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Large granular lymphocyte leukemia and pre-capillary pulmonary hypertension

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Abbreviations:

6MWT: 6-minute walking test

CLPD-NK: Chronic lymphoproliferative disorder of natural killer cells

DISC: Death-inducing signaling complex

EC: Endothelial cell

KIR: Killer immunoglobulin-like receptor

LGL: Large granular lymphocyte

NK: Natural Killer

NKR: Natural Killer Receptor

PAH: Pulmonary arterial hypertension

PH: Pulmonary hypertension

RHC: Right heart catheterization

TTE: Transthoracic echocardiography

Large granular lymphocyte (LGL) leukemia belongs to the chronic mature lymphoproliferative disorders of the T/natural killer (NK) lineage¹. T-LGL leukemia, which accounts for 85% of cases, and chronic lymphoproliferative disorder of NK cells (CLPD-NK) are indolent diseases with the same clinical and biological features¹. Overall 10-year survival in T and NK LGL leukemia patients is about 70% and estimated frequency is 2 to 5% of chronic lymphoproliferative diseases in North America and 5 or 6% in Asia². Clinical presentations are mainly related to recurrent bacterial infections associated with neutropenia and/or anemia. Associated diseases, such as autoimmune cytopenia, B-cell lymphoma, myelodysplastic syndrome, vasculitis and autoimmune disease are commonly observed. Sporadic cases of pulmonary arterial hypertension (PAH) have been reported³⁻⁴⁻⁵⁻⁶⁻⁷ in patients with LGL leukemia. Whether or not this association is fortuitous remains unknown and its incidence seems to be <0.5%.

In group 5 of the pulmonary hypertension (PH) classification, hematological disorders are discussed⁸. However, lymphoproliferative disorders are not mentioned.

This article reports on a descriptive cohort of 9 patients presenting with pre-capillary pulmonary hypertension and LGL leukemia.

Methods

This international cohort included patients compiled retrospectively from the French, Italian and American national LGL registry.

All patients fulfilled the usual LGL leukemia diagnostic criteria⁹ with more than $0.5 \times 10^9/L$ of circulating LGLs. Criteria for T-LGL leukemia included a typical expression of T-LGL surface markers on peripheral blood (CD3+ CD8+ CD57+ and/or CD16+) associated with a T-cell receptor γ gene clonal rearrangement or clonal V β expression on flow cytometry. Criteria for NK-LGL leukemia included expression of NK-LGL markers (CD3-CD8+/CD16+ and/or CD56). The hematologic complete response and partial response criteria were considered after 4 months of treatment according to those previously published⁹.

Pre-capillary hypertension was confirmed by right heart catheterization (RHC) according to criteria published in the European guidelines¹⁰ and updated in the proceedings of the 6th World Symposium on Pulmonary Hypertension⁸. The response of pre-capillary pulmonary hypertension to therapy was assessed, as for PAH follow-up recommended by the European

guidelines¹⁰, by clinical examination, the 6-minute walking test (6MWT), imaging via echocardiography and hemodynamics test assessed by RHC.

Results

This international cohort included 15 patients. Six patients were excluded due to lack of confirmation of pulmonary hypertension by RHC. There were 1 male and 8 females and the median age at diagnosis was 52 years. The median follow-up for LGL leukemia and pre-capillary pulmonary hypertension was 19 and 90 months, respectively. The first diagnosis is PH for four patients, LGL leukemia for 2 patients and concomitant diagnosis for 3 patients. For all LGL leukemia patients, disproportionate dyspnea for anemia is the reason why PH was looking for. Concerning PH patients, LGL leukemia was diagnosed with neutropenia or presence of LGL cells on blood count.

Hemodynamic, clinical and LGL characteristics available at diagnosis and last follow-up are reported in **Table 1**.

- LGL leukemia:

Patients received a median of two lines of therapy (range: 0-3), including methotrexate, ciclosporin and cyclophosphamide with an overall hematological response rate of 3/5 (60%), 2/3 (66%) and 4/7 (57%), respectively. The CLPD-NK patient did not receive any treatment given the absence of cytopenia.

- Pulmonary hypertension:

At the last follow-up assessment, all included patients were alive. Two patients were treated for LGL leukemia only, one (cases 1) responded for both diseases, and the other patient (case 9) had stable LGL leukemia without PH evaluation. Six patients (cases 2, 3, 4, 5, 6 and 7) were concomitantly treated for PH and LGL leukemia, and all of them achieved a hematological and PH response. The one CLPD-NK patient (case 8) received only PH treatment and achieved stable disease.

Finally, in five of the six patients (cases 5, 6, 7, 8 and 9) assessed by RHC at follow-up, four showed a hemodynamic improvement in pulmonary hypertension following hematological treatment. Two patients (cases 3 and 4) of the three who did not undergo RHC assessment at follow-up showed clinical or Transthoracic echocardiography (TTE) improvement.

Overall, seven of the eight evaluable patients showed a clinical, TTE or hemodynamic improvement in pulmonary hypertension following immunosuppressive treatment. No

hemodynamic improvement in pulmonary hypertension occurred in any patient in the absence of a hematological response.

Discussion

We report on a series of 9 patients with concomitant LGL leukemia and pre-capillary pulmonary hypertension. To our knowledge, this combination has been previously reported in sporadic cases³⁻⁴⁻⁵⁻⁶⁻⁷, giving a total number of 15 cases (one patient in our cohort was initially included in a case report⁴). Considering the rarity of both diseases, we speculate a possible link between LGL clonal expansion and development of pre-capillary pulmonary hypertension.

On the basis of the characteristics of these 9 patients, we consider them belonging to group 5 (unclear and/or multifactorial mechanisms) of the PH classification. The main causes of pre-capillary pulmonary hypertension were excluded. However, even if we cannot exclude underlying causes in five out of the nine cases, their involvement is unlikely. Patient 5 showed a right-to-left shunt through a patent foramen ovale but it was considered a consequence and not a cause of pre-capillary pulmonary hypertension. Patients 9 and 11 had positive anticardiolipin antibodies but without signs of thromboembolic disease on ventilation/perfusion lung and computed tomography pulmonary angiography scans, ruling out group 4 chronic thromboembolic pulmonary hypertension. Three patients had anti-nuclear antibodies (33%), which correspond to the estimated frequency of 40% in LGL leukemia patients², although without anti-ENA or anti-DNA specificity. Autoimmune hemolytic anemia was reported in patient 4 and the direct antiglobulin test was positive in patient 7 but no active hemolysis was objectively documented. Any patient present signs of pulmonary veno-occlusive disease after control of CT pulmonary angiogram. We cannot eliminate underlying thrombotic process. However, any characteristic of chronic pulmonary thromboembolism was seen for the seven patients with ventilation/perfusion scan available. The benefit of specific therapy for LGL leukemia led to significant hemodynamic improvement in four of the six RHC-evaluable patients. In addition, four patients showed clinical improvement with NYHA functional class improvements and/or better 6MWT scores but without significant hemodynamic changes. None of the patients died with a median follow-up of 90 months.

We cannot eliminate an inflammatory or auto-immune component as observed in PAH associated with systemic lupus erythematosus. However, we suppose that the favorable evolution of PH following immunosuppressive therapy was mainly due to an improvement of the endothelium dysfunction, as reported for PH associated with tyrosine kinase inhibitor¹¹. It may also explain the reversibility of hemodynamic parameters by treating the trigger. In addition, that can explain why the use of cyclophosphamide is effective on PH in these case despite potential endothelial toxicities¹².

PAH is considered to be a vasculopathy with endothelial cell dysfunction leading to abnormal vascular homeostasis¹³. We suggest that LGL leukemia could be involved in the development of PAH. The proposed pathogenetic mechanism is depicted in **Figure 1**.

Direct cytotoxicity of CD3+ T cells on pulmonary endothelial cells (ECs) has been suggested. Leukemic LGLs, expressing high levels of activating Natural Killer Receptors (NKR), acquire the ability to directly lyse pulmonary artery ECs¹⁴ via migration of lytic granules containing perforin and granzyme B towards the contacted cell-like ECs¹⁵⁻¹⁶. A specific inhibitor of NKRs pathway, tipifarnib, was tested on a patient who had both LGL leukemia and PAH⁵ with report of improvement in mean pulmonary artery pressure. The cytotoxicity of leukemic LGLs involves also dysregulation of the Fas/Fas-ligand (FasL) apoptotic pathway¹⁷⁻¹⁸. In the murine model, Fas-mediated EC apoptosis induces PAH-typical vascular lesions¹⁹.

The limited size of this retrospective cohort limits the potential conclusions. Further studies are required to better understand pathogenetic mechanisms and to determine the optimal therapeutic option for such patients. The initial PAH workup should include a T-cell clonality assessment when clinical/biological abnormalities are suggestive of underlying LGL leukemia in patients presenting with pre-capillary pulmonary hypertension.

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Dr. Loughran is on the scientific advisory boards of Bioniz Therapeutics and Keystone Nano.

All other authors declare no competing interests.

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Appendix

Captions for table 1:

Abbreviations: 6MWT, 6-minute walking test; ANA, antinuclear antibody; CLPD-NK, chronic lymphoproliferative disorder of NK cells; CT, computerized tomography; DAT+, positive direct antiglobulin test; DLCO, diffusing capacity of the lungs for carbon monoxide; F, female; LGL, large granular lymphocyte; M, male; NA, not available; mPAP: mean pulmonary artery pressure; PAWP, pulmonary arterial wedge pressure; PH, pulmonary hypertension; PTRV, peak tricuspid regurgitation velocity; PVR, pulmonary vascular resistance; TTE, transthoracic echocardiography; WHO, World Health Organization; WU, Wood unit.

Captions for figure 1:

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

Fas/FasL Pathway: Trimerization of Fas (CD95) induced by interaction with Fas-L leads to DISC formation. First, the adaptor FADD binds via its own death domain to the death domain in CD95. FADD recruits procaspase-8 (also known as FLICE) to 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 and two large subunits. Active caspase-8 cleaves various proteins in the cell including procaspase-3, which results in its activation and completion of the cell death program. Another pathway, called the intrinsic pathway, may be induced. Caspase-8 cuts the Bcl-2 family member Bid; t-Bid 'activates' the mitochondria which release pro-apoptotic molecules such as cytochrome c. Together with the apoptosis protease-activating factor Apaf-1 and procaspase-9 in the cytoplasm, these molecules form the apoptosome, the second initiator complex of apoptosis which again leads to apoptosis.

Abbreviations: DISC, death-inducing signaling complex; FADD, Fas-associated death domain protein; LGL, large granular lymphocyte; NKR, Natural Killer Receptor; t-Bid, truncated Bid. Adapted from Epling-Burnette PK⁵ and Krammer PH¹⁶.

Table 1b

Patient n°	1	2	3	4	5
CT pulmonary angiogram	Normal	Normal	Normal	Normal	NA
Ventilation/Perfusion Scan	Normal	NA	Normal	Normal	Normal
Spirometry	Normal	Normal	Normal	Normal	Normal
Abdominal Ultrasound	Normal	Normal	Normal	Normal	Normal
Biology	ANA (1/1280)	Negative	ANA (1/320)	Negative	ANA (1/640) + DAT+
Patient n°	6	7	8	9	
CT pulmonary angiogram	Normal	Normal	Normal	Normal	
Ventilation/Perfusion Scan	NA	Normal	Normal	Normal	
Spirometry	NA	Normal	Normal	NA	
Abdominal Ultrasound	Normal	Normal	Normal	Normal	
Biology	Negative	Anticardiolipin +	Anticardiolipin +	NA	

Table 1c

Patient n°	1	2	3	4	5
LGL therapy					
1st Line	Ciclosporine	Methotrexate	Methotrexate	Cyclophosphamide	Cyclophosphamide
2nd Line	Cyclophosphamide		Cyclophosphamide		
3rd Line					
Pulmonary Hypertnesion therapy					
1st Line	No Treatment	Epoprostenol	No Treatment	Ambrisentan + Sildenafil	Bosentan
2nd Line					
Patient n°	6	7	8	9	
LGL therapy					
1st Line	Methotrexate	Methotrexate	No treatment	Methotrexate	
2nd Line	Cyclophosphamide	Ciclosporine		Cyclophosphamide	
3rd Line	Ciclosporine	Cyclophosphamide			
Pulmonary Hypertnesion therapy					
1st Line	Bosentan	Ambrisentan	Bosentan + Sildenafil + Nifedipine	No Treatment	
2nd Line	Sildenafil				

Figure 1: Two potential mechanisms of endothelial cell lysis by LGL cells.

