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Large granular lymphocyte leukemia and pulmonary hypertension : an unusual association

Brieuc Chérel

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THÈSE D'EXERCICE / UNIVERSITÉ DE RENNES 1
sous le sceau de l'Université Bretagne Loire

Thèse en vue du

DIPLÔME D'ÉTAT DE DOCTEUR EN MÉDECINE

présentée par

Brieuc CHEREL

Né le 01/02/1988 à Ploërmel

**Leucémie à grands
lymphocytes
granuleux et
hypertension
pulmonaire : une
association rare**

**Thèse soutenue à Rennes
le 25/10/2017**

devant le jury composé de :

Marc HUMBERT

Professeur – AHP – Hôpital de Bicêtre /
rapporteur

Céline CHABANNE

Praticienne hospitalière – CHU Rennes /
rapporteur

Stéphane JOUNEAU

Professeur – CHU Rennes / *examineur*

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Praticien hospitalier – CH Vannes / *examineur*

Olivier DECAUX

Professeur - CHU Rennes / *président du jury*

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Leucémie à grands lymphocytes granuleux et hypertension pulmonaire : une association rare

Large granular lymphocyte leukemia and pulmonary hypertension: an unusual association

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Résumé

Français

La leucémie à grands lymphocytes granuleux (LGL) est un syndrome lymphoprolifératif rare caractérisé par une expansion de cellules T/NK associée à des cytopénies et d'éventuelles pathologies auto-immunes. Cette étude internationale rétrospective présente 9 cas de patients atteints d'une hypertension pulmonaire prouvée associée à une leucémie LGL. Sur les 5 patients réévalués par cathétérisme cardiaque droit, 4 présentent une amélioration de l'hypertension pulmonaire sous traitement immunosuppresseur spécifique de la leucémie LGL. Avec un suivi médian de 88 mois, tous les patients sont vivants. Les mécanismes pathogéniques liant les deux pathologies sont discutés. Cette étude suggère de compléter le bilan initial d'une HTAP par la recherche d'une population clonale LGL en cas de perturbations hématologiques évocatrices. Si le diagnostic de leucémie LGL est confirmé, l'institution d'un traitement immunosuppresseur peut être proposée.

Abstract

English

Large granular lymphocyte (LGL) leukemia is a rare lymphoproliferative disorder characterized by a clonal expansion of T/NK cells associated with cytopenia and immune diseases. This retrospective international cohort reports 9 cases of patients presenting with pulmonary hypertension (PH) proved by right heart catheterization (RHC) and LGL leukemia. Among the 5 patients revalued by RHC, 4 patients showed hemodynamic PH improvement following immunosuppressive treatment of LGL Leukemia. With a median follow-up of 88 months, all patients are alive. Pathogenesis mechanisms uniting both diseases are discussed. These data suggest completing the initial PAH work-up with T cell clonality detection and to propose starting immunosuppressive therapy if LGL leukemia is concomitantly diagnosed.

Key words: Large granular lymphocyte (LGL) leukemia, Pulmonary hypertension (PH), Pulmonary arterial hypertension (PAH), Right heart catheterization (RHC), Endothelial cells, Immunosuppressive agents

Abbreviations:

6WMT: 6-minutes walking test

AIHA: Auto-immune hemolytic anemia

ANA: Antinuclear antibodies

ANC: Absolute neutrophil count

CI: Cardiac index

CLPD-NK: Chronic lymphoproliferative disorder of natural killer cells

CR: Complete response

DISC: Death-inducing signaling complex

ECs: Endothelial cells

ERS: European respiratory society

ESC: European society of cardiology

FPHN: French Pulmonary Hypertension Network

HIV: Human immunodeficiency viral

KIR: Killer immunoglobulin-like receptor

LGL: Large granular lymphocyte

MHC: Major histocompatibility complex

NK: Natural Killer

NKRs: Natural Killer Receptors

NO: Nitric oxide

PAH: Pulmonary arterial hypertension

PAPm: Mean pulmonary arterial pressure

PAWP: Pulmonary artery wedge pressure

PCR: Polymerase chain reaction

PH: Pulmonary hypertension

PR: Partial Response

PTRV: Peak tricuspid regurgitation velocity

PVR: Pulmonary vascular resistance

REVEAL: Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management

RHC: Right heart catheterization

TCR: T-cell receptor

TNF: Tumor necrosis factor

TTE: Transthoracic echocardiography

WHO: World health organization

WU: Woods units

Introduction

Large granular lymphocyte (LGL) leukemia belongs to the chronic mature lymphoproliferative disorders of the T/natural killer (NK) lineage¹. According to the WHO classification, there are three types of LGL leukemia². The T-LGL leukemia, representing 85% of the cases, and the chronic lymphoproliferative disorder of NK cells (CLPD-NK), which are indolent diseases and share the same clinical and biological presentation³. While aggressive NK cell leukemia, is rarer and characterized by a poor clinical outcome⁴. Overall survival of T and NK LGL leukemia at 10 years is about 70%⁵ and their frequency is estimated at 2% to 5% of the chronic lymphoproliferative diseases in North America and up to 5% to 6% in Asia⁶. The diagnosis of LGL leukemia requires the presence of a T or NK LGL clonal expansion, with an LGL count $> 0.5 \times 10^9/L$ lasting more than six months⁷. The immunophenotyping characteristics of T-LGL leukemia are those of activated T-cells: CD3⁺, TCR $\alpha\beta$ ⁺, CD4⁻, CD5^{dim}, CD8⁺, CD16⁺, and CD57⁺. The CLPD-NK LGL display a CD2⁺, sCD3⁻, TCR $\alpha\beta$ ⁻, CD4⁻, CD8⁺, CD16⁺ et CD56⁺ phenotype⁸. Evidence of the T-LGL clonality is assessed using T-cell receptor (TCR) polymerase chain reaction (PCR) and V β TCR repertory analysis⁹. Because NK cells do not express TCR, restricted expression of Killer immunoglobulin-like receptor (KIR) is used as marker for monoclonal expansion of NK-LGL. Constitutive activation of Stat3 via a gain-of-function mutation is demonstrated in up to 75% of T-LGL leukemia and 50% of CLPD-NK¹⁰. Other recurrent mutations have been reported such as Stat5b¹¹, TNFAIP3, and correlation between the presence of a Stat3 mutation and auto-immune manifestations have been suggested¹². Clonal LGL expansion arises from chronic antigenic stimulation which induces dysregulation of apoptosis, mainly due to constitutive activation of survival pathways⁵. Clinical presentations are mainly related to recurrent bacterial infections associated to neutropenia and/or anemia. B signs and splenomegaly are also reported. Only one third of patients are asymptomatic at diagnosis. Associated diseases, such as auto-immune cytopenia, B cell lymphoma, myelodysplastic syndrome, vasculitis and auto-immune disease, are common. Rheumatoid arthritis occurs in 10 to 18% of patients¹³. Few observations of pulmonary hypertension (PH) has been reported¹⁴⁻¹⁵⁻¹⁶⁻¹⁷⁻¹⁸. This association is further remained controversial.

PH is a syndrome based on diverse etiologies and pathogenesis, defined as an increase in mean pulmonary arterial pressure (PAPm) ≥ 25 mmHg assessed by right heart catheterization (RHC). PH is classified into 5 major groups. In the group 5 defined, as “unclear and/or multifactorial mechanisms”, PH may be associated with hematological disorders such as chronic anemia, myeloproliferative disorders and splenectomy¹⁹. However, lymphoproliferative disorders are not mentioned.

This study reports on a descriptive cohort of 9 patients presenting with PH and LGL leukemia and supports a proposal for pathogenesis.

Materiel & Methods

Cases have been compiled retrospectively from 4 French centers (Dax Hospital, Nantes Hotel-Dieu University Hospital, Bicêtre Hospital AP-HP and Rennes Pontchaillou University Hospital) based on the national French LGL registry, from the Italian LGL registry (Azienda Ospedaliera Università, Padova) and from American LGL registry (University of Virginia Cancer Center, Charlottesville, VA).

Inclusion criteria:

- LGL Leukemia:

Patients fulfilled the usual LGL leukemia diagnostic criteria³⁻⁶⁻²⁰, with more than $0.5 \times 10^9/L$ of circulating LGL. LGL Leukemia must have been proved by typical immunophenotyping on peripheral blood (CD3+ CD8+ CD57+ for T-LGL and CD3- CD8+ CD16- for CLPD-NK) with T-LGL clonality assess by TCR. Patients had bone marrow aspirate, V β TCR repertory analysis and Stat3 mutation detection based on Sanger sequencing. Associations with autoimmune disorders or cancer were notified.

- Pulmonary Hypertension:

Criteria for the PH diagnosis were those recently published²¹. PAPm ≥ 25 mmHg assessed by RHC was required. According to the European society of cardiology (ESC) and European respiratory society (ERS) guidelines, pulmonary arterial hypertension (PAH) refers to a group of PH patient who presents a pre-capillary PH characterized by a pulmonary artery wedge pressure (PAWP) ≤ 15 mmHg and a pulmonary vascular resistance (PVR) > 3 Woods units (WU) in the absence of others causes of pre-capillary PH. To eliminate any other causes of PH, an initial checkup was performed. It usually included an electrocardiogram, an echocardiography, a pulmonary function test, a high-resolution tomography with pulmonary angiography, a ventilation/perfusion lung scan, an abdominal ultrasound scan and blood tests including arterial blood gazes, thyroid function, immunology (antinuclear antibodies (ANA), antiphospholipid antibodies, anticardiolipin antibodies and lupus anticoagulant), hemolysis search and HIV serology.

Response to therapy:

- LGL Leukemia:

Response to treatment was evaluated by blood count after 4 months of treatment. Hematologic complete response (CR) was considered achieved when blood counts returned to normal ranges (eg, platelets > 150 G/L, absolute neutrophil count (ANC) $> 1500/mm^3$, hemoglobin > 12 g/dL, and lymphocytes $< 4G/L$) and circulating LGL in the normal range. In addition, complete molecular remission was considered achieved when T-cell clone was undetectable using PCR. Partial response (PR) was defined as an improvement in blood counts (eg, ANC > 500 or decreasing transfusion requirements) in the absence of CR⁶⁻²².

- Pulmonary hypertension:

Response in pulmonary hypertension was assessed by clinical examination (signs of right heart failure, syncope and WHO functional class), functional test as 6-minutes walking test (6MWT), cardiopulmonary exercise testing with oxygen consumption (VO₂) measurement, biological test as BNP/NT-pro-BNP plasma levels, imaging via echocardiography or cardiac magnetic resonance (Right atrial measure and presence of pericardial effusion) and hemodynamics test assessed by RHC (Right atrial pressure, cardiac index (CI) and mixed venous oxygen saturation)²¹. Transthoracic echocardiography (TTE) provided an estimate of systolic pulmonary arterial pression in the absence of RHC evaluation during follow-up but should not be considered as a robust substitute. On TTE, systolic pulmonary arterial pression

is estimated from the peak tricuspid regurgitation velocity (PTRV) considering right atrial pressure as described by the simplified Bernoulli equation²³.

According to ESC/ERS, these data allowed classifying patients into 3 risk groups; low risk, intermediate risk and high risk. The estimated 1-year mortality was < 5%, 5-10% and > 10% respectively²¹. The « Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management » (REVEAL) risk score was also calculated. Five risk groups were obtained through score calculation; low risk (score 1-7), average risk (score = 8), moderate high risk (score = 9), high risk (score = 10-11) and very high risk (score > 12). The estimated 1-year mortality was > 95%, 90-95%, 85-90%, 70-85% and < 70% respectively²⁴⁻²⁵. Main treatment goal in patients with PH is to achieve a low-risk status. Recent guidelines adopt also as treatment objective an improvement of 6MWT with distance > 440 m²⁶.

Results

This international cohort collected 13 patients, among them 4 patients were excluded due to lack of PH confirmation by RHC. The median age at diagnostic was 53 years and the sex ratio was 2/7. The median follow-up for LGL Leukemia and PH was 88 months. The clinical and biological characteristics of the population at diagnostic are described in **Table 1**.

Initial presentation:

- LGL leukemia:

8 patients had T-LGL Leukemia and 1 patient had CLPD-NK. 3 patients suffered from an associated immune pathology; Behçet's disease, auto-immune hemolytic anemia (AIHA) and arthritis. LGL leukemia was diagnosed before PH for 5 patients and both diseases were concomitantly detected for 2 patients.

- Pulmonary Hypertension:

Among the 9 patients assessed by RHC, 7 patients met PAH criteria. 2 patients (cases 2 and 3) were classified PH because their PVR measures were < 3 WU and presented different hemodynamic characteristics with elevated cardiac index (4.15 and 4.6 L/min/m², respectively). The initial research of secondary causes of PH noticed moderate interstitial lung pneumonia associated to Methotrexate for a patient and positive anticardiolipin antibodies for 2 patients. Three patients presented positive antinuclear antibodies but without specificity. At diagnostic, median PAPm was 43 mmHg (27-63), median PVR was 7.5 mmHg (2.3-12.7) and median cardiac index was 2.6 L/min/m² (2.17-4.6). About clinical consequences of PH, the median 6MWT was 360 m (285-544). Right heart cavity dilatation at TTE was viewed at PH diagnosis for 7 patients. One patient showed pericardial effusion.

Response to therapy:

Hemodynamic, clinical and LGL characteristics available at diagnosis and at last news are reported in **Table 2**.

- LGL Leukemia:

The median line of hematological treatment was 2 (0-3). Immunosuppressive therapy included methotrexate (n=6), ciclosporin (n=4) and cyclophosphamide (n=5) with an overall response rate of 4/6 (67%), 3/4 (75%) and 3/5 (60%), respectively. The complete response rate for the three drugs was 1/6 (16%), 0/4 (0%) and 1/5 (20%), respectively. The unique CLPD-NK patient did not receive any treatment because of absence of hematological criteria.

- Pulmonary Hypertension

At last news, all patients included were alive. Oxygen withdrawal was obtained for the 2 initially oxygen-dependent patients. Right heart cavity dilatation regression was achieved for 3 of the 7 patients and stability for 3 others. Among the 5 evaluable patients assessed by RHC during follow-up, 4 (Cases 2, 5, 6 and 7) showed PH hemodynamic improvement following hematological treatment. Only one patient achieved a complete hemodynamic normalization. Immunosuppressive therapy included cyclophosphamide in 3 cases and ciclosporin in one case. Two responder patients received also PH specific therapy, one bosentan then sildenafil and the other ambrisentan. The patient, who was stable on sildenafil, presented PH amelioration after ciclosporin initiation. The non-responder patient was diagnosed with CLPD-NK and did not receive any immunosuppressive therapy but combination of bosentan, sildenafil and nifedipine. About the 4 patients without RHC assessment during follow-up, 3 (Cases 1, 3 and 4) showed clinical or TTE improvement. Overall 7 of 9 patients showed a clinical, TTE or hemodynamic improvement of PH on immunosuppressive treatment.

- Associated response:

Associated response of LGL Leukemia and PH on immunosuppressive treatment was observed in 4 patients, as detailed in **Table 3** and illustrated in **Figure 1** (cases 1 and 2). No patient showed hemodynamical improvement of PH without a LGL leukemia response.

Safety:

No toxicity was reported for ciclosporin. An ANC decrease with bacterial dermo-hypodermatitis was related to cyclophosphamide leading to treatment cessation. Methotrexate was associated with an immune-allergic interstitial lung disease with treatment cessation requirement and in two cases with hepatitis leading to dosage adjustment.

Discussion

We report on a series of 9 patients suffering from concomitant LGL leukemia and PH. To our knowledge, this has been previously described in 7 sporadic cases¹⁴⁻¹⁵⁻¹⁶⁻¹⁷⁻¹⁸, making a total number of 15 cases. One patient of our cohort was initially reported in a case report¹⁵. Considering the rarity of both diseases, it is tempting to postulate a possible link between LGL clonal expansion and the development of PH.

Based on the clinical characteristics of these 9 PH patients, we may consider that they rather belong to the group 5 of PH classifying. The main causes of PH have been excluded: heritable, drugs, portal hypertension, HIV infection, connective tissue disease, congenital or left heart disease and chronic thromboembolic disorders. However, we cannot exclude the implication of underlying causes in 5 out of the 9 cases. Patient 2 developed interstitial pneumonitis on methotrexate which could result in PH. Nevertheless, PH persisted after pneumonitis resolution and finally decreased following further immunosuppressive therapy. Patient 5 showed a right-left shunt by patent oval foramen but it was considered as consequence and not causality of PH by cardiologic staff. Patients 7 and 8 had positive anticardiolipin antibodies but without thromboembolic disorders signs on CT pulmonary angiogram and ventilation/perfusion scan and we assume that this was not related to the development of PH. AIHA was reported in patient 4, hemolytic disorders can induce PH via significant reduction in nitric oxide (NO) availability due to free hemoglobin and accompanying platelet activation²⁷ but no active hemolysis was objectified at PH diagnosis and during follow-up.

The benefit from specific therapy of LGL leukemia led to significant hemodynamical PH improvement in 4 of the 5 RHC evaluable patients. In addition, three patients showed clinical improvement with NYHA score decrease or 6MWT enhancement but without significant hemodynamic change. None of the patients died with a median follow-up of 88 months.

If we are focusing on the 7 patients with PAH associated to LGL leukemia, their clinical evolution and survival appeared to be different from PAH group 1 patients. Group 1 includes idiopathic and heritable PAH, PAH associated with human immunodeficiency viral infection (HIV), schistosomiasis, congenital heart defect, connective tissue diseases, portal hypertension and drug induced PAH²¹. The most important American cohort REVEAL showed a 1-year survival at 91% for patients from group 1²⁴. The 1-year survival was < 70% for very high-risk patients. 40% and 26.2% of patients received combination PAH therapies or an intravenous prostacyclin analog. In the French cohort « French Pulmonary Hypertension Network » (FPHN), also including group 1 patients, the 1-year survival was 88%²⁸. Our cohort had favorable initial hemodynamics and clinical features than those of REVEAL and FPHN cohorts. However, the PAH evolution following immunosuppressive therapy and the absence of PAH related mortality over a long-term follow-up suggests a different condition. Of note, we cannot exclude that 4 patients could have benefit from concomitant administration of PAH specific therapy with LGL leukemia treatment. In two cases, the PAH response did not appear to be correlated with PAH therapy; patient 4 could achieve dosage reduction of epoprostenol during methotrexate therapy and patient 6, who was stable on sildenafil, showed PAH improvement after ciclosporin initiation, suggesting a potential role of immunosuppressive therapy on PAH.

PAH is considered as a vasculopathy with endothelial dysfunction leading to abnormal vascular homeostasis. Dysregulated levels of vasodilator molecule as NO and vasoconstrictor molecule as Endothelin-1, associated with cell survival and proliferation constitute constrictive lesion, which include medial hypertrophy and intimal and adventitial thickening, and complex lesion as plexiform lesion, very characteristic of PAH²⁹. This pathological vascular remodeling leads to an increase in pulmonary artery pressures and eventual right ventricular (RV) dysfunction. We suggest that LGL leukemia is responsible for PAH development. In fact, LGL leukemia seems to precede PAH development and PAH improvement seems to be correlated to LGL leukemia response to immunosuppressive therapy. Furthermore, improvement of hemodynamic data allowing PAH therapeutic de-escalation is quite unusual due to the supposed irreversibility of PAH histological lesions. Pathogenesis mechanism proposal is described in **Figure 2**.

A direct cytotoxicity of CD3+ T cells on pulmonary endothelial cells (ECs) is suggested. This cytotoxicity is controlled by balance between activating Natural Killer Receptors (NKR) and inhibiting NKRs recognizing major histocompatibility complex (MHC) class I proteins³⁰. Leukemic LGLs, expressing elevated levels of activating NKRs, acquire the ability to directly lyse pulmonary artery ECs³⁰. Activating bonding of NKRs drives a cytoplasmic pathway leading a direct cytotoxicity via migration of lytic granules containing perforin and granzyme B toward the contacted cell like CE³¹⁻³². Because the activation of NKRs leads on Ras/MAPK signaling pathway, a farnesyltransferase inhibitor Tipifarnib, was tested in a patient who presented association between LGL leukemia and PAH¹⁶. After 4 courses of treatment, an arterial pulmonary pressure improvement associated with a decreased tumor marrow infiltration was reported.

The cytotoxicity of leukemic LGLs involves NKRs and dysregulation of Fas/Fas-Ligand (FasL) apoptotic pathway³³⁻³⁴. LGLs are resistant to Fas-mediated apoptosis through a secretion of soluble Fas (sFas) which acts as a decoy receptor³⁵ and elevated c-FLIP

expression³⁶. LGLs express surface FasL and secrete high level of soluble FasL, considered as a potential cause of auto-immune symptoms and neutropenia in LGL leukemia³⁷. ECs express also Fas and FasL promoting the inhibition of leukocyte extravasation and protecting vascular endothelium from local inflammation. ECs have also an elevated expression of c-FLIP, inhibiting the Fas/FasL mediated apoptosis³⁸⁻³⁹⁻⁴⁰. However, local expression of cytokines such as TNF α , expressed in high level in LGLs environment⁴¹, decreases the ECs expression of FasL making ECs susceptible to cytotoxicity⁴². A study reported 2 patients with association of LGL leukemia and PAH, who expressed high level of circulating FasL¹⁸. Lung biopsy described LGL cells infiltration expressing FasL with pneumocyte apoptosis mimicking pathological changes of graft-versus-host disease. Patients clinical improvement was correlated with decreased of circulating FasL levels on LGL leukemia therapy.

In murine model, ECs-Fas mediated apoptosis induces PAH typical vascular lesion⁴³. This could explain why hemodynamic normalization could not be observed in patients with PAH and LGL leukemia. The only patient who achieved hemodynamic normalization was classified PH because of PVR < 3WU, pleading for two distinct mechanisms of PH associated with LGL leukemia. Firstly, vascular remodeling induced by LGL leads to elevated vascular resistance characterizing PAH. Secondly, moderate vascular resistance in the absence of definitive vascular lesions, and elevated cardiac index linked to significative anemia, cause reversible PH.

The association between LGL leukemia and PH does not appear fortuitous regarding their respecting low incidence and the unusual association of both diseases. Moreover, the improvement of PH on immunosuppressive therapy strengthens the relationship between these both diseases. It suggests supplementing the current recommendations for initial work-up with a T clonality search in context of clinic-biological abnormalities evoking an underlying LGL leukemia. A better understanding of pathogenesis mechanisms is required to adapt the optimal therapeutic option for such patients. In absence of gravity signs of PH, we propose to initiate only immunosuppressive therapy with a close monitoring of PH evolution.

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TITRE DE LA THESE d'EXERCICE

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an unusual association**

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Appendix

Table 1: Patients characteristics, n=9.

	1	2	3	4	5	6	7	8	9
Patient n°	53	59	55	52	82	58	33	39	38
Age at diagnosis (Years)	F	F	M	F	F	F	F	F	M
Sexe (M/F)	LGL								
LGL WHO classification	T-LGL Leukemia	T-LGL Leukemia	T-LGL Leukemia	T-LGL Leukemia	T-LGL Leukemia	T-LGL Leukemia	T-LGL Leukemia	CLPD-NK	T-LGL Leukemia
Immunophenotyping									
CD3	+	+	+	+	+	+	+	-	+
CD8	+	+	+	+	+	+	+	-(dim)	+
CD16	+	-	-	+	+/-	+	+	+	-
CD56	-	-	-	-	+	-	-	+	-
CD57	-	+/-	+/-	+	+	+	+	-	+
CD4	-	-	-	-	-	-	-	-	-
Clonality	TCR								
Vbeta repertory	alpha/beta Polyclonal Y640G	alpha/beta Monoclonal NA	alpha/beta Monoclonal D661Y	alpha/beta Monoclonal NA	alpha/beta Monoclonal Wild type	alpha/beta Polyclonal Y640F	alpha/beta Monoclonal D566N ex 19	Polyclonal	alpha/beta NA
STAT3 Mutation	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NA
Bone Marrow Infiltration	No	No	No	Telengectasia + AIHA	No	No	Seronegative Arthritis	Migrant palindromic arthritis	Belçet's
Associated AI disease	311	115	10	209	8	88	265	19	5
LGL Follow-up (months)	2	2	1	1	2	3	3	0	2
Nb. Of therapy lines	Neutropenia	Tumoral syndrome + B signs	Neutropenia + PH	Neutropenia + PAH	PAH	Recurrent infection	Symptomatic Anemia	No	Neutropenia + PAH
Indication of treatment	Pulmonary Hypertension								
PH classification	PAH	PH	PH	PAH	PAH	PAH	PAH	PAH	PAH
Differential diagnosis	CT pulmonary angiogram								
CT pulmonary angiogram	Normal	ILP MTX-associated	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Ventilation/Perfusion Scan	Normal	NA	NA	NA	Normal	NA	Normal	Normal	Normal
Spirometry	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Abdominal Ultrasound	Normal	ANA (1/100)	Normal	Normal	ANA (1/320)	Normal	Anticardiolipin +	Normal	Normal
Biology	ANA (1/1280)	ANA (1/100)	Normal	Normal	Normal	Normal	Anticardiolipin +	Anticardiolipin +	NA
PH Follow-up (months)	49	88	7	229	8	90	179	141	51
PH associated therapy	No	No	No	Yes	No	Yes	Yes	Yes	No
REVEAL Risk Score	9	6	3	8	5	6	6	7	NA
REVEAL Risk Group	Moderately high	Low	Low	Average	Low	Low	Low	Low	NA
ESC/ERS Risk Group	High risk	Intermediate risk	Intermediate risk	High Risk	Intermediate risk	Intermediate risk	Low risk	Intermediate risk	High Risk

Abbreviations: AIHA, auto-immune hemolytic anemia; AI, auto-immune; CLPD-NK, chronic lymphoproliferative disorder of NK cells; CT, computerized tomography; F, female; ILP, interstitial lung pneumonia; LGL, large granular lymphocyte; M, male; MTX, methotrexate; N°, number; NA, no available; PAH, pulmonary arterial hypertension; PH, pulmonary hypertension; ANA, antinuclear antibodies; WHO; world health organization.

Table 2: PH and LGL leukemia characteristics available at diagnosis then on treatment at last news, n=9.

Patient n°	1		2		3		4		5	
LGL	At diagnosis	On treatment	At diagnosis	On treatment	At diagnosis	On treatment	At diagnosis	On treatment	At diagnosis	On treatment
Hemoglobin (g/dL)	12,2	14	13,3	12,9	10,1	9,1	9,9	12,9	12,8	13
Neutrophils (/mm3)	350	790	380	4300	960	1300	800	1720	3780	3000
Lymphocytes (/mm3)	1790	650	3750	945	4600	3400	2000	1000	4150	2570
LGL (%)	39	NA	90	NA	45	NA	9	0	25	18
LGL therapy										
1st Line	Ciclosporine		Methotrexate		Ciclosporine		Methotrexate		Methotrexate	
2nd Line	Cyclophosphamide		Cyclophosphamide						Cyclophosphamide	
3rd Line										
Follow-up (months)	311		115		12		209		8	
Pulmonary Hypertension										
At diagnosis	On treatment	At diagnosis	On treatment	At diagnosis	On treatment	At diagnosis	On treatment	At diagnosis	On treatment	On treatment
Right Heart Catheterisation										
PAPm (mmHg)	27	NA	34	12	33	NA	57	NA	35	26
PCWP (mmHg)	7	NA	10	NA	11	NA	10	NA	3	NA
PVR (WU)	4,4	NA	2,4	1,5	2,3	NA	12,7	NA	4,4	4
Cardiac Index (L/min/m ²)	2,97	NA	4,15	3,4	4,6	NA	2,2	NA	2,2	2,78
TTE										
PTRV (m/S)	3,7	3,3	3,7	NA	3,5	3	4,1	4,5	3,1	NA
Estimated PAPs (mmHg)	65	56	62	39	65	39	81	88	45	NA
Right cavity dilatation	Yes	No	Yes	No	Yes	No	Yes	Yes	No	No
Clinical Impact										
Dyspnea NYHA Score	4	2	2	1	2	1	4	1	3	3
Right heart failure signs	No	No	No	No	No	No	No	No	No	No
6MWT (meters)	285	357	300	420	544	NA	290	475	420	395
Ambient Air PaO ₂ (mmHg)	62	93	NC	NA	62	NA	63	NA	69	NA
O ₂ -therapy Withdrawal	Yes		No need		No need		Yes		No need	
PH Therapy										
1st Line	No Treatment		No Treatment		No Treatment		Epoprostenol		No Treatment	
2nd Line										
Follow-up (months)	49		88		7		229		8	
Patient n°	6		7		8		9			
LGL	At diagnosis	On treatment	At diagnosis	On treatment	At diagnosis	On treatment	At diagnosis	On treatment		
Hemoglobin (g/dL)	12,5	12,9	8,3	11	15	NA	13,3	10,6		
Neutrophils (/mm3)	400	3000	1050	600	5780	NA	0	10		
Lymphocytes (/mm3)	5330	3320	2960	4060	1530	NA	16500	4600		
LGL (%)	80	15	70	69	22	NA	65	NA		
LGL therapy										
1st Line	Methotrexate		Methotrexate		No treatment		Methotrexate			
2nd Line	Cyclophosphamide		Ciclosporine				Cyclophosphamide			
3rd Line	Ciclosporine		Cyclophosphamide							
Follow-up (months)	88		265		19		5			
Pulmonary Hypertension										
At diagnosis	On treatment	At diagnosis	On treatment	At diagnosis	On treatment	At diagnosis	On treatment			
Right Heart Catheterisation										
PAPm (mmHg)	55	37	46	36	63	52	43	NA		
PCWP (mmHg)	11	NA	10	NA	12	NA	10	NA		
PVR (WU)	8,3	5,8	9,2	4,2	7,5	NA	9,4	NA		
Cardiac Index (L/min/m ²)	2,98	3	2,6	4,3	2,17	3,6	2,15	NA		
TTE										
PTRV (m/S)	4	2,5	2,6	3,6	5,1	NA	NA	NA		
Estimated PAPs (mmHg)	70	35	42	60	115	NA	NA	NA		
Right cavity dilatation	Yes	Yes	Yes	Yes	Yes	NA	NA	NA		
Clinical Impact										
Dyspnea NYHA Score	NA	2	NA	1	NA	2	NA	NA		
Right heart failure signs	No	No	No	No	Yes	No	Yes	NA		
6MWT (meters)	450	400	NA	708	NA	595	NA	NA		
Ambient Air PaO ₂ (mmHg)	NA	NA	NA	NA	NA	NA	NA	NA		
O ₂ -therapy Withdrawal	No need		No need		No need		NA			
PH Therapy										
1st Line	Bosentan		Ambrisentan		Bosentan + Sildenafil + Nifedipine		No Treatment			
2nd Line	Sildenafil									
Follow-up (months)	90		179		141		51			

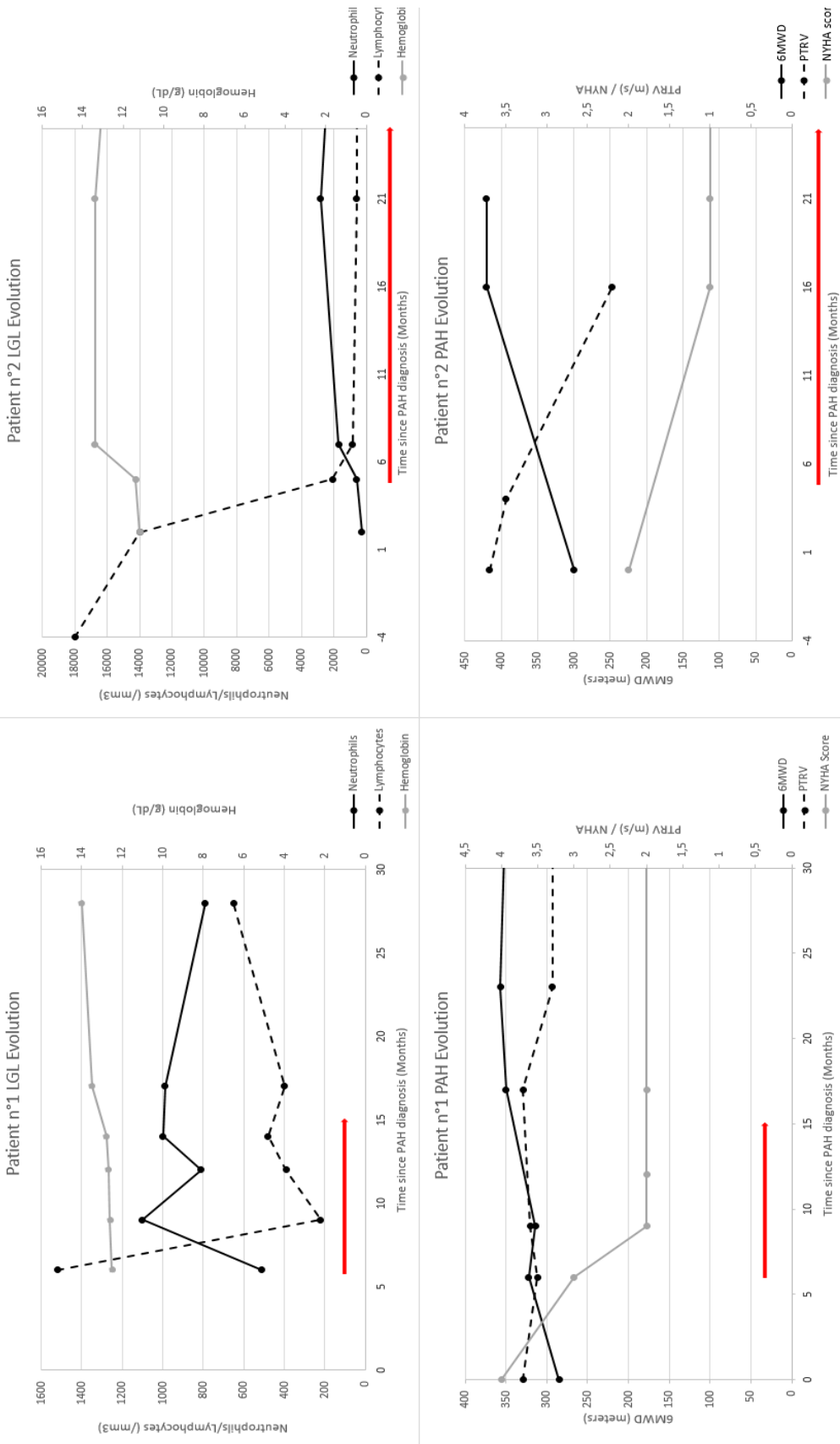
Abbreviations: 6MWT, 6-minutes walking test; LGL, large granular lymphocyte; NA, not available; PAPm: mean pulmonary arterial pressure; PCWP, pulmonary arterial wedge pressure; PH, pulmonary hypertension; PTRV, Peak tricuspid regurgitation velocity; PVR, pulmonary vascular resistance; TTE, transthoracic echocardiography; WU; Wood units.

Table 3: Association of LGL leukemia and PH response on immunosuppressive treatment.

		PH Evaluation	
		R	SD/PD
LGL Evaluation	R/PR	4 patients	/
	SD/PD	/	1 patient

Abbreviations: CR, complete response; LGL, large granular lymphocyte; NA, No available; PD, progression disease; PH, pulmonary hypertension; PR, partial response; R, response; SD, stable disease.

Figure 1: Example for 2 patients (cases 1 and 2) of LGL leukemia and PH evolution on immunosuppressive treatment



Red arrow indicates time on cyclophosphamide. Abbreviations: 6MWT, 6-minutes walking test; LGL, large granular lymphocyte; PAH, pulmonary arterial hypertension; PTRV, Peak tricuspid regurgitation velocity.

Figure 2: Two potential mechanisms of endothelial cell lysis by LGL cells.

NKR Pathway: Activating NKR such as NKG2D, KIR2DS, KIR3DS, NKG2C, NKp44, NKp46, FcR III (CD16), TCR, and NKp30 act through 3 adaptor proteins either in a homodimeric or heterodimeric form (DAP12, DAP10, and CD3). These signals lead to PI3K/MEK/ERK activation then granule redistribution and movement. Granule exocytosis releases Granzyme B who activates caspases in the target cell.

Fas/FasL Pathway: the trimerization of Fas (CD95) induced by interaction with Fas-L leads formation of the DISC. First, the adaptor FADD binds via its own death domain to the death domain in CD95. FADD recruits procaspase-8 (also known as FLICE) into the DISC. Next, procaspase-8 is activated proteolytically and active caspase-8 is released from the DISC into the cytoplasm in the form of a heterotetramer of two small subunits and two large subunits. Active caspase-8 cleaves various proteins in the cell including procaspase-3, which results in its activation and the completion of the cell death programme. Another pathway called intrinsic, may be induced. Caspase-8 cuts the Bcl-2 family member Bid; T-Bid 'activates' the mitochondria who release pro-apoptotic molecules such as cytochrome C. Cytochrome C with the apoptosis protease-activating factor Apaf-1 and procaspase-9 in the cytoplasm form the Apoptosome, the second initiator complex of apoptosis who leads again to apoptosis.

Abbreviations: DISC, Death-inducting signaling complex; FADD, Fas-associated death domain protein; LGL, large granular lymphocyte; NKR, Natural Killer Receptor; T-bid, Truncated Bid.

Adapted from Epling-Burnette PK⁴ and Kramer PH³⁴.

