There were 3 cases of bullous pemphigoid, 1 pemphigus vulgaris, and 2 PF. Five cases showed muscle weakness, and only our case was diagnosed with CADM. The interval between the onset of DM and ABD was 2 weeks to 4 years. All cases with skin eruptions showed Gottron's papules and facial erythema. The facial eruption was not described as a heliotrope rash; however, details such as the distribution were not reported in most cases. Facial erythema was observed in 67% of DM cases (8). Therefore, facial erythema excluding heliotrope rash may have an association with the induction of ABD Abs.

While the Ab of DM was unknown in 5 of the reported cases, anti-NXP2 Ab was identified in our case. Anti-NXP2 Ab, formerly known as anti-MJ Ab, is one of the myositis-specific Abs and can be found in juvenile DM patients with cutaneous calcinosis. In contrast, only limited cases with anti-NXP2 Ab have been reported in adult DM patients. Recently, it was reported that anti-NXP2 Ab was found in 1.6% of adult DM patients and associated with malignancy (2). In our case, malignancy and calcinosis have not been detected for 6 years.

We speculate that the coexistence of DM and ABD is not coincidental. It has been suggested that the Abs of ABD are caused by epidermal damage resulting from DM (5). In our case, anti-NXP-2 Ab was detected. NXP-2, also known as MORC3 (microrchidia family CW-type zinc finger 3), which is one of the MORC-family nuclear proteins, has specific RNA binding function and plays important roles in various nuclear functions, including RNA metabolism and maintenance of nuclear architecture. NXP-2 is localised in the nucleus, distributed to the nuclear matrix, and ubiquitously expressed (9). NXP-2 also regulates the activity of tumour suppressor protein, p53, and its localisation into promyelocytic leukaemia-nuclear bodies (10). Although NXP-2 is reported to have a possible role in SUMO (small ubiquitin-like modifier)-mediated transcriptional repression and the SUMO pathway may play a potential role in the pathogenic mechanisms of DM (11), the exact pathogenic role of NXP-2 in DM is still unclear. One possibility concerning the association between anti-NXP-2 Ab and anti-Dsg Ab is that DM and pemphigus can be linked to malignancy because anti-NXP-2 Ab is reported to be associated with malignancy (2). However, the clinical presentation of our patient does not preempt paraneoplastic pemphigus, and malignancy has not been found. Moreover, ABD followed the onset of DM in all reported cases, and there was only one case of ABD associated with polymyositis, another idiopathic inflammatory myopathy (12) (Table SI[1]). Based on the these results, the occurrence of anti-Dsg Ab seems to be more likely associated with constant epidermal damage in DM than with anti-NXP-2 Ab.

Regarding the Abs following epidermal damage, it has been reported that circulating Abs for the basement membrane zone were detected by immunoblot in 24% of sera from graft-versus-host disease (GVHD) patients after haematopoietic cell transplantation (HCT) (13). However, the types of ABD after GVHD and DM do not appear to be similar. Although almost all reported cases of ABD after HCT were subepidermal blistering diseases (14), half of the cases of ABD after DM were pemphigus (Table SI[1]). Further investigations including those on DM Abs are needed to clarify the association between ABD and DM.

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Review

T helper subsets in Sjögren's syndrome and IgG4-related dacryoadenitis and sialoadenitis: A critical review

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ABSTRACT

IgG4-related disease (IgG4-RD) is a systemic disease characterized by the elevation of serum IgG4 and infiltration of IgG4-positive plasma cells in multiple target organs, including the pancreas, kidney, biliary tract and salivary glands. In contrast, Mikulicz's disease (MD) has been considered a subtype of Sjögren's syndrome (SS) based on histopathological similarities. However, it is now recognized that MD is an IgG4-RD distinguishable from SS and called as IgG4-related dacryoadenitis and sialoadenitis (IgG4-DS). Regarding immunological aspects, it is generally accepted that CD4+ T helper (Th) cells play a crucial role in the pathogenesis of SS. Since it is well known that IgG4 is induced by Th2 cytokines such as interleukin (IL)-4 and IL-13, IgG4-DS is speculated to be a unique inflammatory disorder characterized by Th2 immune reactions. However, the involvement of Th cells in the pathogenesis of IgG4-DS remains to be clarified. Exploring the role of Th cell subsets in IgG4-DS is a highly promising field of investigation. In this review, we focus on the selective localization and respective functions of Th cell subsets and discuss the differences between SS and IgG4-DS to clarify the pathogenic mechanisms of these diseases.

Sjögren's syndrome (SS) is an autoimmune disease characterized by lymphocytic infiltration into the salivary and lacrimal glands with concomitant autoantibody production and destruction of the glandular tissue. Patients typically experience symptoms of dry mouth (xerostomia) and dry eyes (keratoconjunctivitis sicca). Because of its characteristic lymphocytic infiltration and destruction of the salivary and lacrimal glands, SS is considered to be an ideal disease for studying patterns of cytokine production at the site of organ-specific autoimmune damage [1]. SS occurs alone as primary SS, or as secondary SS when underlying other connective tissue diseases [2]. Immunohistochemical studies demonstrated that the salivary glands are predominantly infiltrated by CD4+ T helper (Th) cells at an early stage of SS, and these cells are therefore thought to play a crucial role in the induction and/or maintenance of the disease [3]. In advanced stage, B cells predominate and these infiltration extends to occupy the acinar epithelium and further progress to hypergammaglobulinemia and B cell lymphoma [4]. Recent studies have suggested a central role of the epithelium in orchestrating the immune reaction by expressing HLA antigens, adhesion and costimulatory molecules, cytokines, and chemokines. Therefore, SS has been proposed as an etiological term "autoimmune epithelitis" [4–7], and it is of interest to examine the involvement of interaction between CD4+ Th cells and the epithelium in the initiation and progression of the disease process. Th cell populations comprise functionally distinct subsets characterized by specific patterns of cytokines and transcription factors. At least six Th subsets exist: Th0, Th1, Th2, Th17, regulatory T (Treg), and follicular helper T (Tfh) cells [8], which are suggested to be involved in the pathogenesis of SS [9–12].

On the other hands, Mikulicz's disease (MD) has been considered to be a subtype of SS based on histopathological similarities between the two diseases [13]. However, MD has a number of differences compared with typical SS including: 1) difference of gender distribution (MD occurs in both men and women, while SS occurs mainly in women); 2) persistent enlargement of lacrimal and salivary glands; 3) normal or mild salivary secretion dysfunction; 4) good responsiveness to corticosteroid treatment; 5) hypergammaglobulinemia and low frequency of anti SS-A and SS-B antibodies by serological analyses; and 6) multiple GC formation in

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glandular tissue (Table 1). Previously, we reported that SS was characterized by periductal lymphocytic infiltration with atrophy or severe destruction of the acini, while MD showed non-periductal lymphocytic infiltration with hyperplastic GCs and mild destruction of the acini (Fig. 1) [14]. Fifteen of 66 patients with SS (23%) and 12 of 20 patients with MD (60%) showed ectopic GC formation in labial salivary glands (LSGs). Patients with MD showed a significantly higher frequency, higher number and larger size of GCs compared with SS patients [15]. In addition, Yamamoto et al. [16–18] reported that patients with MD had elevated levels of serum IgG4 and infiltrating IgG4-positive plasma cells in the gland tissues. Similar findings have been observed in autoimmune pancreatitis (AIP) [19], sclerosing cholangitis [20], tubulointerstitial nephritis [21], Ridel's thyroiditis [22] and Küttner's tumor [23]. These diseases are now referred to as IgG4-related disease (IgG4-RD) [24,25]. We recently described the concept of IgG4-RD and provided up-to-date information regarding this emerging disease entity [26]. Recent studies have referred to MD as IgG4-related dacryoadenitis and sialoadenitis (IgG4-DS) [15,27] (Table 2).

IgG4 molecules are symmetrical homobivalent antibodies that can exchange half-molecules (heavy and light chain) specific for two different antigens ("Fab-arm exchange"), which results in losing the ability to cross-link antigens and to form immune complexes [28]. In addition, IgG4 also can bind the Fc fragment of other IgG molecule, particularly other IgG4 molecules ("Fc–Fc interactions"). These IgG4 Fc–Fc interactions proceed to Fab-arm exchange reaction and may contribute to the anti-inflammatory activity, which includes a poor ability to induce complement and cell activation caused by low affinity for C1q (Fig. 2) [29]. Another characteristic is that IgG4 is a Th2-dependent immunoglobulin and has low affinity for its target antigen. interleukin (IL)-4 directs naive human B cell immunoglobulin isotype switching to IgG4 and IgE production [30]. We previously reported that peripheral CD4+ Th cells from patients with IgG4-DS revealed a deviation in the Th1/Th2 balance to Th2 and elevated expression of Th2-type cytokines [15,31,32]. Therefore, IgG4-DS is suggested to have a Th2-predominant phenotype. This review article will emphasize recent studies seeking to understand the role of Th cell subsets in primary SS and IgG4-DS.

1. Cytokine profiles of CD4+ Th cells

1.1. Th1/Th2 paradigm

Th1 cells support cell-mediated immunity and produce IL-2, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α, which induce inflammatory responses responsible for killing intracellular parasites and perpetuating autoimmune responses. However, excessive inflammatory responses can lead to uncontrolled tissue damage. Th2 cells produce IL-4, IL-5, and IL-13, which provide help for humoral immunity and promote IgE secretion and eosinophilic responses. Th2 responses can counteract Th1-mediated microbicidal action. Thus, the Th1/Th2 balance plays an important role in immunoregulation. In contrast, Th0 cells are characterized by the production of both Th1 and Th2 cytokines and are considered precursors of Th1 and Th2 cells. Several studies have revealed that autoimmune diseases are caused by disruption to the Th1/Th2 balance [33,34]. The relationship of Th1/Th2 imbalance to the pathogenesis of SS has been widely investigated. Polarized Th1 responses were associated with the immunopathology of SS [9]. High numbers of IFN-γ-positive CD4+ T cells were detected in the salivary glands of SS patients and intracellular cytokine analysis demonstrated the polarization of Th cells to a Th1 phenotype [35]. Furthermore, we reported that IL-2 and IFN-γ were consistently detected in all SS patients, while IL-4 and IL-5 were only detected in patients with high levels of B cell accumulation in the salivary glands [10,36]. Recently, Theander et al. [37] reported that the detection of GC-like structures (B cell accumulation) in LSG biopsy specimens from primary SS patients could be used as a highly predictive and easy-to-obtain marker for B cell lymphoma development. Taken together, these studies suggest that Th1 cytokines are essential for the induction and/or maintenance of SS, whereas Th2 cytokines may be involved in disease progression, especially local B cell activation. Our clinical data was demonstrated that Th1 and Th2 cytokine concentrations were significantly higher in saliva from SS patients than from controls, and the levels of Th2 cytokines were closely associated with increased lymphocytic accumulation in LSGs. Thus, the measurement of cytokines in saliva may be useful for diagnosis and to reveal disease status [12].

IgG4-DS patients frequently have a history of bronchial asthma and allergic rhinitis with severe eosinophilia and elevated serum IgE levels [38]. It is well known that allergic immune responses are induced by allergen-specific Th2 cytokines, such as IL-4 and IL-13, which promote the secretion of IgG4 and IgE by B cells [39]. Recent studies indicated that Th2 immune reactions contributed to IgG4-DS [15,32,40] and IgG4-related tubulointerstitial nephritis [31,41]. The expression profile of cytokines suggested that IgG4-DS was characterized by a deviation of the Th1/Th2 balance to a Th2 phenotype and elevated expression of Th2 cytokines. Contrary to our results, Ohta et al. [42] reported a strong predominance of Th1 and cytotoxic type 1 cells in the salivary glands from IgG4-DS patients. They concluded that disruption of the Th1/Th2 balance might be due to differences in the specimens examined or the severity of the disease.

Chemokines are important for leukocyte activation and chemotaxis. Interactions between chemokines and chemokine receptors promote the selective local infiltration of specific cells into inflamed areas. Furthermore, chemokines are intimately involved in maintenance of the Th1/Th2 balance and immune responses in cardiac allograft rejection [43], atopic keratoconjunctivitis [44], and cutaneous lupus erythematosus [45]. Chemokines also play a key role in lymphoid neogenesis in target organs [46]. Immunohistochemical staining in our studies indicated that Th2-type chemokines including macrophage-derived chemokine (MDC)/CCL22 and thymus and activation regulated chemokine (TARC)/CCL17, natural ligands for CCR4 on Th2 cells, were detectable in and around the ductal epithelial cells and GCs, while CCR4 was expressed on infiltrating lymphocytes in LSGs in both SS and IgG4-DS patients. Thus, interactions of CCR4 with MDC and TARC may play a critical role in the accumulation of Th2 cells and subsequently, the progression of SS and IgG4-DS [12,32]. In contrast, interferon gamma induced protein 10 (IP-10)/CXCL10, natural ligand for CXCR3 on Th1 cells, was detected in and around the ductal epithelial cells, while CXCR3 was only expressed on infiltrating lymphocytes in LSGs from SS patients [47].

Table 1
Clinical and laboratory findings of Sjögren's syndrome (SS) and IgG4-related dacryoadenitis and sialoadenitis (IgG4-DS). § IgG4 positive plasma cells/IgG positive plasma cells >50%.

	SS	IgG4-DS
Peak age of onset	40's and 50's	60's
Sex	Male ≪ Female	Male ≦ Female
Salivary secretion dysfunction	Moderate or severe	None or mild
Glandular swelling	Recurrent	Persistent
Sialography	Apple-tree sign	Parenchymal defect
$IgG4^+$ plasma cell infiltration§	Positive	Negative
Serum IgG	Often high	High
Serum IgG4	Normal	High
Serum complement	Normal	Often low
Anti SS-A/SS-B antibody (+)	High rate	Rare
Antinuclear antibody (+)	Often	Rare

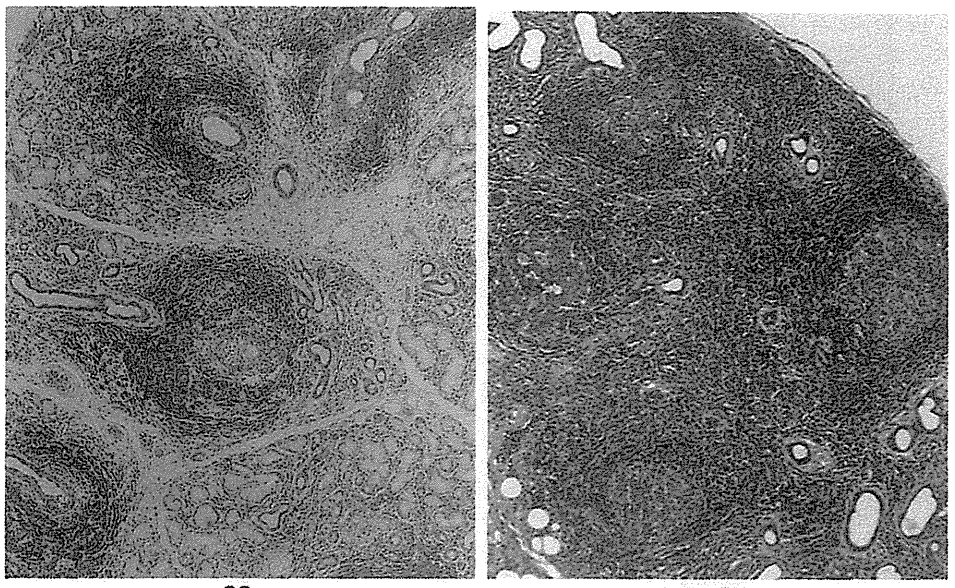

Fig. 1. Histopathological findings in salivary glands from patients with Sjögren's syndrome (SS) and IgG4-related dacryoadenitis and sialoadenitis (IgG4-DS). SS is characterized by periductal lymphocytic infiltration with atrophy or severe destruction of the acini, while IgG4-DS shows non-periductal lymphocytic infiltration with hyperplastic GCs and mild destruction of the acini. Abbreviations: GC, germinal center.

1.2. Th17 cells

The Th1/Th2 paradigm was recently expanded by the identification of Th17 cells, a subset of CD4+ Th cells characterized by their ability to produce IL-17. Several studies have reported that IL-17 was detected in epithelial and infiltrating mononuclear cells in LSGs from patients with SS. In addition, Th17 cells are "tissue seeking" and intimately involved in the initiation of SS [48]. Youinou et al. [49] reported that Th17 cells orchestrate autoreactive GCs. However, Our previous data in selectively extracted lesions from LSGs by laser capture microdissection showed that the expressions of Th17-related molecules in infiltrating lymphocytes outside ectopic GCs were higher than inside ectopic GCs [36]. Interestingly, a subset of Th17/Th1 cells identified in the gut of Crohn's disease patients may co-express IFN-γ and IL-17 [50]. Both Th1 and Th17 cells were involved in the pathogenesis of SS [51], and the early induction of a CD4+ Th1/Th17 pathway caused the systemic release of IL-17 in mice [52]. Our previous data suggest that both Th1 and Th17 cells present around the ductal epithelial cells might be of critical importance in the initiation of SS. Furthermore, the destruction of epithelial by Th1 and Th17 cells are thought to play an important pathogenetic role by the occurrence of infiltrating lesions in various epithelial tissues as well as the increased epithelial expression of various immunoactive molecules. Thus, SS has been described as "autoimmune epithelitis" [6]. In contrast, Th17-related molecules were rarely expressed in patients with IgG4-DS [32,36]. As mentioned above, IgG4-DS showed non-periductal lymphocytic infiltration and mild destruction of the epithelial cells. These findings were speculated that IgG4-DS might be a "non- autoimmune epithelitis".

Table 2
Role of Th subsets in IgG4-related disease (IgG4-RD). Abbreviations: Th, T helper; MD, Mikulicz's disease; AID, activation-induced cytidine deaminase; LSG, labial salivary gland; Tc1, T cytotoxic type 1; Tfh, follicular helper T; NLR, nucleotide-binding oligomerization domain-like receptor; TLR, Toll-like receptor; AIP, autoimmune pancreatitis; BAFF, B-cell activating factor belonging to the tumor necrosis factor family; APRIL, a proliferation-inducing ligand; Treg, regulatory T; TGF-β, transforming growth factor β.

Principal findings	Reference
Overexpression of IL-21 by Th2 cells play a key role in germinal center formation and IgG4 production in IgG4-DS.	[15]
Peripheral CD4+ T cells from the patient with MD reveal the deviation of the Th1/Th2 balance to Th2.	[31]
Th2 and regulatory immune reactions play a key role of IgG4 production in MD.	[32]
The production of IgG4 antibodies appears to be driven in part by Th2 cytokines that mediate allergic responses and IgE production.	[38]
Th2 cells are involved in the pathogenesis of IgG4-related lacrimal gland enlargement.	[39]
Overexpressions of IL-10, TGF-β, and AID in LSGs play important roles in the pathogenesis of IgG4-RD, such as IgG4-specific class-switch recombination and fibrosis.	[81]
IgG4-related tubulointerstitial nephritis shows amplification of IL-10 and TGF-β.	[41]
Th1 and Tc1 cell populations and IL-17 expression are involved in the mechanism of pathogenesis of IgG4-related sclerosing sialadenitis.	[42]
IgG4-related interstitial nephritis shows Tfh cells in enhancing a skewed B-cell terminal maturation and of CD20 + B cells in disease progression.	[66]
Activation of NLR and TLR in monocytes from AIP patients induces IgG4 production by B cells.	[76]
BAFF and APRIL are useful markers for predicting disease activity in IgG4-RD.	[78]
The progression and induction of AIP was supported by increased memory Treg and Th2 immune responses.	[80]

1.3. Regulatory T cells

Treg cells, identified by the expression of Foxp3, are essential for the maintenance of immunological self-tolerance and immune homeostasis to prevent the development of various inflammatory diseases. It achieves this either by direct contact with effector immune cells and/or by secreting anti-inflammatory cytokines such as IL-10 and transforming growth factor (TGF)-β. Treg cells exert their effects through the modulation of both T and B cell responses. Two subsets of Treg cells, CD4+ CD25+ Foxp3+ Treg cells [53] and IL-10–producing Tr1 cells [54] are crucial for regulating effector T cell functions. CD4+ CD25+ Foxp3+ Treg cells can prevent

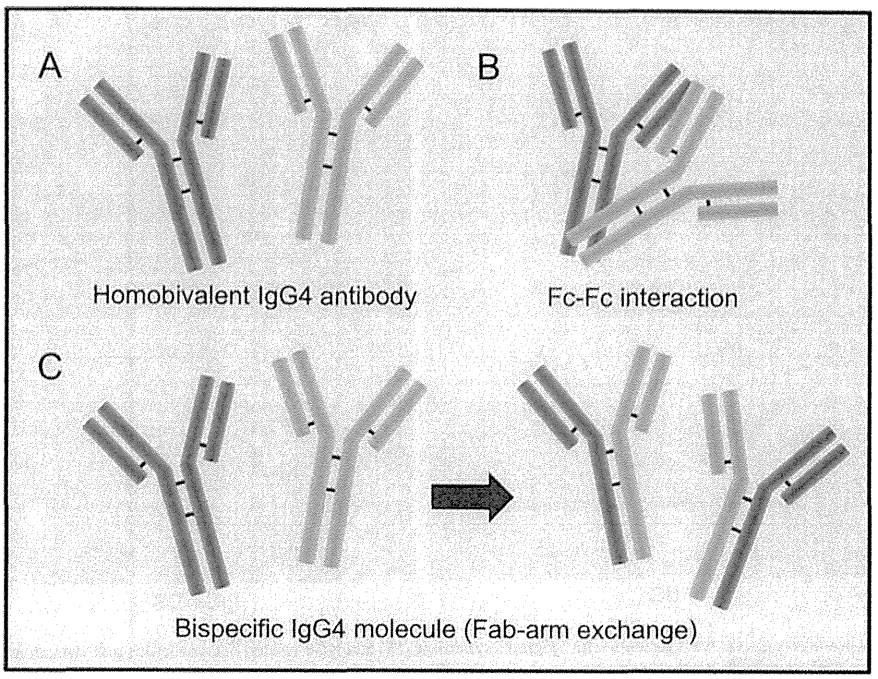

Fig. 2. Unique structure of IgG4 antibody. A, IgG4 antibody consists of two heavy chains and two light chains. B, Fc fragment of IgG4 can interact with the Fc fragment of another IgG4 molecule. C, Exchange of half-molecules (Fab-arm exchange) results in IgG4 combining two different specificities in a single molecule (bispecific antibody).

autoimmune hepatitis and primary biliary cirrhosis [55]. Mice with defects in Treg cell generation often develop T cell-mediated systemic autoimmune responses that affect multiple organs. Kolkowski et al. [56] demonstrated that salivary glands in SS constitutively expressed IL-10 and TGF-β. Other studies reported a significant reduction of Tregs in LSGs and peripheral blood from SS patients that might be involved in the pathogenesis of salivary gland destruction [57,58]. In contrast, Gottenberg et al. [59] reported increased Treg cell numbers in the peripheral blood of SS patients. Therefore, it is unclear whether Tregs are involved in the pathogenesis of SS. According to recent data, Foxp3+ T-regulatory cell frequency in the salivary glands of SS patients correlates with inflammation grade and certain risk factors for lymphoma development [60]. While in early and moderate infiltrations a compensatory control of Tregs in response to Th17 expansion seems to occur, in advanced SS lesions Tregs may fail to control the immune mediated tissue injury [7,61]. Increased levels of Treg cells in salivary glands from SS patients might suggest negative feedback is more active than in healthy subjects. Therefore, Treg cells might be not involved in the initiation of disease.

Zen et al. [62] reported that significant numbers of CD4+ CD25+ Foxp3+ Tregs infiltrated the affected tissues in cases of autoimmune pancreato-cholangitis (AIPC), which is one of IgG4-RD. Furthermore, another study demonstrated that IL-10 decreased IL-4-induced IgE switching but increased IL-4-induced IgG4 production [63]. We found that IL-4, IL-10, and Foxp3 were positively correlated with the IgG4/IgG ratio in the salivary glands from patients with IgG4-DS [32]. These results suggest that Th2 and regulatory immune reactions might play key roles in IgG4 production.

2. Role of IL-21 in SS and IgG4-DS

2.1. Follicular helper T cells

Tfh cells were recently identified as a unique Th phenotype, expressing high levels of CXCR5, a chemokine receptor [64]. Several studies reported that Tfh cells control the functional activity of effector Th cells and promote ectopic GC formation by IL-21, which contributed to impaired B cell differentiation [65,66]. Once GCs are formed, Tfh cells are required for their maintenance and the regulation of B cell differentiation into plasma cells and memory B cells. Several studies in SS patients demonstrated that IL-21 was increased in serum and high levels of IL-21 receptor were present on the surface of most B cells [67]. Furthermore, IL-4 and IL-21 receptors knockout mice have greatly reduced IgG responses, indicating that IL-21 co-operates with IL-4 to regulate humoral immune responses [68]. We previously observed that Tfh-related molecules, CXCR5 and B-cell lymphoma 6 protein (Bcl-6), were highly expressed on infiltrating lymphocytes in ectopic GCs of LSG lesions from both SS and IgG4-DS patients [15,36]. These results provide strong support for Tfh cells in the progression of disease as a lymphoproliferative disorder, particularly in the growth and activation of ectopic GC formation (Fig. 3).

IL-21 was mainly produced by Th2 and Th17 cells in addition to Tfh cells [68,69]. Interestingly, high IL-21 expression was only detected outside ectopic GCs in patients with IgG4-DS in our immunohistological analyses. The expression patterns of Th2-related molecules (IL-4, CCR4 and c-Maf) in LSGs were similar to that of IL-21 in patients with IgG4-DS. In contrast, Th17-related molecules were rarely expressed in patients with IgG4-DS. Furthermore, IL-21 positively correlated with the number of GCs formed in LSGs from patients with IgG4-DS [15]. Taken together, these findings suggest that excessive IL-21 production by Th2 cells in salivary glands from IgG4-DS patients might induce Bcl-6 expression in B cells resulting in multiple GC formation. Furthermore, IL-21 directly inhibited IL-4-induced IgE production [70], and IgG4 class switching was induced by co-stimulation with IL-4 and IL-21 in humans and mice [71]. In addition, IL-21 induced IL-10 production by mitogen-stimulated peripheral blood mononuclear cells in humans [72]. Therefore, we speculate that IL-21 correlates with IL-4 and IL-10 for IgG4 class switching. In the current study, we found that IL-21 positively correlated with the IgG4/IgG ratio in immunohistochemically positive cells

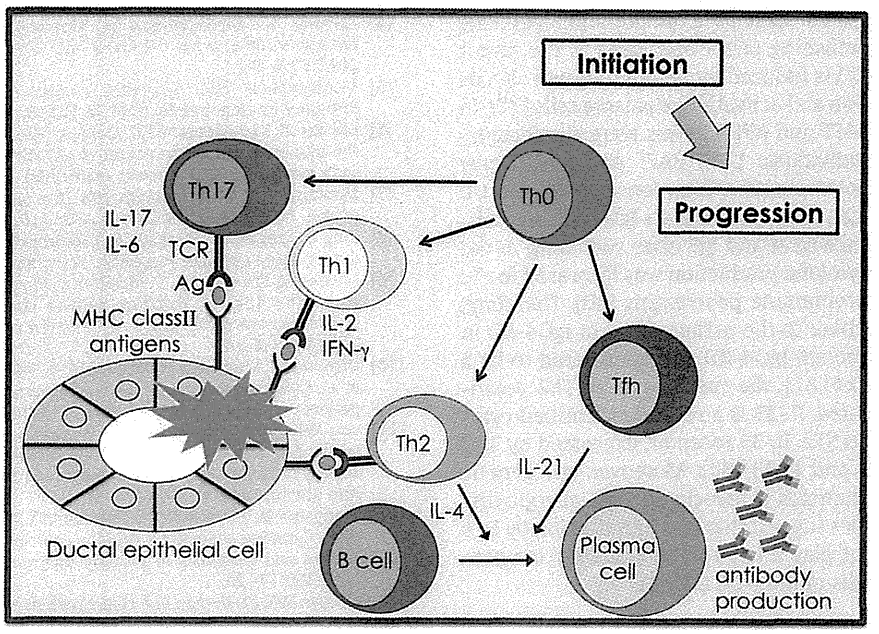

Fig. 3. Schematic model of Th cell network in SS. Th1 and Th17 cells are involved in early stages of disease, while Th2 and Tfh cells are associated with GC formation in the late stage. Abbreviations: Th, T helper; Tfh, follicular helper T.

[15] suggesting that IL-21 might also be involved in the class switching of IgG4 in IgG4-DS [73].

2.2. Innate immunity in IgG4-DS

Macrophages act as cells in the immune response to foreign invaders of the body, by presenting pathogenic antigens to antigen-specific Th cells. Historically, they have been classified into two distinct macrophage phenotypes, "classically activated" proinflammatory (M1) and "alternatively activated" anti-inflammatory (M2) macrophages [74]. M2 macrophages are activated by IL-4, produce high levels of IL-10 and are important for debris scavenging, wound healing and fibrosis. These polarized macrophage populations can also contribute to systemic diseases [75]. Watanabe et al. [76] demonstrated that abnormal innate immune responses induced via Toll-like receptor signaling in macrophages might enhance Th2 immune responses and the immunopathogenesis of IgG4-RD. Our current studies observed that IgG4-DS patients showed predominant infiltration by M2 macrophages that secreted IL-10 and IL-13 in salivary glands.

Dendritic cells (DCs) are professional antigen presenting cells that bridge innate and adaptive immunity. Expression of

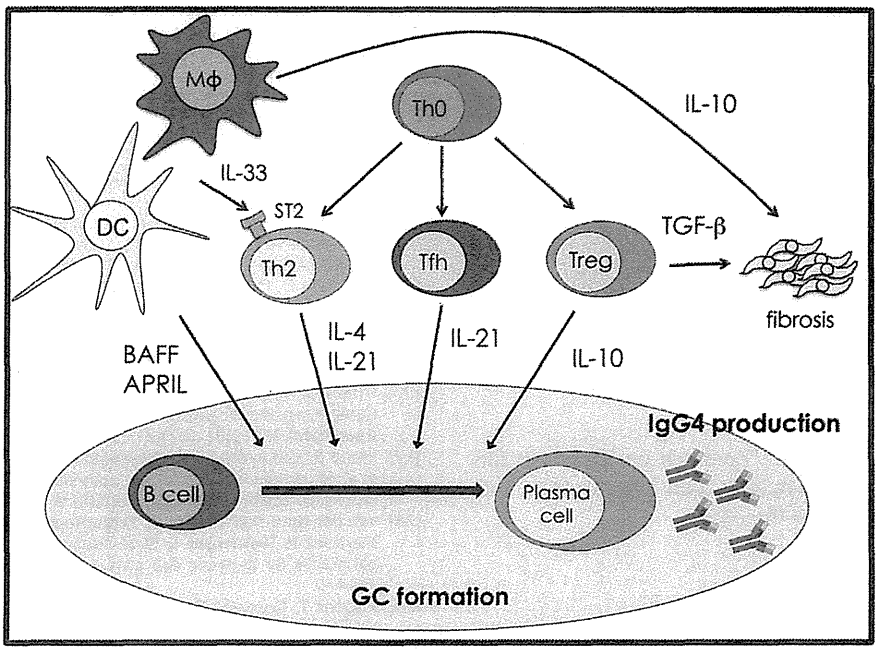

Fig. 4. Schematic model of Th cell and innate immune network in IgG4-DS. Th2, Treg, and Tfh cells play key roles in GC formation and IgG4 production. Dendritic cells and macrophages promote Th2 immune reaction by IL-33 as well as BAFF and APRIL. Abbreviations: Treg, regulatory T; BAFF, B cell activating factor belonging to the tumor necrosis factor family; APRIL, a proliferation-inducing ligand.

DC-derived TNF-family ligands such as a proliferation-inducing ligand (APRIL) and B cell activating factor belonging to the tumor necrosis factor family (BAFF) is induced by innate immune signals to promote the differentiation and activation of plasma cells [77]. In IgG4-RD patients, serum BAFF and APRIL levels were significantly higher than in healthy individuals [78]. BAFF and APRIL may contribute to progressive plasmacyte infiltration and ectopic GC formation in the target organs of patients with IgG4-RD. In addition, BAFF and APRIL enhance IgG4 and IgE class switching in the presence of IL-4 [79]. Th2 cytokine production was increased in the tissues of patients with autoimmune pancreatitis [80]. Therefore, BAFF and APRIL may contribute to the pathogenesis of IgG4-RD in concert with Th2 cells. Although IgG4-RD was considered to be a Th2-dependent disease [40,41,81], the mechanism of Th2 polarization has yet to be elucidated. IL-33 is a recently identified cytokine that directly stimulates ST2, IL-33 receptor, expressed by Th2 cells to produce IL-4, IL-5, and IL-13 [82]. Moreover, the genetic polymorphism of IL-33 in humans is associated with allergic diseases [83]. Our current studies suggest that IL-33 production by DCs and M2 macrophages might play a key role in Th2 cytokine production and the pathogenesis of IgG4-DS (Fig. 4).

3. Conclusions

Research accumulated in recent years makes it increasingly clear that the immunological backgrounds are entirely different between SS and IgG4-DS. However, additional research is required to elucidate further the pathogenesis of IgG4-DS, especially the development of a mouse model of IgG4-DS. Although Glucocorticoids are the standard treatment for IgG4-RD, Yamamoto et al. [84] reported that the relapse rate of IgG4-DS during steroid therapy is 26.8%. A more thorough understanding of the complex mechanisms of IgG4-DS, especially the role of Th subset-related cytokines, could lead to the development of novel pharmacological strategies aimed at disrupting the cytokine network and inhibiting the initiation and/or progression of IgG4-DS. Finally, it should be noted that while this thesis focuses primarily on T cells, that there have recently been other extensive reviews and hypotheses published on Sjogren's syndrome, reflecting its increased interest not only to basic immunologists, but also to rheumatologists [4,85–116].

Competing interests

The authors declare no competing interests.

Author contributions

All authors provided substantial contributions to discussions of content, and to reviewing and editing the manuscript before submission. M Moriyama researched the data and wrote the article.

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Original article

Ultrasonography as an additional item in the American College of Rheumatology classification of Sjögren's syndrome

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Abstract

Objective. In this study we evaluated US as an additional classification item in the ACR classification of SS.

Methods. Of 581 patients classified as either SS ($n=364$) or non-SS ($n=217$) based on the minimum requirements of the American–European Consensus Group (AECG) classification, 184 patients (102 SS and 82 non-SS) who had scored two or more positive or two or more negative results according to the ACR criteria were selected. The AECG classification was used as the gold standard. A parotid and/or submandibular gland that was assigned a score $\geqslant$G1 was designated as SS positive. We evaluated US alone or with varying combinations of the ACR classification items in the diagnosis of SS.

Results. The ACR criteria diagnosed the 184 patients with 91% sensitivity, 90% specificity and 91% accuracy. US alone diagnosed the 184 ACR patients with 79% sensitivity, 90% specificity and 83% accuracy, which was comparable to the results of US diagnosis in the AECG cohort (81%, 86% and 83%, respectively). Incorporating the US criteria as an alternative to one of the three ACR classification items achieved 89–91% sensitivity, 87–96% specificity and 89% or 92% accuracy, which was comparable to that of the original ACR classification. Furthermore, kappa analysis indicated that the results of the original ACR and US-replaced ACR classifications matched completely ($\kappa=0.960$–0.974).

Conclusion. These results suggest that US can be used as an alternative to any of the three ACR classification items.

Key words: Sjögren's syndrome, diagnosis, classification, criteria, ultrasonography, imaging.

Introduction

SS is a chronic inflammatory autoimmune disorder that mainly affects the exocrine glands. The involved glands are characterized histologically by focal lymphocytic infiltration with the resultant gland dysfunction causing dry mouth and/or dry eyes (sicca symptoms). In 1993 the Preliminary European Classification (PEC) criteria for SS were proposed; however, these criteria contained several inherent shortcomings (e.g. the PEC criteria were met in patients without positive results for autoantibodies or labial gland biopsy) [1]. Therefore, in 2002, revised criteria, known as the American-European Consensus Group (AECG) criteria, were proposed [2]. The AECG criteria have been used as the gold standard for diagnosing SS patients and investigating the pathophysiological abnormalities of SS patients.

The ACR recently published new criteria that include three objective measures known to have specificity in the diagnosis of SS: lymphocytic infiltrations in lip biopsy specimens, serum tests for anti-SSA/SSB autoantibodies or ANA and RF and an ocular staining test [3]. However, the ACR classification excludes imaging tools and sicca symptoms and signs. Therefore patients not identified as having salivary gland involvement can be diagnosed with SS. Conversely, patients presenting with

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ocular or oral sicca complaints, positive results for serum autoantibodies or reduction in salivary flow rate—who would certainly be diagnosed with SS clinically by a rheumatologist—do not meet the ACR criteria [4]. Therefore a further validation of the provisional ACR classification criteria may be needed to reach a final agreement on the classification criteria for SS.

US is a non-invasive and inexpensive imaging tool that has been demonstrated to be useful for discriminating between patients with and without SS who are diagnosed based on the AECG criteria [5]. In addition, multivariate logistic regression analysis has shown that only US and sialography are significantly correlated with a positive diagnosis of SS and that US is as effective as sialography in differentiating between patients with and without SS [6]. In this context, several investigators have proposed the use of US to diagnose patients with SS [7, 8]. However, the notion that US can be used as an additional item in the ACR classification criteria has not yet been thoroughly investigated.

Therefore, in the present study we have retrospectively tested whether US can be used as an additional item in the ACR classification of patients with SS. To this end, using a large cohort of SS and non-SS patients classified according to the AECG criteria, we evaluated the usefulness of US as an additional item in the ACR classification system.

Patients and methods

Patients

We retrospectively reviewed the medical records of 2039 patients who presented with clinical symptoms suggestive of SS between March 1993 and March 2013 and underwent diagnostic imaging for the assessment of gland disease in our hospital. Of these, 1956 patients underwent US of the parotid and submandibular glands and, of these, 581 fulfilled the minimum requirements for diagnosing primary/secondary SS or non-SS based on the AECG criteria. Accordingly, these patients were either (i) diagnosed with SS based on the AECG criteria (SS group) or (ii) underwent three or more of the objective (oral, ocular, serological and pathological) examinations but did not fulfil the AECG criteria (non-SS group). The primary study cohort comprised 364 patients with SS [337 women, 27 men; average age 56 years (S.D. 15); 243 primary SS and 121 secondary SS] and 217 non-SS patients [175 women, 42 men; average age 57 years (S.D. 15)]. The co-morbid autoimmune diseases present in patients with secondary SS and non-SS patients are listed in Table 1. These 581 patients were then further categorized into SS and non-SS groups according to the ACR classification system. Consequently 184 patients were selected as those with two or more positive or two or more negative results according to the ACR criteria (the second study cohort that was compatible with the ACR classification). This cohort comprised 117 SS patients [109 women, 8 men; average age 57 years (S.D. 14)] and 67 non-SS patients [50 women, 17 men; average age 58 years (S.D. 14)]. The study was approved by the Nagasaki University Hospital Ethics Committee; written informed consent was waived because of the retrospective nature of the study.

TABLE 1 Characteristics of 581 SS and non-SS patients

Patient group	Co-morbid autoimmune disorders	n
SS		364
Primary		243
Secondary		121
	RA	44
	SLE	34
	SSc	18
	CREST syndrome	6
	Mixed CTD	6
	aPL syndrome	6
	Overlap syndrome	3
	Multiple sclerosis	2
	Progressive myositis	2
Non-SS		217
	RA	24
	SLE	20
	SSc	11
	Mixed CTD	5
	CREST syndrome	3
	Progressive myositis	2
	aPL syndrome	1
	Multiple sclerosis	1
Total		581

CREST syndrome: calcinosis, RP, oesophageal dysmotility, sclerodactyly and telangiectasia syndrome.

Clinical examinations and laboratory tests

Subjective symptoms were considered positive if a patient had complained of a dry mouth and/or dry eyes in the past 3 months. Objective symptoms were evaluated using an unanaesthetized Schirmer's test and/or stimulated Saxon's test. A positive result for the unanaesthetized Schirmer's test was $\leqslant$5 mm/5 min and a positive result for the stimulated Saxon's test was $\leqslant$2 g/2 min; a positive titre for ANA was $\geqslant$1:320 and a positive score for ocular staining was $\geqslant$4 (Rose Bengal) or $\geqslant$3 (fluorescein). Pathological (lip biopsy) examinations were classified as positive or negative based on the AECG criteria [2].

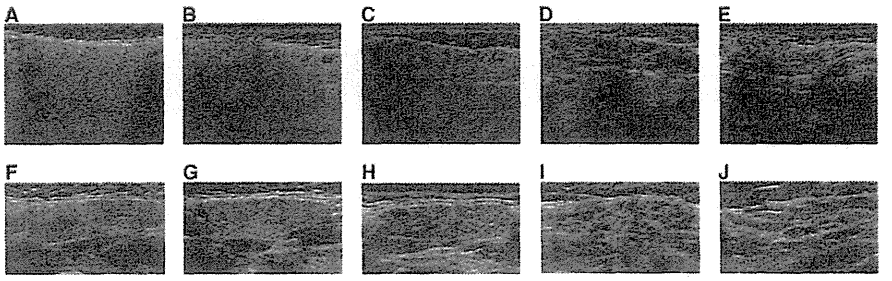
Sialographic classification of gland disease

Sialography of the parotid glands on either side of patients was performed using a non-ionizing contrast medium (Iopamiron; Schering AG, Berlin, Germany). Glands classified as being at stages G0–G4 were diagnosed as positive for SS [9]. Sialograms were categorized by a radiologist with 21 years' experience who was blinded to the clinical and US results.

US classification of gland disease

Grey-scale US of the parotid and submandibular glands was performed at 10 MHz using a Logiq 700 or a Logiq 9 with a wide bandwidth (6–14 MHz) (GE Healthcare,

FIG. 1 US classification criteria



US of the (**A–E**) parotid and (**F–J**) submandibular glands that are classified into grade 0 (G0; **A, F**), grade 1 (G1; **B, G**), grade 2 (G2; **C, H**), grade 3 (G3; **D, I**) or grade 4 (G4; **E, J**). (**A**) A 71-year-old woman without SS. (**B**) A 33-year-old woman with SS. (**C**) A 55-year-old woman with SS. (**D**) A 17-year-old girl with SS. (**E**) A 61-year-old woman with SS. (**F**) A 72-year-old woman without SS. (**G**) A 24-year-old woman with SS. (**H**) A 36-year-old woman with SS. (**I**) A 74-year-old man with SS. (**J**) A 59-year-old woman with SS.

Waukesha, WI, USA). US diagnosis of SS was based on the internal echoes of the parotid and submandibular glands and the gland contours. The gland images were analysed by two radiologists with 17 and 21 years of experience in head and neck radiology who were blinded to the diagnosis (SS or non-SS) and the results of serological, salivary flow, ocular and pathological examinations. The parotid and submandibular glands were categorized by consensus into five grades (G0–G4) as described previously: G0 glands were those with normal US features, G1 glands were those with limited distribution of multiple small and round areas of hypoechogenicity in the parenchyma, G2 glands were those with more extensive distribution of multiple round areas of hypoechogenicity in the parenchyma, G3 glands had generalized distribution of multiple round areas of hypoechogenicity with hyperechogenic bands and irregular gland margins and G4 glands had generalized distribution of multiple irregular areas of hypoechogenicity associated with hyperechoic bands and irregular gland margins (Fig. 1) [10]. A patient with a positive parotid and/or submandibular gland was diagnosed as positive for SS.

Data analysis

The level of agreement between different SS classification systems using different combinations of ACR and US criteria was assessed using κ-values. A κ-value of 0.81–1.00 was interpreted as being very good, 0.61–0.80 as good, 0.41–0.60 as moderate, 0.21–0.40 as fair and 0.00–0.20 as poor. Diagnostic ability was assessed by calculating sensitivity, specificity and accuracy.

Results

Efficacy of clinical and laboratory classification items, ACR classification and US for diagnosing SS

We first assessed the usefulness of individual classification items (sicca symptoms or signs, autoantibodies, lip biopsy, ocular stain, sialography and US) in diagnosing the 581 SS patients as SS or non-SS (Table 2). Both sicca symptoms and signs provided high sensitivity but extremely low specificity. Of the three ACR items, serum antibodies and lip biopsy yielded moderate sensitivity and specificity, but ocular stain yielded low sensitivity and moderate specificity. When combined, the ACR criteria yielded 91% sensitivity, 90% specificity and 91% accuracy in diagnosing the 184 (of 581) patients who fulfilled the ACR criteria (SS patients) or those who had two or more negative results for the classification items (non-SS patients) (Table 3).

Sialographic criteria discriminated between SS and non-SS glands with moderate sensitivity and high specificity (Table 2). US of the parotid and/or submandibular glands differentiated between SS and non-SS patients with moderate sensitivity and specificity when diagnosing SS patients as those with ⩾G1 glands and with low sensitivity and high specificity when diagnosing SS patients as those with ⩾G2 glands (Table 2).

Incorporation of US into the ACR classification system

We next evaluated the efficacy of US in the ACR classification system. Incorporation of US into the ACR classification provided very high sensitivity (98%), but the specificity was low (69%), thus the degree of accuracy was similar to that of the original ACR classification when diagnosing SS patients as those with two or more positive results (any two from lip biopsy, autoantibodies, ocular stain or US) (Table 3). When diagnosing SS patients as those with three or more positive results, the incorporation of US achieved very high specificity (99%) at the expense of sensitivity (71%), whereas the accuracy was again similar to that of the original ACR classification (87%). Differences in the gland types assessed (parotid, submandibular or parotid and/or submandibular glands) did not affect the ability to differentiate patients with SS from those without SS.

TABLE 2 Clinical and imaging profiles of 581 SS or non-SS patients diagnosed based on the AECG classification

Classification items	Patients, *n*	Diagnostic ability, %		
		Sensitivity	Specificity	Accuracy
Sicca symptoms	488	95	22	70
Sicca signs	526	95	26	69
Lip biopsy	260	89	71	83
Autoantibodies	573	81	73	78
Ocular stain	55	47	80	62
Sialography	523	71	92	79
PG and/or SMG US	581			
⩾G1		81	86	83
⩾G2		66	93	76

AECG: American-European Consensus Group; sicca symptoms: dry mouth/dry eyes; sicca signs: stimulated Saxon's test and Schirmer's test; PG/SMG US: US of the parotid and/or submandibular glands; G1: grade 1; G2: grade 2.

TABLE 3 Diagnostic abilities of different combinations of ACR and US classification items

Classification	Patients, *n*	Diagnostic ability, %		
		Sensitivity	Specificity	Accuracy
ACR	184	91	90	91
ACR + US (2/4)[a]				
PG and/or SMG	388	98	69	92
PG alone	360	98	74	93
SMG alone	386	98	70	92
ACR + US (3/4)[b]				
PG and/or SMG	291	71	99	87
PG alone	295	60	100	83
SMG alone	290	70	99	87
ACR replaced with US[c]				
Lip biopsy to US	447	89	89	89
Antibodies to US	210	91	96	92
Ocular stain to US	515	90	87	89

PG: parotid gland; SMG: submandibular gland. [a]US was added as a fourth classification item and patients were diagnosed as SS with two or more positive results for the four items. [b]US was added as a fourth classification item and patients were diagnosed as SS with three or more positive results for the four items. [c]One of the three ACR classification items was replaced with US: lip biopsy to US, lip biopsy replaced with US; antibodies to US, autoantibodies replaced with US; ocular stain to US, ocular stain replaced with US.

Incorporation of US as an alternative third ACR item

Because using US as the fourth ACR classification item did not improve the diagnostic accuracy, we next tested whether substituting US for any one of the three ACR classification items improved diagnostic accuracy. When US was incorporated into the ACR classification as an alternative third item, its diagnostic ability was comparable to that of the original ACR classification, yielding 89–91% sensitivity and 87–96% specificity (Table 3).

In the present study, because not all patients were tested for all three ACR classification items, we could not directly compare the diagnostic efficacy of different classification systems with varying combinations of ACR and US criteria. We therefore assessed interclassification agreement by analysing the κ-values for the study cohorts in which the same set of classification items was used. We found that κ-values were very high (0.961–0.974) between any combination of the original ACR and US-replaced ACR classifications, indicating that the classification results were almost completely matched (Table 4). These results, together with the similar diagnostic abilities achieved by the modified ACR criteria in which one of the three items was replaced with US criteria (Table 3), suggest that US can be a substitute for one of the ACR classification items.

Discussion

The present study demonstrated that the incorporation of US into the ACR classification system as the fourth item or

TABLE 4 Interclassification agreement

Classification	Patients, *n*	κ-value	Mismatch rate
ACR *vs* ACR (lip biopsy to US)	158	0.974	2/158
ACR *vs* ACR (antibodies to US)	156	0.960	3/156
ACR *vs* ACR (ocular stain to US)	182	0.965	3/182

ACR (lip/US), ACR (Ab/US) and ACR (ocular/US) indicate the modified ACR classification in which one of the three original ACR classification items (lip: lip biopsy; Ab: autoantibodies; ocular: ocular stain) was replaced with US.

as an alternative to one of the three ACR classification items did not improve the diagnostic accuracy of the original ACR classification for SS. However, substituting any of the three ACR classification items with US achieved sensitivity and specificity comparable to those of the original ACR classification system. These results suggest that US can be used as an additional item for any of the three items in the ACR classification.

The ACR classification was intended to simplify the AECG classification while retaining a comparable level of diagnostic accuracy [3, 11]. However, labial gland biopsy is invasive and physicians may therefore hesitate to use this method when screening for SS in xerostomia/xerophthalmia patients. This is a serious disadvantage when planning treatment for patients with symptoms or signs suggestive of early stage SS. A patient who does not fulfil the ACR criteria because a physician is reluctant to perform (or the patient is reluctant to undergo) a difficult, invasive examination cannot receive effective treatment, such as oral doses of cevimeline and pilocarpine. Therefore a non-invasive and readily accessible tool for the assessment of gland disease would be a distinct benefit for both patients and physicians. Another problem with the ACR classification is that it may misclassify patients with early stage disease; e.g. those who present with ocular and oral sicca symptoms, positive results for anti-SSA/SSB autoantibodies and decreased salivary or lacrimal flow will not be diagnosed as SS [4]. In addition, we found that the results of ocular staining were often negative in SS patients in the early stages in the present study cohort (data not shown).

US is non-invasive and inexpensive and does not involve radiation. US features of the SS glands are very simple and are characterized by altered parenchymal structures involving lymphocytic infiltration and accumulation in the gland parenchyma. In a previous study, logistic regression analysis using a large cohort of xerostomia patients with or without SS ($n=294$) indicated that among five diagnostic criteria, including sialography, US, Saxon's test, Schirmer's test and serological tests (SSA and SSB), sialography and US were independently significant for the diagnosis of SS patients [6]. In this cohort, US provided diagnostic performance comparable to that of sialography. Ariji *et al.* [10] and, more recently, Cornec *et al.* [7] have shown that the number of hypoechoic areas is a good indicator of the SS grade. Cornec *et al.* [7] further showed that the addition of salivary gland US criteria improved the diagnostic performance of the AECG classification; the addition of US to the AECG items improved sensitivity, while the specificity did not change.

Recently Vitali *et al.* [8], Bootsma *et al.* [12], and Bowman and Fox [13] proposed that US could be included as an additional item in the ACR classification. In the present study we also found that the addition of US as the fourth item in the ACR classification improved sensitivity when diagnosing SS patients with two or more positive results of the four classification items; however, the overall accuracy was not improved due to concomitant decreases in specificity. The incorporation of US improved specificity but decreased sensitivity when diagnosing SS patients with three or more positive results.

Some researchers have previously defined G2 glands as being positive for SS [7]. Early diagnosis of SS is advantageous, as physicians can then initiate treatment with drugs and/or corticosteroid irrigation with high efficacy in improving the salivary flow rate [14, 15]. In this context, we recommend using US criteria to diagnose parotid/submandibular glands classified as $\geqslant$G1 as SS positive. It should also be noted that visual differentiation between G1 and G2 glands, which is based on the number and size of hypoechoic areas, is occasionally difficult and may consequently decrease interobserver agreement. Texture analysis of US images would facilitate the routine use of US evaluation to diagnose SS [10].

Substituting one of the ACR classification items with US retains, but does not improve, the diagnostic performance of the original ACR classification. Therefore, considering US as an additional item in the ACR classification should not be expected to improve one's diagnostic ability; instead, it could replace a more painful or invasive test. In addition, ACR classification lacks the ability to grade SS patients or to evaluate treatment efficacy after oral cevimeline or pilocarpine. There are currently no specific biological markers indicating the disease states for SS glands; however, correlation of US findings in the affected salivary glands with the severity of gland disease, as estimated by sialography, may yield useful results [9].

US scoring systems for diagnosing SS patients have recently been introduced. In these systems, the parotid and submandibular glands are independently and equally scored [7, 16-18]. A previous study showed that echo heterogeneity was more frequently observed in the submandibular glands than in the parotid glands [19]. Although we did not observe any significant differences in the diagnostic accuracy of parotid gland and submandibular gland US in the present study, some SS patients had submandibular glands that were positive for SS in the absence of positive US findings in the parotid glands. Therefore an appropriate US scoring system using findings from both the parotid and submandibular glands may be useful for the accurate diagnosis of SS.

A major limitation of this study is that not all patients underwent all the objective tests for the three ACR classification items. In particular, only 9.5% (55/581) of patients underwent an ocular staining test. Furthermore, lip biopsy was performed in 44.8% (260/581) of patients. A prospective study in a large cohort of patients who underwent all the objective tests is mandatory to reach definitive conclusions concerning the usefulness of US as an additional item in the ACR classification. Another limitation of this study is that the data were collected at a single institution, and this may have further biased the results. Therefore a multicentre study is required in the future. One of the pitfalls of US diagnosis of SS glands is that some gland diseases, including sarcoidosis, HIV infection, lymphoma, juvenile recurrent parotitis and IgG4-related Mikulicz's disease, may mimic the gland disease of SS patients. However, these SS-mimicking diseases can be clinically differentiated from SS in most cases [5, 20–22]. We need more data in larger numbers of patients to address the issue of whether adding US can assist in the diagnosis of SS patients. Even then it is not clear if there will be a definite consensus on the usefulness of US and how to use it. However, the results presented in this report may be helpful in framing some of the difficulties in evaluating US for the diagnosis of SS patients.

In conclusion, the present results suggest that invasive testing (minor salivary gland biopsy) can be replaced with non-invasive testing (parotid and submandibular US) with a relatively minor impact on the quality of the final outcomes of the diagnostic criteria. Furthermore, US can be used to monitor the severity of gland disease and may therefore be useful in assessing treatment efficacy in patients with SS [15].

Disclosure statement: The authors have declared no conflicts of interest.

Rheumatology key messages

- ACR classification can diagnose American-European Consensus Group-compatible SS patients with good accuracy.
- ACR criteria may misdiagnose patients in the early stages of SS owing to limited classification items.
- US may be useful as an alternative to any of the three ACR classification items for the diagnosis of SS.

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Clinical vignette

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Popliteal cyst after yttrium-90 radiosynovectomy—usefulness of delayed PET/CT imaging

A 48-year-old woman with undifferentiated inflammatory arthritis underwent yttrium-90 (^{90}Y) radiosynovectomy of the right knee joint. A PET/CT scan of the knee performed immediately after the procedure showed good, uniform distribution of ^{90}Y activity in the articular space. There was also a slight trace of activity in a previously undiagnosed popliteal cyst (Fig. 1A).

Five days after radiosynovectomy the patient experienced mild pain in the popliteal region and upper calf. A second PET/CT scan revealed sustained, diffuse ^{90}Y distribution in the IA space and high ^{90}Y activity in the popliteal cyst (Fig. 1B). There were no other signs of extra-articular activity and the cyst was not ruptured, which was confirmed with ultrasonography. The patient was ordered additional bed rest and oral NSAIDs. The conducting of PET/CT scans after radiosynovectomy was approved by the Bioethical Committee of the Medical University of Warsaw.

Recent studies [1] have proved that it is possible to obtain good quality images of ^{90}Y-labelled radiopharmaceuticals with the PET/CT technique. Our initial experiences show that even 5 days after ^{90}Y radiosynovectomy, PET scan may be diagnostic. The combined immediate and delayed imaging is of greater clinical significance than immediate imaging alone since it can show the IA dynamics of ^{90}Y distribution. The presented imaging protocol can be a useful tool in providing accurate diagnosis of early complications after radiosynovectomy like ^{90}Y leakage or extra-articular injection.

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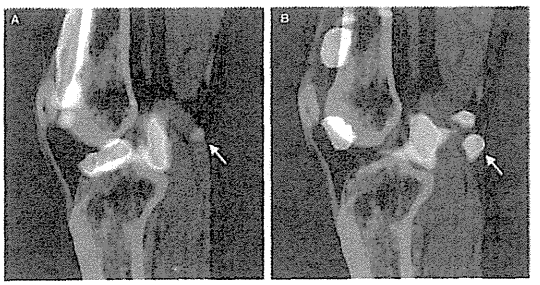
FIG. 1 Popliteal cyst (arrow) (**A**) 30 min and (**B**) 5 days after radiosynovectomy



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ORIGINAL ARTICLE

Serum interferon-α is a useful biomarker in patients with anti-melanoma differentiation-associated gene 5 (MDA5) antibody-positive dermatomyositis

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Abstract

Objective. We have tried to clarify the clinical importance of the measurement of serum type-I interferon (IFN) in patients with anti-melanoma differentiation-associated gene 5 Ab (MDA5 Ab)-positive dermatomyositis (DM).

Methods. We studied 30 patients with DM: 10 were anti-MDA5 Ab-positive and 20 were anti-MDA5 Ab-negative. At each patient's initial visit, serum IFN-α, IFN-β, interleukin 18 (IL-18), ferritin, and the titer of anti-MDA5 Ab were measured using enzyme-linked immunosorbent assays (ELISAs). The associations between the IFNs and with the other variables were examined.

Results. Rapidly progressive interstitial lung disease (RPILD) was confirmed in 10 patients, most of whom were complicated in the anti-MDA5 Ab-positive DM patients. The presence of clinically amyopathic dermatomyositis (CADM) as well as the serum concentrations of IFN-α and ferritin was significantly higher in the anti-MDA5 Ab-positive DM patients. Serum concentration of IL-18 did not differ between anti-MDA5 Ab-positive and anti-MDA5 Ab-negative groups; however, a positive correlation was found between IFN-α and IL-18 in the anti-MDA5 Ab-positive DM patients ($r = 0.8139$, $p = 0.0146$).

Conclusion. Serum IFN-α can be used as a useful biomarker in patients with anti-MDA5 Ab-positive DM, which may reflect the presence of RPILD.

Keywords

IFN-α, anti-MDA5 antibody, CADM, RPILD, Ferritin

History

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Introduction

Dermatomyositis (DM) is a group of chronic inflammatory disorders commonly involved in skeletal muscles and skin. DM is often associated with interstitial lung diseases (ILDs) and is known to cause significant organ damage, adversely affecting the prognosis of ILD patients [1,2]. Clinically amyopathic dermatomyositis (CADM) is a subgroup of DM characterized by various skin manifestations and none-to-mild muscle symptoms [3]. CADM is often complicated with rapidly progressive interstitial lung disease (RPILD), which can be treatment resistant and life threatening [3]. Therefore, the presence of RPILD is of significant importance in ILD found in DM patients, and the identification of biomarkers to identify CADM complicated with RPILD is desirable.

Regarding the clinical diagnosis of CADM complicated with RPILD, anti-CADM 140-kDa polypeptide Abs (anti-CADM-140 Abs) were found in sera from patients with CADM complicated with RPILD [4], and more recently, an RNA helicase encoded by melanoma differentiation-associated gene 5 (MDA5) was identified as a major autoantigen which is targeted by anti-CADM140 Abs [5]. MDA5 is a member of the Rig-I-like receptors (RLR) family that recognizes double-stranded RNA (dsRNA) within the cytosolic compartment and induces the production of inflammatory cytokines and cell surface molecules involved in the antiviral response [6]. In this regard, Sun et al. recently reported that interferon-alpha (IFN-α) in serum is high in CADM patients complicated with ILD; however, they did not investigate the presence of anti-MDA5 Ab or RPILD [7].

One of the characteristics of anti-MDA5 Ab-positive CADM patients is hyperferritinemia [8,9]. These patients often express high serum interleukin 18 (IL-18) [10]. It is possible that the

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production of anti-MDA5 Ab is an epiphenomenon during virus infection that is associated with the onset of CADM and RPILD. Considering that dsRNA virus-mediated innate immune responses might be activated in anti-MDA5 Ab-positive CADM patients, type I IFNs could be crucial biomarkers reflecting organ damage and serologic disease activity.

The present study was undertaken to clarify the clinical importance of the measurement of serum type I IFNs in patients with anti-MDA5 Ab-positive DM. Our present data suggest that serum IFN-α could be used as a biomarker that reflects the disease activity in patients with anti-MDA5 Ab-positive DM complicated by RPILD.

Patients and methods

Patients

The study population consisted of 30 patients (22 females and 8 males) with DM (CADM or classical DM) who had been admitted to our hospital (Nagasaki University Hospital and Kurume University Hospital) between May 2009 and October 2012. The diagnosis of classical DM was based on Bohan and Peter criteria [11,12]. The diagnosis of CADM was based on Sontheimer criteria [13]. The diagnosis of classical DM or CADM was carefully evaluated by Japan College of Rheumatology-certified rheumatologists (M.T., H.N., H.I., T.O., and A.K.) using the patients' medical records. The diagnosis of ILD was based on the results of chest X-ray and high-resolution computed tomography, reported by Japanese board-certified radiologists. RPILD was defined as progressive dyspnea and hypoxemia, and a worsening of interstitial change on chest radiography within 1 month from the onset of respiratory symptoms [4]. Patients gave their informed consent to be subjected to the protocol, which was approved by the Institutional Review Board of Nagasaki University.

Blood samples

Blood specimens were obtained from all patients in this study at their first visit for serological analysis. Blood samples were stored at − 20°C until use, and they were used to measure anti-MDA5 Ab, IFN-α, IFN-β, ferritin, and IL-18. The cut-off value of anti-MDA5 Ab was defined as 8.0 U/mL. An ELISA system using recombinant MDA5 as an antigen source was performed as described [5]. All anti-MDA5 Ab-positive sera samples were positive with anti-CADM140 Abs confirmed using immunoprecipitation. The measurement of IFN-α and IFN-β was performed with the VeriKine™ Human IFN-α ELISA Kit (Product #41100) and the VeriKine™ Human IFN-β ELISA Kit (Product #41410), respectively, following the manufacturer's instructions. The measurement of ferritin was performed with the AssayMax Human Ferritin ELISA Kit (Assaypro, Catalog No. EF 2003-1) following the manufacturer's instructions. The measurement of IL-18 was performed with the Human IL-18 ELISA Kit (Code No. 7620, Medical & Biological Laboratories Co., Nagoya, Japan) following the manufacturer's instructions. In addition to DM patients, serum IFN-α, IFN-β, ferritin, and IL-18 were measured in 36 healthy controls.

Statistical analyses

In order to identify and evaluate differences in variables between the anti-MDA5 Ab-positive DM patient group and the anti-MDA5 Ab-negative DM patient group, we used the Mann–Whitney U-test or the χ^2 test (Fisher's exact probability test when appropriate). Correlations between two serum markers were calculated using the Spearman's rank correlation test. Values of p less than 0.05 were considered significant.

Results

Table 1 summarizes the patients' data. Ten of the thirty patients were positive for anti- MDA5 Ab. In the anti-MDA5 Ab-positive group, CADM was identified in eight patients and classical DM was diagnosed in the other two patients. In the group of 20 patients negative for anti-MDA5 Ab, classical DM was diagnosed in 17 patients, and CADM was diagnosed in the other three patients, indicating the significant prevalence of CADM in the anti-MDA5 Ab-positive group ($p = 0.0006$). Accordingly, ILD, especially RPILD, was preferentially distributed in the anti-MDA5 Ab-positive group ($p = 0.0038$ in ILD, $p < 0.0001$ in RPILD). In fact, all 10 cases of the anti-MDA5 Ab-positive patients were complicated by RPILD. Significantly high levels of anti-MDA5 Ab titer ($p < 0.0001$), IFN-α ($p = 0.0003$) and ferritin ($p = 0.0003$) were found in the anti-MDA5 Ab-positive patients compared to those of the anti-MDA5 Ab-negative patients (Figure 1). In contrast, no significant difference was observed between the two groups in terms of IFN-β or IL-18 (Figure 1). Then we examined the differences of cytokine levels between the anti-MDA5Ab-positive DM and anti-MDA5-negative DM patients complicated with ILD. The result is that IFN-α and ferritin levels were significantly elevated in anti-MDA5 Ab-positive patients than those in anti-MDA5 Ab-negative with ILD (Table 2). Three anti-MDA5 Ab-positive patients died during the observation period. We examined the differences of cytokine levels between the alive and the deceased patients; however, the differences were not statistically significant (Table 3).

We further examined the correlations of the serum concentrations of IFN-α with the other markers in anti-MDA5-positive DM patients (Figure 2). Serum concentration of IL-18 did not differ between anti-MDA5 Ab-positive and anti-MDA5 Ab-negative groups (Figure 1); however, a positive correlation was found

Table 1. Comparison of clinical and serologic manifestations between patients with anti-MDA5 Ab and without anti-MDA5 Ab.

	Anti-MDA5 Ab		
Variable	Positive ($n = 10$)	Negative ($n = 20$)	p value
Age, years (SD)	58.9 (12.38)	44.8 (19.85)	0.0444
Female, n (%)	9 (90%)	13 (62.5%)	0.1512
CADM, n (%)	8 (80%)	3 (15%)	0.0006
ILD, n (%)	10 (100%)	9 (45%)	0.0038
RPILD, n (%)	10 (100%)	1 (5%)	< 0.0001
Anti-MDA5 Ab (U/mL), median (range)	154.423 (16.946–1448.155)	1.255 (0.765–4.782)	< 0.0001
Death, n (%)	3 (30%)	0 (0%)	0.0111

Anti-MDA5 Ab, anti-melanoma differentiation-associated gene 5 (MDA5) antibody; CADM, Clinically amyopathic dermatomyositis; ILD, interstitial lung disease; RPILD, Rapidly progressive interstitial lung disease; IFN, Interferon; IL-18, interleukin-18.

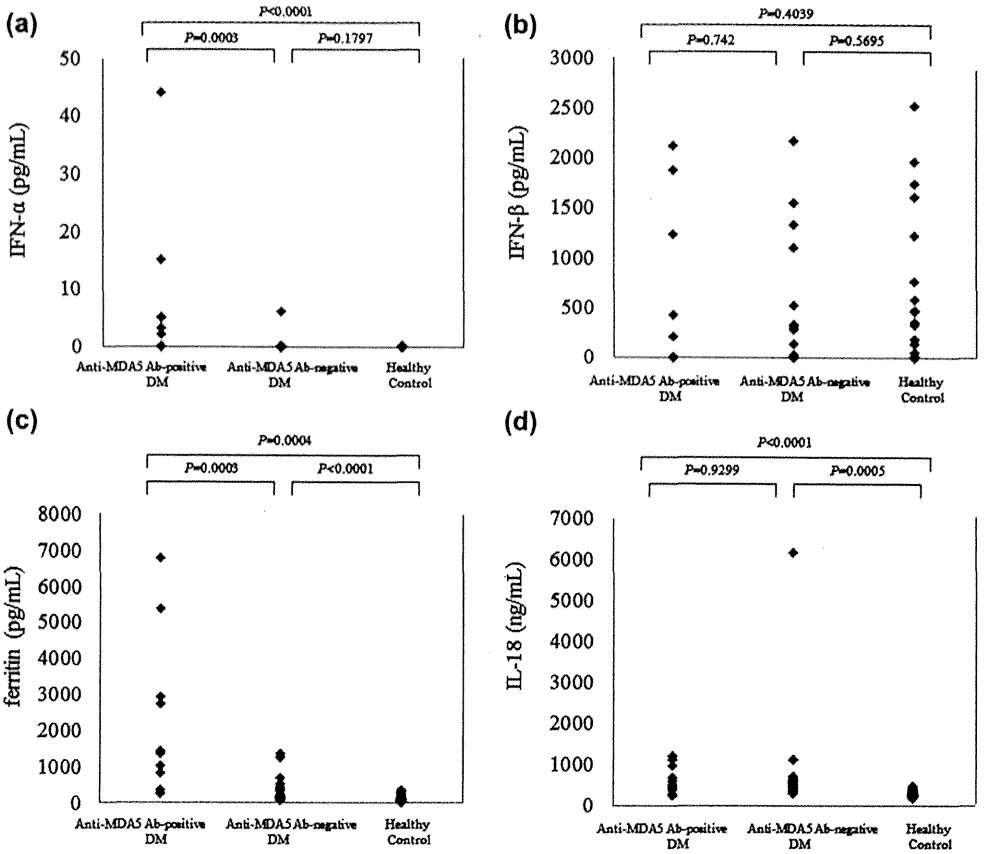

Figure 1. Serum levels of IFN-α, IFN-β, ferritin, and IL-18 in anti-MDA5 Ab-positive DM, anti-MDA5 Ab-negative DM, and healthy controls. *P* value was estimated using the Mann–Whitney U-test.

between IFN-α and IL-18 in these patients (Figure 2). Significant correlations were not observed between INF-α and with the other three variables (Figure 2).

Discussion

The importance of anti-MDA5 Ab in patients with CADM has been established. Authors and other investigators have found an extremely high prevalence of anti-MDA-5 Ab in patients with CADM complicated with RPILD [5,8,14]. In these reports, the titer of anti-MDA5 Ab at the first visit was higher in the CADM patients who died of the disease compared to that of the surviving patients [8], though we did not find the difference probably due to very small sample size. In addition, sustained high levels of anti-MDA5 Ab during therapy were associated with death in patients with anti-MDA5 Ab-positive CADM [8]. Although 2 of the 10 anti-MDA5 Ab-positive DM patients in the present study were classified as having classical DM, the cases of all 10 anti-MDA5 Ab-positive DM patients were complicated by RPILD.

Table 2. Comparison of serum cytokine levels between the anti-MDA5 Ab positive patients and anti-MDA5 Ab negative patients with ILD.

	Patients with ILD ($n = 19$)		
Variable	anti-MDA5 positive ($n = 10$)	anti-MDA5 negative ($n = 9$)	*p*-value
IFN-α (pg/mL), median	4 (0–44)	0 (0–6)	0.0435
IFN-β (pg/mL), median	100 (0–1873)	25 (0–2167)	1.0318
Ferritin (pg/mL), median (normal range 45–163)	1376 (241–6775)	318 (77–1235)	0.003
IL-18 (ng/mL), median (normal range 18–121)	539.5 (239–1210)	617 (439–6170)	0.447

Anti-MDA5 Ab, anti-melanoma differentiation-associated gene 5 (MDA5) antibody; IFN, Interferon; IL-18, interleukin-18, ILD, interstitial lung disease.

Anti-MDA5 Ab is detected in a small group of patients with classical DM, and all these classical DM patients complicate RPILD [15]. Therefore, all 10 anti-MDA5 Ab-positive DM patients in the present study are considered as having the characteristic feature of DM-complicated RPILD. The present study revealed for the first time that serum concentrations of IFN-α are high in the cases of anti-MDA5 Ab-positive DM patients complicated by RPILD compared to those of anti-MDA5 Ab-negative DM patients. It is undeniable that elevated serum IFN-α and ferritin levels in anti-MDA5 Ab-positive patients than in anti-MDA5 Ab-negative DM might associate with ILD. It is known that almost all anti-MDA5 Ab-positive patients complicated ILD [14], especially RPILD, during their clinical courses that is also revealed in the present study. We have found the higher levels of IFN-α and ferritin in anti-MDA5-positive patients with ILD than those in anti-MDA5-negative patients with ILD. Therefore, it might be considered that IFN-α and ferritin levels are reflective toward the presence of anti-MDA5 Ab and RPILD. In addition, Gono et al. have reported the hyperferritinemia with an accumulation of ferritin-producing macrophages in the lung in an autopsied case of anti-MDA5 Ab-positive CADM complicated by RPILD [9], suggesting that some stimuli trigger the activation of macrophages leading to hyperferritinemia. Because MDA5 is expressed in macrophage-lineage cell types [16] and a member of the RLR family that recognizes dsRNA derived from viruses and trigger antiviral response [6], our present data may explain a putative implication of dsRNA virus infection in patients with anti-MDA5 Ab-positive DM. Viral infection may induce the production of IFN-α and previous report suggests that IFN-α

Table 3. Comparison of serum cytokine levels between the alive and the deceased in anti-MDA5 Ab-positive patients.

	Anti-MDA5 Ab positive ($n = 10$)		
Variable	Alive ($n = 7$)	Deceased ($n = 3$)	p value
Anti-MDA5 Ab (U/mL), median	101.841 (16.946–406.473)	207.006 (21.797–1448.155)	0.569
IFN-α (pg/mL), median	5 (0–44)	3 (0–5)	0.667
IFN-β (pg/mL), median	200 (0–1873)	0 (0–2113)	1.000
Ferritin (pg/mL), median (normal range 45–163)	1329 (241–6775)	1423 (1004–5357)	0.667
IL-18 (ng/mL), median (normal range 18–121)	502 (268–1210)	577 (239–967)	0.833

Anti-MDA5 Ab, anti-melanoma differentiation-associated gene 5 (MDA5) antibody; IFN, Interferon; IL-18, interleukin-18.

stimulates the synthesis or secretion of ferritin [17]. Considering IFN-α and ferritin levels were higher in patients with anti-MDA5 Ab-positive DM complicated with RPILD, IFN-α might play an important role in inflammation triggered in the lung.

Serum IL-18 is reported previously to be high in DM patients complicated with ILD compared to those without ILD [10]. In a study of anti-MDA5 Ab-positive DM patients, sustained high IL-18 levels during therapy were observed in the deceased patients compared to those of the survived ones, indicating that IL-18 could also reflect disease activity in patients with DM, especially those who are anti-MDA5 Ab-positive [8]. Interestingly, a positive correlation was found between IFN-α and IL-18 in the anti-MDA5 Ab-positive DM patients of present study, which may support our hypothesis that IFN-α is a useful biomarker in patients with anti-MDA5 Ab-positive DM. In contrast, we did not find the difference of serum IL-18 levels at first visit between anti-MDA5 Ab-positive and anti-MDA5 Ab-negative DM patients, though the serum concentrations of IL-18 from both groups were higher than those of healthy controls. Therefore, the mechanisms to stimulate the production of IL-18 might be different in anti-MDA5 Ab-negative DM patients as compared to those of anti-MDA5 Ab-positive DM patients.

We did not observe a difference in the serum concentrations of another type I IFN, IFN-β, between our anti-MDA5 Ab-positive and anti-MDA5 Ab-negative DM patients. Although IFN-α, IFN-β, and IL-18 are produced in muscle tissues of patients with DM [18,19], the organs responsible for the predominant expression of IFN-α in anti-MDA5 Ab-positive DM patients may not be muscles, since the muscular involvement is less in these patients compared to those of anti-MDA5 Ab-negative DM patients.

In summary, our present data indicate that serum IFN-α is a useful biomarker in patients with anti-MDA5 Ab-positive DM complicated by RPILD. However, other investigators recently reported that serum levels of IFN-α do not correlate with disease activity in DM patients [20]. Since the patterns of organ involvement of DM patients differ widely according to myositis-specific

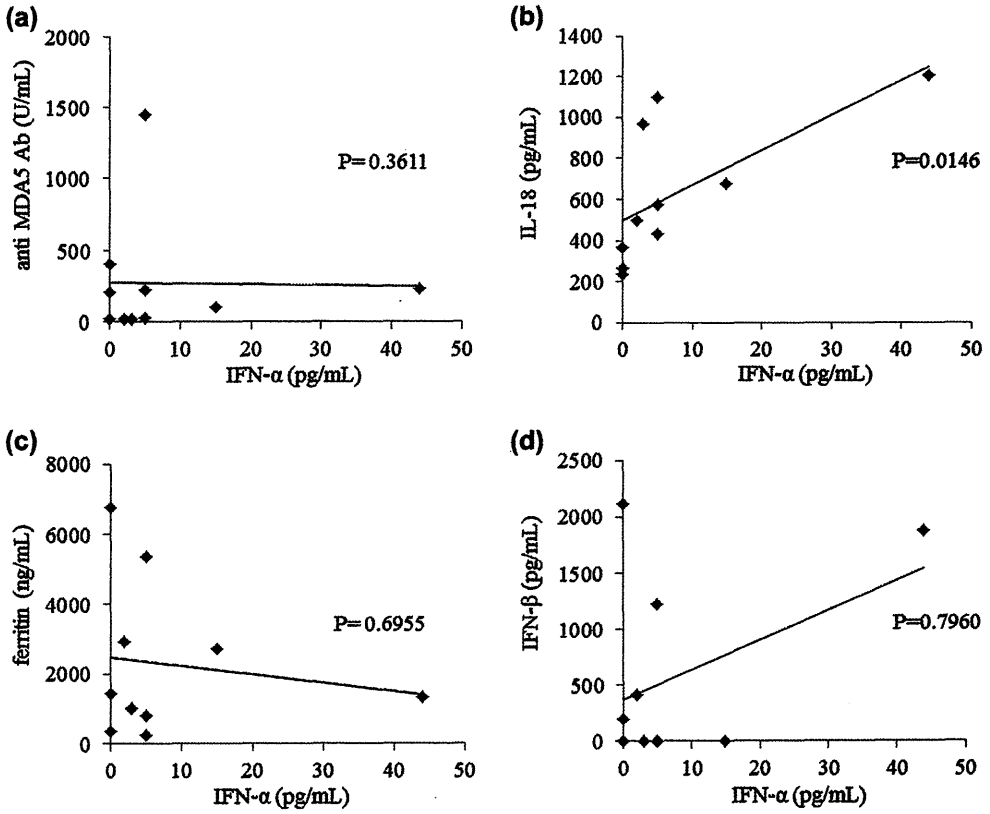

Figure 2. Correlations of serum IFN-α with anti-MDA5 Ab titer, serum IL-18, serum ferritin or IFN-α in the anti-MDA5 Ab-positive DM patients. (a) IFN-α with anti-MDA5 Ab titer, (b) IFN-α and IL-18, (c) IFN-α and ferritin, (d) IFN-α and IFN-β. Positive correlations were found between IFN-α and IL-18 ($p = 0.0146$).