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Clinical and paraclinical correlations in patients with adult T-cell leukemia/ lymphoma

PhD thesis summary

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PhD Thesis Contents

SPECIAL PART

LIST OF PUBLISHED SCIENTIFIC PAPERS

1. **Iuliana Iordan**, Minodora Onisai, Ana-Maria Vladareanu, Cristina Mambet, Elena Cristina Marinescu, Raluca Nistor, Horia Bumbea. Particularities of Neurological Manifestations in Adult T-Cell Leukemia/Lymphoma: Need for a Multidisciplinary Approach – A Narrative Review. *Medicina* 2022, 58(11): 1553, FI – 2.984/ 2022, Q1, Chapter 4, pag. 83-85
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2. **Iuliana Iordan**, Ana-Maria Vlădăreanu, Cristina Mambet, Minodora Onisâi, Diana Cîșleanu, Horia Bumbea. Clinical Features and Survival Outcome in Aggressive-Type Adult T-Cell Leukemia/Lymphoma Patients: Real-Life Experience of a Single Center from an HTLV-1 Endemic Country. *Medicina* 2024, 60(6): 872, FI – 2.4/ 2024, Q1, Chapter 4, pag. 31-69
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3. **Iuliana Iordan**, Ana-Maria Vlădăreanu, Cristina Mambet, Ion Dumitru, Minodora Onisâi, Diana Cîșleanu, Horia Bumbea. Atypical Presentation for Adult T-Cell Leukemia/Lymphoma: a Case Report and Short Review of the Literature. *Maedica – a Journal of Clinical Medicine* 2024, 19(2): 420-425, Pubmed, Chapter 4, pag. 46, 86
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General part – Current state of knowledge

Human T-lymphotropic virus type 1 (HTLV-1)

HTLV-1 is the first oncogenic retrovirus discovered in humans [1]. It was first identified in 1980 [1,2], and later the involvement of the retrovirus in oncogenesis was described [3].

Transmission of HTLV-1 infection occurs through three main routes: parenteral, sexual contact, and vertical transmission. The main transmission route varies depending on the geographical region. For example, in Romania, during the 1980s, the principal route of transmission was parenteral [4], and in Japan it is predominantly vertical, while in Brazil it is mainly through sexual contact [5].

HTLV-1 is not a ubiquitous virus. Romania is the only country in Europe with a high prevalence of HTLV-1 [6]. In 2012, it was estimated that approximately 5–10 million people were infected with HTLV-1, of whom 3,000–15,000 were from Romania [7]. The high prevalence of HTLV-1 in Romania is most likely due to the reuse of non-sterilized intravenous equipment, as well as transfusions with blood untested for HTLV-1 in the 1980s.

HTLV-1 is the etiological agent for two main pathologies, ATLL and HAM/TSP. Both diseases appear after a long latency period of several decades [8]. Most HTLV-1 carriers remain asymptomatic for life; approximately 3–5% develop ATLL and 0.18–1.8% HAM/TSP [8–10]. Rare cases of patients with both pathologies have been reported [11,12].

The pathogenic mechanisms are incompletely understood. Several factors have been identified that may contribute to the development of HTLV-1-associated pathologies: the mode of transmission of infection, proviral load (PVL), the action of viral proteins, cytokines, and host-related factors.

Adult T-cell leukemia/lymphoma

ATLL is a rare and aggressive peripheral T-cell disorder caused by HTLV-1 infection. ATLL was first described in Japan in 1977, before HTLV-1 was identified [13].

Most data on Romanian patients with ATLL come from case reports or case series [14–21]. The first cases in Romania were documented in 1993 [22]. The authors observed a distinctive feature in these patients, namely the younger age at diagnosis, with three of the

six patients being between 22 and 26 years old [22]. The younger age at diagnosis of Romanian patients has been confirmed by more recent case reports and studies [16–21,23,24].

ATLL is a very rare type of lymphoma, accounting for 0.2% of lymphoma cases in non-endemic countries, and up to 36.8% of cases in endemic countries [25]. ATLL affects almost exclusively adults, although cases in children have also been reported [14,26–30]. The median age at diagnosis is approximately 60–70 years, but it varies depending on the region of origin [31–39].

ATLL is classified into four clinical subtypes: acute, lymphoma, chronic, and smoldering [40]. This classification is based on the Shimoyama criteria formulated in 1991 (*Table 1*), which are still valid today [40].

Table 1: Shimoyama diagnostic criteria [40]

	Smoldering type	**Chronic type**	**Lymphoma type**	**Acute type**
Lymphocytes (PB)	<4000/mm^3	≥4000/mm^3	<4000/mm^3	↓/N/↑
Atypical T-cells (%)	≥5%	≥5%	<1%	≥1%
PBS - ”flower cells”	Occasional	Occasional	-	+
Hypercalcemia	-	-	+/-	+/-
LDH ↑	≤1.5xULN	≤2xULN	+/-	+/-
Nodal infiltrate (HP+)	-	+/-	+	+/-
Hepatic infiltrate	-	+/-	+/-	+/-
Splenic infiltrate	-	+/-	+/-	+/-
Cutaneous infiltrate	-	+/-	+/-	+/-
Pulmonary infiltrate	-	+/-	+/-	+/-
CNS infiltrate	-	-	+/-	+/-
Bone infiltrate	-	-	+/-	+/-
Gastrointestinal infiltrate	-	-	+/-	+/-
Ascites	-	-	+/-	+/-
Pleural fluid	-	-	+/-	+/-

Clinical manifestations in ATLL are heterogeneous, including constitutional symptoms, symptoms secondary to cytopenias, metabolic abnormalities—particularly

hypercalcemia, manifestations due to nodal and extranodal infiltration, as well as those secondary to immunodeficiency.

Laboratory abnormalities are more frequent and severe in acute ATLL. Studies have shown significant differences between patients with acute ATLL compared to other disease types regarding leukocytosis with lymphocytosis, serum levels of soluble IL-2 receptor, hypoproteinemia, hypoalbuminemia, hyperbilirubinemia, liver impairment (elevated alkaline phosphatase, ALT, AST), kidney impairment (elevated creatinine and BUN), increased LDH, and hypercalcemia [32,35,40].

The use of prognostic scores is useful for selecting the optimal treatment tailored to risk. Multiple scores exist that include demographic, clinical, and biological variables, with the newest also integrating genetic prognostic factors, such as the m7-ATLPI score [41].

ATLL remains an aggressive disease with short survival, which has not significantly changed since its first description, especially in indolent types [32,34,40]. When the diagnostic criteria for ATLL types were described in 1991, the median survival for the four types of ATLL was 6 months for the acute type, 10 months for the lymphoma type, 38 months for the chronic type, and 38 months for the smoldering type [40]. More recent studies report similar median survival: 8.3 months for the acute type, 10.6–14.3 months for the lymphoma type, 31.5–39.4 months for the chronic type, and 41.2–55.0 months for the smoldering type [34,42].

Causes of death in ATLL patients are disease progression (67–78.6%), infections (10%), and other malignancies aside from ATLL (2.1%) [32,42]. Infections are the second leading cause of death in aggressive and chronic types, while death from other malignancies is the second cause in the smoldering type [32,42].

Prognosis in ATLL has remained unfavorable despite therapeutic advances. In aggressive types, chemotherapy and antiretroviral therapy have limited efficacy, but results are better when combined with allo-HSCT [43]. Newer treatments, such as monoclonal antibodies, immunomodulatory agents, histone deacetylase inhibitors, and EZH1/2 inhibitors, provide additional benefits but are not yet widely available and cannot induce long-term remission without allo-HSCT.

In indolent types, watchful waiting is recommended until transformation into aggressive types or the appearance of adverse prognostic factors, along with treatment with IFN-α and zidovudine or systemic therapy [43,44].

In aggressive types, systemic therapy followed by allo-HSCT is recommended. For the acute type without large tumor masses, first-line treatment is IFN/ZDV, while in lymphoma or acute types with large masses, chemotherapy may be combined with IFN/ZDV [45,46]. For patients ineligible for allo-HSCT, maintenance therapy with low-dose IFN/ZDV or low-dose oral etoposide is recommended [46]. Prophylaxis for CNS involvement is essential, with recommendations for intrathecal therapy, high-dose methotrexate, and/or the use of agents crossing the blood–brain barrier in pre-transplant conditioning regimens, namely busulfan and thiotepa [46].

Enrollment in a clinical trial is recommended from the first line for all ATLL types [45]. Not all patients can receive treatment, most commonly due to poor performance status and comorbidities [37].

Working hypothesis and general objectives

Adult T-cell leukemia/lymphoma is a rare disease, more frequently encountered in endemic regions. Romania is the only country in Europe where HTLV-1 is endemic, resulting in a higher incidence of ATLL in this region.

In Romanian patients with ATLL, distinct features have been observed compared to data reported in international studies, namely younger age at diagnosis, predominance of the lymphoma type, and shorter survival. To date, no extensive studies have analyzed these particularities in a large patient cohort.

The current work presents two distinct studies:

The first study, named "Clinical, biological features and treatment response of patients with ATLL in Romania," aims to characterize the epidemiological, clinical, and biological aspects of ATLL patients diagnosed in Romania, as well as their treatment and response to therapy, in order to better understand disease progression and factors influencing prognosis.

The second study, named "Proinflammatory cytokines in asymptomatic HTLV-1 carriers and patients with ATLL," analyzes the cytokine profile in asymptomatic carriers and patients with aggressive types of ATLL, compared to a control group. This study aims to identify biomarkers that differentiate ATLL patients from asymptomatic carriers and the control group.

The analysis of these particularities is important for a better understanding of disease progression in Romanian patients. Ultimately, this approach may lead to the identification of new therapeutic targets, contributing to the development of more effective and personalized treatment strategies for ATLL patients.

Study 1 - Clinical and biological features and treatment response of patients with ATLL in Romania

Introduction (working hypothesis and specific objectives)

The study "Clinical and biological features and treatment response of patients with ATLL in Romania" aims to: describe the particular characteristics of ATLL patients diagnosed in Romania, study the biological markers associated with the disease course in these patients, identify factors influencing survival, identify risk factors for early death, identify common features of long-term survivors, and develop a prognostic risk model specific to patients in Romania.

Materials and methods

The study is an analytical, observational, prospective, and multicenter study conducted on 58 patients diagnosed with ATLL between 2010 and 2023 at the Bucharest Emergency University Hospital, Colțea Clinical Hospital, and Colentina Clinical Hospital. Adult patients with confirmed HTLV-1 infection and a diagnosis of aggressive type ATLL, who signed informed consent regarding participation in medical research and the use of biological samples for research purposes, were included. Demographic, clinical, and laboratory data, as well as information regarding treatment and treatment response, were obtained from patients' electronic medical records.

Statistical analysis was performed using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA) and Epi Info version 7.2.6.0 (CDC, Atlanta, GA, USA). To verify whether a significant association exists between two nominal variables, the Chi-Square test and Fisher's exact test were used. The Independent-Samples Median Test was used to compare medians. Odds ratios and 95% confidence intervals were calculated to measure the relationship between a risk factor and an outcome. Survival analysis was performed using Kaplan–Meier curves and the Log-rank test. For the development of the prognostic score, ROC curves were used to identify variables associated with 6-month survival, as well as values with the highest sensitivity and specificity and statistical significance. A p-value < 0.05 was considered statistically significant.

Results

Patients were aged between 20 and 72 years, with a median age of 48 years and a mean of 49.84 ± 14.034 years. A slight predominance of females was observed, with a female-to-male ratio of 1.52.

Age at diagnosis appears to show an increasing trend. Patients diagnosed between 2010–2014 had a median age at diagnosis of 43 years, those diagnosed between 2015–2019 had a median age of 47.5 years, and those diagnosed between 2020–2023 had a median age of 57 years (p=0.268) (*Figure 1*).

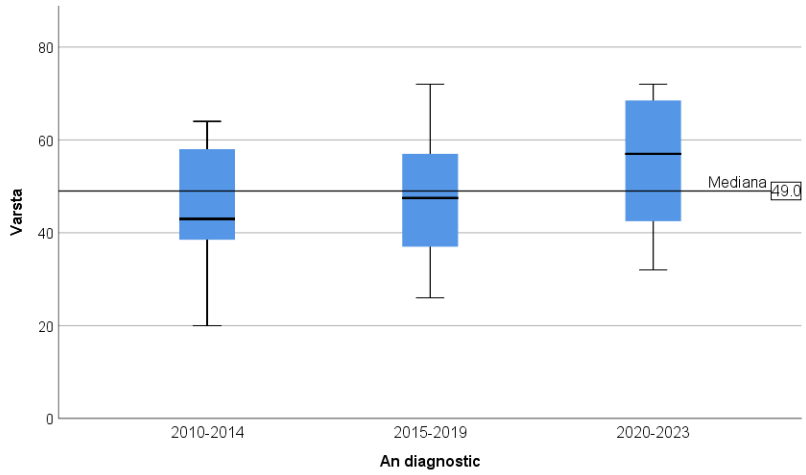

Figure 1: Box plot of age according to the year of diagnosis

The lymphoma type of ATLL (n = 33, 56.90%) was more frequent than the acute type (n = 25, 43.10%). Most patients were diagnosed with aggressive disease types (94.83% Lugano stages 3–4), B symptoms (67.57%), and impaired performance status (45.83%).

The most common complications during disease course included moderate-to-severe cytopenias (41 patients), infections (37 patients), liver cytolysis and/or cholestasis (27 patients), tumor lysis syndrome (14 patients), neurological complications (19 patients), and severe symptomatic hypercalcemia (14 patients).

More than half of the patients (58.62%) had leukocytosis at diagnosis, predominantly those with the acute type (88%). Most patients did not present cytopenias, and among those who did (21.05% – anemia, 14.04% – thrombocytopenia), the grades were mild to moderate, except for one patient with liver cirrhosis and severe thrombocytopenia.

Patients with acute ATLL had significantly higher calcium levels (12.59 vs 9.70 mg/dl, p=0.015), uric acid (8.35 vs 5.10 mg/dl, p=0.007), and renal impairment (creatinine clearance 76.90 vs 97.29 ml/min/1.73 m², p=0.030) compared to those with the lymphoma type, along with trends toward higher LDH and liver involvement.

Coagulation times (PT and APTT) did not differ significantly between the two groups. Serum fibrinogen was significantly lower in patients with the acute type, with a median value of 237.00 mg/dl compared to 344.09 mg/dl (p=0.012).

Flow cytometry immunophenotyping was available for 18 of the 33 patients with the acute type. The typical CD4+CD8- phenotype was the most common, found in 11 patients (64.71%), followed equally by CD4+CD8+ (17.6%) and CD4-CD8- (17.6%). No patients had the CD4-CD8+ phenotype in the studied cohort.

Regarding treatment, all patients received first-line therapy with polychemotherapy (n = 57, 98.3%), either combined (n = 26, 44.83%) or not (n = 31, 53.45%) with antiviral therapy, or antiviral monotherapy (n = 1, 1.7%). The most frequently administered chemotherapy protocols were CHOP (n = 22, 38.60%), CHOEP (n = 14, 24.56%), and LSG15 (n = 11, 19.30%).

Response to first-line treatment was evaluated in 57 patients. The overall response rate (CR + PR) was 16.67% in patients with the acute type and 33.33% in those with the lymphoma type (*Figure 2*).

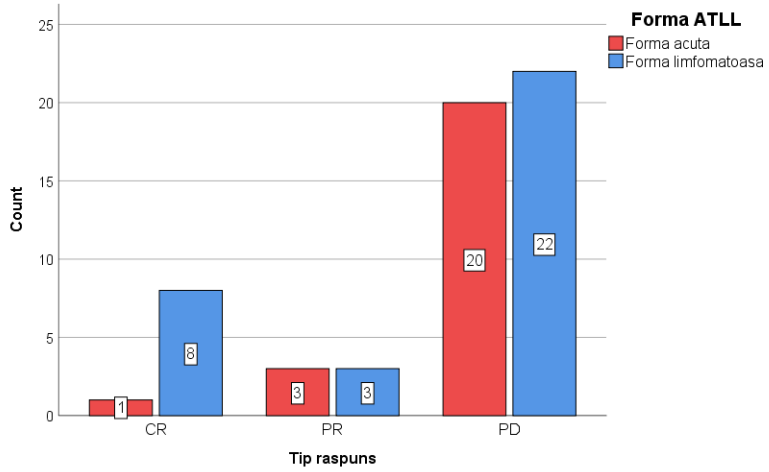

Figure 2. Type of response achieved according to ATLL type

Only four patients received allo-HSCT: one with the acute type and three with the lymphoma type. All patients achieved complete response before allo-HSCT, three after first-line treatment and one after second-line treatment, and no disease relapse was observed before transplantation in any patient. Patient characteristics are presented in *Table 2*.

Table 2. Characteristics of patients who received allo-HSCT

No.	**Age**	**Sex**	**ATLL type**	**1st line treatment**	**Response to 1st line**	**Time to HCT**	**Status (survival)**
1	26 years	M	L	LSG15	CR	11.63months	Alive (106.83 months)
2	57 years	F	L	LSG15	CR	11.4 months	Deceased (49.07 months)
3	40 years	F	L	CHOEP	CR	5.63 months	Deceased (8.30 months)
4	32 years	M	A	CHOP	PR→CR	19.1 months	Alive (106.23 months)

The median follow-up period for patients in the studied cohort was 5.80 months, with a mean of 14.55 ± 28.33 months, ranging from 0.10 to 149.60 months. The median survival was 7.967 months (95% CI 5.771–10.162), and the mean survival was 8.395 months (95% CI 14.189–47.096). Patients diagnosed with the lymphoma type had a higher median survival of 8.300 months compared to 6.633 months for those with the acute type, but this difference was not statistically significant (p=0.227).

Survival was analyzed according to demographic, clinical, biological, and treatment parameters. No significant differences in survival were identified for the following analyzed parameters:

- Clinical: age, sex, Lugano stage, B symptoms, presence of lymphadenopathy
- Laboratory: cytopenias, immunophenotype, uric acid, ALT, AST, alkaline phosphatase, GGT
- Treatment: chemotherapy regimen

Significant differences in survival were identified for:

- Performance status at diagnosis – patients with ECOG 0–1 had a median survival of 9.367 months, while those with higher ECOG had 3.200 months.
- JCOG prognostic index – patients classified in the moderate–high risk group had longer survival compared to those in the high-risk group, namely 29.667 months versus 3.867 months (p=0.000).

- Hepatomegaly and splenomegaly – median survival in the absence of hepatomegaly or splenomegaly was 29.667 months in both cases, whereas patients with hepatomegaly had a median survival of 5.833 months (p=0.001), and those with splenomegaly 6.867 months (p=0.002).
- Severe hypercalcemia at diagnosis – patients with serum calcium corrected for albumin >14 mg/dl had lower survival compared to those without severe hypercalcemia, namely 3.500 months versus 6.167 months (p=0.038).
- Serum LDH – serum LDH levels greater than twice the upper limit of normal were associated with shorter survival (5.767 months) compared to patients with lower LDH, who had a median survival of 9.367 months (p=0.039).
- Total bilirubin – patients with serum bilirubin >1.2 mg/dl had shorter survival (8.00 months) compared to those with normal bilirubin levels (p=0.008).
- Low serum albumin was associated with poorer survival, with a median of 3.60 months versus 8.00 months in patients with albumin within normal limits (p=0.022).
- Renal function – patients with creatinine clearance ≥60 ml/min/1.73 m^2 and BUN ≤24 mg/dl had approximately 5 months longer median survival compared to those with impaired renal function (p=0.039 and p=0.001, respectively).
- Fibrinogen – patients with low fibrinogen levels had shorter survival compared to those with normal fibrinogen, namely 3.50 months versus 7.97 months (p=0.015).
- Achievement of complete or partial response after first-line treatment – patients with CR/PR had a median survival of 49.067 months, much higher than those with progressive disease after first-line therapy, who had 5.067 months (p=0.000) (*Figure 3*). This highlights the importance of achieving therapeutic response after first-line treatment.
- Patients who underwent allo-HSCT also had longer survival, 49.067 months compared to 6.633 months (p=0.045) (*Figure 4*).

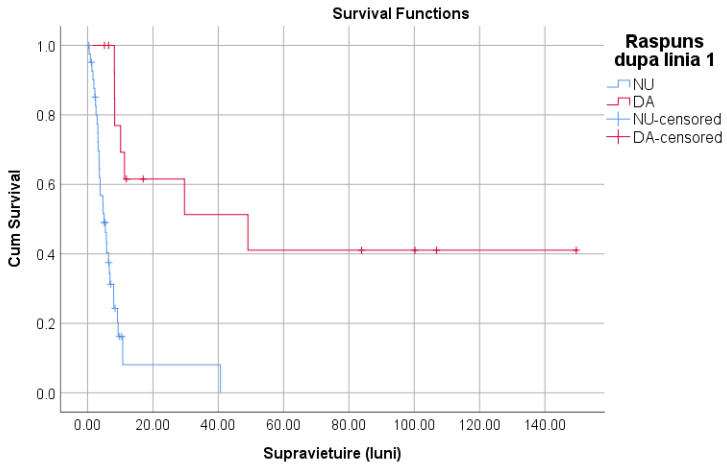

Figure 3. Kaplan–Meier survival curve according to response after first-line treatment

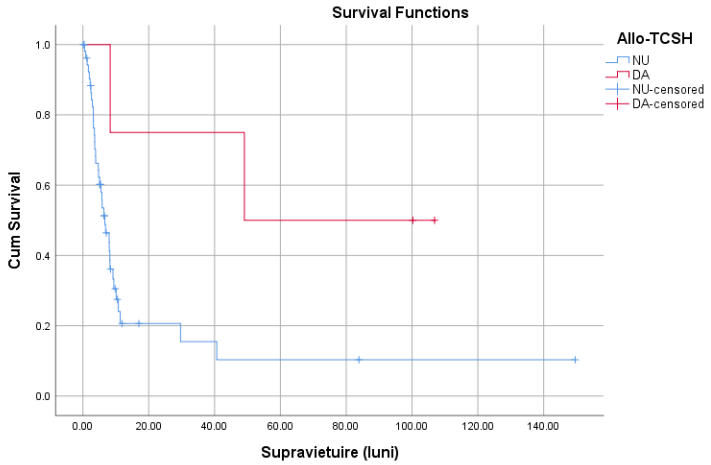

Figure 4. Kaplan–Meier survival curve according to allo-HSCT

Death within 6 months

A univariate analysis was performed to identify risk factors for death within 6 months of diagnosis. Albumin-corrected serum calcium (p=0.009), creatinine (p=0.016), BUN (p=0.005), uric acid (p=0.043), GGT (p=0.045), and albumin (p=0.025) were significantly associated with mortality within 6 months.

A multivariate analysis was conducted to confirm the statistical associations and to identify cutoff values for albumin-corrected calcium, serum creatinine, BUN, and uric acid. Statistically significant p-values were obtained for all analyzed variables. The following cutoff values were identified: albumin-corrected serum calcium >10.40 mg/dl, serum creatinine >0.8 mg/dl, BUN >16.85 mg/dl, and uric acid >6.75 mg/dl. Based on these thresholds, patients were divided into two categories for each variable. For practical application, BUN and uric acid values were rounded to 17 mg/dl and 7 mg/dl, respectively.

The odds ratio was calculated for multiple binary variables, including those identified in the multivariate analysis. No significant associations with survival were found for the following variables: sex (p=0.539), ATLL type (p=0.507), Lugano stage (p=0.092), presence of B symptoms (p=0.873), presence of lymphadenopathy (p=0.873), and splenomegaly (p=0.532). Statistically significant associations were found for: ECOG (p=0.000), hepatomegaly (p=0.039), albumin-corrected serum calcium >10.4 mg/dl (p=0.007), creatinine >0.8 mg/dl (p=0.014), BUN >17 mg/dl (p=0.019), and uric acid >7 mg/dl (p=0.029).

The odds ratio was calculated for each statistically significant variable. To facilitate the use of the prognostic score, points were assigned for the presence of each risk factor according to the odds ratio:

- 2 points for ECOG ≥ 2
- 1 point for albumin-corrected serum calcium ≥ 10.4 mg/dl
- 1 point for serum creatinine ≥ 0.8 mg/dl
- 1 point for BUN ≥ 17 mg/dl
- 1 point for uric acid ≥ 7 mg/dl

A score is obtained that can range from 0 to a maximum of 6 points. Patients were grouped into two prognostic categories: 0–3 points → low risk, 4–6 points → high risk.

Analyzing the association between the proposed prognostic score and mortality within 6 months, statistical significance was maintained (p=0.008). The risk of death within 6 months was 5.94 times higher in patients with high risk.

The risk score was applied to the entire patient cohort. Missing variables were assigned 0 points. However, even if these patients had the risk factors present, they would not have been classified differently in the risk score. Median survival in the high-risk group was 3.50 months, and in the low-risk group 8.30 months, with statistical significance (p=0.001) (Figure 5). Thus, we demonstrated that the score can also be applied for median survival analysis, not only for predicting 6-month mortality risk.

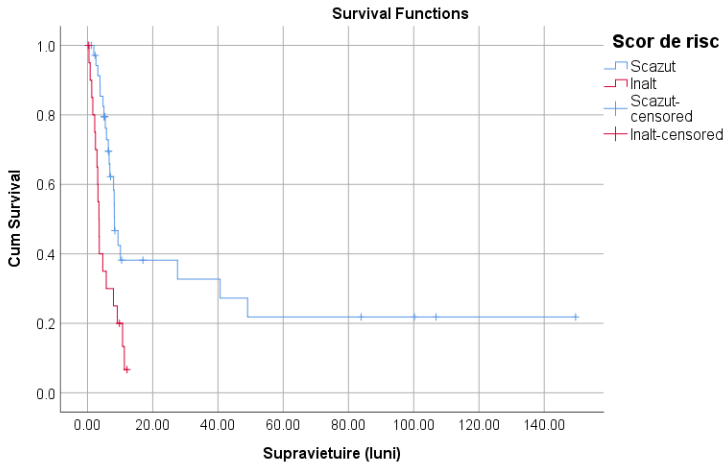

Figure 5. Kaplan–Meier survival curve according to risk group

Discussions

The patients in the studied cohort had a median age of 48 years, lower compared to international studies [32,33]. A rising trend in median age was observed in recent years, reaching 57 years for patients diagnosed between 2020–2023. This aligns with international data and supports the theory that virus transmission occurred predominantly in the 1980s.

In the studied cohort, there was a slight female predominance, with 60.34% of patients being female. This difference likely reflects the transmission route of HTLV-1, as sexual transmission occurs more efficiently from men to women [5].

Compared to Japanese studies, Romanian patients presented with more advanced disease and poorer performance status at diagnosis. Most patients were in Lugano stages III–IV (94.83%) and exhibited B symptoms (65.57%).

ATLL progression is marked by multiple complications, either disease- or treatment-related. Complications can arise from immunodeficiency, nodal or extranodal infiltration, tumor lysis, and paraneoplastic phenomena. In our cohort, a notable finding compared to other hematologic malignancies was the high frequency of neurological complications (CNS infiltration, metabolic encephalopathy, infections, reversible posterior encephalopathy).

Laboratory features also distinguished acute from lymphoma ATLL. Patients with acute ATLL generally exhibited higher leukocyte, neutrophil, and lymphocyte counts, serum calcium, total bilirubin, AST, creatinine, and uric acid. Although not all differences were

statistically significant, acute ATLL patients also showed higher ESR, LDH, ALT, alkaline phosphatase, GGT, and BUN values.

Despite advances in diagnosis, prognostic factor identification, supportive care, and targeted therapy, survival for ATLL patients has not significantly improved since the disease was first described. In 1991, Shimoyama reported median survival of 6 months for acute, 10 months for lymphoma, and 38 months for chronic and smoldering types [40]. Studies from Japonia[32,42] and SUA[34] report minor improvements, whereas studies from Latin America [35,47] and the present study report similar or even lower median survival.

The overall response rate in this study was lower than previously reported [32,34,35]. Imaizumi et al. reported global response rates of 63.6% (14.3% CR, 49.3% PR) for acute and 67.4% (22.3% CR, 45.15% PR) for lymphoma ATLL [32]. In the current study, the global response rate was only 26.31%. Compared to Japanese studies, Romanian patients were diagnosed at a more advanced stage (Lugano stage III-IV), with B symptoms and poorer performance status.

Independent unfavorable prognostic factors identified in this study included ECOG ≥ 2, hepatomegaly and/or splenomegaly, severe hypercalcemia, LDH >2× ULN, low creatinine clearance, elevated BUN, hyperbilirubinemia, hypoalbuminemia, and hypofibrinogenemia. These results align with previously published data [32,40,47,48]. Patients over 60 years had lower median survival (5.467 months) compared to younger patients (8.467 months), though not statistically significant. The results are consistent with previously published data—median survival was 8.37 months for patients under 60 years, 6.68 months for those aged 60–70 years, and 3.48 months for patients over 70 years [48].

In multivariate analysis, continuous variables that remained significant included albumin-corrected calcium, serum creatinine, BUN, and uric acid. Cutoff values with optimal sensitivity and specificity were established. Together with ECOG, these factors were incorporated into a prognostic score, dividing patients into low-risk (median survival 8.30 months) and high-risk groups (median survival 3.50 months).

Most ATLL cases, except in Japan, occur in low- to middle-income countries. Access to advanced diagnostic tests such as NGS or even less expensive tests (soluble IL-2 receptor, beta-2 microglobulin, cytogenetics) is limited in these regions. While current risk stratification trends emphasize genetic analyses, this is not feasible in most areas. Hence,

developing a prognostic score based on widely available tests is useful for broad application, including in resource-limited settings.

Epidemiologic and clinical differences may reflect the heterogeneous global distribution of HTLV-1. In Romania, similar to Spain and Latin America, patients predominantly harbor the transcontinental subtype of Cosmopolitan genotype A, whereas Japan is characterized by a specific Japanese subtype [7,47].

Study 2 - Proinflammatory cytokines in asymptomatic HTLV-1 carriers and patients with ATLL

Introduction (working hypothesis and specific objectives)

HTLV-1 infection and ATLL are associated with an imbalance in cytokine production. Cytokines play an important role in the pathogenesis of ATLL, influencing disease progression and immune response. Analyzing differences in cytokine profiles may lead to a better understanding of the mechanisms that distinguish ATLL patients from asymptomatic carriers and healthy individuals.

The objectives of this study are: to compare the levels of inflammatory cytokines between the control group, asymptomatic carriers, and ATLL patients; to identify cytokines that are significantly higher or lower in asymptomatic carriers compared to the control group; to identify cytokines that could serve as biomarkers for early detection of ATLL in asymptomatic carriers; and to identify cytokines that could be potential therapeutic targets.

Materials and Methods

ATLL patient samples were obtained from the Hematology Departments of the Bucharest University Emergency Hospital, Colțea Clinical Hospital, and Colentina Clinical Hospital. A total of 26 patients with aggressive types of ATLL were included, comprising seven patients with the acute type and 19 patients with the lymphoma type. Samples from asymptomatic HTLV-1 carriers (archived plasma samples, Stefan S. Nicolau National Institute of Virology (INV)) and control samples from HTLV-1 negative individuals were obtained from the National Institute of Blood Transfusion “Prof. Dr. C. T. Nicolau” (INTS) Bucharest.

Samples were processed at the Stefan S. Nicolau Institute of Virology. The Bio-Plex ProTM Human Inflammation Panel^{TM} 1 37-Plex #171AL001M kit (Bio-Rad Laboratories, Inc., Life Science Research Group, Hercules, CA, USA) was used. Results were exported using Bio-Plex Data Pro software (Bio-Rad Laboratories, Inc., Life Science Research Group, Hercules, CA, USA).

The method combines flow cytometry with ELISA (Enzyme-Linked Immunosorbent Assay) technology. Fluorescently colored microspheres coated with antibodies, similar to the ELISA platform, are used. Each antibody is specifically directed against a particular cytokine. The antibodies react with cytokines, followed by a series of washes to remove unbound proteins. A biotinylated antibody specific to a different epitope on the cytokine is added, forming an antibody sandwich around the cytokine. Streptavidin-phycoerythrin is then added, which binds to the biotinylated antibodies. Based on the color and fluorescence of the microsphere, each reaction is identified and quantified. Cytokine concentrations are automatically calculated by Bio-Plex Manager software using a standard curve derived from the analysis of standard samples with known cytokine levels.

Statistical analysis was performed using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as median values. The Kruskal-Wallis test was used to compare medians between different groups. BoxPlot graphs were used to illustrate the data. ROC curves and area under the curve (AUC) were used to determine threshold values for cytokine levels. A p-value <0.05 was considered statistically significant.

For cytokine network and functional enrichment analysis, the STRING platform was used (https://string-db.org/, accessed online on May 1, 2025, version 12.0, European Molecular Biology Laboratory, Heidelberg, Germany).

Results

I compared the cytokine level distribution across all subgroups – controls, asymptomatic carriers, and ATLL patients. No significant differences were identified between the three patient cohorts for gp130/sIL-6Rβ, sIL-6Rα, IL-12 (p70), and MMP-2; therefore, these cytokines will be excluded from pairwise comparisons between patient groups.

No statistically significant differences were found between the control group and the asymptomatic HTLV-1 carriers.

ATLL patients exhibited significantly higher plasma levels than both the control group and asymptomatic carriers for the following cytokines:

- Tumor necrosis factor ligand and receptor family: APRIL/TNSF13, BAFF/TNFSF13B, sCD30/TNFRSF8, sTNF-R1, sTNF-R2; regarding LIGHT/TNFSF14, differences were

observed only between ATLL patients and asymptomatic carriers, but not with the control group; plasma levels of TWEAK/TNFSF12 were higher in the control group and asymptomatic carriers compared to ATLL patients.

- Interferons: IFN-α2, IFN-β, IFN-γ, IL-28A/IFN-λ2, and IL-29/IFN-λ1; median IFN values were nearly double in patients with the acute type compared to the lymphoma type, but without statistical significance.

- Cytokines involved in bone remodeling: osteopontin; ATLL patients had significantly lower median plasma levels of osteocalcin compared to the control group and asymptomatic carriers.

- Interleukins: IL-2, IL-10, IL-11, IL-12 p40, IL-20, IL-22, IL-26, IL-32; plasma interleukin levels were higher in patients with the acute type of ATLL, but without statistical significance.

- Other cytokines: sCD163, pentraxin-3, and TSLP; chitinase 3-like 1 levels were significantly higher in ATLL patients compared to the control group, but not compared to asymptomatic carriers; although median values were higher in patients with the acute type of ATLL, no statistically significant differences were identified.

Determination of cutoff cytokine levels in asymptomatic carriers compared to ATLL patients

To identify threshold values, we first performed a univariate analysis using ROC curves to determine which cytokines showed significantly different levels between the two study groups, namely asymptomatic carriers and ATLL patients (*Figure 6*).

Most of the cytokines analyzed demonstrated a moderate ability to discriminate between ATLL patients and asymptomatic carriers, with sensitivity and specificity values above 70%. Soluble receptors for TNF-α, IFN-α, and IFN-λ, as well as several interleukins, showed high performance (sensitivity and specificity >80%), suggesting potential diagnostic relevance (*Table 3*).

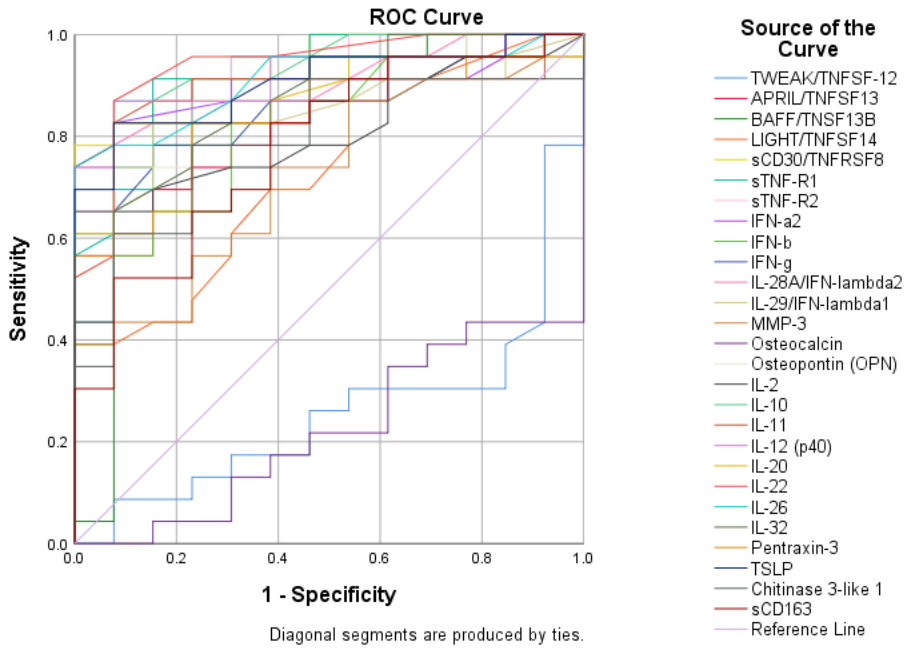

Figure 6. ROC curve

Table 3. The cutoff values for the cytokines that were statistically significant based on the ROC curve analysis

Cytokine	Cutoff (pg/ml)	Sensibility	Specificity
TWEAK/TNFSF-12	NA	↓	↓
APRIL/TNFSF13	159781.278	73.90%	76.90%
BAFF/TNSF13B	10769.54	78.30%	84.60%
LIGHT/TNFSF14	23.33	69.60%	61.50%
sCD30/TNFRSF8	1112.47	87.00%	84.60%
sTNF-R1	593.63	91.30%	84.60%
sTNF-R2	497.72	82.60%	84.60%
IFN- α2	10.69	82.60%	92.30%
IFN-β	33.13	87.00%	69.20%
IFN- γ	30.68	78.30%	84.60%
IL-28A/IFN-λ2	82.95	82.60%	84.60%
IL-29/IFN-λ1	77.29	82.60%	84.60%
MMP-3	14340.03	69.60%	61.50%
Osteocalcin	NA	↓	↓
Osteopontin	34428.51	82.60%	76.90%
IL-2	61.17	69.60%	84.60%
IL-10	22.79	87.00%	84.60%
IL-11	14.03	87.00%	84.60%
IL-12 (p40)	310.49	87.00%	92.30%
IL-20	40.92	82.60%	92.30%
IL-22	34.77	87.00%	92.30%
IL-26	1296.14	78.30%	84.60%
IL-32	70.76	78.30%	76.90%
Pentraxin-3	6793.81	82.60%	76.90%
TSLP	35.65	82.60%	92.30%
Chitinase 3-like 1	8788.20	78.30%	76.90%
sCD163	91398.93	65.20%	76.90%

Discussions

The study analyzes a wide spectrum of cytokines involved in inflammation, apoptosis, tissue and bone remodeling in relation to ATLL. Among all the molecules investigated, only sTNFR1, sTNFR2, sCD30, interferons, osteopontin, IL-2, and IL-10 have previously been described in the context of ATLL, while the rest are presented here for the first time.

ATLL patients exhibited significant alterations in plasma cytokine levels, especially those with the acute type. This profile provides insights for biomarker identification, prognostic stratification, and the development of targeted therapies.

To explore possible interactions between cytokines apparently involved in ATLL pathogenesis, an *in silico* analysis was performed using the STRING platform. This analysis included cytokines that were strongly associated with ATLL based on ROC curve analysis.

The generated network (*Figure 7*) highlighted the significant involvement of cytokines in essential processes for immune regulation and oncogenesis. IL-12 p40, IL-10, IFN-α2, IFN-λ2, and IL-22 are the cytokines with the most connections in the network (more than four each), indicating greater importance compared to the other analyzed cytokines in regulating the immune response in ATLL.

The most significant biological processes identified are illustrated in *Figure 8*. These results suggest that the selected cytokines are involved in activating the JAK-STAT signaling pathway (TSLP, IL-10, IL-12 p40, IL-26) and in regulating the synthesis of proinflammatory and immunomodulatory cytokines (TSLP, IL-10, IL-12 p40, IL-26, sCD30, sTNF-R1, and IFN-α2).

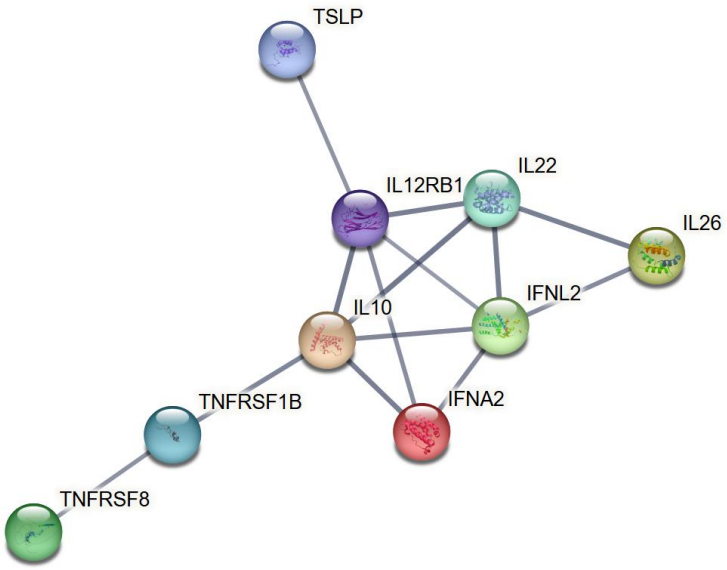

Figure 7. Functional interaction network of cytokines (nodes = proteins, edges = functional or physical interactions)

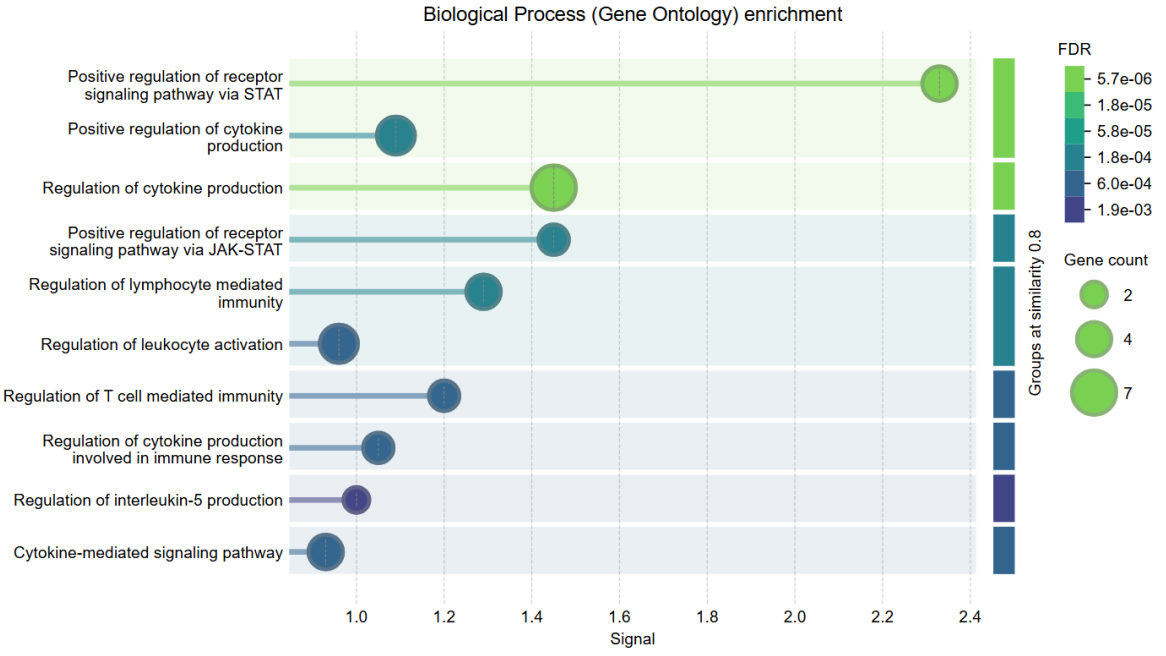

Figure 8. Functional enrichment analysis – Biological processes (Gene Ontology) (terms grouped based on similarity ≥ 0.8, sorted by significance, false discovery rate FDR ≤ 0.05)

Conclusion

Romania is the only country in Europe endemic for HTLV-1. The prevalence among blood donors is much higher than in other developed countries (20–50 times higher than in France or the United States). The infection likely spread in the 1980s, concomitantly with HIV. This contributes to the particular presentation of ATLL patients in Romania compared to those in other endemic regions, such as Japan.

The median age of ATLL patients in Romania is lower compared to patients from other regions, namely 48 years. In recent years, the median age is approaching that reported in international studies, suggesting a possible alignment with global data in the future.

The lymphoma type is more frequent, followed by the acute type. Romanian patients are more often diagnosed at advanced stages (III-IV) and with poor performance status, likely due to the absence of systematic screening strategies.

The survival of Romanian patients is lower than in other regions, probably due to advanced stage at diagnosis, limited access to modern therapies, and lower overall response rates.

The pathogenesis of ATLL is still incompletely understood. Analysis of cytokine profiles and their differences compared to asymptomatic carriers can enhance current knowledge of ATLL pathogenesis, identify new therapeutic targets, and ultimately improve patient survival.

By analyzing the expression of over 30 inflammatory cytokines, a higher plasma level was observed in ATLL patients compared to asymptomatic carriers and the control group, especially in the acute type. The identified biological processes include activation of the JAK-STAT pathway and regulation of the inflammatory response, key mechanisms in ATLL pathogenesis.

Personal contributions

I conducted the most extensive study on ATLL patients in Romania to date, including 58 cases with aggressive types, a significant cohort given the rarity of this disease.

I described the epidemiological characteristics of ATLL patients in Romania:

- Age at diagnosis lower than reported in international studies
- A trend toward increasing age at diagnosis
- Female sex predominance

I analyzed the clinical and biological characteristics and observed that Romanian patients present at the hospital at more advanced stages:

- Over 90% of patients were diagnosed at stages III-IV
- 67% presented B symptoms at diagnosis
- More than one-third of patients had poor performance status
- More than half were classified as high-risk according to JCOG-PI

I outlined the treatments followed by these patients and the outcomes obtained. A suboptimal response to available first-line treatment was observed, lower than results reported in international studies, and a low referral rate for allo-HSCT, with only four patients transplanted.

By analyzing clinical and biological parameters in relation to survival, particularly death within six months, I developed a prognostic score that classifies patients into high- and low-risk groups. The score is simple, includes clinical data and routinely available laboratory analyses, and can be widely applied.

I investigated the role of inflammatory mediators in ATLL pathogenesis by measuring plasma levels of 37 cytokines in three subject categories: patients with acute or lymphoma ATLL, asymptomatic HTLV-1 carriers, and healthy controls. Significant differences in cytokine profiles were observed between ATLL patients and asymptomatic carriers and controls, with marked increases in cytokine levels in the acute type and identified threshold levels with high sensitivity and specificity for ATLL diagnosis.

The contributions presented provide a new perspective on ATLL in Romania and constitute a solid foundation for improving diagnostic, prognostic, and treatment strategies.

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