

Flow Cytometry in Mature T- and NK-cell Neoplasms: A Retrospective Descriptive Study from a Tertiary Care Cancer Centre in Kerala, India

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ABSTRACT

Introduction: Mature T- and NK-cell Neoplasms (MTNKN) constitute around 12% of all non-Hodgkin lymphomas. They have a variable clinical course, ranging from indolent to highly aggressive tumours. Flow cytometry immunophenotyping is crucial for the diagnosis, staging, and classification of MTNKN.

Aim: To determine the frequency, morphologic, and immunophenotypic profile of various subtypes of MTNKN diagnosed by flow cytometry.

Materials and Methods: This was a retrospective descriptive study conducted at the Department of Pathology, Regional Cancer Centre, Thiruvananthapuram, Kerala, India. All cases of MTNKN diagnosed by flow cytometry in peripheral blood, bone marrow aspirates, or body fluids from January 1, 2010 to June 30, 2020 (10.5 years duration) at the cancer centre were studied. The morphology of tumour cells and immunophenotype by flow cytometry were analysed. Clinical parameters (lymphadenopathy, hepatosplenomegaly, skin lesions, effusions, B symptoms) and follow-up details, including Progression-free Survival (PFS) and Overall Survival (OS), were also noted. Descriptive statistics

for continuous variables and frequencies and percentages for categorical variables were obtained. The Kaplan-Meier method for survival plots was used.

Results: Mature T- and NK-cell neoplasms constituted 83 cases. The median age of patients was 56 years. The majority of patients were males (n=49). Adult T-cell Leukaemia/Lymphoma (ATLL) (n=50) constituted the most common subtype, followed by Mycosis fungoides/Sezary syndrome (MF/SS) (n=14), T-cell Large Granular Lymphocytic Leukaemia (T-LGLL) (n=7), T-cell Prolymphocytic Leukaemia (T-PLL) (n=4), Aggressive NK-cell Leukaemia (ANKL) (n=3), Hepatosplenic T-cell Lymphoma (HSTL) (n=3), and Anaplastic Large Cell Lymphoma (ALCL) (n=2). OS and PFS at three years were 22.3% and 16.6%, respectively.

Conclusion: Mature T- and NK-cell neoplasms presenting as leukaemia is rare. ATLL was the most common subtype of MTNKN. Flower cells, Sezary cells, and prolymphocytes are useful morphological clues for diagnosis. Immunophenotyping by flow cytometry, along with clinicopathologic correlation, is crucial for the diagnosis and subclassification of MTNKN. All subtypes except T-LGLL show inferior PFS and OS.

Keywords: Chronic lymphoproliferative disorder, Frequency, Immunophenotype, Subtypes, Survival

INTRODUCTION

The MTNKN are less common than mature B-cell neoplasms, constituting only around 12% of non-Hodgkin lymphomas [1]. There is a higher incidence in Asia, particularly in HTLV-1 endemic regions [2,3]. The primary approach to the flow cytometry diagnosis in MTNKN depends on the demonstration of deviation from the normal immunophenotypic antigen pattern [4-6]. Familiarity with the normal antigen expression pattern in normal and reactive conditions is important, as the reactive T-cell population may also show downregulation of the expression of T-cell antigens. Downregulation of CD5 and CD7 is also reported in reactive T-cell populations. Some mature T-cell lymphoproliferative disorders present primarily in the leukaemic phase, while some present primarily as lymphomas, thus explaining the importance of clinical correlation. MTNKN presenting in the leukaemic phase are ATLL, MF/SS, T-LGLL, T-PLL, HSTL, ANKL, and NK-large granular lymphocytic leukaemia [7]. ATLL is associated with the human retrovirus HTLV-1 and most frequently presents in an acute phase with circulating tumour cells and is morphologically characterised by flower cells. Sezary syndrome is a triad of erythroderma, generalised lymphadenopathy, and the presence of clonally related neoplastic T cells (Sezary cells) in the skin, lymph nodes, and peripheral blood [1,2]. Sezary syndrome and mycosis fungoides are closely related tumours but are considered separate based on the clinical picture. T-PLL classically presents with a markedly high and rapidly rising WBC count and is associated with skin changes, lymphadenopathy, splenomegaly, and effusions. T-LGLL is characterised by a persistent increase in Large Granular

Lymphocytes (LGLs) usually to $2-20 \times 10^9/L$ without a clearly identified cause [1]. ANKL is characterised by systemic proliferation of NK cells associated with an aggressive clinical course [1]. The HSTL is an aggressive extranodal lymphoma characterised by hepatosplenomegaly without lymphadenopathy [1-3]. The present study aimed mainly to determine the frequency of various subtypes of MTNKN diagnosed by flow cytometry, assess the morphologic and immunophenotypic profile, PFS, and OS of these subtypes. Flow cytometry helps in providing a rapid diagnosis in MTNKN, which are generally aggressive. This helps in the early initiation of treatment and thus a better clinical outcome. The present study is the single largest study on MTNKN diagnosed by flow cytometry from Kerala, India.

MATERIALS AND METHODS

This was a retrospective descriptive study that included all cases of MTNKN diagnosed by flow cytometry from 1 January 2010 to 30 June 2020 (a duration of 10.5 years) at Department of Pathology, Regional Cancer Centre, Thiruvananthapuram, Kerala, India, a tertiary care cancer centre in India. The study period was from 1 August 2020 to 31 July 2021. The study was approved by the Institutional Review Board (IRB No: 09/2020/05).

Inclusion criteria: All new cases of MTNKN diagnosed by flow cytometry in peripheral blood, bone marrow aspirates, or body fluids were included in the study.

Exclusion criteria: Relapsed cases of MTNKN were excluded from the study.

Study Procedure

The total number of flow cytometry tests done in the tertiary care cancer centre for the diagnosis of haematologic malignancies during this period was 7574. Mature lymphoid neoplasms in the leukaemic phase, including B-cell and T/NK-cell lineage, accounted for 778 cases. The frequency of MTNKN was calculated based on the total number of mature lymphoid neoplasms of both B- and T- and NK-cell lineage diagnosed by flow cytometry during the period. Samples for flow cytometry included peripheral blood, bone marrow aspirates, or body fluids. The morphology of tumour cells in peripheral blood or bone marrow, including the presence/absence of flower cells, Sezary cells, prolymphocytes, LGLs, was noted. Immunophenotype by flow cytometry was determined using a six-colour flow cytometry analysis with a BD FACS Verse flow cytometer (Becton Dickinson, San Jose, CA, USA). The standard lyse-wash method was used, and a minimum of 10,000 events were acquired for analysis. LCA gating was used to identify the atypical cell population. An antibody panel consisting of CD2FITC (55.2), CD3PerCP (SK7), CD5PE (L17F12), CD7APC (M-T701), CD4 PEcy7 (SK3), CD8FITC (SK1), CD25PerCP (M-A251), CD20APC (L-27), CD34PE (8G-12), CD56PE (MY31), CD16PE (873.1), TCRabFITC (WT31), TCR gdPE (11F2), CD1aAPC (H1149), TdtFITC (E17-1519), and CD45FITC (2D1) was used. Data analysis was done using BDFAC Suite software. Clinical parameters and follow-up details were noted. Serum HTLV-1 assay was done by western blot in all cases suspicious of ATLL. OS was assessed from the date of diagnosis to the date of death (if dead)/closure of the study. PFS was assessed from the date of the 1st treatment to the date of progression/closure of the study.

STATISTICAL ANALYSIS

Statistical analysis was done using Statistical Package for Statistical Sciences (SPSS) software version 28.0 Descriptive statistics, such as the mean and standard deviation for continuous variables and frequency and percentages for categorical variables, were obtained. Statistical associations were assessed using the Chi-square test. The Kaplan-Meier method for survival plot, Log-rank test for statistical significance for survival, and Cox-regression model for risk estimation were utilised. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Frequency: The total number of MTNKN cases diagnosed during the period was 83. MTNKN accounted for 10.67% of mature lymphoid neoplasms, including B-cell and T/NK-cell lineage (n=778). Of the 83 cases, 78 were diagnosed in peripheral blood, four in bone marrow, and one in pleural fluid. ATLL constituted the most common subtype (n=50), followed by MF/SS (n=14), T-LGLL (n=7), T-PLL (n=4), ANKL (n=3), HSTL (n=3), and ALCL (n=2).

Demographic details: The age range of patients ranged from 17 to 83 years, with a median age of 56 years. The majority of patients were males (n=49). The age range of ATLL patients was 32 to 75 years, with the majority (n=33) being males. MF/SS patients were aged between 35 to 72 years, with a male predominance (n=7). T-PLL patients' age range was 53 to 65 years, with an equal distribution between males and females. T-LGLL patients' age ranged from 37 to 68 years. Among the three ANKL patients, two were 17 years old, one was 40 years old, and there was a male predominance (n=2). HSTL patients' age ranged from 26 to 53 years, and all patients were females. Both ALCL patients were males, aged 44 years and 56 years, respectively.

Clinical and laboratory data: Clinical and laboratory features of the various subtypes are presented in [Table/Fig-1]. Among routine haematological parameters, anaemia was present in 36% (n=36), leukocytosis in 94% (n=78), and thrombocytopenia in 35% (n=29) of patients. Analysis of biochemical parameters revealed hypercalcemia in 27% (n=22) and elevated serum lactate dehydrogenase levels in 90% (n=75) of patients. Clinical data analysis revealed lymphadenopathy in 73% (n=61), hepatomegaly

in 40% (n=33), splenomegaly in 49% (n=41), effusions in 24% (n=20), and B symptoms in 46% (n=38) of patients. Effusions and B symptoms were absent in T-LGLL.

Morphology and flow cytometry data: The immunophenotype by flow cytometry of each subtype is shown in [Table/Fig-2].

Clinical and laboratory features	ATLL (n=50)	SS (n=14)	T-PLL (n=4)	T-LGLL (n=7)	ANKL (n=3)	HSTL (n=3)	ALCL (n=2)
Anaemia	21	4	3	1	3	2	2
Leukocytosis	50	13	4	4	2	3	2
Thrombocytopenia	11	4	4	2	3	3	2
Total leukocyte count/mm ³	29k-340k	7.9k-103k	44.9k-147k	7.3k-23.9k	1k-63.3k	12.7k-102k	28.8k-168k
% circulating tumour cells	22-98%	30-92%	64-92%	45-90%	23-82%	85-92%	64-66%
Hypercalcaemia	20	1	1	0	0	0	0
Raised LDH	50	14	4	0	3	2	2
Lymphadenopathy	36	14	4	1	1	3	2
Hepatomegaly	17	4	3	2	2	3	2
Splenomegaly	22	5	3	4	2	3	2
Effusions	11	0	3	0	2	3	1
B symptoms	22	7	3	0	1	3	2

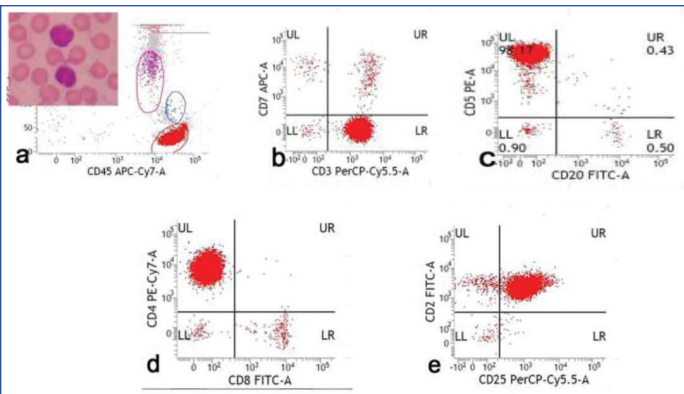
[Table/Fig-1]: Clinical and laboratory parameters of various subtypes are given. Haemoglobin reference range: 13-16 gm% in males, 12-16 gm% in females; total leukocyte count reference range: 4000-11000/mm³; platelet count reference range: 150k-450k/mm³; B symptoms: fever, night sweats, weight loss; LDH: Lactate dehydrogenase reference range: 120-246u/L; calcium reference range: 8.6-10.2 mg/dL

Adult T- cell leukaemia/lymphoma cases (n=50) were diagnosed after correlating immunophenotype by flow cytometry with tumour cell morphology, clinical findings with emphasis on skin lesions, and laboratory findings including serum calcium and LDH levels. All cases showed flower cells in peripheral blood, with flow cytometry performed in all cases. ATLL cases are characterised by positivity for CD2, CD3, CD5 with loss of CD7, and bright expression of CD25. A CD4+CD8- phenotype was noted in 49 cases [Table/Fig-3a-e]. Aberrant immunophenotype included one case showing loss of CD5 and another case showing loss of CD5 and a CD4-CD8- (double negative) phenotype.

CD markers	ATLL (n=50)	SS (n=14)	T-PLL (n=4)	T-LGLL (n=7)	ANKL (n=3)	HSTL (n=3)	ALCL (n=2)
Surface CD3	50	14	3	7	0	3	0
CD2	50	14	4	7	0	3	0
CD5	48	14	4	6	0	0	0/1
CD7	0	0	4	2	0	3	1
CD25	50	10	1	0	0	0	0
CD16	ND	ND	0/1	0	0/1	2	ND
CD56	ND	ND	0	3	3	3	2
TCR ab	50	14	4	7	0	1	0
TCR gd	0	0	0	0	0	2	0
CD4+CD8-	49	13	4	0	0	0	0
CD4-CD8+	0	0	0	0	0	2	0
CD4+CD8+	0	0	0	7	0	0	0
CD4-CD8-	1	1	0	0	0	1	0
cyCD3	ND	ND	1/1	ND	3	ND	0

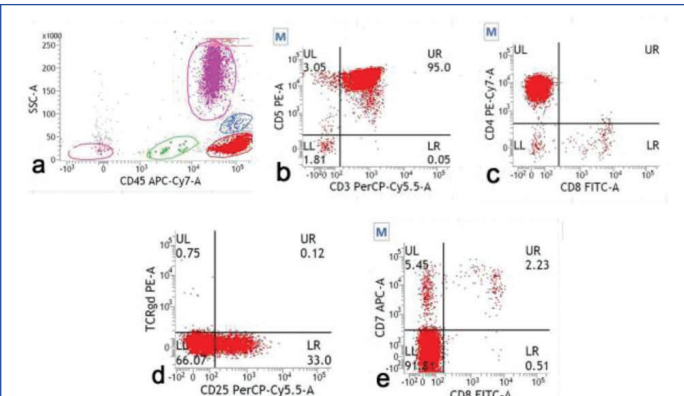
[Table/Fig-2]: Immunophenotype of each subtype of Mature T-and NK-cell Neoplasms (MTNKN). cyCD3: cytoplasmic CD3; ND: Not done; CD: cluster of differentiation; TCR ab: T-cell receptor alpha beta; TCRgd: T-cell receptor gamma delta; ND: Not done

Mycosisfungoides/Sezary syndrome (n=14) was characterised by Sezary cells in all 14 cases, with flow cytometry was done in peripheral blood in all cases. All cases were positive for CD2, CD3, CD5, with loss of CD7. CD25 was positive in three cases, with dim expression compared to ATLL [Table/Fig-4a-e]. A CD4+CD8- phenotype was noted in 13 cases, with one case showing a CD4-



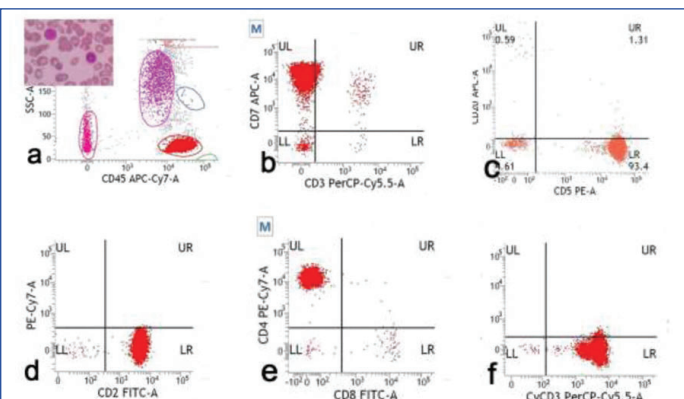
[Table/Fig-3]: (a) Flow cytometry done under LCA gating. Inset showing flower cells. (Giemsa, x1000); (b) Gated population is positive for CD3 and show loss of CD7; (c) Positive for CD5; (d) Positive for CD4; (e) Positive for CD2 and CD25.

CD8- (double negative) phenotype. Serum HTLV1 assay was negative in all 12 cases tested.



[Table/Fig-4]: (a) LCA gating; (b) Gated population is positive for CD3 and CD5; (c) Positive for CD4 and negative for CD8; (d) Dim positive for CD25; (e) Loss of CD7.

T-cell polymorphic leukaemia was diagnosed in four cases, all showing small to medium-sized neoplastic cells with basophilic cytoplasm, round to oval nucleus, and visible nucleoli. Three cases showed typical T-PLL immunophenotype with preservation of T-cell lineage antigens, while one case showed an aberrant immunophenotype with loss of CD3 and preservation of other T-cell lineage antigens, and absence of markers of immaturity [Table/Fig-5a-f]. CD25 was positive in one case.

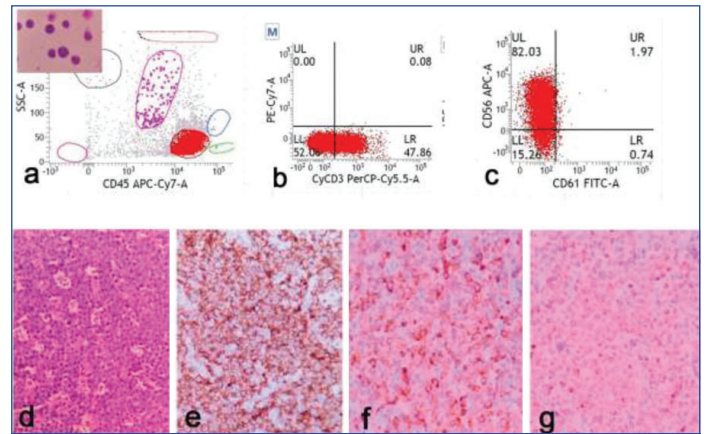


[Table/Fig-5]: (a) LCA gated population. Inset showing polymorphocyte; (b) Gated population is positive for CD7 and show loss of CD3; (c) Positive for CD5; (d) Positive for CD2; (e) Positive for CD4 and negative for CD8; (f) Positive for cytoplasmic CD3.

T-cell large granular lymphocytic leukaemia was seen in seven cases, with one patient diagnosed preceding acute myeloid leukaemia relapse. Flow cytometry was done in peripheral blood for six patients and bone marrow aspirate for one patient. All cases showed a CD8+CD4- phenotype, with three cases positive for CD56.

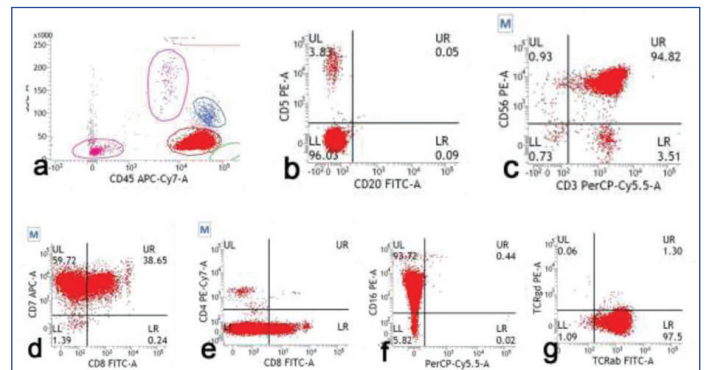
Aggressive NK-cell leukaemia constituted three cases, tumour cells in all cases being cytoplasmic CD3 and CD56 positive and negative

for other T-cell lineage markers [Table/Fig-6a-g]. EBER testing could not be done in all three cases.

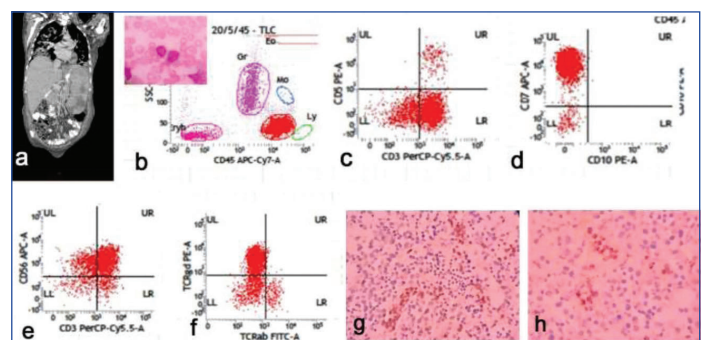


[Table/Fig-6]: (a) LCA gated population. Inset showing the atypical cells in pleural fluid; (b) Gated population is positive for cytoplasmic CD3; (c) Positive for CD57; (d) Biopsy from thigh mass showing sheets of large atypical cells. (H&E, x400); (e) Positive for LCA (IHC, x400); (f) Granzyme (IHC, x400); (g) TIA-1 (IHC, x400).

Hepatosplenic T- cell lymphoma was seen in three cases, with flow cytometry done in bone marrow aspirate for one case and peripheral blood for two cases. All cases showed loss of CD5, with one case exhibiting a TCR alpha beta phenotype [Table/Fig-7a-g] and two cases showing a gamma delta phenotype [Table/Fig-8a-h].



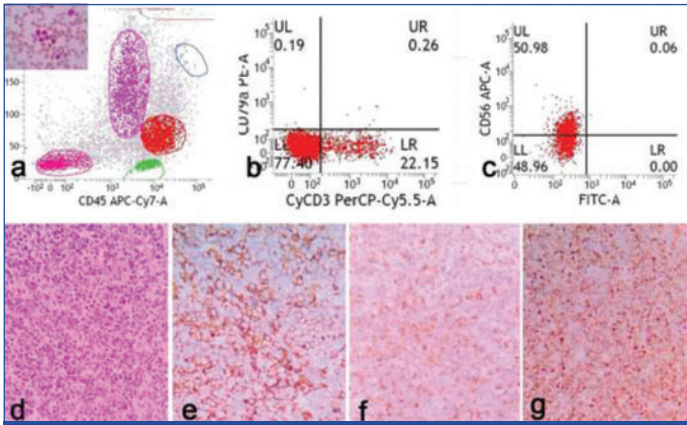
[Table/Fig-7]: (a) Flow cytometry in LCA gating; (b) Gated population is negative for CD20 and CD5; (c) Positive for CD3 and CD56; (d) Positive for CD7; (e) Dim positive for CD8; (f) Positive for CD16; (g) Positive for TCR alpha beta.



[Table/Fig-8]: (a) CT abdomen showing hepatosplenomegaly; (b) Flow cytometry done in LCA gating. Inset shows the tumour cells in peripheral blood. (Giemsa, x1000); (c) Gated population is positive for CD3 and negative for CD5; (d) Negative for CD10 and CD7; (e) Positive for CD3 and CD56; (f) Positive for TCR gamma delta and negative for TCR alpha beta; (g) CD3 in bone marrow biopsy showing intrasinusoidal arrangement of tumour cells. (IHC, x400); (h) Positive for TIA-1(IHC, x400).

Anaplastic large cell lymphoma was seen in two cases. The morphology of tumour cells resembled blasts, prompting an acute leukaemia panel to be conducted in both cases. The first case was positive for CD56 and CD7 only, while the second case was positive for CD56 only and negative for other T-lineage, NK-cell markers, B lineage, and myeloid markers. CD30 and ALK were not present in the flow cytometry panel. Lymph node biopsies were performed in both cases, revealing tumour cells strongly positive

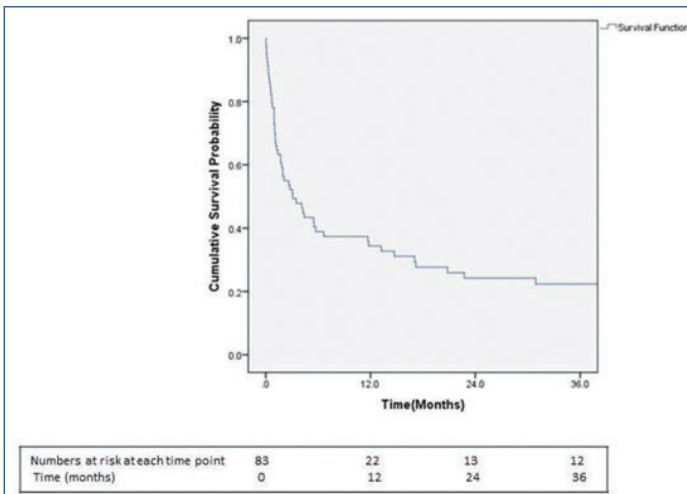
for CD30 [Table/Fig-9a-g]. ALK was positive in the first case and negative in the second case.



[Table/Fig-9]: (a) Flow cytometry done under LCA gating. Inset showing the tumour cells in peripheral blood (giemsa, x1000); (b) Gated population is positive for cytoplasmic CD3; (c) Positive for CD56; (d) Bone marrow biopsy showing atypical lymphoid cells; (e) Tumour cells are CD30 positive (IHC, x400); (f) Positive for CD3 (IHC, x400); (g) Positive for TIA-1 (IHC, x400).

Serum HTLV-1 assay: Serum HTLV-1 assay was done in 24 out of 50 cases suggestive of ATLL by flow cytometry, all of which tested positive.

Survival data: The follow-up of patients at three years was 80.7%, and at four years was 78.3%, with a median follow-up period of 37 months (1-79 months). The Overall Survival (OS) at three years in patients with MTNKN presenting in the leukaemic phase was 22.3% [Standard Error (SE)=5.2%] [Table/Fig-10]. Progression-free Survival (PFS) at three years was 16.6% (SE=4.6%) [Table/Fig-11]. The overall Median Survival (MS) time was 3.1 months (SE=1.22%) [Table/Fig-12].

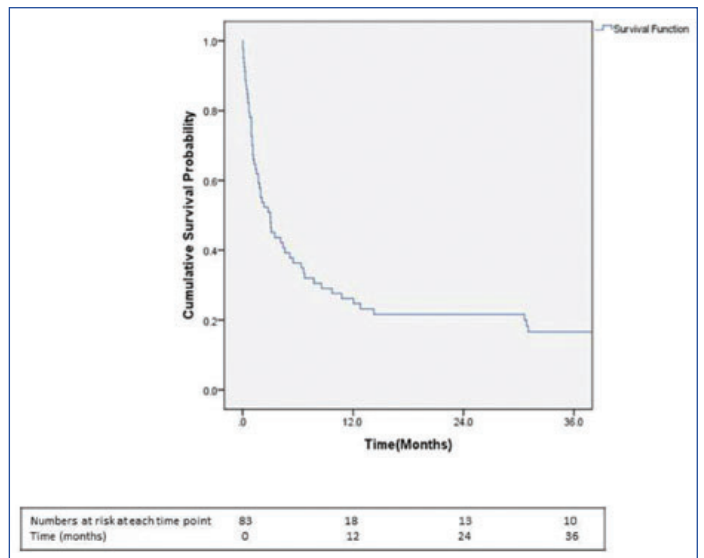


[Table/Fig-10]: Kaplan-Meier curve showing OS at 3 years.

Among ATLL cases, 22 patients were treated with multi-agent chemotherapy regimens based on age and performance status. Four patients received palliative chemotherapy. PFS at three years was 7% (SE=4.6%) [Table/Fig-13,14], and OS was 11.5% (SE=5.8%) [Table/Fig-15,16]. The MS was around 1.3 months (SE=0.42) [Table/Fig-12].

Four patients with MF/SS were treated with multi-agent chemotherapy regimens (CHOP/COP/LSG/MINE) based on age and performance status, while one patient received palliative chemotherapy. PFS at three years was 21.4% (SE=11%) [Table/Fig-13,14]. OS at three years was around 39.7% (SE=13%) [Table/Fig-15,16]. The MS was 20.8 months (SE=8.26) [Table/Fig-12].

Among T-PLL, three patients were treated with a multi-agent chemotherapy regimen (CHOP), and one patient received palliative chemotherapy. All four cases had an aggressive clinical course [Table/Fig-13-16]. The median survival (MS) was 3.5 months (SE=1.77) [Table/Fig-12].

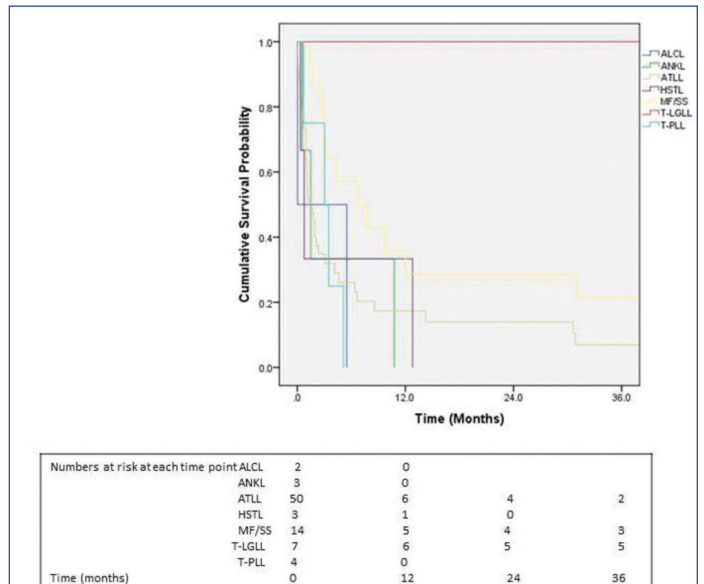


[Table/Fig-11]: Kaplan-Meier curve showing PFS at 3 years.

HPR	Number (n)	No. of events (Event: death)	Median survival (months)	
			Survival time	SE (%)
ALCL	2	2	0.03	0.00
ANCL	3	3	2.63	1.68
ATLL	50	38	1.27	0.42
HSTL	3	3	0.77	0.33
MF/SS	14	10	20.80	8.26
T-LGLL	7	1	0.0	0.00
T-PLL	4	4	3.50	1.77
Total	83	61	3.10	1.22

[Table/Fig-12]: Overall Median Survival (MS) time.

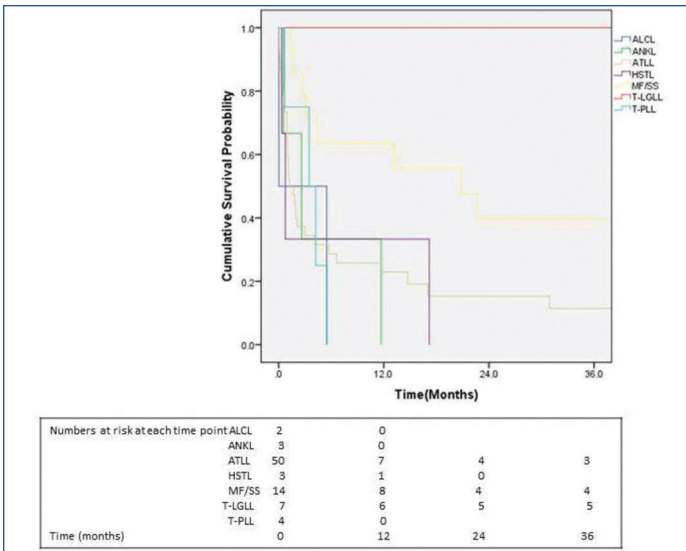
N:number; SE:standard error



[Table/Fig-13]: Kaplan-Meier curve showing PFS at 3 years of various subtypes

HPR	Cumulative survival rate (%)	SE	p-value
ALCL	0	0	0.004
ANKL	0	0	
ATLL	7.0	0.046	
HSTL	0	0	
MF/SS	21.4	0.11	
T-LGLL	100.0	-	
T-PLL	0	0	

[Table/Fig-14]: PFS at three years of various subtypes.



[Table/Fig-15]: Kaplan-Meier curve showing OS at three years of various subtypes.

HPR	Cumulative survival rate (%)	SE	p-value
ALCL	0	0	0.001
ANKL	0	0	
ATLL	11.5	0.058	
HSTL	0	0	
MF/SS	39.7	0.13	
T-LGLL	100.0	-	
T-PLL	0	0	

[Table/Fig-16]: OS at 3 years of various subtypes. Log-rank test applied

In T-LGLL, only two patients required treatment and were managed with a single-agent methotrexate. One of these patients had progressive disease and was then managed with single-agent cyclophosphamide. Progression-free survival (PFS) and overall survival (OS) at three years were both 100% [Table/Fig-13-16].

Patients with ANKL were treated with a multi-agent chemotherapy regimen but had an aggressive clinical course [Table/Fig-13-16]. The median survival was 2.6 months (SE=1.68) [Table/Fig-12].

Among HSTL, two patients were treated with a multi-agent chemotherapy (CVP) regimen, and one patient did not receive treatment. All patients had a dismal outcome [Table/Fig-13-16]. The median survival was 0.8 months (SE=0.33) [Table/Fig-12].

Both patients with ALCL could not begin treatment and had a poor outcome [Table/Fig-13-16]. The median survival was 0.03 months [Table/Fig-12].

The difference in overall survival (OS) between different subtypes was found to be statistically significant (p-value=0.001) [Table/Fig-16]. OS at three years for T-LGLL was 100%, while for non T-LGLL cases it was 14.8% (SE=4.7%), and this difference was statistically significant (p-value=0.001). The progression-free survival (PFS) for the different subtypes was also statistically significant (p-value=0.001) [Table/Fig-14]. PFS at three years for T-LGLL was 100%, compared to 8.8% (SE=3.7%) for non T-LGLL cases, and this difference was statistically significant (p-value=0.001).

DISCUSSION

The MTNKN constituted around 10.67% of mature lymphoid neoplasms diagnosed by flow cytometry. Tembhare PR et al., studied 232 cases of MTNKN diagnosed by flow cytometry out of 4862 patients who were evaluated using flow cytometry for lymphoma diagnosis and staging over a period of seven years [8]. The study correlated flow cytometry results with histopathology and immunohistochemistry. The study also emphasised the role of flow cytometry in confirming bone marrow infiltration in MTNKN,

especially in cases of low-level involvement. The median age of patients was 51 years, showing male predominance with a male-to-female ratio of 2.3:1. Angioimmunoblastic T-cell lymphoma was the most common subtype in the study, constituting 22.4% (n=52).

Gujral S et al., studied the morphology and immunophenotype of MTNKN for a period of four years [9]. The diagnosis was based on the morphology and flow cytometric immunophenotyping of peripheral blood and/or bone marrow samples of cases of MTNKN presenting as leukaemia. MTNKN constituted nine cases, accounting for 4% of mature lymphoid neoplasms diagnosed by flow cytometry. T-LGLL (n=4) was the most frequent subtype, followed by T-PLL (n=2), ATLL (n=2), and primary cutaneous gamma-delta T-cell lymphoma (n=1).

Naseem S et al., studied the spectrum, frequency, morphology, and immunophenotype of newly diagnosed MTNKN over a period of two and a half years [10]. MTNKN constituted 3.1% (n=8) of mature lymphoid neoplasms, with T-PLL (n=4) being the most frequent subtype, followed by chronic lymphoproliferative disorder of NK cells (n=2), T-LGLL (n=1), and HSTL (n=1).

Adult T-cell leukaemia/lymphoma constituted the most common subtype in the present study. The alarming incidence of ATLL in the state of Kerala, India, was also highlighted by Nair RA et al., [11]. CD4/CD8 double negativity is extremely rare [12,13]. Loss of CD2, CD3, and CD5 are also reported. The present study had two cases of ATLL with atypical immunophenotype. Aberrant immunophenotypes may be associated with a more aggressive behaviour in ATLL. Another study from a referral centre in India showed a frequency of 22% (n=2) among mature T and NK-cell neoplasms presenting as leukaemia [9]. The survival of the acute variant of ATLL ranges from two weeks to more than one year [1]. The median survival of ATLL in the present study was 1.3 months.

Mycosis fungoides/Szary syndrome constituted the second most common subtype. The typical immunophenotype of MF/SS is CD3 positive, CD4 positive T cells with aberrant loss of CD7 and CD26. CD25 was expressed in 10 cases in the present study. Compared to ATLL, all the cases showed dim expression of CD25. Bright expression of CD25 would include ATLL as an alternative diagnosis and mandates testing for serum HTLV1. Rare cases of CD4/CD8 double-negative phenotypes are also reported, which was seen in a single case in the present series [14]. The median survival (MS) of SS is around 32 months, whereas the present study showed an MS of 20.8 months (SE=8.26). SS is an aggressive disease with a five-year OS of 10-30% depending on the stage of the disease [1]. The present study also showed similar findings.

T-cell prolymphocytic leukaemia is morphologically characterised by small to medium-sized cells with non granular basophilic cytoplasm, some showing cytoplasmic protrusions or blebs, round/oval/markedly irregular nuclei, and visible nucleoli [1,15,16]. It is characterised by pan T-cell antigen expression without antigen loss. Immunophenotypic aberrancies in CD2, CD3, CD5, and CD7 are also reported. Loss of CD3 is reported in one case series [15]. Loss of CD3 was seen in one case in the present study. Rare immunophenotypes like decreased surface CD3, CD45, as well as the more common CD4 and CD8 double-positive phenotype, may result in a differential diagnosis of T lymphoblastic leukaemia. However, the absence of markers of immaturity like CD1a, CD34, Tdt, along with the morphology of atypical lymphoid cells, will help in the distinction. The course of the disease is aggressive with an MS of 1-2 years [1].

T-cell large granular lymphocytic leukaemia is characterised by Large Granular Lymphocytes (LGLs) in the blood and bone marrow. T-LGLL is typically a disorder of mature cytotoxic T cells. The typical immunophenotype is CD3+, TCRab+, CD4-, CD8+. CD56-positive T-LGLL may have an aggressive clinical course associated with STAT5B mutations [17]. Three cases of T-LGLL in the present study were CD56 positive, and among them, one case had disease progression.

The typical immunophenotype of ANKL is CD2+, CD3 epsilon+, surface CD3-, CD56+. EBER testing could not be performed

in these cases. The median survival of ANKL is less than two months [1]. Similar findings were seen in the present study as well. NK-large granular lymphocytic leukaemia is defined as a persistent increase in NK-cells without a clearly identified cause [1]. It has an indolent behaviour compared to fulminant ANKL. The immunophenotype is similar to ANKL, except that CD16 is more frequently positive. There were no cases of NK-large granular lymphocytic leukaemia in the present series.

Hepatosplenic T-cell lymphoma is characterised immunophenotypically by CD3+, CD5-, usually T-cell receptor gamma delta (TCR gd)+, CD8-/-, CD4-, TIA1+ [1,18-20]. The clinicopathological features of HSTL alpha-beta subtype resemble the gamma-delta subtype, and they can be considered phenotypically heterogeneous subtypes of the same entity [18]. A peculiarity of the three cases was that all the cases presented with lymphocytosis. The maximum total count in peripheral blood reported in the literature is $25.7 \times 10^9/L$. One of the patients in the present study had a total count of $35,900/mm^3$, and another patient had a total count of 1.02 lakh/ mm^3 . The median survival of HSTL is less than two years, as seen in the present study.

Among ALCLs, the leukaemic phase occurs more commonly in ALK-positive ALCL, more often in the small cell variant, and is associated with the presence of cytogenetic abnormalities characterised by t(2;5) [21,22]. Rare ALK-negative cases are also recorded [23]. These patients, regardless of ALK expression, have an unfavourable prognosis. The present study had two cases of ALCL, of which one was ALK-negative ALCL.

Limitation(s)

Molecular studies were not conducted in the present study.

CONCLUSION(S)

The MTNKN accounted for around 10.67% of mature lymphoid neoplasms diagnosed by flow cytometry. ATLL constituted the most common subtype. The presence of flower cells in ATLL, Sezary cells in MF/SS, and prolymphocytes in T-PLL are helpful morphological clues for diagnosis. Morphology should be correlated with clinical, laboratory, and immunophenotypic data. Prognosis was excellent for T-LGLL, while it was inferior for other subtypes. Clinicopathologic correlation is essential for the diagnosis and subcategorisation of these groups of neoplasms.

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AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? No
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. No

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Sep 23, 2023
- Manual Googling: Dec 15, 2023
- iThenticate Software: Jan 27, 2024 (8%)

ETYMOLOGY: Author Origin

EMENDATIONS: 6

Date of Submission: Sep 22, 2023

Date of Peer Review: Dec 07, 2023

Date of Acceptance: Jan 30, 2024

Date of Publishing: Apr 01, 2024