



## RESEARCH ARTICLE OPEN ACCESS

# Lymphocytic Variant Hypereosinophilic Syndrome: Case Series From a Tertiary Referral Center in Canada

Xiu Qing Wang<sup>1</sup>  | Kevin Shopsowitz<sup>1</sup> | Jack Lofroth<sup>2</sup> | Xuehai Wang<sup>1</sup> | Erica Peterson<sup>3</sup> | Andrew P. Weng<sup>1,4</sup> | Luke Y. C. Chen<sup>3,5</sup> 

<sup>1</sup>Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada | <sup>2</sup>Faculty of Medicine, University of British Columbia, Vancouver, British Columbia, Canada | <sup>3</sup>Division of Hematology, Dalhousie University, Halifax, Nova Scotia, Canada | <sup>4</sup>Terry Fox Laboratory, BC Cancer Agency, Vancouver, British Columbia, Canada | <sup>5</sup>Division of Hematology, University of British Columbia, Vancouver, British Columbia, Canada

**Correspondence:** Luke Y. C. Chen ([lchen2@bccancer.bc.ca](mailto:lchen2@bccancer.bc.ca))

**Received:** 14 January 2025 | **Accepted:** 21 January 2025

**Funding:** Luke Chen's research is funded by a philanthropic gift from the Hsu & Taylor Family through the VGH & UBC Hospital Foundation.

**Keywords:** eosinophilia | hypereosinophilic syndrome | lymphocyte immunophenotyping | lymphocytic hypereosinophilic syndrome | T-cell receptor gene rearrangement

## ABSTRACT

**Background:** Lymphocytic variant hypereosinophilic syndrome (L-HES) is a rare disorder characterized by persistent eosinophilia driven by aberrant T-cell populations. Diagnosis remains challenging due to the lack of standardized diagnostic criteria.

**Methods:** We retrospectively analyzed 18 patients diagnosed with L-HES between 2016 and 2023. Comprehensive flow cytometry was performed on peripheral blood samples.

**Results:** Nine patients demonstrated the classic sCD3<sup>+</sup>CD4<sup>+</sup>CD5<sup>+</sup>CD2<sup>+</sup>CD45RO<sup>+</sup>CD45RA<sup>+</sup> immunophenotype, ranging from 0.6% to 70% of total lymphocytes. Two patients showed variant sCD3<sup>+</sup>CD4<sup>+</sup> phenotypes, five had expanded (> 10%) sCD3<sup>+</sup>CD4<sup>+</sup>CD7<sup>+</sup> T-cells, and two displayed aberrant CD8<sup>+</sup> T-LGL populations. Clonality was established in all patients with nonclassic phenotypes by molecular TCR testing or based on uniform TRBC1. We assessed a serial gating strategy to quantify the classic L-HES phenotype and found this to be highly sensitive and specific with an estimated limit of detection of 0.06% of lymphocytes. Using this strategy, we identified decreased but detectable abnormal T-cells in all classic phenotype patients reassessed posttreatment, down to as low as 0.3% of lymphocytes. The identification of T-LGL phenotypes with eosinophilia is a novel finding.

**Conclusion:** Our study highlights the diverse immunophenotypic spectrum of L-HES, emphasizing the importance of comprehensive flow cytometry analysis for accurate diagnosis.

## 1 | Introduction

Hypereosinophilic syndrome (HES) is characterized by persistent peripheral blood eosinophilia associated with end organ damage ( $>1.5 \times 10^9$  /L) [1–3]. Lymphocytic hypereosinophilic

syndrome (L-HES) is a rare subtype of HES characterized by an aberrant T-cell population with gene rearrangements which lead to the overproduction of Th2 cytokines, most commonly Interleukin (IL)-4, IL-5, and IL-13 [4, 5]. In L-HES, the eosinophils are not clonal, but are reactive to eosinopoietic

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2025 The Author(s). eJHaem published by British Society for Haematology and John Wiley & Sons Ltd.

cytokines and chemokines produced by these clonal T-cells.

L-HES presents with a wide range of clinical manifestations, reflecting the involvement of multiple organ systems. These include cutaneous, soft tissue, rheumatological, gastrointestinal, and respiratory symptoms, all of which accompany end organ damage [6]. L-HES is difficult to distinguish from other causes of reactive eosinophilia including vasculitides, autoimmune disease, medications, parasitic infections, and IgG4-related disease [7, 8]. Accurate diagnosis is important for therapy, as patients with L-HES are less responsive to steroids, but more responsive to interferon alpha [9, 10]. JAK inhibitors and IL-5 inhibition are also emerging therapies [11, 12].

There are currently no standardized protocols or criteria for determining what constitutes an L-HES defining aberrancy. The general approach in most centers is to identify and quantify T-cell populations with abnormal marker expression patterns. sCD3<sup>+</sup>CD4<sup>+</sup> T-cells are the most common/specific abnormal T-cell population described in the literature for L-HES [13–15]. A recent study by Carpentier et al. reported that sCD3<sup>+</sup>CD4<sup>+</sup> T-cells in L-HES had other consistent immunophenotypic features, namely bright CD5, bright CD2, and homogeneous CD45RO and CD95 [16, 17]. Other aberrant T-cell immunophenotypes that have been reported in the literature for L-HES include sCD3<sup>+</sup>CD4<sup>+</sup>CD7<sup>+</sup> and sCD3<sup>+</sup>CD4<sup>+</sup>CD8<sup>+</sup> TCR $\alpha\beta$ <sup>+</sup>; however, the sensitivities and specificities of these populations with respect to L-HES is not well established [4, 16, 18]. Notably, these two immunophenotypes are seen as minor T-cell populations in a variety of reactive states where they are often not clonal. It is therefore critical to demonstrate clonality, for example, based on molecular TCR studies or TRBC1 expression [19]. However, even then careful correlation with other clinical and laboratory findings is required [20, 21].

Here we report on a new cohort of 18 patients diagnosed with L-HES at our center. We found half of our patients have a sCD3<sup>+</sup>CD4<sup>+</sup> T-cell population with a similar immunophenotype compared to what has recently been described in the literature. We show that this specific aberrant T-cell population can persist and be detected at low levels after treatment. Importantly we also report on patients without classic sCD3<sup>+</sup>CD4<sup>+</sup> T-cells, but who have other features that support a diagnosis of L-HES. Comparisons between the two groups based on detailed clinical and laboratory characterization are provided, expanding the knowledge base of this rare and diagnostically challenging disease.

## 2 | Methods

At our tertiary referral center, we retrospectively analyzed 18 patients with persistent unexplained eosinophilia who were diagnosed with L-HES between 2016 and 2023. As a control, we also analyzed peripheral blood from patients without eosinophilia found to be negative for a lymphoproliferative disorder by flow cytometry run for indications other than eosinophilia ( $n = 19$ ). The study was approved by the University of British Columbia Clinical Research Ethics Board (H23-03816) as a minimal risk study and did not require patient consent. All patient data were de-identified and unique patient

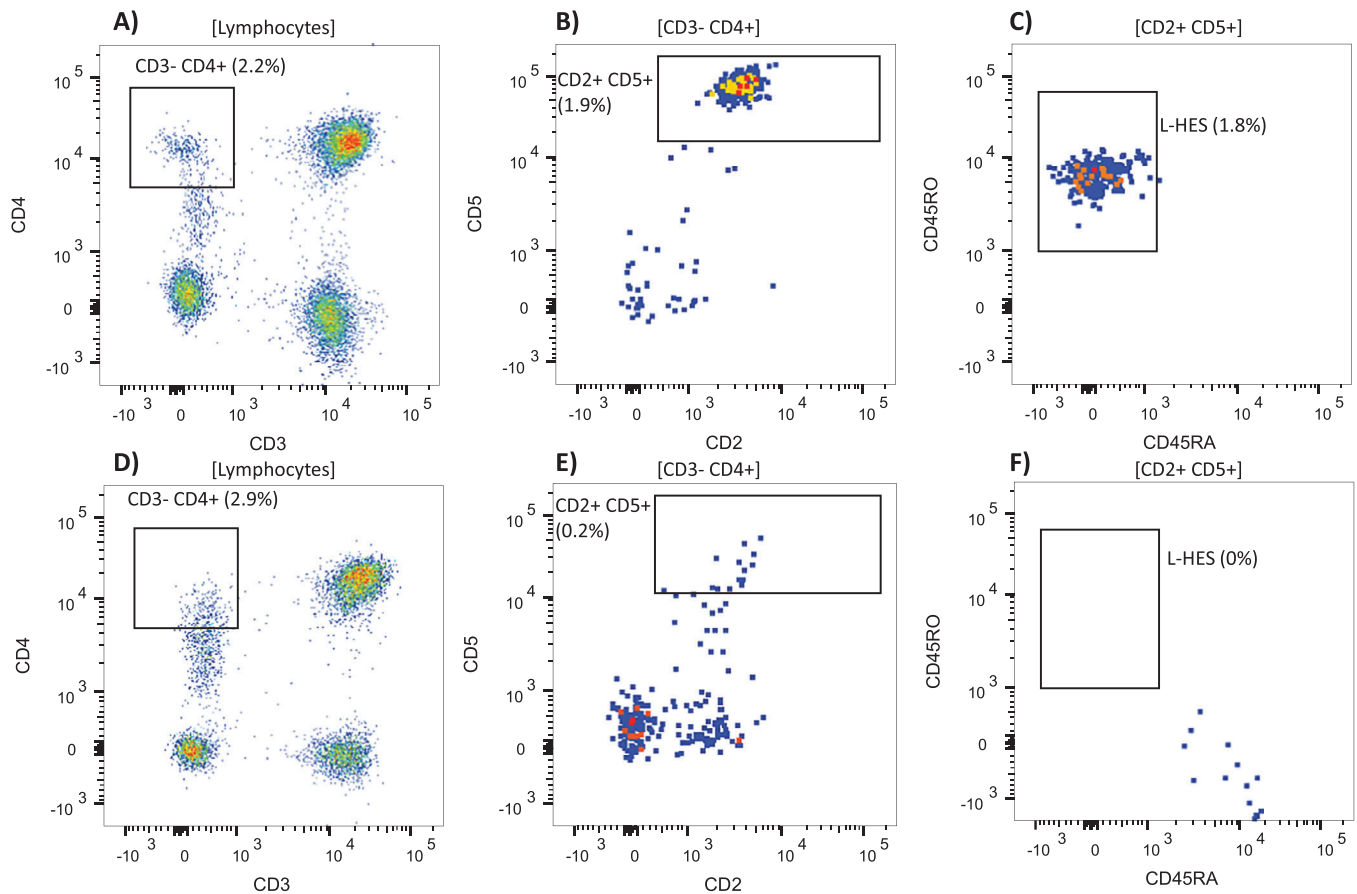
numbers generated for data analysis. All patients were referred to hematology with clinical symptoms suspicious for L-HES, as well as evaluation to exclude reactive and other causes of eosinophilia. Clinical and laboratory data were reviewed.

Flow cytometry was performed on peripheral blood as part of routine clinical evaluation. Lymphocyte populations were analyzed using a three-tube diagnostic panel, with one 13-color tube dedicated to T-cell and NK-cell-associated antibodies: CD2, sCD3, CD4, CD5, CD7, CD8, CD14, CD45, CD45RA, CD45RO, CD56, CD57, and CD279. Samples run after 2021 had an additional color for the marker TRBC1. The remaining two tubes focused on evaluation of B-cell populations; the data are not presented here as the objective of the current study is to quantitate abnormal T cell populations. Data were acquired on a BD FACS Canto II or BD LSRFortessa flow cytometer (Becton Dickinson; San Jose, California), and FCS data files were initially analyzed using BD FACSDiva, Kaluza, FlowJo v10, or a custom multidimensional clinical analysis software. Total lymphocytes were gated on using CD45 versus side scatter followed by a CD14 gate to exclude monocytes and sCD3<sup>+</sup>CD4<sup>+</sup>, sCD3<sup>+</sup>CD4<sup>+</sup>CD7<sup>+</sup>, and sCD3<sup>+</sup>CD4<sup>+</sup>CD8<sup>+</sup> subsets were initially characterized and quantified as part of routine clinical workup. A serial gating strategy to quantify the specific sCD3<sup>+</sup>CD4<sup>+</sup>CD5<sup>+</sup>CD2<sup>+</sup>CD45RO<sup>+</sup>CD45RA<sup>+</sup> L-HES phenotype was developed and retrospectively applied to L-HES patients at multiple time points in order to trend the clone size (minimum cluster size of 10 events to be considered positive, see Figure 1). The same gating strategy was applied to normal controls to assess limit of detection, which was also assessed for other selected phenotypes. The median fluorescence intensities (MFI) for selected markers were compared between the aberrant sCD3<sup>+</sup>CD4<sup>+</sup> populations and normal sCD3<sup>+</sup>CD4<sup>+</sup> T-cells from the same samples (22 samples from seven patients with raw data available for analysis).

The presence of a T-cell receptor (TCR) clonal rearrangement was determined using the BIOMED-2 guideline [22].

## 3 | Results

Clinical and laboratory parameters for the 18 L-HES patients in our cohort are shown in Table 1. The cohort consisted of 11 males and 7 females, with ages at diagnosis ranging from 22 to 91 years. Presenting symptoms varied widely among patients, with the most common being skin-related issues such as pruritus, eczema, and rash. Notably, three patients were asymptomatic at the time of diagnosis. Initial peripheral blood eosinophil counts ranged from 1.7 to  $88 \times 10^9/L$ , with the majority of patients having counts below  $5 \times 10^9/L$ . Initial lymphocyte counts were generally within the normal range ( $0.4\text{--}4.6 \times 10^9/L$ ), with only two patients showing elevated levels. C-reactive protein (CRP) levels varied widely (from  $< 0.2$  to 75.6 mg/L), with several patients exhibiting elevated levels indicative of ongoing inflammation. Serum protein electrophoresis (SPEP) results were predominantly normal, though some patients displayed polyclonal increases in the gamma region, and one patient showed a monoclonal band consistent with MGUS. Notably, immunoglobulin E (IgE) levels ranged from 26 to 45,900 IU/mL, with many patients demonstrating significantly elevated levels. Treatment strategies varied among patients, with most receiving



**FIGURE 1** | Gating strategy to identify classic sCD3<sup>-</sup>CD4<sup>+</sup> L-HES populations. Lymphocytes were initially gated on (not shown) followed by serial gates to identify L-HES cells with the following phenotype: sCD3<sup>-</sup>CD4<sup>+</sup>CD5<sup>+</sup>CD2<sup>+</sup>CD45RO<sup>+</sup>CD45RA<sup>-</sup>. (A–C) L-HES gating for a representative positive case. (D–F) Gating for a representative negative example. Percentages are based on total lymphocytes.

prednisone, pegylated interferon, or a combination of both. Some patients required alternative or additional therapies such as hydroxyurea or imatinib, while a few remained untreated. Only one patient did not respond to treatment (L4).

There were nine patients with an aberrant sCD3<sup>-</sup>CD4<sup>+</sup>CD5<sup>+</sup> (typically bright) CD2<sup>+</sup>CD45RO<sup>+</sup>CD45RA<sup>-</sup> population, which we refer to as the classic phenotype, ranging from 0.6% to 70% of total lymphocytes at diagnosis (Figure 2A–C and Table 2). In addition, there were two patients with variant sCD3<sup>-</sup>CD4<sup>+</sup> immunophenotypes showing dim to negative CD5 and dim or variable CD45RO expression (both  $\geq 5\%$  of lymphocytes), five patients with  $> 10\%$  sCD3<sup>+</sup>CD4<sup>+</sup>CD7<sup>-</sup> T-cells, and two patients with aberrant CD8<sup>+</sup> T-LGL populations with restricted TRBC1 (Table 2). There was corroborating evidence of T-cell clonality by PCR in all but two patients. One had the classic sCD3<sup>-</sup>CD4<sup>+</sup> phenotype (L4) and one with a T-LGL clone (L18). L18 had inconclusive clonality by PCR but showed homogenous TRBC1 expression by flow cytometry. The size of the T-cell clone did not show significant correlation with the eosinophil count at initial diagnosis (Pearson  $r = -0.17$ ;  $p = 0.5$ ).

Comparing the nine patients with the classic L-HES phenotype to those with the nine patients with other T-cell phenotypes. The difference in age between the two groups is statistically significant ( $p < 0.05$ ). Patients with the other phenotype tended to be older than

those with the classic phenotype: the mean age for patients with the classic phenotype is 47.9 years versus 70.3 years for patients with other phenotypes. Comparing the initial peripheral blood eosinophil count using the Mann–Whitney U test, there was no significant difference in eosinophil counts between the two groups. There was no difference in treatment response between the classic and other phenotype groups nor differences in the presence of autoimmune comorbidities between the two groups.

Based on 22 samples measured at various time points from patients with the classic sCD3<sup>-</sup>CD4<sup>+</sup> phenotype, the CD5 MFI was on average 3.4 $\times$  brighter compared to sCD3<sup>+</sup>CD4<sup>+</sup> T-cells from the same samples (95% CI = 3.0 to 3.7), CD2 was on average 1.3 $\times$  brighter (95% CI 1.3 to 1.4), while CD4 was similar between the sCD3<sup>-</sup> and sCD3<sup>+</sup> subsets (mean 1.1, 95% CI 1.0 to 1.1). In addition, CD7 was completely or partially negative in all patients with the classic phenotype. In all samples collected after initial diagnosis (14 samples from 6 patients), a persistent population with the same aberrant phenotype was detectable, often at low levels ( $< 1\%$  in six samples with 0.3% being the lowest level detected) using a standardized gating strategy (Figure 1). Five of these patients had repeat flow cytometry at a time point after receiving treatment, which showed persistence of aberrant T-cells with the same phenotype present at diagnosis at decreased or stable levels in all cases (Figure 2D–F), coinciding with decreases in the eosinophil count (Table 3). As an example, patient L5 showed a decrease in

TABLE 1 | Clinical and laboratory characteristics of 18 patients with lymphocytic variant hypereosinophilic syndrome (L-HES).

Study ID	Gender	Age at diagnosis (years)	Presenting symptom	Eosinophil (giga/L)	Flow	TCR PCR Clonality	Treatments received	Lymph (giga/L)	CRP (mg/L)	SPEP	IgE (µg/L)
L1	M	38	Mild pruritis	2.3	Classic	T-cell clonal	None	1.6	0.7	Normal	449
L2	F	36	Eczema and cervical lymph-adenopathy	2.2	Classic	T-cell clonal	Prednisone followed by PEG INF	2.3	< 0.2	Polyclonal increase in gamma region	305
L3	F	67	Papular eczema	3.3	Classic	T-cell clonal	Prednisone	1.4	1	0.3 g/l IgG lambda MGUS	5300
L4	F	48	Ischemic stroke and fatigue	1.8	Classic	Negative	no response to prednisone or PEG INF	1.1	0.5	Normal	620
L5	M	54	Urticaria	88	Classic	T-cell query clonal	Prednisone followed by PEG INF; Hydroxyurea	0.95	5.8	Normal	197
L6	M	56	No symptoms	25	Classic	T-cell clonal	Prednisone followed by PEG INF	2.1	6.4	Normal	81
L7	M	22	Episodic angioedema	28.8	Classic	T cell clonal	PEG INF	1.3	< 0.3	Normal	26
L8	F	62	Prurigo nodularis	10	Classic	T-cell clonal	PEG INF	4.6	8.7	Normal	5532
L9	F	30	Pneumonitis	1.7	Classic	T-cell clonal	Prednisone	1.9	< 0.3	Normal	45,900
L10	M	60	Urtacaria rash	2	Variant classic	T cell query clonal	Prednisone followed by PEG INF; Imatinib	1.1	0.6	Normal	5153
L11	M	90	Puritic rash	3.8	Variant classic	T cell clonal	Prednisone	1.1	22	Polyclonal increase in gamma region	40,030
L12	M	59	Severe chronic eczema	2.00	Non classic	T-cell clonal	Prednisone	0.9	3.9	Normal	6978
L13	M	91	Extensive erythroderma, progressive renal insufficiency	2.2	Non classic	T-cell clonal	PEG INF	0.5	15.6	Normal	978
L14	F	57	Eosinophilic myocarditis	10.2	Non classic	T cell clonal	Prednisone followed by PEG INF	2.2	24.6	Borderline polyclonal increase in gamma region	128
L15	F	81	No symptoms	2.4	Non classic	T-cell clonal	None	0.4	75.6	Irregular gamma region with inflammatory changes	1367

(Continues)

TABLE 1 | (Continued)

Study ID	Gender	Age at diagnosis (years)	Presenting symptom	Eosinophil (giga/L)	Flow	TCR PCR Clonality	Treatments received	Lymph (giga/L)	CRP (mg/L)	SPEP	IgE (μg/L)
<b>L16</b>	M	70	Pulmonary eosinophilia	2.6	Non classic	T-cell query clonal	Prednisone	1	0.6	Normal	<b>952</b>
<b>L17</b>	M	73	No symptoms	1.9	T-LGL	T-cell clonal	Prednisone followed by PEG INF	3.3	1.5	Normal	61
<b>L18</b>	M	52	Drenching night sweats	16.9	T-LGL	Inconclusive	Prednisone	2.8	<b>7.8</b>	Polyclonal increase in gamma region	54

Note: Bolded values are above the reference range; italicized values are below the reference range.  
Abbreviation: PEG INF: pegylated interferon.

classic sCD3<sup>+</sup>CD4<sup>+</sup> T-cells from 7.2% to 0.3%, with a concurrent decrease in eosinophils from  $88 \times 10^9$  to  $0.1 \times 10^9$ /L. To further validate the significance of such a low level of aberrant T-cells with the classic phenotype, we looked at 19 normal control blood samples run with the same panel/serial gating strategy. This gave an average of 0.014% of lymphocytes residing within the final gate (SD = 0.014%, theoretical limit of detection = 0.06%), with none of the control samples showing a cluster of  $\geq 10$  events with the classic L-HES phenotype. In addition, no clusters of  $\geq 10$  events with the classic phenotype were found in patients identified as having nonclassic phenotypes (10 samples from 8 patients with data available for re-analysis). By contrast, the average percent in the control samples of total sCD3<sup>+</sup>CD4<sup>+</sup> lymphocytes was 1.1% (SD = 0.9%) and sCD3<sup>+</sup>CD4<sup>+</sup>CD7<sup>+</sup> lymphocytes was 3.5% (SD = 1.9%).

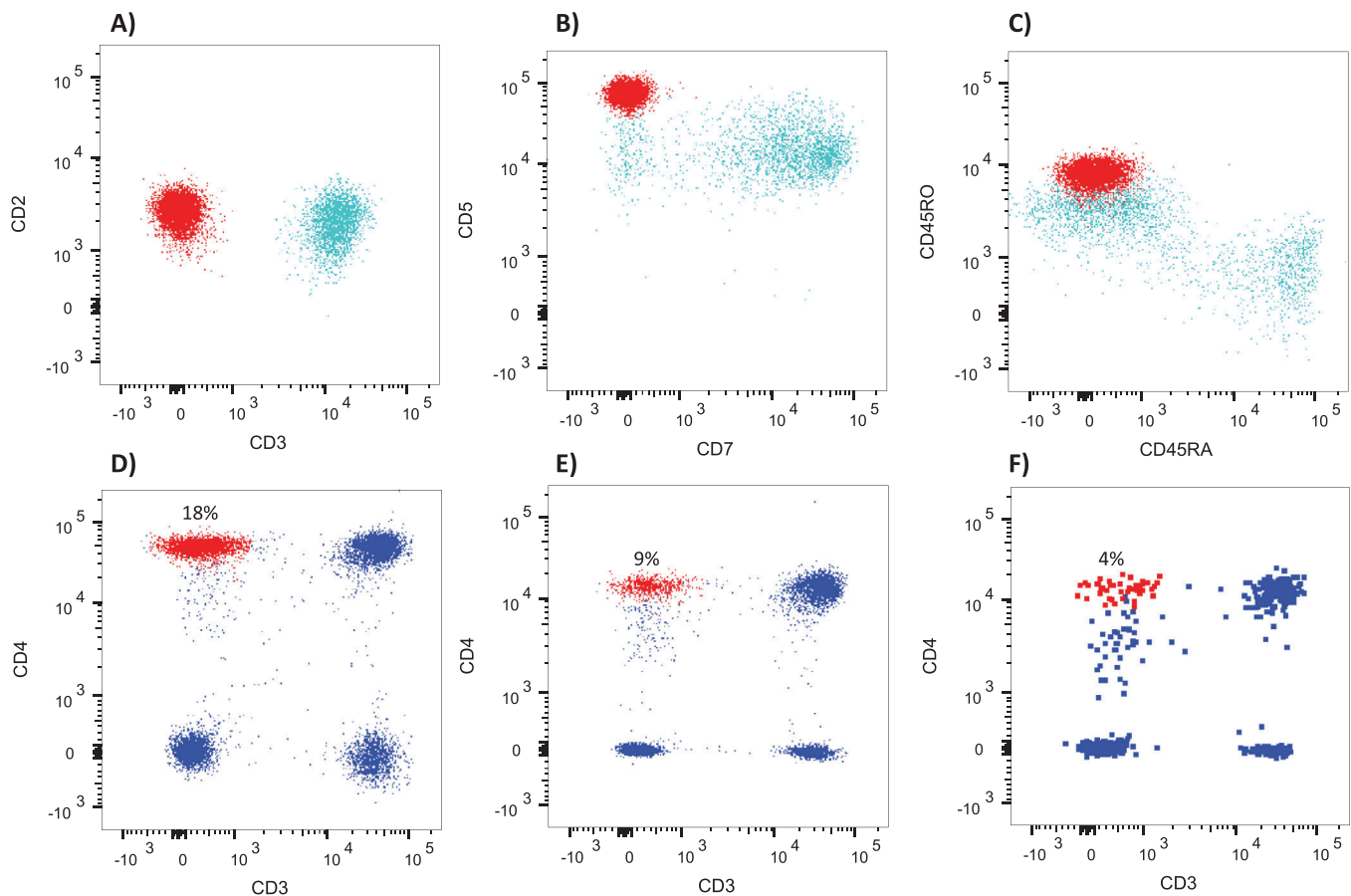
#### 4 | Discussion

Our study highlights the critical role of flow cytometry in the diagnosis of lymphocytic variant hypereosinophilic syndrome (L-HES) yet shows these patients may display a range of immunophenotypes. The classic immunophenotype of sCD3<sup>+</sup>CD4<sup>+</sup>CD5<sup>+</sup>(bright)CD2<sup>+</sup>CD45RO<sup>+</sup>CD45RA<sup>+</sup> emerged as the most common and specific marker for L-HES, consistent with previous findings in the literature [15–18, 23, 24]. We found similar aberrancies as described previously, including bright CD5 and CD2, alongside loss of CD7 [4, 16, 18]. This distinct phenotype was not observed in any of our control samples, highlighting its specificity for L-HES.

In our cohort, half of the patients displayed this classical immunophenotype. We detected populations as small as 0.6% of total lymphocytes at initial diagnosis, which still proved to be clinically relevant. Interestingly, these abnormal populations persisted, albeit for most patients at decreased levels, even after treatment. Given the high specificity of this classic phenotype, we propose that even small populations of cells with this immunophenotype should be considered significant. This underscores the importance of acquiring sufficient events and using appropriate sequential gating strategies to detect these often small abnormal populations with a minimal marker combination including CD45, sCD3, CD4, CD2, CD5, CD45RO, and CD45RA (CD95 has been reported as another marker expressed in classic L-HES but was not included in our panel). For laboratories without dedicated L-HES panels, a serial gating strategy such as the one presented here could be incorporated into existing T-cell panels to ensure these populations are not overlooked. A standalone panel design could also be considered, which may not require as many fluorochromes as a comprehensive lymphoma diagnosis panel. One patient in our cohort with the classic phenotype had negative clonality studies (L4). Interestingly, patient L4 was the only patient in our cohort to not respond to treatment.

While the classic phenotype demonstrated high specificity, our study also identified nonclassic abnormal populations, with sCD3<sup>+</sup>CD4<sup>+</sup>CD7<sup>+</sup> T-cells being the most prevalent. However, these cells are not exclusive to pathological conditions; they can be found in healthy individuals and their frequency tends to increase with age. Moreover, elevated levels of these cells have been observed in a variety of noneosinophilic disorders, particularly those involving chronic inflammation or autoimmune pro-





**FIGURE 2** | (A–C) Immunophenotypic characterization of a representative case of  $sCD3^+CD4^+$  L-HES (red events; CD4 not shown) with slightly bright CD2, bright CD5, loss of CD7, and bright/restricted CD45RO compared to normal  $sCD3^+CD4^+$  T-cells from the same patient (turquoise events). (D–F) Monitoring of a  $sCD3^+CD4^+$  L-HES population (red events) in one patient (L6) over the course of treatment that was identified by the gating strategy shown in Figure 1. Percentages are based on total lymphocytes (blue events = all other lymphocytes).

cesses. Interestingly, there was no significant difference between autoimmune comorbidities when comparing the classic phenotype group to the nonclassic group, however, there was a notable difference in age with the latter group being significantly older.

Distinguishing between a normal variant and a truly abnormal population of  $sCD3^+CD4^+CD7^-$  T-cells often requires additional context and supporting evidence. In our cohort, we had corroborating evidence that expanded  $sCD3^+CD4^+CD7^-$  T-cells were significant based on positive molecular clonality studies and the lack of detectable T-cells with the classic  $sCD3^+CD4^+$  phenotype using a sensitive flow cytometry assay. Based on our findings and the inherent variability of this cell population, we propose a threshold of  $\sim 10\%$  of total lymphocytes for considering a  $sCD3^+CD4^+CD7^-$  population as potentially significant in the L-HES context, although this should likely be validated individual institutions since there could be variability due to patient population differences and technical factors. Incorporating new markers such as TRBC1/TRBC2 to directly assess the clonality of  $sCD3^+CD4^+CD7^-$  T-cell populations identified by flow cytometry could potentially improve specificity, but this requires further study, as the cases we had in our cohort preceded having TRBC1 in our flow cytometry panel. At this time, we strongly emphasize that this phenotype should never be interpreted in isolation: clinicians should always consider it in conjunction with the

patient's clinical presentation, other laboratory findings, and molecular evidence of T-cell clonality.

A novel finding in our study was the identification of patients with aberrant T-large granular lymphocyte (T-LGL) populations. Specifically, we observed T-LGL phenotypes in two patients who presented with clinical features of L-HES. This observation warrants careful consideration in the context of L-HES diagnosis and management. T-LGL leukemia is typically characterized by the clonal expansion of cytotoxic T cells with a  $sCD3^+CD8^+CD57^+$  phenotype, although  $CD4^+$  variants have been reported. The co-occurrence of T-LGL phenotypes with hypereosinophilia presents a diagnostic challenge, as it blurs the lines between distinct hematological entities. Among our two patients, L17's bone marrow showed mild eosinophilia and normal trilineage hematopoiesis. He received first line oral cortical steroids. Clinically, when he was weaned from his steroids, he had rebound eosinophilia and suspected end organ damage in the form of cardiomyopathy. He was subsequently restarted on steroids, followed by second line interferon therapy and responded well. L18's bone marrow showed marked eosinophilia with focal fibrosis with no evidence of *KIT* mutation. L18 only received first line oral cortical steroid therapy and responded well. Both patients had normal karyotypes and negative FISH for *PDGFRA*, *PDGFRB*, *FGFR1*, and *JAK2*. Neither patient had intrasinusoidal infiltrates and

TABLE 2 | Immunophenotypic characteristics of T-cell subsets in 18 L-HES patients at diagnosis.

Study ID	Flow phenotype	Abnormal population immunophenotype	Clone size (% of lymphocytes)	TCR PCR Clonality
L1	Classic	sCD3-CD4+CD5+CD2+CD7-CD45RO+	1.8%	T-cell clonal
L2	Classic	sCD3-CD4+CD5+CD2+CD7-CD45RO+	58.0%	T-cell clonal
L3	Classic	sCD3-CD4+CD5+CD2+CD7-CD45RO+	34.0%	T-cell clonal
L4	Classic	sCD3-CD4+CD5+CD2+CD7-CD45RO+	10.0%	non clonal
L5	Classic	sCD3-CD4+CD5+CD2+CD7-CD45RO+	7.2%	T-cell query clonal
L6	Classic	sCD3-CD4+CD5+CD2+CD7-CD45RO+	15.4%	T-cell clonal
L7	Classic	sCD3-CD4+CD5+CD2+CD7-CD45RO+	0.6%	T-cell clonal
L8	Classic	sCD3-CD4+CD5+CD2+CD7-CD45RO+	70.0%	T-cell clonal
L9	Classic	sCD3-CD4+CD5+CD2+CD7-CD45RO+	1.2%	T-cell clonal
L10	Variant classic	sCD3-CD4+CD5dimCD2-CD7- TRBC1-heterogeneous CD45RA/CD45RO expression	9.4%	T cell query clonal
L11	Variant classic	sCD3-CD4+CD5-CD2+CD7-CD45RO+	5.0%	T cell clonal
L12	Nonclassic	sCD3+CD4+CD7-	11.4%	T-cell clonal
L13	Nonclassic	sCD3+CD4+CD7-	25.7%	T-cell clonal
L14	Nonclassic	sCD3+CD4+CD7-	14.4%	T cell clonal
L15	Nonclassic	sCD3+CD4+CD7-	13.5%	T-cell clonal
L16	Nonclassic	sCD3+CD4+CD7-	10.8%	T-cell query clonal
L17	T-LGL	CD8+CD5-CD56+CD57+ uniform TRBC1+	22.0%	T-cell clonal
L18	T-LGL	CD8+CD5-CD7-CD56+CD57+ uniform TRBC1+	4.8%	inconclusive

TABLE 3 | Comparison of pretreatment and posttreatment data for patients with the classic sCD3<sup>+</sup>CD4<sup>+</sup> T-cell phenotype.

Study ID	Treatment	Pretreatment CD3-CD4+ % of total lymph	Posttreatment CD3-CD4+ % of total lymph	Pretreatment eosinphil count	Posttreatment eosinophil count	T-cell clonality
L2	PrednisonePEG INF	58	1.8	2.2	0	Clonal
L5	Prednisone PEG INF, hydroxyurea	7.2	0.3	88	0.1	Query clonal
L6	Prednisone PEG INF	18	4.1	25	1.2	Clonal
L7	PEG INF	0.6	0.6	28.8	0.3	Clonal
L8	PrednisonePEG INF	70	12	10	0.3	Clonal

Abbreviation: PEG INF: pegalyated interferon.

their clones persisted over time without lymphadenopathy. Future studies should investigate the functional role of T-LGL in hypereosinophilia, particularly their potential cytokine-driven contribution, as seen in sCD3<sup>+</sup>CD4<sup>+</sup> L-HES.

Our findings both support and extend previous research on L-HES. Similar to the studies by Lefevre et al. and Carpentier et al., we observed the classic sCD3<sup>+</sup>CD4<sup>+</sup>CD5<sup>+</sup>CD2<sup>+</sup>CD45RO<sup>+</sup>CD45RA<sup>+</sup> immunophenotype in a significant proportion of L-HES patients [15–17, 23]. We demonstrated that this phenotype can be reliably detected at very low levels in L-HES patients due to its high specificity. Uniquely, our study examined flow cytometry results for patients

with L-HES posttreatment, revealing the persistence of aberrant T-cell populations at decreased or stable levels. This aspect, not addressed in previous studies, provides valuable insights into the durability of these cell populations. Future work could examine whether continued trending of these small residual populations could have clinical value as a form of measurable residual disease testing. Our case series also contained other phenotypes reported in literature for L-HES, including increased sCD3<sup>+</sup>CD4<sup>+</sup>CD7<sup>+</sup> T-cells in patients without an identifiable classic L-HES population. Notably, our identification of T-LGL phenotypes in some L-HES patients represents a novel finding not reported in either previous study. Overall, our study provides a comprehensive perspective on L-HES, highlighting the importance of thorough flow

cytometry analysis both at diagnosis and during follow-up. Our findings suggest that the immunophenotypic landscape of L-HES may be more diverse and persistent than previously recognized. We propose that incorporation of targeted gating strategies or dedicated panels for diagnosis of L-HES could improve detection rates and disease monitoring in clinical practice.

## Acknowledgments

We gratefully acknowledge Dr. Florence Roufosse for her helpful comments and insights for this manuscript. Luke Chen's research is supported by a philanthropic gift from the Hsu & Taylor family through the VGH & UBC Hospital Foundation.

## Conflicts of Interest

L.Y.C. Chen has received consulting fees from Recordati Rare Diseases and from Glaxo-Smith-Kline. None of the other authors have any declarations.

## Data Availability Statement

Data are available upon reasonable request to the corresponding author.

## References

1. P. Valent, A. D. Klion, H. P. Horny, et al., "Contemporary Consensus Proposal on Criteria and Classification of Eosinophilic Disorders and Related Syndromes," *Journal of Allergy and Clinical Immunology* 130 (2012): 607–612.e9.
2. W. Shomali and J. Gotlib, "World Health Organization and International Consensus Classification of Eosinophilic Disorders: 2024 Update on Diagnosis, Risk Stratification, and Management," *American Journal of Hematology* 99 (2024): 946–968.
3. Y. Shi and C. Wang, "What We Have Learned About Lymphocytic Variant Hypereosinophilic Syndrome: A Systematic Literature Review," *Clinical Immunology* 237 (2022): 108982.
4. P. Khoury, "Lymphocytic-Variant Hypereosinophilic Syndromes," *Hematologist* 14, no. 6 (2017).
5. A. K. Williams, C. Dou, and L. Y. C. Chen, "Treatment of Lymphocyte-Variant Hypereosinophilic Syndrome (L-HES): What to Consider After Confirming the Elusive Diagnosis," *British Journal of Haematology* 195 (2021): 669–680.
6. R. L. King, B. Tan, F. E. Craig, et al., "Reactive Eosinophil Proliferations in Tissue and the Lymphocytic Variant of Hypereosinophilic Syndrome," *American Journal of Clinical Pathology* 155 (2021): 211–238.
7. D. Moller, J. Tan, D. T. V. Gauiran, et al., "Causes of Hypereosinophilia in 100 Consecutive Patients," *European Journal of Haematology* 105 (2020): 292–301.
8. M. N. Carruthers, S. Park, G. W. Slack, et al., "IgG4-Related Disease and Lymphocyte-Variant Hypereosinophilic Syndrome: A Comparative Case Series," *European Journal of Haematology* 98 (2017): 378–387.
9. P. Khoury, A. O. Abiodun, N. Holland-Thomas, M. P. Fay, and A. D. Klion, "Hypereosinophilic Syndrome Subtype Predicts Responsiveness to Glucocorticoids," *Journal of Allergy and Clinical Immunology* 6 (2018): 190–195.
10. C. Choi, D. Moller, J. Tan, et al., "Pegylated Interferon Alpha 2a Is an Effective and Well-tolerated Treatment Option for Lymphocyte-Variant Hypereosinophilic Syndrome," *British Journal of Haematology* 188 (2020): e68–e72.
11. S. Faguer, M. Groh, F. Vergez, et al., "JAK Inhibition for CD3-CD4+ Lymphocytic-Variant Hypereosinophilic Syndrome," *Clinical Immunology* 251 (2023): 109275.
12. F. Roufosse, J. E. Kahn, M. E. Rothenberg, et al., "Efficacy and Safety of mepolizumab in Hypereosinophilic Syndrome: A Phase III, Randomized, Placebo-Controlled Trial," *Journal of Allergy and Clinical Immunology* 146 (2020): 1397–1405.
13. H. U. Simon, S. G. Plotz, R. Dummer, and K. Blaser, "Abnormal Clones of T Cells Producing Interleukin-5 in Idiopathic Eosinophilia," *New England Journal of Medicine* 341 (1999): 1112–1120.
14. F. Roufosse, E. Cogan, and M. Goldman, "Lymphocytic Variant Hypereosinophilic Syndromes," *Immunology and Allergy Clinics of North America* 27 (2007): 389–413.
15. G. Lefevre, M. C. Copin, C. Roumier, et al., "CD3-CD4+ Lymphoid Variant of Hypereosinophilic Syndrome: Nodal and Extranodal Histopathological and Immunophenotypic Features of a Peripheral Indolent Clonal T-Cell Lymphoproliferative Disorder," *Haematologica* 100 (2015): 1086–1095.
16. C. Carpentier, L. Schandene, L. Dewispelaere, P. Heimann, E. Cogan, and F. Roufosse, "CD3-CD4+ Lymphocytic Variant Hypereosinophilic Syndrome: Diagnostic Tools Revisited," *The Journal of Allergy and Clinical Immunology: In Practice*, 9 (2021).
17. C. Carpentier, S. Verbanck, L. Schandene, et al., "Eosinophilia Associated with CD3-CD4+ T Cells: Characterization and Outcome of a Single-Center Cohort of 26 Patients," *Frontiers in Immunology* 11 (2020): 1765.
18. Z. Hu, W. Wang, B. Thakral, et al., "Lymphocytic Variant of Hypereosinophilic Syndrome: A Report of Seven Cases From a Single Institution," *Cytometry Part B, Clinical Cytometry* 100 (2021): 352–360.
19. N. D. Novikov, G. K. Griffin, G. Dudley, et al., "Utility of a Simple and Robust Flow Cytometry Assay for Rapid Clonality Testing in Mature Peripheral T-Cell Lymphomas," *American Journal of Clinical Pathology* 151 (2019): 494–503.
20. J. E. Sims, A. Tunnacliffe, W. J. Smith, and T. H. Rabbitts, "Complexity of Human T-Cell Antigen Receptor Beta-Chain Constant- and Variable-Region Genes," *Nature* 312 (1984): 541–545.
21. A. Tunnacliffe, R. Kefford, C. Milstein, A. Forster, and T. H. Rabbitts, "Sequence and Evolution of the human T-Cell Antigen Receptor Beta-Chain Genes," *Proceedings of the National Academy of Sciences of the United States of America* 82 (1985): 5068–5072.
22. A. W. Langerak, P. J. Groenen, M. Brüggemann, et al., "EuroClonality/BIOMED-2 Guidelines for Interpretation and Reporting of Ig/TCR Clonality Testing in Suspected Lymphoproliferations," *Leukemia* 26 (2012): 2159–2171.
23. G. Lefevre, M. C. Copin, D. Staumont-Salle, et al., "The Lymphoid Variant of Hypereosinophilic Syndrome: Study of 21 Patients With CD3-CD4+ Aberrant T-Cell Phenotype," *Medicine* 93 (2014): 255–266.
24. M. Shi, K. L. Rech, G. E. Otteson, et al., "Prevalence and Spectrum of T-Cell Lymphoproliferative Disorders in Patients With Hypereosinophilia: A Reference Laboratory Experience," *Annals of Diagnostic Pathology* 44 (2020): 151412.