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**Title:** IgG4-related disease and lymphocyte-variant hypereosinophilic syndrome: A comparative case series

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**ABSTRACT**

**Objective:** To compare the clinical and laboratory features of IgG4-related disease (IgG4-RD) and lymphocyte variant-hypereosinophilic syndrome (L-HES), two rare diseases that often present with lymphadenopathy, gastrointestinal symptoms, eosinophilia and elevated immunoglobulins/IgE.

**Method:** Comparative case series of 31 IgG4-RD and 13 L-HES patients.

**Results:** Peripheral blood eosinophilia was present in 8/31 IgG4-RD patients compared to 13/13 L-HES patients (median eosinophils 0.4 giga/L vs. 7.0 giga/L,  $P = 0.001$ ) and 12/20 patients with IgG4-RD had increased serum IgE compared to 8/13 L-HES patients,  $P = 0.930$ . Twenty-seven of 30 IgG4-RD patients had elevated serum IgG4 compared to 5/12 L-HES patients (median IgG4 9.6 g/L vs. 0.80 g/L,  $P = 0.002$ ). Flow cytometry demonstrated an aberrant T-cell phenotype in 7/23 IgG4-RD patients and 13/13 L-HES patients ( $P < 0.001$ ). T-cell clonality by PCR was positive in 12/23 IgG4-RD patients vs. 10/13 L-HES patients ( $P = 0.143$ ). Patients in both groups received corticosteroids as first-line therapy. For refractory disease in IgG4-RD, rituximab was the most common steroid-sparing agent, whereas in L-HES it was pegylated interferon- $\alpha$ -2a.

**Conclusion:** The overlapping features of these two diseases with divergent treatment options demonstrate the importance of familiarity with both entities in order to optimize diagnosis and treatment.

**Keywords:** IgG4, IgG4-related disease, eosinophil; hypereosinophilic syndrome, hypergammaglobulinemia

## Introduction

A major challenge in evaluating patients with eosinophilia is that many diseases associated with secondary, or reactive, eosinophilia, are themselves difficult to diagnose. Immunoglobulin G4-related disease (IgG4-RD) is an emerging condition associated with reactive eosinophilia in one third of patients.<sup>1</sup> Common clinical manifestations include dacryoadenitis, sialadenitis, lymphadenopathy, autoimmune pancreatitis, and retroperitoneal fibrosis, although nearly any organ except joints and brain parenchyma can be involved. Forty percent of patients have associated asthma or atopy, often with elevated IgE, although the eosinophilia and increased IgE appears inherent to the IgG4-RD rather than atopic disease.<sup>1,2</sup> Most patients have elevated serum IgG4 often accompanied by elevated total IgG and polyclonal hypergammaglobulinemia on serum protein electrophoresis.<sup>3</sup> Oligoclonal expansion of CD4<sup>+</sup> T effector memory (T<sub>EM</sub>) lymphocytes has been detected using next generation sequencing and is thought to drive the pathophysiology, and in particular, the fibrosis seen in this disease.<sup>4</sup> Despite the protean clinical manifestations, histopathologic features from nearly any organ are similar, and diagnosis of IgG4-RD requires histologic findings including a polyclonal lymphoplasmacytic infiltrate, storiform fibrosis, obliterative phlebitis and increased IgG4+ plasma cells.<sup>3</sup> Treatments for IgG4-RD include glucocorticoids and rituximab.<sup>5</sup>

Hypereosinophilic syndromes (HES) are rare conditions characterized by chronic hypereosinophilia (eosinophils  $\geq 1.5 \times 10^9/L$ ) causing organ damage.<sup>6</sup> Lymphocyte-variant HES (L-HES) is a unique subtype of HES characterized by an immunophenotypically

aberrant T-cell population, often with clonal T-cell receptor gene rearrangements associated with excessive Th2 cytokine production, such as interleukin (IL)-5, IL-4 and IL-13. L-HES typically affects the skin, lungs, and digestive tract, and many patients have asthma and atopic disease.<sup>7</sup> Laboratory features, in addition to the eosinophilia and aberrant T-cell population, include elevated IgE, polyclonal hypergammaglobulinemia and/or small monoclonal bands on serum protein electrophoresis in some patients. The most well-described immunophenotype is CD3<sup>-</sup>CD4<sup>+</sup>, followed by CD3<sup>+</sup>TCR<sup>α/β+</sup>CD4<sup>-</sup>CD8<sup>-</sup> and CD3<sup>+</sup>CD4<sup>+</sup>CD7<sup>-</sup>.<sup>8-10</sup> Like IgG4-RD, L-HES is commonly treated initially with glucocorticoids.<sup>7</sup> The natural history is generally indolent, but some patients may develop aggressive T-cell lymphoma.<sup>11</sup>

The gold standard for diagnosis of IgG4-RD is histopathology, in keeping with the International Consensus Criteria, with corresponding clinical, laboratory and radiological features.<sup>12</sup> Diagnostic criteria for L-HES are not as strictly defined as for IgG4-RD, nor are there a widely accepted set of consensus criteria, but usually involve a combination of clinical findings, hypereosinophilia, immunophenotyping, and T-cell clonality studies.<sup>13</sup> Given that IgG4-RD and L-HES present with overlapping clinical and laboratory features such as asthma, atopy, lymphadenopathy, eosinophilia and elevated serum immunoglobulins, differentiating between these two rare entities is an underappreciated diagnostic challenge.<sup>14,15</sup> We previously published the case of a young woman with polyclonal hyperviscosity syndrome and eosinophilia labeled as idiopathic HES, a diagnosis confirmed prior to publication by international experts in eosinophilia (case I3 in the present study);<sup>16</sup> however, 2 years after publication, she was found to have histologically confirmed IgG4-RD.<sup>17</sup> Given the potential diagnostic confusion between these two entities, and the lack of large studies comparing them, we conducted a comparative case series describing the clinical and laboratory features of patients with IgG4-RD and L-HES. This is the first study to report

T-cell immunophenotyping and T-cell clonality testing in a large number of IgG4-RD patients and the first report of serum IgG4 levels in a large number of L-HES patients.

## Materials and methods

Consecutive patients were retrospectively identified from the practices of author MNC (IgG4-RD) or LYC (IgG4-RD and L-HES) Jul 2008 to Jun 2015. All patient data were de-identified and unique patient numbers generated for data analysis. 44 patients in total were included in this study: 31 with IgG4-RD and 13 with L-HES. All patients with IgG4-RD had histologic confirmation by an expert pathologist (BFS, DFS, GWS, MS) according to the International Consensus Criteria.<sup>12</sup> Total IgG (Siemens©, Tarrytown, USA) and IgG subclasses (IgG1, IgG2, IgG3, IgG4; Binding Site©, Birmingham, UK) were measured by nephelometry, total IgE by chemiluminescent immunoassay (Siemens Vista©). Patients with L-HES had clinical features in keeping with L-HES, exclusion of relevant clonal and reactive causes of eosinophilia, and aberrant T-cell immunophenotype (increased CD3<sup>-</sup>CD4<sup>+</sup>, CD3<sup>+</sup>CD4<sup>-</sup>CD8<sup>-</sup> or CD3<sup>+</sup>CD4<sup>+</sup>CD7<sup>-</sup>) or T cell clonality by PCR.<sup>9</sup> Reference ranges for the T-cell subsets as a percentage of total lymphocytes were established in-house for investigational purposes: CD3<sup>-</sup>CD4<sup>+</sup> 0-2%; CD3<sup>+</sup>CD4<sup>-</sup>CD8<sup>-</sup> 2-15%; CD3<sup>+</sup>CD4<sup>+</sup>CD7<sup>-</sup> 2-10%. Of note, for the “double negative” CD3<sup>+</sup>CD4<sup>-</sup>CD8<sup>-</sup> phenotype, the TCR<sup>αβ</sup> are considered the pathogenic subtype, our flow cytometry panel did not distinguish between membrane expression of TCR<sup>αβ</sup> and TCR<sup>γδ</sup> T lymphocytes. All patients with L-HES were reviewed clinically (by LYC) and immunophenotyping of peripheral blood and/or bone marrow was reviewed (by BID). The presence of a T-cell receptor (TCR) clonal rearrangement was determined using the BIOMED-2 guidelines (see Appendix 1 for primers used). Immunophenotype was considered more important for diagnosis of L-HES than PCR. Clinical data including presenting symptoms and physical exam findings, laboratory results,

pathology and radiology reports, treatment, and outcomes were collected. Subclinical disease with objective radiologic and/or histologic findings were included as organ involvement; for example, radiographic evidence of lymphadenopathy thought to be due to IgG4-RD or L-HES, or typical computed tomography findings of IgG4-related tubulointerstitial nephritis in patients with preserved renal function.

All statistical tests were performed using SPSS software (version 13.0, SPSS Inc., Chicago, IL). Group medians were compared using the Mann-Whitney U test (two independent samples, two tailed) and proportions were compared using the chi-square test. A P value of  $<0.05$  was considered significant for all tests. The study was approved by the University of British Columbia Clinical Research Ethics Board (H15-01433) as a minimal risk study and did not require patient consent.

## **Results**

### **Patients**

Clinical data for IgG4-RD and L-HES patients are shown in Table I. The median age at disease onset (years) was 56 (interquartile range, IQR, 47-64) for IgG4-RD and 53 (IQR 32-68) for L-HES. The median lag time between initial symptoms and diagnosis was 62 months (IQR 16-125) for IgG4-RD and 31 months (IQR 11-59) for L-HES. Most patients with long lag times between initial symptoms and diagnosis presented prior to 2011; in our center, IgG4-RD became widely recognized in 2011, and flow cytometry examining specifically for the aberrant T-cell subsets in L-HES also became available in 2008 (as a modification of a pre-existing lymphoproliferative disorder panel and analytic algorithm). With a median follow-up of 16 months (IQR 9-22) in the IgG4-RD group and 19 months (IQR 11-55) in the L-HES group, four patients died: one with IgG4-RD from cardiovascular

disease, one with IgG4-RD from ischemic bowel, one with L-HES from sepsis, and one with L-HES from intracranial hemorrhage.

### **Organ involvement**

Patients with IgG4-RD had more organs involved than those with L-HES (median 5 vs. 3,  $P = 0.029$ ). There were no differences in constitutional symptoms including weight loss, fevers, night sweats and fatigue, although two patients with IgG4-RD had polyclonal hypergammaglobulinemic hyperviscosity syndrome. The most commonly involved organs in IgG4-RD were the submandibular glands, lymph nodes, and lacrimal glands, all of which were more frequently affected than in L-HES patients (Table I). In L-HES patients, the most commonly symptomatic organs were skin, lungs, lymph nodes, and gastrointestinal tract; salivary and lacrimal glands were not a concern.

### **Histopathologic Features**

Both IgG4-RD and L-HES can have prominent eosinophilic infiltrates in affected tissues.<sup>5</sup> The patients with IgG4-RD had more biopsies of a greater variety of organs, including lacrimal and salivary glands, lymph nodes, bone marrow, skin, digestive tract, pancreas, retroperitoneal fibrosis, and other sites. This reflects the multi-organ involvement of IgG4-RD, the difficulty in arriving at a diagnosis prior to widespread recognition of IgG4-RD, and the importance of histologic confirmation of IgG4-RD. Figure 1 shows a typical lymph node specimen and Figure 2 shows a rare case with bone marrow involvement of IgG4-RD. In contrast, L-HES patients rarely had biopsies other than bone marrow biopsies; a few had skin, lymph node biopsies or gastric/colon biopsies. Hypereosinophilic syndromes often involve the digestive tract, and three L-HES patients in this study had prominent gastrointestinal symptoms, but none had histologically confirmed gastrointestinal

involvement; this may be due in part to patients having received corticosteroids before undergoing endoscopy and biopsy or due to other causes of such as reflux. Nine patients with IgG4-RD had bone marrow biopsies, of which only two had disease involvement with lymphoplasmacytic infiltrate, eosinophilia and increased IgG4+ plasma cells; of note, obliterative phlebitis and storiform fibrosis (not to be confused with myelofibrosis) are not seen in bone marrow specimens and lymph node specimens. All ten of the L-HES patients who had bone marrow biopsies had marrow involvement with eosinophilia (Figure 2).

### Laboratory findings

Laboratory findings are summarized in Table II and Figure 4. Elevated (pre-treatment) serum IgG4 levels were found in 27/30 (90%) IgG4-RD patients tested and 5/12 (42%) L-HES patients tested,  $P = 0.001$  (median 9.6 g/L, IQR 3.1-17.1 vs. 0.8 g/L, IQR 0.30-1.9,  $P = 0.002$ ; Figure 5). One IgG4-RD patient and three L-HES patients died or were lost to follow-up before having serum IgG4 level tested. Polyclonal hypergammaglobulinemia was found in 19/30 (63%) IgG4-RD patients who had a serum protein electrophoresis and 3/13 (23%) of L-HES patients,  $P = 0.015$  (median gamma peak 17.8 g/L, IQR 13.6-30.2 vs. 11.3 g/L, IQR 9.7-12.1,  $P = 0.018$ ). Monoclonal bands on SPEP were seen in 3/13 (23%) of L-HES patients and in none of the IgG4-RD patients ( $P = 0.006$ ). Eight of 31 (26%) IgG4-RD patients had peripheral blood eosinophilia compared to 13/13 (100%) L-HES patients,  $P < 0.001$  (median 0.4 giga/L, IQR 0.2-0.8 vs. 7.0 giga/L, IQR 2.9-17.9,  $P = 0.001$ ; Figure 5). One IgG4-RD patient had severe eosinophilia  $> 5.0$  giga/L, three had moderate eosinophilia 1.5-5 giga/L, and the rest were mild 0.7-1.5 giga/L.

Four patients in the IgG4-related disease group had increased CD4<sup>+</sup>7<sup>-</sup> T cells and two patients had increased CD3<sup>-</sup>4<sup>+</sup> and one patient had CD3<sup>+</sup>4<sup>-</sup>8<sup>-</sup> T cells (Table III). The remainder of the IgG4-RD patients that were tested had normal T-cell immunophenotyping.



Out of patients with an aberrant T cell phenotype, 3 patients also had T cell clonality by PCR; in total, twelve IgG4-RD patients had clonal T-cell receptor gene rearrangements by PCR: eleven in blood or bone marrow and one on a lacrimal gland specimen. All thirteen L-HES patients had abnormal flow cytometry results, including increased CD3<sup>+</sup>4<sup>-</sup>8<sup>-</sup> (n=3), CD3<sup>-</sup>4<sup>+</sup> (n=5), CD4<sup>+</sup>7<sup>-</sup> (n=5). Ten of 13 L-HES patients had clonal T-cell receptor rearrangements by PCR. All L-HES patients were negative for PDGFR $\alpha/\beta$  rearrangements and those tested for BCR/ABL rearrangements (n=9) were negative. Patients with overlapping laboratory features, i.e. IgG4-RD patients with eosinophilia, aberrant T-cell immunophenotype, or T-cell clonality, and L-HES patients with elevated serum IgG4, are summarized in Table III.

## **Treatment**

Twenty-three of 31 patients with IgG4-RD received prednisone and/or rituximab, and seven had not yet received systemic therapy. Four patients were stable and asymptomatic on no medications. Three patients were considering rituximab (n=2) or prednisone (n=1) at the time of the study but had not yet started treatment. Ten of 13 patients with L-HES received prednisone and/or pegylated interferon- $\alpha$ -2a (Appendix 3; see Appendix 4 for summary of treatment of individual patients). Of the three L-HES patients that were not treated, two patients were minimally symptomatic and one patient declined steroids and interferon.

## **Discussion**

This study is the first direct comparison of IgG4-RD and L-HES, rare diseases with significant overlapping characteristics. Both typically present in middle aged and older adults with a slight male preponderance. The variation in clinical manifestations in the IgG4-RD patients reflects the kaleidoscopic nature of the disease, whereas L-HES generally affects fewer target organs (Table I). Patients with IgG4-RD typically present with tumefactive mass

lesions in one or more organs (often leading to suspicion of malignancy), while L-HES patients more often present with skin rash, gastrointestinal symptoms, or reactive airways.

However, some patients may present with features that are not immediately indicative of IgG4-RD or L-HES, particularly early in the course of their illness, as both of these diseases may have a subacute presentation marked by atopy, eosinophilia, and other features that commonly overlap these conditions. Non-invasive testing such as flow cytometry, T-cell clonality by PCR, and serum protein studies including IgG subclasses, are typically the first investigations ordered in such patients. Many frail or elderly patients may not be amenable to the invasive biopsies required to make a histologic diagnosis of IgG4-RD. This study describes for the first time the important overlapping features between IgG4-RD and L-HES clinicians must be aware of in order to make a precise diagnosis.

Seven IgG4-RD patients had an aberrant T-cell immunophenotype, and twelve had T-cell clonality by PCR. Four of the seven patients with aberrant immunophenotype had increased CD4<sup>+</sup>7<sup>-</sup> T cells. Loss of CD7 is seen in a variety of reactive conditions, so this non-specific finding in itself does not necessarily reflect a clonal T-cell population;<sup>19</sup> however, two IgG4-RD patients had increased CD3<sup>+</sup>4<sup>+</sup> cells, and one had increased CD3<sup>+</sup>4<sup>-</sup>8<sup>-</sup> cells, phenotypes which are thought to be more specific for the aberrant T-cells seen in L-HES.<sup>11,20</sup> Five patients with L-HES also had elevated serum IgG4, all with an IgG4/IgG ratio > 0.08. While mild elevations in serum IgG4 are seen in a variety of conditions, two L-HES patients had serum IgG4 levels > 2.8 g/L, a threshold considered over 90% specific for IgG4-RD.<sup>21,22</sup> To our knowledge, two other cases of elevated serum IgG4 in hypereosinophilic syndrome have been reported in the literature.<sup>23-25</sup> The relative paucity of tissue biopsies in L-HES patients in this study is likely related to the ability to diagnose L-HES from peripheral blood and bone marrow samples through immunophenotyping and PCR. Although L-HES can

cause prominent tissue eosinophilia in involved organs along with variable fibrosis, often the tissues biopsied show non-specific reactive changes, e.g. figure 3 shows a reactive lymph node in a patient with L-HES complicated by erythroderma. Only 3/13 (23%) of L-HES patients in this study had lymphadenopathy, compared to 13/21 (62%) of patients in a large L-HES series.<sup>20</sup> This variation in organ involvement may be related to selection bias in this rare disease, as lymphadenopathy did not feature prominently in the original description of L-HES, based on 16 patients recruited from a Dermatology clinic.<sup>26</sup>

Both IgG4-RD and L-HES are typically responsive to first line corticosteroid treatment. Rituximab is often used for relapsed or refractory IgG4-RD (or as first-line therapy in centers where this is readily attainable),<sup>5</sup> whereas Interferon- $\alpha$  is a commonly used second-line therapy in L-HES.<sup>27</sup> The markedly elevated IgE levels seen in some patients with both IgG4-RD and L-HES; although the role of IgE in pathogenesis of these diseases is debatable, this finding raise the possibility of anti-IgE therapy such as omalizumab The numerous overlapping features of these two diseases suggest potentially transferable treatment strategies between them.

This study builds on recent observations in eosinophilic esophagitis. One recent study of 30 patients with eosinophilic esophagitis demonstrated markedly increased IgG4 levels in esophageal tissue homogenates compared to controls. These patients did not respond to omalizumab therapy, leading to the hypothesis that eosinophilic esophagitis may be driven by IgG4 rather than an IgE associated allergy.<sup>28</sup> A separate study of 21 patients with eosinophilic esophagitis confirmed the presence intra-squamous IgG4 deposits in esophageal tissue, and the investigators speculated that the biological basis of eosinophilic esophagitis and IgG4-RD may be similar.<sup>29</sup>

Our study has a number of limitations. Tests such as computed tomography and echocardiography were not routinely performed on all patients, and thus subclinical organ involvement may not have been detected in some patients. We were not able to measure cytokine levels such as IL-5, which might provide more insight into the mechanism(s) of the overlapping features. The cells of interest in the CD3<sup>+</sup>4<sup>-</sup>8<sup>-</sup> phenotype are the TCR<sup>αβ</sup>, but our flow cytometry panel did not distinguish between TCR<sup>αβ</sup> and TCR<sup>γα</sup> cells. In fact, comparing L-HES to IgG4-RD also highlights the lack of widely accepted diagnostic criteria for L-HES, a rare condition wherein flow cytometry is considered the gold standard,<sup>26</sup> yet T-cell clonality by PCR is sometimes used as a surrogate.<sup>18</sup> However, the main strength of this study is that it describes for the first time the potential diagnostic overlap between these two diseases using commonly available laboratory tests such as flow cytometry, T-cell PCR and serum IgG4. Future studies might include more extensive cytokine profiling and next generation sequencing of IgG4-RD and L-HES patients to compare T-cell PCR and flow cytometry with the oligoclonal CD4<sup>+</sup> T<sub>EM</sub> cells which seem to drive IgG4-RD.<sup>30</sup>

In conclusion, both IgG4-RD and L-HES must be considered in all patients presenting with eosinophilia. Both conditions may demonstrate aberrant T-cell immunophenotype, T-cell clonality and elevated serum IgG4 levels. This overlap in laboratory features underscores the importance of careful clinical evaluation; L-HES patients commonly have cutaneous manifestations and the disease rarely affects more than three organs, whereas IgG4-RD patients often have mass lesions and five or more organs involved, including the classic manifestations of autoimmune pancreatitis, retroperitoneal fibrosis, and salivary and lacrimal gland involvement (a.k.a. Mikulicz' disease), which are not seen in L-HES. Ultimately, diagnosis of IgG4-RD requires histologic findings concordant with the International Consensus Criteria,<sup>12</sup> whereas diagnosis of L-HES requires careful evaluation of

clinical findings along with immunophenotyping, PCR, and exclusion of other causes of eosinophilia, such as IgG4-RD.

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**Contribution:** M.C., S.P., and L.C. designed the study and wrote the initial draft of the manuscript. All authors were involved in data collection, data analysis and editing of the manuscript.

**Conflict-of-interest disclosure:** The authors declare no competing financial interests.

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## Tables

**Table I. Clinical characteristics of IgG4-RD and L-HES patients**

|  | IgG4-RD<br>(n=31) | L-HES (n=13) | P      |
|--|-------------------|--------------|--------|
| Age of disease onset, median years (IQR)   | 56 (47-64)        | 53 (32-68)   | 0.433  |
| Age at diagnosis, median years (IQR)       | 66 (55-73)        | 55 (34-77)   | 0.127  |
| Duration of follow-up, median months (IQR) | 16 (9-22)         | 19 (11-55)   | 0.327  |
| Male percentage                            | 68%               | 62%          | 0.692  |
| Number of organs affected, median (IQR)    | 5 (3-7)           | 3 (2-4)      | 0.029  |
| One organ involvement, n patients (%)      | 3 (10)            | 1 (8)        | 0.834  |
| 2 organs                                   | 2 (6)             | 3 (23)       | 0.113  |
| >2 organs                                  | 25 (81)           | 9 (69)       | 0.410  |
| Constitutional symptoms, n (%)             | 15 (48)           | 4 (31)       | 0.282  |
| Organ site involvement, n (%) *            |                   |              |        |
| Bone marrow**                              | 2/6 (33)          | 10/10 (100)  | <0.001 |
| Orbit/lacrimal glands                      | 14 (45)           | 0            | 0.003  |
| Sinuses                                    | 11 (35)           | 1 (8)        | 0.059  |
| Salivary glands                            | 23 (74)           | 0            | <0.001 |
| Lung                                       | 10 (32)           | 4 (31)       | 0.923  |
| Heart/pericardium                          | 2 (6)             | 1 (8)        | 0.882  |
| Gastrointestinal tract                     | 6 (19)            | 3 (23)       | 0.780  |
| Liver                                      | 4 (13)            | 2 (15)       | 0.827  |
| Lymph nodes                                | 19 (61)           | 3 (23)       | 0.021  |
| Skin                                       | 11 (35)           | 10 (77)      | 0.012  |

\*Selected organ sites: see Appendix 2 for full table of organ involvement

\*\*For IgG4-RD patients, only 2 of 6 patients had histologic involvement of bone marrow according to International Consensus Criteria, whereas all 10 L-HES patients who had bone marrow biopsy had involvement by abnormal T-cells (3 elderly L-HES patients declined bone marrow biopsy but had very conclusive peripheral blood findings for L-HES).

Abbreviations: IgG4-RD = Immunoglobulin G4-related disease; L-HES = lymphocyte-variant hypereosinophilic syndrome; IQR = interquartile range;

**Table II. Baseline laboratory values**

| Parameter<br>Median (IQR)          | IgG4-RD<br>(n=31) | L-HES (n=13)     | Normal<br>range | P       |
|------------------------------------|-------------------|------------------|-----------------|---------|
| Serum IgG, g/L                     | 22.8 (18.0-38.1)  | 11.6 (11.0-13.3) | 6.3-14.9        | 0.004   |
| Serum IgG4, g/L                    | 9.6 (3.1-17.1)    | 0.80 (0.3-2.0)   | 0.052-1.25      | 0.002   |
| Serum IgE, µg/L                    | 702 (226-1645)    | 884 (197-2473)   | 0-430           | 0.405   |
| WBC, giga/L                        | 7.6 (5.9-9.05)    | 12.5 (11.2-31)   | 3.9-10.2        | < 0.001 |
| Neutrophils, giga/L                | 4.0 (3.4-5.2)     | 5.8 (3.4-9.0)    | 2.0-7.5         | 0.029   |
| Eosinophils, giga/L                | 0.4 (0.2-0.8)     | 7.0 (2.9-17.9)   | 0.0-0.7         | 0.001   |
| Gamma globulins by on SPEP, g/L    | 17.8 (13.6-30.2)  | 11.3 (9.7-12.1)  | 5.1-15.0        | 0.018   |
| Monoclonal band on SPEP, ratio (%) | 0                 | 3/13 (23) *      | -               | 0.006   |

\*1 patient had <1 g/L IgMκ, 1 patient had 2.8 g/L IgGλ, and 1 patient had <1 g/L IgGκ.

Abbreviations: IgG4-RD = Immuglobulin G4-related disease; L-HES = lymphocyte-variant hypereosinophilic syndrome; IQR = interquartile range; SPEP = serum protein electrophoresis



| <b>Table III. Patients with overlapping laboratory characteristics (n = 16 IgG4-RD patients and 4 L-HES patients)</b> |           |                  |   |     |                 |          |                |
|---|-----------|------------------|---|-----|-----------------|----------|----------------|
| Clinical summary at diagnosis (Study code)  | Diagnosis | Peak AEC* giga/L | Flow cytometry  | PCR | Serum IgG4, g/L | IgG4/IgG | IgE, $\mu$ g/L |
| 42-year-old male with sialadenitis. Dacryoadenitis and hyperviscosity syndrome (I1)                                   | IgG4-RD   | 3.9              | Abnormal (11% CD4 <sup>+</sup> 7 <sup>+</sup> )                       | Neg | 26.9            | 0.69     | 832            |
| 48-year-old male with dacryoadenitis, sinusitis, skin rash and asthma (I2)  | IgG4-RD   | 0.5              | Abnormal (increased NK-cells)   | Pos | 7.5             | 0.36     | 47377          |
| 28-year-old female with hyperviscosity syndrome, sialadenitis and lymphadenopathy (I3)                                | IgG4-RD   | 10.7             | -   | Neg | -               | -        | 912            |
| 73-year-old female with pancreatitis, duodenitis, lymphadenopathy, and membranous glomerulonephropathy (I4)           | IgG4-RD   | 0.7              | Abnormal (16% CD4 <sup>+</sup> 7 <sup>+</sup> and increased NK-cells) | Neg | 25.7            | 0.63     | 1226           |
| 56-year-old female with sclerosing cholangitis, pancreatitis and gastritis (I10)                                      | IgG4-RD   | 0.2              | Abnormal (2.2% CD3 <sup>+</sup> 4 <sup>+</sup> )                      | Pos | 3.02            | 0.13     | -              |
| 78-year-old male with sialadenitis, dacryoadenitis, renal failure, and periaortitis (I11)                             | IgG4-RD   | 3.5              | Normal  | Neg | 25.9            | 0.43     | 4              |

|   |         |     |  |     |      |      |      |
|---|---------|-----|--|-----|------|------|------|
| 72-year-old male with sialadenitis and pancreatitis (I14)   | IgG4-RD | 1.2 | -  | -   | 19.7 | 0.56 | -    |
| 68-year-old male with pancreatitis and bilateral submandibular gland swelling (I18)   | IgG4-RD | 0.9 | Normal   | Neg | 12.2 | 0.53 | 4920 |
| 74-year-old male with bile duct stenosis, orbital myositis, sialadenitis, pancreatitis, multifocal fibrosclerosis, coronary artery peri-arteritis (I21) | IgG4-RD | 1.1 | Normal   | Pos | 11.6 | 0.40 | -    |
| 63-year-old male with sialadenitis and dacryoadenitis (I25)   | IgG4-RD | 0.8 | Normal   | Pos | 44   | 0.73 | 1950 |
| 66-year-old male with sicca symptoms, sialadenitis and pancreatitis (I26)   | IgG4-RD | 0.3 | Abnormal (2.6% CD3 <sup>-</sup> 4 <sup>+</sup> and increased NK-cells) | Pos | 10.4 | 0.46 | 7243 |
| 37-year-old male with intra-abdominal lymphadenopathy and sinusitis (I27)   | IgG4-RD | 0.6 | Normal   | Pos | 26.2 | 0.63 | 298  |
| 74-year-old female with sialadenitis and dacryoadenitis (I28)   | IgG4-RD | 0.1 | Normal   | Pos | 5.43 | -    | -    |
| 66-year-old male with pancreatitis and recurrent biliary obstruction (I30)  | IgG4-RD | 0.4 | Abnormal (15% CD4 <sup>+</sup> 7 <sup>-</sup> )                        | Neg | 1.89 | 0.11 | 571  |
| 68-year-old female with sialadenitis and lymphadenopathy (I31)  | IgG4-RD | 0.2 | Normal   | Pos | 1.87 | 0.12 | -    |

|  |         |       |  |     |      |      |       |
|--|---------|-------|--|-----|------|------|-------|
| 82-year-old male with autoimmune pancreatitis and submandibular swelling (I35) | IgG4-RD | 2.42  | Abnormal (15% CD3 <sup>+</sup> 4 <sup>-</sup> 8 <sup>-</sup> ) | Pos | 27.4 | 0.52 | 460   |
| 44-year-old male with skin rash and recurrent swelling (L5)                    | L-HES   | 17.9  | Abnormal (20% CD3 <sup>+</sup> 4 <sup>-</sup> 8 <sup>-</sup> ) | Pos | 4.01 | 0.21 | 16208 |
| 86-year-old male with ANCA+ CNS vasculitis (L7)                                | L-HES   | 7.6   | Abnormal (20% CD3 <sup>+</sup> 4 <sup>-</sup> 8 <sup>-</sup> ) | Pos | 4.18 | 0.12 | 600   |
| 60-year-old female with eosinophilic cardiomyopathy and asthma (L9)            | L-HES   | 10.2  | Abnormal (14% CD4 <sup>+</sup> 7 <sup>-</sup> )                | Pos | 1.58 | 0.14 | 85    |
| 37-year-old female with skin rash, edema and rheumatoid arthritis (L13)        | L-HES   | 35.69 | Abnormal (11% CD4 <sup>+</sup> 7 <sup>-</sup> )                | Pos | 1.86 | 0.14 | 301   |
| 67-year-old male with pulmonary eosinophilia (L19)                             | L-HES   | 88.83 | Abnormal (11% CD4 <sup>+</sup> 7 <sup>-</sup> )                | Pos | 2.58 | 0.16 | 980   |

\*AEC: Absolute eosinophil count

We defined overlapping patients as IgG4-RD patients with eosinophilia, aberrant T-cell immunophenotype or positive T-cell clonality on PCR, and L-HES patients with elevated serum IgG4 concentrations.

## Figure legends

**Figure 1. Lymph node histology in IgG4-RD.** (A; H&E) Lymph node in patient with IgG4-related disease. The lymph node shows reactive follicular hyperplasia. (B; H&E) The germinal centers contain increased plasma cells. (C) The germinal centers contain greater than 50 IgG4+ plasma cells per high power field. (D) The IgG4+ plasma cells comprise more than 40% of the IgG+ plasma cells.

**Figure 2. Representative bone marrow findings from each cohort, both demonstrating increased eosinophils (including immature forms).** (A) IgG4-RD histology. (B) IgG4-RD smear. (C) L-HES histology. (D) L-HES smear. Abbreviations: IgG4-RD: Immunoglobulin G4 (IgG4) related disease; L-HES: lymphocyte variant hypereosinophilic syndrome.

**Figure 3. Lymph node histology in L-HES.** (A; H&E) The lymph node shows typical features of dermatopathic lymphadenopathy, with a paracortical proliferation of pale cells, consisting of Langerhans cells and interdigitating dendritic cells. (B; H&E) Focal deposition of melanin is present.

**Figure 4. Baseline laboratory results of IgG4-RD and L-HES patients.** Each bar represents the proportion of patients who exceeded normal ranges out of those who were tested for that parameter within each sub-group. Laboratory value ranges for these patients are shown in brackets. Units for IgG, IgG4, gamma peak: g/L; IgE:  $\mu$ g/L; eosinophils: giga/L; T-cell immunophenotypes: %.

**Figure 5. Scatter plots showing differences in laboratory values in IgG4-RD and L-HES patients. (A) Serum IgG4. (B) Eosinophil count.**























