

## Aggressive Variant Large Granular Lymphocytic Leukaemia: A Case Report

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### ABSTRACT

Clonal disorders of LGL may either be CD3+ CD56- or CD3- CD56+ phenotype and these have been designated as T-cell leukaemia (T-LGL) or natural killer cell (NK)-LGL leukaemia respectively. Clonality is usually demonstrated by clonal rearrangement of T-cell receptor gene rearrangement or identified by flowcytometry analysis. Most patients with T-LGL will have an indolent course. In this report we described an aggressiveness of disease in a patient with clonal CD3+ LGL leukaemia whose cells also co-expressed CD56 diagnosed by flowcytometry. The patient responded well to interrupt ALL standard risk protocol however succumbed to her disease while waiting for upfront stem cell transplant. This case highlights on both the classical laboratory findings of rare entity of disease as well as a review of the literature pertaining particularly on its management.

**Keywords:** T-LGL, NK leukaemia, treatment of aggressive variant leukaemia

### INTRODUCTION

Large granular lymphocytes (LGL) comprise 10% to 15% of normal peripheral blood mononuclear cells which morphologically are identifiable subset of peripheral blood lymphocytes. They included natural killer (NK) cells and cytotoxic T-cells. These cells represent in vivo antigens activated T-cells, as a response to viral or other infections.

Clonal disorder CD3+ T-LGL represents 2-3% cases of mature lymphocytic leukaemia.<sup>[1]</sup> Typically, it is a heterogenous indolent disorder characterized by persistent (> 6 months) increase in the number of peripheral blood (PB) large granular lymphocytes usually between 2-20 x 10<sup>9</sup>/L.<sup>[1, 2]</sup> In contrast, CD3- NK-cell leukaemia is an aggressive subtype which affect a younger group of patients. LGL NK-like leukaemia a rare described as an aggressive CD3+, CD56+ variant of T-LGL leukaemia is illustrated here. The literature on the clinic-pathological features and management approaches for this rare, aggressive entity are reviewed.

### CASE REPORT

A 19-year-old Chinese, girl was referred for further investigation and management of pancytopenia. She presented with history of fever, generalized malaise, loss of appetite and significant loss of weight within few months. Her physical examination revealed enlarged liver and massive splenomegaly with generalized lymphadenopathy.

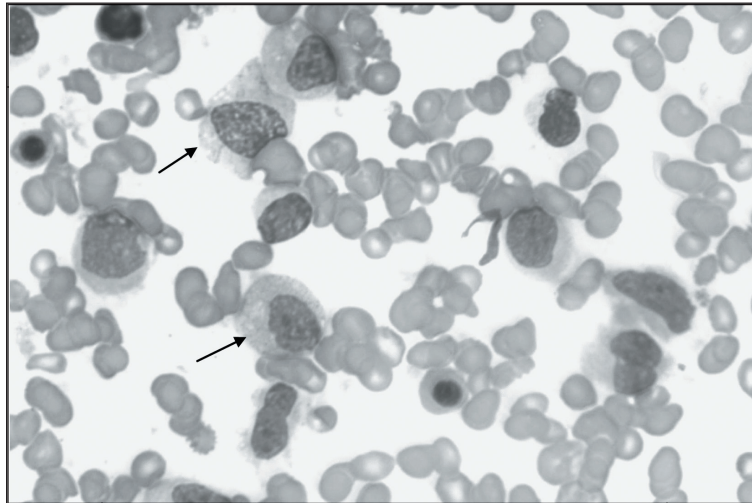
Her complete blood profile revealed a WBC of 1.1 x 10<sup>9</sup>/L (4.0-10.0 x 10<sup>9</sup>/L), haemoglobin of 6 g/dl (12.0-16.0 g/dl) and platelet count of 40 x 10<sup>9</sup>/L (150-400 x 10<sup>9</sup>/L). Pertinent chemistries revealed: normal liver function test, LDH of 785 U/L (211-423) U/L and total bilirubin of 32 µmol/L. Her blood culture was negative. Serology studies showed her blood was positive for EBV IgG but negative for EBV IgM, Hepatitis C, HIV-1 and 2 and CMV IgM. Both rheumatoid factor (RF) and antinuclear antibody (ANA) were negative. Quantitative immunoglobulins were within normal limits.

Blood film examination showed predominantly abnormal looking, large mononuclear/lymphoid cells that constitutes 55% of the white blood cells population. These cells were of varying sizes with abundant irregular cytoplasm, and some having cytoplasmic granules. Subsequent bone marrow aspirate evaluation showed hypercellular marrow with predominantly similar type of mononuclear/lymphoid cells (figure 1). Multiple aggregates of these mononuclear cells, positive for CD3 (figure 2) were seen in her trephine biopsy. Immunophenotyping studies showed the gated cells expressed CD3, CD7, CD8, CD16 and CD56 compatible with aggressive CD3+, CD56+ variant of LGL leukaemia or also known as LGL NK-like leukaemia. Cytogenetic analysis showed normal cells karyotype. Unfortunately specimen sent for TCR gene rearrangement study was unsatisfactory.

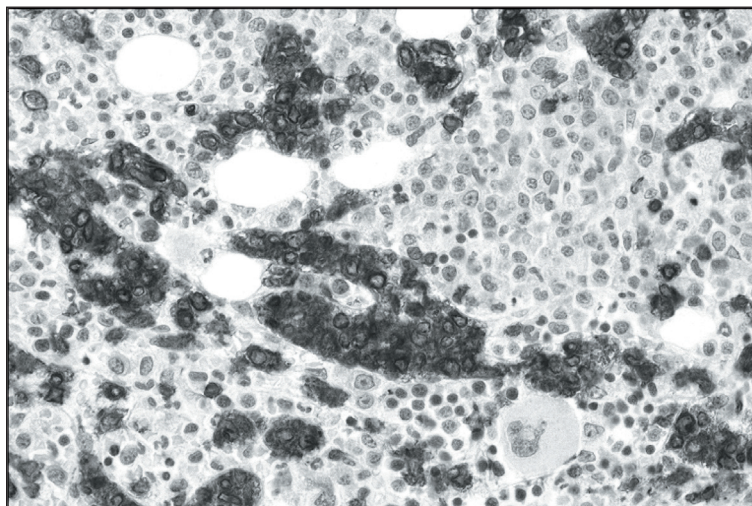
She was treated with interrupted ALL-standard risk protocol. Her disease was temporarily controlled. She had achieved partial clinical and haematological remission after induction. Her spleen had reduced in size and repeat bone marrow assessment showed presence of less than 10% of mononuclear/lymphoid cells. Subsequently, she was treated with another 2 courses of combination cytarabine and mitoxantrone to further control her disease.

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Workout for autologous peripheral blood stem cell transplant was planned. However, during an evaluation for allogenic haemopoietic stem cell transplantation, the patient was noted to have pancytopenia. Subsequent BMA and flowcytometry studies showed she had relapsed with presence of similar types of cells. She succumbed to her illness fourteen months after her initial presentation.



**Figure 1.** BMA MGG X 400 . Circulating large mononuclear/lymphoid cells, some with irregular folding nuclei, open chromatin and distinct nucleoli. The cytoplasm is abundant and some showed azurophilic granules (arrow)



**Figure 2.** Trephine biopsy x 400: multiple aggregates of abnormal lymphoid cells admixed with other haematopoietic elements. These cells have vesicular nuclei with open chromatin pattern and were positive for leukocytes common antigen (LCA) and CD3

## DISCUSSION

Recently, CD56+ entity of haematologic malignancies has attracted attention. The WHO classification of lymphoid malignancies does not recognize an aggressive CD3+ CD56+ T-cell LGL as a subtype of LGL disorders [2]. However, several cases with similar clinicopathological and disease progression as this case have being reported worldwide. [3, 4, 5]

We describe a rare, CD3+, CD56+ LGL leukaemia, which is a variant form of indolent T-LGL leukaemia. It is also described as LGL NK-like leukaemia.[4] Typically, most T-LGL leukaemia have an indolent clinical course. An

absolute lymphocytosis of  $2-20 \times 10^9/L$  accompanied by neutropaenia with or without anaemia and thrombocytopenia are frequent findings.<sup>[1]</sup> Clonality of LGL also has been reported in patients where the absolute lymphocytes count of less than  $2 \times 10^9/L$ .<sup>[2]</sup> Splenomegaly was the main physical finding.<sup>[3, 4]</sup> In comparison, patients with NK-cell leukaemia usually present with constitutional symptoms, organomegaly and leukaemic infiltration in the blood and bone marrow.<sup>[1]</sup> As illustrated in our case, the clinical presentation of CD3<sup>+</sup>, CD56<sup>+</sup> aggressive variant LGL leukaemia was unlike the usual clinical course of T-LGL leukaemia, but remarkably similar to features of NK-cell leukaemia. Macon *et al.*, documented all patients presented with advanced stage of disease, with B symptoms.<sup>[4]</sup> An absolute lymphocytosis (median of  $4.2 \times 10^9/L$ ) was present in all patients at presentation which is relatively higher than values seen in patients with typical, indolent T-LGL leukaemia.<sup>[2, 3]</sup> The marrow, spleen, liver and extranodal involvement was seen in majority of cases.<sup>[3, 4, 5]</sup> Majority of patients (63%) were in a leukaemic phase at their presentation.<sup>[4]</sup>

The LGL cells are identified by their morphology and cell phenotype. Circulating leukaemic cells in aggressive NK-cell leukaemia sometime are indistinguishable from the normal large granular lymphocytes. As seen in this case, the leukaemic cells of NK-like leukaemia often exhibit marked nuclear polymorphism or have a blastic appearance.<sup>[4]</sup> Marrow involvement is more easily established by bone marrow aspirate smears as the tissue section may only show subtle lymphoid infiltrates as seen in this case.<sup>[4]</sup> The common phenotype in T-LGL is CD2<sup>+</sup>, CD3<sup>+</sup>, CD4<sup>-</sup>, CD8<sup>+</sup> with variable expression of CD56.<sup>[1, 2]</sup> In comparison, neoplastic cells in aggressive NK-cell leukaemia are CD2<sup>+</sup>, CD3<sup>-</sup> and CD56<sup>+</sup>. Our patient's immunophenotype analysis showed the leukaemic cells expressed CD3, CD7, CD8, CD16 and CD56 but were negative for intracellular MPO and intracellular CD22. These findings support the suspicion of a rare entity of CD3<sup>+</sup>, CD56<sup>+</sup> variant aggressive LGL leukaemia<sup>3</sup> also described as LGL NK-like leukaemia.<sup>[4]</sup> A variety of cytogenetic abnormalities are much more common in NK-like leukaemia when compared to T-LGL leukaemia.<sup>[1]</sup> Macon *et al.*, suggested this exercise may help to pinpoint patients with poorer prognosis as three out of four patients studied had an abnormal karyotype which include t(2,17), isochromosome 7q and t(8,14)<sup>[4]</sup>. Our patient's cytogenetic analysis showed a normal cell karyotype.

Aggressive T-LGL leukaemia is not considered as a different entity by the latest WHO classification. Aleshkun *et al.*, suggested a diagnosis of aggressive T-LGL leukaemia can be established based upon four clinical and laboratory features which include aggressiveness of clinical presentation (acute onset of B symptoms, hepatosplenomegaly, peripheral cytopenia and lymphadenopathy) together with presence of an absolute large granular lymphocytes count in blood film of more than  $0.5 \times 10^9/L$  (frequently  $> 10 \times 10^9/L$ ).<sup>[5]</sup> Phenotypically the leukaemic cells are positive for CD3, CD8 and CD56 as described in this case. Demonstration of clonally rearranged TCR genes of the leukaemic cells also need to be established.<sup>[1, 4, 5]</sup> Unfortunately, we were unable to prove the clonality by TCR as the sample sent was unsuitable for processing. However, our patient does fulfill the other three clinico-laboratory features as suggested by Todd *et al.* Furthermore; TCR gene rearrangement is also a feature of indolent T-LGL leukaemia. Kojima *et al.*, 1995 reported a case with neither progressive nor aggressive clinical course CD3<sup>+</sup>, CD56<sup>+</sup> LGL with TCR gene rearrangement.<sup>[6]</sup>

The non-progressive nature of typical T-LGL leukaemia probably is better regarded (as clonal of uncertain significance rather than leukaemia). Most patients usually remain asymptomatic even for more than five years.<sup>[1]</sup> Treatment is indicated only in patients who turn aggressive and with symptomatic neutropaenia.<sup>[1, 2]</sup> In contrast, this CD3<sup>+</sup>, CD56<sup>+</sup> aggressive variant LGL leukaemia needed urgent treatment as most untreated cases will die within a months of the diagnosis.<sup>[3, 4, 5]</sup>

Aggressive chemotherapy<sup>[4]</sup> and combination CHOP-like regimen<sup>[3, 4]</sup> have been tried but most patients die within a year of treatments. Gentile *et al.*, described a case which was temporarily controlled after being treated with childhood ALL induction and consolidation regimen therapy. This young female was alive and awaiting for allogeneic stem cell transplant at the time of reporting.<sup>[3]</sup> Successful haematological remission was achieved in the patient treated with hyper-CVAD (fractionated cyclophosphamide with vincristine, adriamycin and dexamethasone) protocol alternating with four cycles of high-dose methotrexate and cytarabine.<sup>[5]</sup> As seen in our case, her disease was temporarily controlled by standard risk ALL regimen chemotherapy. She also was temporarily able to achieve clinical and haematological remission. Unfortunately, while waiting for stem cell transplantation, she succumbed with disease relapse less than 6 months post chemotherapy.

Combination chemotherapy of cyclophosphamide, adriamycin, vincristine and prednisolone (CHOP) also have been used in treating this aggressive disease with variable outcome.<sup>[3, 4]</sup> Gentile *et al.*, reported successful control of disease in a patient treated with CHOP followed by high dose of oral cyclophosphamide.<sup>[3]</sup> This is conflicting with cases reported by Macon *et al.*, where a patient died within few months of his presentation.<sup>[4]</sup> Multiple combination chemotherapy, aggressive chemotherapy with combination of prednisolone with other chemotherapy also failed to control the disease.<sup>[4]</sup> Combination of chlorambucil and whole body irradiation have been tried to treat a patient who presented with generalized maculopapular rash, however this patient also succumbed one year after his presentation.<sup>[4]</sup>

The prognosis of this rare and aggressive disease is poor. As described in this case, recurrence of disease may

occur within few months even after achieving haematological remission<sup>[5]</sup>. There is no data supporting the need of prophylactic intrathecal chemotherapy to control the disease. Studies also show that a long-term remission rarely occurs.<sup>[3, 4, 5]</sup> Upfront autologous haematopoietic stem cell transplantation needs to be considered in managing this aggressive disease entity. Alekshun *et al.*, reported a patient who benefited by this treatment modality even after he had recurrence of leukaemic cells within the cerebral spinal fluid.<sup>[5]</sup> Literature search shows no data on the outcome of patients with aggressive NK-like leukaemia being treated with allogeneic haematopoietic stem cell transplantation.

### CONCLUSION

Awareness among clinician and laboratory haematologists on the existence of this aggressive entity of large granular lymphocytic leukaemia is important in ensuring urgent and intensive treatment can be promptly initiated to control the disease. ALL-like induction chemotherapy protocol followed by haemopoietic stem cell transplant may rescue this rare disorder.

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