patients were found to be significantly older at disease onset than other ALPS-FAS or ALPS-sFAS patients (1).

Moreover, in 7 patient carrying a germline TNFRSF6 mutation, a complementary somatic mutation in the second TNFRSF6 allele was found (23). These somatic mutations were all found in patients carrying germline heterozygous mutations affecting the extracellular portion of Fas. In patients carrying mutations in the intracellular portion complementary somatic mutations were not found. Complementary somatic mutations are therefore not compulsory for developing clinical disease. However, the additional effect of the somatic mutation may account for the higher clinical penetrance seen in patients carrying both mutations in the extracellular portion, as opposed to healthy relatives carrying only the germline mutation.

These findings support the “2-hit” hypothesis, with the second hit being acquired later in life and may validate for the prolonged time to clinical manifestations seen in patients with extracellular mutations.

However, a mutation in the TNFRSF6 gene cannot alone predict the degree of penetrance, other complementary genetic or environmental factors are be required (19).

**Somatic mutations**

Somatic TNFRSF6 mutations are suggested to be the second largest group of known mutations in ALPS, affecting approximately 10% of patients (17). Patients have been described with clinical features identical to ALPS and elevated levels of TCR αβ⁺ DNTs but with normal levels of Fas-mediated apoptosis and no family history of ALPS (24). These patients were found to have heterozygous, somatic mutations in the TNFRSF6 gene limited to the TCR αβ⁺ DNT compartment.

Similar to germline TNFRSF6 mutations, somatic TNFRSF6 mutations exert a dominant negative effect on the Fas signalling pathway (24).
Since somatic TNFRSF6 mutations give rise to clinical and laboratory features which are indistinguishable from germline TNFRSF6 mutations, sequencing of the TNFRSF6 gene in DNA obtained from TCR αβ⁺ DNT cells and control cells could help set the diagnosis (17, 25). Furthermore, some patients with ALPS-U could possibly be identified as carrying somatic TNFRSF6 mutations.

2.2. Perforin/granzyme apoptotic pathway

The predominant method through which cytotoxic T cells (CTLs) induce apoptosis is the Fas/FasL interaction (14). However, an additional cytotoxic T cell-mediated pathway exists that involves secretion of perforin, a pore-forming molecule that is stored in granules of cytotoxic T cells. Perforin forms pores in the target-cell membrane through which granzymes are introduced and apoptosis is induced in the target cell (26-28). Granzyme A induces apoptosis through a caspase-independent apoptotic pathway, but Granzyme B can activate the effector caspase-3 through activation of caspase-10.

It seems that the role of the perforin/granzyme pathway and the Fas/FasL pathway in regulation of peripheral tolerance is overlapping but distinct (29).

Mateo et al. proved that despite resistance to Fas-mediated cell death, Fas-deficient T cells could still undergo apoptosis after repeated T cell receptor (TCR) stimulation (30). Peripheral activated T cells were found to significantly overexpress Granzyme A and Granzyme B in comparison to matched controls. These findings indicate that the perforin/granzyme pathway may partially compensate for Fas deficiency in T lymphocytes in ALPS patients.

However, apoptosis was equivalently detected in both symptomatic patients and their asymptomatic relatives carrying the TNFRSF6 mutations. Compensation by the perforin/granzyme pathway could therefore not account for the variable penetrance seen in patients with ALPS (30).

Nevertheless, other studies found patients with an additional heterozygous mutation in the Prf1 gene together with a heterozygous mutation in the TNFRSF6 gene (26, 31). Both genes are located on chromosome 10 (9).

This combination may lead to more severe and accelerated autoimmune disease, increase susceptibility for ALPS and thus influence disease expression. However it may not be sufficient for the development of ALPS, since healthy relatives carrying both of these mutations have been described (32).
3. Clinical manifestations

Typically ALPS presents in 3 phases: massive lymphoproliferation, autoimmune manifestations and malignancy (33).

3.1. Lymphoproliferation

All patients with ALPS present with chronic nonmalignant lymphadenopathy and/or splenomegaly. This is defined as an enlargement of the lymph nodes and/or spleen for more than 6 months. Some patients also present with hepatomegaly. The degree of lymphadenopathy is variable. Most patients present with multiple lymph nodes larger than 2 cm. Lymhadenopathy often involves the cervical and axillary chains but other anatomic locations can also be involved. Lymphadenopathy usually regresses with age. This is less often the case for splenomegaly.

3.2. Autoimmunity

Autoimmune manifestations or circulating autoantibodies are found in the majority of patients with ALPS (3). Autoimmunity in patients with ALPS displays a typical pattern of exacerbations and remissions or tends to become more severe with age. Autoimmune haemolytic anaemia (AIHA) (<2 years of age: Hb <9.8 g/dl and >2 years of age: Hb <11 g/dl) and autoimmune thrombocytopenia (platelet count < 150 x 10^9/µL) are most frequently found. Neutropenia (Absolute neutrophil count < 1500 / µL) is also seen in ALPS but less frequently. Cytopenias in ALPS can be severe, difficult to treat and even life threatening.

The most commonly found autoantibodies are directed against red blood cells and are positive on the direct antiglobulin test (DAT or direct Coombs test). Moreover, the majority of unaffected relatives do not have a positive direct Coombs test, indicating that RBC autoantibodies can be a useful serologic marker in diagnosing ALPS (34). Platelet antibodies (PA-Ab), human neutrophil antibodies (HNA), and antinucleair antibodies (ANA) are also commonly positive (35, 36). However no association between the detection of neutrophil or platelet antibodies and a clinical history of neutropenia or thrombocytopenia respectively was found (36). This indicates that other, additional factors contribute to the development of cytopenias in ALPS. For instance, cytopenias can be exacerbated by splenic sequestration and hypersplenism (37).
Furthermore, patients can theoretically but infrequently develop autoimmune manifestations in any organ system, such as recurrent urticarial rashes consistent with autoimmune vasculitis, arthritis or uveitis. Patients with glomerulonephritis and Guillain-Barré syndrome have also been described (38). These clinical features could be due to an immune hyperreactivity and deposition of antigen-autoantibody complexes (9).

3.3. Malignancy

It is estimated that 10 to 20% of patients with germline TNFRSF6 mutations develop malignancies, most commonly lymphoma (39). Moreover, an increased risk of malignancy has been reported in asymptomatic relatives of patients with ALPS (40). The average age of lymphoma diagnosis was 28, whereas the average age of ALPS onset was 5 years (40). Long-term surveillance for malignancy in patients with ALPS and their relatives is therefore important.

A significant feature of lymphoma in ALPS is its diversity: B cell and T cell lymphomas of diverse types have been found in multiple ALPS kindreds (40). Mutations affecting the intracellular Fas-death domain (DD) are associated with the highest risk of developing lymphomas (40, 41). Abnormal and prolonged lymphocyte survival could allow additional oncogenic mutations to accumulate and lead to secondary malignancies (42).

Furthermore, Gronbaek et al. found somatic TNFRSF6 gene mutations in 11% (16/150) of sporadic B cell and T cell Non-Hodgkin lymphomas (42). These findings suggest that the TNFRSF6 gene may function as a tumour-suppressor gene (43). Both germline and somatic TNFRSF6 mutations and subsequent loss of tumour-suppressor function may therefore predispose to the development of lymphomas in ALPS (41).
4. Laboratory findings

The most common laboratory findings in ALPS are increased levels of circulating TCR αβ⁺ DNTs and polyclonal hypergammaglobulinemia (IgG, sometimes associated with IgA). Elevated CD3⁺CD4⁻ CD8⁻ T cells that express the αβ⁺ T-cell receptor (TCR) (≥ 1.5% of total lymphocytes or ≥ 2.5% of CD3⁺ lymphocytes) in the setting of normal or elevated lymphocyte levels are a required diagnostic criterion for ALPS. Elevations above 3% of the total lymphocytes (or ≥ 5% of CD3⁺ lymphocytes) are pathognomonic for ALPS (44-46). DNTs are found to express the α/β TCR rather than the γ/δ TCR. However, ALPS can infrequently lead to the proliferation of TCR γ/δ DNTs in the affected lymph node and in the circulation (9, 47, 48). It may be that the nature of the initial trigger defines which TCR will dominate (48). It is hypothesized that development at young age of lymphadenopathy in combination with an unidentified bacterial agent may contribute to the accumulation of TCR γ/δ DNTs (48).

Furthermore, T cells display an in vitro defective Fas-mediated lymphocyte apoptosis (9). The in vitro lymphocyte apoptosis assay measures the percentage of activated lymphocytes undergoing apoptosis upon activation with Fas, which is subsequently impaired in patients with defects in the Fas apoptotic pathway. However, several problems exist with the diagnostic value and execution of this assay. It is therefore no longer considered as a required criterion for the diagnosis of ALPS (Table 1 and Table 2).

Consistently high levels of vitamin B12 are found in patients with ALPS and were recently incorporated in the diagnostic criteria of ALPS (49). The mechanism for this elevation is unclear.

ALPS is also associated with several cytokine abnormalities, such as increased serum levels of IL-10, IL-18 and soluble FAS ligand (sFASL) (49). Caminha et al. found that sFASL is a sensitive biomarker for ruling out TNFRSF6 mutation, as healthy controls and patients with ALPS-U or ALPS-related disorders showed only modest elevations of sFASL (49). In contrast, patients with ALPS-FAS or ALPS-sFAS were found to have significantly higher levels sFASL (> 200 pg/ml). These biomarkers are also currently included in the diagnostic criteria of ALPS.

Overall low levels of IL-18 were found to protect against development of autoimmune diseases in MRL/lpr mice (50). Therefore, it is hypothesized that IL-18 could contribute to the pathogenesis of ALPS and reducing IL-18 levels can be a therapeutic strategy in ALPS (50).
4.1. TCR αβ⁺ DNTs, IL-10 and B cell lymphocytosis

The origin and function of TCR αβ⁺ DNTs in ALPS is poorly understood, partly because these cells do not grow in vitro and are therefore difficult to study. It is unknown whether TCR αβ⁺ DNTs drive the disease or are merely an epiphenomenon.

It has been postulated that TCR αβ⁺ DNTs are derived from cytotoxic CD8+ T-cells that have lost their CD8+ expression in a process regulated at the transcriptional level (47, 51-53).

TCR αβ⁺ DNTs were found to have a distinct cytokine production profile, producing significantly more IL-10 than CD8+ T cells (53). Upon in vitro stimulation, a fraction of the CD8+ T cells were found to acquire a cytokine production profile similar to TCR αβ⁺ DNTs, supporting the hypothesis that CD8+ T cells can differentiate into IL-10 producing TCR αβ⁺ DNTs. High levels of IL-10 were found to correlate with greater disease expression (54, 55). As a result, TCR αβ⁺ DNTs may play a role in ALPS pathogenesis by producing IL-10 which is thought to directly stimulate B-cell proliferation, leading to the selective accumulation of autoimmune B cells and subsequent autoimmunity (56). Moreover, high levels of IL-10 induce the upregulation of the antiapoptotic proto-oncogene Bcl-2 in B and T cells (57). Together this may exacerbate the apoptotic defects already inherent to ALPS and predispose to malignancies.

In patients with ALPS, B cell lymphocytosis (CD19) is commonly found (58). These autoreactive B cells may account for the polyclonal hypergammaglobulinemia, autoantibodies and autoimmunity found in patients with ALPS (58).

This expansion might also be related to an abnormal activation state of B cells. Autoreactive B cells can be eliminated by expressing Fas and therefore become sensitive to killing by Fas ligand-expressing T cells (38, 59). Thus, defective Fas-mediated apoptosis may lead to the accumulation of autoreactive B cells.

Other recent data suggests that TCR αβ⁺ DNTs may be dysregulated regulatory T cells (60). Regulatory T cells are involved in downregulating the immune system and tolerance to self-antigens. Dysregulated TCR αβ⁺ DNTs may be unable to downregulate the immune responses and consequently lead to the development of autoimmune disease (61, 62). It has been shown that TCR αβ⁺ DNTs display low copies of TREC, which is considered an accurate marker for newly produced thymic
Moreover, TCR αβ⁺ DNTs were found to lack some DNA rearrangement events, crucial in the differentiation of CD8⁺ or CD4⁺ T cells. This contradicts the findings that TCR αβ⁺ DNTs are an expansion of CD4⁺ or CD8⁺ cells. TCR αβ⁺ DNTs may result from reduced thymic activity, in addition to the apoptotic defect in peripheral lymphocytes.

5. Diagnostic criteria

Diagnosis of this uncommon syndrome can be a challenge. Clinical features and laboratory findings are unspecific and fluctuate over time.

In 1999 criteria were suggested for the diagnosis of ALPS by the National Institute of Health (NIH) (Table 1). Since then there have been significant advances in the understanding of ALPS. This has led to revisions to the existing diagnostic criteria and classification in 2009 (Table 2) (63).

Several problems with the diagnostic criteria from 1999 were detected. The lymphocyte apoptosis assay is only available in selected centres, difficult to perform and not sensitive for patients with somatic Fas or germline FasL mutations. TCR αβ⁺ DNTs do not survive in vitro, therefore only Fas function and the ability to undergo apoptosis in non-TCR αβ⁺ DNTs is measured. Patients with somatic mutations, limited to de double-negative compartment will therefore have a false negative lymphocyte apoptosis assay. Therefore, this test is no longer considered mandatory for the diagnosis of ALPS. If the in vitro lymphocyte apoptosis assay is performed, it is considered abnormal if the patients cells show consistently (≥ 2 assays) 50% or less of the apoptosis observed in healthy controls.

Furthermore, genetic information and recently identified biomarkers for ALPS were incorporated in the criteria.

In accordance with the revised diagnostic criteria, for an absolute ALPS diagnosis a patient has to meet both required criteria and one of the primary accessory criteria. A probable ALPS diagnosis requires both the required criteria and one of the secondary accessory criteria (63).
Table 1: Diagnostic criteria for ALPS in 1999

<table>
<thead>
<tr>
<th>Required Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Chronic nonmalignant lymphadenopathy and/or splenomegaly</td>
</tr>
<tr>
<td>2. Increased peripheral TCR αβ⁺ DNTs</td>
</tr>
<tr>
<td>3. Defective lymphocyte apoptosis assay</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Supporting Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Family history of ALPS</td>
</tr>
<tr>
<td>2. Characteristic histopathology</td>
</tr>
<tr>
<td>3. Autoimmune manifestations</td>
</tr>
</tbody>
</table>


Table 2: Revised diagnostic criteria for ALPS in 2009

<table>
<thead>
<tr>
<th>Required Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Chronic (&gt; 6 months), non-malignant, non-infectious lymphadenopathy and/or splenomegaly</td>
</tr>
<tr>
<td>2. Elevated TCR αβ⁺ CD3⁺ CD4⁻ CD8⁻ DNTs (≥ 1.5% of total lymphocytes or (≥ 2.5% of CD3⁺ lymphocytes) in the setting of normal or elevated lymphocyte counts</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Accessory criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary</strong></td>
</tr>
<tr>
<td>1. Defective lymphocyte apoptosis assay (in 2 separate assays)</td>
</tr>
<tr>
<td>2. Somatic or germline pathogenic mutations in Fas, FasL or caspase-10</td>
</tr>
<tr>
<td><strong>Secondary</strong></td>
</tr>
<tr>
<td>1. Elevated plasma sFasL levels (&gt; 200 pg/ml) OR elevated plasma IL-10 levels (&gt; 20 pg/ml) OR elevated serum or plasma vitamin B12 levels (&gt; 1500 ng/ml) OR elevated plasma IL-18 levels (&gt; 500 pg/ml)</td>
</tr>
<tr>
<td>2. Typical immunohistological findings as reviewed by an experienced haematopathologist</td>
</tr>
<tr>
<td>3. Autoimmune cytopenias (haemolytic anaemia, thrombocytopenia or neutropenia) AND elevated immunoglobulin G levels (polyclonal hypergammaglobulinemia)</td>
</tr>
<tr>
<td>4. Family history of a non-malignant/non-infectious lymphoproliferation with or without autoimmunity</td>
</tr>
</tbody>
</table>

5.1. Classification

In the 2009 NIH conference recommendations about the molecular classification of ALPS have also been introduced (63). Patients carrying both heterozygous and homozygous TNFRSF6 mutations were unified under ALPS-FAS. Patients carrying somatic Fas mutations should be classified as ALPS-sFAS, patients with FasL mutations as ALPS-FASLG and patients with caspase-10 mutations as ALPS-CASP10.

It is likely that new genetic defects will be discovered in the group of patients with ALPS-U.

<table>
<thead>
<tr>
<th>Previous Nomenclature</th>
<th>Revised Nomenclature</th>
<th>Gene</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALPS type 0</td>
<td>ALPS-FAS</td>
<td>Fas</td>
<td>Germline homozygous Fas mutations</td>
</tr>
<tr>
<td>ALPS type Ia</td>
<td>ALPS-FAS</td>
<td>Fas</td>
<td>Germline heterozygous Fas mutations</td>
</tr>
<tr>
<td>ALPS type Is</td>
<td>ALPS-sFAS</td>
<td>Fas</td>
<td>Somatic Fas mutations</td>
</tr>
<tr>
<td>ALPS type Ib</td>
<td>ALPS-FASLG</td>
<td>FasL</td>
<td>Germline FasL mutations</td>
</tr>
<tr>
<td>ALPS type IIa</td>
<td>ALPS-CASP10</td>
<td>Caspase-10</td>
<td>Germline caspase-10 mutations</td>
</tr>
<tr>
<td>ALPS type III</td>
<td>ALPS-U</td>
<td>Unknown</td>
<td>Genetic defect is undetermined</td>
</tr>
</tbody>
</table>


5.2. Imaging

Periodic ultrasounds are commonly used in the diagnostic work-up and follow-up of lymphadenopathy and splenomegaly in patients with ALPS. Periodic CT scans of the chest, abdomen and pelvis can also be useful but caution is warranted with the use of radiation, especially in children.

5.3. Histopathology

Typically, marked paracortical expansion is seen in lymph node biopsies of patients with ALPS (47). Increased numbers of TCR αβ⁺ DNTs are present in these paracortical regions. Other features include follicular hyperplasia, polyclonal plasmacytosis and prominent vascularity of the interfollicular areas (47).
The combination of follicular hyperplasia and paracortical expansion by an infiltrate containing TCR αβ⁺ DNTs is pathognomonic for ALPS. Together with an overall intact architecture of the lymph nodes, these features can help in the differential diagnosis with other lymphoproliferative diseases and malignant lymphomas (3, 41).

5.4. Differential diagnosis

Patients with ALPS present with clinical and laboratory findings, such as elevated TCR αβ⁺ DNTs and defective in vitro lymphocyte assay, that have an important overlap with many other paediatric haematological diseases. These include T-cell lymphoma, common variable immunodeficiency (CVID), systemic lupus erythematosus (SLE), X-linked lymphoproliferative disease (XLP), familial hemophagocytic lymphohistiocytosis (FHLH) and Rosai-Dorfman disease or sinus histiocytosis with massive lymphadenopathy (SHML) (64-66). As some patients with ALPS display comorbidity with CVID, differential diagnosis can be even more difficult. Moreover, some patients with ALPS develop lymphoma, imposing additional challenges for the differential diagnosis between lymphoid hyperplasia and lymphoma. Clinical awareness of constitutional symptoms (e.g. weight loss, fever, malaise) is necessary in making the decision to investigate for malignancy.

However, only patients with ALPS have markedly elevated levels of TCR αβ⁺ DNTs above 3% of the total lymphocytes (or ≥ 5% of CD3⁺ lymphocytes) and TCR αβ⁺ DNT expansion usually remains polyclonal and non-malignant (44, 45). Furthermore, histopathology can help differentiate as it is often specific for ALPS.

ALPS-related apoptosis disorders (ALD)

This group of disorders is currently classified separately from ALPS. It consists of patients with Caspase-8 deficiency syndrome (CEDS), patients with somatic mutations in NRAS or KRAS, patients with Dianzani autoimmune lymphoproliferative disease (DALD) and patients with X-linked lymphoproliferative syndrome (XLP1) (37, 63). TNFRSF6 gene mutations are not seen in ALD.

Patients with germline caspase-8 mutations present with chronic lymphadenopathy, splenomegaly and defective Fas-mediated apoptosis (67). However, unlike patients with ALPS, patients with CEDS also display defects in the activation of T and B cells and NK cells. Moreover, they suffer from recurrent Herpes Simplex and bacterial sinopulmonary infections.
NRAS and KRAS genes encode for the Ras superfamily, which are small GTPases that transmit intracellular signals and can regulate apoptosis (68). Patients with somatic mutations in NRAS or KRAS present with autoimmunity and lymphoproliferation but normal levels of TCR αβ⁺ DNTs and lymph node histopathology different from patients with ALPS (69, 70). In addition, these patients display aberrations in the myeloid compartment. These patients are now classified as RAS-associated autoimmune leukoproliferative disorder (RALD).

Dianzani et al. first described patients with autoimmune manifestations, lymphoproliferation, defective in vitro lymphocyte apoptosis assay but without elevated TCR αβ⁺ DNTs (71). Patients display defects downstream in the Fas signalling pathway. The genetic defect is unknown, but relatives of these patients display defective Fas function, so a germline component is assumed (72). These patients are now classified as Dianzani autoimmune lymphoproliferative disease (DALD).

X-linked lymphoproliferative syndrome (XLP1) is a rare immunodeficiency associated with mutations in the SH2D1A gene. Upon infection with Epstein - Barr virus, patients display dysregulated immune responses with severe infectious mononucleosis, acquired hypogammaglobulinemia and/or malignant lymphoma (73).

**Evans syndrome**

Evans syndrome is a haematological disorder defined by autoimmune destruction of at least 2 peripheral haematological cell types, after exclusion of other diagnoses (74). In addition to autoimmune manifestations, patients with Evans syndrome may present with lymphadenopathy and hepatosplenomegaly (75).

The underlying pathophysiology is unclear but is thought to be secondary to generalized immune dysregulation (45). Based on these clinical similarities, it has been postulated that a subset of patients may be misdiagnosed with Evans syndrome and may actually have ALPS (44, 45). Elevated TCR αβ⁺ DNTs, in vitro defective lymphocyte apoptosis assay and hypergammaglobulinemia, which are highly suggestive for ALPS, were indeed found in a subset of patients enrolled in the studies. These findings suggest that ALPS may be more common than previously thought. Furthermore it is recommended that patients suspected with Evans syndrome should be screened for ALPS by evaluation of TCR αβ⁺ DNT levels as a part of their diagnostic evaluation (44, 45).
6. Treatment

Lymphoproliferative symptoms (e.g. lymphadenopathy and splenomegaly) do not warrant the consistent use of immunosuppressive drugs, unless they are associated with obstructive symptoms or hypersplenism. This is due to observations that lymphoproliferation tends to reoccur after termination of the immunosuppressive drugs and usually regresses with age (38). Patients with lymphadenopathy but no cytopenias usually only require close clinical and imaging follow-up. Furthermore, genetic counselling and advice concerning splenic rupture is important.

Treatment for ALPS is mainly administered for autoimmune manifestations and malignancies. However, only a few effective and tolerable drugs are available for patients who do require treatment. Corticosteroids remain first-choice treatment although newer drugs are being developed and tested. Recent studies have shown that therapies which are commonly used in other refractory autoimmune cytopenias, such as rituximab and splenectomy are relatively contraindicated in ALPS.

6.1. Corticosteroids and immunosuppressants

Short high-dosed courses of corticosteroids remain the treatment of first choice for autoimmune cytopenias in ALPS and are usually highly effective (37). Other immunosuppressant, such as azathioprine and mercaptopurine, may also be effective in the treatment of autoimmune cytopenias.

However, prolonged use of corticosteroids is not recommended because of the high prevalence of adverse effects and patients’ young age. Moreover, chronic exposure to immunosuppressive agents bears a theoretical risk for development of secondary malignancies, which would lead to an additional malignancy risk in patients with ALPS (75).

6.2. Mycophenolate mofetil (MMF)

Mycophenolate mofetil is an inhibitor of the de novo synthesis of purine. It specifically inhibits proliferating T and B cell and is currently used to prevent acute rejection of transplanted organs (76). Furthermore, it is used as a second-line immunosuppressive treatment for many autoimmune diseases.

MMF was found to be effective in the treatment of chronic, refractory cytopenias in patients with ALPS (77). Moreover, a reduction in the required dose of other immunosuppressive agents was observed.
However, patients did not have a normalisation of TCR αβ⁺ DNT levels or improvement of lymphoproliferation. Furthermore, many patients have shown only partial response. Therefore, MMF may be used to reduce the usage of corticosteroids in patients with ALPS. However, it is recommended that MMF is not used as a first-line treatment and only as a steroid-sparing agent (37).

6.3. mTOR inhibitor (Rapamycin, Sirolimus)

Mammalian target of Rapamycin (mTOR) is a protein kinase that regulates cell growth, proliferation and survival. Targeting the mTOR pathway induces apoptosis in activated lymphocytes through activation of the intrinsic or mitochondrial pathway and additionally increases peripheral blood regulatory T cells (78). A significant decrease in TCR αβ⁺ DNTs, lymphoproliferation and autoantibodies was seen in MRL/lpr mice after treatment with Rapamycin (79, 80). In comparison to MMF, Rapamycin showed a greater reduction of lymphoproliferation. Teachey et al. have shown that Sirolimus is effective in reducing lymphoproliferation, autoimmune cytopenias, non-haematological autoimmune manifestations and normalising TCR αβ⁺ DNT levels refractory to corticosteroids (78). Moreover, the use of Sirolimus could allow for a decrease in the required dose of corticosteroids or even a complete stop. However, haematological side effects such as thrombocytopenia, leucopenia anaemia have been described (81). Close therapeutic monitoring to maximize the therapeutic effect and avoid toxicity is therefore required, given the haematological abnormalities already inherent to ALPS.

6.4. Fansidar (pyrimethamine/ sulphadoxine)

Fansidar consists of two components; pyrimethamine and sulphadoxine. Each component inhibits a different enzyme in the de novo synthesis of folic acid (82). In vitro apoptosis was detectable for each component alone in stimulated T lymphocytes from both MRL/lpr mice and patients with ALPS. However, pyrimethamine was found to be far more potent than sulfadoxine in inducing apoptosis (82, 83). Van der Werff et al. reported a marked shrinkage of lymphadenopathy, a decrease in TCR αβ⁺ DNTs and IL-10 and reduction of autoimmune cytopenias upon treatment with Fansidar (82, 84). These features reappeared after cessation of Fansidar. Possible mechanisms for the observed improvement of autoimmune manifestations could be associated with the observed decrease in IL-10 levels.
As previously noted, a decrease in IL-10 may lead to both a decrease in autoreactive B cells and induction of apoptosis through downregulation of Bcl-2 (86). However, two out of the 7 patients enrolled in this study do not meet the strict criteria for the diagnosis of ALPS, having normal TCR αβ⁺ DNT levels.

Nevertheless, in another clinical trial both pyrimethamine and Fansidar failed to show a significant reduction of lymphoproliferation in MRL/lpr mice (83). Moreover, lymphoproliferation did not improve significantly in seven patients with ALPS upon treatment with pyrimethamine alone (83). Furthermore, no significant changes in TCR αβ⁺ DNTs, plasma IL-10 or serum IgG were found. *In vitro* apoptosis showed no difference in Fas-mediated apoptosis defects in patients.

However, primary endpoints in this study were the safety of pyrimethamine in patients and the ability to diminish lymphoproliferation. Autoimmune cytopenias was not a reason for treatment initiation. Two out of 7 patients displayed decreased levels of platelet counts and/or absolute neutrophil counts prior to treatment. Upon treatment with pyrimethamine, both platelet counts and absolute neutrophil counts normalised. Three other patients had received concomitant treatment with corticosteroids within 3 months prior to treatment with pyrimethamine. Therefore, the efficacy of treatment with Fansidar is still a matter of debate.

Severe hypersensitivity reactions have been associated with pyrimethamine/ sulphadoxine (85, 86). However, in the former studies pyrimethamine and Fansidar were found to be well tolerated (82, 83). Observed adverse effects consisted only of mild to generalised rashes.

6.5. Anti-CD20 monoclonal antibodies (Rituximab)

Rituximab depletes the CD20+ B cells, which would subsequently reduce the autoantibodies found in patients with autoimmune cytopenias. Rituximab is currently used in difficult-to-control B-cell expansions, autoimmunity and B-cell lymphomas.

In ALPS, Rituximab has been successfully used in the management of refractory autoimmune thrombocytopenia (87, 88). However, none of the patients with autoimmune haemolytic anaemia responded to treatment with Rituximab (87). Furthermore, the use of Rituximab did not produce clinically significant shrinkage of lymph nodes or spleen.

Observed adverse effects include prolonged neutropenia, profound and prolonged hypogammaglobulinemia and absent antibody response to polysaccharide vaccines up to 4 years (87). These toxicities predispose to an additional infection risk, especially in asplenic patients.
Moreover, 5-10% of patients develop common variable immunodeficiency (CVID) (87). Caution is warranted since patients with ALPS are already predisposed to developing CVID. Empirical use of Rituximab is therefore not recommended. It should be reserved for patients who fail other therapeutic agents.

6.6. Splenectomy

Nearly 50% of patients with ALPS have undergone splenectomy as a successful treatment for severe, refractory cytopenias and/or hypersplenism. Furthermore, a splenectomy is sometimes performed because of discomfort and traumatic splenic rupture. However, in ALPS splenectomy is associated with a long-term risk of recurrent cytopenias and pneumococcal sepsis with significant morbidity and mortality (3). This complication cannot be avoided by vaccination and treatment with penicillin, as opposed to patients with non-ALPS autoimmune disease. Splenectomy should therefore be avoided in patients with ALPS whenever possible. For patients with chronic, refractory life-threatening cytopenias, partial splenectomy or splenic embolisation should be considered (37).

6.7. IV immunoglobulin G

In patients with ALPS, autoimmune thrombocytopenia usually does not respond sufficiently to IV immunoglobulin G infusions, unlike many other non-ALPS autoimmune manifestations (37).

6.8. Pentostatin

Pentostatin activates the intrinsic apoptotic pathway through accumulation of deoxyadenosine-5’-triphosphate (dATP), which becomes cytotoxic at high concentrations. Bajwa et al. reported the use of pentostatin in a patient with ALPS with cytopenias refractory to splenectomy and immunosuppressive agents (89). Lymphocytosis was found to decrease upon the administration of pentostatin. Pentostatin was well tolerated and effective in keeping the patient in remission until a hematopoietic stem cell transplant (HSCT) was performed.

These findings suggest that pentostatin may be useful in other patients with ALPS who are refractory to MMF and/or Sirolimus.
6.9. Hematopoietic stem cell transplantation (HSCT)

HSCT has been successfully performed in 2 patients with ALPS (90, 91). Sleight et al. described a patient carrying a homozygous TNFRSF6 mutation, resulting in an extreme phenotype of ALPS (90). The patient was diagnosed with non-Hodgkin lymphoma and showed no response to several courses of corticosteroids, G-CSF, α-interferon and a splenectomy. Two years after HSCT was performed, the patient remained free of disease, no signs or symptoms of ALPS were evident. In the second patient, control of lymphoproliferation and autoimmune thrombocytopenia was achieved (91).

However, since the role of stem cell transplantation in ALPS is unclear, there is no need for the routine use of HSCT and it should be reserved for patients with highly refractory disease. Furthermore most patients with ALPS respond well to other therapies (37).

6.10. Arsenic trioxide (As$_2$O$_3$)

It has been postulated that arsenic trioxide eliminates autoreactive cells through induction of the intrinsic or mitochondrial pathway. Arsenic trioxide was found to significantly reduce autoimmune and lymphoproliferative manifestations and decrease TCR αβ$^+$ DNT levels in MRL/lpr mice (92). Arsenic trioxide is thought to specifically activate caspases in the abnormal TCR αβ$^+$ DNTs. This leads to the elimination of TCR αβ$^+$ DNTs but leaves the total number of T cells unchanged. Furthermore, markedly reduced levels of anti-DNA autoantibodies, FasL, IL-10 and IL-18 were observed. As previously noted, reducing IL-18 levels could account for the positive effects seen after treatment with arsenic trioxide. The reduction in IL-10 levels paralleled the decrease in TCR αβ$^+$ DNT levels.

6.11. Valproic Acid (VPA)

Valproic acid (VPA) is a histone deacetylase (HDAC) inhibitor, which is thought to induce apoptosis through increased expression of Fas and FasL, increased caspase-3 activation and increased release of cytochrome C (93).

A significant reduction in lymphoproliferative symptoms and TCR αβ$^+$ DNTs was found after treatment with VPA both in vitro and in MRL/lpr mice (94). VPA is currently being studied in a clinical trial as a treatment for ALPS.
**AIM**

ALPS might be more common than previously thought. The clinical and laboratory manifestations are highly variable and often difficult to differentiate from other haematological paediatric diseases. The aim of this study was to describe the clinical, immunological and laboratory findings in a group of patients diagnosed with ALPS in order to raise clinical awareness. Furthermore, patients’ clinical outcome upon treatment was analyzed. Existing literature in patients with ALPS was reviewed. These results were compared retrospectively with our patient cohort for similarities and differences.

Moreover, a second patient cohort who underwent TCR αβ⁺ DNT assessment based on clinical suspicion of ALPS for the period 2009-2012 at UZ Ghent was identified. The purpose of this additional inquiry was to estimate in how many patients ALPS was included in the differential diagnosis from 2009 to 2012 in UZ Ghent, how often TCR αβ⁺ DNT levels were subsequently elevated and further diagnostic evaluation was warranted.

Additionally, the effect of treatment with Fansidar (25 mg phylimetamine/500 mg sulphadoxine) on clinical outcome and laboratory results in the patient cohort is retrospectively described. In 1998, Van der Werff et al. first reported normalisation of clinical and laboratory features upon the administration of Fansidar in a patient with ALPS. The anti-malaria drug was initially used as chemoprophylaxis for Pneumocystis carinii during lymphopenia. Since then, Fansidar has been consistently used as a first-line treatment for lymphoproliferative and autoimmune manifestations associated with ALPS in some Belgian centres.

Finally, a clinical practice algorithm will be provided describing the clinical and laboratory features when ALPS should be considered, the diagnostic tests that should be performed and corresponding sequence, suggestions for the management of autoimmune cytopenias and long-term follow-up care. Consequently, the study aims to contribute to making the correct diagnosis and choosing the most appropriate treatment option.
METHODS AND MATERIALS

1. Study population

1.1. Background of study population and recruitment

Nine patients were receiving or had received follow-up care at the department of Paediatric Haematology-Oncology at UZ Ghent. Through their medical records, two relatives of the initial 8 patients with phenotypic expression were identified and included in the study. Their medical records were retrieved from the adult department of haematology at UZ Ghent. Another patient, also receiving follow-up care at the adult department and diagnosed with ALPS at infancy, was included.

Other medical centres in Belgium were contacted and their cooperation was asked for the inclusion of more patients with ALPS, followed at these centres. This led to the inclusion of one more patient followed at QFCUH in Brussels, bringing the total initial population size to 13 patients (10 male and 3 female).

Informed consent was obtained for all patients or their legal guardians (Appendix 2). UZ Ghent’s Ethics Committee approval was obtained for the retrospective data collection in medical records (Appendix 3).

1.2. Inclusion and exclusion criteria and sample size

In one patient TCR αβ⁺ DNT levels were not assessed, this patient was therefore not included in the study. As a result, the total study population consisted of 12 patients.

All patients included in the study (n=12) presented with chronic (> 6 months) non-malignant non-infectious lymphoproliferation and elevated TCR αβ⁺ DNT levels (≥ 1.5% of total lymphocytes or ≥ 2.5% of CD3⁺ lymphocytes) fulfilling the required diagnostic criteria of ALPS (Table 4).

Six out of 12 patients met one or more of the primary accessory criteria and have an absolute ALPS diagnosis. Six patients have a probable ALPS diagnosis, meeting one or more of the secondary accessory criteria.
2. Data collection and study conduct

Existing literature on ALPS was reviewed, starting with five relevant and recent articles on ALPS (1, 37, 63, 95, 96). Novel insights into pathophysiology, genetics, penetrance, diagnostic criteria and treatment are described and reviewed herein.

Background information on the immune system and the physiological role of Fas in apoptosis was found in the following medical textbooks: the 8th edition of Clinical Medicine by Kumar and Clark, Review of Medical Physiology by Ganong and The Immune System by Parham.

Furthermore, a website based-search was performed in the online database of biomedical literature by the NCBI. The search string "Autoimmune Lymphoproliferative Syndrome"[Mesh] was used. At the time, this query produced 46 articles. Articles were selected based on relevancy of the abstract, full text availability and study design and purpose. Related citations were reviewed and retained if they contained additional information. More specific searches were performed on: haploinsufficiency and penetrance, the origin and function of TCR αβ⁺ DNTs, the perforin/granzym pathway and effect on disease penetrance, newly discovered biomarkers, differential diagnosis with Evans syndrome and histopathologic features. The final literature review referred to 96 articles.

For the experimental section, information on patients’ clinical and laboratory features, relevant for the diagnosis and characterisation of ALPS was collected. The data was collected by reviewing medical charts and reports, ranging from 1998 until present. The data was summarised and subdivided into 4 spreadsheets: Population Characteristics, Clinical Manifestations, Laboratory Results and Therapy (Appendix 1).

On population characteristics and clinical manifestations, the following data was collected: age at presentation and diagnosis, family history of ALPS, presence and duration of lymphoproliferation and presence of malignancies.

The following laboratory features were evaluated: autoimmune manifestations (autoimmune cytopenias and autoantibodies), TCR αβ⁺ DNT levels, in vitro lymphocyte apoptosis assay (if performed), soluble FasL levels and biomarker levels (vitamin B12, IL-10 and IL-18). Furthermore, imaging (ultrasound, CT, PET-CT) and histopathology results were reviewed. If patients had undergone genetic testing, these results were also retained.
For patients undergoing treatment, reasons for treatment initiation with corresponding date and dosage were collected. If applicable, any intermediate augmentation or treatment cessation was also assessed. The efficacy results of the treatment over time was evaluated by the degree of lymphoproliferation, platelet count, haemoglobin levels, absolute neutrophil count and TCR αβ+ DNT levels as a percentage of CD3+ lymphocytes.

Test results for all patients who underwent assessment of TCR αβ+ DNT levels for the period 2009-2012 were collected by entering the search query “TCRABB AND OPCDBL” in UZ Ghent’s clinical laboratory database. TCR αβ+ CD3+ CD4− CD8− DNTs were analysed by flow cytometry and expressed as a percentage of CD3+ lymphocytes. Additional data on TCR αβ+ DNT levels of the initial study population (n=12) was incorporated in the spreadsheets.

Descriptive and comparative analyses (mean values, range, standard deviations) were performed on the collected patient data. The population size (n=12) was too small to perform statistical analyses.
## RESULTS

Table 4: Demographic and clinical features of study population

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Gender</th>
<th>Age at presentation (yrs)*</th>
<th>Age at diagnosis (yrs)**</th>
<th>Lymphadenopathy†</th>
<th>Splenomegaly‡</th>
<th>Hepatomegaly</th>
<th>Family history</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>12</td>
<td>13</td>
<td>++</td>
<td>+ ++ + (S: 14 yrs)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>4</td>
<td>6</td>
<td>+</td>
<td>+ ++ +</td>
<td>-</td>
<td>Son of patient no.10</td>
</tr>
<tr>
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<td>13</td>
<td>+</td>
<td>+</td>
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<td>-</td>
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<td>++</td>
<td>+</td>
<td>+</td>
<td>Father: autoimmune cytopenias Nephew of patient no. 5</td>
</tr>
<tr>
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<td>43</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Nephew of patient no. 4</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>12</td>
<td>12</td>
<td>+</td>
<td>+ ++ + (S: 12 yrs)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>6</td>
<td>8</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>Brother of patient no. 8</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>6</td>
<td>6</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>Brother of patient no. 7</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>2</td>
<td>4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>9</td>
<td>34</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Mother of patient no. 2</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>7</td>
<td>14</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>Father: germline TNFRSF6 mutation, defective in vitro apoptosis assay</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>2</td>
<td>2</td>
<td>+</td>
<td>+ ++ +</td>
<td>+</td>
<td>Mother: germline TNFRSF6 mutation, no clinical symptoms Maternal uncle: cytopenias, lymphoproliferation and non-Hodgkin lymphoma</td>
</tr>
</tbody>
</table>

*Median age at presentation was 7 years.
** Median age at diagnosis was 10 years.
† Lymphadenopathy: + 1-2 cm nodes; ++ 2-4 cm nodes.
‡ Splenomegaly: +, 1-2 cm; ++, 3-4 cm; +++ 5-6 cm; ++++, >6 cm below costal margin.
S: splenectomy (years).
1. Genetics

Three out of 12 patients underwent genetic testing. Patient no. 5 was found to carry a germline TNFRSF6 nonsense mutation in exon 5, encoding the extracellular domain. Patient no. 12 was found to carry a heterozygous missense TNFRSF6 mutation in exon 9, encoding the Fas-death domain (DD). In patient no. 11, a germline TNFRSF6 nonsense mutation in exon 7 was found. Exon 7 is known to encode the intracellular domain of Fas.

Patient 11 displayed defective in vitro lymphocyte apoptosis assay, whereas patient no. 5 did not. No other differences in presence of lymphoproliferation, autoimmune manifestations or other ALPS related morbidity could be found between these 3 patients. However, age at disease onset was markedly later in patient no.5 carrying the extracellular TNFRSF6 mutation as opposed to patient no.11 and patient no. 12 carrying intracellular TNFRSF6 mutation.

Family history was positive in 8 out of 12 patients. Patient no. 10 was diagnosed at the age of 34 after the diagnosis of ALPS was suspected and confirmed in her son, patient no 3. Patient no. 5 and patient no. 6 are nephews on the paternal side. Moreover, the finding that the father of patient no. 5 displayed autoimmune cytopenias at infancy additionally supports the hypothesis of paternal inheritance.

Patient no. 7 and patient no. 8 are siblings.

The father of patient no. 11 was found to carry the same germline TNFRSF6 nonsense mutation and displayed defective in vitro lymphocyte apoptosis assay in 2 separate assays. However, he has not displayed any lymphoproliferative or autoimmune manifestations.

The mother of patient no. 12 was found to carry the same heterozygous TNFRSF6 missense mutation. To date, she has not displayed any lymphoproliferative or autoimmune features. However, a maternal uncle of patient no. 12 displayed thrombocytopenia and lymphoproliferative symptoms in early childhood. A lymph node biopsy revealed sinus histiocytosis, suggestive for Rosai-Dorfman disease (SHML). He was splenectomised at the age of three. At the age of 29, he developed Non-Hodgkin lymphoma, for which he was treated with localised radiotherapy. He is currently in remission.
2. **Clinical manifestations**

All patients (n=12) presented with lymphoproliferative symptoms and one or more autoimmune cytopenias. The median age of clinical onset was 7 years (range: 2-39). The median age at diagnosis was 10 years (range: 2-43). A median diagnostic delay of 1.5 years is observed in the study population (range: 0-25 years, SD: 7.0). At present, patients’ median age is 20 years (range: 11-48) and median follow-up time is 7 years.

2.1. **Lymphoproliferation**

All patients (n=12) displayed chronic lymphadenopathy, present from over 6 months up to 24 years. Multiple enlarged (1-4 cm) lymph nodes were observed, most frequently affecting the cervical (8/12) and axillary (7/12) lymphatic chains. Other affected locations were the inguinal (6/12), submandibular (4/12) and mesenteric (4/12) chains.

At presentation, splenomegaly was observed in all patients with an average spleen span of 15.2 cm on ultrasound (range: 10 - 30 cm) or 6.5 cm below costal margin (range: 2.5-20 cm). Hepatomegaly was present in 4 out of 12 patients (2-3 cm below costal margin).

2.2. **Autoimmunity**

All patients (n=12) presented with autoimmune manifestations and/or hypergammaglobulinemia at first presentation (Table 5).

Ten out of 12 patients displayed one or more autoimmune cytopenias at initial presentation. Nine patients presented with mild to severe thrombocytopenia (mean platelet count ± SD: 4 x 10^3 ± 35 x 10^3/µL; range: 5-101 x 10^3/µL). Additionally, 6 of these 9 patients also displayed mild to severe neutropenia (mean neutrophil count ± SD: 750 ± 388.2/µL; range: 247-1280/µL). Three out of 9 patients presented with haemolytic anaemia in addition to thrombocytopenia.

One patient displayed only haemolytic anaemia at initial presentation. No patient displayed only neutropenia or pancytopenia at initial presentation.

Seven out of 8 remaining patients without haemolytic anaemia at initial presentation eventually developed haemolytic anaemia after a median duration of 3 years (SD: 2.5; range: 1-8).

In summary, 9 patients displayed thrombocytopenia, 6 patients displayed neutropenia and 11 patients (n=12) displayed haemolytic anaemia at initial presentation or in the course of the disease.
Table 5: Autoimmune manifestations and laboratory results of study population

<table>
<thead>
<tr>
<th>Patient no</th>
<th>Autoimmunity</th>
<th>Autoimmunity features</th>
<th>Autoantibodies</th>
<th>TCR αβ+</th>
<th>Serum Ig‡</th>
<th>In vitro apoptosis assay§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>PA-Ab - HNA - DAT - ANA -</td>
<td>18%</td>
<td>++</td>
<td>↓</td>
</tr>
<tr>
<td>1</td>
<td>Thrombocytopenia* +</td>
<td>Neutropenia* + + +</td>
<td>Haemolytic anaemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Thrombocytopenia + + +</td>
<td>Neutropenia +</td>
<td>Haemolytic anaemia</td>
<td>2,7%</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>3</td>
<td>Thrombocytopenia + +</td>
<td>Neutropenia + +</td>
<td>Haemolytic anaemia</td>
<td>21,4%</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Thrombocytopenia + +</td>
<td>Neutropenia +</td>
<td>Haemolytic anaemia</td>
<td>4%</td>
<td>+</td>
<td>N</td>
</tr>
<tr>
<td>5</td>
<td>Thrombocytopenia + +</td>
<td>Neutropenia +</td>
<td>Haemolytic anaemia</td>
<td>3,7%</td>
<td>N</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Thrombocytopenia + +</td>
<td>Neutropenia +</td>
<td>Haemolytic anaemia</td>
<td>5,4%</td>
<td>N</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Thrombocytopenia + +</td>
<td>Neutropenia +</td>
<td>Haemolytic anaemia</td>
<td>3,4%</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Thrombocytopenia + +</td>
<td>Neutropenia +</td>
<td>Haemolytic anaemia</td>
<td>6%</td>
<td>++</td>
<td>N</td>
</tr>
<tr>
<td>9</td>
<td>Thrombocytopenia + +</td>
<td>Neutropenia +</td>
<td>Haemolytic anaemia</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Thrombocytopenia 0</td>
<td>Haemolytic anaemia</td>
<td>Glomerulonephritis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Haemolytic anaemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Haemolytic anaemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ITP, idiopathic thrombocytopenic purpura; DAT, direct antiglobulin test (Coombs); PA-Ab, platelet antibodies; HNA, human neutrophil antibodies; ANF, antinuclear antibodies; NE, not evaluated. Other antibodies not evaluated if not mentioned.

* Thrombocytopenia: +, mild (platelet count 100-150 x 10^3/µL); ++, moderate (50-100 x 10^3/µL); ++++, severe (<50 x 10^3/µL); 0, platelet count not registered in medical record.
"Neutropenia: +, mild (absolute neutrophil count: 1000-1500 /µL); ++, moderate (500-1000 /µL), +++, severe (<500 /µL).

†TCR αβ+ CD3+ CD4+ CD8− DNTs expressed as a percentage of total CD3+ lymphocytes. TCR αβ+ DNT levels (mean ± SD) were 7.2% ± 6.0%.

‡ Ig serum level: normal ranges vary according to age. N, in normal range; +, >+ 2-4 SD; ++, > + 4 SD; -, <2 SD.

§ In vitro lymphocyte apoptosis assay: 0, normal >50%; ↓, defective.

Out of 11 patients who displayed haemolytic anaemia, 4 patients had a positive direct antiglobulin test (DAT) and 5 patients did not. For the remaining 2 patients, the antiglobulin test was not performed. Platelet antibody titers (PA-Ab) were only assessed in one patient with thrombocytopenia and were negative.

Out of 5 patients with neutropenia, human neutrophil antibodies (HNA) were assessed in 3 patients and were all negative. Antinuclear antibody titers (ANA) were negative in all 4 patients in which they were evaluated.

Two out of 12 patients displayed autoimmune manifestations in other organs, such as glomerulonephritis and urticarial rash at initial presentation and arthritis after 3 years.

2.3. Malignancy

No patient has developed malignancy. A maternal uncle of patient no. 12 developed Non-Hodgkin lymphoma at the age of 29. A germline TNFRSF6 mutation has not been confirmed but is suspected because of the patients’ clinical history. Furthermore, a heterozygous TNFRSF6 missense mutation was found in his sister (mother of patient no. 12) and niece (patient no. 12). Upon local treatment with radiotherapy, the patient is in remission.
3. Laboratory findings

All patients presented with elevated TCR αβ⁺ CD3⁺ CD4⁻ CD8⁻ DNT levels. Mean TCR αβ⁺ DNT levels as a percentage of total CD3⁺ lymphocytes were 7.2% (SD: 6.0%, range: 2.7-21.4%).

Eight out of 12 patients displayed polyclonal hypergammaglobulinemia (IgG). Gamma globulin levels vary according to age but IgG levels of 5 out of 8 patients were 2 to 4 times higher than the standard deviation of normal age distribution. Immunoglobulin G levels of 3 out of 8 patients were more than 4 times higher than the standard deviation of normal age distribution. Immunoglobulin G levels of one out of 4 patients who did not display hypergammaglobulinemia only just remained in the normal age-dependent reference range (11.5 g/dl, reference range: 3.9-11.7 g/dl).

Hypergammaglobulinemia was associated with hyperIgA in one patient and hypoIgA in 4 out of 12 patients.

The *in vitro* lymphocyte apoptosis assay was performed in 6 out 12 patients and defective in 2 separate assays in 4 out of these 6 patients. Soluble FasL levels were elevated (264 pg/ml) in one patient with a proven TNFRSF6 germline mutation. In the other patients, sFasL levels were not evaluated.

One out of 12 patients (patient no. 6) displayed elevated levels of vitamin B12 (>2000 ng/ml). Vitamin B12 levels were normal in 4 patients and not assessed in 7 other patients.

IL-10 and IL-18 levels were not determined in any patient.

Two out of 12 patients displayed B cell lymphocytosis. Normal ranges vary according to age, but both percentages were 2 times higher than the standard deviation of normal age distribution.

From 2009 to 2012, TCR αβ⁺ DNT levels were assessed and expressed as a percentage of CD3⁺ lymphocytes in 124 patients at UZ Ghent on clinical suspicion of ALPS. Median age at assessment was 10.25 years. Of all 124 patients, 46.8% were female and 53.2% were male.

In 10 cases, TCR αβ⁺ DNT levels were elevated above 5% of total CD3⁺ lymphocytes, which is pathognomonic for ALPS. Two of these 10 patients are included in the study population described herein.

In 18 cases, TCR αβ⁺ DNTs were elevated above 2.5% of total CD3⁺ lymphocytes, fulfilling one of the required criteria for ALPS. One of these 18 patients is included in the study population described herein.
In total 22.6% of the requested TCR αβ⁺ DNT level assessments were positive from 2009 to 2012 at UZ Ghent. Out of 28 patients, 25% were female and 75% were male. In 8 cases, TCR αβ⁺ DNT levels did not meet the strict criteria (range: 2-2.4 %). However, results were suggestive of ALPS so further diagnostic evaluation is needed. In 88 cases TCR αβ⁺ DNTs were not elevated above 2.5% of total CD3⁺ lymphocytes. Therefore, no immunophenotypic arguments for ALPS were found.

4. Imaging and histopathology

Ultrasound was consistently used in all patients (n=12) for confirming the degree of lymphoproliferation at diagnosis and in follow-up care. A CT scan was performed in 7 out of 12 patients. Multiple lymphadenopathies and splenomegaly were present in all 7 patients. Hepatomegaly was present in 3 out of 12 patients. Accessory PET-CT scans were performed in 6 out of these 7 patients. In 5 patients elevated FDG-avidity was seen in the enlarged lymph nodes with moderate to intense captation. However no arguments for malignancy were found, all lymphadenopathies were classified as benign. Lymph node biopsies were performed in 4 out of 12 patients. In only one patient typical histopathologic features, such as paracortical expansion, follicular hyperplasia and polyclonal plasmocytosis were found. Follicular hyperplasia was present in another patient. Finally, the last two biopsies revealed a non-specific lymphadenitis.
5. Treatment

All 12 patients required treatment for autoimmune cytopenias and/or lymphoproliferative symptoms (Table 6).

Table 6: Most recent treatment and corresponding outcome in study population

<table>
<thead>
<tr>
<th>Patient no</th>
<th>Reason for initiation</th>
<th>Most recent agent</th>
<th>Dose</th>
<th>Median treatment duration (yrs)</th>
<th>Lymphoproliferation</th>
<th>Autoimmunity</th>
<th>TCR αβ+ DNTs/CD3+ lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lymphoproliferation, Pancytopenia</td>
<td>Fansidar</td>
<td>2 tab weekly</td>
<td>?</td>
<td>Incomplete regression</td>
<td>?</td>
<td>Normalisation</td>
</tr>
<tr>
<td>2</td>
<td>Lymphoproliferation</td>
<td>Fansidar</td>
<td>2 tab weekly</td>
<td>7.2</td>
<td>Complete regression</td>
<td>n/a</td>
<td>Normalisation</td>
</tr>
<tr>
<td>3</td>
<td>Lymphoproliferation, Thrombocytopenia, Neutropenia</td>
<td>Fansidar</td>
<td>3 tab weekly</td>
<td>1.3</td>
<td>Incomplete regression*</td>
<td>Incomplete regression†</td>
<td>Normalisation</td>
</tr>
<tr>
<td>4</td>
<td>Lymphoproliferation, Pancytopenia</td>
<td>MMF</td>
<td>2x250mg daily</td>
<td>5.25</td>
<td>Complete regression</td>
<td>Complete regression</td>
<td>No effect</td>
</tr>
<tr>
<td>5</td>
<td>Thrombocytopenia, Neutropenia</td>
<td>Corticosteroids</td>
<td>64 mg daily</td>
<td>7 days</td>
<td>n/a</td>
<td>Complete regression</td>
<td>No effect</td>
</tr>
<tr>
<td>6</td>
<td>Lymphoproliferation, Haemolytic anaemia</td>
<td>Fansidar</td>
<td>2 tab weekly</td>
<td>1.7</td>
<td>Complete regression**</td>
<td>Complete regression</td>
<td>No effect</td>
</tr>
<tr>
<td>7</td>
<td>Lymphoproliferation, Thrombocytopenia, Neutropenia</td>
<td>Fansidar</td>
<td>2 tab weekly</td>
<td>4.8</td>
<td>No effect</td>
<td>Complete regression</td>
<td>Incomplete regression</td>
</tr>
<tr>
<td>8</td>
<td>Lymphoproliferation, Thrombocytopenia, Neutropenia</td>
<td>Rapamycin</td>
<td>3mg daily</td>
<td>1.4</td>
<td>Incomplete regression‡</td>
<td>Complete regression</td>
<td>No effect</td>
</tr>
<tr>
<td>9</td>
<td>Pancytopenia</td>
<td>MMF</td>
<td>2x250mg daily</td>
<td>7.1</td>
<td>Complete regression</td>
<td>Complete regression</td>
<td>No effect</td>
</tr>
<tr>
<td>10</td>
<td>Lymphoproliferation</td>
<td>Fansidar</td>
<td>1 tab weekly</td>
<td>8.0</td>
<td>Complete regression</td>
<td>n/a</td>
<td>Normalisation</td>
</tr>
<tr>
<td>11</td>
<td>Lymphoproliferation</td>
<td>Fansidar</td>
<td>2 tab weekly</td>
<td>3.4</td>
<td>Incomplete regression*</td>
<td>n/a</td>
<td>No effect</td>
</tr>
<tr>
<td>12</td>
<td>Lymphoproliferation, Haemolytic anaemia</td>
<td>Immuno-suppresants</td>
<td>?</td>
<td>1.3</td>
<td>Incomplete regression‡</td>
<td>Complete regression</td>
<td>No effect</td>
</tr>
</tbody>
</table>

MMF: mycophenolate mofetil

? : no additional data on median treatment duration, lymphoproliferation and/or autoimmunity was available in medical records.

*Spleen size was no longer palpable below costal margin. Lymphadenopathies regressed but remained palpable.

**Patient no. 6 was splenectomised prior to treatment with Fansidar.

‡ Lymphadenopathies were no longer palpable at clinical examination. Spleen size regressed but remained palpable below costal margin.

† Platelet count normalised but absolute neutrophil count remained decreased.
**Fansidar** (Table 7)

Eleven out of 12 patients described herein were treated with Fansidar, containing 25 mg pyrimethamine and 500 mg sulfadoxin, as a first-line treatment or in the course of the disease. In 9 out of 11 patients, Fansidar was administered as a first-line treatment. In one out of 11 patients, Fansidar was initiated for cytopenias refractory to IV Immunoglobulin G administration and splenectomy. In another patient Fansidar was added to corticosteroids because of persistent haemolytic anaemia and splenomegaly. Doses ranged from once to twice weekly. Median treatment duration for all patients (n=11) is 2.2 years (range: 1 week-7 years).

In these 11 patients, reasons for treatment initiation were obstructive lymphoproliferative symptoms in 3 patients, autoimmune cytopenias in 1 patient and both autoimmune cytopenias and lymphoproliferative symptoms in 7 other patients.

| Table 7: Clinical and laboratory outcome upon treatment with Fansidar |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Lymphoproliferation | Autoimmune cytopenias | TCR αβ⁺ DNTs/ CD3⁺ lymphocytes |
| Lymph adenopathy | Splenomegaly‡ | Platelet Count | ANC | Hb level | |
| Complete Regression/ Normalisation | 4 / 10* | 4 / 9 | 3 / 6 | 2 / 6 | 1 / 4 | 4 / 11§ |
| Incomplete Regression | 2 / 10** | 2 / 9° | n/a | n/a | n/a | 1 / 11† |

Abbreviations: ANC: absolute neutrophil count

Median treatment duration for all patients is 2.2 years (range: 1 week-7 years).

Lymphoproliferation: 11 patients were treated for lymphadenopathies and/or splenomegaly.

Autoimmune cytopenias: out of 9 patients treated for autoimmune cytopenias: 2 were treated only for haemolytic anaemia, 4 for both thrombocytopenia and neutropenia and 2 for pancytopenia.

*No lymphadenopathies palpable at clinical examination.

**Lymphadenopathies regressed (median: 60.3%) but remained palpable.

†One out 10 patients has undergone a splenectomy.

° Spleen size decreased (median: 37.5%) but remained palpable below costal margin.

§ TCR αβ⁺ DNTs/ CD3⁺ lymphocytes decreased with a median of 57% (range: 20-66.7%).

† TCR αβ⁺ DNTs/ CD3⁺ lymphocytes remained slightly elevated (2.9%).
After treatment with Fansidar was interrupted, autoimmune cytopenias and/or lymphoproliferation reappeared in 4 out of 11 patients after a median medication-free period of 18.5 weeks (range: 3 weeks-3.5 years). Median treatment duration when Fansidar was discontinued was 32 weeks (range: 4.9 weeks-4.6 years). After Fansidar was restarted once to twice weekly, autoimmune cytopenias and/or lymphoproliferation completely regressed again.

In summary, complete remission (lymphoproliferation and autoimmune cytopenias) was seen in 4 out of 11 patients upon the use of Fansidar after a median treatment duration of 3.5 years (range: 20 months-7 years). Median response duration after therapy is 2.4 years (range: 6 months-12 years). However clinical and ultrasound follow-up is still required. Significant improvement of lymphoproliferation and/or autoimmune cytopenias was seen in two out of 11 patient, who are still receiving treatment with Fansidar to date (median treatment duration: 4.7 years). One out of 11 patients is still undergoing treatment with Fansidar but no additional data on laboratory markers or lymphoproliferation was available.

In one out of 11 patients, a generalised urticarial rash appeared one week after the initiation of Fansidar and treatment was discontinued. In 3 out of the remaining 10 patients clinical and laboratory markers did not or insufficiently improve upon treatment with Fansidar and treatment was discontinued (median treatment duration: 11.5 months, range: 7 months-3.8 years). Three of these 4 patients were subsequently treated with mycophenolate mofetil, immunosuppressants and/or Rituximab. One out of 4 patients was treated with an mTOR inhibitor.
**Corticosteroids**

Short high-dosed (64mg daily during 7 days) courses of corticosteroid therapy (Methylprednisolone) were administered in 4 out of 12 patients for autoimmune cytopenias, concomitantly with Fansidar or as monotherapy. In 3 out of these 4 patients, haemolytic anaemia or autoimmune thrombocytopenia were treated with a combination of Fansidar and short courses of corticosteroids. Autoimmune cytopenias regressed upon treatment but reappeared when corticosteroids were tapered in 2 out of 3 patients. Treatment with corticosteroids concomitantly with Fansidar was successful in one out of 3 patients. Monotherapy with corticosteroids was also successful in one out of 4 patients. To date, both patients are medication-free and in remission. Although it was not the reason for treatment initiation, lymphoproliferation completely regressed in one out of 4 patients upon treatment with corticosteroids. Lymphoproliferation persisted in the 3 other patients.

**Splenectomy**

Two out of 12 patients have undergone a splenectomy due to hypersplenism or refractory cytopenias. The first patient was given Erythromycin 500mg twice weekly, as post-splenectomy prophylaxis. However, recurrent post-splenectomy cytopenias (thrombocytopenia and neutropenia) were seen. The patient was subsequently treated successfully with Fansidar. No additional data on laboratory markers or lymphoproliferation were available for the second patient who has undergone a splenectomy due to cytopenias refractory to Fansidar. No pneumococcal sepsis was observed in these 2 patients.

**Other**

Mycophenolate mofetil (Cellcept) was administered in 3 out of 12 patients. Reasons for treatment initiation were autoimmune cytopenias refractory to Fansidar in 2 out of 3 patients (median treatment duration: 11.5 months). In one out of 3 patients, MMF was initiated because of an allergic reaction to Fansidar within the first week of treatment. MMF was administered at a dose of 2 x 250 mg daily. Platelet counts and absolute neutrophil counts normalised upon treatment with MMF in 2 out of 3 patients. Lymphoproliferation also regressed upon MMF. To date, both patients are still undergoing treatment with MMF (median duration time: 6 years).
In one patient, insufficient increase in haemoglobin levels was seen (Hb level: 9.7 mg/dl). The patient was subsequently treated with the immunosuppressants azathioprine (Imuran) and mercaptopurine (Purinethol). Haemolytic anaemia regressed (Hb level 12.4 g/dl) and immunosuppressive agents were discontinued after 3.25 years. To date the patient has been in remission and medication-free for 16 months.

One out of 12 patients was treated with an mTOR inhibitor (Rapamycin) because of autoimmune cytopenias refractory to both Fansidar and short courses of corticosteroids (Methylprednisolone). Rapamycin was started at a dose of 2mg daily and increased to 3 mg daily under therapeutic monitoring. Platelet count and absolute neutrophil count normalised and lymphoproliferation significantly improved. TCR αβ+ DNT levels remained elevated upon treatment with Rapamycin. No haematological adverse effects were observed in this patient.

Rituximab was administered in one out of 12 patients for haemolytic anaemia refractory to Fansidar. However, Hb levels did not normalise upon treatment. To date, no adverse effects of treatment with Rituximab have been observed.

One out of 12 patients was treated with immunoglobulin G IV because of hypergammaglobulinemia. A small but insufficient decrease in spleen size (21.4%) was observed. Platelet counts remained reduced (100 x 10^3/μL).
DISCUSSION

Genetics
In Belgium, genetic testing for ALPS is not performed, samples have to be sent abroad. These costs are not refundable. Therefore, many patients hesitate to undergo the test. Nevertheless, identifying a mutation can indisputably confirm the diagnosis of ALPS. Furthermore, knowledge of the specific location of the mutation on the TNFRSF6 gene can be useful in predicting future penetrance, severity of clinical phenotype and risk of lymphoma. A minority of patients (3/12) described herein have undergone genetic testing. Two patients were found to carry heterozygous, germline TNFRSF6 mutations affecting the intracellular portion of Fas. The third patient was found to carry a heterozygous, germline TNFRSF6 mutation affecting the extracellular portion. However, with the exception of a normal in vitro lymphocyte apoptosis assays in the patient with the extracellular mutation, no difference in clinical or laboratory features was observed between these 3 patients. However, as described in literature, age of clinical onset was markedly later in the patient carrying an extracellular TNFRSF6 mutation. In accordance with the findings of Magerus-Chatinet, a complementary somatic mutation in the second TNFRSF6 allele next to the germline TNFRSF6 extracellular mutation found in our patient, might account for the similarity in clinical phenotype (23).

Family history of autoimmune cytopenias with or without non-malignant non-infectious lymphoproliferation was positive in 2 out of 12 patients. Furthermore, 2 first-degree relatives were found to carry the same germline TNFRSF6 mutation, but did not display any clinical or immunologic features. These asymptomatic carriers are not considered as patients with ALPS but do require life-long surveillance because of the increased risk of malignancy.

Moreover, 6 out 12 patients described herein are related (first- and second-degree).

Clinical features
All patients presented with chronic, non-malignant non-infectious lymphoproliferation (lymphadenopathy and/or splenomegaly). A median diagnostic delay of 1.5 years was observed. However, this median value comprises of a wide range in delay. A longer delay to diagnosis was more frequently seen in the oldest medical records, when the pathophysiology and characteristics of ALPS were just being discovered.
As described in previous studies, the degree of lymphoproliferation was variable, ranging from lymph nodes of 1 cm up to 4 cm and spleen length from 2.5 to 20 cm below costal margin. However, no correlation was observed between the degree of lymphadenopathy and the degree of splenomegaly. In one patient, splenomegaly was the predominant manifestation of lymphoproliferation. Moreover, splenomegaly regressed in the same degree as lymphadenopathy, independent of therapy.

Over the course of the disease, haemolytic anaemia was most frequently observed of all autoimmune cytopenias (11/12). However, at initial presentation thrombocytopenia was most frequent (9/12). Neutropenia was seen least frequently (5/12). Interestingly, neutropenia was never present alone, but always in association with thrombocytopenia at initial presentation.

Autoantibodies were not consistently assessed in every patient. Any conclusion is therefore to be considered with caution. The DAT test was most frequently performed (9/12). In accordance with other literature data, patients with haemolytic anaemia did not have consistent positive results on the DAT (4/9). Furthermore, if other antibodies were assessed (PA-Ab, HNA, ANA), no association with a clinical history of thrombocytopenia or neutropenia was found.

Autoimmune manifestation in other organs were infrequently seen (2/12).

No patient described herein (n=12) has developed malignancy. In one patient, family history was positive for Non-Hodgkin lymphoma. However, median follow-up time is 7 years to date and 9 out 12 patients are less than 25 years old (median age: 20 years, range: 11-48 years). Malignancy is known to develop later in life. It is important to communicate this life-long risk to the patient and close clinical and imaging follow-up is required. Furthermore, differential diagnosis between newly appearing lymphadenopathies and lymphoma can be a challenge. Patient education of constitutional symptoms (e.g. weight loss, fever, malaise) can be important for an early diagnosis.
Laboratory findings

All patients presented with elevated TCR αβ+ DNTs as a percentage of CD3+ lymphocytes (mean 7.2%). However standard deviation was relatively high (SD: 6.0%) indicating that TCR αβ+ DNT levels were spread out over a large range.

Eight out of 12 patients displayed polyclonal hypergamaglobulinemia. It is important to assess IgG levels, as presence of both polyclonal hypergamaglobulinemia and autoimmune cytopenias is required to fulfil the secondary accessory criteria.

The in vitro lymphocyte assay was performed in 6 out of 12 patients and was found to be defective in 4 out of 6 patients. According to the criteria proposed in 1999, the 2 patients in which the assay was negative in 2 separate assays, would not fulfil the criteria for a diagnosis with ALPS. However, both patients meet one of more of the secondary accessory criteria as proposed in the revised diagnostic criteria of 2009.

IL-10 and IL-18 were not determined in any patient although both are included in the diagnostic criteria and were found to be sensitive biomarkers for ALPS. Furthermore, other biomarkers such as sFasL and vitamin B12 were not consistently assessed in every patient (1/12 and 5/12 respectively). However, diagnostic criteria were only recently revised, whereas medical records ranging from 1998 to present were reviewed.

It is recommended that in the future IL-10, IL-18, sFasL and vitamin B12 are included in the standard diagnostic work-up for ALPS. Additional studies to determine whether IL-10, IL-18 and/or vitamin B12 can be used in evaluating therapeutic efficacy need to be considered.

From 2009 to 2012, in 124 cases ALPS was part of the differential diagnosis. TCR αβ+ DNT levels were found to be elevated in 22.6% (28/124) of all patients. Interestingly, only three out of 28 patients who were found to have elevated TCR αβ+ DNT levels from 2009 to 2012 at UZ Ghent are included in the initial study population (n=12) described herein. This finding supports the hypothesis that ALPS might be more common than previously thought. A subsequent study on the clinical phenotype, other laboratory features and final diagnosis in the remaining 25 patients is recommended.

A male predominance is seen in this descriptive case report study (9/12). However, this finding could be due to small sample size. Interestingly in the larger cohort of 28 patients who were found to have elevated TCR αβ+ DNT levels at UZ Ghent from 2009 to 2012, a male predominance was also seen (75%).
Imaging and histopathology

Periodic ultrasounds are a safe and reliable tool for evaluating the degree of lymphoproliferation. It is therefore recommended for the diagnosis and follow-up in all patients with ALPS. Because ALPS usually first manifests in early childhood, caution is warranted with the use of radiation and repeated CT scans.

At UZ Ghent, a PET-CT scan is performed if an isolated lymph node enlarges, which is suspicious for malignancy. It is not performed for generalised lymphadenopathy.

In a maternal uncle of one patient a lymph node biopsy revealed sinus histiocytosis suggestive for Rosai-Dorfman disease (SHML). However, SHML is included in the differential diagnosis of ALPS. Therefore, clinical awareness is important if clinical and laboratory features are suggestive for ALPS.

Treatment

All patients (n=12) described herein required treatment. Eleven out of 12 patients were treated with Fansidar as a first-line treatment or in the course of the disease. One out of 12 patients required only treatment with a high-dosed course of corticosteroids (Methylprednisolone).

Contradictory to recommendations found in literature, lymphoproliferative symptoms were the main reason for treatment initiation with Fansidar in 3 out of 11 patients. Fansidar was well tolerated. The only toxicity observed was a generalised urticarial rash in one out of 11 patients.

In 4 out of 11 patients, lymphoproliferation and/or autoimmune cytopenias reappeared after cessation of Fansidar and completely regressed again after treatment was restarted. Median treatment duration when Fansidar was discontinued was 32 weeks (range: 4.9 weeks-4.6 years). In other clinical studies on the efficacy of treatment with Fansidar in ALPS, total treatment duration was 12 weeks.

It is possible that the positive evolution of lymphoproliferative symptoms (complete or significant regression) seen upon treatment with Fansidar in 5 out of 11 patients is biased by the natural regression of lymphoproliferation with age. Furthermore, 3 out of 11 patients were receiving concomitant treatment with corticosteroids. It is therefore difficult to determine the individual effect of both treatments on clinical and laboratory features.

Treatment with Fansidar did lead to a complete or incomplete remission of autoimmune cytopenias in one-third of all patients (3/11). Upon treatment with Fansidar, TCR αβ⁺ DNT
levels improved completely or significantly in 5 out of 11 patients. However, larger prospective cohorts or RCTs are needed to determine whether these effects are statistically significant. These studies should preferably include data on sFasL, IL-10, IL-18 and/or vitamin B12.

The observation that corticosteroids are effective in treating autoimmune cytopenias but relapses occur upon tapering of corticosteroids, emphasize the need for effective and well-tolerated therapeutic agents with a long-term effect.

Recent studies have shown that splenectomy and Rituximab, which are commonly used in other refractory autoimmune cytopenias, are relatively contraindicated in ALPS. In the study population described herein, recurrent post-splenectomy cytopenias were seen in one out 2 patients who underwent a splenectomy. Consistent with other literature data, autoimmune cytopenias in 2 out of 12 patients did not respond to treatment with Rituximab or IV immunoglobulin G.

In 3 out of 4 patients with autoimmune cytopenias refractory to Fansidar, clinical and laboratory features normalised upon the use of mycophenolate mofetil or Rapamycin. In the last patient, third-line treatment with the immunosuppressants azathioprine and mercaptopurine led to the regression of autoimmune cytopenias.
CONCLUSION

ALPS is an inherited disorder of abnormal lymphocyte survival that could be more common than originally thought. Patients present with variable clinical phenotype and laboratory findings which can fluctuate over time. Clinical features include lymphadenopathies, hepatosplenomegaly and/or autoimmune cytopenias. Patients also have an increased risk of malignancy.

Over the last decades significant progress has been made in understanding the pathophysiology of ALPS and in developing new diagnostic criteria and therapeutic agents. In the future, more novel insights into the effects of location of the TNFRSF6 gene mutation and the perforin/granzyme pathway on disease penetrance, the origin and function of TCR αβ⁺ DNTs and the utility of biomarkers in diagnosis and therapeutic effect are expected.

ALPS is often misdiagnosed, partially because of its variable phenotype and low awareness in clinical practice. Differential diagnosis with other paediatric haematological disease can also be a challenge. However, making the correct diagnosis of ALPS is important because recent studies have shown that some therapeutic agents commonly used for other refractory non-ALPS autoimmune cytopenias are associated with significant morbidity and mortality in ALPS. Moreover, ALPS is associated with a life-long risk of malignancy. In the development of future therapeutic agents, the long-term effect, positive or negative, on the risk of malignancy should always be taken into account.

Clinical awareness is needed in any child with a combination of unexplained lymphoproliferation and/or autoimmune manifestations and positive family history of autoimmune cytopenias with or without non-malignant non-infectious lymphoproliferation dating back to infancy. Furthermore, ALPS should also be considered as part of the differential diagnosis of T-cell lymphoma, common variable immunodeficiency (CVID), familial hemophagocytic lymphohistiocytosis (FHLH), X-linked lymphoproliferative disease (XLP), systemic lupus erythematosus (SLE), Rosai-Dorfman disease or sinus histiocytosis with massive lymphadenopathy (SHML) and Evans syndrome.

In Figure 2, an algorithm is proposed providing the clinical phenotype suspicious for ALPS and subsequent laboratory tests to diagnose and confirm ALPS.

Assessment of TCR αβ⁺ DNT levels is recommended for initial screening. Elevations of TCR αβ⁺ DNT levels as a percentage of CD3⁺ lymphocytes above 5% are considered pathognomonic for ALPS. In any case where TCR αβ⁺ DNT levels as a percentage of CD3+ lymphocytes are elevated above 2.5% further diagnostic evaluation is recommended.
Polyclonal hypergammaglobulinemia is necessary to fulfil the secondary accessory criteria, in addition to autoimmune cytopenias.

Figure 2: Proposed diagnostic algorithm. Assessment of TCR αβ⁺ DNT levels is recommended for initial screening. Assessment of IgG is necessary to fulfil the secondary accessory criteria, in addition to autoimmune cytopenias. Subsequently, assessing biomarkers is needed to confirm a probable ALPS diagnosis. Additional tests can be performed to confirm an absolute diagnosis but are not required.

Further diagnostic evaluation should consist of assessing sFasL levels, IL-10, IL-18 and/or vitamin B12 levels. Out of these biomarkers, vitamin B12 levels is the easiest and most inexpensive test to perform. The in vitro lymphocyte apoptosis assay may be performed because a positive result may confirm an absolute diagnosis of ALPS. However, the test is not mandatory for a probable diagnosis of ALPS.
Since the costs of genetic testing are not refundable in Belgium and confirmation of a germline or somatic mutation is not mandatory for a probable diagnosis of ALPS, genetic testing is not likely to be performed in all patients. However, genetic testing should always be proposed because it can confirm an absolute diagnosis of ALPS. Furthermore, knowledge of the specific location of the mutation on the TNFRSF6 gene can be useful in predicting future penetrance, severity of clinical phenotype and risk of lymphoma. Histopathologic features are usually specific for ALPS. However, lymph node biopsy is an invasive procedure and should not be performed merely on clinical suspicion.

Furthermore, periodic ultrasound follow-up is recommended for all patients with ALPS. Because ALPS usually first manifests in early childhood, caution is warranted with the use of radiation and repeated CT scans. Furthermore, a PET-CT scan should only be performed when an isolated lymph nodes becomes suspicious for malignancy (enlargement, painful). Fansidar (25 mg phyrimetamine/ 500 mg sulphadoxine) is an anti-malaria drug first used as a treatment for ALPS by Van der Werff et al. (84). In this descriptive case report study a retrospective analysis of effect of treatment with Fansidar on clinical outcome and laboratory results was made. However, several problems are associated with this study design: no additional data on laboratory markers or lymphoproliferation was available for some patients and the effect of time and concomitant use of other therapeutic agents cannot be predicted. Moreover, the sample size was too small to conduct statistical analyses. Nevertheless, complete remission (lymphoproliferation and autoimmune cytopenias) and normalisation of TCR αβ⁺ DNT levels was seen in approximately one-third of all patients (3/11). Larger prospective cohorts or RCTs are needed to determine whether these effects are statistically significant. These studies should preferably include data on sFasL, IL-10, IL-18 and/or vitamin B12.

Based on the findings described herein, for mild to moderate autoimmune cytopenias and lymphoproliferation with obstructive symptoms treatment with Fansidar could be attempted as a first-line treatment. Fansidar has been effective in the treatment of autoimmune cytopenias in some patients. Furthermore, no severe toxicities were described in this descriptive case report study or in other studies investigating the use of Fansidar as a treatment for ALPS. The required dose varies according to weight: for children weighing less than 20 kg, Fansidar should be initiated at a dose of 1 tab per week. For children weighing more than 20 kg, the initial dose should be 2 tab per week. If adverse effects occur, treatment should be discontinued. If autoimmune cytopenias do not improve within
the first 2 weeks of treatment, the dose should be increased with 1 tab. weekly. If autoimmune cytopenias do not regress after one month of treatment with Fansidar, treatment should be discontinued. Subsequently MMF can be administered, which has also proven to be effective in the treatment of autoimmune cytopenias in ALPS. However, patients were found to have only partial response. If autoimmune cytopenias persist upon treatment with MMF, Rapamycin could be attempted as a third-line treatment. Rapamycin is associated with haematological side effects so caution is warranted with the use of Rapamycin in the treatment of ALPS. In patients presenting with severe autoimmune cytopenias, the administration of a high-dosed course of corticosteroids for 1 week is recommended because autoimmune cytopenias can be life-threatening. Concomitantly, treatment with Fansidar could also be attempted. Corticosteroids should be tapered very slowly over 8 to 12 weeks. If autoimmune cytopenias reappear during treatment with Fansidar and/or upon tapering of corticosteroids, corticosteroids should be increased. Furthermore, Fansidar should be discontinued and replaced by Mycophenolate mofetil. Slow tapering of corticosteroids should be reattempted. If autoimmune cytopenias persist upon treatment with Mycophenolate mofetil or upon tapering of corticosteroids, Rapamycin could be attempted as a third-line treatment. Rituximab and splenectomy should only be considered as a fourth-line treatment for highly refractory autoimmune cytopenias. Furthermore, close clinical follow-up for the sometimes life-threatening autoimmune cytopenias, genetic counselling for the recurrence risk in descendants and patient education of constitutional symptoms for the life-long risk of malignancy are important in patients with ALPS.
REFERENCES


93. Kawagoe R Fau - Kawagoe H, Kawagoe H Fau - Sano K, Sano K. Valproic acid induces apoptosis in human leukemia cells by stimulating both caspase-dependent and -independent apoptotic signaling pathways. (0145-2126 (Print)).
APPENDICES

Appendix 1

Patient Data
1. Population Characteristics

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Mededelingen

- V: vrouw
- M: man
- 

Table showing patient data with columns for patient number, birthdate, gender, and family history.
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Note: ALT = Alanine Transaminase, ALP = Alkaline Phosphatase, AST = Aspartate Transaminase, HDL = High-Density Lipoprotein, LDL = Low-Density Lipoprotein.
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Appendix 2

Informed consent

Toestemmingsformulier voor het verzamelen en gebruiken van weefselstalen voor wetenschappelijk onderzoek.

Informatie voor de ouders of voogd van een minderjarige

Beste ouder,

De kans op genezing van een kind met kanker, bloedziekte of ernstige auto-immuunziekte met hematologische weerslag (anemie, trombopenie en/of neutropenie) is de laatste jaren veel verbeterd. Eén van de redenen hiervoor is dat wetenschappers en dokters in het laboratorium onderzoek verrichten op weefselstalen. Weefselstalen zijn cellen die verkregen zijn uit bloed, urine, beenmerg en andere lichaamsvochten van een patiënt, evenals stukjes weefsel verkregen na een operatie (biopsie). Door dit onderzoek komt men te weten waaruit de zieke cellen ontstaan, waarom ze kunnen groeien en hoe ze kunnen vermijd worden. Het hoofddoel is dat de gegevens uit het onderzoek kunnen bijdragen tot een betere kennis en hopelijk een verbetering van de genezing van kanker, bloedziekten en ernstige auto-immuunziekten met hematologische weerslag (anemie, trombopenie en/of neutropenie).

Vanaf de diagnose en gedurende de volledige behandeling van uw kind kunnen, indien u als ouder of voogd toestemt, weefselstalen voor onderzoek verzameld worden. Het verzamelen van eventuele extra weefselstalen zal echter enkel gebeuren op de momenten waarop ook het behandelingsschema een staa名牌 vraagt. Op deze manier wordt het kind niet extra belast onder de vorm van een extra prik.

Tijdens de behandeling en nabehandeling worden een heleboel gegevens van uw kind, zijn of haar ziekte, het onderzoek op de weefselstalen, de behandeling en de reactie van uw kind op zijn of haar therapie, nauwkeurig opgeschreven en bijgehouden.

We vragen aan U als ouder(s)/voogd de toestemming voor:

1. het verzamelen en bewaren van de gegevens i.v.m. de ziekte en de behandeling, evenals voor het gecodeerd verslag uitbrengen bij de gegevensbank.
2. het doorgeven van deze gegevens, eens ze gecodeerd zijn, aan andere artsen en wetenschappers.
3. het gebruik van beenmerg, bloed, urine en andere lichaamsvochten, alsook weefselbioplen voor wetenschappelijk onderzoek dat in relatie staat met hematologie/oncologie en dit na overleg met de behandelende hemato-/oncoloog.
4. het publiceren van resultaten, voortvloeiend uit wetenschappelijk onderzoek, mits de anonimiteit gerespecteerd wordt.

Deze gegevens zullen bij verwerking vertrouwelijk behandeld worden, m.a.w. de persoonlijke gegevens van uw kind (naam, adres, enz.) zullen niet bekend zijn voor de onderzoeker. Men gebruikt een letter/rijfcode om de stalen te identificeren en enkel de behandelende arts kan deze code aan de naam van de patiënt koppelen. De Europese Unie heeft immers richtlijnen die de verzameling en de bewaring van gegevens regelt. De bescherming van de persoonlijke gegevens wordt gewaarborgd door de Belgische wetten van 8 december 1992 over de bescherming van het privé-leven en van 22 augustus 2002 over de rechten van de patiënt.

Een afzonderlijk protocol voor wetenschappelijk onderzoek zal opnieuw worden voorgelegd aan de ethische commissie. Indien U geen toestemming geeft, zal dit geen enkele invloed hebben op de verdere behandeling van uw kind of jullie relatie met de behandelende arts.

Als U meer informatie wil, aarzel dan niet om uw arts te contacteren op volgend telefoonnummer:

.......................................................................................................

1 Weefselstalen zijn bloed, urine, beenmerg en andere weefselvochten, alsook weefselbioplen.
2 Zie cascade minderjarigen.
Cascade minderjarigen

De aangewezen volgorde van de personen die als vertegenwoordiger van een minderjarige toestemming kunnen geven, is als volgt:

1. Indien het kind twee ouders heeft:
   • De toestemming van beide ouders is nodig.
   • De toestemming van één ouder volstaat indien:
     * de andere ouder redelijkerwijze niet kan bereikt worden.
     * de andere ouder in de onmogelijkheid verkeert om zijn wil te kennen te geven.
     * de andere ouder ontzet is uit de ouderlijke macht.

2. Indien het kind slechts één ouder meer heeft:
   • De toestemming van deze ouder volstaat, tenzij deze ontzet is uit de ouderlijke macht.

3. Indien het kind geen ouders meer heeft of beiden in de onmogelijkheid verkeren om hun wil te kennen te geven of beiden ontzet zijn uit de ouderlijke macht:
   • De toestemming van de voogd is noodzakelijk.

4. Indien het redelijkerwijze onmogelijk is om de ouders te bereiken en in situaties waar (nog) geen voogd is aangesteld:
   • Voor dringende therapeutische experimenten kan eventueel beroep gedaan worden op de toestemming van familieleden waar het kind bij verblijft.
   • In niet dringende gevallen dient de aanstelling van een voogd of een voogd ad hoc uitgesloten te worden.

5. Bij minderjarigen ouder dan 12 jaar is steeds ook hun persoonlijke toestemming noodzakelijk, naast de toestemming van de hierboven beschreven personen.

Uit het toestemmingsformulier voor de voogd, verwant, of wettelijke vertegenwoordiger van de patiënt dient men duidelijk te kunnen afleiden dat deze volgorde wordt gerespecteerd. Daarom dient bij de naam van de voogd, verwant of wettelijke vertegenwoordiger van de patiënt de relatie tot de patiënt vermeld te worden, alsook de reden waarom deze volgorde niet werd gerespecteerd. Daarom is het goed om in het informed consent de te respecteren lijst op te nemen.
Toestemmingsformulier

................................. heeft mij mondeling/schriftelijk\(^3\) informatie gegeven over het verzamelen, bewaren en doorgeven van weefselstalen voor wetenschappelijk onderzoek.

Graag aankruisen:

1. Ik/Wij bevestigen dat we de informatie gekregen, gelezen en verstaan hebben. □

2. Ik/Wij vinden de informatie voldoende. □

3. Ik/Wij stemmen toe dat de gegevens gecodeerd worden verzameld en bewaard in de gegevensbank. □

4. Ik/Wij stemmen toe dat de gecodeerde gegevens, onder voorwaarden zoals vermeld in de informatiebrief, worden doorgegeven. □

5. Ik/Wij gaan akkoord dat bloed, urine, beenmerg en andere lichaamsvochten, alsook weefselbiopsten, gebruikt mogen worden voor wetenschappelijk onderzoek, na goedkeuring van het ethisch comité. □

6. Ik/Wij gaan akkoord dat, indien wij wensen dat de stalen van ons kind niet meer mogen gebruikt worden, ik/wij dat laten weten aan de behandelende arts in het behandelingscentrum. □

7. Ik/Wij gaan akkoord dat resultaten van het onderzoek anoniem gepubliceerd mogen worden. □

\(^3\) Schrappen wat niet van toepassing is.
1. Naam van beide ouders bij minderjarigen:

naam ouder 1: ............................................. naam ouder 2: .............................................
datum: ...................................................... datum: ......................................................
handtekening ouder 1: ...................................................... handtekening ouder 2: ......................................................

2. Naam voogd/verantwoordelijke/wettelijk vertegenwoordiger:

naam: ........................................................................
datum: ........................................................................
relatie tot patiënt: ..........................................................
reden van aanstelling: ......................................................
handtekening: ..............................................................

3. Arts:

naam: ........................................................................
datum: ........................................................................
handtekening: ..............................................................

---

4 Zie cascade minderjarigen.
Instemmingsformulier voor het verzamelen en gebruiken van weefselstalen voor wetenschappelijk onderzoek.

Informatie voor een minderjarig kind (12-17 jaar)

Beste jongere,

De kans dat je van kanker, een bloedziekte of een ernstige auto-immunziekte met een weerslag op je bloedbestandstelling (te kort aan rode bloedcellen, bloedplaatjes en/of witte bloedcellen) geneest, is de laatste jaren veel groter geworden. Eén van de redenen hiervoor is dat wetenschappers en dokters in het laboratorium zieke cellen kunnen onderzoeken. Men onderzoekt cellen van zieke kinderen uit bloed, urine, beenmerg en andere lichaamsvochten. Ook biopaten (stukjes weefsel verkregen na een operatie) worden onderzocht. Zo komen ze te weten hoe de zieke cellen ontstaan, waarom ze kunnen groeien en hoe ze kunnen vernietigd worden. Het doel van het onderzoek is meer te weten te komen over de ziekte en zo de genezingskansen van de patiënten te vergroten.

Onze vraag naar jou toe is of we wat gegevens en weefselstalen van jou mogen verzamelen. Dit start bij het stellen van de diagnose en loopt verder gedurende de volledige behandeling. De afname van zo'n staal gebeurt tijdens standaard onderzoeken van de diagnose en behandeling en zal dus nooit leiden tot een extra prik of pijnlijke gebeurtenis. Alle gegevens en stalen worden nauwkeurig bijgehouden voor verder wetenschappelijk onderzoek. De resultaten worden steeds gecodeerd (dit wil zeggen zonder dat jouw naam aan anderen bekend gemaakt wordt) doorgegeven en gepubliceerd.

We vragen aan jou toestemming voor:

1. het verzamelen, bewaren en gecodeerd verslag uitbrengen van gegevens over jouw ziekte en behandeling.
2. het gecodeerd gebruiken van deze gegevens door andere artsen en wetenschappers waarbij enkel jouw behandende arts jouw naam kent.
3. het gebruiken van jouw bloed, urine, beenmerg en andere lichaamsvochten, alsook weefselbiopaten voor wetenschappelijk onderzoek dat in relatie staat met kanker, kwaadaardige bloedziekten of ernstige auto-immunziekten en dit steeds na het vragen van goedkeuring van het ethisch comité.
4. het anoniem publiceren van resultaten van het onderzoek waardoor niemand jouw naam kent.

Indien je geen toestemming geeft, zal dit geen invloed hebben op jouw verdere behandeling of de relatie met jouw behandelende arts.

Heb je hierover nog vragen, dan kan je altijd terecht bij jouw dokter:

........................................................................................................................................

5 Weefselstalen zijn bloed, urine, beenmerg en andere weefselvochten, alsook weefselbiopaten.
Instemmingsformulier

........................................................................................................................................................................
heeft mij mondeling/schriftelijk\(^9\) informatie gegeven over het verzamelen, bewaren en doorgeven van weefselstalen voor wetenschappelijk onderzoek.

Graag aankruisen:

1. Ik bevestig dat ik de informatie gekregen, gelezen en verstaan heb. □

2. Ik vind de informatie voldoende. □

3. Ik stem toe dat de gegevens gecodeerd worden verzameld en bewaard. □

4. Ik stem toe dat de gecodeerde gegevens, onder bepaalde voorwaarden zoals vermeld in de informatiefbrief, worden doorgegeven. □

5. Ik ga akkoord dat bloed, urine, beenmerg en andere lichaamsvochten, alsook weefselbijecten, gebruikt mogen worden voor wetenschappelijk onderzoek, na goedkeuring van het ethisch comité. □

6. Ik ga akkoord dat, indien ik wens dat mijn stalen niet meer mogen gebruikt worden en ik meerderjarig geworden ben, ik dat zelf laat weten aan de behandelende arts in het behandelingscentrum. □

7. Ik ga akkoord dat resultaten van het onderzoek anoniem mogen gepubliceerd worden. □

naam minderjarige ouder dan 12 jaar

........................................................................................................................................................................

naam arts

........................................................................................................................................................................

\(^9\) Schrappen wat niet van toepassing is.
Appendix 3

UZ Ghent’s Ethics Committee approval