Large Granular Lymphocyte Leukemia and Precapillary Pulmonary Hypertension

To the Editor:

Large granular lymphocyte (LGL) leukemia belongs to the chronic mature lymphoproliferative disorders of the T/natural killer (NK) lineage.[1] 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 features.[1] Overall 10-year survival of patients with T- and NK-LGL leukemia is about 70%, and the estimated frequency is 2% to 5% of chronic lymphoproliferative diseases in North America and 5% to 6% in Asia.[2] Clinical presentations are related mainly 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 reported[3-7] 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, hematologic disorders are discussed.[8] However, lymphoproliferative disorders are not mentioned.

This article reports on a descriptive cohort of nine patients presenting with precapillary pulmonary hypertension and LGL leukemia.

Methods

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

All patients fulfilled the usual LGL leukemia diagnostic criteria,[9] with circulating LGLs exceeding 0.5×10^9/L. Criteria for T-LGL leukemia included 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 published.[9]

Precapillary hypertension was confirmed by right heart catheterization (RHC) according to criteria published in the European guidelines[10] and updated in the proceedings of the 6th World Symposium on Pulmonary Hypertension.[8] The response of precapillary pulmonary hypertension to therapy was assessed, as for PAH follow-up recommended by the European guidelines,[10] by clinical examination, the 6-min walking test, imaging via echocardiography, and by RHC.

Results

This international cohort included 15 patients. Six patients were excluded because of lack of confirmation of pulmonary hypertension by RHC. There were one man and eight women, and the median age at diagnosis was 52 years. The median follow-up for LGL leukemia and precapillary pulmonary hypertension was 19 and 90 months, respectively. The initial diagnosis was PH for four patients, LGL leukemia for two patients, and concomitant diagnoses for three patients. For all patients with LGL leukemia, disproportionate dyspnea for anemia was the reason why a search was begun for PH. Concerning patients with PH, LGL leukemia was diagnosed with neutropenia or the presence of LGLs on blood count.

Hemodynamic, clinical, and LGL characteristics available at diagnosis and last follow-up are reported in Tables 1 through 3.

LGL Leukemia

Patients received a median of two lines of therapy (range, none to three), including methotrexate, cyclosporine, and cyclophosphamide, with an overall hematologic response rate of three of five (60%), two of three (66%), and four of seven (57%), respectively. The patient with CLPD-NK 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

TABLE 1] Pulmonary Hypertension and Large Granular Lymphocyte Parameters at Diagnosis and on Treatment: n = 9

Characteristic	Patient No. 1		Patient No. 2		Patient No. 3		Patient No. 4		Patient No. 5	
Age at diagnosis, y	53		52		82		73		46	
Sex, M/F	F		F		F		F		F	
LGL										
LGL WHO classification	T-LGL leukemia		T-LGL leukemia		T-LGL leukemia		T-LGL leukemia		T-LGL leukemia	
Follow-up, mo	311		209		8		18		15	
Hematologic parameters	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	9.9	12.9	12.8	13	15.1	12.8	9.4	12.9
Neutrophils, /mm^3	350	790	800	1,720	3,780	3,000	290	2,530	1,380	2,137
Lymphocytes, /mm^3	1,790	650	2,000	1,000	4,150	2,570	4,500	510	1,960	3,071
LGL, %	39	NA	9	0	25	18	70	0	NA	1
Pulmonary hypertension										
Follow-up, mo	49		229		8		18		15	
Right heart catheterization	At diagnosis	On treatment	At diagnosis	On treatment	At diagnosis	On treatment	At diagnosis	On treatment	At diagnosis	On treatment
mPAP, mm Hg	27	NA	57	NA	35	26	43	NA	49	25
PCWP, mm Hg	7	NA	10	NA	3	NA	10	NA	15	12
PVR, Wood units	4.4	NA	12.7	NA	4.4	4	11	NA	7.5	3
Cardiac index, L/min/m^2	2.97	NA	2.2	NA	2.2	2.78	1.9	3	2.7	4.3
TTE										
PTRV, m/s	3.7	3.3	4.1	4.5	3.1	NA	4	3.3	NA	3.1
Estimated sPAP, mm Hg	65	56	81	88	45	NA	70	51	75	31
Right cavity dilation	Yes	No	Yes	Yes	No	No	Yes	Yes	Yes	No
Clinical impact										
Dyspnea NYHA score	4	2	4	1	3	3	3	1	2	2
D_{LCO}, %	34	51	54	NA	50	NA	60	60	47	NA
Right heart failure signs	No	No	No	No	No	No	Yes	No	Yes	No
6MWT, m	285	357	290	475	420	395	450	501	376	480

(Continued)

TABLE 1] *(Continued)*

Characteristic	Patient No. 1		Patient No. 2		Patient No. 3		Patient No. 4		Patient No. 5	
Ambient air Pao_2, mm Hg	62	93	63	NA	69	NA	78	NA	NA	NA
O_2 therapy withdrawal	Yes		Yes		No need		No need		No need	

Characteristic	Patient No. 6		Patient No. 7		Patient No. 8		Patient No. 9	
Age at diagnosis, y	58		33		39		38	
Sex, M/F	F		F		F		M	
LGL								
LGL WHO classification	T-LGL leukemia		T-LGL leukemia		CLPD-NK		T-LGL leukemia	
Follow-up, mo	88		265		19		5	
Hematologic parameters	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, /mm^3	400	3,000	1,050	600	5,780	NA	0	10
Lymphocytes, /mm^3	5,330	3,320	2,960	4,060	1,530	NA	16,500	4,600
LGL, %	80	15	70	69	22	NA	65	NA
Pulmonary hypertension								
Follow-up, mo	90		179		141		51	
Right heart catheterization	At diagnosis	On treatment	At diagnosis	On treatment	At diagnosis	On treatment	At diagnosis	On treatment
mPAP, mm Hg	55	37	46	36	63	52	43	NA
PCWP, mm Hg	11	NA	10	NA	12	NA	10	NA
PVR, Wood units	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

(Continued)

TABLE 1] *(Continued)*

Characteristic	Patient No.							
	6		7		8		9	
TTE								
PTRV, m/s	4	2.5	2.6	3.6	5.1	NA	NA	NA
Estimated sPAP, mm Hg	70	35	42	60	115	NA	NA	NA
Right cavity dilation	Yes	Yes	Yes	Yes	Yes	NA	NA	NA
Clinical impact								
Dyspnea NYHA score	NA	2	NA	1	NA	2	NA	NA
D_{LCO}, %	NA	NA	NA	NA	NA	NA	NA	NA
Right heart failure signs	No	No	No	No	Yes	No	Yes	NA
6MWT, m	450	400	NA	708	NA	595	NA	NA
Ambient air Pao_2, mm Hg	NA	NA	NA	NA	NA	NA	NA	NA
O_2 therapy withdrawal	No need		No need		No need		NA	

6MWT = 6-minute walking test; CLPD-NK = chronic lymphoproliferative disorder of NK cells; D_{LCO} = diffusing capacity of the lungs for carbon monoxide; F = female; LGL = large granular lymphocyte; M = male; mPAP = mean pulmonary artery pressure; NA = not available; NYHA = New York Heart Association; PCWP = pulmonary capillary wedge pressure; PTRV = peak tricuspid regurgitation velocity; PVR = pulmonary vascular resistance; sPAP = systolic pulmonary artery pressure; T-LGL = T-cell LGL; TTE = transthoracic echocardiography; WHO = World Health Organization.

TABLE 2] Pulmonary Hypertension Differential Diagnosis: n = 9

Investigations	Patient No. 1	2	3	4	5
CT pulmonary angiogram	Normal	Normal	Normal	Normal	NA
V̇/Q̇ scan	Normal	NA	Normal	Normal	Normal
Spirometry	Normal	Normal	Normal	Normal	Normal
Abdominal ultrasound	Normal	Normal	Normal	Normal	Normal
Biology	ANA (1/1,280)	Negative	ANA (1/320)	Negative	ANA (1/640) + DAT^+

Investigations	Patient No. 6	7	8	9	
CT pulmonary angiogram	Normal	Normal	Normal	Normal	
V̇/Q̇ scan	NA	Normal	Normal	Normal	
Spirometry	NA	Normal	Normal	NA	
Abdominal ultrasound	Normal	Normal	Normal	Normal	
Biology	Negative	Anti-cardiolipin$^+$	Anti-cardiolipin$^+$	NA	

ANA = anti-nuclear antibody; DAT^+ = positive direct anti-globulin test result; NA = not available.

TABLE 3] Pulmonary Hypertension and Large Granular Lymphocyte Leukemia Treatment Details: n = 9

Therapies	Patient No. 1	2	3	4	5
LGL therapy					
First line	Cyclosporine	Methotrexate	Methotrexate	Cyclophosphamide	Cyclophosphamide
Second line	Cyclophosphamide	…	Cyclophosphamide	…	…
Third line	…	…	…	…	…
Pulmonary hypertension therapy					
First line	No treatment	Epoprostenol	No treatment	Ambrisentan + sildenafil	Bosentan
Second line	…	…	…	…	…

Therapies	Patient No. 6	7	8	9
LGL therapy				
First line	Methotrexate	Methotrexate	No treatment	Methotrexate
Second line	Cyclophosphamide	Cyclosporine	…	Cyclophosphamide
Third line	Cyclosporine	Cyclophos phamide	…	…
Pulmonary hypertension therapy				
First line	Bosentan	Ambrisentan	Bosentan + sildenafil + nifedipine	No treatment
Second line	Sildenafil	…	…	…

LGL = large granular lymphocyte.

only, one (case 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 hematologic and PH response. The one patient with CLPD-NK (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 hemodynamic improvement in pulmonary hypertension after hematologic treatment. Two patients (cases 3 and 4) of the three who did not undergo RHC assessment at follow-up showed clinical or transthoracic echocardiography improvement.

Overall, seven of the eight evaluable patients showed clinical, transthoracic echocardiography, or hemodynamic improvement in pulmonary hypertension after immunosuppressive treatment. No hemodynamic improvement in pulmonary hypertension occurred in any patient in the absence of a hematologic response.

Discussion

We report on a series of nine patients with concomitant LGL leukemia and precapillary pulmonary hypertension. To our knowledge, this combination has been previously reported in sporadic cases,[3-7] giving a total number of 15 cases (one patient in our cohort was initially included in a case report[4]). Considering the rarity of both diseases, we speculate a possible link between LGL clonal expansion and the development of precapillary pulmonary hypertension.

On the basis of the characteristics of these nine patients, we consider them as belonging to group 5 (unclear and/or multifactorial mechanisms) of the PH classification. The main causes of precapillary pulmonary hypertension were excluded. However, even if we cannot exclude underlying causes in five of the nine cases, their involvement is unlikely. Patient 3 showed a right-to-left shunt through a patent foramen ovale, but it was considered a consequence and not a cause of precapillary pulmonary hypertension. Patients 7 and 8 were positive for anti-cardiolipin

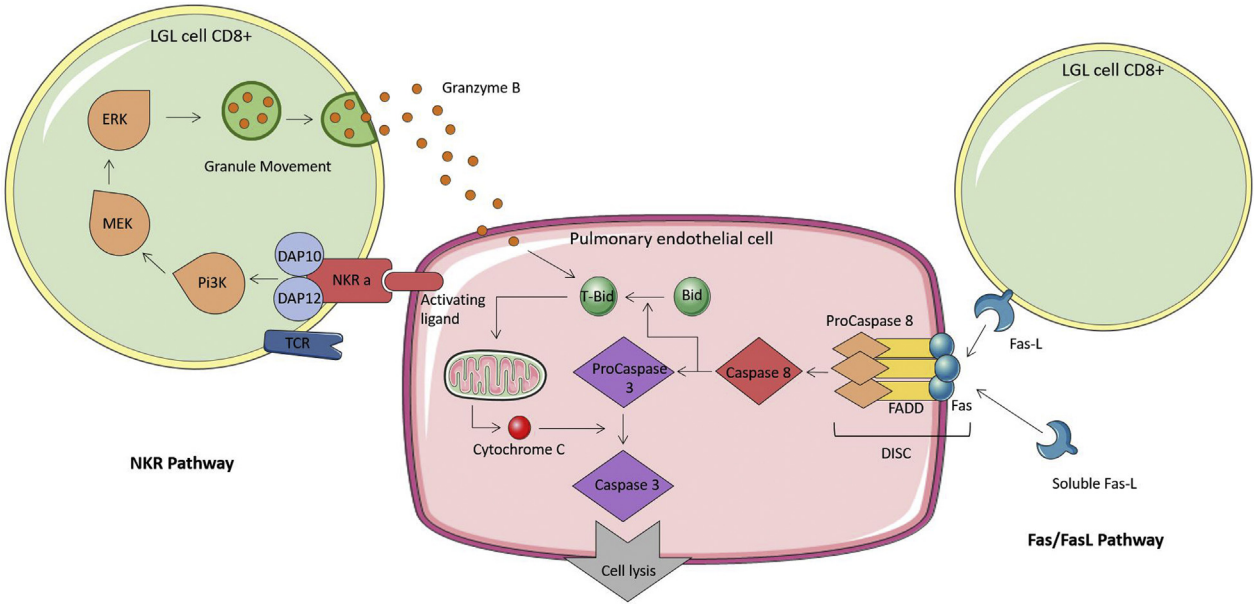

Figure 1 – *Two potential mechanisms of endothelial cell lysis by LGL cells. Natural killer receptor (NKR) pathway: Activating NKRs such as NKG2D, KIR2DS, KIR3DS, NKG2C, NKp44, NKp46, Fc receptor III (CD16), TCR, and NKp30 act through three adaptor proteins in either homodimeric or heterodimeric form (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 proapoptotic 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. DAP10, DAP12 = DnaX-activating proteins 10 and 12; DISC = death-inducing signaling complex; ERK = extracellular signal-regulated kinase; FADD = Fas-associated death domain protein; FasL = Fas ligand; LGL = large granular lymphocyte; MEK = MAPK (mitogen-activated protein kinase)/ERK kinase; PI3K = phosphatidylinositol 3-kinase; t-Bid = truncated Bid. (Adapted with permission from Epling-Burnette et al[5] and Krammer.[18])*

antibodies but without signs of thromboembolic disease on $\dot{V}/\dot{Q}$ lung and CT 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 patients with LGL leukemia,[2] although without anti-ENA (extractable nuclear antigen) or anti-DNA specificity. Autoimmune hemolytic anemia was reported in patient 4, and the direct anti-globulin test produced a positive result in patients 2 and 5, but no active hemolysis was objectively documented. No patients present signs of pulmonary venoocclusive disease after control of CT pulmonary angiogram. We cannot eliminate an underlying thrombotic process, but no characteristic of chronic pulmonary thromboembolism was seen for the seven patients with $\dot{V}/\dot{Q}$ 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 New York Heart Association functional class improvements and/or better 6-min walking test scores but without significant hemodynamic changes. None of the patients died (median follow-up of 90 months).

We cannot eliminate an inflammatory or autoimmune component as observed in PAH associated with systemic lupus erythematosus. However, we suppose that the favorable evolution of PH after immunosuppressive therapy was due mainly to an improvement in endothelial dysfunction, as reported for PH associated with tyrosine kinase inhibitor.[11] 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 in PH in these cases despite potential endothelial toxicities.[12]

PAH is considered to be a vasculopathy with endothelial cell dysfunction leading to abnormal vascular homeostasis.[13] We suggest that LGL leukemia could be involved in the development of PAH. The proposed pathogenic mechanism is depicted in Figure 1.

A direct cytotoxic effect of $CD3^+$ T cells on pulmonary endothelial cells (ECs) has been suggested. Leukemic LGLs, expressing high levels of activating natural killer receptors (NKRs), acquire the ability to directly lyse pulmonary artery ECs[14] via migration of lytic granules containing perforin and granzyme B toward the contacted ECs.[15,16] A specific inhibitor of the NKR pathway, tipifarnib, was tested on a patient with both LGL leukemia and PAH; an improvement in mean pulmonary artery pressure was reported.[5] The cytotoxicity of leukemic LGLs also involves dysregulation of the Fas/Fas ligand apoptotic pathway.[17,18] In a murine model, Fas-mediated EC apoptosis induces vascular lesions typical of PAH.[19]

The size of this retrospective cohort limits potential conclusions. Further studies are required to better understand pathogenic mechanisms and to determine the optimal therapeutic options 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 precapillary pulmonary hypertension.

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