The challenge of early diagnosis of autoimmune lymphoproliferative syndrome in children with suspected autoinflammatory/autoimmune disorders

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ABSTRACT

Objectives: to test the usefulness of an extended panel of lymphocyte subsets (LS) in combination with Oliveira's diagnostic criteria for the identification of autoimmune lymphoproliferative syndrome (ALPS) in children referred to a pediatric rheumatology center.

Methods: patients referred from 2015 to 2018 to our Rheumatology Unit for an autoimmune or autoinflammatory condition were retrospectively analyzed. Oliveira's required criteria (chronic lymphoproliferation and elevated DNT) were applied as first screening. Flow cytometry study included double negative CD4-CD8-TCR αβ+T lymphocytes (DNT), CD25+CD3+, HLA−DR+CD3+T cells, B220+T-cells, and CD27+B cells. Data were analyzed with an univariate logistic regression analysis, followed by a multivariate analysis. Sensitivity and specificity of the Oliveira's required criteria were calculated.

Results: 264 patients were included in the study and classified as: i) autoimmune diseases (26); ii) juvenile idiopathic arthritis (JIA) (35) iii) monogenic systemic autoinflammatory disease (SAID) (27); iv) PFAPA syndrome (100); v) systemic undefined recurrent fever (SURF) (45); vi) undetermined-SAID (14); vii) ALPS (17). Oliveira's required criteria displayed a sensitivity of 100% and specificity of 79%. When compared to other diseases the TCRαβ+B220+ lymphocytes were significantly increased in ALPS patients. The multivariate analysis revealed 5 clinical/laboratory parameters positively associated to ALPS: splenomegaly, female gender, arthralgia, elevated DNT and TCRαβ+B220+lymphocytes.

Conclusions: Oliveira's required criteria are useful for the early suspicion of ALPS. TCRαβ+B220+ lymphocytes should be added in the diagnostic work-up of patients referred to pediatric rheumatology unit for a suspected autoimmune or autoinflammatory condition, providing a relevant support in the early diagnosis of ALPS.

Keymessages

1. ALPS might present a prevalent inflammatory phenotype and should be considered in the differential diagnosis
2. Required Olivera's criteria might help in the preliminary screening for suspected ALPS
3. An enlarged cytofluorimetric panel should be included in the diagnostic work-up in tertiary referral centers

Keywords: autoimmune lymphoproliferative syndrome, ALPS, lymphocyte subsets, autoimmune diseases, autoinflammatory syndromes

Introduction:

Autoimmune lymphoproliferative syndrome (ALPS) is a rare hematological disorder characterized by an altered lymphocyte homeostasis due to a defective apoptotic mechanism leading to an abnormal lymphoproliferation, autoimmunity, and an increased risk of lymphoma (1). It usually presents in childhood, with signs of lymphoproliferation (lymphoadenopathies, hepatosplenomegaly), and autoimmune cytopenias (autoimmune haemolytic anemia, neutropenia or thrombocytopenia) (2).This condition is characterized by the accumulation of abnormally active lymphocytes in lymphoid organs and to the persistance of autoreactive cells, with an increase in TCRαβ+CD4-CD8- double negative T (DNT) lymphocytes (3;4). These cells are a hallmark of ALPS and other ALPS-like disorders. Other laboratory abnormalities are: elevated gamma-globulins, vitamin B12, interleukin-10 (IL-10), interleukin-18 (IL-18) and soluble Fas ligand (sFASL) (5). The diagnosis of ALPS (definitive or probable) is based on the criteria revised by Oliveira et al. in 2009 (6). Genetic mutations in the Fas pathway are the main cause of ALPS. The most frequent cause is represented by germline autosomic dominant mutations of FAS gene (ALPS-Fas), followed by Fas somatic mutations (ALPS-sFAS), germline mutations in Fas

Ligand (ALPS-FASLG) and in Caspase 10 (ALPS-CASP10). Patients meeting all diagnostic criteria but with no genetic mutations are classified as undefined ALPS (u-ALPS) (6).

Beside the well established ALPS cases, a large number of patients present clinical phenotypes overlapping with ALPS and carrying mutations of other genes. This broad category is often named as ALPS-like disorder and includes: Caspase-8 deficiency state,CEDS; Ras-associated autoimmune leukoproliferative disorder, RALD; Dianzani autoimmune lymphoproliferative disease,DALD; Xlinked lymphoproliferative disease,XLP1; p110delta activating mutation causing senescent T cells, lymphadenopathy, and immunodeficiency, PI3K delta syndrome; *CTLA-4* haploinsufficiency with autoimmune infiltration,CHAI; gain-of-function *STAT3* mutations; *LRBA* deficiency with autoantibodies; regulatory T-cell defects, autoimmune infiltration, and enteropathy, LATAIE (5-9).

In ALPS, flow cytometry plays a pivotal diagnostic role. Beside increased DNT cells, mandatory for the diagnosis, other LS result altered in ALPS patients. An elevation of B220+ T cells (1;10;11), together with an expansion in HLA-DR+ T cells (7;12), CD8+/CD57+ T cells, CD5+ T cells, and a decrease of CD4+/CD25+ T cells and CD27+ B cells has been reported (2;7;12).

For this reason, an extended flow cytometry panel has been proposed for ALPS, including DNT cells, CD25+CD3+/HLA−DR+CD3+ cells ratio, B220+ T-cells, and CD27+ memory B cells (13).

In a pediatric rheumatology Unit, the diagnosis of ALPS is rather challenging due to the frequent overlap of the main clinical manifestations (lymphoprolipheration) with many autoimmune and autoinflammatory diseases.

Oliveira's criteria (6) are commonly used for the clinical diagnosis of ALPS. However primary (apoptosis test, genetic analysis for fas) and secondary accessory criteria (IL-10,

IL-18, sFas) are not easily available in real life because few laboratories can routinely provide them.

In the present study we evaluate the incidence of ALPS in the context of a third line pediatric rheumatology clinic according to the Oliveira's diagnostic criteria and their usefulness in ALPS early identification. A novel enlarged panel of lymphocyte subsets (LS) has been also evaluated.

Patients and Methods

The clinical data of patients referred to the pediatric rheumatology Unit of Giannina Gaslini Hospital for a suspicion of autoimmune or autoinflammatory disease from October 2015 to April 2018, were retrospectively analyzed. Data on clinical manifestations, laboratory workup including LS, genetic analysis, treatments and final diagnosis were collected.

The clinical features and laboratory parameters at the moment of the diagnostic work-up and before the initiation of any continuous treatment with disease modyfing drugs or steroids were retrieved from the clinical charts.

Additional laboratory data were: complete blood count (CBC), Coombs direct test, acute phase reactants, B12 vitamin and cytolysis markers. Whenever requested, a lymphocyte apoptosis test was performed, as already described (14).

Whenever present, data about genetic studies were collected. For the common autoinflammatory diseases sanger sequencing or 10 or 40-gene Next Generation Sequencing (NGS) panels were used (15-17). For the ALPS/ALPS-like related genes, a 315 genes panel including the main immune dysregulation syndromes, autoinflammatory diseases and primary immunodeficiencies was applied (18).

The response to the different treatments used during patients follow-up was also recorded. According to previous studies (19-20), response to treatment was classified as: i)

complete: clinical remission in monotherapy and normalization of acute phase reactants; ii) partial: amelioration of the clinical and laboratory parameters or need of additional treatment (NSAIDs, steroids) to achieve a complete remission; iii) failure: lack of response.

Oliveira's criteria: Oliveira's required criteria (lymphoproliferation, DNT) (Supplementary Table 1) were retrospectively applied for a rapid evaluation of all patients. Once applied to all the 264 patients, sensitivity and specificity of the required criteria were calculated. In those who presented a positivity of the required criteria, primary and secondary accessory criteria were applied whenever possible.

Cytofluorimetric analysis: LS were routinely performed during the diagnostic work-up. Cytometric evaluation of LS was performed by an eight-color immunostaining panel and by *lyse and wash* procedure. Briefly, 50 µl of EDTA anticoagulated whole blood was incubated with surface fluorochrome-labelled monoclonal antibodies (mAbs) for 20 min at 4°C and lysed with FACS Lysing solution (Becton Dickinson, BD) for 10 min at room temperature (RT). Data acquisition and analysis was performed on a FACSCanto II flow cytometer (BD) equipped with three lasers a Blue (488-nm, air-cooled, 20-mW solid state), a Red (633-nm, 17-mW HeNe), a Violet (405-nm, 30-mW solid state) and FACS Diva™ software (BD). Peripheral LS were evaluated using the following RUO mAbs: CD3, CD4, CD8, CD16-56, CD19, CD20, CD27, TCRαβ, TCRγδ, HLA-DR, CD25, CD45, CD45RO, CD45RA, CD45B220 (10) (all BD).

Fluorochromes differently combined for eight colors antibody panels were: APC, APC-H7, fitc, pe, pe-Cy7, PerCP-Cy5.5, V450, V500.

With various combinations of the listed antibodies, we analyzed in particular four parameters that, though not part of the classical diagnostic criteria, are however described as common laboratory findings and suggested as additional screening markers in other centres (13):

1. $TCR\alpha\beta^{+}CD4^{-}CD8^{-}$ (DNT) > 1,5% of total T lymphocytes

2. $B220^+$ αβ DNTCs > 60%
3. $CD3^+CD25^+$ / $CD3^+$HLA DR^+ Ratio <1
4. $CD27^+$ B cells <15%

Patients classification. According to the final diagnosis, patients were allocated into 7 groups. 1. Autoimmune diseases (AID); 2. Juvenile Idiopathic Arthritis (JIA); 3. Monogenic Systemic Autoinflammatory diseases (M-SAID); 4 Periodic Fever, Aphthous stomatitis, Pharyngitis and Adenitis syndrome (PFAPA)(21); 5.Systemic Undefined Recurrent Fever (SURF) (17); 6. Undefined Systemic AutoInflammatory Diseases (UND-SAID), clinical conditions characterized by systemic chronic o subchronic inflammatory features of undefined nature, not necessarily associated with fever; 7. ALPS definitive or ALPS-probable, diagnosed according to Oliveira's criteria (6)

Exclusion criteria: patients lost at follow up, with insufficient clinical and laboratory data were excluded. Moreover, patients with other diseases that could not be included in the 7 groups above were also excluded (i.e uveitis, alopecia, pericarditis, primary immunodeficiencies, metabolic diseases).

Statistical analysis: Descriptive statistics are reported as medians (range) and means (± standard deviation) for continuous variables and as absolute frequencies and percentages for categorical variables.

Comparisons of disease characteristics between patient groups were performed by chi-square test, or the Fisher's Exact test, as appropriate, in case of categorical data, while a one-way analysis of variance (ANOVA) for normally distributed variables, or a Kruskall Wallis test, when the assumption of normality was not satisfied, were used. Post-hoc comparison test was used for inter-group comparison (Bonferroni or Dunn tests, as appropriate).

A logistic regression analysis was used to assess the influence of the demographic, clinical, laboratory, genetic and treatment regimen characteristics on the probability to

achieve an ALPS diagnosis. Correlation between all potential prognostic factors were tested to assess collinearity. The presence of collinearity was managed by eliminating from the analyses the factor that was judged to be the least important from a clinical perspective. Only factors significantly associated with the outcome at univariate analysis ($p<0.05$) were included in a multivariate model with a stepwise procedure. All analyses were carried out using the SAS software version 9.3 (Institute Inc., Cary, NC, USA).

Results

The data of 475 patients referred to our Department for a suspicion of an autoimmune or an autoinflammatory disease from October 2015 to April 2018, were analyzed. In total, 211 patients were excluded from the study (see Figure 1), while the remaining 264 patients were stratified in the following 7 groups.

1. **AID:** 26 patients (10 juvenile systemic lupus erythematosus (SLE); 3 Mixed Connective Tissue Diseases (MCTD) ; 6 juvenile dermatomyositis (DM); 5 Behçet disease; 1 Sjogren syndrome; 1 Kawasaki disease);
2. **JIA:** 35 patients (8 oligoarticular; 16 polyarticular; 11 systemic);
3. **M-SAID:** 27 patients: 17 Familial Mediterranean fever (FMF); 3 Mevalonate Kinase deficiency Disease (MKD); 1 TNF-receptor associated Periodic Syndrome; 4 Deficiency of Adenosine Deaminase 2; 2 STING-Associated Vasculopathy with onset in Infancy;
4. **PFAPA syndrome**: 100 patients;
5. **SURF**: 45 patients;
6. **UND-SAID**: 14 patients;
7. **ALPS** or **ALPS-probable**: 17 patients (5 ALPS-definitive, 12 ALPS-probable).

The demographic characteristics of the patients are are shown in Table1.

The clinical and laboratory features observed in each group are reported in Supplementary Table 2. For all clinical manifestations, the $\chi 2$ test revealed a significant statistical difference among the 7 groups ($p<0.001$), except for the cardio-respiratory manifestations

(p<0.14). Arthralgia was observed in 76% of ALPS patients. Lymphoadenopathy and splenomegaly were observed in all ALPS patients and with a lesser extent in other subgroups (Supplementary Table 2). Hematological features were present in 69% of ALPS patients, but also in UND-SAID (60%) and AID (58%). A defective lymphocyte apoptosis test was found in 5 ALPS patients (ALPS definitive).

Genetics: M-SAID were identified by Sanger analysis or NGS-based gene panels, as previously reported (15-18). Five out of 17 ALPS patients were found to carry mutations each in one of the following genes: FAS (p.Cys129Arg), CTLA4 (p.Cys58Serfs*13), TNFRSF13B (p.Cys104Arg), PIK3CD (p.Ser312Cys), and NRAS (a somatic p.Gly13Asp mutation) (Papa et al in preparation). .

Lymphocyte subsets: ALPS patients displayed a significantly lower absolute number of TCD3+ lymphocytes, TCD4+ helper lymphocytes, TCD8+ cytotoxic lymphocytes and B lymphocytes (Supplementary Table 3). Similary, patients with ALPS and autoimmune diseases showed a lower absolute number of T memory lymphocytes and circulating T regulatory cells when compared to the other groups (Supplementary Table 4).

The behaviour of the expanded LS specific for ALPS for each disease group is reported in Figure 2.

As expected, DNT were elevated in all ALPS patients (required criterion) with a median value of 2.6% (range 1.6-6.3). However, increased DNT were also observed in other groups, such as UND-SAID (60%), AID (42%), M-SAID (36%), and PFAPA (27%), even if with a lower percentage compared to ALPS patients (Figure 2).

Among the LS recently proposed as additional markers for ALPS (13), increased TCRαβ+B220+ lymphocytes and a reduced CD3CD25+/CD3HLADR+ ratio were observed in 82% and 76% of ALPS patients respectively (Supplementary table 5). When compared to other groups, increased TCRαβ+B220+ lymphocytes were the only LS significantly

higher in ALPS patients (p0.006). Conversely, B memory CD27+ lymphocytes did not differentiate ALPS from the other groups (Figure 2).

Performance of Oliveira's criteria: a total of 68 patients met the required criteria (lymphoproliferation and elevated DNT): 3 Behçet, 2 LES, 1 MCTD, 1 SJIA, 4 FMF, 1 MKD, 27 PFAPA, 9 SURF, 3 UND and, of course, all 17 ALPS patients (Figure 3). Sensitivity and specificity of the required criteria were 100% and 79% respectively.

Among ALPS patients, 5 met the primary accessory criteria to be defined ALPS-definitive (defective apoptosis test or FAS mutation), and 12 met the secondary accessory criteria to be defined ALPS probable. In the 51 non-ALPS patients the accessory criteria were retrospectively applied only in those patients who did not have a clear alternative diagnosis, such as patients with undefined autoinflammatory diseases.The 3 UND-SAID patients that met the Oliveira's required criteria had a relevant and persistent inflammatory phenotype with recurrent periodic fevers and rash and, even if in presence of lymphoproliferation and elevated DNT, did not meet the secondary accessory criteria: they had a normal apoptosis test and underwent genetic analysis for FAS and other ALPS related disorders that resulted negative. They did not respond to MMF or sirolimus but had a better response to anti IL-1 or anti IL-6 treatment. All patients with SURF displayed an optimal response to colchicine, that is not characteristic of ALPS.

No statistical significance was observed in the expression of the additional marker for ALPS (CD3CD25+/CD3HLADR+, TCRαβ+B220+ lymphocytes, B memory CD27+ lymphocytes) between the patients positive or negative for the Oliveira's criteria (Supplementary Table 6). A trend towards an higher expression of B220+ T lymphocytes, was observed in patients positive for Olivera's criteria, suggesting the hypothesis of an over-expression of this marker in patients with lymphoproliferation and increased DN T lymphocytes. However, among patients fulfilling the Olivera's criteria, B220+ T

lymphocytes were significantly increased in ALPS patients compared to patients with other diagnosis (Supplementary Table7).

Identification of clinical and laboratory features suggestive of a possible ALPS

We subsequently analyzed which clinical and laboratory variables could be predictive of ALPS in patients referred to a pediatric rheumatology clinic. The results of the univariate analysis are shown in Supplementary Table 8. Splenomegaly (OR=52.85), lymphoid manifestations (OR=17.88), periorbital oedema (OR=11.61) and generalized lymphadenopathy (OR=11.46) were positively associated to ALPS, whereas the absolute number of CD3+ lymphocytes, CD3+TCRalfabeta+ lymphocytes and total lymphocytes were negatively associated to ALPS (OR=0.999).

The multivariate analysis revealed 5 clinical/laboratory parameters that showed the higher independent association with ALPS in the analyzed cohort of patients: splenomegaly (OR 626.05, 95%CI: 14.55-1000), female gender (OR=104.39, 95%CI: 2.82-860.18), elevated DNTs (OR=8.04, 95%CI:2.11-30.62), arthralgia (OR=63.62, 95%CI: 1.46-385.42) and elevated αβ+B220+ T lymphocytes (OR=1.23, 95%CI: 1.07-1.43) (Table 2). Notably, cytopenia and haemolytic anemia were predictive of ALPS only in the univariate analysis, wheras they were not confirmed in the multivariate analysis.

Response to treatment in ALPS patients: In Supplementary Figure 1, the therapeutic strategies adopted in the 7 groups is reported. The response to treatment of the 17 ALPS patients is reported in Figure 4. Steroids were only partially effective. Sirolimus and MMF were the most effective drugs, with a complete or partial response in all treated patients. Two ALPS-probable patients, characterized by a severe inflammatory phenotype, also received biologics DMARD (anti-IL-1 and anti-TNF), with no clear effect. All of them displayed a good subsequent response to MMF and Sirolimus.

Discussion

Our study reports on the incidence of ALPS and ALPS-like syndrome in a tertiary pediatric rheumatology center, on the usefulness of the Oliveira's required diagnostic criteria and of an expanded panel of LS in the diagnostic work-up of patients referred for a suspicion of an autoinflammatory or autoimmune disorder.

The diagnosis of ALPS in the context of a pediatric rheumatology Unit is rather challenging: when considering patients with inflammatory manifestations, the spectrum of conditions that fall in the differential diagnosis is very broad, ranging from a number of autoimmune and autoinflammatory diseases to haemato-oncological and infectious conditions.

The results of our study show that ALPS might present with a prevalent inflammatory phenotype without clear hematological manifestations in many patients. Therefore ALPS must be carefully considered in the differential diagnosis of many autoimmune and autoinflammatory conditions. In 3 years of observation,17 of 264 patients (approximately 6%), received the final diagnosis of definitive or probable ALPS.

Usually, ALPS is mainly observed in a pediatric hematological setting, being typically characterized by the association of cytopaenia and lymphoproliferative manifestations. Our study shows that a significant number of ALPS patients may present with a consistent inflammatory phenotype (recurrent fever) or with symptoms frequently found in rheumatic and autoinflammatory conditions, such as lymphoid organ enlargement and arthralgia. Therefore, it is important to include the search for ALPS in the diagnostic work-up of patients referring to a pediatric rheumatology unit. In this line, the systematic search of the Oliveira's required diagnostic criteria (lymphoproliferation and elevated DNT) and of an extended panel of lymphocyte subsets, may represent a valid initial screening for the identification of a possible ALPS.

In our study 68 patients (20% of the whole cohort) satisfied the required Olivera's criteria raising the suspicion of a possible ALPS. Signs of lymphoproliferation may be observed in

different rheumatic or autoinflammatory diseases, such as lymphoadenopathies in PFAPA syndrome (22) and splenomegaly in many autoimmune conditions and in systemic onset JIA. Moreover, it is known that the expansion of DNT, for a long time considered as a pathognomonic marker of ALPS, can be found also in other disorders including rheumatic diseases such as SLE, MCTD and JIA (23). This was also observed in our study, with 30% of patients (74/264) displaying DNT levels greater than 1.5% of total lymphocytes. These findings suggest that, at least in the context of inflammatory and autoimmune conditions, the cut-off proposed by Oliveira et al. (6) could be increased, in order to obtain a higher specificity.

In this study we also analyzed the possible usefulness of an expanded panel of LS, as suggested by Lenardo et al (13). Increased B220+T lymphocytes were significantly associated with ALPS, and the good specificity of this marker is confirmed by its inclusion as independent variable in the multivariate analysis.This marker is expressed by T cells in the phases immediately preceding cellular apoptosis (24). This result may suggest that this parameter should be included in the standard of care diagnostic work-up to raise the suspicion of ALPS and to increase the specificity of the cytofuorimetric analysis.

Besides the presence of signs of lymphoproliferation and cytofluorimetric variables (increased DNT and B200+ T lymphocytes), the multivariate analysis indicated that female gender and arthralgia are factors that may further guide the suspicion of a possible ALPS. Also the presence of cytopenia, even if observed in several other autoimmune conditions, should be considered as a further sign of suspicion.

In daily practice, the combination of the required Oliveira's criteria and the expanded LS might help identifying those patients that most likely are affected by ALPS. In these patients, in the absence of an evident alternative diagnosis, the work-up should be completed with additional laboratory examinations (test for fas-mediated apoptosis, genetics, vitamin B12 etc) that are requested to achieve the diagnosis of ALPS.

Our study presents some limitations. The retrospective analysis might be associated to some bias in the patients' selection. Due to referral bias, our series selected ALPS patients with a prevalent inflammatory and lymphoproliferative component, in which cytopenia has rarely represented a prevalent or persistent feature. Moreover, even if LS were performed in a routinary diagnostic setting, it is conceivable that, in some disease groups such as JIA with a clear and isolated articular involvement, the test was not performed in all patients during the diagnostic work-up, and this has surely over-estimated the incidence of ALPS in our population. In this retrospective setting, having applied Oliveria's required criteria only for a rapid use in a real life setting might be also considered as a limiting bias. However, this choice was made in order to verify the possible usefulness of this simple approach which may be easily employed in daily practice in reference centers.

An other limitation of the study is the lack of a more detailed analysis for the possible search of somatic mutations in "genetically-negative" ALPS patients, especially in the FAS gene (25). Only one patient was found to be carrier of a somatic mutation of NRAS, initially suspected after whole exome sequencing and then confirmed by Sanger sequencing in DNA sampled from different sources. Deep targeted sequencing analysis on DNT is in progress to verify this possibility.

In conclusion**,** on top of Oliveira's criteria, the routinary use of the cytofluorimetric analysis, including DNT and B220+T lymphocytes, might provide a further relevant support for the diagnosis of ALPS in the context of a pediatric population presenting with a more prominent inflammatory phenotype and may prompt the use of the most specific and effective treatments, namely MMF and sirolimus (9;26-29).

Funding: No specific funding was received from any bodies in the public, commercial or not-for-profit sectors to carry out the work described in this article

Competing interests: The authors declare no conflict of interests.

Ethical approval information The study was approved by the Istituto Giannina Gaslini ethical review board .

Data sharing statement: All data relevant to the study are included in the article or uploaded as supplementary information. Data are available upon reasonable request

Patient and Public Involvement. The local patients' association (AMRI) was involved in the design of the study and in the illustration of the main results.

Acknowledgments : the work was supported by Ricerca Corrente Ministeriale of the Italian Minister of Health. LOM was supported from the European Society of immunodeficiencies for long term fellowship in autoinflammatory diseases at Giannina Gaslini Hospital. IRCCS G. Gaslini is member of the European Reference Network for Rare Immunodeficiency, Autoinflammatory and Autoimmune Diseases -Project ID No 739543."

Contributorship

Concept and design: MG, MM, LOM, CD

Acquisition of data: CMC, LOM, MB, FC

Analysis, or interpretation of data: CMC, LOM, FC, PT, FB, IC, AG, SV, RC, MB, FF, EP, DG, MM, MG, CD, AR

Drafting of the manuscript: CMC, LOM, MG, MM, CD,

Critical revision of the manuscript for important intellectual content: All co-authors

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Figure Legends:

Figure 1. Flowchart of the diagnostic work up of 475 patients enrolled in a Pediatric Rheumatology tertiary referral center

Figure 2. Lymphocyte subsets: ALPS-specific parameters in all the groups. Normal Values: CD3+TCRαβ+ CD4-CD8- <1,5%; B CD27+>15%; TCD3CD25+/CD3HLADR >1%; TCR αβ+ B220+<60%, Heterogeneity test §p=0.006, # p<0.0001; *statistically significant differences in the post-hoc analysis between ALPS and the other groups

Figure 3. Performance of the Oliveira's criteria in all patients. SLE, Systemic lupus erythematosus; MCTD,mixed connective tissue disease; sJIA, systemic juvenile idiopathic arthritis; MKD, mevalonate kinase deficiency; PFAPA, Periodic Fever, Aphthous stomatitis, Pharyngitis and Adenitis syndrome; SURF, Systemic Undefined Recurrent Fever; UND-SAID Undefined Systemic AutoInflammatory Diseases

Figure 4. Treatments used in ALPS/ALPS-like patients. MMF Mofetil Mycofenolate, MTX methotrexate, CsA cyclosporine A, AZA azathioprine, Biologics: anti IL-1 and anti-TNF

Supplementary Figure 1. Treatments used. Immunosuppressive Agents: MTX, Azathioprine, Cyclosporine, MMF, Sirolimus; Biological Drugs: anti IL-1: Anakinra, Canakinumab; anti-TNF**:** Infliximab, Etanercept, Adalimumab; AIV autoimmune diseases and vasculitis; JIA juvenile idiopathic arthritis; M-SAID monogenic systemic

autoinflammatory diseases; PFAPA Periodic Fever, Aphthous stomatitis, Pharyngitis and Adenitis syndrome; SURF Systemic undifferentiated recurrent fevers; UND-SAID Undetermined systemic autoinflammatory diseases; ALPS autoimmune lymphoproliferative syndrome

Table 1. Demografic features

	N° pts	***Male***	***Female***	***Age of Onset Median***	***Age of Onset Interval***	***Duration of Illness - Median***	***Duration of Illness - Interval***
AID	26	8 (31%)	18 (69%)	9.3	0.5-15.3 a	3.5	<1-10.0 y
JIA	35	8 (23%)	27 (77%)	4.0	0.5-15.3 a	3.5	<1-20.0 y
M-SAID	27	16 (59%)	11 (41%)	2.0	0.3-15.7 a	5.0	<1-20.0 y
PFAPA	100	61 (61%)	39 (39%)	1.6	0.0-16.6 a	2.0	<1-9.0 y
SURF	45	22 (49%)	23 (51%)	2.0	0.3-12.0 a	3.0	<1-16.0 y
UND-SAID	14	7 (50%)	7 (50%)	8.0	0.3-15.6 a	8.0	<1-23.0 y
ALPS	17	4 (24%)	13 (76%)	4.0	1.0-11.0 a	4.0	1.0-11.0 y

Table 2. Multivariate analysis. *Area under the curve 0.990*

Variable	***OR (95% CI)***
Splenomegaly	626.05 (14.55-1000)
Female Sex	104.86 (2.82-860.18)
CD3+TCR αβ CD4-CD8-	8.37 (2.11-30.62)
Arthralgia	63.62 (1.46-385.42)
TCR αβ+ B220+	1.23 (1.07-1.42)

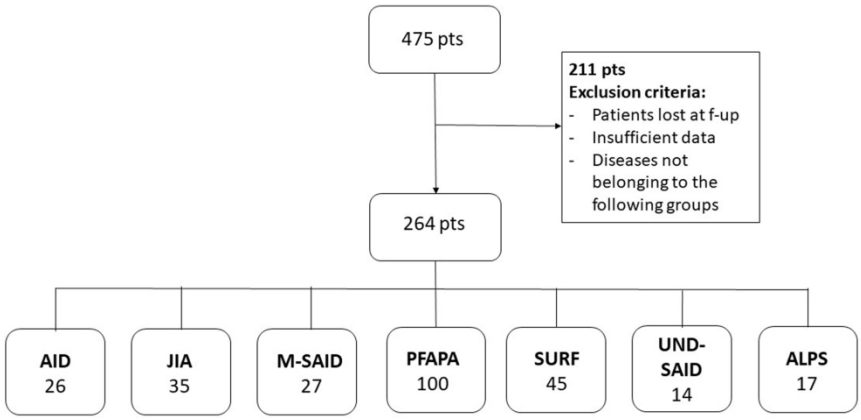

Figure 1

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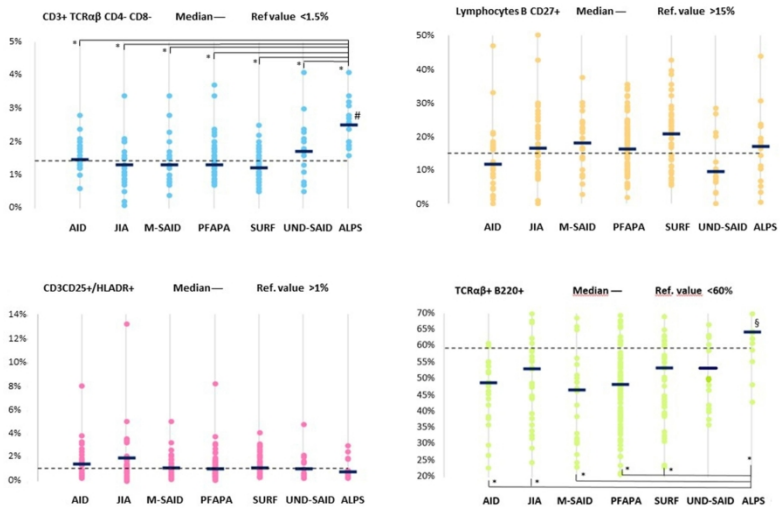

Figure 2

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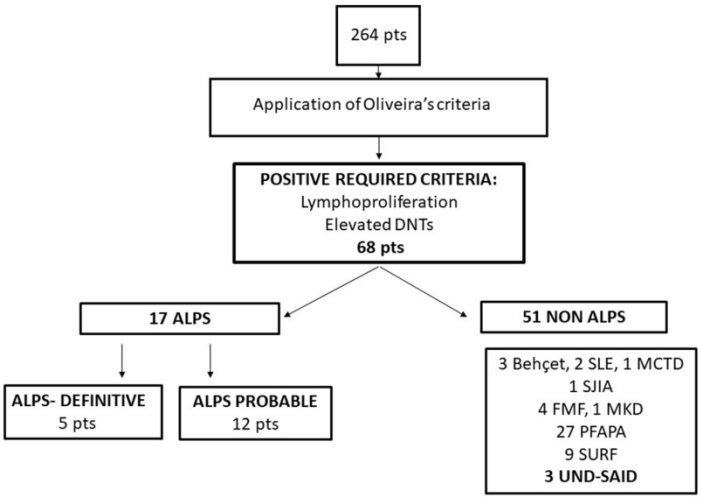

Figure 3

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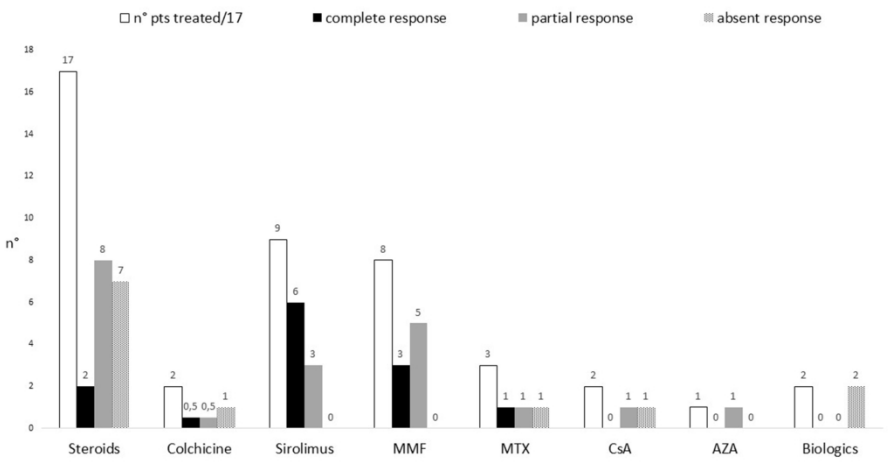

Figure 4

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