A Challenging Case of Gamma Delta T-Cell Lymphoma with Precursor T-Cells and Marked Eosinophilia: A Case Report

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Abstract
Gamma-delta (γδ) T-cell lymphomas are very rare and aggressive neoplasms. We describe here a challenging case of γδ T-cell neoplasm composed of γδ mature T-cells and γδ precursor T-cells with marked eosinophilia that is inapplicable to the current 2016 World Health Organization (WHO) classification. A 3-year-old female child who was presented with fever and marked leucocytosis. Peripheral blood smear showed marked lymphocytosis, marked eosinophilia, neutrophilia, monocytes, and 5% circulating blasts. CT scan showed anterior mediastinal mass, lymphadenopathy, and hepatosplenomegaly. The patient underwent a bone marrow examination and a biopsy taken from the mediastinal mass. Peripheral blood and bone marrow findings were consistent with a γδ T-cell neoplasm with increased blasts and eosinophilia. The patient was sequentially treated with imatinib (tyrosine kinase inhibitor), acute lymphoblastic leukemia protocol (BFM 2009) then shifted to lymphoma protocol (LMP 96). In conclusion, we report a unique rare case of γδ T-cell neoplasm with a combination of mature and immature γδ T-cells and eosinophilia that is inapplicable to the current 2016 WHO classifications. This case raises a challenging concept of a mature T-cell lymphoma arising in an immature T-cell neoplasm. It also highlights the need to target all neoplastic components to eradicate the disease.

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Introduction

There are two classes of T-cells depending on the structure of the T-cell receptor (TCR), alpha-beta T-cells (αβ T-cells) and gamma delta T-cells (γδ T-cells). γδ T-cells are <5% of all normal T-cells, found mainly in the red pulp of the spleen and in epithelial sites including the intestinal epithelium [1]. Most cases of T-cell lymphoma develop from αβ T-cells. γδ T-cell lymphomas are aggressive and rare diseases with very unfortunate prognosis [2].

Based on location, mature γδ T-cell neoplasms can be classified into 3 groups: 1 – hepatosplenic: hepatosplenic γδ T-cell lymphoma (HSγδTL); 2 – cutaneous: peripheral cutaneous γδ T-cell lymphoma (PC γδ TCL); and 3 – nonhepatosplenic/noncutaneous γδ peripheral T-cell lymphomas (NHNC γδ PTCL).

NHNC γδ PTCL are a miscellaneous group of discrete T-cell lymphomas of which only sporadic cases have been documented [3]. The incidence of γδ T-cell acute lymphoblastic leukemia (ALL) is reported to comprise 9–12% of T-lymphoblastic leukemia/lymphoma [4]. Currently, we report an interesting challenging case of γδ T-cell neoplasm with a combination of a mature γδ T-cell lymphoma/leukemia associated with a γδ precursor T-cells and marked eosinophilia.

Case Report

A 3-year-old female child from Sri Lanka presented with a history of fever and cough for 10 days, initially treated with oral antibiotics with no improvement in symptoms. Complete blood count at presentation showed mild anemia (Hb 10 g/dL), mild thrombocytopenia (platelets 127 × 10³/μL), and very high white blood cell count (WBCs 133 × 10³/μL).

Laboratory workup including virology screen for HIV, HBV, HCV, EBV, varicella zoster, adenovirus was negative. Rotavirus and parasitic ova in stool were negative. CMV PCR was positive (1,506 IU/mL). The autoimmune screen including ANA and ANCA was negative. The patient had normal IgG, high IgM 225 mg/dL (48–168) and high total IgE 53 mg/dL (<23).

Computerized tomography (CT) scan showed an anterior mediastinal mass (7 × 6 × 2.6 cm) with axillary, right paratracheal, cervical, inguinal, and external iliac lymphadenopathy, and severe hepatosplenomegaly. Peripheral smear showed marked leukocytosis with marked lymphocytosis, the majority of lymphoid cells were small to medium looking lymphocytes with many atypical forms. There was also neutrophilic leukocytosis with left shift and dysplastic features in neutrophils (hyposegmentation and hypogranulation), marked eosinophilia with many immature forms (at myelocytes stage), and many atypical dysplastic forms (abnormal segmentation, hypogranulation, and vacuolization) in addition to monocytosis.

Fig. 1. Peripheral blood smear (composite) shows marked leukocytosis, marked lymphocytosis (majority are mature looking with many atypical forms), 5% blasts, dysgranulopoietic features (hyposegmentation and hypogranulation), marked eosinophilia with left shift & dysplastic features (hypo/non/abnormal segmentation and abnormal granulations) (Wright stain, ×100).
and 5% blasts. Differential count showed: neutrophils 9%, lymphocytes 57%, monocytes 4%, eosinophils 22%, metamyelocytes 1%, myelocytes 2%, and blasts 5% (Figure 1).

The patient underwent a bone marrow (BM) examination. BM aspirate showed a marked increase in eosinophils (23%) with left shift and atypical dysplastic forms, increased lymphocytes (46%) with many atypical forms, and 12% blasts (Figure 2). BM biopsy was hypercellular (100%cellularity) and showed extensive diffuse lymphoid infiltration with increased eosinophils (Figure 3). Immunohistochemistry (IHC) stains showed diffuse lymphoid infiltration positive for CD3, CD5, CD7, CD4, and CD8 with increased positivity for TdT and CD99 and decreased positivity for CD2 (partial loss of CD2) (Figure 4).

Flow cytometry immunophenotyping was performed on BM aspirate and showed an abnormal population of T-cells (41%), expressing γδ TCR. The T-cells included two subpopulations: one major subpopulation (30%), expressing CD45, sCD3, cCD3, CD5, CD7, TIA, and γδ TCR with a partial expression of CD8 and CD2 (partial loss of CD2) and one small subpopulation (7%) of precursor T-cells expressing CD45, sCD3, cCD3, CD5, CD7, γδ TCR, TdT, CD1a (partial) and showed double positivity for CD4 and CD8 (Figure 5). This small immature subpopulation was negative for CD34, CD10, CD56, CD57, CD16, CD25, CD33, CD117, MPO, and HLA-DR. The whole γδ T-cells were negative for CD10, CD56, CD57, CD25, CD34, CD1a, CD33, CD15, CD16, MPO, and CD117.

Fig. 2. Bone marrow aspirate shows 12% blasts (arrows), marked increase in eosinophils (23%), and increased lymphocytes (46%) with many atypical forms (Wright stain, ×100).

Fig. 3. Bone marrow biopsy is hypercellular (100%) showing diffuse lymphoid infiltration, many with irregular nuclear borders, many primitive-looking cells (open chromatin and prominent nucleoli) and prominent eosinophils (hematoxylin-eosin, ×50).
Cytogenetic FISH analysis for BCR-ABL1 and 4q12 (FIP1L1-PDGFRα), TRA/D, FGFR1 (8p11.2), ABL2, CHIC2, PDGFRB, and ABL1 was normal.

Chromosomal analysis showed abnormal clone (13/60 cells) with a complex karyotype and multiple structural abnormalities including the deletion of 5q.

Karyotype: (46,X,?del(X)(p21),der(5)t(5;15)(q22;q15),?inv(8)(p23q13),der(10)add(10)(p13)del(10)(q24),del(11)(q21q22),add(13)(q34),del(15)(q21)13]/46,XX[47]).

The molecular analysis for TCR gene rearrangement demonstrated a clonal population (a partial T-beta (D-J) rearrangement and T-gamma rearrangements). Molecular analysis for JAK2 mutation was negative.

The child had thoracoscopic excisional mediastinal lymph node biopsy which showed effacement of architecture by the diffuse lymphoid proliferation of small to medium-sized pleomorphic lymphoid cells with irregular nuclei, clumped chromatin, inconspicuous nucleoli, and rare mitoses. There were many eosinophils and scattered larger lymphoid cells (Figure 6A). An expanded IHC panel was done. The atypical lymphoid cells were positive for CD3 and CD5 with markedly reduced expression of CD2, CD4, and CD8 and showed patchy TdT positivity. Ki-67 showed >85% proliferation rate (Figure 6B).

Flow cytometry immunophenotyping performed on mediastinal lymph node showed abnormal γδ T-cell population (59%) and (8%) γδ T-cell precursors.

**Fig. 4.** IHC stains show diffuse infiltration by CD3-positive cells (A; ×10) and diffuse CD99 positivity and increased TdT positivity (B; ×40).
The overall findings were indicative of a T-cell neoplasm; however, with the presence of these three components of mature and precursor T-cells and dysplastic eosinophilia, reaching a precise diagnosis was very challenging. All the pathological materials were then sent abroad for an expert second opinion, and the feedback came confirming lymph node, peripheral blood, and bone marrow involvement by a T-cell neoplasm, best interpreted as involvement by T-lymphoblastic leukemia/lymphoma.

The patient received first intrathecal chemotherapy and started initially on imatinib awaiting further genetic and molecular results and continued on Imatinib for another 20 days. There was a significant reduction in WBC count down to $30 \times 10^3/\mu$L, and the patient was clinically stable. A follow-up BM examination was performed on day 8 after the first presentation for further evaluation before starting on BFM protocol which showed an increase in blast count up to 19%. Flow cytometry performed on this BM aspirate showed persistence of the abnormal mature $\gamma\delta$ T-cell population (48%) with (8%) $\gamma\delta$ T-cell precursors. Subsequently, the child was started on BFM 2009 Protocol for T-cell lymphoblastic lymphoma/

**Fig. 5.** Flow cytometry immunophenotyping performed on the bone marrow aspirate dot blot shows an abnormal population of T-cells comprising $\sim 41\%$ (blue colored), expressing CD45, sCD3, cCD3, CD5, and TCR $\gamma\delta$ with a partial expression of CD2 and CD8. There is another small population (7%) (red color) of precursor T-cells expressing sCD3, cCD3, CD5, TCR $\gamma\delta$, TdT, partial CD1a and show double positivity for CD4 &CD8.
leukemia. She continued to have fluctuated persistent high WBC count between (20–30 × 10^3/μL) predominately lymphocytes.

BM evaluation on day 15 was hypercellular with 2% blasts and many atypical mature lymphoid cells. Flow cytometry showed the persistence of the abnormal mature γδ T-cells (71%). CT scan showed a significant reduction of lymph nodes, mediastinal mass, and spleen size.

BM evaluation on day 33 was hypocellular with 2% blasts and many atypical mature lymphoid cells. Flow cytometry showed persistence of the abnormal mature γδ T-cells (38%) and did not reveal any lymphoblastic population. CT scan showed no significant change in a lymph node or spleen size.

As the patient was still having the persistent disease with the abnormal mature γδ T-cell population, the decision was taken to shift to LMP 96 Lymphoma Protocol and was treated according to group C treatment plan to target the mature T-cell population.

After starting the lymphoma protocol, follow-up BM evaluation showed a dropping of the abnormal mature γδ T-cell population down to 3% and did not reveal any lymphoblastic population.

Minimal residual disease after starting lymphoma protocol diagnostic was sent abroad in the context of lymphoma/leukemia therapy, and the patient showed residual tumor load (leukemia), at time point 1 (before starting BFM2009), at time point 2 (day 15), at time point 3 (day 33), and at time point 4 (after starting lymphoma protocol).

The patient’s course was complicated with many chemotherapy-related complications including febrile neutropenia, fungal infection, right internal jugular vein thrombosis, electrolyte disturbance, mucositis, and recurrent vomiting and diarrhea. The child's parents decided not to continue treatment (after 2 courses of lymphoma protocol) and took the child back to the home country to plan for stem cell transplantation. We lost the child to follow-up after traveling and later found out that she passed away before having hematopoietic stem cell transplantation.
Discussion

The 2008 World Health Organization (WHO) classification acknowledged two major groups of γδ T-cell lymphoma: Hepatosplenic γδ T-cell lymphoma (HSγδTL) and Primary cutaneous gamma-delta TCL (PCγδTCL) [5]. The 2016 WHO classification of γδ T-cell lymphoma combined a few more subcategories: (1) Hepatosplenic γδ T-cell lymphoma (HSγδTL); (2) Primary cutaneous gamma-delta TCL (PCγδTCL); (3) Monomorphic epitheliotropic intestinal TCL (MEITL); (4) Gamma-delta large granular lymphocytic leukemias (γδT-LGL) [1].

HSγδTL is defined in young men exposed to chronic immunosuppressants. Splenomegaly and hepatomegaly are the most common findings and there is no lymphadenopathy [6, 7].

The mucocutaneous γδ TCLs can be classified into those localized to the bowel, respiratory tract, thyroid, and nasal cavity and those involving the skin [8].

PCγδTCL lymphoma constitutes <1% of all primary cutaneous lymphomas, occurring in adults with cutaneous manifestations [9, 10].

γδ T-LGL is a very rare indolent disease of patients presenting with splenomegaly and lymphocytosis with mature lymphocytes having a particular morphology (large lymphocytes with abundant cytoplasm and conspicuous azurophilic granules) in both peripheral blood and bone marrow with rare lymphadenopathy [11].

T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL) is rare and infrequently shows T-cell differentiation or TCR expression [12]. γδ T-cell lymphoblastic leukemia is rare representing a small percentage of T-ALL cases [4, 13].

γδ T-cell neoplasms including HSTL, PCγδTCL, γδ T-LGL, and γδ T-ALL/LBL subtypes differ in their presentation, clinical course, response to treatment, and prognosis [13].

Although the WHO classification classifies and describes lymphoma subtypes, the literature contains some case reports of rare gamma-delta T-cell lymphoma variants that are still hard to classify. Consequently, clinical suspicion and diagnosis are usually deferred, and the treatment remains inconsistent.

The initial differential diagnosis of our case included myeloid/lymphoid neoplasms associated with eosinophilia and rearrangements of PDGFRA, PDGFRB or FGFR1, γδ T-lymphoblastic leukemia/lymphoma, and HSγδ TL.

Based on the laboratory and pathologic data including BM, peripheral blood, and mediastinal biopsy findings, our patient was diagnosed as a case of γδ T-cell lymphoma/leukemia with eosinophilia. Nevertheless, her clinical findings do not match any precise subtype of the current 2016 WHO classification, as she presented with lymphadenopathy, hepatosplenomegaly, mediastinal mass with no cutaneous lesions. Also, she presented in the leukemic phase with an immature blastic component in addition to the presence of marked eosinophilia. Moreover, our patient is a 3-year-old who does not fit the age range of the γδ T-cell lymphoma subtypes and does not have a prior history of the immunocompromised state.

The case reported hereby is unique, and no such case has been reported in the literature to the best of our knowledge. This case showed three abnormal components at diagnosis: a large population of mature γδ T-cells, a minor population of immature γδ T-cells (T-lymphoblasts), and marked eosinophilia.

This case also raises a challenging concept of a mature T-cell lymphoma arising in an immature T-cell neoplasm in a subset of lymphomas. It is known that the development of T-cells starts in the thymus from early precursors, the pro-T cell when they still have the potential to differentiate into other lineages like NK cells and myeloid cells. Subsequently, after TCR rearrangement, the precursor thymocyte becomes designated to the T-cell lineage and ends its maturation in the thymus to give rise to mature αβ T-cells and γδ T-cells before
migrating to the periphery [14]. One study published in 2015 indicated that T-cell lymphoma, in particular anaplastic large cell lymphoma, may originate in immature T-cell precursors in the thymus [15].

In our patient, it seems that the three components including immature T-cells, mature T-cells, and eosinophils originated from a neoplastic early precursor cell that can give rise to different cell types. This is important in order to identify effective treatment and prevent relapse by eradicating all lymphoma cells including the neoplastic immature precursor cells.

Traditionally, γδ T-ALL/LBL has been treated like B lymphoblastic lymphoma/leukemia (B-ALL). Nevertheless, the appropriate induction regimen and consolidation policy for this exclusive entity remain unidentified. Despite aggressive therapy for patients diagnosed with this unique disease, the prognosis is poor and long-term survival is hardly achieved [13].

Our patient had a partial response on imatinib alone which may suggest undiscovered molecular abnormalities causing clonal eosinophilia that respond to tyrosine kinase inhibitors. She did not achieve complete remission response to the aggressive acute leukemia therapy and continued to show persistent mature γδ T-cell lymphoma component and started to achieve a good response when she was shifted to the lymphoma protocol to target the mature component. Although the cytogenetic/molecular testing was negative for clonal eosinophilia, the partial response to imatinib raises an assumption that the eosinophilia may be clonal and associated with the γδ T-cell neoplasm originating from any of the abnormalities enclosed within the complex karyotype of the patient or undiscovered molecular abnormality not included yet within the known genetic causes for clonal eosinophilia. Whether this case represents two separate diseases or differentiation of a malignant clone secondary to an acquisition of a secondary genetic mutation requires further molecular studies on the two cell clones which unfortunately is unavailable in our center.

**Conclusion**

We present an exceptional case of a γδ T-cell neoplasm with 3 abnormal components at diagnosis including a large population of mature γδ T-cells, a small population of γδ T-lymphoblasts, and marked eosinophilia in a 3-year-old child. To the best of our knowledge, this is the first described case in the literature with such findings. This case does not fit into any of the γδ T-cell neoplasms of the current WHO classification. The presence of a combination of mature γδ T-cells with immature γδ T-cell precursors at diagnosis raises a question about the relationship between those two components. Are the mature and immature neoplastic T-cells arising from a common precursor or represent two different neoplastic clones causing two different neoplasms at the same time?

Are the mature lymphoma cells evolving to immature cells (lymphoblastic transformation) or the neoplastic lymphoblasts are differentiating to mature T-cells?

In addition, the response to imatinib in this patient raises a question whether the eosinophilia, in this case, is part of the neoplastic clone. Further studies are needed to answer those questions and to better understand, diagnose, classify, and manage those rare unclassifiable γδ T-cell neoplasms.

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Statement of Ethics

The authors have no ethical conflicts to disclose. The case report was conducted ethically in accordance with the guideline of the Medical Research Center (MRC) of Hamad Medical Corporation (HMC), and after obtaining their approval. Written informed consent was obtained from the legal guardians of the patient (parents) for publication of this case report and any accompanying images.

Conflict of Interest Statement

The authors have no conflicts of interest to declare. The case report was operated ethically in agreement with the guideline of the Medical Research Center (MRC) of Hamad Medical Corporation (HMC), and after getting their approval.

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Author Contributions

Dr. Samah Kohla did the literature review and wrote the hematopathology part of the manuscript. Dr. Ahmad Al-Sabbagh and Dr. Feryal Ibrahim reviewed the hematology part of the manuscript. Dr. Ilham Bilal wrote the clinical part of the manuscript. Dr. Einas AL-Kuwari wrote and reviewed the anatomic pathology part of the manuscript.

References

1. Swerdlow SH, Campo E, Pileri SA, Lee Harris N, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127(20):2375–90.
2. Sindhu H, Chen R, Chen H, Wong J, Chaudhry R, Xu Y, et al. Gamma-delta (γδ) T-cell lymphoma—another case unclassifiable by World Health Organization classification: a case report. J Med Case Rep. 2017 Jun 19;11(1):163.
3. Markow M, Mirza A-S, Perez L, Shao H, Horna P, Anasetti C, et al. Transformation of T-cell acute lymphoblastic lymphoma to peripheral T-cell lymphoma: a report of two cases. Case Rep Hematol. 2018;2018:1–11.
4. Foppoli M, Ferreri AJ. Gamma-delta T-cell lymphomas. Eur J Haematol. 2015 Mar;94(3):206–18.
5. Wei EX, Leventaki V, Choi JK, Raimondi SC, Azzato EM, Shurtleff SA, et al. γδ T-cell acute lymphoblastic leukemia/lymphoma: discussion of two pediatric cases and its distinction from other mature γδ T-cell malignancies. Case Rep Hematol. 2017;2017:1–7.
6. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, Fourth Edition - WHO - OMS - [Internet]. [cited 2019 Dec 23]. Available from: https://apps.who.int/bookorders/anglais/detart1.jsp?codlan=1&codcol=70&codch=4002
7. Ioannito E, Tripodo C: How I diagnose and treat splenic lymphomas. Blood. 2011 Mar 3;117(9):2585–95. doi: 10.1182/blood-2010-09-271437.
8. Petri HT, Soollay R, Shortman K. Commitment to the T cell receptor-αβ or -γδ lineages can occur just prior to the onset of CD4 and CD8 expression among immature thymocytes. Eur J Immunol. 1992 Aug;22(8):2185–8.
9. Endly DC, Weenig RH, Peters MS, Viswanatha DS, Comfere NI. Indolent course of cutaneous gamma-delta T-cell lymphoma. J Cutan Pathol. 2013 Jan;40(10):896.
10. Berti E, Cerri A, Cavicchini S, Delia D, Soligo D, Alessi E, et al. Primary cutaneous gamma/delta T-cell lymphoma presenting as disseminated pagetoid reticulosis. J Invest Dermatol. 1991 May;96(5):718–23.
11. Chen YH, Chadburn A, Evens AM, Winter JN, Gordon L, Chenn A, et al. Clinical, morphologic, immunophenotypic, and molecular cytogenetic assessment of CD4-/CD8-γδ T-cell large granular lymphocytic leukemia. Am J Clin Pathol. 2011;136(2):289–99.
Matos DM, Rizzatti EG, Fernandes M, Buccheri V, Falcão RP. Gammadelta and alphabeta T-cell acute lymphoblastic leukemia: comparison of their clinical and immunophenotypic features. *Haematologica*. 2005;90(2):264–6.

Paluri R, Donnellan W, Mineishi S, Wicker J. Gamma-delta T cell acute lymphoblastic leukemia: a single-center experience. *Glob J Cancer Ther*. 2016 Dec 29;2(1):026–9.

Bhandoola A, Sambandam A. From stem cell to T cell: one route or many? *Nat Rev Immunol*. 200;6(2):117–26.

Moti N, Malcolm T, Hamoudi R, Mian S, Garland G, Hook CE, et al. Anaplastic large cell lymphoma-propagating cells are detectable by side population analysis and possess an expression profile reflective of a primitive origin. *Oncogene*. 2014 May 12;34(14):1843–52.