Subclinical labial salivary gland involvement in IgG4-related disease affected with vital organs

Sirs,

IgG4-related disease (IgG4-RD) is a new disease entity characterised by elevated serum IgG4 and infiltration of $IgG4^+$plasma cells into various tissues (1-2). Although biopsy of the affected organ is desirable for histological diagnosis (3), target organ biopsy is often difficult to perform unless the affected organs are located in a superficial region and places the patient, often a middle-aged or elderly man with comorbidities, at risk for complications. Lip biopsy, a minimally invasive and convenient procedure, that does not require general anesthesia, has been shown useful for pathological diagnosis of IgG4-RD in a certain population of patients with sensitivity of 57–69% (4-6). However, patient selection to perform lip biopsy can be controversial, since detectable abnormality with physical examination or imaging of the labial salivary glands (LSGs) is rare (4-6). Therefore, we reviewed the cases of our six IgG4-RD patients (Table I) without apparent involvement of LSGs on physical examination or imaging who had undergone lip biopsy, in order to identify any biomarkers that might guide patient selection for this minimally invasive procedure. This study was approved by the ethics committee of our institution and informed consent was obtained from all patients.

All patients had been diagnosed based on the 2011 comprehensive IgG4-RD diagnostic criteria (3). Sjögren syndrome was excluded. Plasmablasts were defined as $CD19^+CD20^-CD27^+CD38^+$cells. T follicular helper cells (Tfh) were defined as $CD3^+CD4^+CXCR5^+CD45RA^-$ cells and subdivided into Tfh1 ($CXCR3^+CCR6^-$), Tfh2 ($CXCR3^-CCR6^-$) or Tfh17 ($CXCR3^-CCR6^+$) (7) and detected by flow cytometry. Extensive IgG4-positive plasma cell infiltration of the LSGs was identified in five cases (83.3%). Evaluation of serum IgG, IgG4, IgE, soluble IL-2 receptor, and quantification of circulating plasmablasts revealed that of all five measurements, the absolute number of circulating plasmablasts best correlated with the percentage of $IgG4^+/IgG^+$ plasma cells in the LSGs ($R^2 = 0.010, 0.176, 0.024, 0.213$ and 0.531, respectively – see Table). On the other hand, Tfh is a distinct population of $CD4^+$T cells that induces the differentiation of B cells into plasmablasts. There are three Tfh subsets: Tfh1, Tfh2, and Tfh17. Tfh2 and Tfh17 are known to be involved in plasmablasts differentiation (7). We recently identified an increased number of Tfh2 cells correlating with elevations in serum IgG4 and plasmablast numbers in IgG4-RD, suggesting that Tfh2 is the underlying pathological T cell subset in IgG4-RD (8). Additional analysis of Tfh and their subsets has revealed that the number of Tfh2 cells correlated more closely with the percentage of $IgG4^+/IgG^+$ plasma cells in LSGs compared to the number of Tfh and Tfh1 ($R^2 = 0.283, 0.082$, and 0.092, respectively). In this study we demonstrated that the number of circulating Tfh2 cells showed a positive correlation with the percentage of $IgG4^+/IgG^+$ plasma cells in LSGs, while Tfh17 numbers showed a negative correlation. This further supports the idea that elevated numbers of Tfh2 cells are associated with $IgG4^+$plasma cell infiltration of affected tissues, such as LSGs. The number of circulating plasmablasts also positively correlated with the percentage of $IgG4^+/IgG^+$plasma cells in LSGs, supporting the recent identification of the plasmablast as a biomarker for diagnosis and disease activity in IgG4-RD (9). However, $IgG4^+$ plasma cells in LSGs might be in the normal range despite increased Tfh2 and plasmablasts, as was seen in patient #6. It should also be kept in mind that LSG biopsy may not be useful to rule out malignant lymphoma (10), so further workup may be necessary if the diagnosis of IgG4-RD is in question.

We conclude that circulating Tfh2 cells and plasmablasts, and not serum IgG4 levels, reflect $IgG4^+$ plasma cell infiltration in LSGs, suggesting this finding could be a predictive marker for patient selection and positive pathological findings on lip biopsy in IgG4-RD.

Table I. Analysis of biomarkers showing the association of plasmablasts, Tfh2, and Tfh17 with $IgG4^+$ plasma cell infiltration in LSGs.

No.	$IgG4^+/IgG^+$plasma cells (%) in LSGs biopsy	Age (year)	Sex	Symptom duration (month)	Affected organs*					
					LG	PG	SMG	LN	Orbit	Kidney
1	90%	38	M	4	+	+	+	+	–	–
2	50%	71	F	26	–	+	+	+	–	–
3	50%	61	M	28	–	+	+	–	+	–
4	50%	64	F	4	+	+	+	+	–	+
5	47%	60	M	6	–	–	+	+	–	+
6	Negative	63	F	48	+	+	+	–	–	+

Association with $IgG4^+/IgG^+$plasma cells (%) in LSGs (R^2)

No.	Paraaortic	Bile duct	Pancreas	Lung	Retroperitoneal fibrosis	LSG	Comorbidity	Allergic disorders	ANA	Anti-Ro/SS-A swelling
1.	–	–	+	+	+	None	Dyslipidaemia	Asthma sinusitis	–	–
2.	–	+	–	–	–	None	IPAH	Sinusitis	–	–
3.	–	–	+	–	–	None	–	Asthma sinusitis	–	–
4.	–	–	–	+	+	None	Dyslipidaemia	Allergic rhinitis	+	–
5.	+	–	–	–	–	None	Dyslipidaemia	Allergic rhinitis	–	–
6.	+	–	+	–	–	None	Diabetes mellitus	Allergic rhinitis	–	–

No.	Anti-La/SS-B	IgG (mg/dL)	IgG4 (mg/dL)	IgE (IU/mL)	Soluble IL-2 receptor (U/ml)	plasmablasts ($\times 10^3$cells/ml)	Tfh ($\times 10^3$cells/ml)	Tfh1 ($\times 10^3$cells/ml)	Tfh2 ($\times 10^3$cells/ml)	Tfh17 ($\times 10^3$cells/ml)
1.	–	4431	1870	580	1270	25.2	138.5	41.7	73.4	16.2
2.	–	1914	829	2200	616	6.99	86.8	2.16	35.8	21.8
3.	–	1551	840	2600	721	NA	NA	NA	NA	NA
4.	–	2107	232	150	1415	2.16	96.0	30.5	19.2	29.8
5.	–	1856	314	110	295	1.52	90.6	32.9	23.8	25.8
6.	–	2516	992	210	650	3.72	158.7	54.9	38.3	44.0
		0.010	0.176	0.024	0.213	0.531	0.082	0.092	0.283	0.900

*Affected organs were detected by physical examination, computed tomography, positron-emission tomography and/or magnetic resonance imaging.

LG: lachrymal gland; PG: parotid gland; SMG: submandibular gland; LN: lymph node; LSG: labial salivary gland; NA: not available; IPAH: idiopathic pulmonary hypertension; Tfh: T follicular helper cells; Tfh1: T follicular helper 1 cells; Tfh2: T follicular helper 2 cells; Tfh17: T follicular helper 17 cells.

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