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ORIGINAL ARTICLE

Clinical Spectrum of Ras-Associated Autoimmune Leukoproliferative Disorder (RALD)

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Abstract

Ras-associated autoimmune leukoproliferative disorder (RALD) is a clinical entity initially identified in patients evaluated for an autoimmune lymphoproliferative syndrome (ALPS)-like phenotype. It remains a matter of debate whether RALD is a chronic and benign lymphoproliferative disorder or a pre-malignant condition. We report the case of a 7-year-old girl diagnosed with RALD due to somatic *KRAS* mutation who progressed to a juvenile myelomonocytic leukemia phenotype and finally evolved into acute myeloid leukemia. The case report prompted a literature review by a search for all RALD cases published in PubMed and Embase. We identified 27 patients with RALD. The male-to-female ratio was 1:1 and median age at disease onset was 2 years (range 3 months–36 years). Sixteen patients (59%) harbored somatic mutations in *KRAS* and 11 patients (41%) somatic mutations in *NRAS*. The most common features were splenomegaly (26/27 patients), autoimmune cytopenia (15/16 patients), monocytosis (18/24 patients), pericarditis (6 patients), and skin involvement (4 patients). Two patients went on to develop a hematopoietic malignancy. In summary, the current case documents an additional warning about the long-term risk of malignancy in RALD.

Keywords Autoimmunity · KRAS · NRAS · malignancy · Ras-associated autoimmune leukoproliferative disorder

Introduction

The autoimmune lymphoproliferative syndrome (ALPS), the most common genetic disorder of lymphocyte apoptosis, is caused by germline or somatic mutations in the gene encoding *FAS* (a cell-surface receptor from the TNF receptor superfamily), germline mutations in Fas ligand (*FASLG*), or caspase 10 (*CASP10*) [1–3]. Patients develop early-onset chronic splenomegaly frequently associated with lymphadenopathy and present a high incidence of autoimmunity (mainly cytopenias) as well as an increased risk for the development of hematological malignancies, including both Hodgkin and non-Hodgkin lymphoma [3].

In 2007, Oliveira et al. reported a patient with an ALPS-like disease caused by a somatic mutation in *NRAS* [4]. Later, two additional patients with similar phenotype of non-malignant lymphoproliferation and autoimmune cytopenias were found to have mutations in *KRAS* [5]. Previously designated ALPS type IV, these patients are now reclassified under a new clinical entity termed RALD, for Ras-associated autoimmune leukoproliferative disorder.

Somatic mutations in the 3 RAS genes (*HRAS*, *KRAS*, *NRAS*) are found in almost 30% of human cancers, solid as well as hematological where they act as oncogenes [6]. The *NRAS* and *KRAS* mutations observed in RALD patients are heterozygote-dominant and gain-of-function mutations, similar to the ones described in tumors and in a subpopulation of patient with juvenile myelomonocytic leukemia (JMML) [7] with which RALD shares some clinical and laboratory findings. However, the question arises as to whether RALD is a chronic and benign lymphoproliferative disorder or a pre-malignant state.

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We describe a case of RALD evolving into myeloid malignancy. We review all RALD patients reported so far in the literature to broaden the current understanding of the clinical aspects, laboratory features, and outcome of the disease.

Case Description

A girl born from non-consanguineous Caucasian parents was first noted to have hepatosplenomegaly with lymphadenopathy when admitted at the age of 15 months for fever and purpura. Her past medical history was characterized by recurrent otitis media, stomatitis, panniculitis, and gastro-enteritis, and one episode of mastitis. Blood analysis revealed the presence of hemolytic anemia with positive direct Coombs test, thrombocytopenia, and neutropenia. Lymphocytes count was normal and monocytosis was noted (1420/μL). Serum immunoglobin levels were normal. Immunophenotyping of peripheral blood lymphocytes revealed increased B cell percentage (CD20+, 3055/μL) and increased TCRα+β+ CD4-/CD8- double-negative T (DNT) cells (3.0%). Biomarkers typically associated with ALPS, such as IL-10 and sFASL, were normal, and Sanger sequencing failed to show a mutation in *FAS*. Bone marrow aspiration revealed normal cellularity without clonal cytogenetic abnormalities. A diagnosis of ALPS-like disease was proposed.

Whole exome sequencing showed a c.38 G>A (p.Gly13Asp) *KRAS* somatic mutation in peripheral blood mononuclear cells evoking a diagnosis of RALD. Interestingly, an inherited microduplication 22q11.2 was also observed, although the patient has no apparent physical or intellectual disabilities related to this condition.

During the following 4 years, the clinical course was complicated by persistently recurrent infections including pneumonia (5 episodes, one with pleural effusion) and cellulitis (4 episodes), requiring hospitalizations. The patient subsequently developed severe autoimmune and autoinflammatory manifestations, including arthritis and recurrent pericarditis. She was treated sequentially with methylprednisolone, mycophenolate mofetil, sirolimus, and later with methotrexate.

At the age of 5.5 years, bone marrow aspiration showed evolution into a mixed myelodysplastic and myeloproliferative syndrome without blast excess. Because of progression into JMML phenotype in addition to serious autoimmune manifestations and hematologic disease, hematopoietic stem cell transplantation (HSCT) was considered.

One month later, a biological work-up revealed hyperleukocytosis with a white cell count of 86.000/μL. Bone marrow aspiration showed a diagnosis of acute myeloid leukemia (AML) with 57% of blast cells. Karyotype was normal, 46 XX. A comprehensive genetic characterization of AML samples was performed by a 50 genes panel PCR-based next generation sequencing platform (TruSight™ Myeloid Sequencing Panel, Illumina, San Diego, CA, USA) and identified a mutation in NPM1 gene. The patient was treated according to the NOPHO-DBH AML2012 protocol [8] and achieved complete remission. She received a HSCT from her haploindentical father following a conditioning regimen consisting of thiotepa-busulfan-fludarabine. She received post-transplant cyclophosphamide followed by tacrolimus and mycophenolate mofetil as graft versus host disease (GVHD) prophylaxis. The HSCT was complicated by a BK virus-associated hemorrhagic cystitis and a grade II GVHD. The immunosuppressive therapy was stopped 14 months post-HSCT. At last follow-up, 26 months post-HSCT, the patient has a full donor chimerism with no signs of AML or RALD.

Literature Review

We searched all cases diagnosed as RALD in PubMed and Embase published between May 2007 and February 2020. The keywords used were "Ras-associated autoimmune leukoproliferative disorder," "NRAS (and) autoimmunity," and "KRAS (and) autoimmunity." Publications in English or French were considered. The data set regarded demographic characteristics (age and gender), clinical phenotype including autoimmune and lymphoproliferative complications, laboratory explorations, *RAS* mutation, management, and outcome.

Demographics and Initial Presentation

Our review identified 14 publications describing 27 RALD patients [4, 5, 9–20]. The clinical and laboratory characteristics of the patient population are outlined in Table 1. The male-to-female ratio was 1.1. The median age at disease onset was 2 years (range, 3 months to 36 years): 9 patients had onset within the first year of life (33%) and only 2 of the patients had late-onset disease (i.e., 13 and 36 years). Median follow-up was 7 years with a range of 1 to 56 years.

Sixteen out of these 27 patients (59%) had somatic mutations in *KRAS* and 11 (41%) had somatic mutations in *NRAS*, involving the 12th or 13th codon. These mutations were present only in cells of the hematopoietic lineage (myeloid and lymphoid) but not in skin-derived fibroblasts or mouth swab samples. The initial age of presentation of the RALD patients who were subsequently found to have a *NRAS* mutation was younger (median 9 months) than of the *KRAS* patients (median 3 years old).

The first disease manifestations were cytopenia and lymphoproliferation ($n = 10$), cytopenia ($n = 2$), lymphoproliferation ($n = 2$), or cutaneous involvement ($n = 1$). A history of recurrent infections that began months prior the onset of autoimmune and/or lymphoproliferative manifestations was reported in 5 patients and included bronchitis, bronchiectasis,

Table 1 Clinical and laboratory features of RALD patients

	Reference(s)	Gender	Age of onset, y	Age at FU, y outcome	Mutation	ADP	SMG	Cytopenia	Positive autoantibodies	IgG (mg/dL)	Absolute monocyte count (/μL)	HbF (%)	αβ-DNT (%)	Treatment(s)	Complications
1	4, 9	M	0.5	57, alive	NRAS G13D	+	+	ND	DAT, ANA, ACA, FR	1310	3700	ND	2.0	ND	acute leukemia at 4 yo/LNH at 32 yo
2	5	M	0.75	1.75, alive	KRAS G13D	+	+	HA, T	DAT, ANA, APA, dsDNA, ACA	3067	760	0.6	1.4	CS	
3	5	F	0.5	ND, alive	KRAS G13D	ND	+	HA, T	DAT, ANA, dsDNA, AN	1562	510	1.9	1.1	CS, IV Ig, cytarabine, HSCT	
4	9, 10	F	4	13, deceased	KRAS G13C	+	+	HA, T	ACA, AN, AP	2130	1500	ND	0.8	ND	Pericarditis/recurrent infections/death of indetermined cause at 13 y
5	9, 10	F	5	12, alive	KRAS G12D	+	+	HA, T, N	LA, ACA, ANA	2220	1900	0,7	1,4	ND	Cutaneous involvement
6	9	F	1	20, alive	KRAS G13D	ND	+	ND	DAT, ANA, ACA	2450	49,400	ND	ND	ND	
7	9	F	4	10, alive	KRAS G13C	ND	+	ND	DAT, LA, ANA	1410	2100	ND	ND	ND	
8	9, 11	F	36	43, alive	KRAS G12A	ND	+	ND	DAT, LA, ANA	1430	10,000	ND	ND	CS, MMF	Cutaneous involvement/bowel inflammation
9	9	M	3	13, alive	KRAS G12S	ND	+	ND	DAT, ACA, ANA	1610	1800	ND	ND	ND	
10	9	F	2	12, alive	KRAS G13C	ND	+	ND	DAT, LA	1370	1500	ND	ND	ND	Pericarditis
11	9	M	0.25	5, alive	NRAS G12V	ND	+	ND	ND	1570	14,300	ND	ND	ND	
12	9	M	0.25	6, alive	NRAS G13D	ND	+	ND	DAT, ACA, ANA	2100	4800	ND	ND	ND	
13	9	M	0.25	9, alive	NRAS G13D	ND	+	ND	DAT, LA	2750	2100	ND	ND	ND	Glomerulonephritis
14	9	M	2	19, alive	NRAS G13D	ND	+	ND	DAT, LA, ACA, ANA	2190	3700	ND	ND	ND	
15	9	M	0.75	5, alive	NRAS G12S	ND	+	ND	ND	1030	4200	ND	ND	ND	
16	12	F	5	20, deceased malignancy	KRAS G13C	+	+	HA, T	ND	8400	ND	ND	ND	CS, IV Ig, splenectomy, rituximab	Pericarditis/arthritis/colic B cell proliferation
17	13	F	0.5	ND, alive	NRAS G13D	+	+	HA, T, N	ANA, ACA, dsDNA, APA	2013	432	ND	6.5–12.7	CS, cyclosprorine	
18	13	F	1	ND, alive	NRAS G13D	+	+	HA, T	LA, ACA, ANA, APA, anti-DsDNA	2270	5544	3.8	0.6	no therapy	Recurrent fever
19	14, 15	M	3	ND, alive	KRAS G13C	–	–	HA, T	Négatif	1018	336	0.8	2.75	CS, cyclosporine, adalimumab	Recurrent HSP/arthralgia/intestinal Behçet disease
20	15	M	13	25, alive	KRAS G13C	+	+	HA, T	Positif	2749	690	ND	ND	CS, MMF	Ulcer at colon
21	16	M	4	15, alive	KRAS G13C	+	+	HA, T, N	ANA, DsDNA, DAT	2500	ND	ND	normal	CS, rituximab, CP, azathioprine, MMF	Pericardial effusion/headaches/inflammatory arthritis
22	17	F	0.75	6, alive	KRAS G13A	+	+	HA, T	DAT	1650	1610	ND	2.6	IV Ig, CS, rapamycin, splenectomy	Recurrent infections
23	18	F	2	11, alive	KRAS G13C	+	+	HA, T	DAT, AP, dsDNA, ANA, LA	5777	ND	ND	2.9	CS, CP, MMF, rituximab, IV Ig	Recurrent infections, pericarditis
24	19	M	6	8, alive	KRAS G12S	+	+	–	ANA	–	+	ND	ND	topical CS	Cutaneous involvement
25	20	M	3	4, alive	NRAS G13D	+	–	HA, T	DAT, dsDNA, ANA, ACA	2331	1790	ND	4.9	CS, IV Ig	Arthritis, cutaneous involvement, pericarditis, recurrent infections
26	20	M	1	7, alive	NRAS G13C	+	+	T	DAT, dsDNA, ANA	1629	830	ND	7.2	CS, platelet Tf, Leflunomide	Arthritis, recurrent infections, bronchiectasis
27	20	F	4	6, alive	NRAS G13D	–	+	T	DAT, dsDNA, ANA	2158	2400	ND	ND	CS, IV Ig, MMF, hydroxychloroquine	Cutaneous involvement, arthritis

N.D. not determined, *HA* hemolytic anemia, *T* thrombocytopenia, *N* neutropenia, *DAT* direct antibody test, *LA* lupus anticoagulant, *ACA* anticardiolipin antibodies, *ANA* antinuclear antibodies, *dsDNA* anti-dsDNA antibody, *AP* anti-platelet antibody, *AN* anti-nutrophil antibody, *CS* corticosteroids, *Ig* immunoglobulins, *MMF* mycophenolate mofetil, *CP* cyclophosphamide, *HSCT* hematopoietic stem cell transplantation

pneumonia, cellulitis, otitis, and severe viral infections (e.g., herpes simplex virus and cytomegalovirus).

The initial diagnosis retained included ALPS ($n = 4$), systemic lupus erythematosus ($n = 4$), JMML ($n = 3$), Evans syndrome ($n = 2$), immune thrombocytopenic purpura ($n = 2$), and hemophagocytic lymphoplasmocytosis ($n = 1$).

Autoimmune, Lymphoproliferative, and Cutaneous Manifestations

Splenomegaly was documented in almost all patients (26/27, 96%) and generally occurred early in the course of the disease. Five patients underwent splenectomy, because of hyperplenism, pain, or large splenic infarct. No bacterial sepsis after splenectomy has been reported. Chronic lymphadenopathy was also frequent (14/16, 88%).

At initial presentation, cytopenias were found in 13 out of 16 patients for whom data are available: autoimmune hemolytic anemia (AIHA) in 1 patient, immune thrombocytopenia (ITP) in 4 patients, and simultaneous AIHA and ITP in 8 patients. In 3 of these patients, a second cytopenia only developed afterwards. A concurrent neutropenia was reported in 3 cases. Two other patients with initial normal blood test developed Evans syndrome during disease progression.

Gastrointestinal manifestations were recorded in 4 *KRAS* patients. Colonoscopy was performed in all of them and displayed B cell proliferation ($n = 1$), colic inflammation requiring resection ($n = 1$), and ulcerations ($n = 2$). Patient 19 was diagnosed as Behçet colitis and treated with adalimumab associated with clinical benefit.

Other autoimmune and/or inflammatory complications were pericarditis and/or pericardial effusion ($n = 6$), arthralgia and/or inflammatory arthritis ($n = 5$), and glomerulonephritis ($n = 1$). All but one of these patients harbored a *KRAS* mutation. Neurological findings, of uncertain relation with RALD, were reported in patient 21 and consisted in headaches with microinfarcts as demonstrated by MRI.

Four patients (2 with *KRAS* mutation and 2 with *NRAS* mutation) developed recurrent or persistent unspecified erythematous and edematous plaques at onset ($n = 1$) or during the course of the disease ($n = 3$). Histologic examination, performed in 2 cases, demonstrated perivascular and interstitial infiltrate of immature myeloid cells. Interestingly, molecular studies performed on extracted DNA from skin histologic slides reported same *KRAS* mutation that in hematopoietic cells [19]. One patient developed recurrent Henoch-Schonlein purpura.

Malignancy

A total of 3 malignancies occurred in 2 patients: (i) patient 1 presented an atypical history of two hematopoietic malignancies: an acute leukemia (not subclassified) in childhood and a non-Hodgkin's B cell lymphoma in early adulthood; and (ii) patient 16 developed acute and fatal myeloproliferation with pulmonary infiltration diagnosed as JMML at the age of 20 years old.

Laboratory Results

Initial blood examination showed normal or mildly increased total lymphocyte numbers. Monocytosis (> 1000/μL) was observed in all but six patients (18/24, 75%). Most patients exhibited hyper-gamma-globulinemia (range 13–84 g/L). DNT cells were modestly increased (> 1.5%) in 7 out of 14 patients (median 2.9%, range 2.0-12.7%). B cell lymphocytosis was frequently noted. Increased plasmatic vitamin B12 was reported in 2 out of 5 patients. Twenty-three of the 24 cases tested (96%) showed positivity for various autoimmune antibodies, mainly antinuclear antibody, anti-double-stranded DNA, and direct Coombs test. Decreased complement (C3 and C4) has been reported in 4 out of 5 patients tested.

Evaluation of the intrinsic pathway of apoptosis was performed in 18 patients and revealed defective IL-2 withdrawal-induced apoptosis associated with the markedly decreased expression of pro-apoptotic protein BIM. A multiplex inflammatory cytokine immunoassay was used to quantify circulating levels of cytokines in the plasma of 9 RALD patients. Compared with healthy donors, the levels of IL-2, IL-6, and IL-10 were found significantly increased in RALD patients [21].

Bone marrow aspirate performed in 19 patients showed normal or hypercellular marrow without blast excess. Cytogenetic analyses revealed a normal karyotype in all cases. *BCR/ABL* rearrangement was not detected when assessed. Granulocyte-macrophage colony-stimulating factor hypersensitivity of myeloid progenitors was positive in 7 of 11 patients tested (64%) while some had increased hemoglobin F for age.

Lymph node biopsy specimens from 4 patients were studied for histology. Two revealed reactive follicular hyperplasia with non-specific proliferation of histiocytes, but without the extrafollicular expansion of DNT cells typical for ALPS. In contrast, samples from patients 16 and 20 showed histological features of Rosai-Dorfman disease. This rare non-Langerhans cell histiocytosis is characterized by sinus expansion of large histiocytes and plasma cells associated with expression of the histiocytic markers CD68 and S100 upon immunological staining.

Treatment and Outcome

Thirteen of the 15 patients for whom we have information about disease management received a first-line treatment with corticosteroids. Ten patients underwent second-line immunomodulatory treatments in order to control cytopenias and/or autoimmune manifestations. These included mTOR

inhibitors, mycophenolate mofetil, cyclosporine, cyclophosphamide, leflunomide, and rituximab. Conversely, patient 18, who displayed AIHA and ITP, exhibited progressive improvement, without receiving any specific therapy.

Other therapeutic interventions included intravenous immunoglobulin, cytarabine, hydroxycholoroquine, platelet transfusion, and topical steroids. The latter were sufficient to induce remission in patient 24, who presented with late-onset disease (6 years) limited to skin manifestations and mild lymphoproliferation. Patient 3 was treated with HSCT due to steroid-refractory autoimmune manifestations. Outcome was unknown.

Median age at last follow-up was 10 years (range 2 to 57 years), and all but 2 patients were alive (25/27, 93%). The two deaths were reported in patient 6 who experienced an aggressive form of JMML and patient 4 who passed away suddenly at age 13.

Discussion

RALD, an intrinsic lymphocyte apoptosis defect, is extremely rare, with only twenty-seven patients described in the literature. All patients with RALD described so far harbor somatic gain of function mutations in *KRAS* or *NRAS*, which occur in hematopoietic progenitors or stem cells and are subsequently found in lymphocytes and monocytes. Lymphoproliferation and autoimmune manifestations, including immune cytopenia, are the main clinical features while hypergammaglobulinemia and monocytosis are frequently present. A history of recurrent infections is reported in a few patients, suggestive of immune deficiency. Differential diagnosis includes ALPS, JMML, and systemic lupus erythematosus. In particular, distinguishing between RALD and ALPS may be challenging because RALD patients may display several diagnostic markers of ALPS including elevated DNT cells and increased plasmatic IL10 and vitamin B12. The patient we report extends the clinical phenotype. In addition to lymphoproliferation and autoimmune cytopenia, our patient evolved into acute myeloid leukemia, a complication that, to the best of our knowledge, has not been reported so far.

Cellular death is regulated to a large extent by pro- and anti-apoptotic sensors of the BCL2 family, especially the pro-apoptotic protein BIM [22]. The balance of these factors determines whether mitochondria become depolarized, leading to formation of a mitochondria-derived complex called the apoptosome that in turn promotes caspase activation for cell death [23]. RAS mutations are responsible for a hyperactivation of the MAPK (RAF/MEK/ERK) pathway inducing the phosphorylation and destruction of the pro-apoptotic protein BIM [24]. Consequently and contrary to ALPS, RALD patients exhibit normal Fas-mediated apoptosis but present a specific defect in the intrinsic pathway of apoptosis characterized by persistent proliferation after IL-2 withdrawal [4].

Autoimmunity is a key feature of RALD. Combinations of autoimmune cytopenias, concomitantly and/or sequentially, are typical, with AIHA and ITP as the most common combination. These findings are associated with autoantibodies including frequently a positive direct Coombs test. Among the other autoimmune manifestations, pericardial effusion seems to be the main life-treating condition and is mostly reported in patients harboring *KRAS* mutations. As previously described in patients with RASopathies such as type-1 neurofibromatosis or Noonan syndrome [25], patients with RALD meet some or full classification criteria for systemic lupus erythematosus [26] including low C3 and C4 levels, antinuclear, lupus anticoagulant, and anti-dsDNA antibodies. In a study evaluating the clinical and laboratory autoimmune features in patients affected by RASopathies, autoimmune diseases and autoantibodies were observed in 14 and 52%, respectively, suggesting an association between those entities [27]. These observations strongly suggest that a genetic defect leading to overactivation of the RAS pathway could predispose to the development of autoimmunity.

The incidence and pattern of malignancies in RALD are not clearly defined. Herein, we report the second case of evolution into myeloid malignancy, the first one describing a RALD patient who progressed to severe and fatal JMML after more than 15 years of indolent lymphoproliferation [12]. Consequently, with a median follow-up of 7 years, it cannot be excluded that other patients of this series may develop cancer later in life. Closely monitoring of RALD patients is therefore recommended.

JMML is a rare, aggressive myeloproliferative/myelodysplastic disorder unique to childhood, characterized by increased infiltration of the peripheral blood, bone marrow, and various organs by abnormal myelomonocytic cells. Patients show hepatosplenomegaly, lymphadenopathy, skin rash, fever, and pallor [28]. Although spontaneous remission may occur in some patients [29], the prognosis of JMML is usually poor, with a median survival of children who do not undergo HSCT of less than 12 months [28].

The diagnosis of JMML is usually made using the World Health Organization classification of myeloid neoplasms [30]. Interestingly, the majority of RALD patients described to date fulfill all required diagnostic criteria of JMML that, in addition to mutations of Ras pathway genes, include peripheral blood monocyte count > 1000/μL, blast percentage in peripheral blood and bone marrow < 20%, splenomegaly, and absence of t(9;22)*BCR/ABL* fusion gene. Hypersensitivity of JMML myeloid progenitor cells to granulocyte-macrophage colony-stimulating factor (GM-CSF), a hallmark of the disease and important diagnostic tool for many years [31], is still needed to establish the diagnosis for the patients without a detectable known molecular defect. In RALD, hypersensitivity to GM-

CSF was present in 64% of the patients tested. While all RALD patients have normal bone marrow karyotypes, patients with JMML may harbor monosomy 7 or other karyotypic abnormalities [32]. However, normal karyotypes are described in about two-thirds of JMML cases [28]. Consequently, RALD is difficult to distinguish from normal karyotype JMML even if the new JMML criteria are applied. Based on this, Meynier et al. performed functional in vitro apoptosis assays in T cells from patients with JMML harboring *KRAS* or *NRAS* mutations [33]. Compared with RALD, the JMML patient's cells displayed normal intrinsic apoptosis pathway including normal levels of BIM expression but a defect in the extrinsic apoptosis pathway related to a FASL downregulation. These observations hold out the prospect of functional apoptosis assays as an accurate diagnostic discrimination between RALD and JMML.

About 85% of JMML patients harbor somatic and/or germline mutations in genes involved in RAS/MAPK signaling pathway such as *PTPN11*, *NRAS*, *KRAS*, *NF1*, and *CBL* [32]. In particular, patients with RASopathies are prone to develop JMML [34, 35]. Interestingly, RALD and up to 25% of patients with JMML share identical somatic *KRAS* and *NRAS* mutations. Lanzarotti et al. therefore hypothesize that these clinico-biological entities are not distinct diseases but opposite ends of the same spectrum [12]. Various additional genetic or epigenetic events (in addition to *RAS* pathway activation) could therefore contribute to the autoimmune or malignant phenotype [24]. Takagi et al. reported 6 JMML patients harboring *KRAS* or *NRAS* mutations who experienced spontaneous regression of proliferative myelomonocytic disease but with persistence of *RAS*-mutated clones and autoimmunity, years after remission [36]. These patients showed hypergammaglobulinemia, positivity for autoimmune antibody, autoimmune cytopenia, and/or persistent monocytosis. These findings are compatible with a RALD diagnosis. Unlike what is observed in other tumors, heterozygous somatic Ras mutations have long been considered to be the initiating and unique event in JMML, even though the five mutational targets alone do not fully explain the phenotypic diversity of JMML [37]. The application of NGS and deep sequencing of target genes in JMML patient cohorts recently revealed new insights into the pathogenesis and progression of JMML. Secondary genetic mutations within and outside of the Ras pathway have been reported and are associated with a worse prognosis. Mutations occurred in genes involved in DNA methylation as well as Ras pathway genes, spliceosome members, transcription factors, and *SETBP1* [38–41]. Noticeably, very high levels of methylation were associated with disease progression and significantly poorer survival [39]. Duplication of oncogenic *KRAS* from acquired uniparental disomy was also associated with aggressive transformation of JMML [42]. Based on these observations, the initial hypothesis of mutually exclusive RAS pathway mutations in JMML would be rebutted. These findings confirm the urgency to identify early genetic or epigenetic markers of leukemic development to enable early treatment.

While some authors recommend avoiding aggressive therapy due to the hypothesized benign clinical course of the RALD [9], optimal treatment is unknown. Most patients require corticosteroid and/or immunosuppressive drugs to treat autoimmune complications including refractory cytopenia and pericardial effusion. Premature death occurred in patients reported in the literature due to malignancy [12] or unexplained sudden death [9]. HSCT, the only curative treatment, has been applied in one patient who suffered from steroid-refractory autoimmunity [5]. It is likely that there may be several subpopulations of RALD patients with different predisposition to develop hematological malignancy, even after years of non-malignant clinical course [43]. Improve understanding of the molecular defects that underlying these evolutions will help to determine the indication and optimal timing of HSCT in treatment of RALD. Small molecule inhibitors of kinase effectors of Ras-GTP have been developed as anticancer agents (e.g., phosphoinositide 3-kinase inhibitor, RAF inhibitor). In RALD, Oliveira and colleagues showed that the use of specific inhibitors of MEK, a downstream component of the Raf/MEK/ERK pathway, corrected the apoptotic defect in vitro and rescued BIM protein expression in *NRAS* mutated-human lymphocytes [4]. Moreover, ERK inhibition also restored apoptosis after IL-2 withdrawal. All this suggests encouraging prospects for the use of RAS-inactivating drugs in the treatment of disorders of lymphocyte homeostasis in addition to cancer.

In summary, this is the first review and largest case series of patients with RALD that provides further details about the clinical spectrum and outcome of this new clinical disease, which lies between ALPS and JMML. The current case documents an additional warning about the long-term risk of malignancy. Further studies are needed to establish clinical and biological hallmarks that could differentiate RALD from JMML, predict evolution, and determine the optimal treatment such the potential role of HSCT for RALD patients.

Authorship Contribution

Q.N. designed the research, performed review, collected and analyzed the data, and wrote the manuscript; C.B., A.B., M.D., I.M., and A.V.D. participated in the clinical care of the patient; L.M. and I.M. performed whole exome sequencing; B.B. participated in the clinical care and wrote the manuscript. All authors contributed to manuscript revisions, read, and approved the submitted version.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Ethics Statement Written informed consent was obtained from the patient's parents in accordance with the 1975 Declaration of Helsinki. Approval was granted by the Ethics Committee of the University Hospitals Leuven (S58466).

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