

# Th1 Cytokines Signature in 2 Cases of IgA Nephropathy Flare after mRNA-Based SARS-CoV-2 Vaccine: Exploring the Pathophysiology

Laura Martinez Valenzuela<sup>a</sup> Laia Oliveras<sup>a</sup> Montserrat Gomà<sup>b</sup> Eugenia Quiros<sup>b</sup>  
Paula Antón-Pámpols<sup>a</sup> Francisco Gómez-Preciado<sup>a</sup> Xavier Fulladosa<sup>a</sup> Josep  
Maria Cruzado<sup>a</sup> Juan Torras<sup>a</sup> Juliana Draibe<sup>a</sup>

<sup>a</sup>Nephrology Department, Bellvitge University Hospital, Bellvitge Biomedical Research Institute (IDIBELL), Hospitalet de Llobregat, Barcelona, Spain; <sup>b</sup>Department of Pathology, Bellvitge University Hospital, Hospitalet de Llobregat, Barcelona, Spain

## Keywords

Immunology · T cells · SARS-CoV-2 · IgA nephropathy · Vaccine

## Abstract

mRNA-based vaccines have dramatically shifted the course of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. IgA nephropathy (IgAN) flare is the most reported renal adverse effect after the administration of these vaccines. Unraveling the mechanistic pathways leading to these flares is necessary to confirm a causal association. Herein, we report 2 cases of IgAN flare after SARS-CoV-2 vaccination in patients previously diagnosed with IgAN. We describe and compare the clinical and analytical features of the disease at the time of the diagnostic with the post-vaccine flare. In addition, we obtained serum and urine of these patients at the moment of the flare and determined the levels of IL-2, TNF- $\alpha$ , and IFN $\gamma$  using a multiplex bead-based assay. As diseased controls, we included  $n = 13$  patients diagnosed with IgAN who had available serum and urine samples at the moment of the diagnostic stored in our biobank. We also included 6 healthy controls. Compared to the first episode, postvaccination flares were more severe in terms of peak serum creatinine, albuminuria, and urinary

erythrocyte count. The histological lesions found at the biopsy performed during the post-vaccine flare were similar to those found at the diagnostic. One of the patients who suffered a post-vaccine flare showed increased serum IL-2 and TNF $\alpha$  compared to the IgAN-diseased controls and the healthy controls. In conclusion, although several cases of post-vaccine IgAN flares have been reported, there are no mechanistic studies on the occurrence of these flares. We here suggest that hyperactivation of the Th1 pathway may be involved, but larger studies with more refined methods for numerical and functional Th1 lymphocytes evaluation are required.

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## Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has emerged as one of the three most pathogenic human coronaviruses of the last century. Although it is less deadly than SARS-CoV or the Middle East respiratory syndrome coronaviruses, its transmissibility has led to an unprecedented worldwide health crisis with more than 200 million cases diagnosed to date [1].

Coronavirus disease-19 (COVID-19) is the denomination of the clinical syndrome associated with SARS-CoV-2 infection. It mainly manifests with mild to severe respiratory disease, but multiorgan involvement has been reported. The spike (S) glycoprotein is key to the fusion of the viral and host cell membranes after recognizing angiotensin-converting enzyme 2 (ACE2). The occurrence of acute kidney failure (AKI) among infected patients ranges between 1 and 42% of cases depending on the series and the definition of AKI [2]. Some authors suggest that SARS-CoV-2 can damage the different kidney compartments [3, 4], despite tubular cells being the most frequently affected. This may be due to their high expression of ACE2 [5].

Among the glomerular diseases reported after SARS-CoV-2 infection, collapsing glomerulopathy and IgA nephropathy (IgAN) flares are among the most frequent [6]. It has been hypothesized that hyperproduction of interferon gamma (IFN $\gamma$ ) due to viral infection leads to APOL1 gene risk variant overexpression, thus resulting in podocyte dysregulation and collapsing glomerulopathy. In a case series of 240 patients with SARS-CoV-2-associated AKI reported by May et al. [6], 2.9% of biopsied cases were diagnosed with IgAN. Zhang et al. [7] suggest that IgAN flares may be related to an aberrant and long-lasting IgA response to the infection, with high levels of specific anti-SARS-CoV-2 IgA antibody levels in serum.

Administration of mRNA vaccines targeted against the S glycoprotein, mRNA-1273 and BNT162, have been associated with the occurrence of IgAN flares (summarized in Table 1). Despite IgA production being majorly triggered by mucosal infections, mRNA vaccines also elicit serum S-specific IgA response that may be related to these IgAN flares. In preclinical studies, mRNA vaccines trigger CD4+ lymphocyte production of IFN $\gamma$ , tumor necrosis alpha (TNF $\alpha$ ), and interleukin-2 (IL-2) [8]. Sahin et al. [9] reported a predominance of the T-helper (Th) 1 over the Th2 response in patients immunized with BNT162b1 mRNA vaccine based on the cellular higher production of IFN $\gamma$  and IL-2 but not interleukin-4 or interleukin-5. Some authors reported the polarization towards a Th1 phenotype in IgAN [10], and Han et al. [11] demonstrated higher IFN $\gamma$  serum levels in IgAN compared to healthy controls. Thus, vaccination with mRNA-based vaccines and IgAN flares may share humoral and cellular pathways [12].

Herein, we report 2 cases of IgAN flare that occurred after vaccination in patients previously diagnosed with the disease. We compare the clinical features of these vaccination-related flares to their first episode of IgAN.

Thereafter, we analyze a Th1-related cytokine panel in serum and urine of these cases compared to a cohort of nonvaccine-related IgAN cases and healthy controls in order to deepen the knowledge of the pathophysiology of these vaccine-related flares.

## Case Reports

### *Patient 1*

A 36-year-old female was diagnosed with IgAN in January 2020. At the moment of the diagnostic biopsy, her creatinine was 115  $\mu\text{mol/L}$ , her 24-h albuminuria was 1.4 g/day, and she presented microhematuria. Treatment with prednisone and mycophenolate mofetil was prescribed. In May 2021 her creatinine and 24-h albuminuria had improved to 80  $\mu\text{mol/L}$  and 0.7 g/day, respectively, but microhematuria persisted. In July 2021, she was admitted to the emergency room claiming gross hematuria, fever, and malaise few hours after the administration of the second dose of mRNA-1273 SARS-CoV-2 vaccine. Her serum creatinine had risen from 80  $\mu\text{mol/L}$  to 159  $\mu\text{mol/L}$ . Her urinalysis showed an increase in her pre-existing persistent hematuria, and her proteinuria rose from 0.7 g/day to 1.5 g/day at admission. Under the clinical assumption of IgAN flare, a new renal biopsy was performed. Light microscopy showed diffuse moderate mesangial hypercellularity and extracapillary proliferation. A mild interstitial inflammatory infiltrate was present. Immunofluorescence staining was predominantly IgA positive with a mesangial pattern. Those findings confirmed IgAN flare. At the moment of the admission, she was being treated with mycophenolate mofetil 250 mg twice a day and 2.5 mg of prednisone/day. After the kidney biopsy, prednisone dose was increased to 0.5 mg/kg body weight. Two months later, kidney function improved to creatinine 96  $\mu\text{mol/L}$  and proteinuria decreased under 0.5 g/day. Currently, she is being treated with mycophenolate mofetil 250 mg twice a day and 5 mg of prednisone per day.

### *Patient 2*

A 50-years-old male had been diagnosed with IgAN in 2001 by kidney biopsy. He did not receive immunosuppressive treatment. He was infected with SARS-CoV-2 in 2020, presenting mild symptoms. In March 2021, his creatinine was 88  $\mu\text{mol/L}$  and his albuminuria was 0.7 g/day. He referred gross hematuria 72 h after the first dose of BNT162 SARS-CoV-2 vaccine on a routine outpatient visit in June 2021. At that moment, his renal function showed a mild deterioration to serum creatinine 107  $\mu\text{mol/L}$ , and his albuminuria severely increased to 3.3 g/day without associated hypoalbuminemia. His urinalysis showed an increase in his pre-existing persistent hematuria. A new kidney biopsy was performed. Light microscopy showed mesangial hypercellularity affecting more than 50% of the glomeruli, associated with mild interstitial infiltrate. Immunofluorescence staining demonstrated IgA deposition. Serum creatinine and proteinuria spontaneously recovered to their baseline values.

We therefore compared the clinical and analytical parameters of both patients at the moment of the diagnosis and at the moment of the postvaccination flare. In both cases, the clinical presentation was similar, without extrarenal organ involvement. Interestingly, during the flare, peak serum creatinine, urinary albumin to creati-

**Table 1.** IgA nephritis flares after mRNA-based SARS-CoV-2 vaccination reported in the literature

Reference	Sex	Age	Vaccine manufacturer	Dose	Days after dose	Medical history	Clinical picture	Lab tests	Biopsy findings	Treatment	Outcomes
Kudose et al. <i>Kidney Int.</i> 2021 Jun 16	W	50	mRNA-1273	2	2	APL syndrome Baseline SCreat 1.3 mg/dL Baseline P/C 1.3 g/g Pre-existent microscopic hematuria	Gross hematuria Low grade fever Generalized body aches	SCreat 1.7 mg/dL P/C 2 g/g Gross hematuria	12 glomeruli Mild diffuse mesangial hypercellularity 1/12 fibrinoid necrosis 1/12 cellular crescent Mesangial staining for IgA (3+), C3 (1+)	Not treated	Gross hematuria resolved within 5 days
Kudose et al. <i>Kidney Int.</i> 2021 Jun 16	M	19	mRNA-1273	2	2	Pre-existent microscopic hematuria	Gross hematuria	SCreat 1.2 mg/dL P/C negative Gross hematuria	25 glomeruli Mild diffuse mesangial hypercellularity 2/25 segmental endocapillary hypercellularity 1/25 segmental fibrous crescent 1 to 2+ granular global mesangial staining for IgA, C3	Not treated	Gross hematuria resolved within 5 days
Anderegg et al. <i>Kidney Int.</i> 2021 Jun 1	M	39	mRNA-1273	2	Shortly	Hypertension	Severe fever Flu-like symptoms Gross hematuria Nephritic syndrome	AKI	Severe crescentic IgA nephritis Mesangial IgA + deposits	High-dose glucocorticoids and cyclophosphamide	Serum creatinine normalized Proteinuria decreased Microhematuria persisted
Rahim et al. <i>Kidney Int.</i> 2021 Jul;100(1):238	W	52	BNT162	1	1	Prior biopsy-proven IgAN with SCreat in the normal range and <1 g/day proteinuria	Gross hematuria Fever Myalgias Body aches Lower back pain	P/C 4.2 g/g SCreat 0.8 mg/dL	Not performed	Not treated	Gross hematuria resolved within 5 days and P/C improved
Negrea et al. <i>Kidney Int.</i> 2021 Jun;99(6):1487	W	38	mRNA-1273	2	1	Prior biopsy-proven IgAN with SCreat in the normal range and <1 g/day proteinuria	Gross hematuria Body aches Headache Fatigue Fever and chills	Proteinuria increase to 1.4 g/day No AKI	Not performed	Not performed	
Negrea et al. <i>Kidney Int.</i> 2021 Jun;99(6):1487	W	38	mRNA-1273	2	1	Prior biopsy-proven IgAN with SCreat in the normal range and <0.5 g/day proteinuria	Gross hematuria Body aches Headache Fatigue Fever and chills	No AKI No increase in proteinuria	Not performed	Not performed	
Perrin et al. <i>Kidney Int.</i> 2021 Jun 1;S0085-2538(21)00564-0	M	22	mRNA-1273	1 and 2	2	Prior biopsy-proven IgAN Normal baseline kidney function	Gross hematuria Arthralgia	Proteinuria 3 gr/gr	Not performed	Not performed	
Perrin et al. <i>Kidney Int.</i> 2021 Jun 1;S0085-2538(21)00564-0	W	41	BNT162	1	2	Prior biopsy-proven IgAN Kidney transplant	Gross hematuria	Proteinuria 0.47 g/g	Not performed	Not performed	
Perrin et al. <i>Kidney Int.</i> 2021 Jun 1;S0085-2538(21)00564-0	W	27	BNT162	2	2	Prior biopsy-proven IgAN Hemodialysis	Gross hematuria Urticaria Abdominal pain	Proteinuria 1.9 g/g	Not performed	Not performed	

**Table 1 (continued)**

Reference	Sex	Age	Vaccine manufacturer	Dose	Days after dose	Medical history	Clinical picture	Lab tests	Biopsy findings	Treatment	Outcomes
Tan et al. 2021 May 23;50085-2538(21)00504-4	W	41	BNT162	2	1	Unremarkable	Gross hematuria Generalized myalgia Headache Hypertension	SCreat 153 umol/L P/C ratio 2.03 g/g	36 glomeruli 6% cellular and 8% fibrocellular crescents	Pulse methylprednisolone, followed by oral prednisolone, cyclophosphamide	N/A
Park et al. <i>Kidney Int Rep.</i> 2021 Jun 19	W	22	mRNA-1273	2	2	Prior biopsy-proven IgA vasculitis	Gross hematuria	Mild proteinuria No rise in serum creatinine	Not performed		Complete resolution
Park et al. <i>Kidney Int Rep.</i> 2021 Jun 19	W	39	mRNA-1273	2	2	Unremarkable	Gross hematuria	Mild proteinuria No rise in serum creatinine	Not performed		Complete resolution
Park et al. <i>Kidney Int Rep.</i> 2021 Jun 19	M	50	mRNA-1273	2	1	Chronic kidney disease and mild proteinuria at baseline	Gross hematuria	SCreat 3.56 mg/dL (baseline 2.40 mg/dL)	13% active crescents	ACE inhibition	Partial resolution
Park et al. <i>Kidney Int Rep.</i> 2021 Jun 19	M	67	mRNA-1273	1	30	Chronic kidney disease and mild proteinuria at baseline	Gross hematuria Maculopapular rash	SCreat 1.40 mg/dL (baseline 1.20 mg/dL)	Skin biopsy showed IgAV	Steroids	Partial resolution
Hanna et al. <i>Kidney Int. Sep.</i> 2021 Sep;100(3):705-6	M	13	BNT162	2	1	IgAN. Subnephrotic proteinuria, normal creatinine	Gross hematuria	SCreat: 1.31 mg/dL A/C: 1.6 mg/mg	Not performed	None	Complete resolution
Hanna et al. <i>Kidney Int.</i> 2021 Sep;100(3):705-6	M	17	BNT162	2	1	Unremarkable	Gross hematuria	SCreat: 1.78 mg/dL A/C: 1.75 mg/mg	M1 E1 S1 T1 C1	Steroids	Partial resolution (day 22 after treatment)
Plasse et al. <i>Kidney Int.</i> 2021 Oct;100(4):944-5	M		BNT162	2	5	Prior biopsy-proven IgAN	Gross hematuria Myalgia Chills	SCreat: 3.53 mg/dL A/C: 3 mg/mg	Not performed	Steroids	Recovery to baseline renal function at 1 month
Plasse et al. <i>Kidney Int.</i> 2021 Oct;100(4):944-5	M		BNT162	2	1	Prior biopsy-proven IgAN	Gross hematuria	SCreat: 1.16 mg/dL A/C: 3.2 mg/mg	Not performed	None	Complete resolution
Abramson et al. <i>Kidney Med.</i> 2021 Jul 14	M	30	mRNA-1273	2	1	Unremarkable	Gross hematuria Fever Chills	SCreat: 1.03 mg/dL A/C: 0.8 g/g	M1-E0-S1-T0-C0	ACE inhibition	Complete resolution

nine ratio, and urinary erythrocyte count were higher compared with the values of these variables at diagnostic in the 2 cases reported. Tables 2 and 3 show the analytical and clinical features of the diagnostic and the post-vaccine IgA flares of these patients.

Regarding the kidney biopsy, the histological lesions found at the biopsy performed during the post-vaccine flare were similar to those present at the diagnostic, as shown in Table 4. Figure 1 shows light microscopy images of the kidney biopsies performed in the post-vaccine IgAN flare cases.

We obtained serum and urine samples from the 2 patients who presented with an IgAN flare temporarily associated with mRNA SARS-CoV-2 vaccine. As diseased controls, we included  $n = 13$  patients diagnosed with IgAN who had available serum and urine samples at the moment of the diagnostic stored in our biobank. Exclusion criteria were as follows: documented urinary tract infection, sepsis, or evidence of other superimposed glomerular or autoimmune disease. We also recruited  $n = 6$  healthy controls. Healthy controls were volunteers from our institution without any known medical condition.

**Table 2.** Analytical parameters of the two mRNA vaccine-associated IgA nephritis flares at diagnosis

	Patient 1	Patient 2
Sex	Female	Male
Age	36	50
Serum creatinine, $\mu\text{mol/L}$	159	107
CRP, mg/L	29	<0.6
Serum albumin, g/L	29	42
Hemoglobin, g/L	89	143
Serum IgA, mg/L	2,174	4,809
Serum anti S protein, UI/mL	>2,500	>2,500
Serum anti N protein, UI/mL	0	Positive
Albuminuria, g/mol	203	329
Urine erythrocyte count, cells/ $\mu\text{L}$	363	170

Serum and urine samples were obtained at the moment of the diagnostic and prior to the initiation of the treatment, centrifuged at 2,000 rpm 10 min and stored at  $-80^{\circ}\text{C}$  until cytokine assessment. Based on the literature review, we selected three cytokines associated to the Th1 response: IFN $\gamma$ , IL-2, and TNF $\alpha$ . We determined the serum and urinary concentration of these cytokines using a customized Multiplex Immunoassay commercial kit (Invitrogen ProcartaPlex; ThermoFisher Scientific, Waltham, MA, USA). We used a Luminex MAGPIX<sup>®</sup> reader to retrieve the results.

Low levels of IL-2 were detected in serum and urine from patients diagnosed with IgAN, which were similar to the levels noticed in healthy controls. Serum and urine IFN $\gamma$  and TNF $\alpha$  were undetectable both in IgAN patients and healthy controls. Interestingly, patient 1 showed an increase in serum IL-2 and TNF $\alpha$  compared to the IgAN cohort and healthy controls. In contrast, patient 2 showed similar levels of cytokines compared to IgAN patients and healthy controls. Table 5 shows mean serum and urine concentration of the cytokines among groups.

## Discussion

The availability of effective vaccines has dramatically decreased the severity and mortality of COVID-19. Nonetheless, the massive vaccination of worldwide population has led to the notification of multiple adverse effects. It is necessary to identify mechanism of these supposed adverse effects in order to establish the individual risk profile of IgAN flare.

There is scarce data about the incidence of de novo IgAN associated with mRNA vaccination. According to the United Kingdom Medicines and Healthcare products Regulatory Agency's report, 1.4 million first doses and approximately 1.2 million second doses of the mRNA-

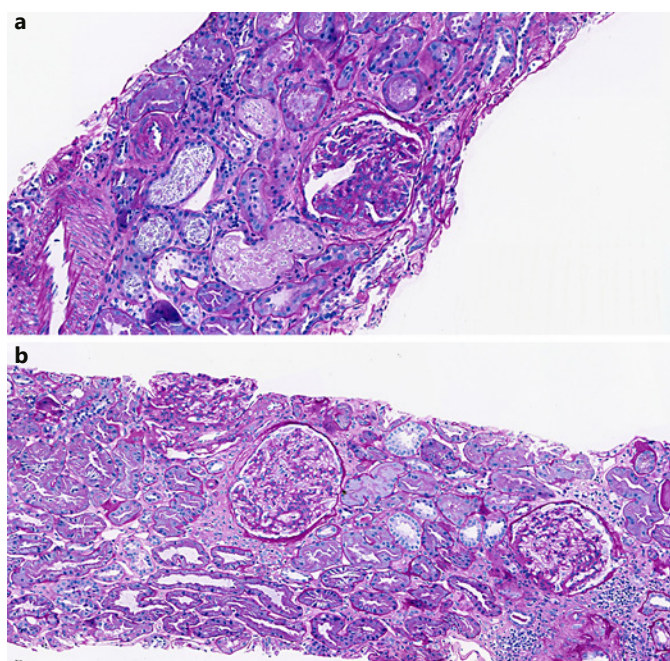
**Table 3.** Clinical and analytical parameters at the diagnostic and at the post-vaccine flare of the 2 cases reported

	Patient 1		Patient 2	
	diagnostic	post-vaccine flare	diagnostic	post-vaccine flare
Hematuria	Present	Present	Present	Present
Hypertension	Absent	Absent	Present	Present
Arthralgia	Absent	Absent	Absent	Absent
Abdominal pain	Absent	Absent	Absent	Absent
Fever	Absent	Present	Absent	Absent
Skin rash	Absent	Absent	Absent	Absent
Peak serum creatinine, $\mu\text{mol/L}$	115	158	96	107
Urinary albumin/creatinine ratio, g/mol	138.2	203.6	107.2	329.2
Urinary erythrocyte count, cells/ $\mu\text{L}$	40	363	1	170
C-reactive protein, g/L	1.1	29	N/A	2.5
Serum IgA, mg/L	3,229	2,174	3,760	4,809
Hemoglobin, g/L	123	92	150	143
Hypocomplementemia	Absent	Absent	Absent	Absent



**Table 4.** First and post-vaccine flare kidney biopsy findings of the 2 reported cases

	Patient 1		Patient 2	
	diagnostic	post-vaccine flare	diagnostic	post-vaccine flare
Mesangial hypercellularity	Diffuse, moderate	Diffuse, moderate	Diffuse, moderate	Diffuse, moderate
Endocapillary hypercellularity	20% glomeruli	Absent	Absent	Absent
Glomerular sclerosis	Absent	15% glomeruli, segmentary	Absent	Absent
Tubular atrophy/interstitial fibrosis	Absent	Absent	Absent	Mild
Crescents	15–20% glomeruli	15% glomeruli	Absent	Absent
IgA deposits (immunofluorescence intensity, localization)	3+, mesangial	3+, mesangial	3+	2+
IgG deposits (immunofluorescence intensity, localization)	Negative	Negative	Negative	Negative
IgM deposits (immunofluorescence intensity, localization)	1+, mesangial	1+, mesangial	+/-, mesangial	Negative
C3 deposits (immunofluorescence intensity, localization)	3+, mesangial	2+, mesangial	3+	2+
C4 deposits (immunofluorescence intensity, localization)	Negative	Negative	Negative	Negative
C1q deposits (immunofluorescence intensity, localization)	Negative	Negative	Negative	Negative



**Fig. 1.** Light microscopy examination of the two IgA nephritis flares registered after vaccination. **a** Patient 1, representative glomerulus with segmental fibroepithelial reaction and mesangial proliferation (periodic acid–Schiff, original magnification,  $\times 34$ ). **b** Patient 2, representative glomeruli with mesangial hypercellularity (periodic acid–Schiff, original magnification,  $\times 25$ ).

1273 vaccine, and 19.4 million and 24.0 million second doses of the BNT162 vaccine have been administered until September 24<sup>th</sup>, 2021, in the UK. Among the individuals who received the mRNA-1273 vaccine, 2 IgAN have

been notified. Regarding the ones receiving the BNT162 vaccine, there have been 2 IgAN cases notified, 18 nephrotic syndromes, 6 minimal change disease, and 2 rapidly progressive glomerulonephritis cases [13]. This registry does not include information regarding the previous diagnostic of glomerular disease in these cases.

Although being the most frequently reported renal adverse effect of mRNA-based SARS-CoV-2 vaccines in the medical literature, the overall incidence of flares among patients previously diagnosed with IgAN is low. Lim et al. [14] reviewed 145 patients diagnosed with IgAN in the past 5 years in active follow-up at their institution. Of those, none of the 61.5% of vaccinated patients was diagnosed with a flare and did not present de novo gross hematuria.

Regarding the severity of the flares, our 2 cases presented higher peak serum creatinine and albuminuria compared to their first episode. The findings of the kidney biopsy performed on patient 1 at the moment of the post-vaccine flare were similar to those in her first diagnostic biopsy, including active inflammatory lesions such as cellular crescents that required the intensification of the immunosuppressant treatment. In contrast, post-vaccine biopsy in patient 2 showed low inflammatory activity, which was consistent with the mild elevation of the creatinine and with the self-limiting clinical course of the flare in this patient. Importantly, at 3 months both patients recovered to their baseline creatinine. The clinical picture of the vast majority post-vaccine IgAN flares cases published (summarized in Table 1) consists of the occurrence of gross hematuria with scarce elevation of serum creatinine. Only in 3 of the cases, cellular crescents were present in the kidney biopsy. Nevertheless, kidney

**Table 5.** Serum and urine concentration of the evaluated cytokines among groups

	Post-vaccine IgAN flare (n = 2)	IgA nephropathy (n = 13)	Healthy controls (n = 6)
Serum IL-2 mean ± SD, pg/mL	11.96±2.16	10.36±0.28	10.44±0.23
Serum IFN $\gamma$ mean ± SD, pg/mL	Undetectable	Undetectable	Undetectable
Serum TNF $\alpha$ mean ± SD, pg/mL	Patient 1 – 12.35 Patient 2 – undetectable	Undetectable	Undetectable
Urine IL-2 mean ± SD, pg/mL	10.44±0.39	10.61±0.42	10.18±0.52
Urine IFN $\gamma$ mean ± SD, pg/mL	Undetectable	Undetectable	Undetectable
Urine TNF $\alpha$ mean ± SD, pg/mL	Undetectable	Undetectable	Undetectable

IgAN, immunoglobulin A nephropathy; IL-2, interleukin-2; IFN $\gamma$ , interferon gamma; TNF $\alpha$ , tumor necrosis factor alpha; SD, standard deviation.

biopsy was performed only in 7 out of the 19 cases of IgAN flares reported.

IgAN is a mesangioproliferative glomerulonephritis characterized by the presence of immunocomplex deposits composed of a form of aberrantly glycosylated IgA1 and antibodies specific against these forms. Mesangial deposition of IgA1-containing immune complexes activates the complement system and leads to glomerular damage. Aside from elevated aberrantly glycosylated IgA1 serum levels, genetic, dietetic, and environmental factors are involved in the pathogenesis of the disease [15]. IgA is the most profusely produced immunoglobulin isotype, and the main one involved in mucosal defense. Thus, IgA dominates the early neutralizing response to SARS-CoV-2 [16]. Wisniewski et al. [17] longitudinally monitored the IgA response after mRNA SARS-CoV-2 vaccine in 4 healthcare workers without prior history of infection. The vaccine elicited a specific anti-S IgA response in addition to the IgG response with a similar kinetic but a faster decline after the first dose [17]. Some authors expressed their concern that a nonmucosal trigger such as mRNA vaccine could be able to develop an IgA response that could lead to an IgAN flare, but the results of the study from Wisniewski et al. [17] confirm that this link is possible. IgA serum levels were only over the normal range in the case of patient 2 (cutoff for the normal values in our laboratory is 4,400 mg/L), as seen in Table 2. Whether the elevation of IgA levels is in the setting of the IgAN flare or a response to the vaccine remains inconclusive. Nevertheless, galactose-deficient IgA1 and glycan-specific IgG and IgA autoantibodies are the antibodies involved in IgAN pathogenesis, and their serum levels have been proposed as disease biomarkers, aside from the total IgA serum levels [18].

After a review of the literature, we centered our cytokine studies on a set of Th1 cytokines: IL-2, IFN $\gamma$ , and TNF $\alpha$ . IL-2 controls the differentiation and homeostasis of T cells [19]. Schena et al. [20] reported higher IL-2 production in IgAN patients compared to controls. Zhang et al. [21] also found a significant increase in IL-2 and IFN $\gamma$  in serum from IgAN patients at the moment of the diagnostic kidney biopsy before treatment. IFN $\gamma$  is critical for the Th1 response to infection: enhances antigen presentation, participates in macrophage activation, and increases lymphocyte recruitment. Some authors suggest the role of the hyperproduction of IFN $\gamma$  in IgAN [22]. TNF $\alpha$  is mainly produced by macrophages, but in inflammatory situations can be produced by Th1 lymphocytes, and has a role in the orchestration of the inflammatory immune response. Circulating TNF $\alpha$  levels have been associated with the severity of the disease course of IgAN [23–25]. In contrast, Ruzskowski et al. [26] found higher proportions of circulating Th2, Th17, and Th22 with lower Th1 and Treg cell populations.

Thus, some authors found a predominance of the Th1 activity, meanwhile other authors described a preponderance of the Th2 subset in IgAN. We were not able to confirm the elevation of the Th1 cytokines in the patients with IgAN. The predominance of these subsets may vary depending on the activity of the disease, the course of the disease, and the received treatment. There is a myriad of factors that can influence the level of the evaluated cytokines. The bulk of the studies do not take into account the temporal changes in the Th1/Th2 cytokines, and the cohorts of patients studied are majorly heterogenous. One must also take into account the plasticity of the Th subsets, which can acquire different phenotypes and produce different cytokines depending on the signals present in

the cell environment. Another source of variability may be the different methodology used in the evaluation of the levels of the cytokines, which include ELISA, CLIA and in our case, bead-based assays in the Luminex platform.

mRNA-based COVID-19 vaccine's response is characterized by a Th1 and also a Th17 increased response [27]. Bergamaschi et al. [28] evaluated the serum concentration of 52 cytokines and chemokines in healthy individuals receiving the BNT162 vaccine and found a rise in IFN $\gamma$  and TNF $\alpha$  after administration, and no differences in IL-2 levels. This finding is in line with the reports from Taborska et al. [29], who reported the presence of spike-specific IFN and TNF producing T cells after BNT162 vaccination.

In patient 1, we found serum IL-2 and TNF $\alpha$  slightly elevated in comparison to healthy controls and patients with IgAN flare not related to the vaccine. We were not able to detect IFN $\gamma$  in the serum or urine in any cohort. Differences with the findings reported by Bergamaschi et al. [28] may be due to the different techniques used to evaluate the concentration of the analytes, together with the potential effects of the immunosuppressive therapy in the case of *patient 1*.

The existing evidence is insufficient to confirm a causal association between mRNA-based SARS-CoV-2 vaccines and IgAN. Studies based on international multicentric registries are necessary in order to establish the real incidence of IgAN flares among previously diagnosed patients, but also the real incidence of IgA de novo diagnosis in general population after vaccine administration. At this moment of the pandemic, we consider that the benefits of vaccination are greater than the risk of IgAN flares based on the number of notified cases and their severity. The administration of a third vaccine dose or the combination of the different available vaccines may be a potential source of IgAN flares in the future. Finally, there is a lack of mechanistic studies that could help in the understanding of the disease process. We here suggest that hyperactivation of the Th1 pathway may be involved, but larger studies with more refined methods for numerical and functional Th1 lymphocytes subpopulations are needed to ascertain this hypothesis.

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## Statement of Ethics

The Clinical Research Ethics Committee of Bellvitge University Hospital approved the study protocol. Patients signed informed consent prior to inclusion. The patients included in the diseased controls cohort provided informed written consent to have data from their medical records used in research and stored samples for this purpose.

## Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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## Author Contributions

Laura Martinez Valenzuela and Juliana Draibe conceptualized the study. Laia Oliveras, Paula Antón, and Francisco Gómez reviewed the medical records. Laura Martinez Valenzuela performed cytokine determination and elaborated the manuscript. Montserrat Gomà and Eugenia Quiros evaluated and captured the light microscopy images. Xavier Fulladosa, Josep Maria Cruzado, Juan Torras, and Juliana Draibe critically supervised the study.

## Data Availability Statement

All data are available under request to the corresponding author.

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