CASE REPORT

Unexplained lymphadenopathies: autoimmune lymphoproliferative syndrome in an adult patient

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SUMMARY
Autoimmune lymphoproliferative syndrome (ALPS) is characterised by massive enlargement of the lymphoid organs, autoimmune cytopenias and a predisposition to develop lymphoid malignancies. The basic defect is a disturbance of the lymphocyte apoptosis, and a high number of circulating TCRαβ CD3−CD4−CD8+ T-cells (double-negative T cells (DNT cells)). We describe a case of a 41-year-old man with fever, hepatosplenomegaly, multiple lymphadenopathies, autoimmune haemolytic anaemia and severe thrombocytopenia. Peripheral blood immunophenotyping revealed elevation of the characteristic DNT cells in 8% and high levels of interleukin 10. Histopathological analysis of lymph nodes showed lymphadenitis with paracortical hyperplasia. It was assumed as a probable diagnosis of ALPS, and the procedure was to medicate the patient with steroids. As a result, a significant clinical improvement was achieved, and he has been in remission for 2 years. To our knowledge, this is the first case reported in a Portuguese adult patient.

BACKGROUND
Autoimmune lymphoproliferative syndrome (ALPS), also known as the Canale-Smith syndrome, is a rare disorder. The increase in DNT cells, interleukin 10 (IL-10) and polyclonal elevation of gamma globulin (IgG) can be diagnostically relevant in patients under suspicion of this syndrome. The genetic defect found in most patients is a mutation in the FAS gene, which encodes a cell surface receptor, on stimulation, and induces a programmed cell death. This entity typically develops in childhood and, in contrast, there are a few reported adult-onset cases.1–3

CASE PRESENTATION
A 41-year-old man, with a history of pulmonary tuberculosis and with no family history of lymphoproliferative and autoimmune diseases, was taken to the emergency department and exhibited multiple cervical, axillary and inguinal lymph node enlargements, and epistaxis with more than a 6-month history. He had had an intermittent fever lasting for 4 months. There was no loss of weight or appetite, neither night sweats nor any other localising symptoms, namely pulmonary symptoms or signs. On physical examination, he had pallor, petechial and oral haemorrhagic bullae.

OUTCOME AND FOLLOW-UP
We have been closely following him up every month, and his lymphadenopathy and hepatosplenomegaly have regressed (ultrasound liver 15.7 cm and spleen 10.8 cm). The axillary nodes have disappeared, and his lymphadenopathy and hepatosplenomegaly have regressed (ultrasound liver 15.7 cm and spleen 10.8 cm). The axillary nodes have disappeared, and the cervical nodes have been just palpable for 2 months. After a 24-month follow-up without any treatment, the patient is asymptomatic and with no evidence of recurrence. It was programmed a lymphocyte apoptosis assay and a positron emission tomography (PET) scans to keep the patient under surveillance.

INVESTIGATIONS
According to our research on this patient, we found out that he had bicytopaenia with a haemoglobin of 8.7 g/dL and platelet count of 4000/μL, total leukocyte count was 6650/μL with normal differentials. Liver and renal function tests, prothrombin time and vitamin B12 were all within normal range. Biochemical evidence of haemolysis was supported by serum decreased haptoglobin level, increased indirect bilirubin and lactate dehydrogenase levels. Direct anti-globulin test was positive (IgG3; C3d1+). Abdominal ultrasound revealed a hepatomegaly (17 cm), splenomegaly (15,4 cm), as well as retroperitoneal and external iliac adenomegaly about 3×1 cm. Thoracic CT showed axillary adenomegaly of 2×2.5 cm. The infection diagnostic work-up, including blood and urine screening, cures for tuberculosis, HIV, toxoplasmosis, parvovirus, cytomegalovirus, Brucella and hepatitis, was all negative. The autoimmune work-up was also negative. The patient’s bone marrow biopsy was normal. In addition, ganglion excisional biopsy excluded malignancy and mycobacteriology cultures were negative for Mycobacterium tuberculosis or other nontubercular Mycobacterium. Flow cytometry of peripheral blood reported that 8% of the lymphocytes were DNT cells. Plasma concentrations of soluble interleukin-10 (IL-10) were increased, but none FAS and FASL mutations were detected (tables 1 and 2). A probable ALPS diagnosis was made.

TREATMENT
In the acute phase, 1 mg/kg of IgG was first administered and 10 mg/kg methylprednisolone over 3 days, followed by maintenance dose of 1.5 mg/kg per day prednisolone. He began to show a response after 2 weeks and the platelet count was normal in just 1 month. A complete remission of the clinical and laboratory abnormalities was achieved after a prolonged prednisolone tapering over the period of 12 months.

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this case, patient showed a haptoglobin decrease, an indirect bilirubin and a lactate dehydrogenase increased and a positive direct antiglobulin test (IgG 3+; C3d1+), compatible with autoimmune haemolytic anaemia. The incidence of lymphomas is quite significant. Therefore, a bone marrow aspiration was performed which showed no suggestive lymphoproliferative disorders of marrow involvement. In ALPS diagnosis, the peripheral blood immune phenotyping is essential to find the DNT cells elevation. Moreover, regarding our patient, it was observed a flow cytometry which showed 8% of the lymphocytes total to be DNT cells, one the required criterion (to have more than 1.5% in the setting of normal lymphocyte counts). When this condition is suspected, mutation molecular genetic test of FAS, FASL and CASP10 genes are necessary, because they have diagnostic and prognostic value, as there is an increased risk of lymphoma development. The pathogenesis of ALPS in the majority of patients results in defective apoptosis of lymphocytes (table 2) mediated through the FAS/FAS ligand (FASL) pathway or mutations in CASP10. In cases where no mutation was identified, a functional deficiency of FAS-mediated apoptosis was also observed. In our case, we did not search into two separate assays the lymphocyte apoptosis or searched deletions or duplications in the FAS gene and sequencing of FASL or CASP10 genes, as required in the ALPS additional primary criteria (box 1). However, it is no longer considered essential for the diagnosis of ALPS, as patients with somatic or germline FAS mutations, FASL mutations can present with normal in vitro FAS-induced apoptosis assays. These findings led the authors in the National Institutes of Health Clinical Center Group to propose a classification scheme based on the several different molecular abnormalities. Patients who fulfil ALPS diagnostic criteria and have germline homozygous or heterozygous mutations in FAS, should be named as ALPS-FAS; if they have somatic FAS mutations as ALPS-SFAS; if they have FAS ligand mutations as ALPS-FASL or if they have caspase-10 mutations, they should be classified as ALPS-CASP10. However, when patients have an indeterminate genetic defect, like in your patient’s case, they are categorised as ALPS-U (undetermined).

Some biomarkers, which are increased in ALPS, include soluble FASL, circulating IL-10 and vitamin B12, whose biological origin is unknown. The combinations of these markers can be highly likely to diagnose ALPS instead of FAS sequencing. The new consensus ALPS classification and the inclusion of these biomarkers in diagnostic criteria will expedite ALPS diagnosis.

According to reviewed ALPS criteria, the lymphocyte apoptosis defects detection and somatic or germline mutation identification (FAS, FASL, or CASP10 genes) are primary additional criteria (box 1). Whereas an elevation of biomarkers, anatomopathological findings, autoimmune cytopenias with an elevated IgG levels and family history disease are considered as secondary additional ALPS diagnostic criteria (box 1). In our patient’s case, no autoimmunity or lymphadenopathy has occurred in his family background. Also, we observed haemolytic anaemia with polyclonal hypergammaglobulinaemia and an increased IL-10.

Although some patients do not need treatment, most of them require immunosuppressive therapy, mainly those who develop cytopenias. The treatment is based on high doses of glucocorticoids and G-immunoglobulin intravenous associated with a good response. The glucocorticoids should be used only in exacerbations, but discouraged as chronic therapeutic due to its association with many side effects and complications. In exacerbation disease, the methylprednisolone pulses (5–10 mg/kg) must be used for 7–10 days, the prednisolone maintenance therapy (1–2 mg/kg)

### DISCUSSION

ALPS is a disease characterised by immune dysregulation due to an inability to regulate lymphocyte homeostasis through abnormalities in lymphocyte apoptosis or programmed cell death. The required clinical criteria are the presence of chronic lymphadenopathy and splenomegaly, present for more than 6 months, which may be asymptomatic and incidentally identified during routine physical examinations. In our case, the patient presented with hepatomegaly, splenomegaly and multiple lymph node enlargements during a period of 6 months. Patients with ALPS may present initially with episodes of fatigue, pallor and icterus due to haemolytic anaemia. They may also be more likely to have easy bruising and mucocutaneous bleeding caused by thrombocytopenia. In our case, the patient was taken to the emergency room with pallor, petechial, oral haemorrhagic bullae and a platelet count of 4000/μL.

Patients with ALPS can also have multilineage cytopenias, more typically in adults, which are chronic and can be refractory to therapy. The autoimmune haemolytic anaemia is also a common manifestation while immune neutropenia is rare. In

### Table 1 Results of laboratory tests released to patient during the study

<table>
<thead>
<tr>
<th>Test</th>
<th>Patient values</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bilirubin</td>
<td>2.07 mg/dL</td>
<td>0.1–1.1</td>
</tr>
<tr>
<td>Direct bilirubin</td>
<td>0.69 mg/dL</td>
<td>0.1–0.3</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>618 U/L</td>
<td>135–225</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>&lt;10.0 ng/mL</td>
<td>30–200</td>
</tr>
<tr>
<td>Direct antiglobulin test</td>
<td>IgG 3+; C3d1+</td>
<td></td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
<td>44 U/L</td>
<td>4–33</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td>50 U/L</td>
<td>4–50</td>
</tr>
<tr>
<td>γ-glutamyl transferase</td>
<td>71 U/L</td>
<td>5–61</td>
</tr>
<tr>
<td>C reactive protein</td>
<td>3.79 mg/dL</td>
<td>0–0.5</td>
</tr>
<tr>
<td>Polyclonal</td>
<td>2710 mg/dL</td>
<td></td>
</tr>
<tr>
<td>hypergammaglobulinaemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activated partial thromboplastin time</td>
<td>42.4 s</td>
<td>28.0–40.0</td>
</tr>
<tr>
<td>Peripheral blood assays</td>
<td>IL-10: 22.8 pg/mL</td>
<td>≤20</td>
</tr>
<tr>
<td>FASL: 143 pg/mL</td>
<td>≤200</td>
<td></td>
</tr>
<tr>
<td>Immunophenotyping of peripheral blood lymphocytes</td>
<td>8% TCRab CD3+CD4+CD8+ T-cells</td>
<td></td>
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</tbody>
</table>

Table 2 Results of histopathological and genetics analysis released during the study

<table>
<thead>
<tr>
<th>Test</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow biopsy</td>
<td>Anatomopathological examination: 3 haematopoietic lines were observed. The erythroid line was increased with normoblasts and megalakercytic line with many dysplastic elements. No changes to granulocytic line. Without malignancy characteristics. Immunophenotyping: without changes.</td>
</tr>
<tr>
<td>Axillary lymph node excisional biopsy</td>
<td>Anatomopathological examination: preservation of lymph node architecture and reactive character changes without malignancy characteristics; Acid-alcohol resistant bacillus: negative; Mycobacteriology cultures: negative; Immunophenotyping: without changes.</td>
</tr>
<tr>
<td>Fas gene detection</td>
<td>Mutations not detected, but the method used does not exclude mutations outside the FAS gene regions analysed or not detectable by sequencing. Unrealised searching for deletions or duplications in the FAS gene and sequencing of FASL or CASP10 genes.</td>
</tr>
</tbody>
</table>
Box 1 Autoimmune lymphoproliferative syndrome (ALPS) diagnostic criteria based on the first international workshop of ALPS 2009

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

Additional criteria
1. Primary
   1. Defective lymphocyte apoptosis in two separate assays
   2. Somatic or germline pathogenic mutation in Fas, FASL or Casp10
2. Secondary
   1. Elevated plasma sFASL levels (>200 pg/mL), plasma interleukin 10 (IL-10) levels (>20 pg/mL), serum or plasma vitamin B₁₂ levels (>1500 ng/L) or plasma IL-18 levels >500 pg/mL
   2. Typical immunohistological findings as reviewed by a hematopathologist
   3. Autoimmune cytopenias (haemolytic anaemia, thrombocytopenia or neutropenia) with elevated IgG levels (polyclonal hypergammaglobulinaemia)
   4. Family history of a non-malignant/non-infectious lymphoproliferation with or without autoimmunity

Definitive diagnosis: Both required criteria plus one primary accessory criterion.
Probable diagnosis: Both required criteria plus one secondary accessory criterion.

Learning points

- Autoimmune lymphoproliferative syndrome (ALPS) is an uncommon disease in adult patients, and this disorder is associated with abnormalities in lymphocyte apoptosis and a high number of circulating TCRαβ⁺CD3⁺CD4⁺CD8⁻ T-cells (DNT cells).
- Patients do not need treatment, but when they develop cytopenias, they require immunosuppressive therapy. This is based on high doses of glucocorticoids and IgG intravenous, and the experience with other immunosuppressants in ALPS is very limited.
- Genotyping of the genes associated with ALPS are no longer considered essential for the diagnosis but can be helpful in patients with confusing clinical and/or laboratory findings and in prognosis definition.
- The incidence of lymphomas is quite significant and therefore a permanent follow-up is needed.

Contributors All authors had been involved in this patient’s care. FLS and GSC contributed to the drafting of the manuscript. HPC contributed to the interpretation of data. AO revised the content and accepts responsibility for the overall content as a guarantor.

Competing interests None declared.

Patient consent Obtained.

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REFERENCES
