



Increased incidence of Graves' disease during the SARS-CoV2 pandemic

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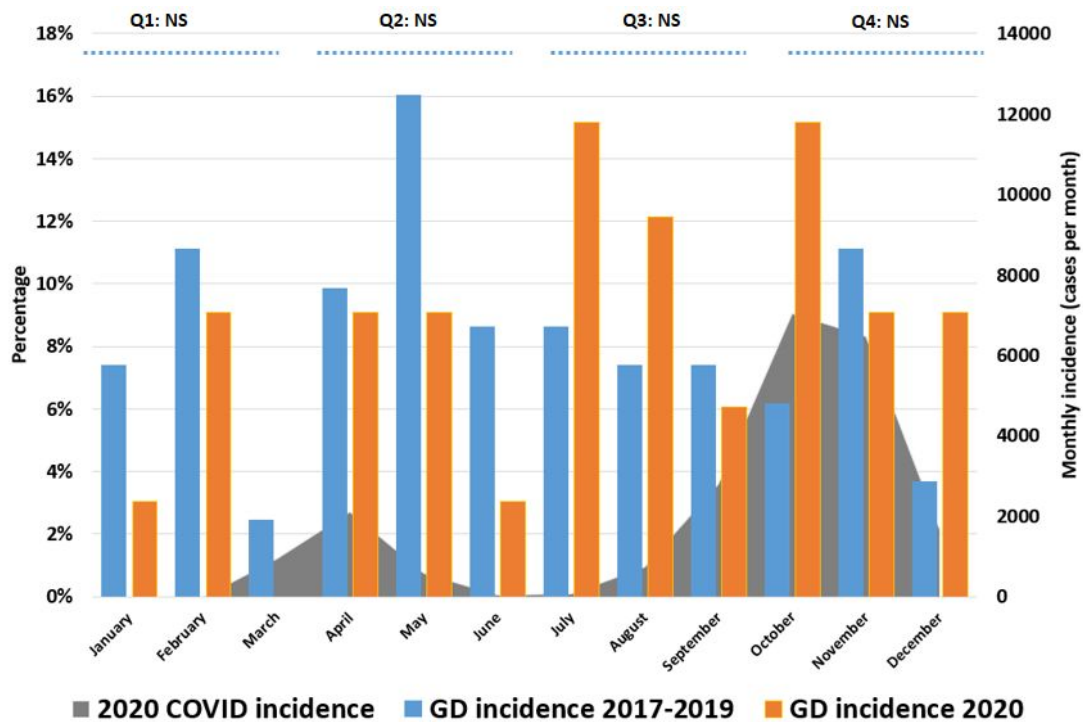


Figure 1. Percentage of cases of Graves' disease by month in 2017–2019 and 2020. COVID monthly incidence in 2020 is shaded.

Q1: January–March; Q2: April–June; Q3: July–August; Q4: October–December. NS: Not significant.

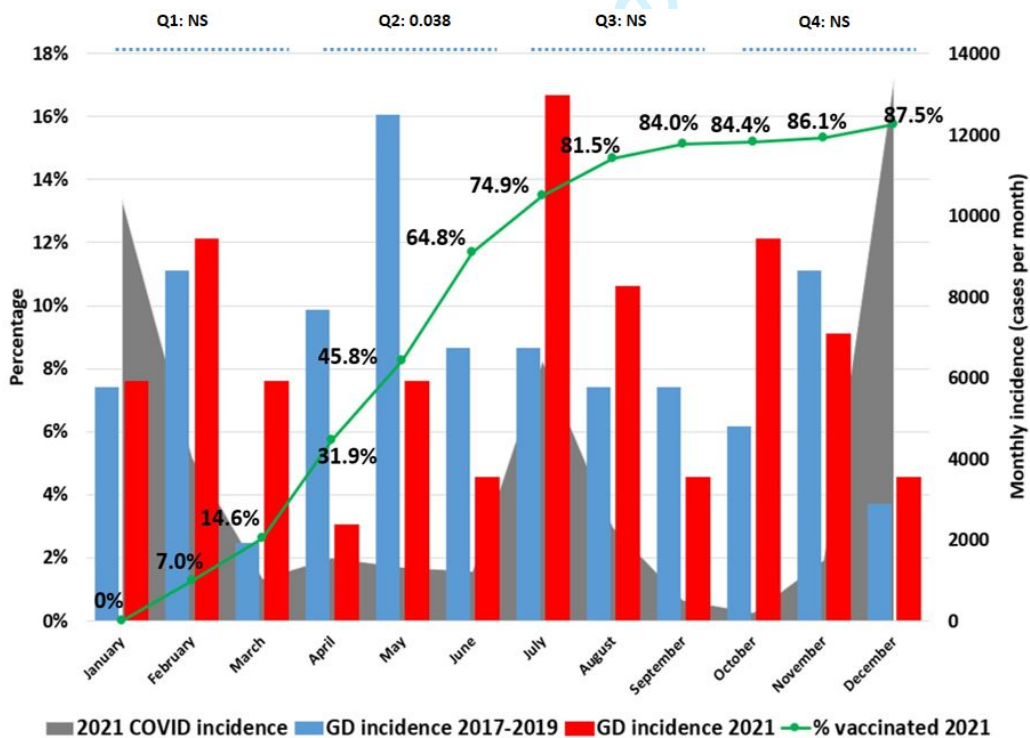


Figure 2. Percentage of cases of Grave's disease by month in 2017–2019 and 2021. COVID monthly incidence in 2021 and total % of population over 18 years vaccinated with complete vaccination

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3 regimen (2 doses for BNT162b2, 2 doses for mRNA1273, 2 doses ChAdOx1-CoV-2 and 1 dose for Ad26-
4 CoV-2) in 2021.
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6 Q1: January–March; Q2: April–June; Q3: July–August; Q4: October–December. NS: Not significant.
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Year	Total cases	Population	Incidence
2017	27	309,886	8.7×10^{-4}
2018	25	308,531	8.1×10^{-4}
2019	29	304,534	9.5×10^{-4}
2020	33	303,102	10.88×10^{-4}
2021	66	300,788	21.94×10^{-4}

Table 1. Graves' disease total number of cases by year in the period 2017–2021

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	Years 2017–2019 (N=81)		Year 2020 (N=33)		Year 2021 (N=66)		Overall p-value	Years 2017–2019 vs. 2020 p-value	Years 2017–2019 vs. 2021 p-value	Year 2020 vs. 2021 p-value
Sex										
Female (%)	70	86.4%	26	78.8%	62	93.9%	0.074	0.311	0.174	0.0387
Male (%)	11	13.6%	7	21.2%	4	6.1%				
Mean age (SD) years	46.3	(17.5)	51.5	(13.5)	48.6	(16.5)	0.234	0.081	0.368	0.453
FT4 (SD) ng/dL	2.43	(1.39)	3.32	(1.89)	3.24	(1.57)	<0.001	0.010	<0.001	0.777
FT3 (SD) ng/dL	0.65	(0.39)	1.11	(0.77)	1.55	(2.56)	0.216	0.370	0.089	0.927
TSH (SD) mIU/L	0.011	(0.022)	0.005	(0.01)	0.006	(0.08)	0.020	0.583	0.006	0.033
TRAbs (SD) IU/mL	21.44	(34.08)	15.91	(16.78)	13.99	(14.90)	0.286	0.360	0.126	0.746
IS supplementation	5	10.4%	1	7.1%	4	9.5%	0.935	0.186	0.803	0.776
No IS supplementation	43		13		38					
Family history of TD	10	27.8%	7	38.9%	14	31.8%	0.710	0.407	0.593	0.695
No family history of TD	26		11		30					
Autoimmune disease	5	6.2%	2	6.1%	8	12.1%	0.428	0.999	0.249	0.488
No autoimmune disease	76		31		58					
Smoker	10	12.3%	13	39.4%	14	21.2%	0.005	0.001	0.148	0.056
No smoker	71		20		52					
SARS-CoV-2 infection	NA		5	15.6%	2	3.0%		NA	NA	0.030
No SARS-CoV-2 infection	NA		21		64					

Table 2. Clinical and demographic characteristics of the population with Graves' disease by period. *IS: Iodine salt, TD: thyroid disease

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Vaccine	Total doses	%	GD cases	%	p
BNT162b2	492,085	73.95	25	56.81	0.033
mRNA1273	93,184	14.00	5	11.37	0.695
ChAdOx1-CoV-2	66,290	9.96	7	15.91	0.146
Ad26-CoV-2	13,860	2.09	7	15.91	<0.001
Total	665,419		44		

Table 3. Graves' disease cases by vaccine in the 90 days prior to symptoms onset in year 2021.

For Peer Review

TITLE: Increased incidence of Graves' disease during the SARS-CoV2 pandemic.

SHORT TITLE: Graves' disease during the covid-19 pandemic.

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ABSTRACT

Introduction

COVID-19 has a wide spectrum of clinical severity and there is evidence that SARS-Cov2 affects several organs and systems. Among the organs affected since the beginning of the pandemic, the relationship between SARS-CoV-2 infection and thyroid involvement has been demonstrated. Novel and highly effective mRNA and DNA-based vaccines have

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3 been rapidly developed to decrease SARS-CoV-2 morbidity and mortality. Early after
4 mass vaccinations, cases of thyroid dysfunction mainly including episodes of subacute
5 thyroiditis, began to be reported like adverse effects. The objective of this study is to
6 determine the impact of the pandemic, both due to SARS-CoV2 infections and
7 vaccinations, on the incidence of Graves' disease.
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10 Methods

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13 Cross-sectional, observational study comparing incidence of Graves' disease in adult
14 population (over 18 years) before (2017-2019) and after (2020-2021) Covid-19
15 pandemic. Only patients with new cases of Graves' disease, no relapsed diseases, were
16 included. SARS-CoV-2 diagnosis was based on nucleic acid amplification tests on
17 nasopharyngeal swabs or measurement of class M and class G antibodies to SARS-CoV-
18 2 by highly specific assays. Data on incidence and vaccination related to SARS-CoV-2
19 infection were obtained from the public records from Castilla y León autonomous
20 regional government.
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24 Results

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27 180 subjects were diagnosed and treated for Graves' disease (GD) during the study
28 period. We observed a notable increase in expected GD cases in 2021 compared to
29 2017-19. The number of GD cases was higher in the second (Q2) quarter. Among 2021
30 GD cases, 42/66 patients (63.6%) had been vaccinated in the 90 days prior to symptom
31 onset, but none of them in the first quarter of the year. 97.7% were women with a mean
32 age of 48.9 (SD 15.6) years. On average they were diagnosed 19.9 (SD 17.6) days after
33 receiving the vaccine. 7/42 (16.67%) had another previously diagnosed autoimmune
34 disease and 11/42 (26.19%) were smokers.
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38 Discussion

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41 Our results show a notable increase in the incidence of GD during the year 2021,
42 specially in women with a history of smoking. Hyper activation of the immune system
43 induced by SARS-Cov2 and by the recently released SARS-COV-2 vaccines has been
44 highlighted in recent months. To assess whether this observed increase in the
45 incidence of GD is sustained in the coming years or has simply been a precipitous
46 trigger for individuals who were already predisposed to develop the disease, future
47 studies will be needed.
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INTRODUCTION

The novel coronavirus disease COVID-19, caused by SARS-CoV-2, was declared a pandemic by the World Health Organization (WHO) in March 2020. Since then, as of 7:33pm CET, 18 March 2022, there have been 464,809,377 confirmed cases of COVID-19 globally, including 6,062,536 deaths, reported to the WHO, with a total of 10,925,055,390 vaccine doses having been administered⁽¹⁾.

SARS-CoV-2 has a wide spectrum of clinical severity and evidence suggests that COVID-19 can affect several organs and systems. Angiotensin-converting enzyme 2 (ACE2) is involved in SARS-CoV-2 internalization into human cells, playing an important role in the pathogenesis of COVID-19. ACE2 is expressed on thyroid follicular and parafollicular cells, allowing the entry of SARS-CoV-2 to the thyroid gland⁽²⁾. Because of this biological plausibility, there has been interest in trying to determine whether a history of thyroid dysfunction might be associated with a worse clinical course of COVID-19 or an increased risk of SARS-CoV-2 infection. Several studies on this subject have apparently concluded that neither a history of hyperthyroidism nor a history of hypothyroidism is associated with worse clinical outcomes⁽³⁾. What does seem to be confirmed is that the direct entry of SARS-CoV-2 into thyroid cells⁽⁴⁾ explains the association between a history of SARS-CoV-2 infection and the development of thyroid diseases, such as subacute thyroiditis⁽⁵⁾. It is also known that viral infections are involved in the pathogenesis of immune-mediated thyroid diseases⁽⁶⁾ which would explain the association described between a history of SARS-CoV-2 infection and new-onset Graves' disease (GD) cases⁽⁷⁾.

The severity of the SARS-CoV-2 pandemic has urged the rapid development of vaccines to decrease the associated morbidity and mortality. To date, in the Spanish healthcare system, the vaccines administered have been BNT162b2 from Pfizer-BioNTech and

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3 mRNA1273 from Moderna Biotech, both using mRNA from viruses that are capable of
4 generating immunity in the host cell, and recombinant human adenovirus vaccines such
5 as ChAdOx1-CoV-2 from AstraZeneca and Ad26-CoV-2 from Johnson and Johnson. Early
6 after mass vaccinations, cases of thyroid dysfunction began to be reported as adverse
7 effects, mainly as episodes of subacute thyroiditis which have been described with both
8 the use of mRNA vaccines^(8,9) and the use of the adenovirus vaccine¹⁰. Somewhat later,
9 there were also published cases of recurrence or new diagnoses of GD in clear relation
10 to the recent history of vaccination; both mRNA vaccines and adenovirus vaccines have
11 been implicated as the possible trigger mechanism^(11,12).

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14 The objective of this study is to determine the impact that the situation derived from
15 the pandemic, both due to SARS-CoV-2 infections and vaccinations against this agent,
16 has had on the incidence of GD.
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METHODS

Study Design and Population

This was a retrospective observational study. From January 2017 to December 2021, 180 subjects were diagnosed and treated for Graves' disease at the University Hospital of León. The referred hospital covers an area of the province of León (Spain) that has more than 300,000 inhabitants⁽¹³⁾ and for which it is the only hospital of reference. The diagnosis of Graves' disease was based on both clinical and biochemical diagnoses, considering the elevation of free T4 with respect to laboratory reference values and TSH receptor antibodies value above the reference cut-off point (>2.7 IU/mL). Patients with a relapse of previously diagnosed disease were excluded.

Patients from the period January 2017 to December 2019 are referred to as pre-SARS-CoV-2. The SARS-CoV-2 diagnosis was based on nucleic acid amplification tests performed on nasopharyngeal swabs or the measurement of class M and class G antibodies to SARS-CoV-2 by highly specific assays. Data on incidence and vaccination related to SARS-CoV-2 infection were obtained from the public records from Castilla y León regional autonomous government;⁽¹⁴⁾ those referring to our healthcare area are shown in Figures 1 and 2.

Data publication was approved by the local institutional review committee, registration number: 21170. In the years 2020 and 2021, the relationship between a history of SARS-CoV-2 infection or vaccination against SARS-CoV-2 and the onset of GD was considered probable if there was a history of SARS-CoV-2 infection or vaccination against SARS-CoV-2 within the previous 90 days. For the SARS-CoV-2 vaccination events, only the first vaccination dose was considered.

Previous autoimmune disease was considered if the patient has an established diagnosis of rheumatoid arthritis, multiple sclerosis, Hashimoto's thyroiditis, type 1 diabetes mellitus, psoriasis, celiac disease, pernicious anemia, myasthenia gravis, Addison's disease or inflammatory bowel disease.

A positive family history of thyroid disease was defined if the patient had first-line relatives with thyroid disease of immune origin: Hashimoto's thyroiditis or Graves' disease.

Laboratory Examinations

Thyroid hormones and TSH were tested using immunoenzymatic assays on the Roche Diagnostic Cobas e801 autoanalyzer. Reference ranges were 0.93–1.7 ng/dL for free T4 (FT4), 0.27–0.42 ng/dL for free triiodothyronine (FT3), and 0.27–4.2 mIU/L for TSH. TSH-

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3 receptor antibodies (TRAbs) were tested by enzyme-linked immunosorbent assay
4 (ElisaRSR TRAb third generation, Cardiff, UK) (positive cut-off >2.7 IU/mL).
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7 Statistical Analysis

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10 Statistical data analysis was performed using the statistical software R version 4.1.2.
11 Continuous variables are presented using their mean and standard deviation, while
12 categorical variables are described with the number of individuals in each category and
13 the corresponding percentage. The Shapiro-Wilk test was used to assess the normality
14 of data distribution of continuous variables. Statistical tests used to compare groups
15 included Student's t-test for normally distributed variables and the Mann-Whitney U
16 test for variables with skewed distribution. The Chi-squared test or Fisher exact test
17 were used to compare counts and frequencies between groups for categorical variables,
18 as appropriate. The Kruskal-Wallis test or one-way analysis of variance (ANOVA) with
19 post-hoc correction were also applied, depending on the distribution of variables.
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RESULTS

Study Population

In total, 180 patients were included: 87.8% were female, with a mean age of 48.11 (SD 16.51) years mean age and 8.3% had a previous diagnosis of another autoimmune disease.

Year Distribution of Graves' Disease Incidence

Table 1 shows a similar number of Graves' disease patients per year throughout the period from 2017 to 2020, while a large increase is observed in 2021 with the total number of cases double that of 2020 ($p < 0.001$) and 2.44 times higher than the average incidence of cases in the 2017–2019 period ($p < 0.001$).

Year	Total cases	Population	Incidence
2017	27	309,886	8.7×10^{-4}
2018	25	308,531	8.1×10^{-4}
2019	29	304,534	9.5×10^{-4}
2020	33	303,102	10.88×10^{-4}
2021	66	300,788	21.94×10^{-4}

Table 1. Graves' disease total number of cases by year in the period 2017–2021.

Seasonal Distribution of Graves' Disease Incidence

a. 2020 vs 2017–19

We observed a similar number of GD patients per year throughout the period from 2017–2020 (Table 1). By seasonal distribution of GD cases, no differences were observed in the distribution by quarter period (Fig. 1). The 3 waves of SARS-CoV-2 of 2021 incidence observed in our department was superimposable to that which happened across the rest of the country (Fig. 1). From our point of view, the number of SARS-CoV-2 cases from the first wave is probably underestimated because of healthcare disruption during the early pandemic.

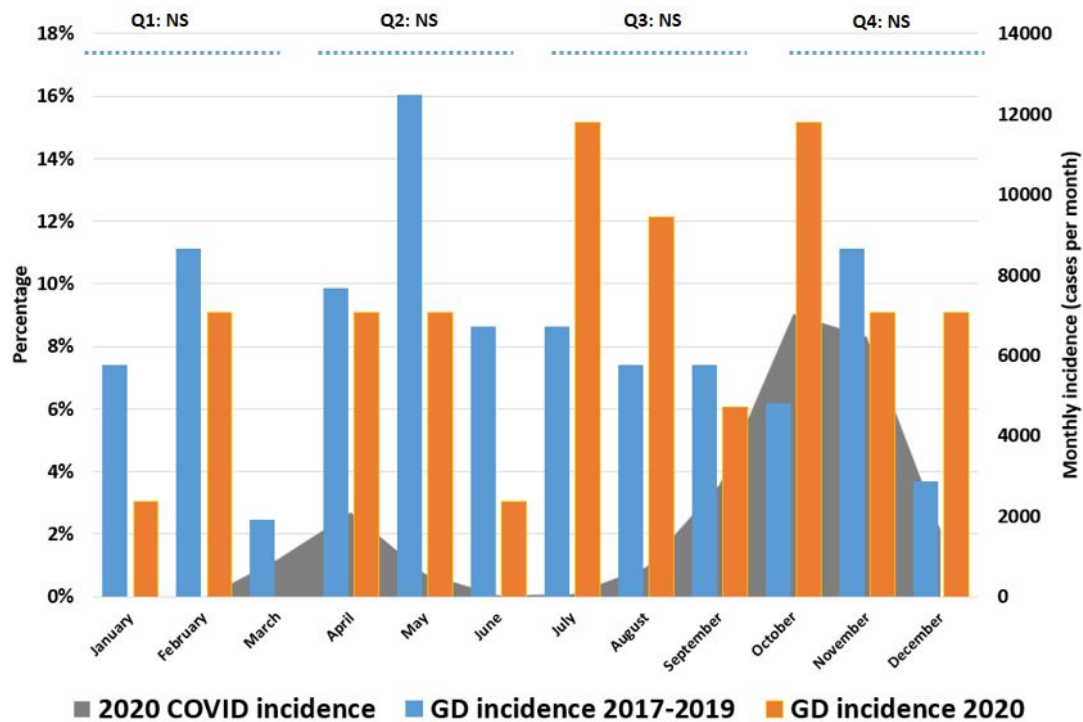


Figure 1. Percentage of cases of Graves' disease by month in 2017–2019 and 2020. COVID monthly incidence in 2020 is shaded.

Q1: January–March; Q2: April–June; Q3: July–August; Q4: October–December. NS: Not significant.

b. 2021 vs 2017-19

We observed a notable increase in expected GD cases in 2021 compared to 2017–19. The seasonal percent distribution of GD cases was different in the second quarter (Q2: April to June). During Q2 of the years 2017–2019, cases were more common compared with 2021 (28/81 vs 10/66) ($p=0.038$); no significant differences were found in the rest of the three-month periods. The 3 waves of SARS-CoV-2 of 2021 incidence observed in our department was superimposable to what happened in the rest of the country (Fig. 2), the valley of the case curve during the year 2021 coincides with a period between SARS-CoV-2 waves. The vaccination curve of the population of the health area expressed as a percentage has also been superimposed.

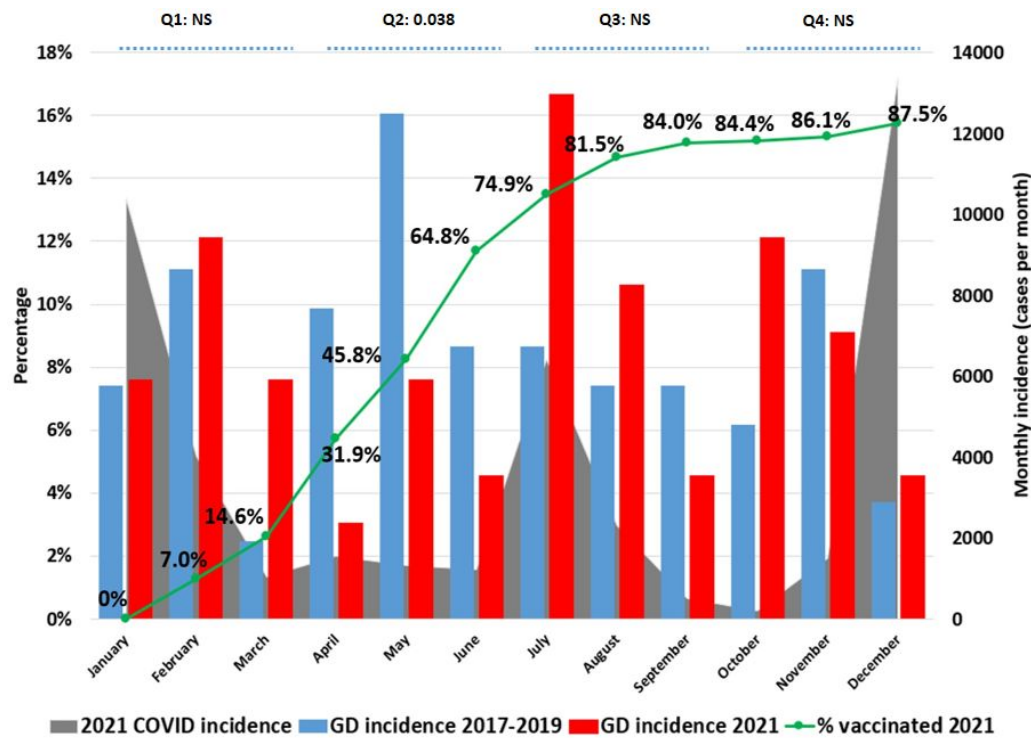


Figure 2. Percentage of cases of Grave's disease by month in 2017–2019 and 2021. COVID monthly incidence in 2021 and total % of population over 18 years vaccinated with complete vaccination regimen (2 doses for BNT162b2, 2 doses for mRNA1273, 2 doses ChAdOx1-CoV-2 and 1 dose for Ad26-CoV-2) in 2021.

Q1: January–March; Q2: April–June; Q3: July–August; Q4: October–December. NS: Not significant.

Clinical and laboratory features

No patient had a history of SARS-CoV-2 infection in 2017-2019. In 2020, 5/33 (15.2%) patients had a history of SARS-CoV-2 infection demonstrated by PCR (polymerase chain reaction) within 90 days prior to symptom onset, while 2/66 (3.0%) patients had a history of PCR-demonstrated SARS-CoV-2 infection in the 90 days prior to symptom onset in 2021 (Table 2). Notable findings include a significant increase in initial FT4 numbers in both 2020 and 2021 compared to pre-pandemic years and a higher proportion of females in 2021 compared to 2020.

Distribution of Graves' Disease Incidence by vaccine

In 2021, 44/66 patients (66.7%) had a history of vaccination in the 90 days prior to symptom onset, although none of these were in the first three months of the year. Of these 44 patients, 23 developed Graves' disease after receiving the first dose of SARS-CoV-2 vaccine, compared to 21 who did so after the second dose. None had a recorded history of SARS-CoV-2 infection. Overall, 43/44 (97.7%) were women with a mean age of 48.9 (SD 15.6) years. On average, they were diagnosed 19.9 (SD 17.6) days after receiving the vaccine. Also, 7/44 (15.9%) had another previously diagnosed autoimmune disease and 11/44 (25%) were smokers.

In total, 25/44 (56.8%) received the Pfizer vaccine (BNT162b2), 5 (11.4%) the Moderna vaccine (mRNA1273), 7 (15.9%) the AstraZeneca vaccine (ChAdOx1-COV-2), and 7 (15.9%) the Janssen vaccine (Ad26-CoV-2). Significant differences were observed with an increase in the GD cases recorded after vaccination with the Janssen vaccine and that expected according to the percentage of administered doses (2.1%, $p < 0.001$). 74.0% of the population was vaccinated with Pfizer's vaccine, while the percentage of observed cases of GD decreased to 59.52%, $p = 0.033$.

Vaccine	Total doses	%	GD cases	%	p
BNT162b2	492,085	73.95	25	56.81	0.033
mRNA1273	93,184	14.00	5	11.37	0.695
ChAdOx1-CoV-2	66,290	9.96	7	15.91	0.146
Ad26-CoV-2	13,860	2.09	7	15.91	<0.001
Total	665,419		44		

Table 3. Graves' disease cases by vaccine in the 90 days prior to symptoms onset in year 2021.

	Years 2017–2019 (N=81)		Year 2020 (N=33)		Year 2021 (N=66)		Overall p-value	Years 2017–2019 vs. 2020 p-value	Years 2017–2019 vs. 2021 p-value	Year 2020 vs. 2021 p-value
Sex										
Female (%)	70	86.4%	26	78.8%	62	93.9%	0.074	0.311	0.174	0.0387
Male (%)	11	13.6%	7	21.2%	4	6.1%				
Mean age (SD) years	46.3	(17.5)	51.5	(13.5)	48.6	(16.5)	0.234	0.081	0.368	0.453
FT4 (SD) ng/dL	2.43	(1.39)	3.32	(1.89)	3.24	(1.57)	<0.001	0.010	<0.001	0.777
FT3 (SD) ng/dL	0.65	(0.39)	1.11	(0.77)	1.55	(2.56)	0.216	0.370	0.089	0.927
TSH (SD) mIU/L	0.011	(0.022)	0.005	(0.01)	0.006	(0.08)	0.020	0.583	0.006	0.033
TRAbs (SD) IU/mL	21.44	(34.08)	15.91	(16.78)	13.99	(14.90)	0.286	0.360	0.126	0.746
IS supplementation	5	10.4%	1	7.1%	4	9.5%	0.935	0.186	0.803	0.776
No IS supplementation	43		13		38					
Family history of TD	10	27.8%	7	38.9%	14	31.8%	0.710	0.407	0.593	0.695
No family history of TD	26		11		30					
Autoimmune disease	5	6.2%	2	6.1%	8	12.1%	0.428	0.999	0.249	0.488
No autoimmune disease	76		31		58					
Smoker	10	12.3%	13	39.4%	14	21.2%	0.005	0.001	0.148	0.056
No smoker	71		20		52					
SARS-CoV-2 infection	NA		5	15.6%	2	3.0%		NA	NA	0.030
No SARS-CoV-2 infection	NA		21		64					

Table 2. Clinical and demographic characteristics of the population with Graves' disease by period. *IS: Iodine salt, TD: thyroid disease

DISCUSSION

Graves' disease (GD) is an organ-specific autoimmune disorder, associated with the presence of circulating TSH-R autoantibodies, and is the most common cause of hyperthyroidism in developed countries. Although GD can affect anyone, it is more common between 30 and 60 years of age, and about 5 to 6 times higher in females than in males⁽¹⁵⁾. Previous studies carried out in other developed countries indicate a variable incidence of 10–50 cases per 100,000 inhabitants/year⁽¹⁶⁾. This variability responds to the strong genetic component in the likelihood of developing GD, which is estimated at 79%⁽¹⁷⁾, but many other risk factors have been associated with autoimmune thyroid disorders involving T-cell dysfunction, among which smoking stands out with an odds ratio of 1.9,¹⁸ or iodine intake⁽¹⁹⁾. Unfortunately, we have a lack of knowledge on the incidence of GD in Spain and only have data about the estimated prevalence of 0.8%⁽²⁰⁾ of the population being diagnosed with hyperthyroidism and 0.14% receiving antithyroid treatment⁽²¹⁾.

Compared with pre-pandemic, the post-SARS-CoV-2 era patients presented some differences from a clinical and epidemiological point of view, with a more severe thyrotoxicosis, an increase in the levels of FT4 and an even greater gender gap than traditionally described, of up 10 times higher in females; in fact, only one man presented GD with the possibility that it was triggered by a history of vaccination. There is also a higher incidence of smoking in the cases observed in 2020; one possibility is that a history of SARS-CoV-2 infection may have triggered a higher number of cases in predisposed subjects, just as a history of smoking is known to be associated with a more severe course of COVID-19⁽²²⁾.

For obvious reasons, no patient in this study had a history of SARS-CoV-2 infection in 2017–2019. It should also be considered that the number of SARS-CoV-2 cases from the first wave in 2020 is probably underestimated because of the disruption to healthcare during the early pandemic. In 2020, 5/33 (15.2%) patients had a history of SARS-CoV-2 infection demonstrated by PCR within 90 days prior to symptom onset, while only 2/66 (3.0%) patients had a history of PCR-demonstrated SARS-CoV-2 infection in the 90 days prior to symptom onset in 2021. Although the real incidence of SARS-CoV-2 infection in 2020 was probably underestimated, the seroprevalence IgG-SARS-CoV-2 data in 2020 was 9.7% in León province⁽²³⁾; this difference contrasts with the fact that the recorded incidence of SARS-CoV-2 infection during 2021 was much higher than that recorded during 2020 (Fig 1, Fig 2) and, while there was only a small increase in GD cases during 2020, the incidence of GD more than doubled during 2021, which suggests that large-scale vaccination has had an impact of its own. It should be considered that the doubled diagnosis of Graves' disease in 2021 might reflect a delayed diagnosis of cases in 2020 due to healthcare disruption which occurred during the first year of the pandemic in addition to vaccination.

The possible interrelationship of vaccines developed for SARS-COV-2 and the activation of the autoimmune system has been highlighted in recent months. The most widely

1
2
3 accepted theory is the "adjuvant-induced autoimmune/inflammatory syndrome"
4 (AISIS); for example, aluminum salts are the adjuvant in the Ad26-CoV-2 from Johnson
5 and Johnson vaccine, Pfizer-BioNTech's BNT162b2 vaccine contains lipid nanoparticles
6 to facilitate the transport of mRNA into the host cell and two of them contain
7 polyethylene glycol (PEG) extracts that can trigger an exaggerated immune response. In
8 addition to AISIS (also known as ASIA syndrome), other possible explanations for Graves'
9 disease being triggered by SARS-CoV-2 vaccinations include molecular mimicry of the
10 spike protein with endogenous thyroid antigens, or a toxic activity of the spike protein
11 due to the interaction with ACE-II, which is highly expressed in the thyroid gland,
12 triggering inflammation and then autoimmunity. Adjuvants are generally well tolerated,
13 but they can cause an autoimmune response in genetically predisposed subjects. This
14 can occur by a variety of mechanisms, including altered host immunity, polyclonal
15 activation of B cells, effects on cell-mediated immunity, virus-induced antibodies and
16 accelerated molecular mimicry ⁽¹⁵⁾.
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22 Although some differences have been observed with an apparently higher relative risk
23 in adenovirus vaccines compared to mRNA vaccines, this could be explained by the fact
24 that adenovirus vaccines were used in the vaccination strategy of young populations
25 that are more susceptible to immunological problems. It should also be taken into
26 consideration that the vaccination strategy in our population was changeable due to the
27 limited availability of new doses and some subjects ended up combining different types
28 of vaccines in the first and second doses, although this was not the case for any of the
29 individuals in this study, but it did occur in the general population.
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34 With the aforementioned limitations, it seems that the results obtained in this work
35 should invite the healthcare community to be especially vigilant about the possible
36 impact of mass vaccination campaigns and/or a history of SARS-CoV-2 infection on the
37 development of this and other autoimmune diseases. In our opinion, it is necessary to
38 perform larger studies that will allow us to deepen our knowledge of the specific risk
39 attributable to each type of vaccine, revaccination or history of infection, and to
40 complement this with data that have been observed in other geographic areas. In addition,
41 long-term studies should be considered to assess differences in the clinical course and
42 therapeutic needs depending on the triggering factor. Finally, it is worth analyzing in the
43 coming years whether the increase observed corresponds to patients with a predisposition
44 to develop the disease, where the precipitating factor has brought the process forward a
45 few years, or whether the increase in incidence will be maintained in the long-term.
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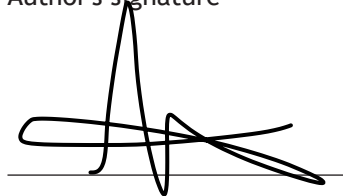
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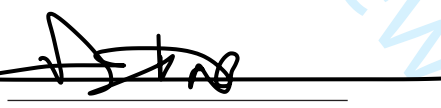
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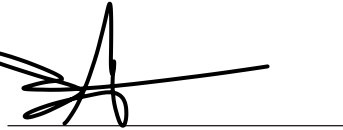
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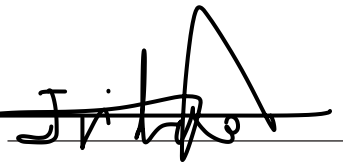
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ABSTRACT

Objective

The objective of this study is to determine the impact of the pandemic, both due to SARS-CoV-2 infections and vaccinations, on the incidence of Graves' disease.

Context

COVID-19 has a wide spectrum of clinical severity and there is evidence that SARS-CoV-2 affects several organs and systems. Among the organs affected since the beginning of the pandemic, a relationship between SARS-CoV-2 infection and thyroid involvement has been demonstrated. Novel and highly effective mRNA and DNA-based vaccines have been rapidly developed to decrease SARS-CoV-2 morbidity and mortality. Early after mass vaccinations, cases of thyroid dysfunction, mainly including episodes of subacute thyroiditis, began to be reported as adverse effects.

Design

This was a cross-sectional, observational study comparing the incidence of Graves' disease in adult population (over 18 years) before (2017–2019) and after (2020–2021) the COVID-19 pandemic.

Patients

Only patients with new cases of Graves' disease, no relapsed diseases, were included. In total, 180 subjects were diagnosed and treated for Graves' disease (GD) during the study period.

Measurements

SARS-CoV-2 diagnosis was based on nucleic acid amplification tests on nasopharyngeal swabs or measurements of class M and class G antibodies to SARS-CoV-2 by highly specific assays. Data on incidence and vaccination related to SARS-CoV-2 infection were obtained from the public records from Castilla y León autonomous regional government.

Results

We observed a notable increase in expected GD cases in 2021 compared to 2017–19. The number of GD cases was higher in the second (Q2) quarter. Among the 2021 GD cases, 42/66 patients (63.6%) had been vaccinated in the 90 days prior to symptom onset, but none of them in the first quarter of the year. Overall, 97.7% were women with a mean age of 48.9 (SD 15.6) years. On average they were diagnosed 19.9 (SD 17.6) days after receiving the vaccine, 7/42 (16.67%) had another previously diagnosed autoimmune disease and 11/42 (26.19%) were smokers.

Discussion

Our results show a notable increase in the incidence of GD during 2021, particularly in women with a history of smoking. Hyperactivation of the immune system induced by SARS-CoV-2 and by the recently released SARS-CoV-2 vaccines has been highlighted in recent months. To assess whether this observed increase in the incidence of GD is sustained in the coming years or has simply been a precipitous trigger for individuals who were already predisposed to develop the disease, future studies are required.

INTRODUCTION

The novel coronavirus disease COVID-19, caused by SARS-CoV-2, was declared a pandemic by the World Health Organization (WHO) in March 2020. Since then, as of 7:33pm CET, 18 March 2022, there have been 464,809,377 confirmed cases of COVID-19 globally, including 6,062,536 deaths, reported to the WHO, with a total of 10,925,055,390 vaccine doses having been administered⁽¹⁾.

SARS-CoV-2 has a wide spectrum of clinical severity and evidence suggests that COVID-19 can affect several organs and systems. Angiotensin-converting enzyme 2 (ACE2) is involved in SARS-CoV-2 internalization into human cells, playing an important role in the pathogenesis of COVID-19. ACE2 is expressed on thyroid follicular and parafollicular cells, allowing the entry of SARS-CoV-2 to the thyroid gland⁽²⁾. Because of this biological plausibility, there has been interest in trying to determine whether a history of thyroid dysfunction might be associated with a worse clinical course of COVID-19 or an increased risk of SARS-CoV-2 infection. Several studies on this subject have apparently concluded that neither a history of hyperthyroidism nor a history of hypothyroidism is associated with worse clinical outcomes⁽³⁾. What does seem to be confirmed is that the direct entry of SARS-CoV-2 into thyroid cells⁽⁴⁾ explains the association between a history of SARS-CoV-2 infection and the development of thyroid diseases, such as subacute thyroiditis⁽⁵⁾. It is also known that viral infections are involved in the pathogenesis of immune-mediated thyroid diseases⁽⁶⁾ which would explain the association described between a history of SARS-CoV-2 infection and new-onset Graves' disease (GD) cases⁽⁷⁾.

The severity of the SARS-CoV-2 pandemic has urged the rapid development of vaccines to decrease the associated morbidity and mortality. To date, in the Spanish healthcare system, the vaccines administered have been BNT162b2 from Pfizer-BioNTech and mRNA1273 from Moderna Biotech, both using mRNA from viruses that are capable of generating immunity in the host cell, and recombinant human adenovirus vaccines such as ChAdOx1-CoV-2 from AstraZeneca and Ad26-CoV-2 from Johnson and Johnson. Early after mass vaccinations, cases of thyroid dysfunction began to be reported as adverse effects, mainly as episodes of subacute thyroiditis which have been described with both the use of mRNA vaccines^(8,9) and the use of the adenovirus vaccine¹⁰. Somewhat later, there were also published cases of recurrence or new diagnoses of GD in clear relation

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3 to the recent history of vaccination; both mRNA vaccines and adenovirus vaccines have
4 been implicated as the possible trigger mechanism^(11,12).

5 The objective of this study is to determine the impact that the situation derived from
6 the pandemic, both due to SARS-CoV-2 infections and vaccinations against this agent,
7 has had on the incidence of GD.
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For Peer Review

METHODS

Study Design and Population

This was a retrospective observational study. From January 2017 to December 2021, 180 subjects were diagnosed and treated for Graves' disease at the University Hospital of León. The referred hospital covers an area of the province of León (Spain) that has more than 300,000 inhabitants⁽¹³⁾ and for which it is the only hospital of reference. The diagnosis of Graves' disease was based on both clinical and biochemical diagnoses, considering the elevation of free T4 with respect to laboratory reference values and TSH receptor antibodies value above the reference cut-off point (>2.7 IU/mL). Patients with a relapse of previously diagnosed disease were excluded.

Patients from the period January 2017 to December 2019 are referred to as pre-SARS-CoV-2. The SARS-CoV-2 diagnosis was based on nucleic acid amplification tests performed on nasopharyngeal swabs or the measurement of class M and class G antibodies to SARS-CoV-2 by highly specific assays. Data on incidence and vaccination related to SARS-CoV-2 infection were obtained from the public records from Castilla y León regional autonomous government;⁽¹⁴⁾ those referring to our healthcare area are shown in Figures 1 and 2.

Data publication was approved by the local institutional review committee, registration number: 21170. In the years 2020 and 2021, the relationship between a history of SARS-CoV-2 infection or vaccination against SARS-CoV-2 and the onset of GD was considered probable if there was a history of SARS-CoV-2 infection or vaccination against SARS-CoV-2 within the previous 90 days. For the SARS-CoV-2 vaccination events, only the first vaccination dose was considered.

Previous autoimmune disease was considered if the patient has an established diagnosis of rheumatoid arthritis, multiple sclerosis, Hashimoto's thyroiditis, type 1 diabetes mellitus, psoriasis, celiac disease, pernicious anemia, myasthenia gravis, Addison's disease or inflammatory bowel disease.

A positive family history of thyroid disease was defined if the patient had first-line relatives with thyroid disease of immune origin: Hashimoto's thyroiditis or Graves' disease.

Laboratory Examinations

Thyroid hormones and TSH were tested using immunoenzymatic assays on the Roche Diagnostic Cobas e801 autoanalyzer. Reference ranges were 0.93–1.7 ng/dL for free T4 (FT4), 0.27–0.42 ng/dL for free triiodothyronine (FT3), and 0.27–4.2 mIU/L for TSH. TSH-receptor antibodies (TRAbs) were tested by enzyme-linked immunosorbent assay (ElisaRSR TRAb third generation, Cardiff, UK) (positive cut-off >2.7 IU/mL).

Statistical Analysis

Statistical data analysis was performed using the statistical software R version 4.1.2. Continuous variables are presented using their mean and standard deviation, while categorical variables are described with the number of individuals in each category and the corresponding percentage. The Shapiro-Wilk test was used to assess the normality of data distribution of continuous variables. Statistical tests used to compare groups included Student's t-test for normally distributed variables and the Mann-Whitney U test for variables with skewed distribution. The Chi-squared test or Fisher exact test were used to compare counts and frequencies between groups for categorical variables, as appropriate. The Kruskal-Wallis test or one-way analysis of variance (ANOVA) with post-hoc correction were also applied, depending on the distribution of variables.

For Peer Review

RESULTS

Study Population

In total, 180 patients were included: 87.8% were female, with a mean age of 48.11 (SD 16.51) years mean age and 8.3% had a previous diagnosis of another autoimmune disease.

Year Distribution of Graves' Disease Incidence

Table 1 shows a similar number of Graves' disease patients per year throughout the period from 2017 to 2020, while a large increase is observed in 2021 with the total number of cases double that of 2020 ($p < 0.001$) and 2.44 times higher than the average incidence of cases in the 2017–2019 period ($p < 0.001$).

Seasonal Distribution of Graves' Disease Incidence

a. 2020 vs 2017–19

We observed a similar number of GD patients per year throughout the period from 2017–2020 (Table 1). By seasonal distribution of GD cases, no differences were observed in the distribution by quarter period (Fig. 1). The 3 waves of SARS-CoV-2 of 2021 incidence observed in our department was superimposable to that which happened across the rest of the country (Fig. 1). From our point of view, the number of SARS-CoV-2 cases from the first wave is probably underestimated because of healthcare disruption during the early pandemic. 2021 vs 2017-19

We observed a notable increase in expected GD cases in 2021 compared to 2017–19. The seasonal percent distribution of GD cases was different in the second quarter (Q2: April to June). During Q2 of the years 2017–2019, cases were more common compared with 2021 (28/81 vs 10/66) ($p = 0.038$); no significant differences were found in the rest of the three-month periods. The 3 waves of SARS-CoV-2 of 2021 incidence observed in our department was superimposable to what happened in the rest of the country (Fig. 2), the valley of the case curve during the year 2021 coincides with a period between SARS-CoV-2 waves. The vaccination curve of the population of the health area expressed as a percentage has also been superimposed.

Clinical and laboratory features

No patient had a history of SARS-CoV-2 infection in 2017-2019. In 2020, 5/33 (15.2%) patients had a history of SARS-CoV-2 infection demonstrated by PCR (polymerase chain reaction) within 90 days prior to symptom onset, while 2/66 (3.0%) patients had a history of PCR-demonstrated SARS-CoV-2 infection in the 90 days prior to symptom onset in 2021 (Table 2). Notable findings include a significant increase in initial FT4

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3 numbers in both 2020 and 2021 compared to pre-pandemic years and a higher
4 proportion of females in 2021 compared to 2020.
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6 7 Distribution of Graves' Disease Incidence by vaccine

8 In 2021, 44/66 patients (66.7%) had a history of vaccination in the 90 days prior to
9 symptom onset, although none of these were in the first three months of the year. Of
10 these 44 patients, 23 developed Graves' disease after receiving the first dose of SARS-
11 CoV-2 vaccine, compared to 21 who did so after the second dose. None had a recorded
12 history of SARS-CoV-2 infection. Overall, 43/44 (97.7%) were women with a mean age
13 of 48.9 (SD 15.6) years. On average, they were diagnosed 19.9 (SD 17.6) days after
14 receiving the vaccine. Also, 7/44 (15.9%) had another previously diagnosed autoimmune
15 disease and 11/44 (25%) were smokers.
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20 In total, 25/44 (56.8%) received the Pfizer vaccine (BNT162b2), 5 (11.4%) the Moderna
21 vaccine (mRNA1273), 7 (15.9%) the AstraZeneca vaccine (ChAdOx1-COV-2), and 7
22 (15.9%) the Janssen vaccine (Ad26-CoV-2). Significant differences were observed with
23 an increase in the GD cases recorded after vaccination with the Janssen vaccine and that
24 expected according to the percentage of administered doses (2.1%, $p < 0.001$). 74.0% of
25 the population was vaccinated with Pfizer's vaccine, while the percentage of observed
26 cases of GD decreased to 59.52%, $p = 0.033$. (Table 3)
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33 DISCUSSION

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35 Graves' disease (GD) is an organ-specific autoimmune disorder, associated with the
36 presence of circulating TSH-R autoantibodies, and is the most common cause of
37 hyperthyroidism in developed countries. Although GD can affect anyone, it is more
38 common between 30 and 60 years of age, and about 5 to 6 times higher in females than
39 in males⁽¹⁵⁾. Previous studies carried out in other developed countries indicate a variable
40 incidence of 10–50 cases per 100,000 inhabitants/year⁽¹⁶⁾. This variability responds to
41 the strong genetic component in the likelihood of developing GD, which is estimated at
42 79%⁽¹⁷⁾, but many other risk factors have been associated with autoimmune thyroid
43 disorders involving T-cell dysfunction, among which smoking stands out with an odds
44 ratio of 1.9,¹⁸ or iodine intake⁽¹⁹⁾. Unfortunately, we have a lack of knowledge on the
45 incidence of GD in Spain and only have data about the estimated prevalence of 0.8%⁽²⁰⁾
46 of the population being diagnosed with hyperthyroidism and 0.14% receiving
47 antithyroid treatment⁽²¹⁾.
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52 Compared with pre-pandemic, the post-SARS-CoV-2 era patients presented some
53 differences from a clinical and epidemiological point of view, with a more severe
54 thyrotoxicosis, an increase in the levels of FT4 and an even greater gender gap than
55 traditionally described, of up 10 times higher in females; in fact, only one man presented
56 GD with the possibility that it was triggered by a history of vaccination. There is also a
57 higher incidence of smoking in the cases observed in 2020; one possibility is that a
58 history of SARS-CoV-2 infection may have triggered a higher number of cases in
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3 predisposed subjects, just as a history of smoking is known to be associated with a more
4 severe course of COVID-19⁽²²⁾, but another consideration is that smoking may be a
5 predisposing factor for developing Graves' disease after SARS-CoV2 infection.
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8 For obvious reasons, no patient in this study had a history of SARS-CoV-2 infection in
9 2017–2019. It should also be considered that the number of SARS-CoV-2 cases from the
10 first wave in 2020 is probably underestimated because of the disruption to healthcare
11 during the early pandemic. In 2020, 5/33 (15.2%) patients had a history of SARS-CoV-2
12 infection demonstrated by PCR within 90 days prior to symptom onset, while only 2/66
13 (3.0%) patients had a history of PCR-demonstrated SARS-CoV-2 infection in the 90 days
14 prior to symptom onset in 2021. Although the real incidence of SARS-CoV-2 infection in
15 2020 was probably underestimated, the seroprevalence IgG-SARS-CoV-2 data in 2020
16 was 9.7% in León province⁽²³⁾; this difference contrasts with the fact that the recorded
17 incidence of SARS-CoV-2 infection during 2021 was much higher than that recorded
18 during 2020 (Fig 1, Fig 2) and, while there was only a small increase in GD cases during
19 2020, the incidence of GD more than doubled during 2021, which suggests that large-
20 scale vaccination has had an impact of its own. It should be considered that the doubled
21 diagnosis of Graves' disease in 2021 might reflect a delayed diagnosis of cases in 2020
22 due to healthcare disruption which occurred during the first year of the pandemic in
23 addition to vaccination.
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30 The possible interrelationship of vaccines developed for SARS-COV-2 and the activation
31 of the autoimmune system has been highlighted in recent months. The most widely
32 accepted theory is the "adjuvant-induced autoimmune/inflammatory syndrome"
33 (AISIS); for example, aluminum salts are the adjuvant in the Ad26-CoV-2 from Johnson
34 and Johnson vaccine, Pfizer-BioNTech's BNT162b2 vaccine contains lipid nanoparticles
35 to facilitate the transport of mRNA into the host cell and two of them contain
36 polyethylene glycol (PEG) extracts that can trigger an exaggerated immune response. In
37 addition to AISIS (also known as ASIA syndrome), other possible explanations for Graves'
38 disease being triggered by SARS-CoV-2 vaccinations include molecular mimicry of the
39 spike protein with endogenous thyroid antigens, or a toxic activity of the spike protein
40 due to the interaction with ACE-II, which is highly expressed in the thyroid gland,
41 triggering inflammation and then autoimmunity. Adjuvants are generally well tolerated,
42 but they can cause an autoimmune response in genetically predisposed subjects. This
43 can occur by a variety of mechanisms, including altered host immunity, polyclonal
44 activation of B cells, effects on cell-mediated immunity, virus-induced antibodies and
45 accelerated molecular mimicry⁽¹⁵⁾.
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52 Although some differences have been observed with an apparently higher relative risk
53 in adenovirus vaccines compared to mRNA vaccines, this could be explained by the fact
54 that adenovirus vaccines were used in the vaccination strategy of young populations
55 that are more susceptible to immunological problems. It should also be taken into
56 consideration that the vaccination strategy in our population was changeable due to the
57 limited availability of new doses and some subjects ended up combining different types
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3 of vaccines in the first and second doses, although this was not the case for any of the
4 individuals in this study, but it did occur in the general population.
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7 With the aforementioned limitations, it seems that the results obtained in this work
8 should invite the healthcare community to be especially vigilant about the possible
9 impact of mass vaccination campaigns and/or a history of SARS-CoV-2 infection on the
10 development of this and other autoimmune diseases. In our opinion, it is necessary to
11 perform larger studies that will allow us to deepen our knowledge of the specific risk
12 attributable to each type of vaccine, revaccination or history of infection, and to
13 complement this with data that have been observed in other geographic areas. In addition,
14 long-term studies should be considered to assess differences in the clinical course and
15 therapeutic needs depending on the triggering factor. Finally, it is worth analyzing in the
16 coming years whether the increase observed corresponds to patients with a predisposition
17 to develop the disease, where the precipitating factor has brought the process forward a
18 few years, or whether the increase in incidence will be maintained in the long-term.
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