thyroiditis [32], it is still unclear whether Riedel's thyroiditis is a type of IgG4RD.

IgG4-related lymphadenopathy and Castleman's disease

Concomitant lymphadenopathy is common in patients with IgG4RD, and there have been several reports dealing with the morphological and immunohistological findings of lymph node lesions [55–57]. Although IgG4-related lymphadenopathy is occasionally characterized by systemic lymphadenopathy, polyclonal hyperimmunoglobulinemia, especially elevated IgG and IgE concentrations, and positivity for various autoantibodies, patients with IgG4RD with generalized lymphadenopathy should only be evaluated for lymphoma, sarcoidosis, multicentric Castleman's disease, and other malignancies.

IgG4-related lymphadenopathy can be characterized into five histological subtypes: Castleman's disease-like morphology (type I), reactive follicular hyperplasia (type II), interfollicular plasmacytosis and immunoblastosis (type III), progressive transformation of germinal center-like (type IV), and inflammatory pseudotumor-like morphology (type V) [57]. In addition, IgG4-related lymphadenopathy can be classified into two types based on the infiltrative patterns of IgG4-positive cells: interfollicular plasmacytosis (types I, II, III, and V) and intragerminal center plasmacytosis (type IV). Patients with systemic IgG4-related lymphadenopathy were significantly older (68.8 vs. 43.3 years) and had significantly lower C-reactive protein (0.29 vs. 8.71 mg/dl) and interleukin (IL)-6 (8.45 vs. 34.82 pg/ml) concentrations than patients with multicentric Castleman's disease [56].

IgG4-related retroperitoneal fibrosis (IgG4-related RPF)

RPF is a chronic inflammatory condition with marked fibrosis in retroperitoneal tissue. In patients with advanced RPF a retroperitoneal mass covers the abdominal aorta and compresses the ureters, leading to urinary obstruction. Its etiology is unknown, but it has many causes, including infection, radiation, drugs, malignant tumor, and trauma. Three patients with RPF and elevated serum IgG4 have been described [58], and the histology of all 12 patients with RPF was reported to be similar to that seen in AIP, including fibrosis, intense inflammatory cell infiltration with plasma cells, venulitis, and obliterative arteritis [59]. Of 17 patients with RPF, 10 had both elevated serum IgG4 and histopathological features typical of IgG4RD, suggesting that RPF could be categorized as IgG4-related [60]. However, in RPF, fibrosis gradually progresses during chronic inflammation, with lymphocyte infiltration predominant during the early stages and a fibroinflammatory process occurring later. Therefore, determining the stage of illness seems important for diagnosis and prediction of response to steroid treatment [61].

IgG4-related aortitis

There have been several recent reports of inflammatory aneurysms in the abdominal or thoracic aorta [62–64]. For example, 40% of inflammatory abdominal aortic aneurysms (AAAs) were IgG4RD, with elevated IgG4 in serum and abundant infiltration of IgG4+ plasma cells and obliterative phlebitis [62]. These findings suggested that inflammatory AAAs can be classified into 2 groups: IgG4-related and IgG4-unrelated [62]. Although IgG4RD shows good response to steroid therapy, treatment with the anti-CD20 monoclonal antibody, rituximab, may result not only in clinical improvement, but in the tapering or discontinuation of steroids or other drugs [65].

Pathogenesis and pathophysiology of IgG4RD

At present, the pathogenetic mechanism and underlying immunological abnormalities in IgG4RD remain unclear. The elevated serum IgG4 concentration and tissue infiltration of IgG4-positive plasma cells are characteristic features of IgG4RD. Because IgG4 antibodies are dynamic molecules that can exchange Fab arms by swapping a heavy chain and attached light chain, IgG4 can form bi-specific antibodies, as well as functioning as a monovalent molecule [66, 67]. These properties may protect against type I allergy by inhibiting IgE functions, and may prevent type II and III allergy by blocking the Fc-mediated effector functions of IgG1 and inhibiting the formation of large immune complexes. The predominant expression of IgG4 under conditions of chronic antigen exposure is compatible with the clinical features of IgG4RD, including its slow progression and relatively weak immune response.

Some autoantibodies, including those to pancreatic trypsin inhibitor (PSTI), lactoferrin (LF), and carbonic anhydrase (CA), have been detected in patients with IgG4RD, especially in those with IgG4-related AIP [34]. Although IgG4 from the patients was able to bind the normal epithelia of the pancreatic ducts, gallbladder, and salivary gland ducts [68], IgG4-type autoantibodies have not been detected in patients with IgG4RD.

Aberrant immunological findings have been observed in patients with IgG4RD. For example, the Th2-dominant immune response and the production of Th2-type cytokines, such as IL-4, IL-5, IL-10, and IL-13, are increased [69–71]. Furthermore, the numbers of regulatory T cells (Treg) expressing CD4+CD25+Foxp3 are significantly higher in the affected tissues and peripheral blood of

patients with IgG4RD than the numbers in patients with autoimmune and nonautoimmune diseases [72–74]. Overexpression of the regulatory cytokines IL-10 and transforming growth factor β (TGF-β) has also been reported in patients with IgG4RD [74, 75]. IL-10 and TGF-β have potent activities in directing B cells to produce IgG4 and induce fibroplasia, respectively. IL-4, IL-5, and IL-13 are important for class switching to IgE production and eosinophil migration. Therefore, abnormalities in the production of these cytokines may be involved in the pathogenesis of IgG4RD.

Perspectives on IgG4RD

Although IgG4RD is a novel clinical entity, it is not a rare disease. Despite the effectiveness of steroid therapy, for IgG4RD, the condition has often been misdiagnosed as a malignant tumor, lymphoma, Sjögren's syndrome, or other diseases. To date, the clinical diagnostic criteria for IgG4RD have not been established. Because IgG4RD may occur in a variety of organs throughout the body, comprehensive discussions with the cooperation of many clinicians from various specialized fields is needed to establish uniform diagnostic criteria. At present, the diagnostic criteria for IgG4-MD (Table 2) [8] and those for IgG4-AIP type 1 (Table 5) [14] have been established.

Consensus has been reached on two diagnostic criteria for IgG4RD: (1) serum IgG4 concentration >135 mg/dl, and (2) >40% of IgG-positive plasma cells being IgG4-positive. The MHLW Japan team has proposed guidelines for the diagnosis of IgG4RD; these are shown in Table 3. The formulation of organ-specific (i.e., kidney and pulmonary) diagnostic criteria for IgG4RD requires cooperation with the relevant societies. Although IgG4RD responds well to steroid therapy, recurrence and relapse occur following the early reduction or withdrawal of prednisone. Therefore, it is necessary to develop treatment guidelines to establish initial doses of steroids, tapering procedures, and maintenance doses. The MHLW Japan team is currently pursuing a "Prospective study for creating IgG4-related disease treatment guidelines", and unified clinical guidelines are expected in the near future.

Table 5 Clinical diagnostic criteria of autoimmune pancreatitis; revised proposal in Japan (2006) [79]

1. Diffuse or segmental narrowing of the main pancreatic duct with irregular wall and diffuse or localized enlargement of the pancreas on imaging modalities, such as abdominal ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI)
2. High-serum F-globulin, IgG, or IgG4, or the presence of autoantibodies, such as antinuclear antibodies and rheumatoid factor
3. Marked interlobular fibrosis and prominent infiltration of lymphocytes and plasma cells into the periductal area, with occasional lymphoid follicles in the pancreas

For diagnosis, criterion 1 must be present, together with criteria 2 and/or 3

However, it is necessary to exclude malignant diseases such as pancreatic and biliary cancers

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Conflict of interest None.

Appendix

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Associations Between Salivary Gland Histopathologic Diagnoses and Phenotypic Features of Sjögren's Syndrome Among 1,726 Registry Participants

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Objective. **To examine associations between labial salivary gland (LSG) histopathology and other phenotypic features of Sjögren's syndrome (SS).**

Methods. **The database of the Sjögren's International Collaborative Clinical Alliance (SICCA), a registry of patients with symptoms of possible SS as well as those with obvious disease, was used for the present study. LSG biopsy specimens from SICCA participants were subjected to protocol-directed histopathologic assessments. Among the 1,726 LSG specimens exhibiting any pattern of sialadenitis, we compared biopsy diagnoses against concurrent salivary, ocular, and serologic features.**

Results. **LSG specimens included 61% with focal lymphocytic sialadenitis (FLS; 69% of which had focus scores of ≥1 per 4 mm^2) and 37% with nonspecific or sclerosing chronic sialadenitis (NS/SCS). Focus scores of ≥1 were strongly associated with serum anti-SSA/SSB positivity, rheumatoid factor, and the ocular component of SS, but not with symptoms of dry mouth or dry eyes. Those with positive anti-SSA/SSB were 9 times (95% confidence interval [95% CI] 7.4–11.9) more likely to have a focus score of ≥1 than were those without anti-SSA/SSB, and those with an unstimulated whole salivary flow rate of <0.1 ml/minute were 2 times (95% CI 1.7–2.8) more likely to have a focus score of ≥1 than were those with a higher flow rate, after controlling for other phenotypic features of SS.**

Conclusion. **Distinguishing FLS from NS/SCS is essential in assessing LSG biopsies, before determining focus score. A diagnosis of FLS with a focus score of ≥1 per 4 mm^2, as compared to FLS with a focus score of <1 or NS/SCS, is strongly associated with the ocular**

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and serologic components of SS and reflects SS autoimmunity.

The Sjögren's International Collaborative Clinical Alliance (SICCA) is a US National Institutes of Health–supported international Sjögren's syndrome (SS) registry, funded from 2003 through 2013. Through 2009, it comprised research groups in Buenos Aires, Argentina; Beijing, China; Copenhagen, Denmark; Kanazawa, Japan; London, UK; and San Francisco, California, US, where the SICCA data coordinating center and specimen repository are located (at the University of California, San Francisco [UCSF]). The goals of SICCA include: 1) designing and implementing an international clinical data and biospecimen repository; 2) providing these resources for future studies of SS; and 3) developing standardized, universally acceptable classification criteria for SS. The SICCA registry prospectively enrolls individuals using broad eligibility criteria to establish a cohort ranging from participants with symptoms of possible SS to those with established disease. More information about the SICCA registry is available in previously published articles (1,2) and online at http://sicca.ucsf.edu. SICCA collaborators in addition to those who are authors of the present report are listed in Appendix A.

The observation and assessment of lymphocytic infiltration in minor salivary glands has long been associated with SS (3,4). The first prospective study to include semiquantitative histopathologic examination of labial salivary glands (LSGs) was of 40 patients diagnosed with SS, 4 different types of arthritis, or scleroderma and 60 postmortem specimens (5). Despite much study, the utility and application of focus scoring in the setting of focal lymphocytic sialadenitis (FLS) is still not universally accepted. The range of opinions include the view that it is a specific and accurate assessment of the salivary component of SS (6–11), that it is an alternative in the clinical assessment of SS (12–18), or that it is only a scientific assessment for research purposes (19).

In addition to FLS, other morphologic patterns of chronic inflammation occur commonly in LSG biopsy specimens: nonspecific chronic sialadenitis (NSCS) and sclerosing chronic sialadenitis (SCS) (8). LSG biopsies with FLS and focus scores of >1 focus per 4 mm^2, rather than these other patterns, are associated with the diagnosis and severity of the ocular manifestations of SS (keratoconjunctivitis sicca [KCS]) (11). However, the specificity of FLS as compared to NSCS or SCS (NS/SCS) in relation to other phenotypic features of SS has not yet been established. Furthermore, a narrow range of focus score values has been used as the significance threshold for diagnosing the salivary component of SS, including a focus score of >1 (5–8), a focus score of ≥ 1 (15–18), and a focus score of ≥ 2 (10) per 4 mm^2, but use of a focus score of <1 has not been studied.

The specific aim of this study was to improve diagnostic applications of LSG biopsy by using data from the large, prospective SICCA cohort to 1) distinguish FLS from NS/SCS in LSG biopsies from patients with suspected SS by analyzing their associations with specific ocular, serologic, and salivary phenotypic features of SS; and 2) compare FLS focus score values of <1 to those of ≥ 1 to assess the traditionally used threshold. These unique assessments are possible because in the SICCA registry, an LSG biopsy is performed on all participants as part of their comprehensive baseline study visit.

PATIENTS AND METHODS

Study population. In the SICCA registry, examinations and specimen collections are performed according to a standardized protocol that is identical and consistently applied across all 6 research sites. Adherence to the standardized protocol is ensured by ongoing specimen examination and quality assurance site visits. Eligibility criteria for enrollment require that a participant be at least 21 years of age and have at least 1 of the following: patient-reported dry eyes or dry mouth; a previous suspicion or diagnosis of SS; elevated serum level of antinuclear antibody (ANA), rheumatoid factor (RF), SSA, or SSB; bilateral parotid enlargement in the clinical setting of SS; a recent increase in dental caries; or a diagnosis of rheumatoid arthritis or systemic lupus erythematosus and possible secondary SS (1). The present analysis is based on a cohort of participants who had been enrolled in the SICCA registry and for whom biopsy results and all other data were available for analysis as of September 20, 2010. Informed consent was obtained in compliance with the Helsinki Declaration, and the study was approved by the UCSF Committee on Human Research. Additional reviews and approvals were provided by local institutional review boards at each of the participating institutions.

Variables and measures. SICCA participants undergo baseline evaluation starting with questionnaires that record, among other information, demographic data, oral and ocular symptoms, and medical history. Three specialty examinations then follow: ocular (including lissamine green and fluorescein ocular surface staining to establish the presence or absence of KCS [the ocular component of SS, described in detail in ref. 2]), oral/salivary (including 5-minute unstimulated whole salivary flow rate and LSG biopsy), rheumatologic, and serologic. In aggregate, 9 types of biospecimens are collected from each participant, including formalin-fixed and frozen LSGs. Two-year followup evaluations include the same clinical examinations and biospecimen collections; the results of these longitudinal analyses will be published in a separate manuscript. All SICCA questionnaires, data collection forms, and clinical and specimen protocols are available for review at http://sicca.ucsf.edu.

Table 1. Distribution of histopathologic diagnoses and focus scores in LSG biopsy specimens collected from 1,787 baseline participants in the SICCA registry*

Histopathologic diagnosis	
FLS†	1,093 (61)
NSCS‡	372 (21)
SCS§	296 (17)
Within normal limits (no lymphocytes)¶	22 (1)
Granulomatous inflammation#	3 (<1)
Marginal-zone (MALT) lymphoma**	1 (<1)
Focus scores among the 1,058 participants with FLS††	
>1 foci per 4 mm^2	693 (66)
1 foci per 4 mm^2	37 (3)
<1 foci per 4 mm^2	328 (31)
Presence of germinal centers‡‡	115 (11)

* Values are the number (%). LSG = labial salivary gland; SICCA = Sjögren's Syndrome International Collaborative Clinical Alliance; SCS = sclerosing chronic sialadenitis; MALT = mucosa-associated lymphoid tissue.

† Presence of 1 or more dense aggregates of 50 or more lymphocytes (usually several hundred or more), usually located in perivascular or periductal locations. The foci are located adjacent to normal-appearing mucous acini in gland lobes or lobules lacking duct dilation or interstitial fibrosis and contain no more than a minority proportion of plasma cells. This diagnosis is assigned when these foci are the only inflammation present in a specimen, or the most prominent feature. Focus scores are then assigned by assessing the glandular area in each and calculating the number of lymphocytic foci present, per 4 mm^2 of glandular area (20).

‡ Scattered or focal infiltrates of lymphocytes, macrophages, and plasma cells that are not adjacent to normal-appearing acini and located in gland lobules that exhibit some combination of acinar atrophy, interstitial fibrosis, duct dilation, and luminal inspissated mucus.

§ Considered to be an advanced stage of nonspecific chronic sialadenitis (NSCS) in which interstitial fibrosis, various patterns of chronic inflammation, and acinar atrophy predominate.

¶ Diagnosed in minor salivary glands with normal-appearing architecture and scattered plasma cells, but without acinar atrophy and few if any lymphocytes.

Clusters of CD-68 positive macrophages, with or without occasional multinucleated giant cells and without necrosis.

** Diagnosed in minor salivary glands exhibiting diffuse lymphocytic infiltration with loss of glandular architecture and composed of sheets of CD20-positive cells without follicular distribution, few scattered CD3-positive cells, and few if any follicular dendritic (CD21- or CD23-positive) cells.

†† In the present study, 1,058 specimens were large enough (i.e., ≥4 mm^2) for focus score assessment. Focus score percentiles among the 1,058 participants with focal lymphocytic sialadenitis (FLS) ranged from 0.1 to 13.5; scores by percentile were as follows: 1st percentile 0.1, 25th percentile 0.8, 50th percentile 1.8, 75th percentile 3.7, 99th percentile 11.6.

‡‡ Germinal center presence is estimated based on the appearance of a cluster of relatively clear staining cells within a lymphocytic focus in hematoxylin and eosin–stained sections. More specific identification of germinal centers requires immunohistochemical staining for follicular dendritic cells with anti-CD21 or anti-CD23.

LSG biopsy samples are obtained at the time of the SICCA baseline evaluation on all participants, or a previous LSG biopsy specimen is accepted if it was obtained no more than 3 years previously and the microscopic slides are available for examination. LSG biopsies are performed, after local anesthetic infiltration, to harvest 5–10 glands (8,20), some of which are fixed in neutral buffered formalin while others are quickly frozen in liquid nitrogen. Three to five formalin-fixed LSGs are processed by the local pathology departments (paraffin embedding, sectioning, and hematoxylin and eosin [H&E] staining) and remaining glands are frozen and stored in liquid nitrogen. All biospecimens, including paraffin-embedded and frozen salivary glands, are shipped quarterly to the SICCA coordinating center at UCSF.

H&E-stained sections of each specimen are evaluated initially by 1 of 3 pathologists (TED, DC, and RJ), who are blinded with regard to the participants' demographic, clinical, and serologic characteristics and who assign 1 of 6 possible diagnoses: FLS, NSCS, SCS, granulomatous inflammation, marginal-zone (mucosa-associated lymphoid tissue [MALT]) lymphoma, or within normal limits. All diagnoses are defined in the footnote to Table 1, and illustrative photomicrographs of FLS are provided in Figures 1 and 2. If FLS is diagnosed in any specimen, the focus score is then determined (20,21). Specimens must have a glandular area of at least 4 mm^2 (preferably 10–20 mm^2, because focus scores can be overestimated in smaller specimens) and have lymphocytic foci of ≥50 cells (Figure 2A), but most are larger (Figures 1, 2B, and 2C). FLS may include hyperplasia and lymphocytic infiltration of ductal epithelium or lymphoid germinal centers (Figure 2C). A focus score of 12 foci per 4 mm^2 is usually the highest that can be counted; above that number of foci, infiltrates become confluent.

Each specimen is then independently reevaluated by a second observer, and any differences are resolved by consensus between the first two, or with a third observer. This approach also provides an ongoing calibration of the examiners' findings. We also conducted a formal assessment of the interexaminer agreement rate on 56 biopsy specimens that had been read independently by the 2 main pathologists. We computed the kappa statistics to assess agreement rate on their diagnoses, and the interclass correlation coefficient (ICC) to assess agreement rate for diagnosis, number of foci, and focus score.

Specimens exhibiting other patterns of chronic inflammation, as defined in Table 1, are classified as NSCS or SCS depending on the presence of interstitial fibrosis, atrophic or

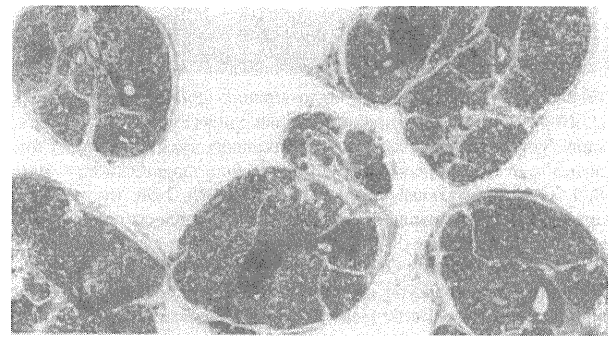

Figure 1. Hematoxylin and eosin–stained labial salivary glands exhibiting focal lymphocytic sialadenitis. Approximately 10 focal lymphocytic infiltrates can be seen in this image. Under the microscope, there was a total glandular area of 24 mm^2, yielding a focus score of 2 foci per 4 mm^2. Original magnification × 2.

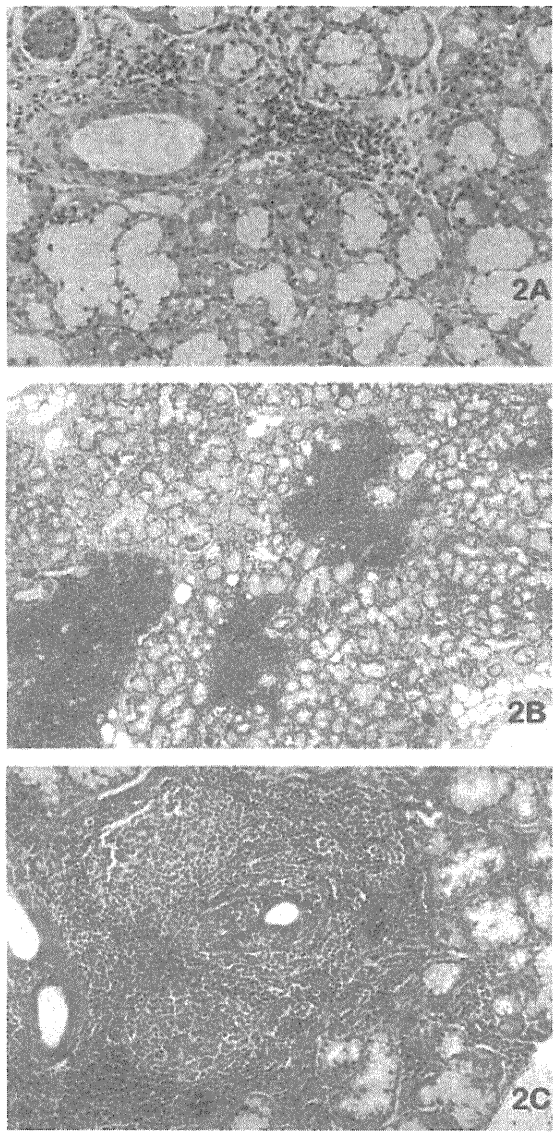

Figure 2. Hematoxylin and eosin–stained labial salivary glands (LSGs) exhibiting focal lymphocytic sialadenitis (FLS). **A,** LSG with a small lymphocytic aggregate that is minimally sized (>50 cells) for inclusion in a focus score calculation. Original magnification × 100. **B,** LSG with 4 variously sized lymphocytic foci. Note the normal-appearing acini immediately adjacent to the lymphocyte aggregates, a characteristic feature of FLS. The entire specimen had a focus score of 3 foci per 4 mm^2. Original magnification × 16. **C,** LSG with 2 prominent lymphocytic germinal centers and ductal hyperplasia within a large lymphocytic focus. Original magnification × 40.

absent acini, and scattered (Figure 3A) or focal chronic inflammation (Figures 3B and C). These aggregates are not counted for a focus score because of the absence of adjacent normal acini. Specimens containing epithelioid histiocytes and occasional Langhans-type giant cells forming noncaseating granulomas are further examined by immunohistochemistry to detect the pattern of CD68 antigen expression. In such cases, the absence of acid-fast bacilli in the specimen would lead to a recommendation that the participant be evaluated for sarcoidosis or other chronic granulomatous disease. Some specimens with no apparent lymphocytic infiltration or other inflammation are classified as being within normal limits.

Statistical analysis. We computed proportions to explore the distribution of histopathologic diagnoses from the LSG biopsies and of focus scores (among those with FLS), after categorizing the focus score as >1, 1, or <1 foci per 4 mm^2, and we ascertained the presence of germinal centers within specimens with FLS and an assessable focus score. Among specimens found to exhibit any form of sialadenitis, we explored the associations between 3 categories of LSG diagnosis (FLS with focus score ≥1, FLS with focus score <1, and NS/SCS) and other phenotypic characteristics of SS, using a contingency table approach (with chi-square testing). We used a nonparametric approach (Wilcoxon's rank sum test) to compare focus score as a continuous variable by presence/absence of each phenotypic feature of SS, with focus scores presented as the median and range accordingly. We then fitted a logistic regression model to explore the explanatory role of various phenotypic features of SS in relation to the outcome "having FLS with focus score ≥1 as compared to focus score <1 or NS/SCS," among participants with sialadenitis. We present adjusted odds ratios with 95% confidence intervals (95% CIs) from this analysis.

RESULTS

We analyzed LSG biopsy specimens from 1,787 participants who were enrolled in the SICCA registry as of September 20, 2010. Approximately one-fourth of the participants (26%) were enrolled from the US, 20% from Denmark, 17% from Argentina, 15% each from Japan and China, and 7% from the UK (since 2007). The majority of the participants (93%) were women, and the median age was 54 years (range 21–90). Eighty-seven of the participants (5%) were classified as having secondary SS since they had confirmed diagnoses of rheumatoid arthritis, systemic lupus erythematosus, or, in a few cases, scleroderma or mixed connective tissue disease.

There were a mean ± SD of 4.7 ± 1.6 minor glands per LSG specimen, with a total mean glandular area of 14.4 ± 7.8 mm^2 per specimen. Table 1 summarizes histopathologic diagnoses from all baseline specimens. A total of 1,093 specimens (61%) were diagnosed as showing FLS, 668 (37%) were diagnosed as showing 1 of 2 other forms of chronic sialadenitis, and 26 (1%) were given other diagnoses, including 22 determined to be within normal limits, 3 cases of granulomatous inflammation, and 1 case of marginal-zone (MALT) lymphoma. Histopathologic grading criteria are included in Table 1. Among the 1,093 specimens with FLS, 35 (3%)

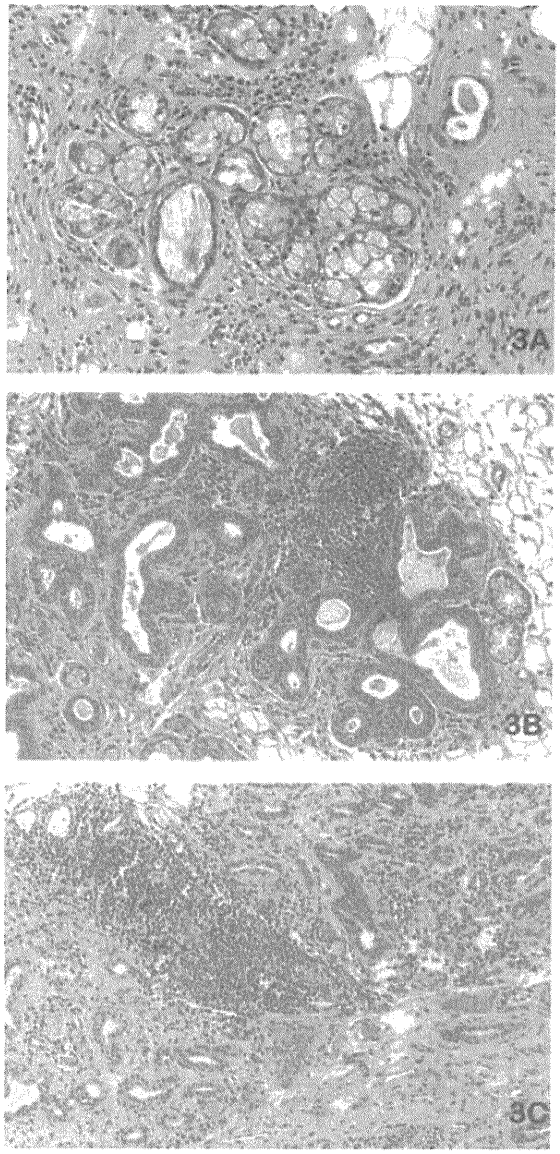

Figure 3. Hematoxylin and eosin–stained labial salivary glands exhibiting nonspecific chronic sialadenitis (NSCS) and sclerosing chronic sialadenitis (SCS). These patterns do not represent the salivary component of Sjögren's syndrome, and all of these specimens are from participants who were negative for anti-SSA/SSB and rheumatoid factor. **A,** NSCS and SCS with scattered lymphocytes and plasma cells and prominent interstitial fibrosis. Original magnification × 100. **B,** SCS with duct dilation, interstitial fibrosis, and a prominent lymphocytic infiltrate, but without adjacent normal-appearing acini. Original magnification × 50. **C,** SCS with severe interstitial fibrosis, a lymphocytic aggregate, many duct-like structures, and no normal-appearing acini. Original magnification × 50.

were too small to enable calculation of a focus score. Among the remaining 1,058 specimens, 66% had a focus score of >1, 3% had a focus score of 1, and 31% had a focus score of <1; 11% included germinal centers. The focus score ranged from 0.1 to 13.5, with a median of 1.8. Given the small proportion of FLS specimens with focus scores of 1 (3%), we combined them with specimens with focus scores of >1 in our analyses.

In a calibration exercise based on 56 slides reviewed independently by the 2 main pathologists, we found high agreement rates with respect to diagnosis (κ = 0.98 [95% CI 0.91–1.00]), number of foci (ICC 0.97 [95% CI 0.96–0.99]), and focus score (ICC 0.96 [95% CI 0.94–0.99]).

Among the 1,093 specimens diagnosed as exhibiting FLS, 266 also included generally small areas of periductal sclerosis. Prior to combining this subset with the 827 specimens that did not exhibit such sclerosis, we ruled out any statistical differences between these 2 FLS subgroups with respect to serologic measures of autoimmunity (elevated serum ANA, RF, SSA, or SSB), controlling for focus score. However, age was found to be associated with periductal sclerosis: the median age among the group with FLS and sclerosis was 61 years, compared to 51 years in the FLS only group ($P < 0.001$), both among those with focus scores of ≥1 and among those with focus scores of <1.

Among 1,787 LSG biopsy specimens, 1,726 had some form of sialadenitis (and an assessable focus score). Within this group of 1,726 participants, we found a high proportion with focus scores of ≥1 (as compared to focus scores of <1 or to NS/SCS) among those with positive serum SSA/SSB (76%) and/or RF (72%), ANA (titer >1:320) (72%), hypergammaglobulinemia (73%), ocular staining score ≥3 (50%), or unstimulated whole salivary flow rate <0.1 ml/minute (53%) (Table 2). Strong statistical associations were observed between the 6 phenotypic features of SS and the pattern of sialadenitis (focus score ≥1 versus focus score <1 versus NS/SCS), all with P values of <0.0001. There were no significant associations or only weak associations between any pattern and participants' symptoms of dry mouth or dry eyes.

Nonparametric analysis performed to explore focus score as a continuous variable in relation to each phenotypic feature of SS confirmed the associations displayed in Table 2, where focus score was categorized as ≥1, <1, and no focus score. The median focus score among participants with positive anti-SSA/SSB serology was 2.8 (range 0.1–12.5), versus 0.9 (range 0.1–13.5) among those with negative anti-SSA/SSB ($P < 0.0001$). Participants with other abnormal serologic results, such as positive RF, ANA titer ≥1:320, and hypergammaglobulinemia (IgG >1,445 mg/dl), also had a higher median focus score (3, 2.8, and 3, respectively) than

Table 2. Bivariate analysis exploring patterns of sialadenitis and focus scores by phenotypic features of Sjögren's syndrome in 1,726 SICCA registry participants with sialadenitis*

Phenotypic feature of Sjögren's syndrome	Sialadenitis pattern: FLS with focus score ≥1 (n =730)	FLS with focus score <1 (n =328)	NS/SCS (no focus score) (n = 668)	P†
Serum anti-SSA/SSB				
Positive	487 (76)	63 (10)	91 (14)	
Negative	243 (22)	265 (24)	575 (53)	<0.0001
Rheumatoid factor				
Positive	458 (72)	64 (10)	113 (18)	
Negative	270 (25)	264 (24)	555 (51)	<0.0001
Ocular surface staining score				
≥3	630 (50)	206 (16)	415 (33)	
<3	99 (21)	121 (26)	253 (53)	<0.0001
Antinuclear antibody				
≥1:320	477 (72)	68 (10)	115 (17)	
<1:320	253 (24)	260 (24)	552 (52)	<0.0001
IgG				
>1,445 mg/dl	424 (73)	54 (9)	104 (18)	
≤1,445 mg/dl	305 (27)	273 (24)	561 (49)	<0.0001
Unstimulated whole salivary flow rate				
<0.1 ml/minute	502 (53)	148 (15)	306 (32)	
≥0.1 ml/minute	228 (30)	179 (23)	362 (47)	<0.0001
Dry mouth symptoms				
Present	669 (43)	292 (19)	595 (38)	
Absent	60 (36)	35 (21)	70 (42)	0.3
Dry eye symptoms				
Present	624 (43)	292 (20)	549 (37)	
Absent	105 (41)	35 (14)	117 (46)	0.01

* Among 1,787 LSG biopsy specimens analyzed, 1,726 had some form of sialadenitis, i.e., FLS or NS/SCS. Values are the number (%). See Table 1 for definitions.
† By chi-square analysis.

those with negative test results (1, 1.1, and 1, respectively). Similarly, Wilcoxon's nonparametric rank sum test revealed statistically significant associations between focus score and each of these serologic features. The median focus score was also elevated in those with abnormal ocular surface staining (score ≥3) and unstimulated whole salivary flow rates of <0.1 ml/minute (2.2 and 2.3, respectively, compared to 0.9 and 1.1 in those without abnormal ocular surface staining or unstimulated whole salivary flow rate) ($P < 0.0001$). Finally, the median focus score was 4.3 (range 0.8–13.5) among specimens with germinal centers and 1.5 (range 0.1–12.5) among those without germinal centers. Wilcoxon's nonparametric rank sum test, performed to compare focus scores in the 2 groups, revealed a statistically significant association between focus score and the presence of germinal centers ($P < 0.0001$).

Next, we stratified the contingency table analysis according to whether participants were or were not using one or more of the many prescription drugs that could reduce salivary secretion. Among participants who were not taking such drugs, 50% of those reporting symptoms of dry mouth had a focus score of ≥1, versus 17% with a focus score of <1 and 33% with NS/SCS ($P = 0.02$). Among those who were taking such drugs, there was no association between the pattern of sialadenitis and symptoms of dry mouth ($P = 0.7$), suggesting that anticholinergic drug use was an effect modifier. Similarly, responses to more specific questions such as "Do you need to sip liquids to swallow dry foods?" or "Does your mouth feel dry when eating a meal?" (22) were not associated with the pattern of sialadenitis among those taking these drugs, but an association was found among those not taking such drugs.

We found that participants who were positive for anti-SSA/SSB were 9 times more likely to have a focus score of ≥1 (95% CI 7.4–11.9) than those with negative SSA/SSB serology, after controlling for abnormal ocular surface staining, abnormal unstimulated whole salivary flow, and dry mouth/dry eyes symptoms. Similarly, those with either abnormal ocular surface staining or abnormal unstimulated whole salivary flow were more than twice as likely to have focus scores of ≥1 compared to those without these characteristics (Table 3).

Table 3. Multivariate model exploring the explanatory role of various phenotypic features of Sjögren's syndrome in relation to the outcome FLS with a focus score of ≥1, as compared to FLS with a focus score of <1 or NSCS or SCS, among 1,716 SICCA registry participants with sialadenitis*

Phenotypic feature of Sjögren's syndrome	Adjusted OR (95% CI)	P†
Positive anti-SSA/SSB serology	9.4 (7.4–11.9)	<0.0001
Ocular surface staining score ≥3	2.2 (1.6–2.9)	<0.0001
Unstimulated whole salivary flow rate <0.1 ml/minute	2.2 (1.7–2.8)	<0.0001
Reported dry mouth symptoms	1.2 (0.8–1.8)	0.5
Reported dry eye symptoms	1.0 (0.7–1.4)	0.9

* Ten participants among the 1,726 with sialadenitis had a missing observation on at least 1 of the independent variables. OR = odds ratio; 95% CI = 95% confidence interval (see Table 1 for other definitions).
† By chi-square analysis.

DISCUSSION

This is the first large-scale prospective cohort study to analyze and confirm the importance of distinguishing FLS from NS/SCS in LSG biopsies from patients with suspected SS and to demonstrate their associations with phenotypic features of the disease. We also compared focus score thresholds of <1 versus ≥1 foci per 4 mm^2 and found no basis to change the traditional threshold value of ≥1. This analysis of >1,700 LSG biopsy specimens revealed that FLS with a focus score of ≥1 was strongly associated with the main phenotypic features of SS, including positive anti-SSA/SSB and RF serology, high ANA titers and IgG concentration, presence of KCS (ocular staining score ≥3), and unstimulated whole salivary flow rates <0.1 ml/minute. FLS with a focus score of ≥1 was not associated with symptoms of dry mouth or dry eyes.

LSG biopsy has played an important role in SS because of its disease specificity, wide availability, minimal invasiveness, and the opportunity to assess autoimmune disease–active cells within an SS target organ. This study has shown that the presence of FLS was highly associated with both the serologic and ocular components of SS and was significantly more specific to the salivary component of SS than is an unstimulated salivary flow rate of <0.1 ml/minute. LSG biopsy can yield histopathologic information about the extent and nature of the disease process. The greatest weakness of LSG biopsy is inconsistent histopathologic assessment, which can be overcome by following the protocol described herein and on the SICCA web site (20).

The focus score threshold of >1 was first suggested in 1968 (5) and has since been applied in several large patient series (7,8,11). In 1993, a focus score threshold of ≥1 was proposed (15), and this recommendation continued through 2002 (18). Among the participants in our cohort who had FLS, only 3% had focus scores of exactly 1. It is therefore somewhat arbitrary as to whether these specimens should be combined with the specimens with focus scores of >1 or with those with focus scores of <1 for classification purposes. To maintain consistency with more recent studies conducted by others, we decided to combine specimens with focus scores of 1 with those with focus scores of >1 for analysis. Table 2 shows that among participants with FLS and focus scores of <1, the proportions who had phenotypic features of SS were significantly lower than among those with focus scores of ≥1. Thus, this analysis confirms that FLS with a focus score of ≥1 represents a distinct entity from FLS with a focus score of <1 or NS/SCS and is strongly associated with the phenotypic features of SS.

In 1933, Henrik Sjögren first noted symptoms of hyposalivation in almost half of his 19 study patients and observed significant lymphocytic infiltration of the parotid, sublingual, and accessory salivary glands upon examination of 1 postmortem case (3). Thus began the uncertainty about the nature of the salivary component of SS. Should it be considered present based on a symptom of dryness, or a secretory threshold value, or results from salivary scintigraphy, sialographic imaging, or histopathologic assessment?

In an early study, SS was defined as the presence of 2 of 3 from "the triad of keratoconjunctivitis sicca [KCS] ('dry eyes'), xerostomia ('dry mouth'), and rheumatoid arthritis or other connective tissue disease" (23). Unfortunately, the term "xerostomia" was and continues to be applied, often indiscriminately, to either symptoms or signs of dry mouth, with no consensus on how to assess either. This confusion 35 years ago led to defining the salivary component of SS as FLS (with a focus score of >1) in an adequate LSG biopsy specimen, instead of "xerostomia" (7). Other methods to define the salivary component of SS have been introduced, including defined whole salivary or parotid flow rates, with or without stimulation, sialographic imaging of a major salivary gland, measuring technetium uptake and secretion by salivary

scintigraphy, and ultrasound imaging of the glands. However, the specificity of these assessments to SS has not been clearly established. Meanwhile, a strong association was shown between the presence and severity of the ocular component of SS (KCS) and FLS in LSG biopsies (11), confirming the relevance of FLS as a disease-specific measure of the salivary component of SS.

Symptoms of dry mouth have been proposed as components of classification criteria for primary SS since 1993 (15,18). However, SICCA study participant responses to the questions "Does your mouth feel dry?" or "Do your eyes feel dry?" were not statistically associated or only weakly associated with the presence of focal lymphocytic sialadenitis (focus score >1), serum anti-SSA/SSB, or ocular staining ≥ 3 (indicating KCS) (1,24) (Table 2). Furthermore, the presence of an association between the pattern of sialadenitis and symptoms of dry mouth among those not taking anticholinergic drugs, and absence of association among those taking these medications, suggests the presence of a statistical interaction. Thus, these findings confirm that symptoms of dry eyes or dry mouth may be nonspecific and can be due to causes other than SS in a significant proportion of patients. We have also shown that unstimulated whole salivary flow rates were significantly associated with FLS and focus scores of ≥ 1, but at a much lower adjusted odds ratio than positive anti-SSA/SSB serology (Table 3).

The Chisholm and Mason grading scale for assessing inflammation in LSG biopsies applied both qualitative and semiquantitative assessments of lymphocytic infiltration to LSGs that were still embedded in mucosal epithelium and connective tissue (5). It introduced the useful SS-associated threshold value of "more than one focus of 50 or more lymphocytes per 4 mm^2 of salivary tissue," but its grades 0–4 are now obsolete. It is a nonlinear scale, with grades 0 and 1 assessed qualitatively, grade 2 assessed qualitatively *or* semiquantitatively (focus scores <1 per 4 mm^2), and grades 3 and 4 assessed semiquantitatively (grade 3, focus scores of 1 per 4 mm^2 and grade 4, focus scores of >1 per 4 mm^2). It can provide a useful severity threshold assessment, but does not further consider severity levels above focus scores of 1 (6) and, most importantly, does not distinguish between different patterns of chronic LSG inflammation (i.e., FLS versus NS/SCS), as described herein and previously (8,11). Previous studies have not examined the associations of SS components with LSGs with focus scores of <1, which we report here to be very similar to the specimens with NS/SCS and significantly different from those with focus scores of ≥ 1.

Based on reviews of previously diagnosed LSG biopsy specimens, some pathologists do not perform the semiquantitative part of LSG biopsy assessment to arrive at a focus score, or do so incorrectly (25). LSG biopsy samples must first be diagnosed qualitatively to assess the presence of FLS versus NS/SCS: if FLS is present then focus score assessment should follow, but if NS/SCS is present a focus score is unnecessary and would be misleading if given.

We observed that specimens with FLS exhibiting periductal sclerosis were from older participants (median 61 years) than those with FLS without sclerosis (median 51 years). However, this age difference existed whether the focus score was ≥ 1 or <1, suggesting that while age is associated with periductal sclerosis, it is not a confounding variable in the focus score analysis. The presence of sclerosis is consistent with an earlier observation of a proportional increase in salivary gland fibrous tissue with increasing age (26).

The presence of germinal centers within lymphocytic infiltrates of LSGs was observed in 17% of a series of specimens from patients with SS (27), indicating lymphoid neogenesis within these SS target organs. In the present study, the median focus score was higher in specimens with evidence of germinal center formation (4.3) compared with those without (1.5), and there was a strong association between higher focus scores and the presence of germinal centers. The small difference between the 17% prevalence of LSG germinal centers in the previous study and 11% in the present study is most likely a result of the earlier investigators' use of various immunohistochemical markers to identify germinal centers and our result based on their presence in H&E-stained sections.

Assessment of LSG biopsy specimens in the setting of SS can be subject to several types of misinterpretation. These include failing to determine a focus score on specimens exhibiting FLS and attempting to apply a focus score to specimens having nonspecific patterns of inflammation (25). Based on the typical irregular distribution of lymphocytic foci in LSGs, another diagnostic pitfall is assessment when too little tissue is present (e.g., only 1 gland or fragments of several glands), which can result in an overestimation of the focus score. This can be avoided by using a standardized protocol for assessment of LSGs that dictates minimum size of the salivary gland tissue specimen prior to focus scoring.

In conclusion, LSG biopsies with focus scores of ≥ 1, as compared to those with focus scores of <1 or with NS/SCS, are strongly associated with phenotypic ocular

and serologic components of SS. An LSG biopsy focus score of ≥1 is not a gold standard for diagnosing SS, but remains the best method for diagnosing its salivary component and assessing an important site of autoimmune activity.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Daniels had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Daniels, Cox, Shiboski, M. Schiødt, Wu, Umehara, De Souza, Criswell, Jordan, Greenspan.

Acquisition of data. Daniels, Cox, M. Schiødt, Wu, Lanfranchi, Umehara, Zhao, Challacombe, Lam, De Souza, J. Schiødt, Holm, Bisio, Gandolfo, Sawaki, Zhang, Varghese-Jacob, Ibsen, Keszler, Kurose, Nojima, Odell, Criswell, Jordan, Greenspan.

Analysis and interpretation of data. Daniels, Cox, Shiboski, Umehara, Lam, Li, Varghese-Jacob, Ibsen, Criswell, Greenspan.

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APPENDIX A: ADDITIONAL COLLABORATORS IN THE SJÖGREN'S INTERNATIONAL COLLABORATIVE CLINICAL ALLIANCE

Collaborators in the Sjögren's International Collaborative Clinical Alliance, in addition to the authors, are as follows: at the University of California, San Francisco, K. Sack, D. Lee, J. Whitcher,

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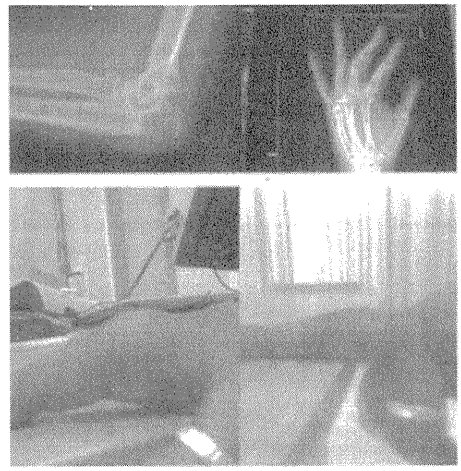
Clinical Images: Infliximab therapy of polyarticular small joint sarcoid arthritis



The patient, a 43-year-old woman, initially presented with bilateral spontaneous fractures of the olecranon processes. Plain radiography revealed an osteolytic lesion as the cause of a fracture of the left olecranon process (top left). Erythema nodosum, cervical lymphadenopathy, and tenderness were noted in the small joints of the hands and feet. Tuberculosis was ruled out, and bone and lymph node biopsy confirmed a diagnosis of skeletal and lymphoreticular sarcoidosis. Chronic sarcoid arthritis developed, skeletal sarcoidosis progressed, and an osteodestructive lesion developed in the third proximal phalanx of the left hand (top right and bottom left), despite treatment with high-dose corticosteroids and methotrexate over 3 years. She was then prescribed infliximab (3 mg/kg of body weight; interval of 2 weeks between the first and second infusions, 4 weeks between the second and third infusions, and every 8 weeks thereafter), which resulted in dramatic improvement in the signs and symptoms after the third infusion (bottom right). Early morning stiffness decreased from 30 minutes to 5 minutes, and the Health Assessment Questionnaire score decreased from 0.5 to 0. The swollen and tender joint count decreased from 3 to 0. Case–control trials have proven the efficacy of infliximab in the treatment of pulmonary disease, which relapsed in >80% of patients after the treatment was stopped. Tumor necrosis factor α maintains the integrity of established granulomas, along with other cytokines such as interleukin-2 and interferon-γ. This is the first report using imaging and validated patient- and clinician-reported outcomes for inflammatory arthritis to quantify the clinical response of polyarticular sarcoid arthritis to treatment with intravenous infliximab.

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CLINICAL STUDY

Donor–recipient gender difference affects severity of dry eye after hematopoietic stem cell transplantation

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Abstract

Purpose **To determine whether the incidence rate and severity of dry eye after hematopoietic stem cell transplantation varies with donor *vs* recipient gender.**

Methods **We limited this study to patients received bone marrow transplantation (BMT). In all, 172 patients received BMT at Keio University School of Medicine between January 2000 and May 2007. Of them, 136 recipients who survived at least 70 days were studied prospectively. We classified the 136 patients according to the gender of the donor and the recipient (group I: female to female; group II: male to male; group III: male to female; group IV: female to male). The incidence and severity of chronic graft-*vs*-host disease-associated dry eye were determined for each group. The donor gender was masked when we assessed dry eye and calculate the incidence.**

Results **The incidence of dry eye was 47.4% for group I, 37.5% for group II, 58.6% for group III, and 42.9% for group IV. The percentage of patients with severe dry eye was 44.4, 50.0, 35.3, and 77.8% respectively. There was a significant difference between the percent severe dry eye/total dry eye incidences in groups III and IV ($P = 0.0375$) (odds ratio, 7.6; 95% confidence interval, 1.00–101.01).**

Conclusions **Close attention must be paid to the development of dry eye in cases of female to male BMTs, because the ratio of severe/total dry eye is more common in cases of female to male BMTs than in other gender combination.**

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Keywords: dry eye; allogeneic hematopoietic stem cell transplantation; chronic graft-*vs*-host disease; gender-mismatched transplantation; minor histocompatibility antigens

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Introduction

Graft-*vs*-host disease (GVHD) is a major complication subsequent to hematopoietic stem cell transplantation (HSCT). Many HSCT recipients become long-term survivors of their disease, and chronic GVHD (cGVHD) occurs in 30–70% of allogeneic HSCT recipients.[1,2] As a result of this, assuring patient quality of life (QOL) and addressing late complications after HSCT have become increasingly important. The eye, mouth, liver, lung, skin, and intestine are preferential targets of cGVHD.[3] Dry eye has emerged as a major complication of systemic cGVHD as well as ocular cGVHD that strongly affects patient QOL.[4] So far, neither radical treatment nor prophylaxis has been established for cGVHD-related dry eye. This study was intended to learn which patients are at the higher risk for developing progressive dry eye.

It is generally known that a donor–recipient gender mismatch in HCST often leads to severe GVHD.[5] In particular, GVHD in male recipients of female donors tends to be especially severe.[6–9] Given the current knowledge about gender mismatch and GVHD, it seems likely that HSCT with a female donor and male recipient could increase the patient's risk of developing cGVHD-related severe dry eye. However, no report on donor–recipient gender mismatch affecting ocular GVHD has been published in the ophthalmologic literature.

In this retrospective study, we focused on donor–recipient gender only in cases treated by bone marrow transplantation (BMT), and

investigated the incidence and severity of dry eye associated with matched and mismatched gender in donor–recipient pairs.

Patients and methods

Study design

This was a retrospective survey evaluating the late effects of BMT. All research and measurements followed the tenets of the Declaration of Helsinki. The study was approved by the ethics committee of the Keio University School of Medicine. All patients underwent standardized clinical and ophthalmological evaluation, as described below, before BMT and 3, 6, 9, 12, 18, 24, and 30 months after BMT, as well as on additional occasions as indicated.

Between January 2000 and May 2007, 172 patients underwent BMT at Keio University School of Medicine. We selected patients who survived for at least 70 days after BMT for this study. This criterion selected patients who survived beyond the time of acute GVHD (aGVHD) development and who could potentially develop cGVHD. We excluded patients under the age of 15 and those who underwent re-transplantation. In total, 36 of the 172 recipients were excluded from this study, and the remaining 136 patients were studied prospectively. When we examined BMT patients, the donor genders were masked. We classified the 136 patients according to the genders of the donor and recipient, and determined the incidence rate and severity of dry eye among these groups ((donor to recipient) group I: female to female; group II: male to male; group III: male to female; group IV: female to male).

Clinical evaluation

Ocular surface vital staining
The fluorescein and rose bengal stain scores for the ocular surface were obtained using the double-vital staining method.[10–13] Two microliter of a preservative-free solution of 1% rose bengal and 1% fluorescein was instilled into the conjunctival sac by a micropipette.[10] The van Bijsterveld scoring system was used for the rose bengal staining.[14] For the rose bengal score, the ocular surface was divided into the three zones: nasal conjunctival, corneal, and temporal. A score of 0–3 points was used for each zone, with a minimum possible score of 0 and a maximum possible score of 9. Scarce punctuate staining was given 1 point. Denser staining not covering the entire zone was given 2 points. Denser staining over the entire zone was given 3 points. For fluorescein staining, the cornea was divided three equal upper, middle, and lower zone. Each zone has a staining score ranging from 0 to 3 points, as with the rose bengal stain, and a minimum and maximum score was 0 and 9, respectively. The presence of scarce staining in a zone was scored 1; frequent puncta not covering the entire zone was scored as 2 points; and puntate staining covering the entire zone was scored as 3 points.[15]

Tear function test
Tear film break-up time (TBUT) was measured three times at the time of double staining, and the median value was calculated.[11] The Schirmer test was performed using standard strips (Alcon, Fort Worth, TX, USA) placed in the lower conjunctival sac for 5 min without anesthesia. For the Schirmer test with nasal stimulation, the standard strips were placed in the conjunctival sac for 5 min, while a cotton–wool swab was inserted into the nose until the tip reached the nasal membrane of the ethmoid sinus. The middle turbinate was touched with the cotton–wool swab, which was kept in place for 5 min. We then measured the length of the moistened part of the standard strip from the conjunctival sac.[16]

Diagnostic criteria

Dry eye was diagnosed as a disorder of tear film caused by tear deficiency and/or excessive tear evaporation, which cause the damage of ocular surface with or without symptom.[12] Dry eye was diagnosed when the tear film of patients showed disturbance of tear dynamics (TBUT $\leq$5 s, Schirmer test $\leq$5 mm) and the ocular surface was abnormal (rose bengal score $\geq$3, fluorescein score $\geq$1).[13] Severe dry eye was defined as reduced reflex tearing (Schirmer test with nasal stimulation $\leq$10 mm) and abnormality of the ocular surface (rose bengal score $\geq$3 and/or fluorescein score $\geq$3)[14] and/or a grade of 3 or 4 according to the dry eye workshop (DEWS) report 2007.[17] Briefly, DEWS proposed the classification of dry eye severity level, which is classified according to the grade of symptom, ocular surface findings, and tear dynamics. The severity level 3 or 4 was regarded as the sign of severe frequent or constant and/or disabling symptom, severe ocular surface damage accompanied by marked injection, filamentary keratitis, mucous clumping, tear debris, and ulceration. When trichiasis, keratinization, and symblepharon along with sign of symptom of dry eye were present, dry eye was also regarded as grade 3 and 4. In addition, TBUT and Schirmer scores are $\leq$5 s and $\leq$5 mm for grade 3 and immediate and $\leq$2 mm for grade 4, respectively.

Mild dry eye was defined as abnormality of the ocular surface (rose bengal score $\geq$3, fluorescein score $\geq$1) without reduced reflex tearing (Schirmer test with nasal stimulation $>$10 mm). According to the grading system of the severity level of dry eye based on DEWS report,

grade 1 and 2 were regarded as mild to moderate stress, and none to mild, or variable conjuntival injection, conjunctival or corneal staining or corneal/tear signs. In addition, TBUT and Schirmer scores are variable for grade 1, and $\leq$10 s and $\leq$10 mm for grade 2, respectively. Patients who had dry eye before BMT were considered to have a sustained a dry eye incident only when the severity of the dry eye worsened after the transplantation.

Statistical analysis

Fisher's direct method was used to evaluate differences among the groups. Statistical analyses were performed using *R* statistical software (Free Software Foundation, Boston, MA, USA). *R* is available freely on web site. This is an open source software for statistical analysis.[18,19]

In this article, version-2.9.1 was available. Significant difference was defined as $P \leq 0.05$.

Results

The 136 subjects survived for at least 70 days after BMT and were evaluable for the presence and severity of dry eye. The median age of the patients was 44 years (range 18–61 years). The median age of the donors was 35 years (range 18–80 years). Clinical characteristics of the 136 patients are shown in Table 1. There were no significant differences between patient and donor age in any group. The percentage of unrelated donors was higher than that of related donors in all groups. There was no statistical significant when we carried out a statistical analysis concerning related/unrelated parameters of donor, that is, the genetic influence in all four groups (Table 1).

We showed the baseline profile of pre-BMT and the serial change of ophthalmic findings at pre- and post-BMT (Tables 2 and 3, Figure 1). There were significant differences of the clinical variables of ocular surface findings and tear dynamics between at the pre- and post-BMT (Figure 1).

Table 1 Patient characteristics in the four donor–recipient gender groups

	I (F→F) n=*38*	*II (M→M)* n=*48*	*III (M→F)* n=*29*	*IV (F→M)* n=*21*
Patient age (range)	39.5 (20–60)	44.5 (18–59)	43 (25–61)	47 (18–59)
Donor age (range)	36 (33–63)	33 (17–58)	38 (21–80)	36 (23–52)
Donor relation				
Unrelated (%)	27 (71)	36 (75)	23 (79.3)	17 (81)
Related (%)	11 (29)	12 (25)	6 (20.7)	4 (19)

Abbreviations: F, female; M, male.

The incidence rate of mild dry eye, severe dry eye, and total dry eye was not significantly different in any of the four groups (Table 4). However, the percentage of patients with dry eye that became severe was greatest in group IV (77.8%), and the incidence was significantly higher than in group III (35.3%) ($P=0.0375$). Odds ratio (OR) was 7.6 (95% confidence interval: 1.00–101.01). The severity of dry eye was more prevalent in group IV more than group III (Table 4, Figure 2). We double checked the severity of dry eye using diagnostic criteria 2007, but the severity determinations were the same for our usual criteria[12] and the DEWS criteria.[17]

We then evaluated the results by pooling the two gender-matched groups and the two gender-mismatched ones. The incidence rate for total, mild, and severe dry eye was slightly higher in the gender-mismatched group, although significant differences were not observed between these two groups (Figure 3).

Discussion

Here, we investigated the incidence rate and severity of dry eye associated with matched and mismatched genders in donors and recipients after HSCT, in patient groups with few differences in patient characteristics (Table 1).

The efficacy of HSCT is improved by the graft-*vs*-leukemia (GVL) effect, a result of moderate aGVHD, but the downside of the GVL effect is an increased risk of cGVHD. Therefore, it is important to control the degree of GVHD. It is known that host rejection and GVHD occur even in HLA-matched donor–recipient pairs, because of mismatches in the minor histocompatibility antigens.[6,8,9]

We found that, if dry eye occurred at all, it usually became severe among male BMT recipients whose donor was female (OR, 7.6). Conversely, there was much less progression to severe dry eye in female BMT recipients of donor tissue from males. These findings are consistent with previous reports on the severity of systemic GVHD, in which male HSCT recipients of female donor tissue had a significantly higher probability of developing systemic GVHD compared with the recipients of other recipient–donor gender combinations.[6–9] These results indicate that dry eye well reflects systemic cGVHD, suggesting that dry eye could be a hallmark of cGVHD based on the assessment of dry eye parameters compared with pre-BMT (Figure 1). There was no difference in the overall incidence of dry eye among the four groups. However, there was a significant difference in the percentage of severe dry eye/total dry eye between the two gender mismatched groups, suggesting that gender-related factors affect the severity of dry eye after

Table 2 Baseline profile ocular surface findings and tear dynamics of pre-BMT

Pre-F	*RB*	*TBUT*	*S*	*S(N)*	*MGD*
0.49 ± 1.09 ($n = 136$)	0.19 ± 1.07 ($n = 135$)	9.36 ± 1.05 ($n = 117$)	12.95 ± 1.72 ($n = 129$)	19.02 ± 10.31 ($n = 84$)	0.86 ± 1.18 ($n = 40$)

Abbreviations: F, fluorescein score; MGD, meibomian gland dysfunction; RB, rose bengal score; S, value of Schirmer test; S (N), value of Schirmer test with nasal stimulation; TBUT, tear film break-up time.

Table 3 Summary of clinical evaluation at pre- and post-BMT

	F	*RB*	*TBUT*	*S*	*S(N)*	*MGD*
Pre						
I ($n = 38$)	0.5 ± 0.9(38)	0.1 ± 0.5 (37)	9.0 ± 2.1 (34)	14 ± 11.8 (35)	18.0 ± 10.4 (20)	0.8 ± 1.1 (8)
II ($n = 48$)	0.5 ± 1.2 (48)	0.1 ± 0.5 (48)	9.7 ± 1.1 (38)	11.9 ± 10.5 (48)	16.1 ± 9.0 (34)	0.9 ± 1.3 (15)
III ($n = 29$)	0.3 ± 0.7 (29)	0.1 ± 0.6 (29)	9.6 ± 1.1 (27)	11.8 ± 8.5 (29)	20.4 ± 11.2 (20)	0.8 ± 0.7 (12)
IV ($n = 21$)	0.8 ± 1.5 (21)	0.4 ± 0.9 (21)	9.2 ± 1.6 (18)	14.2 ± 12.0 (20)	21.6 ± 11.3 (10)	1.0 ± 1.3 (5)
Post						
I ($n = 38$)	2.1 ± 2.6 (38)	1.7 ± 2.2 (35)	5.7 ± 3.5 (34)	5.6 ± 7.9 (17)	6.4 ± 5.6 (9)	1.9 ± 1.0 (12)
II ($n = 48$)	2.1 ± 2.4 (47)	1.6 ± 2.3 (44)	7.0 ± 3.1 (46)	6.8 ± 5.2 (18)	12.1 ± 8.6 (15)	1.7 ± 0.9 (9)
III ($n = 29$)	2.1 ± 2.1 (28)	1.5 ± 2.1 (27)	6.2 ± 3.3 (27)	6.1 ± 6.0 (12)	8.9 ± 7.6 (7)	2.3 ± 0.7 (7)
IV ($n = 21$)	2.0 ± 2.1 (21)	2.0 ± 2.2 (20)	5.9 ± 3.2 (21)	6.9 ± 7.5 (11)	9.4 ± 6.5 (10)	3.0 ± 0.0 (3)

Abbreviations: F, fluorescein score; MGD, meibomian gland dysfunction; Pre, pre-BMT; Post, post-BMT; RB, rose bengal score; S, value of Schirmer test; S (N), value of Schirmer test with nasal stimulation; TBUT, tear film break-up time.

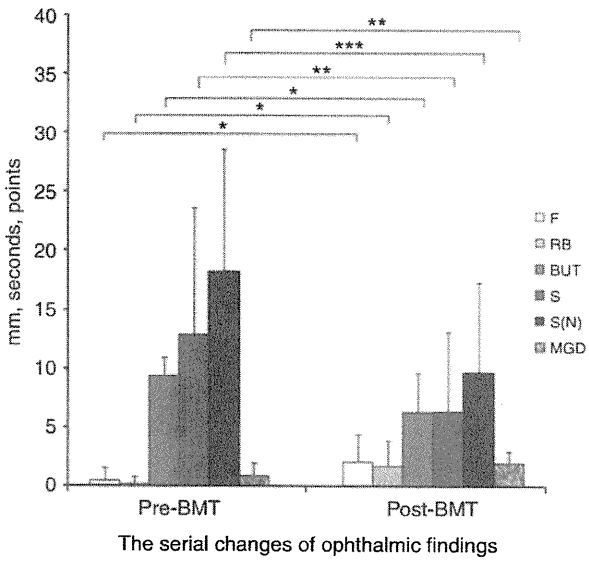

Figure 1 Clinical variables for dry eye parameters at pre- and post-BMT. There were significant differences in ophthalmic findings of pre- and post-BMT. Ocular surface findings and tear dynamics were deteriorated after BMT. F, fluorescein score; RB, rose bengal score; TBUT, tear film break-up time; S, value of Schirmer test; S(N), value of Schirmer test with nasal stimulation; MGD, meibomian gland dysfunction. *$P < 0.001$, **$P < 0.01$, ***$P < 0.05$.

BMT (Figure 2). Particularly, male BMT recipients from female donors have possibility of progressing of severer dry eye.

Table 4 Incidence rate of dry eye, mild dry eye, severe dry eye, and severe/total dry eye in the four groups

	I	*II*	*III*	*IV*
Mild type (%)	26.3	18.8	37.9	9.5
Severe type (%)	21.1	18.8	20.7	33.3
Total dry eye (%)	47.4	37.5	58.6	42.9
Severe/total dry eye (%)	44.4	50	35.3	77.8*

*$P = 0.0375$.

Copelan[20] reported that minor antigens encoded by genes on the Y chromosome account for the higher incidence of GVHD and lower rate of patient relapse among male recipients of marrow transplants from female donors than among male recipients of transplants from male donors. Miklos *et al* proposed that, because female donor T cells have not been exposed to unique epitopes on the Y chromosome, thymic maturation does not delete the T cells capable of recognizing the H-Y antigens.[21] Besides the T cells, antibodies against the H-Y antigen made by donor B cells probably also contribute to GVHD.[21,22] There are some reports that male recipients whose female donors had previous pregnancies or blood transfusions are at increased risk of developing GVHD,[23–25] apparently because of B-cell sensitization to the H-Y antigen. In this study, the previous donor pregnancies and transfusions was not checked. Although many factors contribute to the severity, there is a possible that our study supports these earlier findings, showing a higher percentage of cGVHD-associated dry eye

progressing to the severe form in male recipients of bone marrow from a female donor.

We also pooled the groups to compare all gender-matched pairs with all gender-mismatched pairs. We found the incidence of dry eye in the gender-mismatched pairs to be slightly higher than in gender-matched pairs, although the difference was not significant (Figure 3). This finding also agrees with previous studies in the medical literature, in which the occurrence of GVHD in gender-mismatched pairs was higher than in gender-matched pairs.[5] However, some other reports have not shown a significant difference in GVHD incidence between gender-mismatched and gender-matched pairs.[24,26] In this study, we found no significant differences in patient age in these two groups, ruling out age as a contributing factor. However, the diagnosis, stage of the disease, and genetic relatedness between the donor and the recipient, which we did not take into account, all seem to affect the incidence of GVHD.

The only significant difference was the percentage of patients with dry eye who developed severe dry eye, and this difference reached significance only in the group III *vs* group IV comparison. However, patient refusal of the Schirmer test with nasal stimulation may have distorted the data from this test (Table 3). We therefore, double checked the severity level of dry eye according to the DEWS report 2007.

Our study indicates that there was a significant association between severe dry eye and female to male BMT. We are unaware of any previous report on the severity of dry eye correlating with a gender-donor mismatch, although we searched PubMed and MEDLINE reviews. Until now, little attention has been paid to the possible risk for severe dry eye after HSCT associated with donor–recipient gender mismatch. Our study indicates that careful observation is needed for male BMT recipients of female donors. As dry eye well reflects systemic cGVHD, suggesting that dry eye could be a hallmark of cGVHD. As severe dry eye can lead to blindness and poor QOL, early detection to prevent dry eye progression is important.

In this study, we investigated the effect of donor–recipient mismatch on dry eye and severe dry eye incidence, without taking into consideration the relatedness of donor and recipient, previous donor pregnancies, or the recipient's disease diagnosis or stage. Further study will be required to understand the relative impact of these various factors, and will lead to a better understanding of cGVHD.

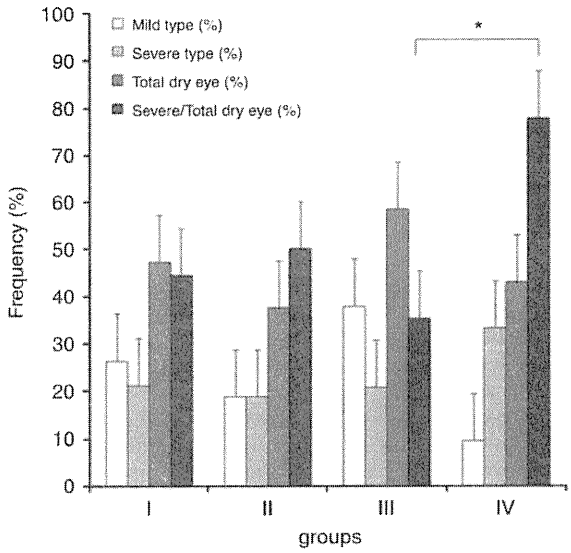

Figure 2 Comparison of the frequency of various type dry eye among four groups. The patients with dry eye that became severe was greatest in group IV (77.8%), and the incidence was significantly higher than in group III (35.3%) (*$P = 0.0375$). OR was 7.6 (95% confidence interval: 1.00–101.01). The severity of dry eye was more prevalent in group IV more than group III.

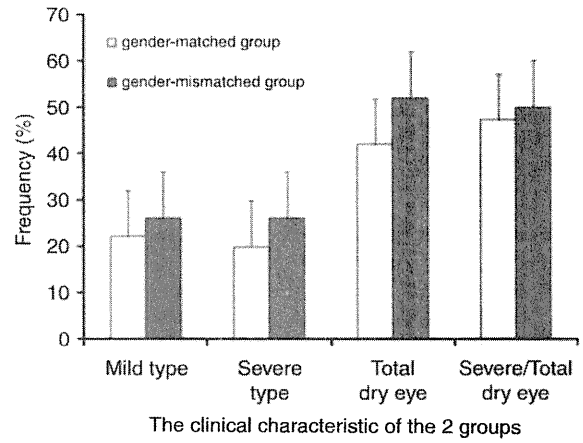

Figure 3 Comparison of the frequency of various type dry eye between gender-matched and gender-mismatched groups. There were no significant differences between gender-matched and gender-mismatched group. However the incidence rate of total, mild, and severe dry eye was slightly higher in gender-mismatch group.

Summary

What was known before

- It is generally known that a donor–recipient gender mismatch in HCST often leads to severe systemic GVHD. In particular, GVHD in male recipients of female donors tends to be especially severe.

What this study adds

- So far, no report on donor–recipient gender mismatch affecting ocular GVHD has been published in the ophthalmologic literature. Our study indicates that there was a significant association between severe dry eye and female to male BMT.