

# Retrospective comparison of thyroid hormone levels in patients following a SARS-CoV-2 (COVID-19) infection

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**UNIVERSITY OF SPLIT  
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**RETROSPECTIVE COMPARISON OF THYROID HORMONE LEVELS IN  
PATIENTS FOLLOWING A SARS-COV-2 (COVID-19) INFECTION**

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## **LIST OF ABBREVIATIONS**

Ab – antibody  
ADAMT17 – ADAM metalloproteinase with thrombospondin motif 17  
AITD – autoimmune thyroid diseases  
AKI – acute kidney injury  
ANOVA – analysis of variance  
ARDS – acute respiratory distress syndrome  
AT1 – alveolar type 1 cells  
AT1R – angiotensin I receptor  
AT2 – alveolar type 2 cells  
BALF – bronchoalveolar lavage fluid  
cAMP – cyclic adenosine monophosphate  
CaSR – calcium-sensing receptors  
CCS – COVID-19 cytokine storm  
COPD – chronic obstructive pulmonary diseases  
CoV – Coronavirus  
COVID-19 – Coronavirus disease-19  
CRP – c-reactive protein  
D1 – type 1 deiodinases  
D2 – type 2 deiodinases  
D3 – type 3 deiodinases  
DABK –  
DAD – diffuse alveolar damage  
DATP – damage associated transient progenitors  
DIT – diiodotyrosine  
DMV – double membrane vesicle  
DVT – deep vein thrombosis  
dsRNA – double-stranded RNA  
eNOS – endothelial nitric oxide synthase  
ER – endoplasmic reticulum  
fT3 – free tri-iodothyronine  
fT4 – free tetra-iodothyronine  
GD – Grave's Disease  
hACE2 – human angiotensin- converting enzyme 2  
HLA – human leukocyte antigen

HPT axis – hypothalamic-pituitary-thyroid axis  
HT – Hashimoto’s thyroiditis  
IFG-I – insulin-like growth factor I  
IFN – interferons  
IQR – interquartile range  
MERS-CoV – Middle east respiratory syndrome coronavirus  
MHC – major histocompatibility complex (MHC)  
MIT monoiodotyrosine  
mRNAs – messenger RNA  
NET – neutrophilic extracellular traps  
NF –  $\kappa$ B nuclear factor  $\kappa$ B  
NIS – sodium iodine symporter  
NTIS – non-thyroidal illness syndrome  
pDCs – plasmacytoid dendritic cells  
PE – pulmonary embolisms  
PRR – pattern recognition receptor  
PVN – paraventricular nucleus  
RAAS – Renin-Angiotensin Aldosterone System  
RBD – receptor-binding domain  
RBM – receptor binding motif  
RdRp – RNA-dependent RNA polymerase  
RIG-I-like receptors – retinoic acid-inducible gene-I-like receptors  
RNA – ribonucleic acid  
ROS – reactive oxygen species  
RT3 – reverse T3  
RTC – replication and transcription complex  
ssRNA – single-stranded ribonucleic acid  
SARS- CoV – severe acute respiratory syndrome coronavirus  
SARS-CoV-2 – severe acute respiratory syndrome coronavirus- 2  
SAT – subacute thyroiditis  
SPINA – structure parameter interference approach  
TBG – thyroxine-binding globulin  
TF – tissue factor  
TG – glycoprotein thyroglobulin



TH – thyroid hormone  
TNF-a – tumor Necrosis Factor alpha  
TGF- $\beta$  – transforming growth factor- $\beta$   
TLR7 – Toll-like receptor  
TMPRSS2 – type II Transmembrane serine protease  
TPO – thyroidperoxidase  
TR – thyroid receptor  
TRH - thyrotropin-releasing hormone  
TSH – thyroid-stimulating hormone  
TSH- R – thyroid-stimulating hormone receptor  
TSI – thyroid-stimulating immunoglobulin  
TTR – transthyretin  
VOC – variants of concern  
VTE – venous thromboembolism  
WHO – World Health Organization

## **1. INTRODUCTION**

At the end of December 2019, first cases of a novel virus causing a potentially life-threatening pneumonia emerged and spread globally in a matter of only a few weeks (1). It had tremendous disruptive socio-economic, but also psychological effects and ushered in incommensurable impacts on healthcare systems (2). On February 11<sup>th</sup> in 2020, the World Health Organization (WHO) officially stated the causative agent of corona disease 2019 (COVID-19) being the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (3). On March 11<sup>th</sup> 2020, the WHO followed suit with a declaration classifying the infectious situation as pandemic (4). This would be the third time a zoonotic highly pathogenic coronavirus is introduced to human population (5). Phylogenetic data linked SARS-CoV-2 to severe acute respiratory syndrome coronavirus (SARS- CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV). These three viruses are able to replicate in the lower respiratory tract ultimately leading to a fatal acute respiratory distress syndrome (ARDS) (4,6).

### **1.1. Virology**

Coronaviruses (CoV) are a highly diverse family of single-stranded (ssRNA), non-segmented, enveloped RNA viruses (5). Phylogenetic analysis proved SARS-CoV-2 to be closely related to  $\beta$  coronaviruses, one of the four ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ) genera of the subfamily Coronavirinae and is part of the Orthocoronavirinae, subgenus of the Coronaviridae family (3,5). SARS-CoV-2 is characteristically a pneumotropic virus and has been first discovered in bronchoalveolar lavage fluid (BALF) samples taken from patients in Wuhan, Hubei province, China (7).

The virion genome encodes several structural and non-structural proteins (4). One of the structural entities is a surface projection protein, the triple spike (S) protein, which is a common feature among coronaviruses (3). It allows assessment of tropism and viral transmissibility. The spike (S) protein exists in a metastable pre-fusion conformation and upon binding undergoes major rearrangements (9). Functionally, the glycoprotein is constituted of two subunits. As seen in Figure 1 the first subunit (S1) contains a receptor-binding domain (RBD) with which it interacts with the host cell receptors and is thereby pivotal in facilitation of viral entry. The second subunit (S2) contributes to membrane fusion. Therefore, the triple spike (S) protein holds a major function in initiation of infection. Other proteins include a membrane/ matrix glycoprotein (M) which is responsible for the assembly of viral particles. Additionally, it is involved in budding of viral particles by means of interacting with nucleocapsid (N) proteins and accessory proteins. Phosphoprotein nucleocapsid (N) is participating in pathogenesis due to its function to bundle the genome into a helical ribonucleoprotein complex and subsequent

replication, virion formation and moreover circumvention of the hosts immune system. The envelope (E) protein enables production, maturation, and release of viral particles via endocytosis (8,10–12). Moreover, non-structural proteins are encoded which construct replicase-transcriptase complex enabling viral replication, assembly and transcription processes (8). The intercommunication between structural and non- structural proteins contributes to the viruses ability to infect and replicate within host cells (5).

Over the last years, multiple strains of coronaviruses have been discovered in avian hosts, as well as in mammals, such as bats, pangolins, ferrets and minks (10,13). Early investigations suggested a direct transmission via bats or via intermediate hosts/animal reservoir as possible transmissions (10). Although it has to be mentioned that Corman *et al.* argue SARS- like CoVs are unable to directly infect humans unless a mutation or recombination in animal hosts as intermediates occurred (14).

Around six different corona species are known to cause common cold or enteric symptoms in humans (6,7,15). Two strains however, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), were proven to be zoonotic in origin, and have been causative agents of previous endemics (7). With a high degree of pathogenicity, their infection lead to severe, even life- threatening respiratory pathologies as well as lung injuries (5). Additionally, genome sequencing determined a close relation between SARS-CoV, MERS-CoV, and SARS-CoV-2 which would indicate a similar route of transmission of the virus to humans (8,16). It is essential to acknowledge that RNA viruses have a higher mutational load caused by a shorter replication time (17). They undergo mutations more often in comparison to DNA viruses which could have been the reason for transmission between species (10). Although a possible origin and several intermediate hosts are in discussion, for this pandemic human to human transmission remains the main route of transferring viral particles (18). Contractions between humans enable the virus to undergo various genetic mutations (3). The spread primarily occurs via respiratory droplet secretions and aerosols when an infectious person coughs, sneezes, or talks (6,10). SARS-CoV19 is an enveloped RNA virus (16). Thereby, it creates a possibility for persistence and transmission also via contaminated surfaces by contact alone (3,10).

## **1.2. Pathology and pathogenesis**

### **1.2.1. Intracellular pulmonary pathology: infection and replication cycle**

The initial step in invasion is the attachment of previously mentioned subunit S1 of spike (S) protein to the cellular entry receptor human angiotensin- converting enzyme 2 (hACE2) located on cell surfaces via receptor- binding domain (RBD) of S1 (S1-RBD) (5). This reaction can be seen in Figure 1. The receptor is present in multiple mammalian organ tissues, principally in the lungs, kidneys, heart, liver, blood vessels and the brain (19,20). It has been revealed that the conservation and minor substitutions in spike glycoproteins of SARS-CoV-2 is more adaptable to ACE2 receptors in comparison to SARS-CoV and thereby, it has an enhanced transmission to human host cells. In other words, SARS-CoV-2 RBD has a higher binding affinity to human receptor ACE2 (hACE2) in comparison to SARS-CoV (10,21). Mutations occurring in spike (S) protein and non-structural proteins are of vital importance since they contribute to virulence and differentiation mechanisms (22). Subsequently, they also play a role in development of neutralizing antibodies and vaccines (10). Following the attachment of S1-RBD to ACE2 receptor, the spike (S) protein is cleaved by host cell- derived proteases for conformational changes necessary for membrane fusion (11,23). Figure 1 depicts additionally the role of the cell-surface Type II Transmembrane serine protease (TMPRSS2) which is one of the proteases mediating spike (S) protein activation and initiating viral entry, ultimately leading to infection (24).

Primary route of infection is through the upper respiratory tract or facial mucosal epithelial surfaces. Reason being, hACE2 and TMPRSS2 are abundantly expressed in these tissues contributing to replication and pathogenesis. The expression and distribution in tissues of ACE2 as well as the abundance of TMPRSS2 proteases determine in turn viral tropism and pathogenicity (5,25–27). The wide distribution of hACE2 expression might be explaining the multiorgan presentation (10). Following the binding to ACE2 receptors, viral particles are internalized by receptor-mediated endocytosis (10). Thereby, the viral RNA alongside with its translational polyproteins is being released into the host cell cytoplasm. Afterwards, the viral particles are assembled into replication/ transcription complexes with virus-induced double membrane vesicles (DMVs) (11). 16 non-structural proteins are the main constituents of SARS-CoV-2 replication and transcription complex (RTC). Noteworthy here are the RNA-dependent RNA polymerase (RdRp) and several auxiliary factors (28). A complex program of viral gene expression is being set in motion (5). Concisely, genomic RNA and host ribosomes form the RNA-ribosome unit and translation into proteins occurs. Through this mechanism viral genomic

RNA and subgenomic mRNAs are generated. The viral particles then are translocated to host cell organelles such as the endoplasmic reticulum and Golgi apparatus and afterwards released into neighboring cells by exocytosis (10).

The foremost cells targeted by the virus are multiciliated ones located in the nasopharynx or trachea. Another possible route of entry are the sustentacular cells in the olfactory mucosa of the nose (26,27). It is hypothesized that an infection of these cells prompts the loss of ciliation and a failure of upward movement of mucous in airways, trapping the virion particles in alveoli leading to transportation of particles to the lower respiratory system (29). Concomitantly, innate and adaptive immune responses are activated. If these mechanisms should be insufficient in clearing the viral particles, they spread to the lower respiratory tract and migrate to alveoli or disseminate down the tracheobronchial tree (6).

Upon an infection of the alveoli the pathological changes cause inflammation and limit gas exchange. SARS-CoV-2 seems to be targeting alveolar type 2 (AT2) cells in particular due to their abundant expression of ACE2 and TMPRSS2. Their physiological role is production and secretion of pulmonary surfactant which is necessary for reduction of surface tension in the alveoli during respiration. The generation of surfactant has been shown to decrease due to diminished surfactant gene expression (6,20). Another mechanism postulated which causes a decline in surfactant results simply from the destruction and damage of alveolar cells (30). The decrement of surfactant leads to a rise of surface tension and consequently to a predisposition for ARDS (30). Moreover, AT2 cells are progenitors of alveolar type 1 (AT1) cells. The importance of this is that there has been data collected which indicates that a failure of differentiation occurred after SARS-CoV-2 virus entered AT2 cells. The cells have been trapped into what has been termed damage associated transient progenitors (DATPs). The relative number of DATPs is increased in patients with COVID-19 and has a pathological significance in the fibrotic phase of ARDS. To summarize, generation of immune response with subsequent local inflammatory changes and loss of surfactant are through to be direct effects of viral entry (6,20).

Viruses depend for their survival on the host cell machinery and in turn drive cellular machinery to the generation of molecules for virion replication. Especially mitochondrial functions in various tissues have been suppressed inducing a dynamic in mitochondrial gene expression and energetics of cells as the virus disseminates to organs. In order to compensate for the substantial shift towards replication of viral constituents availability and synthesis of amino acids were shown to be altered in the first 48 hours after infection, alongside with depletion of intracellular glucose and folate concentrations with a concomitant increase of

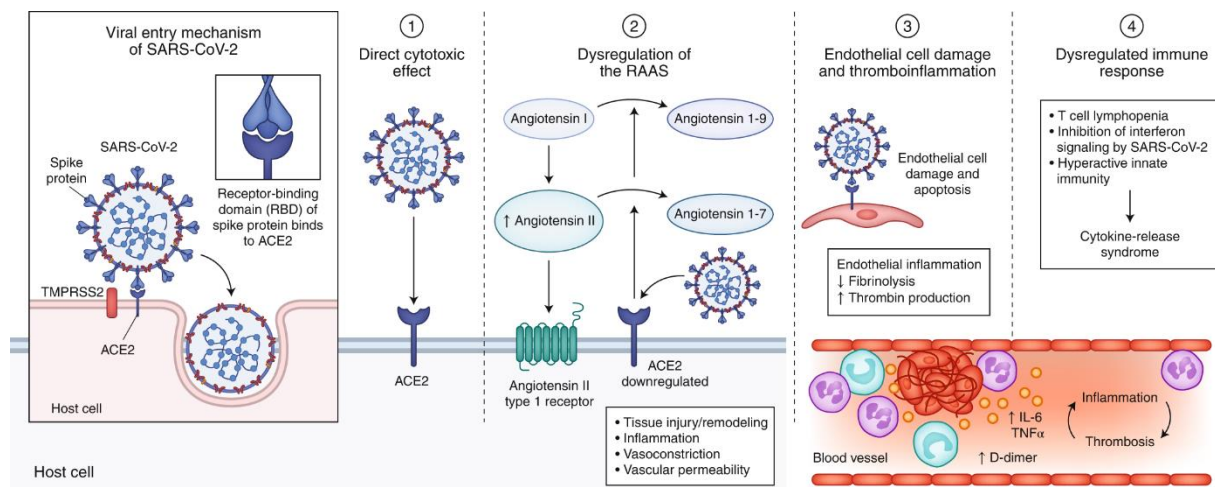
lactate levels. These findings were suggestive for diversion or redirection of cellular metabolic pathways by virus causing an increased glycosylation but decrease of oxidative phosphorylation. These effects are accompanied by an increased production of reactive oxygen species (ROS) and reactive nitrogen species with various long-term pathophysiological sequelae yet to be determined (20).

### **1.2.2. Extrapulmonary manifestation**

Primary viremia follows replication and invasion of pneumocytes. Propagation of viral particles throughout the body occurs most likely via blood stream and secondary replication ensues with subsequent pyroptosis (10,30). Severe forms of COVID-19 might lead to alterations and manifestations in a multitude of organs since the viral particles disseminate to tissues with ACE2 receptor expression accumulating in rhabdomyolysis, coagulopathy, and shock (31). Concisely, T cell-mediated inflammatory reactions develop as a consequence, releasing cytokines, chemokines and interleukins, potentially leading up to development of COVID-19 cytokine storm (CCS) (10,30). The entirety of inherent mechanisms leading to specific extrapulmonary manifestations have not been fully established yet (6,32,33).

As previously stated, mode of access is by binding of viral spike (S) protein- S1 subunit to a transmembrane glycoprotein angiotensin-converting enzyme-2 (ACE2) receptor with enzymatic activity located on pneumocytes belonging to the Renin-Angiotensin Aldosterone System (RAAS). The primary physiological systemic role of RAAS is modulation of blood pressure and osmolarity in the setting of hormonal feedback loops. In essence, pressure sensitive cells are located in the renal juxtaglomerular apparatus and release upon stimulus renin which in turn results in conversion of angiotensin into angiotensin I (20,30). Local role of RAAS is the mediation of pro-inflammatory, pro-thrombotic and profibrotic events mediated by angiotensin I (AT1R) receptor. On the other hand, this cascade is antagonized by binding of ACE2 receptor which cleaves angiotensin I and II and thereby fulfilling protective cardiovascular functions such as vasodilatation and control of endothelial permeability. Moreover, it reduces blood pressure, heart frequency, and alveolar surface tension (30). Following viral entry, a downregulation of ACE2 receptor expression caused by its internalization has been described. An alternative proposed mechanism of downregulation is mediated by cleavage of cellular proteases such as disintegrin and metalloproteinase with thrombospondin motif 17 (ADAMT17). These effects can be seen in Figure 1 and result in a disruption of RAAS simultaneously upregulating the angiotensin II-AT1 axis and leading to

inflammation and circulatory dysfunction (16, 17).



**Figure 1.** Pathophysiology of COVID-19

Source: Fig. 1 Pathophysiology of COVID-19. | Nature Medicine. [cited 29.01.2024]; Available from: <https://www.nature.com/articles/s41591-020-0968-3/figures/1>

### 1.3. Immunology: host response

Understanding of the immune system responses in previous endemics caused by other coronaviruses, namely SARS-CoV and MERS-CoV, helped in advancing research. Current data indicates an involvement of innate as well as adaptive immune responses. Both are interconnected by various feedback loops regulating complex immune reactions (10,33). The innate immune system encompasses the first line of defense and consists of initially nonspecific responses, involving a myriad of cell populations. Upon an active SARS-CoV-2 infection activation seems to be led by neutrophils with concomitant elevations of reactive oxygen species (ROS) and neutrophilic extracellular traps (NETs). Furthermore, a decreased level of ACE2 and DABK seems to promote neutrophil infiltration. This process is aggravated by chemotaxis of macrophages and monocytes leading to further inflammation and pulmonary fibrosis (33). Various pattern recognition receptors (PRRs) are another mechanism by which the innate immune system detects viral particles. The primary PRR detecting SARS-CoV-2 is thought to be MDA5 which initiates transcription of type I and III interferons (IFN) (6). PRR signaling recruits NK cells which detect and subsequently eliminate cells infected by pathogens. Nonetheless, NK cells have been depleted and rendered dysfunctional in severe cases of COVID-19 due to exposure to transforming growth factor- $\beta$  (TGF- $\beta$ ) which impairs their antiviral activity. A second possibility to ascertain and recognize endocytosed double-stranded



(dsRNA) or single-stranded (ssRNA) are specialized plasmacytoid dendritic cells (pDCs) utilizing Toll-like receptor 7 (TLR7). Other cell types express TLR3 and TLR8 which fulfill the inherent same function. A second opportunity arises during viral replication inside the host cell. During that process intermediate products of dsRNA are recognized by cytosolic RNA sensors or RIG-I-like receptors (RLRs). Once the signaling cascade interacts with TLRs and RLRs it instigates production of IFN type I and III alongside with nuclear factor  $\kappa$ B (NF- $\kappa$ B)-dependent proinflammatory cytokines and chemokines (35). With the significant local hyperinflammatory state a persistent impairment of lung function, diminished exercise capacity and impairment of quality of life has been seen in multiple follow-ups, which lead to the hypothesis of impairment in lung regeneration due to alterations in cellular composition, transcription and translation mechanisms, and disrupted cell-to-cell interactions (33).

On the other hand, adaptive immunity mediates humoral antibody (Ab) and cell-mediated immune reactions mediated by B and T cells. Essentially, it is a much more specific response to pathogen infection (33). Abs work by neutralization of SARS-CoV-2 since they prevent association of viral spike (S) protein with ACE2 receptor. In detail, Abs bind to the RBD of S1 subunit which express the receptor binding motif (RBM). Furthermore, they are involved in promotion of effector function since they involve the complement and Fc receptors. Quantity and quality of adaptive Ab mediated immune response is dictated by concentration of antigens and temporal extent of germinal center reaction. Cellular mediated adaptive immune response of CD4<sup>+</sup> and CD8<sup>+</sup> T cells is directed against a variety of structural and nonstructural proteins. A limitation of T cell response to acknowledge is the substantial lymphopenia (lowered CD4 and CD8 numbers) affecting immune mechanisms (33). Especially the emergence of SARS-CoV-2 variants of concern (VOC) and their evasion of neutralizing Abs and mutations in RBM, prompted an increased binding affinity to ACE2, evoking difficulties for an adequate viral clearance.

Coronaviruses developed multiple means by which they avoid detection by host immune systems. First, the survival of SARS-CoV virions is ensured by evasion resulting from a lack of pathogen-associated molecular patterns on DMVs and failure of detection PRRs (35). Surrounding epithelial and local immune cells start producing interferons upon detection of SARS-CoV-2 by the means of Toll-like receptors (TLRs) or paracrine effects (6). Further, a significant observation is the heavy glycosylation of coronavirus spike (S) proteins, which promotes immune evasion by shielding epitopes from neutralizing antibodies (5). It is important to consider, that accessory proteins which have a role in replication of virion are also involved with immunoevasive activities and block several of the pathways by various means.

Additionally, several non-structural proteins impede with IFN-I responses by induction of degradation of host mRNA along with repression of transcription factors. As a result, lower concentration of IFN-I and IFN-III are detected (6,11,35). In later stages and more severe ones multiple immune system constellations have been reported. Later stages show a correlation of prolonged IFN secretion and severity of outcome which might result from continuous recruitment of inflammatory mediators. Another phenomenon already described above is the depletion and mitigation of NK-cell function and their inability to clear viral infected cells. Furthermore, T cells have been measured to be dramatically depleted in patients with a severe course of the infection (35).

#### **1.4. Systemic manifestation and clinical presentation: general symptoms**

Coronaviruses cause a variety of symptoms ranging from an asymptomatic, mild or moderate infection to severe and critical cases. These divisions were made in accordance with the degree of infection and severity of symptoms which could be affecting several organ systems causing among others respiratory, enteric, hepatic, and neurological diseases (6,9,30). Approximately 5 days following an infection patients showed symptoms of COVID-19. Duration of displayed symptoms was shown to be closely linked to the immune status as well as age of patients. In mild to moderate cases initially, fever, cough, myalgia or fatigue were the most common findings. Gastrointestinal signs are reported to be second most prevalent in an infection with coronaviruses (15). Alongside sputum production, headaches, nausea, vomiting, general weakness, anosmia, and ageusia were described. Pharyngalgia might also be a symptom of upper respiratory tract infection. In patients with a more severe course of an infection, dyspnea resulting from hypoxemia is the most common symptom (1,6,35–37).

Next to pulmonary symptoms, extrapulmonary manifestations of the viral infection have been reported and viral presence has been detected in several organ systems such as the small intestine, skin, pancreas, kidney, brain, and heart (20). A study conducted by Mao *et al.* described three categories of nervous system involvement. Some showed signs of central nervous system manifestations such as dizziness, impaired consciousness, as well as ataxia and seizures. Signs affecting the peripheral nervous system such as taste, and smell impairment constitute the second category. Thirdly, skeletal muscle injuries were described (38). Moreover, non- pulmonary complications included among others hepatic injury, acute myocardial injury, acute kidney injury (AKI), septic shock, leading up to multiple organ failure (3). Several risk factors for development of an aggravated advancement of an infection haven been determined

and include advanced age (immunity and comorbidities), obesity (higher ACE2 expression in adipose tissue and comorbidities), male sex (ACE2 expression in testicular tissue). Alongside, co-morbidities such as hypertension, heart failure, cardiac arrhythmia, diabetes, kidney failure and chronic obstructive pulmonary diseases (COPD) were connected with an exacerbated and more severe progression of COVID-19. Furthermore, multiple gen loci have been ascertained to be connected to an excessive unfolding of the disease (6,20,30).

## **1.5. Complications**

### **1.5.1. Cytokine storm**

Stemming from a physiological immunological reaction by the innate immune system evidence pointed to a state of systemic hyperinflammation with the consecutive release of pro-inflammatory cytokines, including an elevation of reactive inflammatory markers namely, D-dimer, ferritin, and C-reactive protein (CRP) amassing into a state termed COVID-19 Cytokine Storm (CCS). A selective development of immune mediated COVID-19 cytokine storm (CCS) has been described, which is a key aspect of an infection with the virus and depicted in Figure 1. It has been noted that in a subgroup of patients a hyperimmune pro-inflammatory response occurs which develops secondary to an overproduction of cytokines by a dysregulation of immune system responses (6,16,30,39).

Clinically, it can cumulate to coagulopathies, multi-organ failure and ARDS (30,40) The cytokine polypeptides normally function as intercellular mediators of the immune system and are involved in processes fundamental for survival, such as inflammation, tissue repair, necrosis, and coagulation. Regulatory mechanisms which create a balance between production of pro- and anti-inflammatory cytokines fail. Subsequently, a previously local reaction develops into a systemic one (40). Albeit, if produced in excess a systemic hyperinflammatory state develops with autoinflammatory diseases, primary and secondary hemophagocytotic, multicentric Castelman disease, and sepsis. It is noteworthy, that these findings previously have been described for SARS and MERS (24). The innate as well as adaptive immunity are involved in the production of a myriad of inflammatory cytokines such as Tumor Necrosis Factor alpha (TNF- $\alpha$ ), interleukins (IL) such as IL1, 6, 8, and 18, and many more. Their presence has been proven to correlate with the disease severity and are independent predictors of patients survival (41). Clinical presentation in these individuals was determined by the underlying increased vascular permeability (leaky state) leading to secondary cardiocirculatory instabilities. Following, the hypercoagulable state leads to venous and arterial thromboembolism, tissue

inflammation and destruction with cumulation into acute respiratory distress syndrome (ARDS) (1,6,41,42).

### **1.5.2. ARDS**

A complication manifesting with worsening of the aforementioned symptoms includes acute respiratory distress syndrome (ARDS) with subsequent respiratory failure (3). As previously mentioned, the most frequent symptom in cases of severe COVID-19 is dyspnea resulting from hypoxemia. The Berlin definition in 2012 determined several criteria of ARDS: The patients exhibit various degrees of hypoxemia, ranging from mild to severe. Moreover, radiographic severity showing bilateral opacities within the first seven days of exposure. ARDS is exhibiting pulmonary affliction distinguished by inflammatory processes, pulmonary vascular permeability, and ensuing in a depletion of aerated lung parenchyma (6,16). These pathologies were histologically discovered in lung specimens of a patient who initially demonstrated fever, cough and dyspnea. The patient subdued to the infection in hospital in Beijing in January 2020. Biopsy samples taken in the setting of an autopsy which was performed on this patient reported bilateral diffuse alveolar damage with an exudate of cellular fibromyoxid. Furthermore, histological examination of lung tissue with desquamation of pneumocytes alongside with hyaline membrane formation which indicates an early phase of ARDS. Interstitial inflammatory together with viral cytopathic-like changes were seen in the intra-alveolar spaces (37). The predominant pattern of injury fit the categorization of diffuse alveolar damage (DAD). In general, it exhibits an initial exudative phase characterized by oedema, necrotic cells, hyaline membranes and inflammation. Following, in a proliferative phase AT2 cells undergo hyperplasia in order to regenerate the alveoli. As previously mentioned the AT2 cells fail to differentiate fully into their descendent cell line cumulating to a state referred to as damage- associated transient progenitors (DATPs). Alveolar damage leads to a disruption of epithelium and causes an imbalance of the coagulation and fibrinolytic cascade, ultimately leading to formation of hyaline membranes. These membranes are sealing off the alveoli in a protective manner, but limit as a consequence effective gas exchange. Patients presenting with a fibrotic phase in ARDS exhibit clinical, radiographic and histologic hallmarks of pulmonary fibrosis. The degree is in direct correlation with the duration of COVID-19 (6,43,44).

### **1.5.3. Prothrombotic state**

Concurrently, a prothrombotic state as a reminiscent of immunothrombosis develops due to an imbalance in coagulation and fibrinolysis. In its basic mechanism it can be seen in Figure 1. Trigger for an unfolding of such state are said to be hypoxia, immune modulatory cytokines and chemokines which induce a leaky state in endothelium and epithelium. Upon disruption of the endothelium tissue factor (TF) comes in contact with coagulation factors and the exposed extracellular matrix sets the stimulus for initiation of extrinsic coagulation pathway. Subsequently, it causes formation of fibrin, which is a component of clots and seen in hyaline membranes formed during ARDS as described in the section prior. The tissue damage can also initiate intrinsic coagulation pathway stimulated by extracellular RNA, DNA and collagen again leading to fibrin deposition (6).

The endothelial integrity is being disrupted and can thereby be predisposition for venous thromboembolism (VTE) disease resulting in formation of deep vein thrombosis (DVT), pulmonary embolisms (PE), and strokes. Concomitantly, disseminated intermittent coagulopathy has been reported in hospitalized patients (30).

### **1.6. Perspective**

In conclusion, the pandemic reflected sensitive balances in a globally connected and complex ecosystem compromising not only humans, but also animals, pathogens and their environment. In combination with an extreme efficiency transmission alongside its enormous level of morbidity and mortality, SARS-CoV-2 -pandemic made these sensibilities evident (45). The British biologist Richard Dawkins pointed out in his book “The Selfish Gene” published in 1967 that evolution arises at the level of gene competition. Subsequently, the human species is in constant rivalry with microbes and is pressed to adopt immediate and long-term control strategies to ensure long-term survival (46). One has to be aware of the current climate change with its effect on wildlife, ecosystem health, urbanization and globalization and of a certain probability that zoonotic coronaviruses might occur more often in the future (6). Therefore, it is crucial to recognize that successful eradications go hand in hand with controlling existing diseases as well as their prevention and control of emerging diseases. These aspects require a tremendous amount of effort in the fields of scientific research, public health systems and politics in order to address the upcoming emergence of infectious diseases (45).

## **1.7. Thyroid gland**

The primary function of the gland lies in the production and secretion of iodothyronine hormones, namely tri-iodothyronine (T3) and thyroxine or tetra-iodothyronine (T4) as well as reverse T3 (rT3). These hormones have a crucial role in cell differentiation, organogenesis, metabolic processes, and exert control over principal vital physiological mechanisms (47–49). The impacts manifested by thyroid hormones are complex and depend on the expression of cell and tissue-specific transporters, various isoforms of the thyroid receptor (TR), and the interactions with corepressors and coactivators (50). Moreover, several mechanisms of intercommunication with a myriad of other signaling pathways have been described (51). The gland itself is an integral part of the hypothalamic-pituitary-thyroid (HPT) axis and is in constant communication through negative feedback mechanisms which regulate synthesis along with secretion of hormones (52,53).

### **1.7.1. Development**

In the development of this endocrine gland, several embryogenic cell lines are involved. Noteworthy is that it is embryonically the earliest gland to appear. During the third week towards the end of the fourth week, proliferating foregut endoderm cells migrate through the foramen cecum down the thyroglossal duct toward their final destination, inferior to the cricoid cartilage. These cells ultimately form the thyroid gland. This conglomeration of cells merges into two lobes which are interconnected by a parenchymal bridge, the isthmus. Moreover, neural crest cells invade the primordial thyroid mass and generate connective tissue of the gland. Another set of tissue are ultimobranchial bodies which mature into parafollicular cells (C cells) and eventually produce the polypeptide hormone calcitonin (47,49,54).

### **1.7.2. Cellular level**

In the end, thyroid parenchyma consists of numerous thyroid follicles enclosing a central lumen which lost all luminal connections with other parts of the body (55). These follicles come in a variety of size ranges and constitute the functional units of the gland. The central lumen is filled with eosinophilic acidic colloid and serves as a reservoir for synthesized thyroid hormones. It is lined by a monolayered polarized epithelium. Histologically, active glands show a more columnar epithelium whereas glands which are hypoactive appear more squamous (56). The primary cell type of this epithelium are thyrocytes which are involved in the production of

thyroid hormones (TH). On the acinar surface of the parenchymal cells, villi reach inside the colloid. Polarization and internal tension enable storage of substrate alongside with storage and synthesis of hormones (48). A second cell type contained in the thyroid are neuroendocrine C-cells which produce calcitonin. They are arranged in parafollicular or intrafollicular fashion and in response to extracellular  $\text{Ca}^{2+}$  concentration sensed in the capillary network they react by activation of calcium-sensing receptors (CaSR), inducing the release of calcitonin. Subsequently, it decreases the calcium concentration in serum, thereby antagonizing parathyroid hormone (47). The endocrine gland is highly vascularized and capillaries surround each follicle enabling release of synthesized hormones for distribution throughout the body (56).

### **1.7.3. HPT axis**

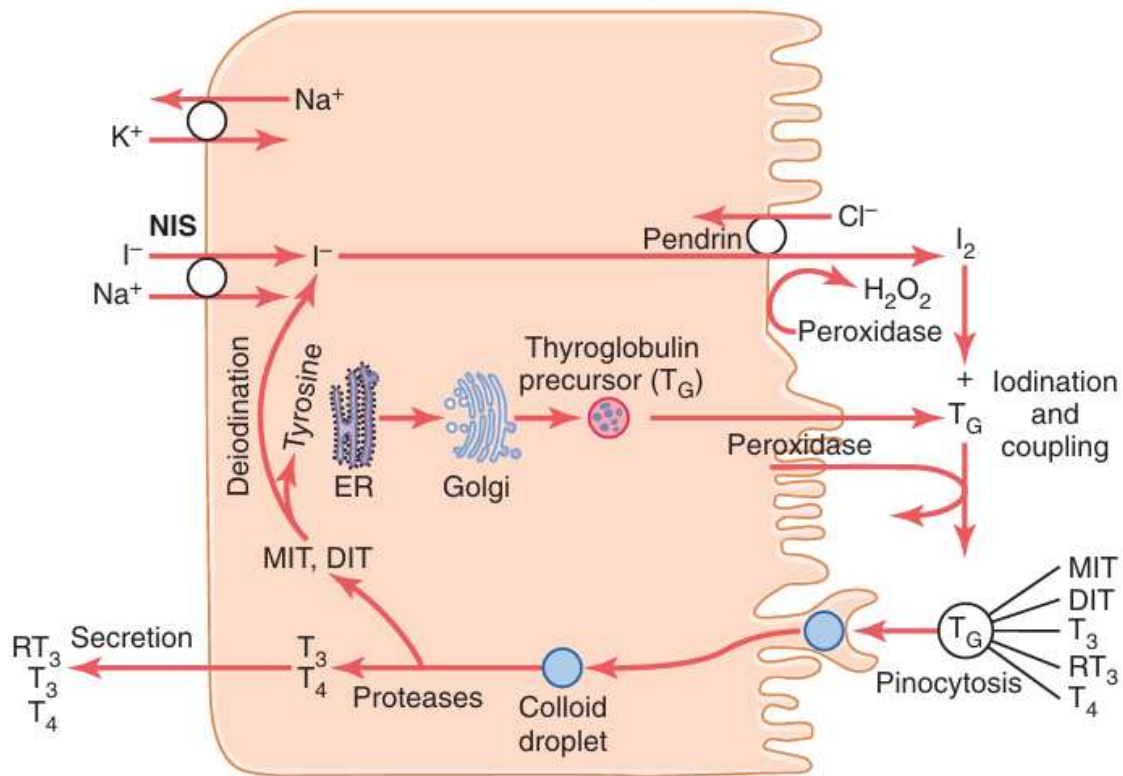
This capillary network is used as a pathway for complex communication and is essential for intact functional processes of the endocrine system. Numerous studies have elucidated the importance of intricate negative and positive feedback loops involving the hypothalamus, pituitary gland, and thyroid gland collectively termed hypothalamic-pituitary-thyroid (HPT) axis. The process begins in the hypothalamus, where the tripeptide amide thyrotropin-releasing hormone (TRH) is synthesized in the paraventricular nucleus (PVN) and released in response to various physiological cues such as exposure to cold and emotional reactions (49,53,57). Afterwards, TRH travels via the hypophyseal portal system in the median to the adenohypophysis (anterior pituitary gland), stimulating the output of thyroid-stimulating hormone (TSH), alternatively termed thyrotropin. The peptide TSH in turn, acts on its basal cell receptor (TSH-R) and increases the activity of iodide pump, thereby increasing iodide uptake, biosynthesis, alongside with a potentiation of secretory functions of follicular cells (47,49,52,53,57). Upon binding to the receptor, it activates adenylate cyclase which elicits an increase of intracellular cyclic adenosine monophosphate (cAMP) levels ultimately leading to dissemination of thyroid hormones (TH) (53). Negative feedback mechanisms by TH are exerted on the pituitary and hypothalamus with subsequent regulation of TSH synthesis. Even though TRH is the primary signal for TSH synthesis, a significant regulatory role is elicited by TH at the level of the pituitary gland. TSH hormones establish the set point of axis (49). Furthermore, intracellular T3 concentration is a second important determinant of TRH regulation. Other hormones including insulin-like growth factor I (IFG-I), epidermal growth factor, transforming growth factor  $\beta$  (TGF- $\beta$ ), endothelin and numerous cytokines are also

reported to have effects especially evident in other dysfunctional physiologic states on the thyroid gland (49). Moreover, external factors regulate the gene expression of TRH genes, for instance, temperature. Integrative mechanisms combine external factors and internal signals of different hormone levels and ultimately lead to an intricate system which plays an essential role in homeostasis (57).

#### **1.7.4. Hormone synthesis**

In the process of hormone synthesis, the first stage of formation is the active absorption of iodide ( $I^-$ ) by thyrocytes (follicular cells) through a basolateral sodium-iodide symporter (NIS) which is secondarily driven by a sodium-potassium adenosine triphosphate ( $Na^+/K^+$ -ATPase) pump. Low levels of iodine enhance sodium iodide symporter (NIS) expression and thereby increase the amount of iodide inside the cells. Contrary effects are elicited by high levels of iodide (49). As seen in Figure 2, it couples inward translocation of  $Na^+$  downstream its electrochemical gradient with inward translocation of  $I^-$  against its gradient. The process of concentrating iodide inside the cell is termed iodide trapping. Multiple factors influence the degree of accumulation, the most relevant being TSH concentration. Following this, the active transport of iodide into the follicular lumen via the apical ion channel pendrin, a chloride/iodide exchanger, occurs. Thyroid epithelial cells additionally transport the amino acid tyrosine in form of thyroglobulin into the follicles which serves as binding portion for iodine (53,56). Concomitantly, the glycoprotein thyroglobulin (TG) is generated and subsequently transported into the follicular lumen through the Golgi apparatus via exocytosis. This glycoprotein has no hormonal activity itself but contains tyrosine amino acids essential for synthesis of thyroid hormones (56). During the next processes, iodine is formed by oxidation of the iodide ion in the presence of membrane bound thyroidperoxidase (TPO) and hydrogen peroxide. Thereby enabling iodine to bind to tyrosine. The binding of iodine with the thyroglobulin is a process called organification catalyzed by TPO. By this mechanism, precursors mono- and diiodotyrosine (MIT and DIT) are being constructed and subsequently stored in the follicular lumen. Solely the possibility to store its hormones is a distinctive feature of the thyroid gland made possible by thyroglobulin molecules which is being able store up to 30 thyroxine molecules. These complex and intricate interrelating mechanisms are depicted and visualized for a direct understanding in Figure 2 (49,58).





**Figure 2.** Thyroid cellular mechanisms

Source: Figure 77-2: Thyroid cellular mechanisms for iodine transport, thyroxine and triiodothyronine formation, and thyroxine and triiodothyronine release into the blood. Abbreviations: DIT, diiodotyrosine; ER, endoplasmic reticulum; I, iodide ion; I<sub>2</sub>, iodine; MIT, monoiodotyrosine; NIS, sodium-iodide symporter; RT<sub>3</sub>, reverse triiodothyronine; T<sub>3</sub>, triiodo thyronine; T<sub>4</sub>, thyroxine; TG, thyroglobulin. | Hall JE, Guyton AC. Guyton and Hall textbook of medical physiology. 13th edition. Philadelphia, Pa: Elsevier; 2016. 952 p. (Student consult). [cited 2024 Feb 07]

The hormones are developed 2-3 months in advance and stored until physiological requirements prompt their release. In case of deficiencies, it might take several months for clinical effects to be noticed. Iodide from unused hormone precursors MIT and DIT is released by deiodinase and made available again to the intracellular iodide pool. In order to release stored hormones, iodinated TG is endocytosed into thyrocytes involving fluid-phase pinocytosis and receptor-mediated endocytosis (58). Afterwards, the vesicles fuse with lysosomes intracellularly of thyrocytes. Next, proteases degrade thyroglobulin thereby unbinding T<sub>3</sub> and T<sub>4</sub>. The hormones are secreted in endocrine manner into the blood. Thyroxine-binding globulin (TBG), transthyretin (TTR, prior named thyroxine-binding prealbumin or TBPA) and albumin covalently bind the hormones distributing them to peripheral tissues (49,53,56). These proteins

increase the sera concentration of hormones, postpone their clearance and regulate the hormone delivery (49). Notable findings pointed out that especially T3 shows inhibitory effects on hypothalamus and pituitary gland, participating in negative feedback loop along the HPT axis (59).

The less active form of the thyroid hormones, T4/thyroxine, is released in a much higher portion (93%) than the more potent T3 (7%). In conclusion, T4 serves as a biologically inert storage form of thyroid hormones. Eventually, thyroxine is peripherally converted by deiodinase into the biologically active T3 in the blood and cytosol of most body cells by deiodinase. Functionally, both hormones are the same, although they differ in rapidity, half-life, degree of potency and both hormones exert a negative log-linear feedback on TSH levels. In detail, if the concentration of T3/T4 is elevated, TSH is subsequently lowered and vice versa (49,53,60).

Although both hormones are involved, it has to be noted that T3 is the predominant hormone inhibiting TSH secretion (59). The relationship between serum TSH and level of free hormones is termed set point and fluctuates around this intrapersonal range. It has been found to present with a greater interindividual variability than intraindividual. This finding suggests a certain genetic influence on the thyroid hormone pathway related to D2 polymorphisms. (61).

### **1.7.5. Physiological functions of thyroid hormones**

Thyroid hormones are an integral part in normal development, growth, neural differentiation, and metabolic regulatory pathways in mammals (62). In general, they interact with an extensive abundance of signaling pathways, modulated by nutritional and iodine status, in nearly all cells of the body. At the target tissues, the hydrophobic hormones pass the cellular membrane by passive diffusion, although not solely, since they utilize organic anion-transporting polypeptide (OATPs) 1C1, mono-carboxylase 10 transporter (MCT10), and the MCT8 (located on X chromosome) for an uptake (49,62,63).

### **1.7.6. Deiodinases**

Furthermore, within the cytoplasm of peripheral target cells, deiodinases convert T4 into the much more active T3. In total the human organism has three distinct types D1-D3 (64). Their principal mechanism is to convert thyroid hormones into active or inactive metabolites. Local conversion of circulating T4 occurs by type 2 deiodinases (D2) located on the endoplasmic reticulum (ER) near the nucleus into the active metabolite T3. Cells with an

enhanced expression of D2 potentiate local signal transduction by thyroid hormone and thereby energy expenditure. D3 on the other hand, converts T3 into the inactive reverse T3. Moreover, D3 mediates the conversion of T3 into the inactive form T2. In other words, it terminates the action of thyroid hormone (60,62). These deiodinases play a principal role in expression of genes and shift the focus of systemic modulation by thyroid hormone to a cellular, local level. It has been found in a study by Andersen *et al.* that in patients without any thyroid diseases their hormone levels seldom fluctuate. Therefore, the expression of deiodinases, either D2 with its stimulatory or D3 with an inhibitory effect, regulate cellular processes independently to thyroid hormone levels. In conclusion, cells individualize and customize by these mechanism the effects of thyroid hormone according to their homeostatic state (60,61). As an additional mechanism of control, the various types of deiodinases have a highly different tissue expression. Thyroid, liver, and kidneys express primarily type I enzymes. On the other hand, Type 2 is found to a higher degree in the pituitary gland, cerebrum, brown fat adipose tissue, as well as the thyroid gland (49).

Subsequent to the deionization processes, T3 acts on nuclear thyroid hormone receptors (TRs) enhancing or inhibiting gene transcription and thereby participating in various metabolic and ionic cycles, and increased mitochondrial respiration which all lead to an accelerated energy expenditure (49,53,60,65). In this framework, thyroid hormone receptor and Retinoid X Receptor form heterodimeric complex and in combination act at designated response elements located on the DNA double helix (49,53). There exist several nuclear receptor isoforms of the thyroid hormone receptor (TR) which are activated upon binding. These isoforms, TR $\alpha$  and TR $\beta$  with their respective subcategories, have a varying degree of expression in tissues which ultimately generate corresponding physiological changes (50,62). It has to be mentioned that the TR isoforms not only recognize thyroid hormones but also interact with several other transcriptions factors. This poses an important interaction between multiple metabolic pathways (51).

Furthermore, it has been found that also nongenomic cellular effects are exerted by T3 as well as T4. Among these effects an involvement in the process of angiogenesis and formation of endothelial nitric oxide synthase (eNOS) have been reported concomitantly, with effects on muscle function (66,67). The site of nongenomic outcomes concerns plasma membranes, cytoplasm, along with mitochondria and other cell organelles (53).

The interaction of T3 with the nuclear receptor and transcription of proteins result in a myriad of clinical effects in different organ systems which become enhanced or suppressed in different thyroid pathologies (60).

### **1.7.7. Diagnostics**

Laboratory measures are a widely accessible approach with TSH in serum being the first-line screening test in the majority of patients with a suspected thyroid problem. Concomitantly, T3 and T4 values aid in assessment especially if they are used to distinguish between overt and subclinical hyperthyroidism. Furthermore, their sera level can aid to distinguish between primary and secondary diseases and if an autoimmune etiology is suspected, the thyroid autoantibodies directed against TG, TPO and or TSH receptor respectively, should be evaluated (53,68). Additionally, thyroid function can be evaluated using multiple diagnostic approaches. Sonography, scintigraphy, elastography, thyroid suppression tests, and fine-needle aspiration are possible *in vivo* methods and are implemented depending on indications, clinical presentation and personal history of patients (69).

### **1.7.8. Ascertainment of the functional dynamism of the thyroid gland**

The assessment of homeostasis of thyroid function and the proficiency of conversion of the hormones can not only be accomplished by the quantification of TSH, fT3, fT4, and by the ratio of fT3 to fT4 (denoted as the T3/T4 ratio) (70). However, individual variations remain a challenge in diagnostics. Especially, the setpoint of equilibrium between TSH and fT4, representing the equilibrium within the HPT axis, is highly individual. This setpoint can be modulated by the aforementioned feedback mechanisms when external synthetic hormones are supplemented and mutations are considered (70). In addition, a varying degree of deiodinase activity based on polymorphisms play a role (70,71). In 2016 Dietrich *et al.* (72) published research in which they developed advancements in this regard. The article presents formulas based on a structure parameter interference approach (SPINA). Further constructed fundamental properties of the thyroid gland can be calculated, including secretory capacity of the thyroid gland (SPINA- GT), sum activity of peripheral deiodinases, especially 5'-deiodinase (SPINA- GD), and Jostel's TSH index (TSHI) for an estimation of thyrotropic pituitary function. These algorithms enable a holistic approach and distinction of euthyroid state and a pathological level of hormones (72).

## **1.8. Pathophysiology of the thyroid gland**

### **1.8.1. Hypothyroidism**

Hypothyroidism is a common endocrine disorder with a deficiency of thyroid hormone. The condition is in general more frequently observed in people older than 65 years and in association with autoimmune diseases including diabetes mellitus type 1, autoimmune gastritis atrophy, as well as constituent of endocrinopathies (73).

Per definition, a primary endocrine disease is originating at the level of the gland itself. In case of hypothyroidism the thyroid hormone deficiency is rooted in synthesis or release by the thyroid gland. If the adenohypophysis produces low levels of TSH, due to an interconnection along the HPT axis, a lack of stimulation of thyroid follicular cells follows causing secondary thyroid disorders. In this context, tertiary endocrine disorders are located even higher at the level of the hypothalamus with an insufficiency of TRH. Peripheral forms such as an anomalous expression of deiodinase 3 are as scarce as central forms of hypothyroidism which are comprised of secondary and tertiary etiologies simultaneously. The latter pathophysiologic disorder is in half of the cases induced by a pituitary adenoma which presents with low or lowered TSH concentrations in the serum, alongside with a low concentration of thyroxine. Other origins of central forms include, for example, dysfunctions of pituitary gland or hypothalamus by trauma to the head and Sheehan's syndrome (73,74).

Additionally, other factors can lead to different forms of hypothyroidism like the chronic autoimmune inflammation known as Hashimoto's thyroiditis which is later described in more detail. Iodine overloading might occur during the treatment with amiodarone or other iodine-containing medications, which lead through the so called Wolff-Chaikoff effect to a blockage of thyroid iodide organification to a subsequent inhibition of hormone synthesis by the thyroid gland and ultimately to hypothyroidism (49,73,75).

The clinical presentation of hypothyroidism is often overt and especially elderly do not present with typical symptoms (73). Independent of the etiology the physiological effects are the same. Neonatal screening is obligatory in countries of the Global North and performed within first three days following birth to detect any congenital forms of hypothyroidism (49). Most commonly patients present with increased fatigue, lethargy and somnolence due to a decreased metabolic rate. Circulatory changes include a decreased heart rate with diminished cardiac output and lowered blood volume. Moreover, symptoms such as depression, decreased peripheral reflexes, entrapment syndromes and constipation are consequences due to the lack of activated thyroid hormone on various tissues and organs of the body. Weight gain is most

often related to the fluid retention in myxedematous interstitial spaces (49,53). One of the complications in patients with a total lack of thyroid hormone function is the development of a generalized edematous state termed myxedema. In this state, the total quantity of interstitial fluid increased by an augmentation of hyaluronic acid and chondroitin sulfate (53). Furthermore, a rise of cholesterol levels resulting from altered fat and cholesterol metabolism lead to an increased risk for atherosclerosis and its sequelae such as coronary artery disease (53,76).

### **1.8.2. Hyperthyroidism**

As for hypothyroidism, the same distinction based on the anatomic location of disturbance is made in hyperthyroidism. The primary form of hyperthyroidism increased concentrations of T3 and T4 are produced which suppress through negative feedback inhibition TSH secretion from the anterior pituitary. Furthermore, in secondary hyperthyroidism, the anterior pituitary produces large amounts of TSH, which, in turn, stimulate the thyroid follicular cells to secrete thyroid hormones in excessive amounts.

An important conceptual distinction to be made is the following: An increased synthesis and secretion of thyroid hormone is referring to hyperthyroidism, which can be overt or subclinical. On the other hand, thyrotoxicosis entails an elevation of thyroid hormones in serum, unrelated to etiology (77).

Clinical symptoms of hyperthyroidism with increased secretion of thyroid hormones present as an augmentation of their physiological effects. For example, some of the patients often complain of anxiety, sleeping disturbances and diarrhea disrupting their everyday life leading to permanent exhaustion. Constitutional symptoms such as hyperhidrosis, heat intolerance, and polydipsia are caused by an increased basal metabolic rate. Cardiovascular manifestations are also reported by patients include palpitations due to an increased resting heart rate and cardiac output (53).

### **1.8.3. Autoimmunity and autoimmune thyroid diseases (AITD)**

Immunologic tolerance is defined as an unresponsiveness toward antigens, and it represents a fundamental principle to recognize self-antigens. In case of autoimmunity, the immune system is failing to differentiate between cells of the host and autologous antigens (78,79). In more detail, the disbalance of effector and regulatory immune responses are caused by a defective elimination and inability to control self-reactive lymphocytes (80). Subsequently,

pathogenic inflammatory reactions occur inside of tissues (organ-specific) or several organ systems (non-organ-specific) (79). As previously mentioned, a variety of factors can contribute to a failure of self-tolerance and the mechanisms are quite complex. A combination of multiple hereditary susceptibility genes, human leukocyte antigen (HLA) linkage as well as environmental variables, for example, infections, Vitamin D or selenium deficiencies has been named as causation (68,73,78,79).

Research supported a multitude of AITD entities all with a wide range of symptoms and clinical presentations which makes a diagnosis more challenging. A significant role in those intricate AITDs represent major histocompatibility complex (MHC) class II genes which encode the HLA glycoproteins. Several associations of HLA with different clinical pictures have been detected and other autoimmune conditions such as polyendocrine autoimmune syndrome, diabetes type 1, pernicious anemia and mood disorders are linked to the occurrence of AITDs (68,81). However, even with profound insights into molecular patterns, genetic tools which enable clinical prediction of risk for development of any autoimmune conditions have yet to be constructed (82). The principal antigens in autoimmunity are TG, TSHR, and TPO. Some minor antigens which have been detected include the sodium/iodide symporter (NIS) and other various cell components for example pendrin (68,79,83). The subsequent formation of antibodies due to a multitude of etiologies cause an interconnected involvement of humoral, cell-mediated and complement pathways (83). The presence of antibodies to each of the antigens and their concentration in sera relative to each other aids in differentiation between those entities previously mentioned alongside with clinical, and imaging findings (79).

First and foremost, Hashimoto's thyroiditis is presenting with a decreased function of thyroid gland in the setting of autoimmunity. It is the most common AITD in epidemiological regions with sufficient Iodine concentrations and characterized by lymphocytic infiltrations (49). Immunologic reactions are associated with TPO-antibodies as well as anti-TG antibodies (73,79). Furthermore, these trigger a destruction of the gland, although it has to be mentioned that the exact pathophysiological mechanisms are not known up to this day. In the thyroid parenchyma, lymphocytic infiltration leading to recurrent inflammation, fibrosis, and subsequently parenchymal atrophy has been described. These changes are dominated by humoral antibody mediated responses which elicit a progressive deterioration concomitantly with a decrease in thyroid hormone levels (53,79). The inability to synthesize thyroid hormone is causing an enlargement of the endocrine gland in an attempt to compensate and initially, the patients are asymptomatic and present in later stages with more pronounced organ destruction. In 90% of the patients TPO- antibodies are present in their sera, along with TG-Antibodies

(positive in 50% of patients) (68,79).

The most common form associated with hyperthyroidism is Graves' disease (GD) which represents the second major autoimmune entity of the thyroid gland. Etiologic studies discovered a multifactorial contribution, including decline or loss of immunologic tolerance with a combination of external as well as genetic factors. In this setting a generation of IgG-thyroid-stimulating immunoglobulins (TSIs) against TSH-receptor occurs and causes upon binding three different effects. The first and most frequently with GD associated configuration can induce a continuous activation of cAMP messenger system ultimately leading to an attenuated hormone production (49,53,77,79,84). Alternatively, blockers or Abs with neutral effects can be generated but have a role of lesser importance in GD (79). Etiologically, HLA-DR genes play a significant role in development of GD (81). Next to the typical vegetative and metabolic clinical presentation specific for Graves' the Merseburger Trias has been constructed. In this constellation an enlarged gland, tachycardia as well as exophthalmos are seen (85). The endocrine orbitopathy is seen in hyperthyroidism independent of etiology. In detail, the pathophysiological mechanism is instigated by TSH-receptor antibody activation of T cells and additionally stimulates fibroblast proliferation with concomitant accumulation of glycosaminoglycans in the extraocular muscles and retroocular connective tissue. As a consequence, edematous swelling and degenerative changes of extraocular muscles develop which ultimately facilitate a proptosis of the eyes with diplopia (53,77). TG has been detected in patients with thyroid ophthalmologic involvement which could lead to a cross-reaction in GD since the presence of TG has not been confirmed in healthy individuals (68). Other, much rarer symptoms in GD include extrathyroidal dermatopathy located in the pretibial areas as well as acropachy (77).

Multiple variations of AITDs have been reported to exist including transient entities only present during pregnancy or post-partum. Other forms are subclinical presentations of hypothyroidism or neonatal forms of both and they might be variants of the principal AITD illustrated prior. These syndromes are all interconnected by their similarities in pathology, immune reactions, hereditary clustering, alongside with an intrapersonal transition from one clinical picture into another (68,83).

In conclusion, measuring the concentrations of Abs aids in etiological differentiation of possible entities of AITDs. Additionally, they can be used for prognosis and prevention of multiple complications arising from surplus or deficiency of thyroid hormones. It is essential as a clinician to be aware of the subtypes and wide variety of entities AITDs can present themselves (79).



## **1.9. Conjunction**

Following the introduction of both topics, their interconnectedness caused by viral effects on the thyroid gland/thyroid-related diseases, the respective changes and clinical consequences of a SARS-CoV-2 infection have become increasingly relevant. Those dependencies and chain of causalities have not been unraveled in entirety. First of all, the significance was evident in light of multi-organ involvement leading to cardiovascular system, central nervous system, metabolic system, kidney and liver-related symptoms as well as a late-engagement of the endocrine system with subsequent effects (30,86,87). Especially the pituitary-thyroid axis is considered to be a susceptible structure with direct and indirect damage resulting in secondary hypothyroidism (88).

### **1.9.1. Hypothesis and theories of pathogenesis of SARS-CoV-2 in relation to thyroid gland**

In accordance with in-depth- insights of SARS-CoV-2 pathogenesis, various theories were established as to how the thyroid gland could be affected. One proposed mechanism is by receptor expression. As previously stated, the virus causing COVID-19 attaches with RBD-S1-subunit to the transmembrane carboxypeptidase ACE2 and enables membrane fusion and entry of viral particles into cells (86,88). Furthermore, the serine protease TMPRSS2 is necessary for entry. Therefore, both entities need to be present on cells for an infection to occur. Based on this, tissue distribution and abundance of ACE 2 would determine the pathological effects of SARS-CoV-2. A multitude of endocrine tissues are expressing ACE2 receptors, especially important in light of the focus of this thesis are the centrally located hypothalamus, pituitary, and thyroid gland (89). Rotondi *et al.* identified a high concentration of messenger RNA (mRNA) for ACE-2 receptors inside thyroid follicular cells (90).

A second hypothesis for endocrine involvement has been proposed to be the activity of systemic immune reactions as the infection activates innate as well as adaptive immunity (33,91). Thyroid hormones trigger cytokine production and potentiate the antiviral activity of IFN- $\gamma$ . This could provide an explanation for cytokine and hyperactivity of T1-helper cells (90).

Thirdly, direct cytotoxic effects are exerted by the virus ultimately lead to the cytokine storm which in turn induces NTIS and transient pituitary dysregulation. The latter is caused by direct cytotoxic effects of the virus on the pituitary gland (91).

Taking the thyroid gland with its function into consideration, the virus could have significant effects on its hormone synthesis and secretion and therefore, on a multitude of

physiologic pathways in the organism (88).

Previously conducted studies that focused on the thyroid gland reported a diminished thyroid gland and HPT axis function. Thyroid-related diseases which are hypothesized to be triggered by COVID-19 include the following: Subacute thyroiditis, Non-thyroidal illness syndrome (NTIS), thyrotoxicosis, Hashimoto's thyroiditis, Grave's disease (86).

As previously established, the influence of thyroid hormones and involvement in various physiological mechanisms is a highly complex matter with dysregulations leading to several clinical consequences (87).

## **2. OBJECTIVES**

## **2.1. Aims of the study**

The aim of this study is to investigate to which degree a thyroid dysfunction represented by thyroid hormones and subsequent inflammatory state exacerbate hormone deficiency or oversupply in patients with an active SARS-CoV-2 infection. This study therefore investigates the prevalence of patients with abnormal thyroid parameters (outside the range of reference). Furthermore, the association of COVID-19 infection on thyroid gland secretion based on laboratory parameters of routine clinical data was analyzed and assessed by SPINDA GD model.

## **2.2. Hypothesis**

1. Patients with an active infection of SARS-CoV-2 exhibit thyroid dysfunction in form of alterations in thyroid parameters, namely TSH/ T3/ T4 levels, measured upon admittance to the hospital as later assessed by the SPINA-GD model.
2. The severity and degree of thyroid dysfunction in connection with the thyroid hormones is more pronounced following a COVID-19 infection.
3. A positive or negative association of COVID-19 infection on thyroid gland secretion based on laboratory parameters of routine clinical data could be present.
4. A preexisting state of AITDs, namely Hashimoto's thyroiditis and Graves' disease, are considered to be more likely connected with a more severe change in thyroid hormone levels and assessed by SPINA- GD model.

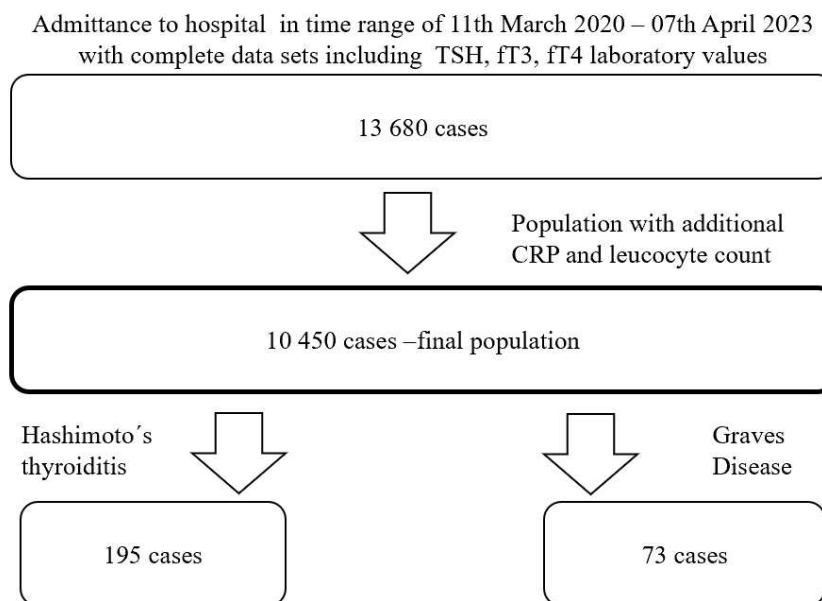
### **3. SUBJECTS AND METHODS**

### 3.1. Ethical approval

This retrospective study earned its approval by the IRB of the Medical School Regiomed Coburg on 15.02.2024.

### 3.2. Study design

For this retrospective observational study, routine clinical data between the 11<sup>th</sup> March 2021 (Date the pandemic was declared by WHO) and 7<sup>th</sup> April 2023 (official end of pandemic in Germany) of patients admitted to the REGIOMED Hospital Coburg, Upper Franconia region in Bavaria, Germany (single-site) were included. Recruitment and case selection was based on the following inclusion and exclusion criteria. As seen in Figure 3 the entirety of data included 13 680 cases of which in total 10 450 cases met the following mentioned inclusion and exclusion criteria. 195 of those cases were also positive for Hashimoto's Thyroiditis. On the other hand, 93 were included based on their ICD code in the group for Grave's disease.



**Figure 3.** Recruitment and Overview of selection process

Data presents the selection process, created by author

### **3.3. Inclusion and exclusion criteria**

For this study to be included the patients had to be at least 18 years old at the time of admission. Furthermore, it was required that patients had their laboratory values (TSH, T3, T4, CRP and leukocytes) measured. If multiple laboratory values were available, the one closest to admission was used for statistical analysis. Only patients which were being treated at the REGIOMED Hospital Coburg with a proven SARS-CoV19 (ICD code ICD U07.1 “COVID-19, Virus nachgewiesen”) infection were included independent of the severity of their respiratory symptoms. SARS-CoV2 detection was performed by real time PCR (RT-PCR) if a previous rapid antigen test was positive.

Further inclusion criteria for the subgroups included subjects with an already diagnosed Hypo- or Hyperthyroidism, only in form of the AITDs Hashimoto’s thyroiditis and Grave’s Disease. Using their respective ICD codes E06.3 (Hashimoto’s thyroiditis) and E05.0 (Grave’s Disease) the data bank Orbis (Orbis®, Dedalus Healthcare, Bonn, Germany) has been searched. This method of selection was used in order to ensure a definite diagnosis. Reason being that autoantibodies directed towards components of the thyroid gland would only be positive in 90-95% of patients with an active AITD. Additionally, in some cases they are not measurable in every phases of the disease (92). There was not a standardization of antibody titers are considered pathological and could appear in normal physiologic conditions and reference ranges vary.

Exclusion criteria are incomplete data sets in form of missing laboratory variables or data sets that are no longer traceable. Patients with co-morbidities have not been excluded. No distinction has been made for patients with pregnancy or any malignancy.

### **3.4. Data collection**

Over the course of this study, the databank of REGIOMED Hospital Coburg was searched. Thereby, patients with a SARS-CoV19 diagnosis (ICD U07.1 “COVID-19, Virus nachgewiesen”) and their thyroid hormone levels of ft3 and ft4, and TSH levels are being assessed. The values are derived from blood samples taken upon the patients’ admission by trained personnel of the hospital. The data was already available and taken from the laboratory system as well as their cooperative partner Synlab. Furthermore, patients with the already established diagnosis of hypo -and hyperthyroidism, namely Hashimoto’s thyroiditis and Grave’s disease were included. The entirety of cases has been anonymized.

### 3.5. Laboratory analysis and reference values- definitions

Upon admittance to the hospital during the COVID-pandemic it was required to take a swab for an antigen test, regardless if patients showed respiratory symptoms. In case of a positive test result, a PCR test for SARS-CoV19 was performed. Measurements of TSH, fT3 and fT4 were done by the laboratory of REGIOMED hospital Coburg with their respective machinery.

### 3.6. Statistical analysis

The statistical analysis was performed using RStudio (Version2023.09.1+494, Posit Software, PBC). For building figures, and graphs Microsoft Excel for Windows was being used. The calculation of the SPINA GD, which denotes the sum activity of peripheral deiodinases, was conducted by using the formula provided by Dietrich et al. (72). According to the units given it has been converted into the following formula:  $SPINA\ GD = ((8 \cdot 10^{-6}) \cdot (500 + (FT4 \cdot 10^{-12})) \cdot (1 + ((2 \cdot 10^9) \cdot 300) \cdot (FT3 \cdot 10^{-12}))) / (0,026 \cdot (FT4 \cdot 10^{-12}))) \cdot (10^{-9})$ .

The reference values for SPINA GD are predetermined by the essay used and typically set between 20-60 nmol/L (72). The prevalence analysis includes a summary of all metric and non-metric variables in their common descriptive form. The 95%- confidence interval was calculated with the exact binominal test by Clopper & Pearson (1934). To research an association between an infection with SARS-CoV19 in patients with comorbidities an analysis of variants using ANOVA has been implemented as well as a post-hoc testing with Bonferroni adjustment for p-values. The statistical significance of p value was set at <0.05.



## **4. RESULTS**

In this study, a total of 10 450 cases are included. Table 1 presents multiple characteristics of the sample population. The characteristics are divided into the categories age, sex, TSH, fT3, fT4, c-reactive protein (CRP), leukocyte count, and diseases including CoV-19, Hashimoto's thyroiditis and Grave's disease. The median age of the population is 71 years, with an interquartile range (IQR) from 59 to 81 years. The gender ratio is almost balanced with 52% females and 48% males. Furthermore, 1.9% of the cases are with Hashimoto's thyroiditis. Only 73 patients or 0.7% of the population has a proven Grave's disease. Within the given time frame 512 people of the sample population have an active COVID-19 infection.

**Table 1.** Baseline characteristics of the entire sample (n=10 450)

Characteristics		n (%) [95%-CI]
Age (Years)	mean±SD	68.31±16.35
	Median (Q1 - Q3)	71 (59 - 81)
	≥ 65	6871 (65.8) [64.9 - 66.7]
SEX	female (n)	5385 (52.0) [50.5 - 52.4]
TSH (μU/ml)	mean±SD	3.71±6.58
	> 4.2	3719 (35.6) [34.7 - 36.6]
	< 0.27	1760 (16.9) [16.1 - 17.6]
fT3 (pmol/l)	mean±SD	3.79±1.25
	> 6.0	302 (28.9) [25.8 - 32.3]
	< 2.43	1235 (11.8) [11.2 - 12.5]
fT4 (pmol/l)	mean±SD	17.14 ±4.20
	> 19.1	2811 (26.9) [26.1 - 27.8]
	< 9.1	200 (1.9) [1.7 - 2.2]
CRP (mg/l)	mean±SD	38.75±67.41
	> 5.0	6141 (58.8) [57.9 - 59.8]
Leucocytes (10 <sup>3</sup> /μl)	mean±SD	9.33±10.98
	> 10.9	2440 (23.4) [22.6 - 24.2]
SPINA-GD (nmol/s)	mean±SD	31.27±13.83
Diseases	covid infection confirmed (n)	512 (4.9) [4.5 - 5.3]
	Hashimoto (n)	195 (1.9) [1.6 - 2.1]
	Graves (n)	73 (0.7) [0.5 - 0.9]

\*Data are presented as mean±standard deviation or as number (%)

In Table 2, groups are divided into patients with either a confirmed COVID infection (“COVID YES” n=512) and on the other hand with no proven COVID infection (“COVID NO” n=9938). In both groups the distribution of their respective characteristics is evaluated in terms of age, TSH, fT3, fT4, and CRP and SPINA GD values. The data is presented with mean and standard deviation alongside the calculated *P*-values. There were significant differences in age, TSH, fT3, fT4, CRP, and SPINA GD between patients with and without COVID-19 (*P*-values < 0.001). In greater detail, the mean age is seen to be slightly higher in patients who were proven to be tested positive for COVID-19 (72.06 years vs. 86.11 years, *P* < 0.001). Patients with a confirmed COVID-19 infection had lower TSH levels (3.01 μU/ml) in comparison to those with no infection (3.74 μU/ml). Furthermore, fT3 and fT4 values are varying between both groups. fT3 (μU/ml) levels are statistically significant lower in patients with a COVID-19 infection than those without (3.14 μU/ml vs. 3.82 μU/ml, *P* < 0.001). The same applies to fT4 (μU/ml) levels. They are significantly lower in patients who had contracted the infection (16.49 μU/ml) than those who belong to COVID NO group (36.71 μU/ml). As in the previous case, this result is statistically significant (*P* < 0.001). Also, CRP levels (mg/l) are observed to be higher in patients with an active infection (78.12 mg/l) than in patients without the infection (36.71 μU/ml). Similar to the previous characteristics, SPINA GD values in those with the confirmed infection (28.35 nmols/s) are lower in comparison to the COVID NO group (31.42 nmols/s), with a statistically significant difference (*P* < 0.001). On the other hand, leucocytes (10<sup>3</sup>/μl) are the only characteristic of this group which are not seen to be significantly different between the two groups (*P* = 0.5).

Additionally, it is seen that the group with a proven COVID- 19 infection has a higher prevalence of patients with TSH levels < 2.43 μU/ml in the COVID YES group (65.2%) compared to patients with no proven-COVID infection (56.2%). These differences are statistically significant (*P* < 0.001, Table 2). Other characteristics including Hashimoto’s thyroiditis, Grave’s disease, abnormal TSH levels (TSH < 2.43 & > 4.2), abnormal FT3 levels, and abnormal FT4 levels, are not shown to have a statistically significant difference between the two groups (*P* > 0.05). Going into greater detail, it can be seen that the prevalence of Hashimoto's thyroiditis is similar between the two groups (1.8% vs. 1.9%) with no statistically significant difference (*P* = 0.866). Furthermore, the prevalence of Grave’s disease is lower in COVID-19 patients (0.2%) compared to NO\_ COVID-19 patients (0.7%). As in the previous case, this difference is not statistically significant leading to the conclusion that no difference between the groups has been detected (*P* = 0.268). Additionally, no statistical significant results are seen in relation to the prevalence of abnormal TSH levels (< 2.43 & > 4.2, *P* = 0.396). The

prevalence of abnormal FT3 levels is lower in COVID-19 patients (1.8%) in comparison to NO\_COVID (3.0%). The difference was not statistically significant ( $P = 0.136$ ). Moreover, abnormal FT4 levels were similar between the two groups (28.8% vs. 27.7%).

**Table 2.** Structured breakdown of group characteristics

Characteristics	Groups (mean)		P-value
	COVID NO (n = 9938)	COVID YES (n = 512)	
Age (Years)	68.11	72.06	t(579.88) = -5.929; $P < 0.001^*$
TSH ( $\mu\text{U/ml}$ )	3.74	3.01	t(608.91) = 3.185; $P < 0.001^*$
fT3 (pmol/l)	3.82	3.14	t(587.92) = 14.196; $P < 0.001^*$
fT4 (pmol/l)	17.17	16.49	t(567.1) = 3.622; $P < 0.001^*$
CRP (mg/l)	36.71	78.12	t(540.92) = -10.542; $P < 0.001^*$
Leukocytes ( $10^3/\mu\text{l}$ )	9.54	9.54	t(641.34) = -0.659; $P = 0.5^*$
SPINA GD (nmols/s)	31.42	28.35	t(655.89) = 7.4868; $P = < 0.001^*$
<b>Prevalences (%) [95%-CI]</b>			
Hashimoto (n)	185 (1.8) [1.6 - 2.1]	10 (1.9) [0.9 - 3.6]	0.8663 †
Basedow (n)	72 (0.7) [0.6 - 0.9]	1 (0.2) [0.0 - 0.4]	0.2678 †
TSH < 2.43 & > 4.2	9168 (92.3) [91.7 - 92.7]	478 (93.3) [90.8 - 95.4]	0.3955 †
FT3 < 0.27 & > 6.0	294 (3.0) [2.6 - 3.3]	9 (1.8) [0.8 - 3.3]	0.1359 †
FT4 < 9.1 & > 19.1	2870 (28.8) [28.0 - 29.8]	142 (27.7) [23.9 - 31.8]	0.6169 †
TSH < 2.43	5590 (56.2) [55.3 - 57.2]	334 (65.2) [60.9 - 69.3]	< 0.001 †

other combinations not significant

\* Welch Two Sample t-test

† Fishers-Exact Test

In Table 3, the independent variable (TSH values) is set in correlation onto several models each with a different confounder. Model 1 describes that having a COVID-19 infection increases the odds of the outcome by 46% (OR =1.46 (95%-CI: 1.21 - 1.76) with  $P < 0.001$ . Concomitantly, each additional year of age significantly increases the odds by 1% (OR = 1.01 (95%-CI: 1.00 - 1.01),  $P < 0.001$ ). The third independent variable in this model is the sex with 1.21, 95% CI [1.12 - 1.31], and  $P < 0.001$ . This demonstrates being male significantly increases the odds by 21% compared to being female. These findings were consistent across all models.

In terms of model performance, the R<sup>2</sup> is quite low across all models (0.002 for Model 1, and 0.015 for models 2, 3, and 4), therefore only explaining a small portion of the variance in the outcome. Furthermore, the Brier Score is similar across all models (0.245 for Model 1, and 0.243 for models 2, 3, and 4), suggesting that the models' predictive performance is consistent but limited.

In summary, COVID-19, age, and sex (male) are significant predictors of the outcome in all models, with COVID-19 patients having higher odds of the outcome. CRP and Leucocytes are not significant predictors. The models explain a small proportion of the variance (low R<sup>2</sup> values) and have similar Brier Scores, indicating the models' predictive performance is consistent but limited.

The most prevalent findings of this calculation indicate that a past COVID-19 infection increases the likelihood of a reduced TSH value (below the reference) by 40% (OR 1.40 [95%-CI: 1.16 – 1.69]) following an adjusting for CRP and leukocyte count.

**Table 3.** Results of logistic regression models (showing only significance for COVID disease)

Independent variable	Confounder (OR) [95%-CI]	model 1	model 2	model 3	model 4
TSH *	COVID (ref=no)	1.46 (1.21 - 1.76)	1.39 (1.16 - 1.68)‡	1.40 (1.16 - 1.69)†	1.40 (1.16 - 1.69)†
	Age		1.01 (1.00 - 1.01)§	1.01 (1.00 - 1.01)§	1.01 (1.00 - 1.01)§
	SEX (ref= female)		1.21 (1.12 - 1.31)§	1.21 (1.12 - 1.31)§	1.21 (1.12 - 1.31)§
	CRP			0.99 (0.99 - 1.00)	0.99 (0.99 - 1.00)
	Leucocytes			0.99 (0.99 - 1.00)	0.99 (0.99 - 1.00)
	R <sup>2</sup>	0.002	0.015	0.015	0.015
	Brier- Score	0.245	0.243	0.243	0.243

\* TSH < 0.27 µU/ml

† P-value <0.01

‡ P-value <0.001

§ P-value <0.001

Table 4 presents the results of an ANOVA test conducted across four different subgroups. The mean TSH levels across different subgroups related to COVID-19 and Hashimoto's disease status are compared. Mean TSH levels vary among the subgroups. The highest mean TSH level had been observed in the "COVID & Hashimoto" subgroup (7.53), while the lowest mean TSH level is seen in the "COVID & No Hashimoto" subgroup (2.92). The "No COVID & Hashimoto" subgroup has a mean TSH level of 4.89, which in comparison to subgroup "No COVID & No Hashimoto" subgroup (3.72).  $F=0.5$  suggests that there is a small ratio of the variance between the groups. The differences in mean TSH levels are not proven to be statistically significant ( $P= 0.495$ ). Therefore, the observed differences are likely due to random chance rather than a true effect of the conditions (SARS-CoV-2 infection and Hashimoto's disease).

**Table 4.** Analysis of Variances (ANOVA) in subgroups

Subgroup	n	TSH mean*	ANOVA-Result
no COVID & no Hashimoto	9753	3.72 (6.62)	F(1,10448) = 0.5: P = 0.495
COVID & no Hashimoto	502	2.92 (4.85)	
no COVID & Hashimoto	185	4.89 (8.01)	
COVID & Hashimoto	10	7.53 (8.77)	

\* Standard deviation [SD]

Following, an ANOVA test with SPINA GD as quantitative variable in association with Hashimoto and COVID infection had been performed with the results listed in Table 5. It can be seen that mean SPINA-GD levels vary among the subgroups. The highest mean SPINA-GD level is observed in the "No COVID & Hashimoto" subgroup (33.86), while the lowest mean SPINA-GD level is in the "COVID & Hashimoto" subgroup (26.7). The "No COVID & No Hashimoto" subgroup has a mean SPINA-GD level of 31.38, which is higher than the "COVID & No Hashimoto" subgroup (28.38). There is a significant ratio of variance between the groups to the variance within the groups  $F(3,10446) = 10.03, P < 0.001$ . These findings are reflecting a significant difference in mean SPINA-GD-levels.

**Table 5.** Analysis of Variances (ANOVA) in subgroups for SPINA GD in patients with Hashimoto’s thyroiditis

Subgroup	n	SPINA GD mean [SD]	ANOVA-Result
no COVID & no Hashimoto	9753	31.38 (10.89)	F(3,10446) = 10.03: p < 0.001
COVID & no Hashimoto	502	28.38 (9.047)	
no COVID & Hashimoto	185	33.86 (14.73)	
COVID & Hashimoto	10	26.7 (6.469)	

\* Standard deviation [SD]

Since the ANOVA of the previous subgroups was significant, a post-hoc analysis followed suit with their results presented in Table 6. The analysis revealed significant differences in SPINA-GD levels between several subgroups. Hashimoto's thyroiditis does not significantly impact SPINA-GD levels in patients who have COVID-19 ( $P = 1.000$ ).

**Table 6.** Post hoc- analysis in subgroups for SPINA GD in patients with Hashimoto’s thyroiditis

	NO_covid_NO_Hashimoto*	NO_covid_YES_Hashimoto*	YES_covid_NO_Hashimoto*
NO_covid_YES_Hashimoto	0.092 <sup>†</sup>		
YES_covid_NO_Hashimoto	< 0.001 <sup>†</sup>	< 0.001 <sup>†</sup>	
YES_covid_YES_Hashimoto	1.000 <sup>†</sup>	0.661 <sup>†</sup>	1.000 <sup>†</sup>

\*post-hoc analysis

<sup>†</sup>  $P$ -value <0.001

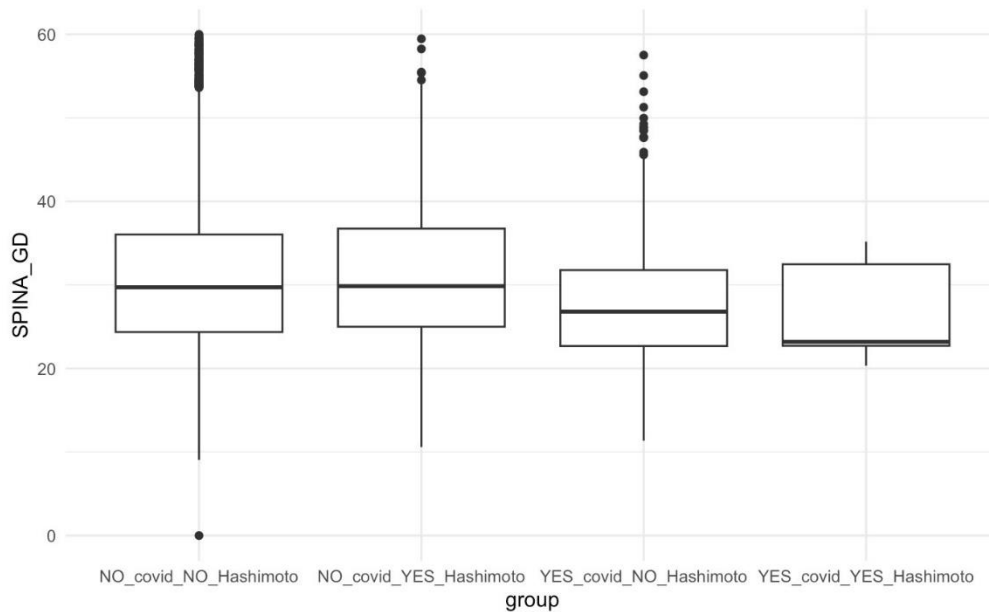
The post hoc analysis using Cohen's D reveals that there are significant differences in SPINA-GD levels between the subgroups as presented in Table 7. The comparison between "YES COVID & NO Hashimoto" and "NO COVID & NO Hashimoto" shows a statistically significant small to medium effect size (Cohen's  $D = 0.258$ ,  $P < 0.001$ ). The comparison between "YES COVID & NO Hashimoto" and "NO COVID & YES Hashimoto" shows a statistically significant medium effect size (Cohen's  $D = 0.334$ ,  $P < 0.001$ ). These results suggest that COVID-19 has a notable impact on SPINA-GD levels.

**Table 7.** Post-hoc analysis in subgroups for SPINA GD based on Cohens D in patients with Hashimoto’s thyroiditis

Effects size (Cohens D) between groups		Cohens D	p-value
YES_covid_NO_Hashimoto	NO_covid_NO_Hashimoto	0.258	< 0.001
YES_covid_NO_Hashimoto	NO_covid_YES_Hashimoto	0.334	< 0.001

The box-plot graph visualizes the data distribution of the dataset (Figure 4). The four different groups are represented on horizontal plot. In this setting, each box plot represents the distribution of SPINA- GD levels within their respective combination. For visualization purposes the upper limit was set at SPINA GD values of 60 nmol/s. The first subgroup "NO COVID & NO Hashimoto" had the highest median SPINA-GD level (30 nmol/s) and a relatively wider IQR, indicating a more varied distribution of SPINA-GD levels. Secondly, the "NO COVID & YES Hashimoto" subgroup showed a slightly higher median SPINA-GD level (32 nmol/s) and a similar IQR to the first group, suggesting that the presence of Hashimoto's disease in non-COVID patients slightly increases SPINA-GD levels. The "YES COVID & NO Hashimoto" subgroup presented with a lower median SPINA-GD level (25 nmol/s) with a narrower IQR. This indicates lesser variability and a significant reduction in SPINA-GD levels compared to non-COVID groups. The "YES COVID & YES Hashimoto" subgroup also has a median SPINA-GD level of approximately 25 and a similar IQR to the previous group, suggesting that the combined presence of COVID-19 and Hashimoto's disease results in lower SPINA-GD levels, comparable to the COVID-only group. Most prevalent is the finding that COVID-19 is associated with lower SPINA-GD levels, regardless of the presence of Hashimoto's thyroiditis. Non-COVID groups have higher SPINA-GD levels, with the presence of Hashimoto's disease slightly elevating these levels. The presence of outliers in each group suggests some individual variability. This visualization complements the statistical findings, reinforcing the impact of COVID-19 on reducing SPINA-GD levels





**Figure 4.** Box-plot representation of analysis of variances (ANOVA) in subgroups for SPINA GD in patients with Hashimoto’s thyroiditis

The same tests were used on the subgroups in combination with Grave’s Disease. However, the sample size was too small to carry out any of the previous calculations. Table 8 shows the mean SPINA-GD levels which were found to vary among the subgroups. The highest mean SPINA-GD level is observed in the "NO COVID & NO Graves" subgroup (31.34 nmol/s), while the lowest mean SPINA-GD level is in the "COVID & NO Graves" subgroup (28.24 nmol/s). The third subgroup, "NO COVID & Graves", had a mean SPINA-GD level of 29.83 nmol/s. The "COVID & Graves" subgroup has only one participant, so the mean SPINA-GD level is not available for this group.

There is a significant ratio of the variance between the groups to the variance within the groups ( $F = 14.07$ ). Furthermore, there was a statistically significant difference seen in mean SPINA-GD levels across the three subgroups (excluding the "COVID & Graves" subgroup due to the insufficient sample size) ( $P < 0.001$ ).

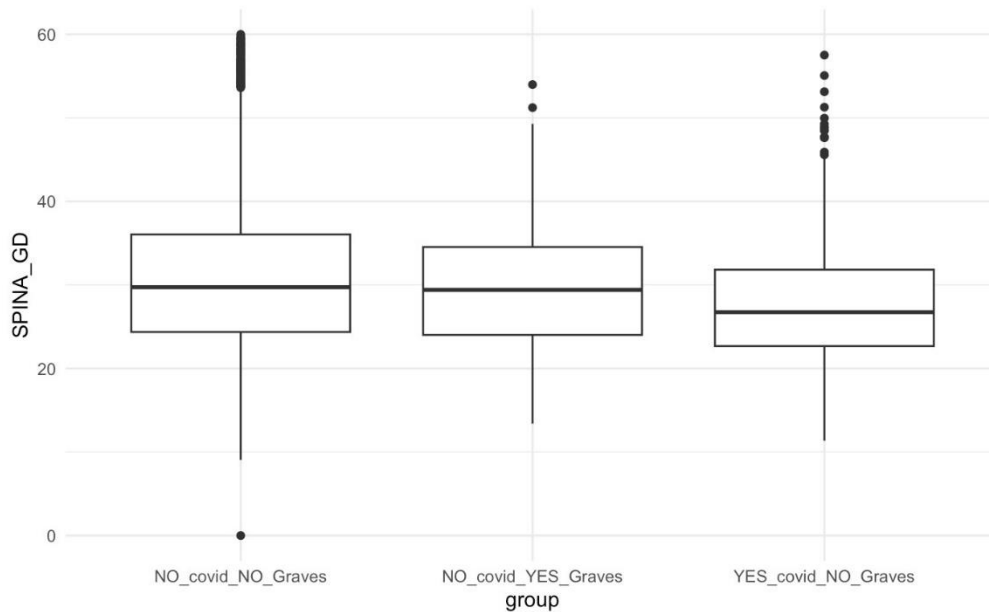
Neither a post hoc- analysis in the subgroups for SPINA GD in patients with Grave’s Disease nor one based on Cohen’s D was possible to conduct owed to a limited number of patients.

**Table 8.** Analysis of Variances (ANOVA) in subgroups for SPINA GD in patients with Grave's Disease

Subgroup	n	SPINA GD mean*	ANOVA-Result
no COVID & no Graves	9866	31.34 (10.97)	F(3,10446) = 14.07: <i>P</i> < 0.001
COVID & no Graves	511	28.24 (8.81)	
no COVID & Graves	72	29.83 (10.18)	
COVID & Graves	1	N/A	

\* Standard deviation [SD]

Again, a box-plot graph depicts the distribution of SPINA- GD levels within their respective combination in patients with Grave's disease (Figure 5). The three different subgroups are presented on the horizontal line. As in the previous case, for visualization purposes the upper limit was set at SPINA GD values of 60 nmol/s. In the figure "NO COVID & NO Graves" subgroup with a median SPINA-GD level of 30 nmol/s and a wide IQR, indicating a more varied distribution of SPINA-GD levels. The "NO COVID & YES Graves" subgroup also has a median SPINA-GD level of 30 nmol/s and a similar IQR to the "NO COVID & NO Graves" group. The third subgroup "YES COVID & NO Graves" showed a lower median SPINA-GD level (25 nmol/s) with a narrower IQR, indicating less variability and a reduction in SPINA-GD levels compared to non-COVID groups.



**Figure 5.** Box-plot representation of analysis of variances (ANOVA) in subgroups for SPINA GD and Grave’s disease

The results of the simple linear regression for different models are presented in Table 9. The independent variable was selected to be SPINA GD with various cofounders. Model 1 indicates that the presence of COVID-19 is associated with a statistically significant decrease in SPINA-GD levels by approximately 3.07 ( $P < 0.001$ ). This model accounts for 0.23% of the variability in SPINA-GD levels ( $R^2 = 0.0023$ ). Both the presence of COVID-19 and advancing age are associated with statistically significant decreases in SPINA-GD levels, by approximately 2.41 and 0.17 per year, respectively (both  $P < 0.001$ ). This model explains 4.19% of the variability in SPINA-GD levels ( $R^2 = 0.042$ ). Furthermore, adding sex into the model there are statistically significant changes in SPINA-GD levels, with decreases of approximately 2.45 and 0.17 per year for COVID-19 and age, respectively, and an increase of approximately 2.11 for males (all  $P < 0.001$ ). This model explains 4.78% of the variability in SPINA-GD levels ( $R^2 = 0.045$ ). Lastly, adding higher CRP levels, and higher leucocyte counts seem to be significant predictors of SPINA-GD levels. Specifically, COVID-19 is associated with a decrease of approximately 1.40 ( $P = 0.022$ ), age with a decrease of 0.16 per year, female sex with an increase of 2.28 units, CRP levels with a decrease of 0.03 units, and leucocyte counts with a decrease of 0.01 units (all  $P < 0.001$ ). Model 4 also accounts for 4.75% of the variability in SPINA-GD levels ( $R^2 = 0.047$ ).

In summary, the absence of a Covid infection shows a statistically significant negative influence on SPINA-GD level for model 1 to 3, but controlling for Leucocytes proves to be insignificant. Gender (female) seems to have a positive influence of 2.28 on SPINA-GD levels. Furthermore, age, leucocyte count, and CRP do have a statistic significant influence on SPINA-GD levels as seen in Table 9. However, only a small proportion of the variability in the outcome is explained by these models, suggesting that there are other factors not included in the models contributing to the variability in SPINA-GD levels.

**Table 9.** Linear regression models

Independent variable	Confounder *	model 1	model 2	model 3	model 4
SPINA-GD	COVID (ref=no)	- 3.0709 (p < 0.001)	-2.405683 (p < 0.001)	-2.45034 (p < 0.001)	-1.400983 (p = 0.022)
	Age		-0.168639 (p < 0.001)	-0.16704 (p < 0.001)	-0.159037 (p < 0.001)
	SEX (ref=female)			2.11274 (p < 0.001)	2.276322 (p < 0.001)
	CRP				-0.026102 (p < 0.001)
	Leucocytes				-0.011510 (p < 0.001)
F- Statistics		F(1,10448) = 24.05, p < 0.001	F(2,10447) = 228.6, p < 0.001	F(3, 10446) = 174.6, p < 0.001	F(5, 10444) = 142.6, p < 0.001
R-squared		0.002296	0.04193	0.04775	0.04748
adjusted R-squared		0.002201	0.04174	0.06391	0.06346

\* Estimated [F-Statistics; P- value]

To summarize the significant and most prevalent results of this study, it was seen that patients with an active COVID-19 infection TSH levels were reduced below reference values by 40% (OR 1.40 [95%-CI: 1.16 – 1.69]) after adjusting for CRP and leukocyte count (Table 3). Based on these findings the conclusion that a viral infection leads to reduced TSH values can be drawn.

Secondly, this study described in Table 5 with the ANOVA that the changed levels of SPINA GD and subsequently the lowered deiodinase activity was associated with the presence of CoV-19 infection and not by autoimmune activity in the setting of Hashimoto’s thyroiditis.

## **5. DISCUSSION**

Due to the influence of the thyroid gland on a multitude of organs an infection and altered mechanism could have significant health consequences (88).

In this study one of the major findings proved to be that a COVID-19 infection increases the likelihood of a reduced TSH value (below reference) by 40% after adjusting for CRP and leukocyte count (Table 3). Accordingly, it can be assumed that the presence of SARS-CoV-2 leads to a reduced TSH secretion. The initial assumption held true. Patients of the Upper Franconian region who were presenting with an active infection of SARS-CoV-2 would exhibit thyroid dysfunction in form of alterations in thyroid parameters, namely TSH levels. Thereby, also the second hypothesis was proven to be valid, since a mild degree of thyroid dysfunction in form of a reduced TSH level was seen.

Other authors reported similar findings in albeit much smaller populations describing reduced levels of TSH parameters amongst other changes (93–96). In more detail, one retrospective study conducted by a research team of Zhejiang University, Hangzhou region in China found similar results. They included a total of 50 COVID-19 patients. In 56% of these, levels of TSH were found to be below the standard range. Furthermore, in comparison to healthy individuals and patients with pneumonia not related to COVID-19, the COVID-19 patients exhibited significantly reduced levels of TSH and TT3 (93). Similar, decreased levels of TSH and fT3 among other laboratory parameters were described in a study examining the basic laboratory indexes in COVID-19 patients with a more milder course (94). Another, a study set in northern Italy found reduced serum TSH levels with a simultaneous varying degree of the other hormone parameters, namely fT3 and fT4. It has to be mentioned that this study was conducted in patients of the high intensity of care units which is subsequently connected with a more severe course of the disease (95). Though, it does show a connection between a milder course and more severe development of an infection and its subsequent pathological influence on the thyroid gland.

Contradictory to the findings of this study and those mentioned previously, a Japanese study conducted by Nakamura *et al.* found that thyroid hormone levels did not change at all during a mild or moderate course. In fact, most often the patients presented as euthyroid (97).

Another significant finding of this study is described by the ANOVA in regards to the SPINA GD in association with Hashimoto's thyroiditis (Table 5). Based on this finding, the calculation suggests that differences between the groups are significant which leads to the conclusion that a reduced deiodinase activity was caused by the CoV-19 infection and not by autoimmune activity in the setting of Hashimoto's thyroiditis. The literature research was done in the following ways: First the selected keywords SPINA-GD, thyroid, SARSCoV-2,

coronavirus, COVID, COVID-19 were used and MeSH terms identified. Next, the PubMed data bank was searched. Since other sites required a payment-based subscriptions they were excluded. The selected articles focussed on the connection between the thyroid gland and the pathophysiological consequences by an active CoV-19 infection. Since no article mentioned SPINA GD values, the results stand for themselves and need further backup by other research papers.

As previously mentioned, this study did not find that any other laboratory values in relation to the thyroid gland were changed. Therefore, no altered thyroid function was detected in the patients of this study which presented with a milder course. Moreover, no statistically significant difference in means of TSH between the groups of patient's with an active Hashimoto thyroiditis was seen. This has not been described by similar studies. They rather found a great variety in the thyroid hormone levels especially in more severe courses of disease which were not included in this study (98). Additionally, a study conducted by Dabas *et al.* reported patients with predominantly low T3 and sick euthyroid syndrome or NTIS (99) and some authors identified concomitant inflammatory states of the thyroid gland, namely NTIS and SAT. The latter is a self-limited inflammation of the thyroid gland and also known as De Quervain's thyroiditis which is especially prevalent following a viral respiratory tract infection caused by a T-cell mediated injury (87). Already prior to the recent corona pandemic it has been verified that viral agents are causing SAT even several months later to the resolution of the infection (87,100). A certain distinctive characteristic for CoV-2 was shown to be that post-Covid SAT was even more severe than others caused by viral agents (87). Affirming this, some studies connected the cytokine storm induced by massive release of these mediators with painless SAT. Several studies also reported ultrasonographic findings, providing confirmatory evidence in form of structural enlargement with concomitant diminished vascularity indicative for SAT (87,101).

Though it has to be mentioned that the direct correlation between a SARS-CoV-2 infection and SAT has not been definitely proven as some authors claimed no increase in seasonal variations and several discrepancies in presented symptoms (87).

### **5.1. Autoimmune related effects**

Resulting from the findings this study did not found any correlation that a preexisting state of AITDs, Hashimoto's thyroiditis and Graves' disease was considered to be connected with a more severe change in thyroid hormone levels. Further assessment of peripheral

deiodinase activity was done by SPINA- GD model and also found to be negative. Neither Hashimoto's thyroiditis nor Graves' disease were associated with severely altered thyroid levels during a CoV-19 infection.

These findings are in accordance to multiple research articles and reviews describing the finding that a preexisting thyroid disease did not increase the risk for an infection (96). Furthermore, a study led by Nguyen *et al.* in 2021 established that neither hypo- nor hyperthyroidism are connected with an enhanced risk of contracting SARS-CoV-2, admission rates, or more severe development (89). Only limited evidence in form of clinical case studies documented a development of Grave's disease following an infection (102).

## **5.2. Confounding factors and limitations**

It is essential to acknowledge the limitations inherent to the study design, samples size and other limitations as it can bias the interpretation and generalizability of findings. As the study's design is retrospective it posed a limitation as the documentation of duration and severity of COVID-19 infections could not be influenced and specific data was not available.

Further, no information pertaining to covariates, concurrent medical conditions, or the status of patients' thyroid hormone replacement therapy was added.

The inclusion of study participants was mainly based on laboratory values and available data sets. The data collection was guided by the main laboratory values taken from Orbis System (Orbis®, Dedalus Healthcare, Bonn, Germany). However, a potential bias exists if the initial documentation is inaccurate or flawed. Logistical and time constraints can have an influence on data as they were collected by personnel upon admission of the patient. It can affect the scope and depth of a study. Unintentional biases during the process of admittance cannot be excluded.

The study did not capture data on hormone levels (Luteinizing Hormone and Follicle-Stimulating Hormone), the precise timing within the menstrual cycle, or the presence of menopause with the concomitant hormonal changes.

We could not analyze all possible confounding factors such as exact body measures, regular alcohol consumption, smoking patterns, sleeping patterns, patterns of daily physical behavior, and detailed nutrition habit such as supplement intake could not be integrated.

Despite mentioned limitations, it has to be emphasized that effort was made to limit possible confounders. Considering these limitations, our study did gain valuable data and insights into pathophysiological effects of a SARS-CoV-2 infection in regards to the thyroid



gland. Effort into decreasing these limitations was made. To minimize this limitation incomplete data sets were excluded from the study and the larger sample size (n=10 450) increases the generalizability of the study findings. The adult population characteristics in Table 1 in Chapter 4 aid generalizability of the study. In order to exclude any other inflammatory conditions leading to an increased CRP and leukocyte count, patients with increased levels of both have been excluded, thereby limiting their influence of concurrent medical conditions. Furthermore, context-specific findings were intended as the results were supposed to represent the population of the Upper Franconian region surrounding Coburg. In this framework it has to be emphasized that the results from this study may not apply to other settings or populations. Reason being that this area has a high incidence of thyroid autoimmune diseases due to regional iodine deficiency in soli causing this population to be of special interest (data not yet published).

Due to heterogeneity of other research articles, further prospective studies have to be conducted to provide further insight into the complex interplay of hormonal axis, the exogenous and endogenous regulation, and modulation in order to overcome these limitations. The intricacy of thyroid endocrinology and broad range of possible influences on every step of the HPT axis need to be taken into consideration.

As outlined prior, the influence of thyroid hormones and involvement in various physiological mechanisms is a highly complex matter with dysregulations leading to several clinical consequences. To contextualize the study within a broader framework and in juxtaposition to other findings, literature research revealed a discordance of results and contradictory conclusions across multiple studies. A pronounced degree of variant results creates a complex task in their comparison. Despite a multitude of studies and various forms of publications haven been conducted, a definite unanimous correlation between thyroid hormones, severity of disease and mortality rate, has not been found. The endeavor to unravel pathophysiological effects on the thyroid gland presents to be another obstacle since contradictory observations have been reported.

## **6. CONCLUSION**

The results of this study provide valuable insights into the in the epidemiological Upper Franconia region of Bavaria, Germany. The findings shed light into an immensely discussed topic with contradictory findings in literature.

The main findings and hypothesis of this work include that patients with an active infection of SARS-CoV-2 did exhibit thyroid dysfunction in form of alterations in thyroid parameters, namely reduced TSH. Other laboratory values did not change with a proven infection.

In this setting, the severity and degree of thyroid dysfunction was only mild as no other thyroid parameter changed following a COVID-19 infection.

Based on these statistical results neither an enhanced or a reduced expression of thyroid gland secretion based on laboratory parameters in association with a COVID-19 was proven.

This study did not detect that a preexisting state of AITDs, here Hashimoto´s thyroiditis and Graves´ disease, was connected with a more severe change in thyroid hormone levels. Further assessment of peripheral deiodinase activity was done by SPINA- GD model which also found no correlation. Neither Hashimoto´s thyroiditis nor Graves´ disease were associated with severely altered thyroid levels during a CoV-19 infection.

However, it was revealed that one of the major findings of this study included the alteration of SPINA GD activity caused by a CoV-19 infection in patients with Hashimoto´s thyroiditis.

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## **8. SUMMARY**

**Objectives:** The objective of this study was to collect regional data of Upper Franconia and observe the association between thyroid parameters thyroid-stimulating-hormone (TSH), free triiodothyronine (fT3), free thyroxine (fT4) and an infection with SARS- CoV- 2. It was set to investigate the prevalence of patients with abnormal thyroid parameters (outside the range of reference), the association of COVID-19 infection on thyroid gland secretion based on laboratory parameters of routine clinical data and a possible increased vulnerability of persons with a history of Hashimoto's thyroiditis and Graves' disease to infection with COVID-19.

**Patients and methods:** For this retrospective observational study, routine clinical data of patients admitted to the REGIOMED Hospital Coburg, Upper Franconia region in Bavaria, Germany (single-site) were included and subsequently analyzed. The admissions were in between the official pandemic declaration of WHO on 11<sup>th</sup> March and ended with the declaration of German government on 07<sup>th</sup> April 2023 ending the pandemic. Data was collected from anonymized sources. A total of 10 450 cases were included of which 195 were also diagnosed with Hashimoto's thyroiditis. Furthermore, 93 were included based on their ICD code in the group for Grave's disease. The statistical analysis was carried out with RStudio.

**Results:** The first and foremost finding of this study indicates that the prevalence of a TSH value below the reference was significantly lower in the group with an active COVID infection in comparison to the non-COVID group. Other laboratory parameters fT3 and fT4 did not change in a mild course of an infection. Secondly, reduced deiodinase activity was seen to be caused by the CoV-19 infection and not by autoimmune activity in the setting of Hashimoto's thyroiditis. Furthermore, neither Hashimoto's thyroiditis nor Graves' disease were seen to be associated with severely altered thyroid levels during a CoV-19 infection.

**Conclusion:** A mild infection with COVID-19 increases the risk of a lower TSH value but no altered thyroid function was seen in these patients. Following the pandemic several studies in the realm of the thyroid gland have been conducted but found widely heterogenic and also conflicting results have been found. Furthermore, a second significant finding of this study described reduced levels of SPINA GD with a subsequently a lowered deiodinase activity. This finding was indicated to be caused by the CoV-19 infection and not by an autoimmune activity in the setting of Hashimoto's thyroiditis. Targeted prospective studies need to follow to evaluate on associations found in our study.



## **9. CROATIAN SUMMARY**

**Ciljevi:** Cilj ovog istraživanja bio je prikupiti regionalne podatke Gornje Frankonije i promatrati povezanost između parametara štitnjače koji stimuliraju štitnjaču (TSH), slobodnog trijodtironina (fT3), slobodnog tiroksina (fT4) i infekcije SARS-CoV-2. Postavljeno je istraživanje prevalencije bolesnika s abnormalnim parametrima štitnjače (izvan raspona referencije), povezanost infekcije COVID-19 na izlučivanje štitnjače na temelju laboratorijskih parametara rutinskih kliničkih podataka i moguće povećane ranjivosti osoba s poviješću Hashimotovog tiroiditisa i Gravesove bolesti na infekciju COVID-19.

**Pacijenti i metode:** Za ovu retrospektivnu opservacijsku studiju uključeni su rutinski klinički podaci pacijenata primljenih u bolnicu REGIOMED Hospital Coburg, regija Gornja Frankonija u Bavarskoj, Njemačka. Priznanja su provedena između službenom pandemijskom deklaracijom izjave WHO-a o pandemiji 11. ožujka, te završilo je izjavom njemačke vlade 07. travnja 2023. o okončanju pandemije. Podaci su prikupljeni iz anonimiziranih izvora. Uključeno je ukupno 10.450 slučajeva, od kojih je kod 195 također dijagnosticiran Hashimotov tiroiditis. Osim toga, 93 su uključena u skupinu za Gravesovu bolest na temelju njihovog ICD koda. Statistička analiza provedena je sa RStudio.

**Rezultati:** Prvi i najvažniji rezultat ove studije pokazuje da je prevalencija TSH ispod referentnih vrijednosti bila značajno niža u skupini s aktivnom COVID infekcijom u usporedbi s nema-COVID skupinom. Ostali laboratorijski parametri fT3 i fT4 nisu se promijenili u blagom tijeku infekcije. Drugo, utvrđeno je da je smanjena aktivnost deiodinaze uzrokovana infekcijom CoV-19, a nema autoimunom aktivnošću u kontekstu Hashimotovog tiroiditisa. Osim toga, nije utvrđeno da ni Hashimotov tiroiditis ni Gravesova bolest nisu povezani s ozbiljno promijenjenom razinom štitnjače tijekom infekcije CoV-19.

**Zaključak:** Blaga infekcija COVID-19 povećava rizik niže vrijednosti TSH, ali u tih bolesnika nije uočena promijenjena funkcija štitnjače. Nakon pandemije provedeno je nekoliko studija na području štitne žlijezde, ali su dale vrlo heterogene i kontradiktorne rezultati. Osim toga, drugi značajan rezultat ove studije opisao je smanjenu razinu SPINA-GD s naknadno smanjenom aktivnošću deiodinaze. Ovaj nalaz uzrokovan je infekcijom CoV-19, a nema autoimunom aktivnošću u kontekstu Hashimotovog tiroiditisa. Ciljane prospektivne studije moraju slijediti kako bi se procijenile asocijacije pronađene u našoj studiji.