Suppression of pituitary hormone genes in subjects who died from COVID-19 independently of virus detection in the gland

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Abstract

Context. Involvement of the pituitary gland in SARS-CoV-2 infection has been clinically suggested by pituitary hormone deficiency in severe COVID-19 cases, by altered serum ACTH levels in hospitalized patients, and by cases of pituitary apoplexy. However, the direct viral infection of the gland has not been investigated.

Objectives. To evaluate whether the SARS-CoV-2 genome and antigens could be present in pituitary glands of lethal cases of COVID-19, and to assess possible changes in the expression of immune-related and pituitary-specific genes.

Methods. SARS-CoV-2 genome and antigens were searched in the pituitary gland of 23 patients who died from COVID-19 and, as controls, in 12 subjects who died from trauma or sudden cardiac death. Real-time RT-PCR, in situ hybridization, immunohistochemistry and transmission electron microscopy were utilized. Levels of mRNA transcripts of immune-related and pituitary-specific genes were measured by the nCounter assay.

Results. The SARS-CoV-2 genome and antigens were detected in 14/23 (61%) pituitary glands of the COVID-19 group, not in controls. In SARS-CoV-2 positive pituitaries, the viral genome was consistently detected by PCR in the adeno- and the neurohypophysis. Immunohistochemistry, in situ hybridization and transmission electron microscopy confirmed the presence of SARS-CoV-2 in the pituitary. Activation of type I interferon signaling and enhanced levels of neutrophil and cytotoxic cell scores were found in virus-positive glands. mRNA transcripts of pituitary hormones and pituitary developmental/regulatory genes were suppressed in all COVID-19 cases irrespective of virus-positivity.

Conclusion. Our study supports the tropism of SARS-CoV-2 for human pituitary and encourage to explore pituitary dysfunction post-COVID-19.

Keywords: SARS-CoV-2, COVID-19, pituitary gland, hypophysis, gene expression, pituitary hormones

Introduction

The Coronavirus Disease-2019 (COVID-19) caused by multiple variants of the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) has been spreading worldwide for over two years provoking deaths and sequelae in the respiratory system and extrapulmonary organs [1]. Ample evidence shows that during the 2003 outbreak of the Severe Acute Respiratory Syndrome (SARS) due to SARS-CoV-1, many patients suffered extrapulmonary sequelae in the gastrointestinal, cardiovascular, immune, nervous, and endocrine systems [2,3].

Confirming previous observations on infection by SARS-CoV-1 (which is strictly linked to SARS-CoV-2), even the COVID-19 pandemic may influence endocrine organs such as the adrenals [4,5], thyroid [6–9], testes [10–12], ovaries [13], pancreatic islets [14–17], and pituitary [5,6,18,19]. In lethal cases of COVID-19, autopsy studies revealed the virus within endocrine cells and explored the alterations of endocrine organs [13,20]. Clinical studies analyzed the endocrine consequences of COVID-19 [2,3,21–23].

As reported by Frara and colleagues [24], pre-existing pituitary disorders may aggravate COVID-19. For instance, Cushing disease and acromegaly predispose to obesity, visceral adiposity, and diabetes mellitus. Untreated growth hormone (GH) deficiency associates with abnormal lipid profile and increased production of proinflammatory cytokines. Hypopituitarism, acromegaly, Cushing disease, adenomas secreting thyroid-stimulating hormone (TSH) are associated with vertebral fractures that aggravate the respiratory dysfunction typical of COVID-19. Also, due to the possible administration of glucocorticoids, hospitalized COVID-19 patients tend to have reduced levels (<30 pg/mL) of adrenocorticotropic hormone (ACTH) while patients with mild to medium severity conditions have ACTH levels higher than normal [19]. Low ACTH levels appear to correlate with disease severity [5] and a dramatic fall of ACTH is a hallmark of severe COVID-19 [19]. In addition, it has been shown that adrenals may be directly infected with SARS-CoV-2 [4]. The resulting cellular damage could predispose COVID-19 patients to adrenal dysfunction [4]. In inflammatory diseases, cytokines tend to enhance ACTH production [25], but, in severe cases, the feedback acting on the hypothalamus-

pituitary-adrenal (HPA) axis is often impaired, and ACTH levels remain low [5]. Impairment of the pituitary-thyroid axis has been documented in the clinics [6], while central diabetes insipidus (ADH deficiency) has been reported as a consequence of COVID-19 [18]. A few cases of pituitary apoplexy have been reported in COVID-19, both in patients with pituitary adenoma [26–29] and in patients with conserved pituitary function [30,31].

Physiological and pathological conditions may influence the secretion of pituitary hormones, including infection. For these reasons, we investigated autoptic COVID-19 cases who died from SARS-CoV-2 infection to assess whether the virus could be found in the pituitary gland. In several cases, the virus was found in cells of the adeno- and neurohypophysis. Pituitary infection was associated with altered transcription of immune- and hormone-related genes.

Materials and methods

Investigated cases

All patients were of Caucasian ethnicity and were recruited between November 2020 and January 2022. Three case groups were investigated: a) controls (i.e., subjects who died from acute causes other than infectious, such as trauma and sudden cardiac death; n=12); b) patients who died from COVID-19 that had pituitary tissue negative for SARS-CoV-2 (n=9); patients who died from COVID-19 that had pituitary tissue positive for SARS-CoV-2 (n=14). Patients were treated according to COVID-19 protocols that were standard at the time of hospitalization [32,33]. Received treatments did not include antivirals or IL1/IL6 blockade [34]. Prior to COVID-19, none of the patients had clinical evidence of pituitary dysfunction.

At autopsy, the pituitary gland was accurately removed, fixed in formalin, and embedded in paraffin for pathological evaluation and for molecular analyses. Tissue samples for transmission electron microscopy were also taken. In 15/23 COVID-19 cases, the neurohypophysis was also sampled. Due to the low amount of material, neurohypophysis tissue was used only for SARS-CoV-2 detection, and gene expression analysis could not be done.

At pathological examination, diffuse alveolar damage, fibrosis, and T-cell infiltration of lung parenchyma was found in all COVID-19 cases, and the pathological report defined COVID-19 as the cause of death. Two lung samples were tested for SARS-CoV-2 genome by real-time RT-PCR. All COVID-19 cases were positive while virus was not detected in controls. In both COVID-19 cases and controls, histopathological examination of pituitaries did not detect neoplastic lesions.

The study was approved by the local Ethics Committee (Comitato Etico Area Vasta Nord-Ovest, Italy; protocol number 17327, 2020-05-14).

Virus detection and gene expression analyses

For each case, RNA was purified from three 10µm-thick unstained sections using the RNeasy FFPE kit (Qiagen, Hilden, Germany). The quality and quantity of RNA were assessed by spectrophotometry (Trinean, Gentbrugge, Belgium). About 250 ng of RNA were used for the one step real-time RT-PCR assay to detect the SARS-CoV-2 genome in lung and pituitary specimens. The Easy SARS-CoV-2 WE kit (Diatech Pharmacogenetics, Jesi, Italy), validated for in vitro diagnostics, was used on a Rotor-Gene Q instrument (Qiagen). The assay has a limit of detection of 5 target copies *per* reaction. The assay steps were as follows: retro-transcription at 50°C for 10 minutes; activation of Taq polymerase at 95°C for 5 minutes; 40 cycles of denaturation at 95°C for 5 seconds and annealing/extension at 58°C for 30 seconds. The amplification of an internal control (*VPS29*) ensured the sample adequacy in terms of amplifiability. A sample was deemed positive when at least one of the two target genes [i.e., nucleocapsid (N) and RNA-dependent RNA polymerase (RdRp) genes] was amplified before the 36th Ct (N gene) and before the 38th Ct (RdRp gene).

In situ hybridization (ISH) for SARS-CoV-2 RNA was performed using the RNAscope Probe V-nCoV2019-S (Advanced Cell Diagnostics, ACD - Bio-Techne, Minneapolis, MN, United States) and the RNAscope Intro Pack 2.5 HD Reagent Kit BROWN (ACD - Bio-Techne) for manual assays. The probe is designed on Wuhan-Hu-1 (NC_045512.2) sequence of SARS-CoV-2 and targets the spike (S) gene (21631 – 23303 nucleotides). The kit includes positive and negative control probes directed against

the human peptidylprolyl isomerase B (*PPIB*) gene and the bacterial dapB gene of *Bacillus subtilis*. Four µm-thick FFPE sections were used; tissue retrieval was performed using specific Target Retrieval Reagents at 100°C for 15 minutes. After washing steps, treatment with proteases was performed at 40°C for 30 minutes. Then, a series of signal amplification steps by hybridization were performed. Signal detection was carried out by DAB staining. Slides were counterstained with hematoxylin.

Gene expression assay was performed using the nCounter system (nanoString Technologies, Seattle, WA, USA). Briefly, about 175 ng of RNA were hybridized with probes at 65°C for 21 hours. Three sets of probes were used: a) the Human Host Response (which contains probes for 770 immune-related genes), b) the Coronavirus Panel Plus targeting genes of common Coronaviruses including the SARS-CoV-2; c) a custom panel of 24 genes: 10 housekeeping genes for normalization purposes (*ABCF1*, *ALAS1*, *GUSB*, *MRPS7*, *NMT1*, *NRDE2*, *OAZ1*, *PGK1*, *SDHA*, *STK11IP*), three interferon stimulated genes (*IFI44*, *OAS1*, *RSAD2*) and 11 pituitary-specific genes (*AVPR1B*, *FSHB*, *GHRHR*, *GNRHR*, *GPR50*, *LHB*, *LHX3*, *POMC*, *POU1F1*, *PRL*, *TSHB*). Pituitary-specific genes were selected based on The Human Protein Atlas (https://www.proteinatlas.org/). The top genes that were reported as tissue-enriched in the human pituitary were selected, and genes that were enriched also in other tissues or organs were filtered out. The complete list of the analyzed transcripts is reported in Supplementary Table S1 [35].

Immunohistochemistry

Three-µm-thick sections were stained with the SARS-CoV-2 nucleocapsid antibody (NB100-56683, Novus Biologicals, Centennial, CO, United States, RRID: AB_838841) and immune cell markers. The following immune cell markers were investigated: CONFIRM anti-CD3 (clone 2GV6, T-cell marker, Ventana Medical Systems, Oro Valley, AZ, United States, RRID: AB_2335978), CONFIRM anti-CD8 (clone SP57, cytotoxic T-cell marker, Ventana Medical System, RRID: AB_2335985), CONFIRM anti-CD68 (clone KP-1, macrophage marker, Ventana Medical System, RRID: AB_2335972), CONFIRM

anti-CD15 (clone MMA, granulocyte marker, Ventana Medical System, RRID: AB_2335952), anti-CD7 (clone SP94, T-cell and natural killer marker, Ventana Medical System, RRID: AB_2336023) and CONFIRM anti-CD20 (clone L26, B-cell marker, Ventana Medical System, RRID: AB_2335956).

Transmission electron microscopy

Small blocks of adenohypophysis of approximately 1 mm^3 were fixed in 2.5% glutaraldehyde in 100 mM sodium cacodylate buffer for 2-4 hours at 4°C. Samples were then processed as described by Cassella and colleagues [36]. Briefly, after post-fixation in 1% osmium tetroxide and dehydration in a graded series of ethanol, they were embedded in an "Epon-Araldite" resin. Ultrathin sections were stained with uranyl acetate and lead citrate and analysed with a Jeol 100 SX transmission electron microscope. Digital images and measurements were acquired using AMT image capture software.

Data analyses

Raw gene expression counts were normalized using the Advanced Analysis module of the nSolver software v.4.0 (nanoString Technologies). Genes with raw counts as low as 20 were omitted from further analyses. Differentially expressed genes (DEG) in COVID-19 virus-positive and virus-negative cases were computed using the controls as baseline and following the procedures of the Advanced Analysis module. Briefly, for each gene, the software uses the best fit among a mixture negative binomial model, a simplified negative binomial model and log-linear model; age and sex were used as confounders. *P*-values were adjusted with the Benjamini-Yekutieli method, and a false discovery rate (FDR) of 0.1 was considered significant. Then, the ranked gene lists were used to run a gene set enrichment analysis (GSEA) following the procedures of the clusterProfiler Bioconductor package v.4.2.0. In details, the Hallmark (H) and the Gene Ontology (C5) collections from the Mutational Signatures Database (MutSigDB) v.7.4 were used as reference [37], and a minimum gene set size of 20 genes was set. Pathway enrichment analysis was performed following the procedures of clusterProfiler Bioconductor package and using the Kyoto encyclopedia of genes and genomes

(KEGG) database as reference. Immune cell scores were calculated using the method described by Middleton [38]; only immune cell scores with at least two markers were considered. The Dunn test for multiple comparisons was used to evaluate differences in terms of immune cell scores and infiltrates. All analyses were performed in R environment v.4.1.2 (https://www.r-project.org/, last accessed January 28, 2022) unless otherwise specified.

Results

Detection of SARS-CoV-2 in the pituitary gland of COVID-19 subjects

Table 1 summarizes the demographic and clinical data of controls and COVID-19 cases. Cases of the COVID-19 cohort were older than controls and more frequently obese or overweight. Comorbidities were highly prevalent in both cohorts, with cardiovascular disease being the most frequent. RNA quality was suitable for further analyses, with A260/A280 ratio of 1.7-2.1, A260/A230 ratio of 1.8-2.4. By real-time RT-PCR, the SARS-CoV-2 genome was detected in 14/23 (61%) pituitary samples from COVID-19 cases. Only five of them showed measurable quantities of SARS-CoV-2 transcripts by the nCounter assay (hybridization assay without gene amplification), suggesting that the majority of COVID-19 cases had low viral loads in the gland. These five cases had the highest viral loads according to Ct values (Ct between 25 and 30). In the neurohypophysis tissue (15 cases), virus detection by real-time RT-PCR was coherent with that of adenohypophysis. None of the controls was virus-positive by either real-time RT-PCR or the nCounter assay. SARS-CoV-2 positivity by real-time RT-PCR was consistently confirmed by IHC staining for the nucleocapsid antigen and ISH for the viral genome (Figure 1). The presence of SARS-CoV-2 was also confirmed by ultrastructural examination that showed virus-like particles inside membrane-bounded vesicles in the cytoplasm of cells. Although morphology was negatively affected by lysis of cells, some virus particles showed clear envelope membrane, faint surface projections (i.e., spike) and electron-dense dots (i.e., nucleocapsids) (Figure 2) [39–42]. Due to the long time elapsed from death to autopsy and the

consequent partial autolysis, the types of infected pituitary epithelial cells could not be identified based on the properties and size of hormone-containing secretory granules.

Differential transcription of immune-related genes

As compared to pituitary samples of controls who died abruptly for trauma or sudden cardiac death, both virus-positive and virus-negative pituitary samples of COVID-19 cases showed a prevalent downregulation of immune-related genes. In COVID-19 cases that were virus-positive in the pituitary, ten genes were significantly deregulated compared to the controls. *FAM30A* (a long non-coding RNA related to the expression of immunoglobulin genes in B-cells [43]) was strongly upregulated, while transcription of *ICAM3*, *FASLG*, *ZAP70*, *ATF4*, *PELI1*, *TXNIP*, *DDOST*, *LAG3* and *IFNW1* was downregulated (Figure 3A). In COVID-19 samples that were virus-negative in pituitary, five genes were significantly downregulated, namely *SCARB2*, *IFI16*, *GK*, *OSM* and *IL1A* (Figure 3B). The complete results of differential gene expression analysis are reported in Supplementary Table S2 [35]. By using the ranked gene lists of differential expression analysis, the Hallmark interferon alpha response (M5911) and the Gene Ontology Biological Process (GOBP) response to type I interferon (GO:0034340) were activated in virus-positive cases, while – compared to controls – no gene set was altered in COVID-19 cases that were virus-negative in pituitary (Figure 3C-D). To find genes uniformly deregulated in virus-positive and virus-negative COVID-19 cases, an FDR<0.25 was selected, and 9 commonly downregulated genes emerged, namely *ICAM3*, *ATF4*, *SCARB2*, *OSM*, *MAP2K4*, *MTOR*, *CCNC*, *GK* and *AHR*. The latter nine genes were tested by pathway enrichment analysis, and two pathways were significantly enriched: growth hormone synthesis, secretion, and action (hsa04935, *q*-value = 0.01) and chemical carcinogenesis – receptor activation (hsa05207, *q*-value = 0.03). The downregulation of genes involved in chemical carcinogenesis by receptor activation could suggest a suppressed signal transduction.

Inflammatory infiltrates in the pituitary

As measured by mRNA transcript levels of immune-related genes, scores of immune cells were not highly different between COVID-19 cases and controls. However, T/NK cytotoxic cells were over-represented in both virus-positive and in virus-negative COVID-19 cases (*P*=0.05 and *P*=0.05 respectively). In addition, polymorphonuclear neutrophils were more abundant in COVID-19 compared to controls (*P*=0.03). Immune cell scores are summarized in Figure 4. Due to the scarcity and the focal nature of infiltrates, the small number of investigated cases, and the limited number of available pituitary sections, the results could not be fully confirmed by IHC. In fact, CD3, CD7 and CD8 staining was limited to scattered cell clusters observed in part of the cases with no significant differences between COVID-19 and controls (Figure 5A-D-E). The B-cell marker CD20 produced no staining in COVID-19 cases nor in controls (Figure 5F). Notably, CD15 (granulocyte) and CD68 (macrophage) markers were largely expressed also by pituitary cells themselves making it difficult to identify possible low levels of infiltrating leukocytes (Figure 5B-C). This made impossible the statistical comparisons of IHC results. Expression of selected CD markers in epithelial cells of pituitary has been reported in the context of pituitary adenomas, but the biological significance and functions of these immune markers remain unclear [44].

Differential transcription of pituitary-specific genes

Results and gene functions are summarized in Table 2. As compared to controls, virus-positive pituitaries (n=14) showed suppression of the Follicle Stimulating Hormone Subunit Beta (*FSHB)* (FDR=0.002), Thyroid Stimulating Hormone Subunit Beta (*TSHB)* (FDR=0.02), Luteinizing Hormone Subunit Beta (*LHB)* (FRD=0.02), Gonadotropin Releasing Hormone Receptor (*GNRHR*) (FRD=0.09), and LIM Homeobox Protein 3 (*LHX3)* (FDR=0.002), a transcription factor required for pituitary development and hormone production. Transcription of the receptor for Growth Hormone Releasing Hormone (*GHRHR*) was slightly enhanced (FRD=0.02). A trend for reduced transcription of POU Class 1 Homeobox 1 (*POU1F1*) (FDR=0.13) was also observed. In virus-negative pituitaries (n=9),

transcription of *LHB* was clearly downregulated (FDR=0.002). In addition, there was a trend for the suppression of *FSHB* (FDR=0.13), *LHX3* (FDR=0.13), *TSHB* (FDR=0.14) and *POU1F1* (FDR=0.14). The activation of interferon response in virus-positive pituitaries was confirmed by the upregulation three interferon-induced genes, namely *IFI44* (FDR=0.00005), *OAS1* (FDR=0.001) and *RSAD* (FDR=0.001) compared to controls. The transcription of these genes was not altered in virus-negative pituitaries compared to controls.

Discussion

Multiple extrapulmonary manifestations of COVID-19 have been described [1]. Following SARS-CoV-2 infection, endocrine defects may become apparent during the acute or the post-acute phases [22]. Previous autopsy studies by our group and others showed that SARS-CoV-2 antigens and genome were present in the thyroid, testis and subcutaneous adipose tissue of individuals who died of COVID-19 [13,20,45,46]. Evidence from the above studies also showed that virus infection of thyroid promotes activation of interferon signalling and immune changes consistent with sub-acute thyroiditis [45]. In COVID-19, additional endocrine dysfunctions have been reported, and post-COVID-19 monitoring is granted in order to administer suitable therapies.

Case reports of pituitary apoplexy in the course of COVID-19 have been documented [26–29]. Besides acute events in patients with pre-existing conditions, hypopituitarism has been hypothesized in severe cases [5,19]. However, the infection of cells of the pituitary gland and changes of gene expression have not been explored in COVID-19. From autopsy cases, evidence emerges for the pituitary tropism of SARS-COV-2. In our series, in all cases that were virus-positive by real-time RT-PCR in the pituitary, the viral genome and antigens could be detected at approximately the same levels both in the adeno- and the neurohypophysis. Pituitary glands of COVID-19 cases showed a

small but consistent down-regulation of immune-related genes. Transcription levels of pituitary-specific genes were also measured. As compared to controls, transcription of the *FSHB*, *TSHB* and *LHB* genes was strongly suppressed in pituitary glands infected by SARS-CoV-2. In addition, the mRNA levels of *LHX3* - a transcription factor that regulates pituitary development and the transcription of pituitary-specific genes [47,48] – were also downregulated. Heterozygous germline mutations in *LHX3* gene determine a combined deficiency of pituitary hormones [49,50]. Regarding *FSHB* and *LHB*, the results hold true also in COVID-19 cases without evidence for virus within the gland tissue, showing that suppressive signals other than those of virus replication are in action during severe COVID-19. These findings are consistent with the possibility of hypopituitarism in the course of infection and of systemic inflammatory disorders [51–53]. Consistently with our previous findings in autoptic thyroid and adipose tissues [45,46], the type I interferon pathway was selectively activated in pituitary glands that were positive for both genome and antigens of SARS-CoV-2. Finally, we observed higher levels of cytotoxic cell and neutrophil scores in virus-positive pituitary of COVID-19 cases. However, focal immune/inflammatory infiltrates were present only in a part of the cases.

Our study has some limitations. First, the number of investigated cases is relatively small due to the risky procedure for collecting the pituitary from sella turcica of infectious COVID-19 cases. Second, immunostaining for CD15 and CD68 markers was not satisfactory for assessing infiltrates of granulocytes and macrophages due to the diffuse expression of these markers in pituitary cells. Additionally, only a few cases presented CD8 and CD3 staining, thus not providing sufficient data for statistical comparisons. All subjects were of Caucasian ethnicity which hindered the ability of modelling ethnical factors. Finally, hormone dosage was not available since it is not a routine practice in hospitalized COVID-19 patients; hence we could not establish whether SARS-CoV-2 infection was associated with any degree of hypopituitarism.

In closing, the study shows that SARS-CoV-2 infection of the pituitary gland may occur at least in severe forms of COVID-19. As in other endocrine tissues [45,46], the infection activates the type I interferon response and appears to reduce the transcription of pituitary-specific hormones irrespective of the direct viral infection of pituitary cells (Figure 6). While COVID-19 infections are more frequently associated with a severe course and higher mortality in men, women appear more predisposed to long COVID [54]. Clinicians should be aware of sexual dimorphism in COVID-19 and the differential long-term consequences of this pandemic virus. Thus, they are advised to follow-up COVID-19 patients for signs of pituitary disorders. As for cardiovascular pathology [55], studies of long-term endocrine outcomes of COVID-19 will better define the wide pathogenic spectrum of SARS-CoV-2 in humans.

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Data availability: Original data generated and analyzed during this study are included in this published article or in the data repositories listed in References.

References

1. Gupta, A.; Madhavan, M.V.; Sehgal, K.; Nair, N.; Mahajan, S.; Sehrawat, T.S.; Bikdeli, B.; Ahluwalia, N.; Ausiello, J.C.; Wan, E.Y.; et al. Extrapulmonary Manifestations of COVID-19. *Nat Med* **2020**, *26*, 1017–1032, doi:10.1038/s41591-020-0968-3.
2. Somasundaram, N.P.; Ranathunga, I.; Ratnasamy, V.; Wijewickrama, P.S.A.; Dissanayake, H.A.; Yogendranathan, N.; Gamage, K.K.K.; de Silva, N.L.; Sumanatilleke, M.; Katulanda, P.; et al. The Impact of SARS-Cov-2 Virus Infection on the Endocrine System. *J Endocr Soc* **2020**, *4*, bvaa082, doi:10.1210/jendso/bvaa082.
3. Kothandaraman, N.; Rengaraj, A.; Xue, B.; Yew, W.S.; Velan, S.S.; Karnani, N.; Leow, M.K.S. COVID-19 Endocrinopathy with Hindsight from SARS. *Am J Physiol Endocrinol Metab* **2021**, *320*, E139–E150, doi:10.1152/ajpendo.00480.2020.
4. Kanczkowski, W.; Evert, K.; Stadtmüller, M.; Haberecker, M.; Laks, L.; Chen, L.-S.; Frontzek, K.; Pablik, J.; Hantel, C.; Beuschlein, F.; et al. COVID-19 Targets Human Adrenal Glands. *Lancet Diabetes Endocrinol* **2022**, *10*, 13–16, doi:10.1016/S2213-8587(21)00291-6.
5. Alzahrani, A.S.; Mukhtar, N.; Aljomaiah, A.; Aljamei, H.; Bakhsh, A.; Alsudani, N.; Elsayed, T.; Alrashidi, N.; Fadel, R.; Alqahtani, E.; et al. The Impact of COVID-19 Viral Infection on the Hypothalamic-Pituitary-Adrenal Axis. *Endocr Pract* **2021**, *27*, 83–89, doi:10.1016/j.eprac.2020.10.014.
6. Ilera, V.; Delfino, L.C.; Zunino, A.; Glikman, P.; Drnovsek, M.; Reyes, A.; Dios, A.; Toibaro, J.; Pachioli, V.; Lannes, N.; et al. Correlation between Inflammatory Parameters and Pituitary-Thyroid Axis in Patients with COVID-19. *Endocrine* **2021**, *74*, 455–460, doi:10.1007/s12020-021-02863-2.
7. Speer, G.; Somogyi, P. Thyroid Complications of SARS and Coronavirus Disease 2019 (COVID-19). *Endocr J* **2021**, *68*, 129–136, doi:10.1507/endocrj.EJ20-0443.

8. Malik, J.; Zaidi, S.M.J.; Waqar, A.U.; Khawaja, H.; Malik, A.; Ishaq, U.; Rana, A.S.; Awan, A.H. Association of Hypothyroidism with Acute COVID-19: A Systematic Review. *Expert Rev Endocrinol Metab* **2021**, *16*, 251–257, doi:10.1080/17446651.2021.1968830.

9. Lui, D.T.W.; Lee, C.H.; Chow, W.S.; Lee, A.C.H.; Tam, A.R.; Pang, P.; Ho, T.Y.; Cheung, C.Y.Y.; Fong, C.H.Y.; Law, C.Y.; et al. The Independent Association of TSH and Free Triiodothyronine Levels With Lymphocyte Counts Among COVID-19 Patients. *Front Endocrinol (Lausanne)* **2021**, *12*, 774346, doi:10.3389/fendo.2021.774346.

10. Moreno-Perez, O.; Merino, E.; Alfayate, R.; Torregrosa, M.E.; Andres, M.; Leon-Ramirez, J.-M.; Boix, V.; Gil, J.; Pico, A.; COVID19-ALC Research group Male Pituitary-Gonadal Axis Dysfunction in Post-Acute COVID-19 Syndrome-Prevalence and Associated Factors: A Mediterranean Case Series. *Clin Endocrinol (Oxf)* **2022**, *96*, 353–362, doi:10.1111/cen.14537.

11. Selvaraj, K.; Ravichandran, S.; Krishnan, S.; Radhakrishnan, R.K.; Manickam, N.; Kandasamy, M. Testicular Atrophy and Hypothalamic Pathology in COVID-19: Possibility of the Incidence of Male Infertility and HPG Axis Abnormalities. *Reprod Sci* **2021**, *28*, 2735–2742, doi:10.1007/s43032-020-00441-x.

12. Duarte-Neto, A.N.; Teixeira, T.A.; Caldini, E.G.; Kanamura, C.T.; Gomes-Gouvêa, M.S.; Dos Santos, A.B.G.; Monteiro, R.A.A.; Pinho, J.R.R.; Mauad, T.; da Silva, L.F.F.; et al. Testicular Pathology in Fatal COVID-19: A Descriptive Autopsy Study. *Andrology* **2022**, *10*, 13–23, doi:10.1111/andr.13073.

13. Wong, D.W.L.; Klinkhammer, B.M.; Djudjaj, S.; Villwock, S.; Timm, M.C.; Buhl, E.M.; Wucherpfennig, S.; Cacchi, C.; Braunschweig, T.; Knüchel-Clarke, R.; et al. Multisystemic Cellular Tropism of SARS-CoV-2 in Autopsies of COVID-19 Patients. *Cells* **2021**, *10*, 1900, doi:10.3390/cells10081900.

14. Müller, J.A.; Groß, R.; Conzelmann, C.; Krüger, J.; Merle, U.; Steinhart, J.; Weil, T.; Koepke, L.; Bozzo, C.P.; Read, C.; et al. SARS-CoV-2 Infects and Replicates in Cells of the Human Endocrine and Exocrine Pancreas. *Nat Metab* **2021**, *3*, 149–165, doi:10.1038/s42255-021-00347-1.

15. Wu, C.-T.; Lidsky, P.V.; Xiao, Y.; Lee, I.T.; Cheng, R.; Nakayama, T.; Jiang, S.; Demeter, J.; Bevacqua, R.J.; Chang, C.A.; et al. SARS-CoV-2 Infects Human Pancreatic β Cells and Elicits β Cell Impairment. *Cell Metab* **2021**, *33*, 1565-1576.e5, doi:10.1016/j.cmet.2021.05.013.

16. Mine, K.; Nagafuchi, S.; Mori, H.; Takahashi, H.; Anzai, K. SARS-CoV-2 Infection and Pancreatic β Cell Failure. *Biology (Basel)* **2021**, *11*, 22, doi:10.3390/biology11010022.

17. Steenblock, C.; Richter, S.; Berger, I.; Barovic, M.; Schmid, J.; Schubert, U.; Jarzebska, N.; von Mässenhausen, A.; Linkermann, A.; Schürmann, A.; et al. Viral Infiltration of Pancreatic Islets in Patients with COVID-19. *Nat Commun* **2021**, *12*, 3534, doi:10.1038/s41467-021-23886-3.

18. Misgar, R.A.; Rasool, A.; Wani, A.I.; Bashir, M.I. Central Diabetes Insipidus (Infundibuloneuro Hypophysitis): A Late Complication of COVID-19 Infection. *J Endocrinol Invest* **2021**, *44*, 2855–2856, doi:10.1007/s40618-021-01627-z.

19. Gu, W.T.; Zhou, F.; Xie, W.Q.; Wang, S.; Yao, H.; Liu, Y.T.; Gao, L.; Wu, Z.B. A Potential Impact of SARS-CoV-2 on Pituitary Glands and Pituitary Neuroendocrine Tumors. *Endocrine* **2021**, *72*, 340–348, doi:10.1007/s12020-021-02697-y.

20. Poma, A.M.; Bonuccelli, D.; Giannini, R.; Macerola, E.; Vignali, P.; Ugolini, C.; Torregrossa, L.; Proietti, A.; Pistello, M.; Basolo, A.; et al. COVID-19 Autopsy Cases: Detection of Virus in Endocrine Tissues. *J Endocrinol Invest* **2021**, doi:10.1007/s40618-021-01628-y.

21. Das, L.; Dutta, P.; Walia, R.; Mukherjee, S.; Suri, V.; Puri, G.D.; Mahajan, V.; Malhotra, P.; Chaudhary, S.; Gupta, R.; et al. Spectrum of Endocrine Dysfunction and Association With Disease Severity in Patients With COVID-19: Insights From a Cross-Sectional, Observational Study. *Front Endocrinol (Lausanne)* **2021**, *12*, 645787, doi:10.3389/fendo.2021.645787.

22. Clarke, S.A.; Abbara, A.; Dhillo, W.S. Impact of COVID-19 on the Endocrine System - a Mini-Review. *Endocrinology* **2021**, bqab203, doi:10.1210/endocr/bqab203.

23. Barrett, C.E.; Koyama, A.K.; Alvarez, P.; Chow, W.; Lundeen, E.A.; Perrine, C.G.; Pavkov, M.E.; Rolka, D.B.; Wiltz, J.L.; Bull-Otterson, L.; et al. Risk for Newly Diagnosed Diabetes >30 Days

After SARS-CoV-2 Infection Among Persons Aged <18 Years - United States, March 1, 2020-June 28, 2021. *MMWR Morb Mortal Wkly Rep* **2022**, *71*, 59–65, doi:10.15585/mmwr.mm7102e2.

24. Frara, S.; Allora, A.; Castellino, L.; di Filippo, L.; Loli, P.; Giustina, A. COVID-19 and the Pituitary. *Pituitary* **2021**, *24*, 465–481, doi:10.1007/s11102-021-01148-1.
25. Silverman, M.N.; Pearce, B.D.; Biron, C.A.; Miller, A.H. Immune Modulation of the Hypothalamic-Pituitary-Adrenal (HPA) Axis during Viral Infection. *Viral Immunol* **2005**, *18*, 41–78, doi:10.1089/vim.2005.18.41.
26. Chan, J.L.; Gregory, K.D.; Smithson, S.S.; Naqvi, M.; Mamelak, A.N. Pituitary Apoplexy Associated with Acute COVID-19 Infection and Pregnancy. *Pituitary* **2020**, *23*, 716–720, doi:10.1007/s11102-020-01080-w.
27. Solorio-Pineda, S.; Almendárez-Sánchez, C.A.; Tafur-Grandett, A.A.; Ramos-Martínez, G.A.; Huato-Reyes, R.; Ruiz-Flores, M.I.; Sosa-Najera, A. Pituitary Macroadenoma Apoplexy in a Severe Acute Respiratory Syndrome-Coronavirus-2-Positive Testing: Causal or Casual? *Surg Neurol Int* **2020**, *11*, 304, doi:10.25259/SNI_305_2020.
28. Santos, C.D.S.E.; Filho, L.M. da C.L.; Santos, C.A.T.; Neill, J.S.; Vale, H.F.; Kurnutala, L.N. Pituitary Tumor Resection in a Patient with SARS-CoV-2 (COVID-19) Infection. A Case Report and Suggested Airway Management Guidelines. *Braz J Anesthesiol* **2020**, *70*, 165–170, doi:10.1016/j.bjane.2020.05.003.
29. Bray, D.P.; Solares, C.A.; Oyesiku, N.M. Rare Case of a Disappearing Pituitary Adenoma During the Coronavirus Disease 2019 (COVID-19) Pandemic. *World Neurosurg* **2021**, *146*, 148–149, doi:10.1016/j.wneu.2020.11.073.
30. Ghosh, R.; Roy, D.; Roy, D.; Mandal, A.; Dutta, A.; Naga, D.; Benito-León, J. A Rare Case of SARS-CoV-2 Infection Associated With Pituitary Apoplexy Without Comorbidities. *J Endocr Soc* **2021**, *5*, bvaa203, doi:10.1210/jendso/bvaa203.

31. Bordes, S.J.; Phang-Lyn, S.; Najera, E.; Borghei-Razavi, H.; Adada, B. Pituitary Apoplexy Attributed to COVID-19 Infection in the Absence of an Underlying Macroadenoma or Other Identifiable Cause. *Cureus* **2021**, *13*, e13315, doi:10.7759/cureus.13315.

32. Ricciotti, E.; Laudanski, K.; FitzGerald, G.A. Nonsteroidal Anti-Inflammatory Drugs and Glucocorticoids in COVID-19. *Adv Biol Regul* **2021**, *81*, 100818, doi:10.1016/j.jbior.2021.100818.

33. Subramaniam, A.; Anstey, C.; Curtis, J.R.; Ashwin, S.; Ponnapa Reddy, M.; Aliberti, M.J.R.; Avelino-Silva, T.J.; Welch, C.; Koduri, G.; Prowle, J.R.; et al. Characteristics and Outcomes of Patients With Frailty Admitted to ICU With Coronavirus Disease 2019: An Individual Patient Data Meta-Analysis. *Crit Care Explor* **2022**, *4*, e0616, doi:10.1097/CCE.0000000000000616.

34. van de Veerdonk, F.L.; Giamarellos-Bourboulis, E.; Pickkers, P.; Derde, L.; Leavis, H.; van Crevel, R.; Engel, J.J.; Wiersinga, W.J.; Vlaar, A.P.J.; Shankar-Hari, M.; et al. A Guide to Immunotherapy for COVID-19. *Nat Med* **2022**, *28*, 39–50, doi:10.1038/s41591-021-01643-9.

35. Poma, A.M.; Proietti, A.; Macerola, E.; Bonuccelli, D.; Conti, M.; Salvetti, A.; Dolo, V.; Chillà, A.; Basolo, A.; Santini, F.; et al. Supplementary Material from: Detection of SARS-CoV-2 in Pituitary of COVID-19 Cases: Suppression of Hormone-Related Genes Independently of Viral Presence in the Gland" 2022.

36. Cassella, L.; Salvetti, A.; Iacopetti, P.; Ippolito, C.; Ghezzani, C.; Gimenez, G.; Ghigo, E.; Rossi, L. Putrescine Independent Wound Response Phenotype Is Produced by ODC-like RNAi in Planarians. *Sci Rep* **2017**, *7*, 9736, doi:10.1038/s41598-017-09567-6.

37. Subramanian, A.; Tamayo, P.; Mootha, V.K.; Mukherjee, S.; Ebert, B.L.; Gillette, M.A.; Paulovich, A.; Pomeroy, S.L.; Golub, T.R.; Lander, E.S.; et al. Gene Set Enrichment Analysis: A Knowledge-Based Approach for Interpreting Genome-Wide Expression Profiles. *Proc Natl Acad Sci U S A* **2005**, *102*, 15545–15550, doi:10.1073/pnas.0506580102.

38. Middleton, G.; Yang, Y.; Campbell, C.D.; André, T.; Atreya, C.E.; Schellens, J.H.M.; Yoshino, T.; Bendell, J.C.; Hollebecque, A.; McRee, A.J.; et al. BRAF-Mutant Transcriptional Subtypes Predict

Outcome of Combined BRAF, MEK, and EGFR Blockade with Dabrafenib, Trametinib, and Panitumumab in Patients with Colorectal Cancer. *Clin Cancer Res* **2020**, *26*, 2466–2476, doi:10.1158/1078-0432.CCR-19-3579.

39. Dittmayer, C.; Meinhardt, J.; Radbruch, H.; Radke, J.; Heppner, B.I.; Heppner, F.L.; Stenzel, W.; Holland, G.; Laue, M. Why Misinterpretation of Electron Micrographs in SARS-CoV-2-Infected Tissue Goes Viral. *Lancet* **2020**, *396*, e64–e65, doi:10.1016/S0140-6736(20)32079-1.
40. Brahim Belhaouari, D.; Fontanini, A.; Baudoin, J.-P.; Haddad, G.; Le Bideau, M.; Bou Khalil, J.Y.; Raoult, D.; La Scola, B. The Strengths of Scanning Electron Microscopy in Deciphering SARS-CoV-2 Infectious Cycle. *Front Microbiol* **2020**, *11*, 2014, doi:10.3389/fmicb.2020.02014.
41. Hopfer, H.; Herzig, M.C.; Gosert, R.; Menter, T.; Hench, J.; Tzankov, A.; Hirsch, H.H.; Miller, S.E. Hunting Coronavirus by Transmission Electron Microscopy - a Guide to SARS-CoV-2-Associated Ultrastructural Pathology in COVID-19 Tissues. *Histopathology* **2021**, *78*, 358–370, doi:10.1111/his.14264.
42. Akilesh, S.; Nicosia, R.F.; Alpers, C.E.; Tretiakova, M.; Hsiang, T.-Y.; Gale, M.; Smith, K.D. Characterizing Viral Infection by Electron Microscopy: Lessons from the Coronavirus Disease 2019 Pandemic. *Am J Pathol* **2021**, *191*, 222–227, doi:10.1016/j.ajpath.2020.11.003.
43. de Lima, D.S.; Cardozo, L.E.; Maracaja-Coutinho, V.; Suhrbier, A.; Mane, K.; Jeffries, D.; Silveira, E.L.V.; Amaral, P.P.; Rappuoli, R.; de Silva, T.I.; et al. Long Noncoding RNAs Are Involved in Multiple Immunological Pathways in Response to Vaccination. *Proc Natl Acad Sci U S A* **2019**, *116*, 17121–17126, doi:10.1073/pnas.1822046116.
44. Saeger, W. CD Markers in Pituitary Adenomas. *Endocr Pathol* **2001**, *12*, 307–313, doi:10.1385/ep:12:3:307.
45. Poma, A.M.; Basolo, A.; Bonuccelli, D.; Proietti, A.; Macerola, E.; Ugolini, C.; Torregrossa, L.; Alì, G.; Giannini, R.; Vignali, P.; et al. Activation of Type I and Type II Interferon Signaling in SARS-CoV-2-Positive Thyroid Tissue of Patients Dying from COVID-19. *Thyroid* **2021**, *31*, 1766–1775, doi:10.1089/thy.2021.0345.

46. Basolo, A.; Poma, A.M.; Bonuccelli, D.; Proietti, A.; Macerola, E.; Ugolini, C.; Torregrossa, L.; Giannini, R.; Vignali, P.; Basolo, F.; et al. Adipose Tissue in COVID-19: Detection of SARS-CoV-2 in Adipocytes and Activation of the Interferon-Alpha Response. *J Endocrinol Invest* **2022**, doi:10.1007/s40618-022-01742-5.

47. Thaler, J.P.; Lee, S.-K.; Jurata, L.W.; Gill, G.N.; Pfaff, S.L. LIM Factor Lhx3 Contributes to the Specification of Motor Neuron and Interneuron Identity through Cell-Type-Specific Protein-Protein Interactions. *Cell* **2002**, *110*, 237–249, doi:10.1016/s0092-8674(02)00823-1.

48. Sheng, H.Z.; Zhadanov, A.B.; Mosinger, B.; Fujii, T.; Bertuzzi, S.; Grinberg, A.; Lee, E.J.; Huang, S.P.; Mahon, K.A.; Westphal, H. Specification of Pituitary Cell Lineages by the LIM Homeobox Gene Lhx3. *Science* **1996**, *272*, 1004–1007, doi:10.1126/science.272.5264.1004.

49. Netchine, I.; Sobrier, M.L.; Krude, H.; Schnabel, D.; Maghnie, M.; Marcos, E.; Duriez, B.; Cacheux, V.; Moers, A. v; Goossens, M.; et al. Mutations in LHX3 Result in a New Syndrome Revealed by Combined Pituitary Hormone Deficiency. *Nat Genet* **2000**, *25*, 182–186, doi:10.1038/76041.

50. Sloop, K.W.; Parker, G.E.; Hanna, K.R.; Wright, H.A.; Rhodes, S.J. LHX3 Transcription Factor Mutations Associated with Combined Pituitary Hormone Deficiency Impair the Activation of Pituitary Target Genes. *Gene* **2001**, *265*, 61–69, doi:10.1016/s0378-1119(01)00369-9.

51. Gao, L.; Guo, X.; Tian, R.; Wang, Q.; Feng, M.; Bao, X.; Deng, K.; Yao, Y.; Lian, W.; Wang, R.; et al. Pituitary Abscess: Clinical Manifestations, Diagnosis and Treatment of 66 Cases from a Large Pituitary Center over 23 Years. *Pituitary* **2017**, *20*, 189–194, doi:10.1007/s11102-016-0757-7.

52. Prodam, F.; Caputo, M.; Mele, C.; Marzullo, P.; Aimaretti, G. Insights into Non-Classic and Emerging Causes of Hypopituitarism. *Nat Rev Endocrinol* **2021**, *17*, 114–129, doi:10.1038/s41574-020-00437-2.

53. Warmbier, J.; Lüdecke, D.K.; Flitsch, J.; Buchfelder, M.; Fahlbusch, R.; Knappe, U.J.; Kreutzer, J.; Buslei, R.; Bergmann, M.; Heppner, F.; et al. Typing of Inflammatory Lesions of the Pituitary. *Pituitary* **2021**, doi:10.1007/s11102-021-01180-1.

54. Bechmann, N.; Barthel, A.; Schedl, A.; Herzig, S.; Varga, Z.; Gebhard, C.; Mayr, M.; Hantel, C.; Beuschlein, F.; Wolfrum, C.; et al. Sexual Dimorphism in COVID-19: Potential Clinical and Public Health Implications. *Lancet Diabetes Endocrinol* **2022**, S2213-8587(21)00346-6, doi:10.1016/S2213-8587(21)00346-6.

55. Xie, Y.; Xu, E.; Bowe, B.; Al-Aly, Z. Long-Term Cardiovascular Outcomes of COVID-19. *Nat Med* **2022**, doi:10.1038/s41591-022-01689-3.

Figure legends

Figure 1. Detection of SARS-CoV-2 in pituitary glands. Scale bars refer to 100 micrometers. A) Hematoxylin and eosin section of a COVID-19 case. Neither adeno- nor neurohypophysis showed marked alterations (original magnification 4x). B) SARS-CoV-2 nucleocapsid staining in adenohypophysis (60x). C) Higher magnification (100x) shows granular perinuclear nucleocapsid staining in pituitary cells. D) Viral nucleocapsid staining was observed also in neurohypophysis (60x). E) Viral genome detected by in situ hybridization (ISH); grey dots show a diffuse viral infection (60x). F) Detection of SARS-CoV-2 genome by ISH in a case with low viral load (60x).

Figure 2. Ultrastructure investigation of anterior pituitary. A) Epithelial cell showing SARS-CoV-2-like particles inside vesicles delimited by membrane (arrows) that separate them from the cytoplasm. m (mitochondrion). Arrowheads indicate the area enlarged in B and C. B-C) Micrographs showing virus-like particles with membrane envelope and electron dense internal structures. Virus particles have an approximate diameter of 100 nanometers (see scale bars).

Figure 3. Activation of IFN in the context of gene downregulation. Both virus-positive (A) and virus-negative (B) pituitary tissues from COVID-19 cases showed a predominance of gene downregulation compared to controls. However, the activation of IFNα/type I IFN signaling is observed in virus-positive tissues (C-D). NES, normalized enrichment score. * $P<0.05$.

Figure 4. Immune cell scores. Pituitary tissues from COVID-19 cases do not show marked immune cell infiltration compared to controls. Only T/NK cytotoxic cell and polymorphonuclear neutrophils scores were slightly higher both in virus-positive and virus-negative tissues.

Figure 5. Staining of CD markers in pituitary glands. Scale bars refer to 100 micrometers. A) CD8 staining shows a cluster of cytotoxic T-cells in the adenohypophysis of a COVID-19 case (original magnification 40x). B) A cluster of T-cell infiltrating the pituitary gland observed by CD3 staining. C)

CD7 staining shows some T and natural killer cells intermingled with pituitary cells. D) Lack of CD20 staining suggests absence of B-cell infiltration in the pituitary gland.

Figure 6. Summary of results. In severe forms of COVID-19, patients may suffer impaired production and release of pituitary hormones, independently of direct SARS-CoV-2 infection of pituitary cells. The type I interferon pathway is activated in pituitary glands positive for the viral genome and antigens.

Table 1. Demographic, clinical and virological features of the investigated autopsy cases.

	Controls (n=12)	COVID-19 patients (n=23)	
	SARS-CoV-2 negative	SARS-CoV-2 negative in the pituitary (n=9)	SARS-CoV-2 positive in the pituitary (n=14)
Detection of SARS-CoV-2 by real-time RT-PCR			
Lungs	negative	positive	positive
Pituitary	negative	negative	positive
Age			
years, median (range)	59 (35-84)	70 (41-91)	68 (30-81)
Sex			
Men	10 (83%)	6 (67%)	12 (86%)
BMI			
kg/m^2, median (IQR)	24.5 (23.1-26.3)	25.6 (23.3-27.2)	24.8 (23.3-31.0)
Previous disease			
Cardiovascular disease	10 (83%)	6 (67%)	5 (36%)
Chronic pulmonary disease	2 (17%)	1 (11%)	2 (14%)
Diabetes	2 (17%)	2 (22%)	6 (43%)
Malignancy	4 (33%)	1 (11%)	1 (7%)
Severe kidney impairment[1]	0	0	1 (7%)
Received a COVID-19 vaccine			
one shot	0	0	1[§] (7%)
two shots	2[§§] (17%)	3[§§] (33%)	3[§§§] (28%)
Respiratory support			
Simple oxygen	NA	1 (11%)	2 (14%)
Mechanical ventilation	NA	4 (44%)	4 (29%)
COVID-19 treatment	NA	Non-steroidal anti-inflammatories (NSAIDs); antibiotics; prophylactic anticoagulation or full dose heparinization. When needed: dexamethasone, antihypertensive drugs, bronchodilator drugs, anti-diabetic drugs, levothyroxine	
Days from initial symptoms to death			
median (IQR)	NA	25 (15-32)	7 (5-10)
Days from death to autopsy			
median (IQR)	3 (2-5)	3 (2-3)	5 (3-7)

[1] Defined as glomerular filtration rate lower than 30 mL/min per 1.73 m^2

[§] Moderna Spikevax

[§§] Comirnaty Pfizer

[§§§] 2 Comirnaty Pfizer, 1 Moderna Spikevax; when two shots were administered, the same vaccine type was used

RT-PCR, reverse transcription polymerase chain reaction; BMI, body mass index; NA, not applicable; IQR, interquartile range.

Table 2. Transcription of pituitary-specific genes compared to controls in SARS-CoV-2-positive and -negative adenohypophysis of COVID-19 cases.

Gene	Function	COVID-19 virus-positive in pituitary vs control		COVID-19 virus-negative in pituitary vs control	
		Log2 FC	FDR	Log2 FC	FDR
FSHB	Beta subunit of follicle stimulating hormone that induces egg and sperm production	-2.29	0.002	-1.66	0.13
LHX3	LIM homeobox 3. Transcription factor required for pituitary development. Mutations in this gene cause combined pituitary hormone deficiency	-0.88	0.002	-0.58	0.13
GHRHR	Receptor for growth hormone releasing hormone (GhRH); binding to its ligand leads to synthesis and release of growth hormone	0.69	0.02	0.44	0.29
LHB	Beta subunit of luteinizing hormone; it promotes spermatogenesis and ovulation by stimulating steroids production in the testes and ovary	-1.38	0.02	-2.47	0.002
TSHB	Beta subunit of thyroid stimulating hormone; it controls thyroid structure and metabolism	-1.66	0.02	-1.49	0.14
GNRHR	Receptor for gonadotropin releasing hormone (GnRH); binding to its ligand stimulates the secretion of the luteinizing and follicle stimulating hormones	-0.87	0.09	-0.73	0.29
POU1F1	POU class 1 homeobox 1. Transcription factor that regulates hormone expression. Mutations in this gene result in combined pituitary hormone deficiency	-0.29	0.13	-0.39	0.14
AVPR1B	Receptor for arginine vasopressin that stimulates ACTH release	0.68	0.28	0.27	0.86
PRL	Prolactin. It is a growth regulator for many tissues, and it is essential for lactation	0.36	0.28	0.04	0.91
GPR50	G protein-coupled receptor 50; it inhibits melatonin receptor	-0.08	0.84	0.09	0.90
POMC	Proopiomelanocortin. It is a preprotein synthetized mainly in corticotroph cells of the pituitary. Adrenocorticotrophin and lipotropin beta are the major end products	0.09	0.84	0.45	0.53

FC, fold change; FDR, false discovery rate.

Figure 1

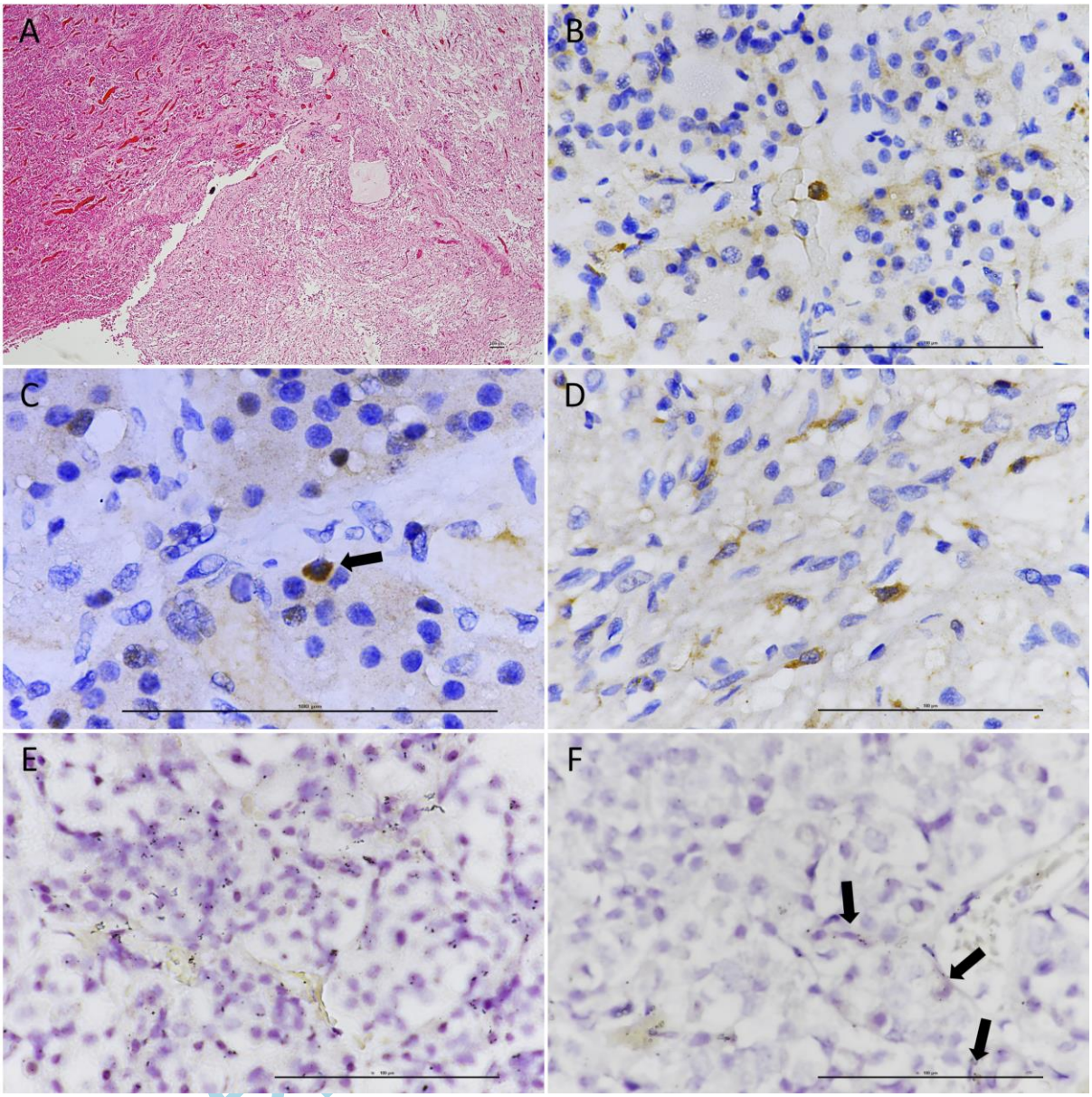


Figure 2

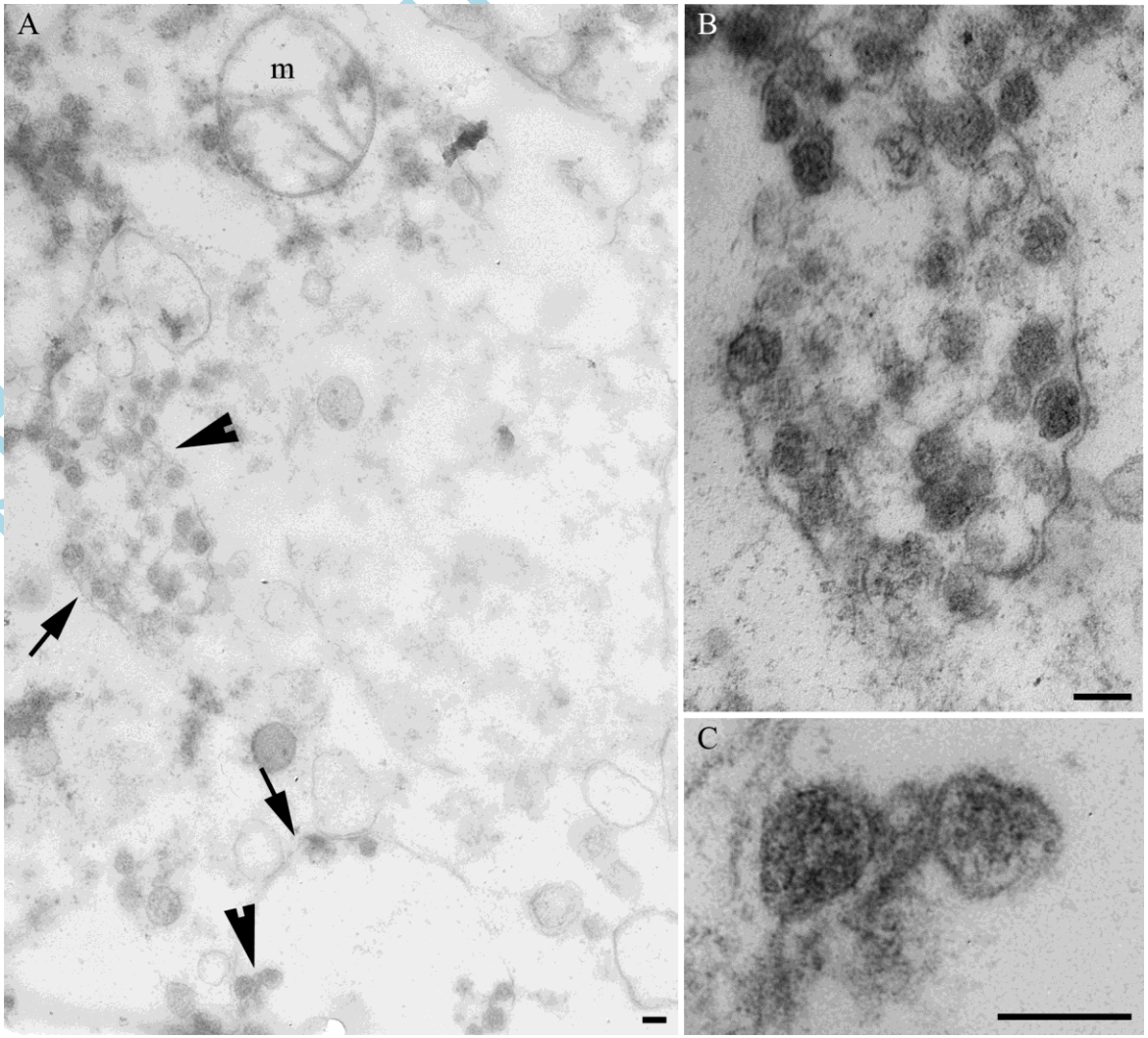


Figure 3

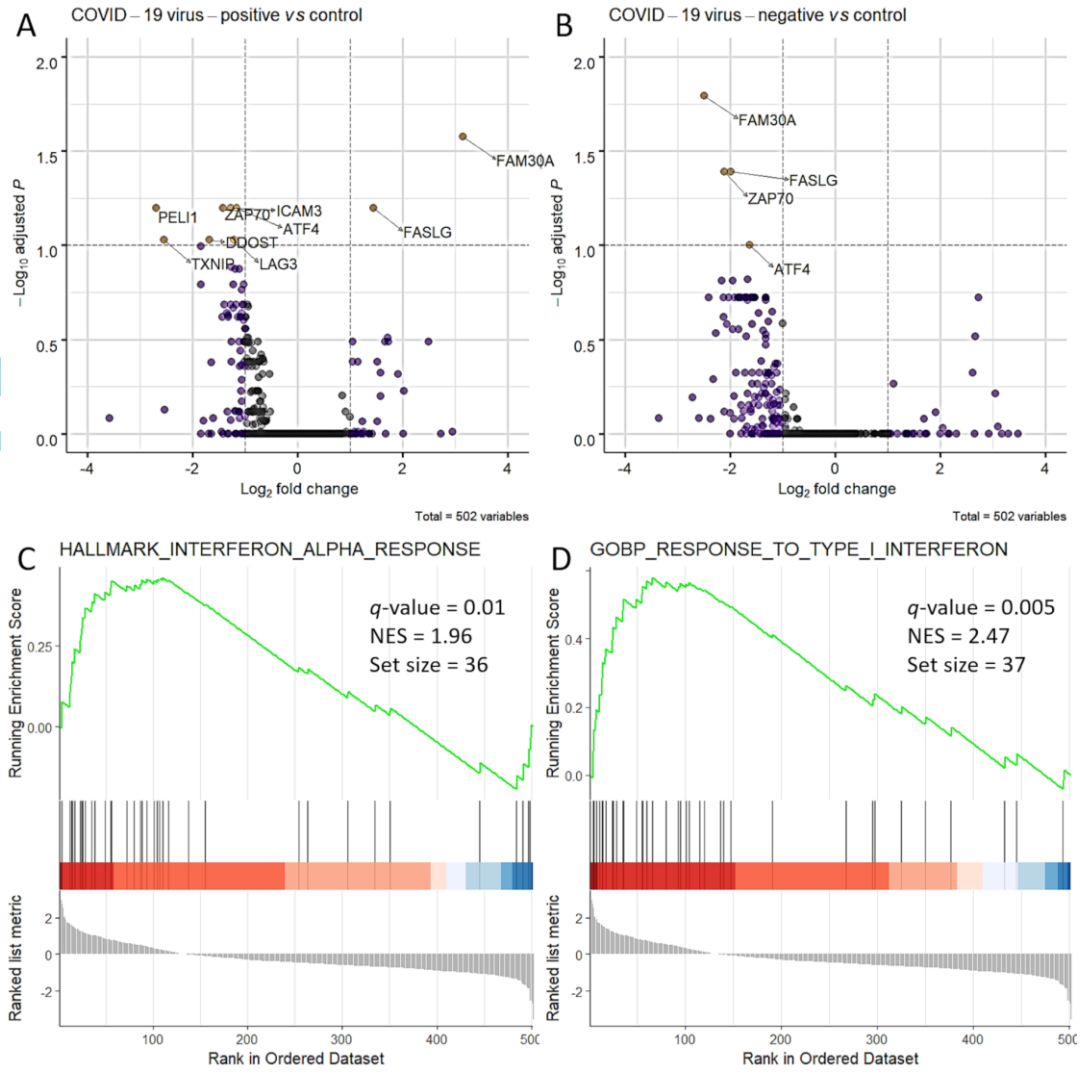

A
COVID – 19 virus – positive vs control
B
COVID – 19 virus – negative vs control
−Log10 adjusted P
Log2 fold change
Total = 502 variables
FAM30A
FASLG
ZAP70
ICAM3
ATF4
PELI1
DDOST
TXNIP
LAG3
C
HALLMARK_INTERFERON_ALPHA_RESPONSE
q-value = 0.01
NES = 1.96
Set size = 36
D
GOBP_RESPONSE_TO_TYPE_I_INTERFERON
q-value = 0.005
NES = 2.47
Set size = 37
Running Enrichment Score
Ranked list metric
Rank in Ordered Dataset

Figure 4

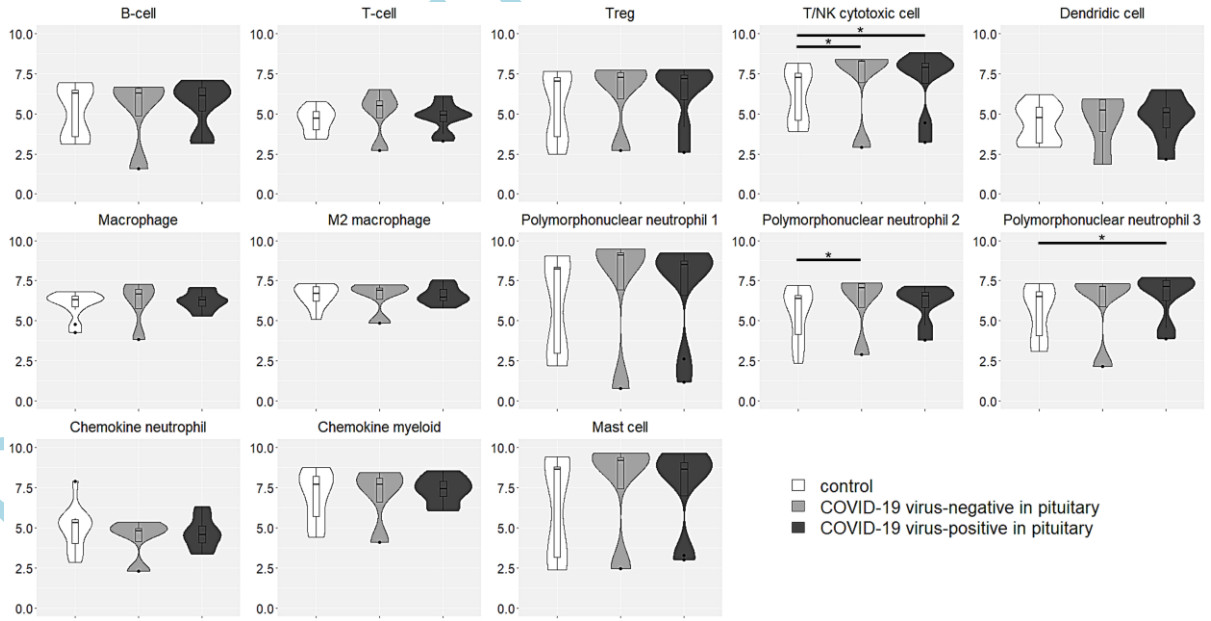


Figure 5

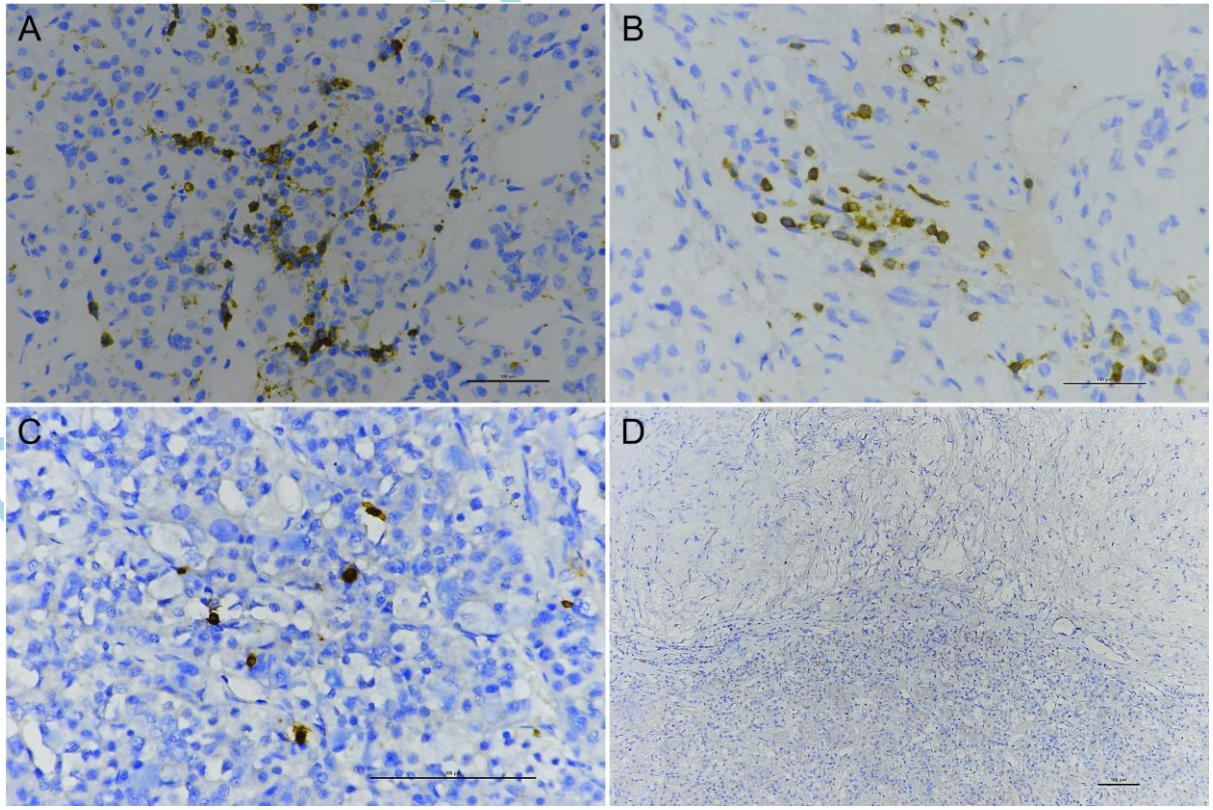
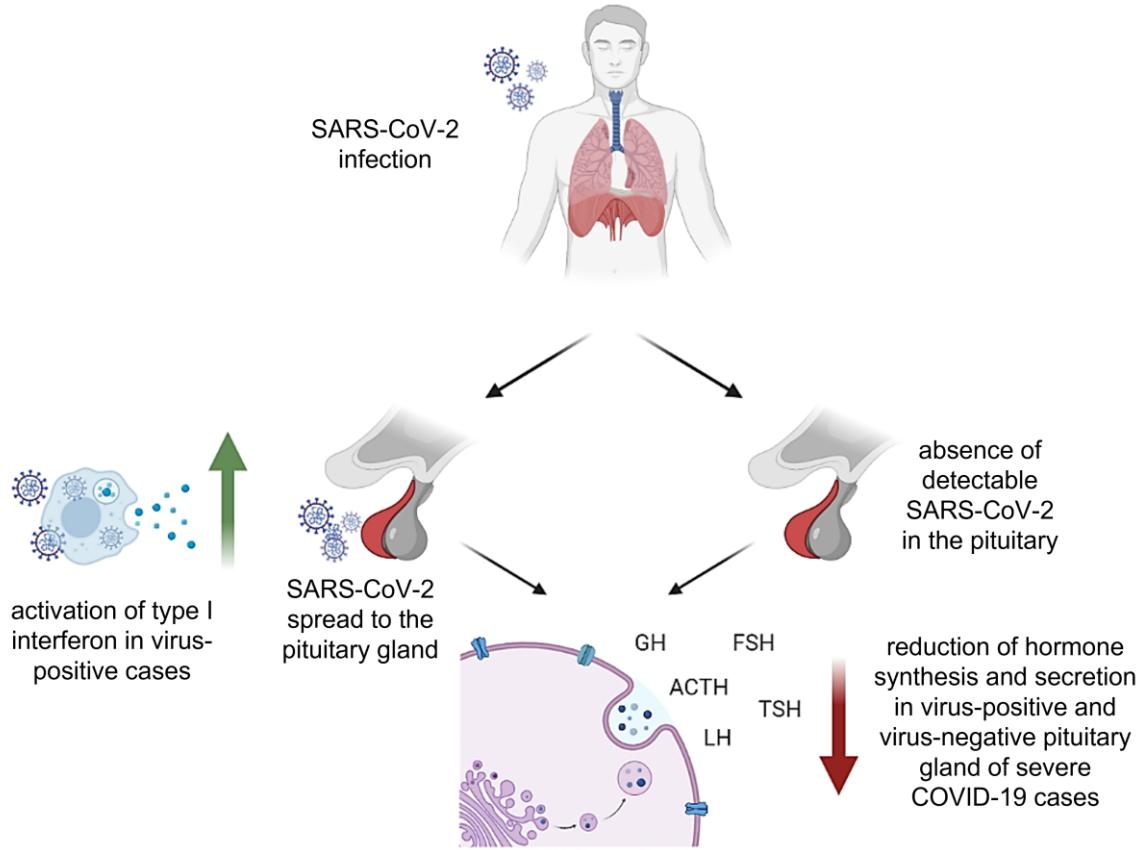


Figure 6


SARS-CoV-2
infection
activation of type I
interferon in virus-
positive cases
SARS-CoV-2
spread to the
pituitary gland
absence of
detectable
SARS-CoV-2
in the pituitary
GH
FSH
ACTH
TSH
LH
reduction of hormone
synthesis and secretion
in virus-positive and
virus-negative pituitary
gland of severe
COVID-19 cases