Clinical Course and Prognosis of the Lymphoproliferative Disease of Granular Lymphocytes

A Multicenter Study

F. PANDOLFI, MD, T. P. LOUGHRAN JR, MD,* G. STARKEBAUM, MD, T. CHISESI, MD, T. BARBUI, MD, W. C. CHAN, MD, J. C. BROUET, MD, G. DE ROSSI, MD, R. W. MCKENNA, MD, F. SALSANO, MD, F. HERRMANN, MD, J. W. VANOOSTVEEN, MD, G. SCHLIMOK, MD, A. CAFARO, MD, R. ZAMBELLO, MD,† M. C. GARCIA RODRIGUEZ, MD, C. H. GEISLER, MD, G. PIZZOLO, MD, R. G. STEIS, MD, J. U. BRISBANE, MD, M. E. KADIN, MD, A. MANTOVANI, MD, S. TAGAWA, MD, A. S. FAUCI, MD, G. GASTL, MD, M. PALUTKE, MD, S. J. PROCTOR, MD, H. F. PROSS, MD, P. MANCINI, BS, F. AIUTI, MD, AND G. SEMENZATO, MD

Lymphoproliferative disease of granular lymphocytes (LDGL) is a recently recognized, relatively rare atypical lymphocytosis characterized by the presence of over 2000 lymphocytes with cytoplasmic azurophilic granules/mm^3 in the peripheral blood. The clinical course is heterogeneous, varying from spontaneous regression to progressive, malignant disease. As a consequence, clinical intervention is not standardized. In a worldwide multicenter study, the authors observed 151 patients with LDGL for a mean follow-up time of 29 months. Forty-three patients were asymptomatic at the time of diagnosis. In the remaining cases, clinical symptoms included fever (41 cases), infections (58), neutropenia (47), anemia (17), and thrombocytopenia (12). In 69 cases, LDGL coexisted with an associated disease. Most patients had a nonprogressive clinical course despite the presence of severe symptoms. In 19 patients, death related to LDGL occurred within 48 months. The authors investigated which features at diagnosis were significantly associated with increased mortality. In the univariate analysis, lymph node and liver enlargement, fever at presentation, skin infiltration, a low (≤ 5000/mm^3) or high (> 20,000/mm^3) peripheral leukocyte count, relatively low (≤ 3000) or high (> 7000/mm^3) absolute peripheral granular lymphocyte (GL) count, and a low (≤ 15%) percentage of HNK-1-positive cells were found to be predictors of increased mortality. In the multivariate analysis, significant independent predictors were fever at diagnosis, a low (≤ 15%) percentage of HNK-1-positive peripheral blood mononuclear cells (PBMC) and a relatively low (≤ 3000) GL count. These results showed that about 25% of the patients with LDGL were diagnosed after a routine blood count and had no clinical symptoms. The remaining patients were symptomatic, with some experiencing a fatal clinical course. The authors' analysis of the significant prognostic features of LDGL may help in understanding the heterogeneous nature of this syndrome.

Cancer 65:341–348, 1990.

From the Departments of Allergy and Clinical Immunology, Human Biopathology, and Clinical Medicine, La Sapienza University of Rome, Rome, Italy; Fred Hutchinson Cancer Research Center, Seattle, Washington; Veterans Administration Medical Center, Seattle, Washington; Hematology Section, Vicenza Hospital, Vicenza, Italy; Department of Hematology, Ospedali Riuniti di Bergamo, Bergamo, Italy; Department of Pathology and Laboratory Medicine, Emory University Hospital, Atlanta, Georgia; Hopital Saint-Louis, Paris, France; Department of Pathology, University of Texas, Dallas, Texas; Department of Hematology, University of Mainz, West Germany; Department of Pathology, Free University Hospital, Amsterdam, The Netherlands; Medical Clinic II, Ausburg, West Germany; Immunology Unit, Hospital La Paz, Madrid, Spain; Medical Department, Finsen Institute, Copenhagen, Denmark; Department of Hematology, University of Verona, Verona, Italy; National Cancer Institute, Frederick, Maryland; Veterans Administration Medical Center, Boston, Massachusetts; Beth Israel Hospital, Boston, Massachusetts; Mario Negri Institute, Milan, Italy; Division of Internal Medicine, Osaka University, Japan; Department of Internal Medicine, University of Innsbruck, Austria; Wayne State University School of Medicine, Detroit, Michigan; Department of Hematology, The Royal Victoria Infirmary, Newcastle upon Tyne, United Kingdom; Department of Microbiology and Immunology, Queen's University, Kingston, Canada; Department of Statistics, University of Rome; and Department of Clinical Medicine, University of Padua, Italy.

Supported in part by MPI (Italy) fondi 60% (1986, 1987); CNR (Italy) Progetto strategico: Farmaci per malattie orfane; grants CA 18221 and HL 32444 awarded by the National Cancer Institute and National Heart, Lung and Blood Institutes, DHHS, and the Leukemia Research Foundation.

* Special fellow of the Leukemia Society of America.

† Fellow of AIRC, Milan.

The authors thank Dr. M. Falcolini (Rome) for skillful assistance in the statistical analysis of the data, and Prof. F. Mandelli (Rome) for his helpful criticisms.

Address for reprints: G. Semenzato, MD, Istituto di Medicina Clinica, dell'Università di Padova, Clinica Medica I, Via Giustiniani 2, 35128 Padova, Italy.

Accepted for publication July 17, 1989.

OVER THE LAST FEW YEARS, a number of investigators have reported patients with an atypical lymphocytosis characterized by an expansion of lymphocytes with cytoplasmic azurophilic granules, usually referred to as granular lymphocytes (GL) or T-gamma cells. Although in the past patients with this lymphocytosis were considered to have a leukemic disease, in recent years it has been shown that the clinical course of patients with GL lymphocytosis is variable. Thus the working designation of lymphoproliferative disease of GL (LDGL) has been established to indicate this heterogeneous but clinically, morphologically, and immunologically distinct syndrome.[1-3]

The diagnosis is made by finding a peripheral lymphocytosis of GL and can be confirmed by light microscopic examination with routine blood smears. Most patients have a chronic GL lymphocytosis without other signs of malignancy and a nonprogressive clinical course, even without treatment.[4-8] In several cases of LDGL, an associated disease (often rheumatoid arthritis (RA), hepatitis, or cancer) has been observed.[6,9-11]

Since GL have cytotoxic activity and are thought to play an important role in host defenses against tumors and infectious agents, several investigators have suggested that in some cases GL lymphocytosis could be the result of a reactive process.[6,9] Other patients, however, develop a more aggressive disease with anemia or, more frequently, neutropenia, with a few cases experiencing an aggressive malignant clinical course.[12-14] Thus, the disease is a heterogeneous disorder ranging from an indolent and, possibly, reactive condition to overt malignancy.[15] Since the prognosis may vary considerably[15-17] and it is often impossible at diagnosis to distinguish the aggressive disease from the indolent form, it seems relevant to define the features with a significant prognostic value at the time of presentation.

Although LDGL has been considered to be a rare disease, increasing awareness has resulted in more frequent diagnoses today. Nevertheless, even the recently reported "larger" series of patients[15,16] have been too small to establish the natural history, clinical course, and prognosis of the disease. This can only be achieved on the basis of a larger collaborative effort. Through a multicenter study involving 25 institutions, we collected clinical and laboratory data from 151 patients with LDGL to determine which factors were associated with, or predictive of, a subsequent aggressive clinical course.

Materials and Methods

Patients and Criteria for Admission

The criterion for admission to this retrospective study was a diagnosis of LDGL made on the basis of a count of at least 2000 GL/mm^3 in the peripheral blood for at least 3 months in the lack of any obvious causative illness such as acute Epstein Barr virus infection or cytomegalovirus infection. The value of 2000 GL/mm^3 represents an increase of five to seven times the normal values (250–460/mm^3). Since the disease is heterogeneous and no criteria are currently available to distinguish idiopathic persistent diseases from cases resulting from reactive proliferations, we included all such cases in this large study. Once general prognostic criteria are established, further studies will help recognize different criteria in different groups of patients with idiopathic or reactive conditions. Clinical and laboratory data from 151 patients with LDGL at diagnosis were collected from 25 institutions in 11 countries, specifically the US (61 cases), Italy (60 cases), West Germany (eight), France (seven), Netherlands (four), Denmark (three), Japan (two), Canada (one), and UK (one).

Immunologic Studies

Peripheral blood mononuclear cells (PBMC) were isolated from freshly drawn heparinized blood by Ficoll-Hypaque gradient centrifugation. The positivity for monoclonal antibodies defining CD2, CD3, CD4, CD8, CD16, and HNK-1 (Leu-7) related antigens was determined using a fluorescent microscope or an analyzer following standard techniques.[18] Natural killer (NK) activity was assessed against the K 562 cell line according to standard techniques.[19]

Statistical Analysis

The univariate statistical analysis was performed using the product-limit survival estimate and Mantel-Cox statistic test, during a follow-up of 48 months.[20] In order to further analyze prognostic factors, we adopted the proportional hazard regression model by Cox (Biomedical Package BMDP, 1981).[21] All of the variables that were significant in the univariate analysis ($P < 0.05$) were used to develop a multiple regression model. The model was elaborated in keeping with the partial likelihood theory and estimated the regression coefficients (β) expressing the link between covariates and survival. A positive coefficient means an increase in the death rate whereas a negative coefficient has the opposite meaning. The regression coefficients are given together with standard measures of significance of the covariates (prognostic factors). The set of variables analyzed in Cox's model were as follows: leukocyte count $\leq$ 5000, 5001 to 20,000, and $>$ 20,000; absolute number of GL/mm^3 $\leq$ 3000, 3001 to 7000, and $>$ 7000; percent HNK-1-positive (HNK-1+) GL $\leq$ 15% and $>$15%.

Results

Clinical Findings

Eighty-six patients were males and 65 females. The age at diagnosis ranged from 5 to 88 years with a mean of 55 years; 12 cases were younger than 30 years, 70 cases were between 30 and 60 years old, and 69 patients were older than 60. The mean period of observation was 29 months. Only 13 patients were observed for less than 6 months and 29 patients had a follow-up of more than 4 years.

Twenty-six patients died after a mean follow-up time of 23 months (range, 1–84 months) (Fig. 1). Five of the deaths were unrelated to LDGL (in four cases the cause of death was a cardiovascular event and in one case a head injury). In the statistical analysis, these five cases were considered as censored at the time of death, *i.e.*, alive but lost to the follow-up. In the remaining 21 cases, eight deaths were due to infections (five with sepsis and three with pneumonia), six deaths to progressive lymphoproliferative disease with increase in the leukocyte count not responsive to chemotherapy, three deaths to concomitant neoplastic disorders (one with a diffuse undifferentiated lymphoma, one with acute myeloid leukemia [AML], and one with squamous cell carcinoma), one death to disseminated intravascular coagulation (DIC), one death to gastrointestinal (GI) bleeding, and two deaths to undetermined causes. In the two patients with lymphoma and in the patient with AML, the neoplasias were discovered after the diagnosis of LDGL. These 21 deaths were considered related to LDGL. The four deaths due to DIC, GI bleeding, and undetermined causes were also included because the two patients who died from DIC and GI bleeding were both thrombocytopenic and in the two who died from undetermined reasons, it was likely that the deaths were related to either anemia (Coomb's positive) or infections.

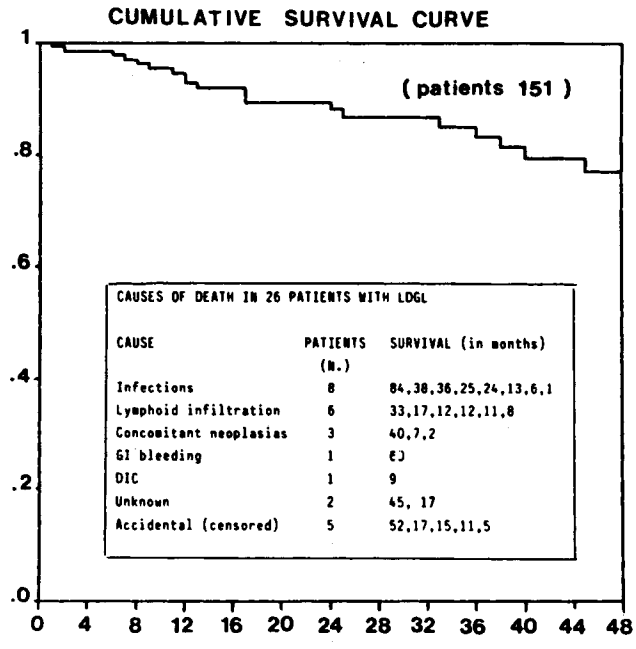

FIG. 1. Cumulative survival curve at 48 months in 151 cases of LDGL and causes of death. Patients whose death was thought to be accidental were censored at the time of death.

An associated disease was reported in 69 patients. The most frequently observed associated diseases were arthritis or RA (27 patients), hepatitis (12 patients), cancer (ten patients, mostly lymphomas or other neoplasias responsive to chemotherapy), pure erythrocyte aplasia (four patients), and hemolytic anemia (two patients). Remaining diseases were represented by a miscellanea of conditions including chronic infections (five patients), autoimmune disorders (two patients), primary immunological defects (two cases), and hematologic disorders (two cases). Although in most cases the associated disease was discovered before the diagnosis of LDGL, we cannot rule out the possibility that, in some cases, its onset was subsequent to LDGL, rather than a primary event.

The clinical status during the follow-up was reported as follows: (1) worsening in 15 cases (13 died within 48 months); (2) improved in 21 cases; and (3) stable in the remaining 115 patients. During the follow-up, the GL lymphocytosis spontaneously improved in 17 patients who received no cytostatic or corticosteroid treatment. In five of these cases, the GL expansion disappeared after a period ranging from 6 months to 4 years.

Univariate Analysis

The univariate analysis was carried out during 48 months of follow-up. Results related to clinical, laboratory, and immunologic data are reported in Tables 1 through 3. Factors present at diagnosis that were predictive of an increased frequency of death within 48 months were (Figs. 2 and 3): lymph node enlargement, liver enlargement, skin infiltrations, and fever at the time of diagnosis. The most commonly observed patterns of fever were (1) high continuous, (2) high remitting, and (3) continuous low grade. Laboratory features associated with poor prognosis were a low ($\leq$ 5000) or high ($>$ 20,000/mm^3) peripheral leukocyte count, a relatively low ($\leq$ 3000), or high ($>$ 7000/mm^3) absolute peripheral GL count, and a low ($\leq$ 15%) percentage of HNK-1+ cells. In addition, a trend toward increasing GL lymphocytosis after diagnosis was also significantly associated with increased mortality within 4 years. As reported in Figure 3, the differences between the various classes of leukocyte and GL counts were also assessed separately for pairs of classes.

TABLE 1. Survival Distributions for Groups With Different Clinical Features

	% Censored*	*P* value
Age (yr) (n = 151)		
<30 (12)	0.75	
30–60 (70)	0.90	
>60 (69)	0.87	0.814
Sex (n = 151)		
Male (86)	0.84	
Female (65)	0.90	0.419
Lymph node enlargement (151)		
No (132)	0.89	
Yes (19)	0.73	0.044
Spleen enlargement (132)†		
No (66)	0.92	
Yes (66)	0.80	0.080
Liver enlargement (151)		
No (100)	0.91	
Yes (51)	0.80	0.026
Clinical symptoms (151)		
Cumulative		
No symptoms (43)	0.95	
Any symptoms (108)	0.84	0.127
Fever		
No (110)	0.91	
Yes (41)	0.75	0.006
Astenia		
No (104)	0.91	
Yes (47)	0.78	0.094
Joint pain		
No (108)	0.88	
Yes (43)	0.83	0.286
Infections		
No (93)	0.88	
Yes (58)‡	0.86	0.922
Liver impairment		
No (134)	0.89	
Yes (17)	0.70	0.063
Skin infiltration		
No (146)	0.89	
Yes (5)	0.40	0.0000
Associated diseases (151)		
Cumulative		
No associated disease (82)	0.89	
Any associated disease (69)	0.85	0.382
Hepatitis		
No (139)	0.87	
Yes (12)	0.83	0.771
Cancer		
No (141)	0.87	
Yes (10)	0.90	0.966
Arthritis or RA		
No (124)	0.87	
Yes (17)	0.88	0.776

RA: rheumatoid arthritis.

Univariate analysis was performed with the Mantel-Cox test. *P* values represent the significance level of the test, showing the difference of mortality in different classes for each variable.

* According to the Mantel Cox test, censored are those patients who survived to the end of the study and those patients who were still alive when lost to observation, before the end of the follow-up (48 mo).

† Nineteen patients who had been splenectomized before diagnosis are not included.

‡ Infections were cutaneous and subcutaneous (27), upper (7) and lower respiratory tract (22), sepsis (8), ORL (7), and urinary tract (4). One patient had tuberculosis. Infections of different type were seen in 18 cases.

TABLE 2. Survival Distributions for Groups With Different Laboratory Data: Univariate Analysis

	% Censored	*P* value
Lymphocytosis before diagnosis of LDGL (151)		
>3 yr (25)	0.86	
<3 yr (126)	0.92	0.523
Hemoglobin (g/l) (151)		
≤8.9 (17)	0.88	
9–10.9 (20)	0.85	
>10.9 (114)	0.87	0.936
Leukocyte count (×1000/mm^3) (151)		
≤5.0 (25)	0.84	
5.001–20.0 (109)	0.92	
>20.0 (17)	0.65	0.004
Absolute number of GL (×1000/mm^3) (151)		
≤3.0 (47)	0.85	
3.000–7.0 (76)	0.97	
>7.0 (28)	0.80	0.042
GL lymphocytosis trend (146)*		
Stable (103)	0.90	
Reduced (28)	0.92	
Increased (15)	0.53	0.0004
Absolute number of neutrophils (/mm^3) (149)		
<500 (55)	0.85	
501–1500 (41)	0.90	
>1500 (53)	0.87	0.603
Platelets (/mm^3) (134)		
<100,000 (12)	0.75	
>100,000 (122)	0.87	0.136
Lymphoid BM infiltration (118)		
<30% (39)	0.89	
≥30% (79)	0.82	0.545

LDGL: lymphoproliferative disease of granular lymphocytes; GL: granular lymphocytes; BM: bone marrow.

* GL trend does not reflect the situation at diagnosis but during the follow-up. Five patients with a short follow-up were excluded from this analysis.

The following factors were not associated with an increased mortality within 4 years: age; sex; spleen enlargement; and the presence of any associated clinical symptoms such as astenia, joint pain, infections, or liver impairment, all independently evaluated. Other factors not significant in predicting mortality included the presence of an associated disease (any) or an association with hepatitis, cancer or arthritis (including RA), diseases which were separately analyzed. Splenomegaly (present in 66 patients) was not associated with short survival, but a few patients with massive spleen enlargement did have a poor prognosis (data not shown). Lymphocytosis present for more than 3 years before diagnosis of LDGL, the levels of hemoglobin, the absolute counts of neutrophils and platelets or the degree of bone marrow infiltration by lymphocytes (see Table 2 for classes) were not significant; the percentage of PBMC reactive with CD2, CD3, CD8,

CD16, and CD11 related reagents of the NK functional activity of PBMC were also not prognostic factors.

Multivariate Analysis

Results of the multivariate Cox's analysis are shown in Table 4. Significant independent predictors of short survival were as follows: (1) fever at diagnosis; (2) a low (≤ 15%) percentage of PBMC positive with HNK-1 monoclonal antibody; and (3) a relatively low (≤ 3000/mm^3) GL count. The Cox's model was also run considering only two ranges for the values of GL and leukocyte counts, *i.e.*, class 1 with low or high GL counts, and class 2 with intermediate levels. Results indicated a significant level of a positive β coefficient for class 1 (low or high levels) *versus* 2 (intermediate levels; data not shown) together with fever and frequency of HNK-1+ cells. Although significant in the univariate analysis, leukocyte count, liver enlargement, lymph node enlargement, and skin infiltration were not found to be significant when analyzed by multivariate techniques, suggesting a correlation between or among variables.

TABLE 3. Survival Distributions for Groups With Different Immunologic Data: Univariate Analysis

	% Censored	*P* value
% of PBMC positive with		
Anti-CD3 (136)		
≤33 (16)	0.87	
34–66 (21)	0.90	
>66 (99)	0.87	0.958
Anti-CD8 (142)		
≤33 (36)	0.80	
34–66 (40)	0.92	
>66 (66)	0.89	0.213
Anti-HNK-1 (121)		
≤15 (12)	0.58	
16–40 (28)	0.92	
>40 (81)	0.91	0.0005
Anti-CD16 (103)		
≤15 (40)	0.90	
16–40 (18)	0.88	
>40 (45)	0.93	0.757
Anti-CD11 (94)		
≤15 (51)	0.86	
16–40 (12)	1.00	
>40 (31)	0.77	0.193
In vitro NK activity (93)		
Reduced or absent (50)	0.78	
Normal (28)	0.96	
Increased (15)	0.88	0.183

PBMC: peripheral blood mononuclear cells; NK: natural killer.

Discussion

In this worldwide multicenter study on LDGL, we analyzed the prognostic factors predictive of mortality related to disease occurring within 48 months from diagnosis (observed in 13.9% of cases in our study). In the univariate analysis, lymph node enlargement, liver enlargement, fever at diagnosis, skin infiltrations, a low (≤ 5000) or high (> 20,000/mm^3) peripheral leukocyte count, a relatively

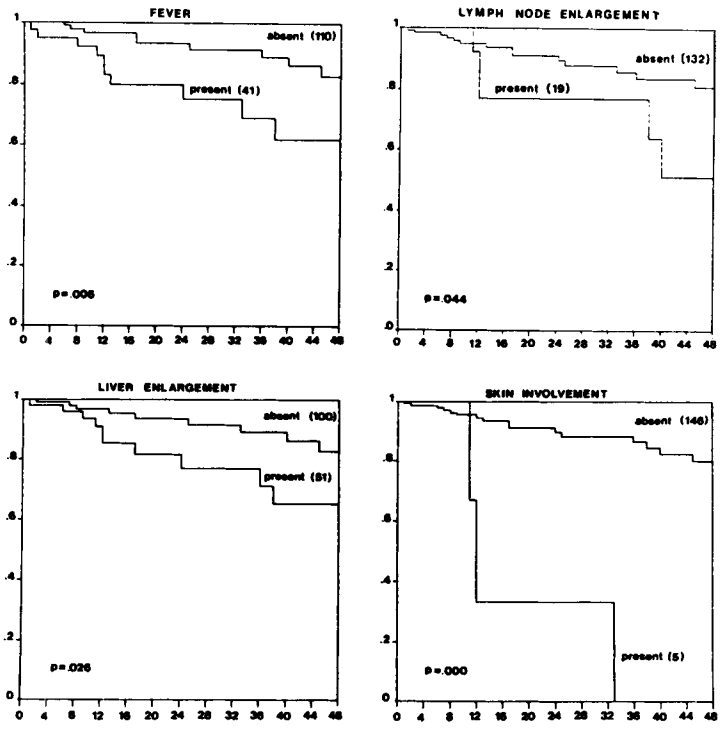

FIG. 2. Survival curves at 48 months significantly different in the univariate analysis, according to different clinical parameters: presence of fever, lymph node enlargement, liver enlargement, or skin involvement.

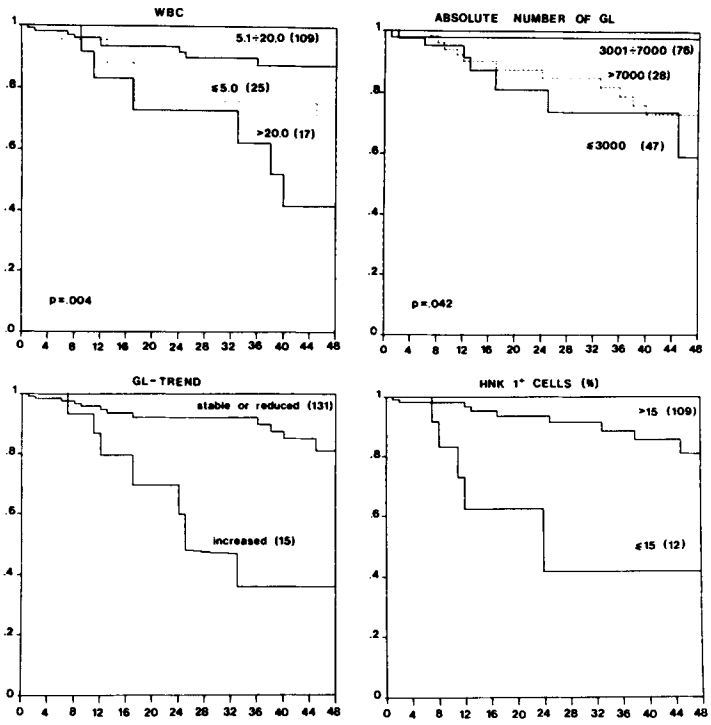

FIG. 3. Survival curves at 48 months significantly different in the univariate analysis, according to different laboratory data: leukocyte counts, GL counts, trend of the GL lymphocytosis, and percentages of HNK-1+ cells on PBMC. For leukocyte and GL counts the univariate test was also run for pairs of class. For leukocyte counts: class 1 (counts ≤ 5000), class 2 (5001–20,000), and class 3 (over 20,000). Results are the following: 1 *versus* 2, $P = 0.083$; 2 *versus* 3, $P = 0.0013$; 1 and 3 *versus* 2, $P = 0.0021$. For GL counts: class 1 (counts ≤ 3000), class 2 (3001–7000), and class 3 (over 7000). Results are the following: 1 *versus* 2, $P = 0.0091$; 2 *versus* 3, $P = 0.028$; 1 and 3 *versus* 2, $P = 0.0158$. For the GL lymphocytosis trend, two classes (with stable or reduced trend) are grouped together because these curves were overlapping and both significantly different from that with increased GL counts. For HNK-1-positive cells, the curve shows only cases with ≤ 15% of positive cells *versus* cases with positive cells > 15%, since no difference was seen between the two classes (16%–40% and over 40%).

low (≤ 3000), or high (> 7000/mm^3) absolute peripheral GL count, and a low percentage of HNK-1+ mononuclear cells were predictors of increased mortality. In the multivariate analysis, significantly independent predictors were fever, low percentages of HNK-1+ cells, and a relatively low GL count.

Lymphoproliferative disease of granular lymphocytes appears to be a heterogeneous syndrome with patients presenting different clinical features. Some patients have an indolent course resembling a reactive leukemoid reaction. In 43 of our patients, the diagnosis of LDGL was established in the absence of clinical symptoms. In other cases, the clinical course was characterized by fever (27.1%), infections (38.4%), severe neutropenia (31.1%), severe anemia (12.3%), and thrombocytopenia (7.9%), as reported in detail in Tables 1 and 2. Nevertheless, most of the patients had a stable, nonprogressive clinical course even in the presence of severe symptoms. A worsening of the general clinical status during follow-up was reported in only 15 patients, most of whom were included in the group who died within 48 months. So far, no clear criteria for separating different groups of LDGL patients are available, particularly at the time of the diagnosis.

TABLE 4. Risk Factors for Mortality in Lymphoproliferative Disease of Granular Lymphocytes

	β*	*P* value
A history of fever at diagnosis	2.35	0.0003
Percentages of HNK-1 positive PBMC†	−3.28	0.002
Absolute numbers of peripheral GL ≤3000	2.54	0.001

PBMC: peripheral blood mononuclear cells; GL: granular lymphocytes. Variables are reported as entered in the model.
* Estimated regression coefficients in Cox's survival model.
† Class 1: ≤15%; class 2: >15%; favorable feature was a percentage >15%.

Analysis of clinical features related to a poor prognosis has shown a significantly increased mortality in patients with liver (present in 33.7% of patients) and/or lymph node enlargement (12.6%). The presence of skin infiltrations, although extremely atypical for this disease (five of 151 patients) also proved to be a poor prognostic finding since three of these five patients died. The skin biopsy, performed in two cases, showed an infiltration of HNK-1+ cells. Both cases reported from Japan had skin infiltrations together with high leukocyte counts and a very aggressive clinical course. This could reflect a geographic aggressive variant of the disease, different from that commonly observed in Europe or in the US, and should be further investigated in light of the recent report describing a possible association of certain cases of LDGL with antibodies against proteins of the human T-cell leukemia virus type I.[22]

Laboratory data showed that mortality significantly correlated with leukocyte and GL counts. By contrast, several features usually found to have prognostic significance in most hematologic malignancies (such as bone marrow infiltration, hemoglobin levels, neutrophils, or platelets) were not associated with increased mortality. Patients with severe anemia (including four cases with

pure erythrocyte aplasia), and patients with severe neutropenia did not show an increased probability of death. In these latter cases a protective role of GL against infections may be hypothesized.[23] Immunologic data proved that mortality was significantly correlated with a low frequency of HNK-1+ cells. No correlation was observed with other surface markers including antigens recognized by CD3, CD8, CD11, CD16, CD2, and CD4 (data of the latter two are not shown), although it should be pointed out that CD16 data were lacking in several of the patients who died within 48 months. Also, there was no correlation found between mortality and NK function activity.

In the multivariate Cox model, three variables were found to be significantly associated with a poor prognosis at 48 months: (1) the presence of fever at diagnosis; (2) low levels of HNK-1+ PBMC (< 15%); and (3) a relatively low (2000–3000/mm^3) GL count. Fever could be related to either undiagnosed infections or to an aggressive variant of LDGL. Due to the lack of prognostic significance of both infections and neutrophil counts in the univariate test, fever is more likely to be related to an aggressive variant of the disease. In this regard, it has been shown[24] that cells from LDGL patients are potent producers of interleukin-1 (IL-1), which acts as an endogenous pyrogen. Thus, it may be speculated that in LDGL, the growth of an IL-1-producing subpopulation is associated with an aggressive disease.

The association of mortality with relatively low GL counts is difficult to explain. The univariate test demonstrated a unique pattern of association in LDGL, *i.e.*, both relatively low counts (between 2000 and 3000) and very high counts (higher than 7000) were associated with a poor prognosis, while intermediate values had a good prognostic association. A possible interpretation of these data may reflect the heterogeneity of this disease. As commonly observed in most hematologic diseases, patients with very high GL counts are more likely to have a leukemic expansion of GL and therefore an aggressive disease. Cases with intermediate values might represent reactive cellular expansions with a defined immunoregulatory role, thus explaining a better prognosis. The association of aggressive disease with a relatively low GL count is more intriguing and difficult to explain. One possibility rests on the fact that patients with relatively low GL counts have a lymphoma-like disease with more visceral involvement. Indeed, in at least one patient with aggressive LDGL, parenchymal infiltration by GL was observed.[13]

The increased survival observed in patients with cell expansions that are HNK-1+ rather than HNK-1 negative, deserves some comments. Through this large series of patients, we have confirmed that LDGL is usually due to the expansion of CD3-positive (CD3+), HNK-1+ GL.[25,26] These GL, present in low percentage in the peripheral blood of normal donors, are commonly referred to as cytotoxic T-lymphocytes (CTL) and are thought to exert a cytotoxic activity against viral or tumor antigens.[27] Since cases with virtually absent HNK-1 cells have a poor prognosis, it may be hypothesized that, in HNK-1+ LDGL, the expanded cells may exert some protective function. Thus, in these cases, LDGL could represent a disease of immune regulation rather than an uncontrolled proliferation of leukemic cells. As a result, the heterogeneity of the clinical course may reflect the outcome of the balance between the efficacy of the regulation and the underlying disturbing condition (an associated disease, including viral infections and neoplasias, was present in 69 cases).

Alternatively, LDGL could be a leukemic disease with a low grade of malignancy and the HNK-1-negative GL may belong to a different, possibly more mature subset and thus grow in a more uncontrolled fashion. Recent data on the genetic rearrangement of the T-cell receptor (TCR) beta and gamma chains have provided evidence for the clonal origin in a consistent number of CD3+ LDGL cases,[15,28–30] but not all of them.[15,31] Although these clonal expansions are likely to represent a neoplastic transformation of immune cells, the possibility cannot be ruled out that they represent a clonal response to a specific antigen, developed as a sequela of the normal immune response. In any case, at least in this disease the monoclonality of expanded cells does not seem to be correlated with a poor prognosis since most patients with monoclonal rearrangements of the TCR beta genes survived longer.[15,32] A crucial point which should be investigated is the *in vivo* function, if any, of the CD3+ HNK-1+ cells and specifically their antigen restriction. On clinical grounds, further studies will clarify whether patients with good prognosis show patterns of survival similar to the control population.

Thus far, the treatment of LDGL has been empirical. In our series, 106 patients received no therapy, 21 cases were treated with steroids, and 24 received cytostatic drugs. In eighteen patients an improvement in the lymphocytosis or neutropenia was observed, although in some cases it was transient. In light of this, a pulse steroid therapy has been reported to be of some benefit in a few patients with neutropenia.[6] According to our series of cases, we found no efficient treatment available for most patients with aggressive LDGL or other abnormalities such as anemia, despite anecdotal reports describing improvement in individual cases of anemia.[7,10,16] In 12 of 151 patients, therapeutic splenectomy was tried. The GL lymphocytosis worsened in nine cases, remained unchanged in two cases and improved, in association with chemotherapy, in only one patient. Although we want to stress that most cases of LDGL should not be treated, we hope that the identification of prognostic factors help define patients in whom therapeutic trials should be attempted.

REFERENCES

1. Reynolds CW, Foon KA. T-gamma-lymphoproliferative disease and related disorders in humans and experimental animals: A review of the clinical, cellular and functional characteristics. *Blood* 1984; 64:1146–1158.
2. Pandolfi F. T-CLL and allied diseases: New insights into classification and pathogenesis. *Diagn Immunol* 1986; 4:61–74.
3. Oshimi K. Granular lymphocyte proliferative disorders: Report of 12 cases and review of the literature. *Leukemia* 1988; 2:617–627.
4. Rumke HC, Miedema F, Ten Berge IJM *et al.* Functional properties of T cells in patients with chronic T gamma lymphocytosis and chronic T cell neoplasia. *J Immunol* 1982; 129:419–426.
5. Aisemberg AC, Wilkes BM, Farris NL, Ault KA, Karey RW. Chronic T-cell lymphocytosis with neutropenia: Report of a case studied with monoclonal antibodies. *Blood* 1981; 58:818–822.
6. McKenna RW, Arthur DC, Gajl-Peczalska KJ, Flynn P, Brunning RD. Granulated T cell lymphocytosis with neutropenia: Malignant or benign chronic lymphoproliferative disorder? *Blood* 1985; 66:259–266.
7. Grillot-Courvalin C, Vinci G, Tsapis A, Dokhelar MC, Vainchenker W, Brouet JC. The syndrome of T8 hyperlymphocytosis: Variation in phenotype and cytotoxic activities of granular cells and evaluation of their role in associated neutropenia. *Blood* 1986; 69:1204–1210.
8. Chan WC, Link S, Mawle A, Check I, Brynes RK, Winton EF. Heterogeneity of large granular lymphocyte proliferations: Delineation of two major subtypes. *Blood* 1986; 68:1142–1153.
9. Semenzato G, Pizzolo G, Ranucci A *et al.* Abnormal expansions of polyclonal large to small size granular lymphocytes: Reactive or neoplastic process? *Blood* 1984; 63:1271–1277.
10. Newland AC, Catovsky D, Linch D *et al.* Chronic T cell lymphocytosis: A review of 21 cases. *Br J Haematol* 1984; 58:433–446.
11. Wallis WJ, Loughran TP Jr, Kadin ME, Clark EA, Starkebaum GA. Polyarthritis and neutropenia associated with circulating large granular lymphocytes. *Ann Intern Med* 1985; 103:357–362.
12. Loughran TP, Kadin ME, Starkebaum G *et al.* Leukemia of large granular lymphocytes: Association with clonal chromosomal abnormalities and autoimmune neutropenia, thrombocytopenia, and hemolytic anemia. *Ann Intern Med* 1985; 102:169–175.
13. Pandolfi F, Pezzutto A, De Rossi G *et al.* Characterization of two patients with lymphomas of large granular lymphocytes. *Cancer* 1984; 53:445–452.
14. Koizumi S, Seki H, Tachinami T *et al.* Malignant clonal expansion of large granular lymphocytes with a Leu-11+, Leu-7- surface phenotype: *In vitro* responsiveness of malignant cells to recombinant human interleukin 2. *Blood* 1986; 68:1065–1073.
15. Semenzato G, Pandolfi F, Chisesi T *et al.* The lymphoproliferative disease of granular lymphocytes: A heterogeneous disorder with a spectrum ranging from indolent to aggressive conditions. *Cancer* 1987; 60: 2971–2978.
16. Loughran TP Jr, Starkebaum G. Large granular lymphocyte leukemia: Report of 38 cases and review of the literature. *Medicine* 1987; 66:397–405.
17. Bassan R, Introna M, Rambaldi A *et al.* A large granular lymphocytes/natural killer cells proliferative disease: Clinical and laboratory heterogeneity. *Scan J Haematol* 1986; 37:91–97.
18. Stites DP, Stobo JD, Wells JV. Basic and Clinical Immunology. Los Altos, CA: Appleton and Lange, 1987.
19. Herberman RB. Natural Cell Mediated Immunity Against Tumors. New York: Academic Press, 1981.
20. Peto R, Pike PC, Armitage P *et al.* Design and analysis of randomized clinical trials requiring prolonged observations of each patient. *Br J Cancer* 1977; 35:27–34.
21. Cox DR. Regression models and life tables. *J R Stat Soc* 1972; 34:187.
22. Starkebaum G, Loughran TP Jr, Kalyanaraman VS *et al.* Serum reactivity to human T-cell leukemia/lymphoma virus type I proteins in patients with large granular lymphocytic leukemia. *Lancet* 1987; 1:596–598.
23. Garcia-Penarrubia P, Koster FT, Kelley RO, McDowell TD, Bankhurst AD. Antibacterial activity of human natural killer cells. *J Exp Med* 1989; 169:99–113.
24. Rambaldi A, Rossi V, Allavena P *et al.* Lymphokine production in T-gamma lymphoproliferative disorders. *Scand J Immunol* 1986; 23: 183–188.
25. Pandolfi F, Semenzato G, De Rossi G *et al.* HNK-1 monoclonal antibody (Leu 7) in the identification of abnormal expansions of large granular lymphocytes. *Clin Exp Immunol* 1983; 52:641–647.
26. Loughran TP Jr, Draves KE, Starkebaum G, Kidd P, Clark EA. Induction of NK activity in large granular lymphocyte leukemia: Activation with anti-CD3 monoclonal antibody and interleukin 2. *Blood* 1987; 69:72–78.
27. Phillips JH, Lanier LL. Lectin-dependent and anti-CD3 induced cytotoxicity are preferentially mediated by peripheral blood cytotoxic T lymphocytes expressing Leu-7 antigen. *J Immunol* 1986; 136:1579–85.
28. Rambaldi A, Pellicci P, Allavena P *et al.* T cell receptor beta chain gene rearrangements in lymphoproliferative disorders of large granular lymphocytes/natural killer cells. *J Exp Med* 1985; 162:2156–2162.
29. Waldmann TA, Davis MM, Bongiovanni KF, Korsmeyer SJ. Rearrangements of genes for the antigen receptor on T cell as markers of lineage and clonality in human lymphoid neoplasms. *N Engl J Med* 1985; 313:776–783.
30. Loughran TP Jr, Starkebaum G, Kidd P, Neiman P. Clonal proliferation of large granular lymphocytes in rheumatoid arthritis. *Arthrtis Rheum* 1988; 31:31–37.
31. Lauria F, Foa R, Migone N *et al.* Heterogeneity of large granular lymphocyte proliferations: Morphological, immunological and molecular analysis in seven patients. *Br J Haematol* 1987; 66:187–192.
32. Aisemberg AC, Krontiris TG, Mak TW, Wilkes BM. Rearrangement of the gene for the beta chain of the T-cell receptor in T chronic lymphocytic leukemia and related disorders. *N Engl J Med* 1985; 313: 529–533.