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Post-COVID Endocrine Disorders: Putative Role of Molecular Mimicry and Some Pathomorphological Correlates

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ABSTRACT

The article is devoted to the problem of autoimmune diseases provocation by coronavirus infection and the role of molecular mimicry in this phenomenon. SARS-CoV-2 can disguise its proteins as human ones in order to avoid immune attack. A bioinformatics analysis of the probable pentapeptide sharing between human autoantigens of endocrinocytes and SARS- CoV-2 spike protein, membrane protein and nucleocapsid protein was performed. Antigen mimicry between S-proteins of all other known human Coronaviruses and typical target autoantigens of endocrinocytes was also explored. Six human-identical regions were found in the SARS-CoV-2 membrane and nucleocapsid proteins, all of them in their immunodominant epitopes. All shared epitopes belong to antigens of endocrine cells commonly targeted during autoimmune endocrinopathies. Moreover, samples of the pituitary, adrenal and thyroid from patients who died from coronovirus infection (COVID-19) were studied morphologically using histochemical methods. A high frequency of SARS-CoV-2 caused inflammation of the studied endocrine organs was found in patients who died from severe COVID-19. At the same time, the abundant expression of virus antigens by the cells of the adenohypophysis was combined with the complete absence of its expression by the cells of the neurohypophysis. SARS-CoV-2 infected cells apparently perished by non-apoptotic pathway. The foci of lesions in endocrine organs contained abundant lymphocytic infiltrates which may witness for the impact of autoimmune processes. The facts revealed emphasize the need of endocrinological diagnostic alertness of a physician while observing patients with post-vaccination and post-COVID-19 health disorders. [3 figures, 6 tables, bibliography: 45 references].

Key words: Antigen mimicry, Autoimmune diseases, SARS-CoV-2, Human Coronaviruses, Thyroid gland, Pancreatic islets, Adrenals, Pituitary.

The novel coronavirus disease pandemic, although currently on the decline, has given rise to a growing problem, namely post-COVID subacute and chronic health disorders, in particular, various variants of post-COVID syndrome. This syndrome is most frequently observed among those who experienced an acute infection in a non-severe and even mild form, and the number of such patients in the world is increasing [1]. Based on the recent literature, the post-COVID cohort is further divided into two categories: (1) subacute or ongoing symptomatic COVID-19, which includes symptoms and abnormalities present from 4 to12 weeks beyond acute COVID-19; and (2) chronic or post-COVID-19 syndrome, which includes symptoms and abnormalities persisting or present beyond 12 weeks of the acute COVID-19 onset and are not attributable to alternative diagnoses [2]. A recent meta-analysis revealed that 80% of the patients infected with SARS-CoV-2 developed one or more long-term symptoms [3]. Neuropsychiatric, rheumatic, respiratory, cardiovascular, gastrointestinal and not infrequently – also endocrine manifestations were identified, and over 40% of patients still reported fatigue during 7–9 months after the acute COVID-19 resolution, and the prevalence of most symptoms has even risen after 7–9 months compared to the early recovery period (3–10 weeks) [4]. Pathological autoimmunity plays a pivotal mechanistic part in post-COVID complications [5]. Another growing cohort includes individuals with post-anti-COVID vaccination adverse manifestations which presumably have an autoimmune/autoinflammatory origin [6]. Quite often, the post-COVID

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syndrome as well as anti-COVID vaccination adverse effects alter the endocrine regulation, including thyroid [7], pituitary [8], adrenal [9], insulin dependent [10], and gonadal [11] mechanisms.

The molecular mimicry between pathogen and host antigens has been long time suspected as a provocative mechanism of pathological autoimmunity triggered by infection [12-13]. That assumption is true for the COVID-19 sequela as well [14-15]. Previously we have demonstrated peptide sharing between immunogenic epitopes of *SARS-CoV-2 spike protein (SP)* and few autoantigens of human endocrine cells [16-17].

In addition to the SARS-CoV-2 SP. several other viral antigens have been proven to be highly significant in the immune response against COVID-19. The *SARS-CoV-2 Nucleocapsid protein (NP)* is its immunodominant antigen, moreover, anti-NP antibodies have the highest titers among all anti-viral specificities in persons previously infected with this virus [18]. The *SARS-CoV-2 Membrane protein (MP)* is essential in the disease pathogenesis due to its role in anti-interferonogenic and pro-apoptotic effects and also is highly immunogenic [19-20]. In this study, we have explored NP and MP for antigen mimicry with several common target antigens of human autoimmune endocrinopathies (hypophysitis, adrenalitis, insulitis and oophoritis/orchitis).

The autoimmune complications were suspected after Coronavirus infections even prior to current pandemic of SARS-CoV-2. For example, in 2004 molecular mimicry was hypothesized between SARS-CoV-1 and pituitary antigens [20a]. Later seasonal Coronaviruses were blamed for possible provocation of CNS lesions *via* autoimmunity [20b]. That's why we also compared the molecular mimicry potential of SPs from all other known human Coronaviruses against typical autoantigens of human endocrinocytes.

Materials and Methods

In order to identify matching amino acid sequences (pentapeptides) between the SPs, MPs and NPs of human coronoviruses and human autoantigens targeted in autoimmune endocrinopathies, and for a comparative analysis of the various Coronaviruses proteome and the proteome of human, we used the original computer program ALIGNMENTAJ, created by M.G.Normatov. The ALIGNMENTAJ works fast and uses an algorithm with local alignments. The data from the UNIPORT database about primary structure of proteins was used.

A comparison of proteins by a consecutive search for regions of one protein in the others, which is essentially a standard task of finding a sub-string in a string, was performed. This algorithm is implemented in standard methods of Python, in which the main program was coded. A bioinformatics analysis of the probable pentapeptide sharing between human endocrinocytes' antigens and various proteins of coronaviruses was based on the NCBI, UniProt and IEDB databases. Matching peptides of human endocrinocytes and SP, MP and NP of various coronaviruses were analyzed using pentapeptides as sequence probes since a peptide grouping formed by the five amino acid (aa) residues defines a minimal immune determinant that can 1) induce highly specific Abs, and 2) determine antigen-Ab specific interaction [13]. A library of human endocrinocyte-associated proteins has been assembled at random from the UniProtKB database [22]. The primary sequence of all viral proteins was dissected into pentapeptides offset by one residue and the resulting viral pentapeptides were analyzed for their occurrences within a primary sequence of human autoantigens explored. Occurrences and corresponding proteins were annotated. The immunological potential of the pentapeptides shared between various coronaviruses and human endocrinocytes was analyzed by searching the Immune Epitope Data Base [23] for the immunoreactive epitopes of various coronaviruses hosting the shared pentapeptides.

In order to search for "wet lab"pathomorphological correlates of bioinformatics findings, a histological analysis of some endocrine organs was carried out. For morphological studies samples of the pituitary, adrenal and thyroid glands taken from deceased adults who died from a new coronavirus infection (COVID-19), confirmed by intravital and post-mortem isolation of SARS-Cov2 nucleotide sequences from biological material by PCR, were fixed with a 10% buffered formalin solution (pH=7.4) within 72 hours. After fixation, the tissue samples were dehydrated using isopropanol, paraffin imbibition, and embedded in paraffin according to conventional methods. Tissue sections 4 μm thick were made from paraffin blocks and placed on glass slides treated with polylysine. Immunohistochemical studies were performed using the A360 Immunohistosteiner (Thermo, USA), the UltraVision Quanto DAB universal immunohistochemical imaging system (Thermo, USA), SARS-CoV-2 Spike rabbit polyclonal antibodies (GeneTex, USA), and mouse monoclonal antibodies to Caspasae3 (clone 3CSP03, Diagnostic BioSystems, USA) in accordance with the recommendations of the reagent manufacturers.

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Results

Bioinformatics analysis

Quantitatively, SP, MP and NP of the human Coronaviruses were found to share totally 79 minimal immune pentapeptide epitopes: 41 in SP, 14 in MP and 24 in NP, - with 18 autoantigens expressed by human endocrinocytes. The shared pentapeptides belong to the proteins of human endocrine cells listed in Table 1, Table 2 and Table 3.

The Immune Epitope Data Base exploration [23] revealed that all of the shared pentapeptides described in Table 4, Table 5 and Table 6 belong to those epitopes of SP, MP or NP from various human coronaviruses that have been experimentally validated as immunoreactive ones.

Table 1. Spike glycoprotein of human Coronaviruses and autoantigens common for autoimmune endocrinopathies: shared pentapeptides.

Spike glycoprotein

Hum. Coronaviruses // autoantigens of endocrine cells	SARS-CoV-2 (P0DTC2)	SARS-CoV (P59594)	MERS-CoV (R9UQ53)	HCoV-HKU-1 (S5YA28)	HCoV-OC43 (P36334)	HCoV-NL63 (Q6Q1S2)	HCoV-229E (P15423)
Thyroid peroxidase (P07202)	RAAEI	RAAEI	-	-	-	SFSKL	-
Thyrotropin receptor (P16473)	LLPLV, ICGDS	ICGDS	DTKIA, ASELS	-	-	ILLVL	VSQTS, IPSLP
Thyroglobulin (P01266)	LDSKT, FNFSQ, SAIGK	FNFSQ, FLLFL	GFGGD	WYQKP	VVSCL, RVSPG	LQENQ, LKSGV	FVNTT, LQENQ
Alpha-enolase (P06733)	-	-	-	-	-	IADLA	IADLA
RPH3L(rabfillin-3a) (Q9UNE2)	-	-	RLDVL, LDVLE, DVLEQ	-	-	-	-
Cytotoxic T-lymphocyte protein 4 (P16410)	-	-	-	-	-	-	-
Prolactin (P01236)	SNLLL	-	-	-	-	TEVRG, NLSSE	-
Steroid 21-hydroxylase (P08686)	LQDVV	LQDVV	GTVII	-	PDLSL	-	-
Steroid 17-alpha-hydroxylase (P05093)	-	-	-	DTLMQ	-	-	EISTL
Glutamate decarboxylase 1 (Q99259)	VGYQP, AGAAL	AGAAL	-	-	-	-	DGDGI
Glutamate decarboxylase 2 (Q05329)	-	-	-	-	-	-	-
Receptor-type tyrosine-protein phosphatase-like N (Q16849)	LPPLL	LPPLL	PLGQS, LVALA	LSTLL, GSSSR	LPPLL, EPALL, LAGVA	RLAAL	RLAAL
Receptor-type tyrosine-protein phosphatase N2 (Q92932)	-	-	AALSA	LMQGV, SSSRS, GAALA	-	-	AVLTY
Islet cell autoantigen 1 (Q05084)	LDPLS, GYQPY	GYQPY, KGYQP, ELLNA	FRKVQ	NASSL	ASLQE	-	-
Insulin (P01308)	-	-	-	-	-	-	-
Insulin receptor (P06213)	-	-	DYYRK, LKELG	FRDLS, TICKS, RKRRS	LKDGV, ENNVV	IVNLL, SNSSS	-
Zinc transporter 8 (Q8IWU4)	-	-	LLSLFS	VSSCA, ALLSI	-	-	-
Carboxypeptidase E (P16870)	SALLA	-	-	-	-	-	-

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Table 2. Membrane protein of human Coronaviruses and autoantigens common for autoimmune endocrinopathies: shared pentapeptides.

Membrane protein

Hum. Coronaviruses // autoantigens of endocrine cells	SARS-CoV-2 (P0DTC5)	SARS-CoV (P59596)	MERS-CoV (R9UNX5)	HCoV-HKU-1 (Q0ZJ82)	HCoV-OC43 (Q01455)	HCoV-NL63 (U3M6Q8)	HCoV-229E (P15422)
Thyroid peroxidase (P07202)	-	-	-	-	-	-	-
Thyrotropin receptor (P16473)	-	-	TSVTA	-	-	-	-
Thyroglobulin (P01266)	-	-	-	-	-	-	LFRRA
Alpha-enolase (P06733)	-	-	-	-	-	-	-
RPH3L(rabfillin-3a) (Q9UNE2)	-	-	-	-	RLPST	-	-
Cytotoxic T-lymphocyte protein 4 (P16410)	-	-	-	-	-	-	-
Prolactin (P01236)	-	-	-	-	-	-	-
Steroid 21-hydroxylase (P08686)	-	-	-	-		-	-
Steroid 17-alpha-hydroxylase (P05093)	-	-	-	-	-	-	-
Glutamate decarboxylase 1 (Q99259)	-	-	-	-	-	-	DGDGI
Glutamate decarboxylase 2 (Q05329)	-	-	-	LWLMW	LWLMW	-	-
Receptor-type tyrosine-protein phosphatase-like N (Q16849)	TRPLL	-	PLVED	-	-	-	EVNAI
Receptor-type tyrosine-protein phosphatase N2 (Q92932)	-	-	-	-	-	-	EVNAI
Islet cell autoantigen 1 (Q05084)	-	-	-	-	-	-	-
Insulin (P01308)	-	-	-	-	-	-	-
Insulin receptor (P06213)	-	-	-	TVIRG	-	-	TVAVP
Zinc transporter 8 (Q8IWU4)	-	-	-	-	-	-	-
Carboxypeptidase E (P16870)	GNYKL	GNYKL	-	-	-	-	-

Table 3. Nucleoprotein of human Coronaviruses and autoantigens common for autoimmune endocrinopathies: shared pentapeptides.

Nucleoprotein

Hum. Coronaviruses // autoantigens of endocrine cells	SARS-CoV-2 (P0DTC9)	SARS-CoV (P59595)	MERS-CoV (T2BBK0)	HCoV-HKU-1 (S5ZBQ7)	HCoV-OC43 (P33469)	HCoV-NL63 (H9EJA4)	HCoV-229E (P15130)
Thyroid peroxidase (P07202)	-	-	-	-	PTSGV	-	AAALK
Thyrotropin receptor (P16473)	-	-	-	-	ILNKP, DVYEL	RGQRV	-
Thyroglobulin (P01266)	-	RVRGG	-	SASNS, FTVST	-	-	-
Alpha-enolase (P06733)	-	-	-	-	GKDAT	-	-
RPH3L(rabfillin-3a) (Q9UNE2)	-	-	-	-	-	-	-
Cytotoxic T-lymphocyte protein 4 (P16410)	PPTEP	PPTEP	-	-	-	-	-
Prolactin (P01236)	-	-	-	-	-	-	-
Steroid 21-hydroxylase (P08686)	-	-	-	-	-	-	-
Steroid 17-alpha-hydroxylase (P05093)	ALLLL	ALLLL	-	-	-	-	-
Glutamate decarboxylase 1 (Q99259)	-	DNVIL	-	-	-	-	-
Glutamate decarboxylase 2 (Q05329)	-	-	-	-	-	-	-
Receptor-type tyrosine-protein phosphatase-like N (Q16849)	-	-	SSSRA, SEPPK	-	SSSRA	-	-
Receptor-type tyrosine-protein phosphatase N2 (Q92932)	SSSRS	SSSRS	-	-	-	REENV	RAPSR
Islet cell autoantigen 1 (Q05084)	-	-	-	-	-	-	LGKFL
Insulin (P01308)	-	-	-	-	-	-	-
Insulin receptor (P06213)	-	-	-	-	-	-	-
Zinc transporter 8 (Q8IWU4)	-	-	-	-	-	-	-
Carboxypeptidase E (P16870)	SSPDD	-	DLQGN	-	-	-	-

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Table 4. Immunoreactive epitopes of human coronaviruses spike glycoprotein containing peptides shared with human endocrinocytes autoantigens.

ID in IEDB	*SP epitope sequence*
534141	tqqli**RAAEI**rasan
1071273	**LLPLV**SSQCVNLTTR
1310877	vdctmy**ICGDS**tecs
1310392	fgtt**LDSKT**qslliv
1087679	pikdfgg**FNFSQ**ilpdps
1071651	nqfn**SAIGK**iqdsls
1069347	dstec**SNLLL**qygsf
1310448	gk**LQDVV**nqnaqaln
1309589	sygfqptng**VGYQP**yrvvvl
531783	g**AGAAL**qipfamqma
1071969	qkfngltv**LPPLL**td
1309593	tttdavdca**LDPLS**etkctl
1309482	**GYQPY**rvvvlsfellhapat
1069137	aqyt**SALLA**gtitsg
51379	qli**RAAEI**rasanlaat
33874	ktsvdcnmy**ICGDS**tec
64888	tlkyfgg**FNFSQ**ilpdp
41504	mfi**FLLFL**tltsgsdld
14208	esltttstalgk**LQDVV**
62872	tagwtfg**AGAAL**qipfa
3983	aqkfngltv**LPPLL**tdd
19657	gfytttgi**GYQPY**rvvv
16417	fknkdgflyvy**KGYQP**I
23437	gyqpyrvvvls**ELLNA**
1411039	an**DTKIA**sqlgm
1411879	aqalakl**ASELS**
1451681	iip**GFGGD**fnlt
1439102	gdiiq**RLDVL**eq
1455525	ivdiiqr**LDVLE**
1439102	gdiiqrl**DVLEQ**
1406455	aaanat**GTVII**s
1419664	dcnl**PLGQS**lca
1503295	ses**AALSA**qlak
1519626	ttnea**FRKVQ**da
1414267	awedg**DYYRK**ql
1422379	d**LKELG**nytyyk
1482114	nltk**LLSLF**mvn
1510167	stl**WYQKP**flsd
1424576	**DTLMQ**gvtlssn
1507146	sncnfn**LSTLL**r
1444184	**GSSSR**sffedll
1424576	dt**LMQGV**tlssn
144485	g**SSSRS**fiedll
1432711	f**GAALA**mekvne
1501401	saafhq**NASSL**a
1462061	ksgan**FRDLS**lk
1448456	h**TICKS**igssrn
1489830	psfsssr**RKRRS**
1423081	dnltdys**VSSCA**
1646363	atafnn**ALLSI**qngfs
1419202	dag**VVSCL**ykrn
1646031	a**RVSPG**lciagdrgia

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1411536	a**PDLSL**dyinat
1441061	gikv**LPPLL**sdn
1408158	afhanss**EPALL**
1412712	**ASLQE**iisrlda
1459900	k**LKDGV**nfnidd
1455023	it**ENNVV**vtstc
1662988	gtcpf**SFSKL**nnfqkf
1410576	alqtdv**LQENQ**k
1411387	ansldn**LKSGV**i
1418283	ctkgls**IADLA**c
1513648	**TEVRG**srrlaqq
1473904	ltyl**NLSSE**lkq
1509536	sssfdc**IVNLL**f
1418007	c**SNSSS**galdtt
1526382	vlvn**VSQTS**ian
1510901	sv**IPSLP**rsgsr
1415918	c**FVNTT**idnett
1471298	**LQENQ**rilaasf
1385058	ls**IADLA**caqyyngim
1427980	**EISTL**enksael
1475433	lyvsws**DGDGI**t
1648957	ctd**AVLTY**ssfgvcad
36723	litg**RLAAL**

Table 5. Immunoreactive epitopes of human Coronaviruses membrane protein containing peptides shared with human endocrinocytes autoantigens.

ID in IEDB	***MP epitope sequence***
1312642	hgtil**TRPLL**eselv
56634	ryri**GNYKL**
1531436	vyhryri**GNYKL**
1488579	plveds**TSVTA**v
1443619	grtvvr**PLVED**s
1646603	a**TVIRG**hlyiqgvklg
1498820	**RLPST**qkgsgmd
1414330	awnp**EVNAI**tvt
1470736	lpeym**TVAVP**st

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Table 6. Immunoreactive epitopes of human Coronaviruses nucleoprotein containing peptides shared with human endocrinocytes antigens.

ID in IEDB	***NP epitope sequence***
25542	idayktf**PPTEP**kkd
2431	al**ALLLL**dr
60669	srggsqassr**SSSRS**r
1075010	NTN**SSPDD**qigyy
51074	qigyyrratr**RVRGG**dgk
33669	ktf**PPTEP**kkdkkkk
19442	getal**ALLLL**
7807	ddkdpqfk**DNVIL**lnk
4782	assr**SSSRS**rgnsrnst
1440437	ggnsq**SSSRA**ss
1423544	dqi**SEPPK**eqrv
1422553	**DLQGN**fgdlkfn
1501942	**SASNS**rpgsrsq
1437039	**FTVST**qpqenti
1411373	ansgnra**PTSGV**
1430757	evrqk**ILNKP**rq
1429244	epqk**DVYEL**ryn
1409816	akl**GKDAT**kpqq
1436882	ftpgkq**SSSRA**s
1429742	erwrmr**RGQRV**d
1490443	pt**REENV**iqcfg
1406449	**AAALK**slgfdkp
1407387	adepds**RAPSR**s
1421399	dhph**LGKFL**eel

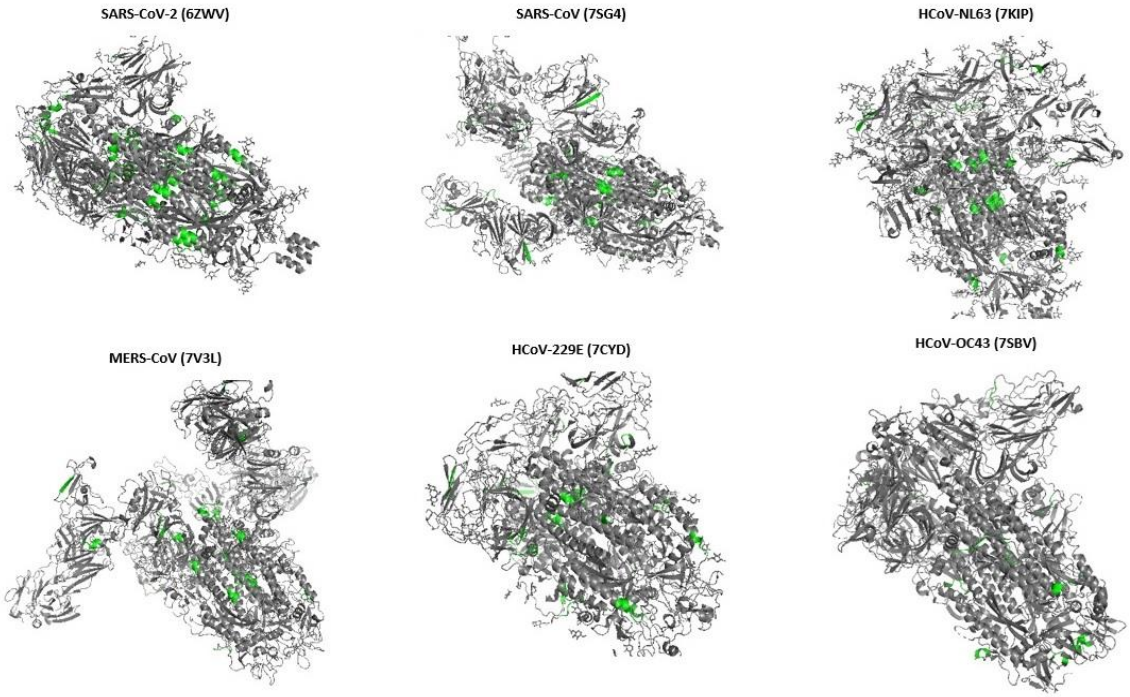

Figure 1. Location of pentapeptides shared with human endocrine autoantigens in 3-D structure spike glycoproteins of all human coronaviruses. (According to databases: PDB [24] and AlphaFold [25]). Pentapeptides are shown with grass-green.

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Pathohistological data

Immunohistochemical study of the organs of the endocrine system (pituitary, thyroid and adrenal glands) taken on autopsies from the patients who had a severe lethal course of COVID-19, showed a high incidence of SARS-Cov2 infection of their endocrine cells. However, the affinity of SARS-Cov2 to endocrine cells was selective. Thus, the abundant expression of viral proteins by the cells of the adenohypophysis was combined with the contrasting complete absence of its expression by the cells of the neurohypophysis (Fig. 2, A-D). Selective SARS-CoV2 infection of endocrine cells was not accompanied by their apoptotic death, as demonstrated by the absence of Caspasae3 expression by degenerative and dying endocrinocytes (Fig. 2, I-H). The latter is most likely due to other mechanisms of death prevailing in cells affected in COVID-19. A similar effect was observed earlier in our studies as well as in works by other researchers [26] and undoubtedly requires further studies.

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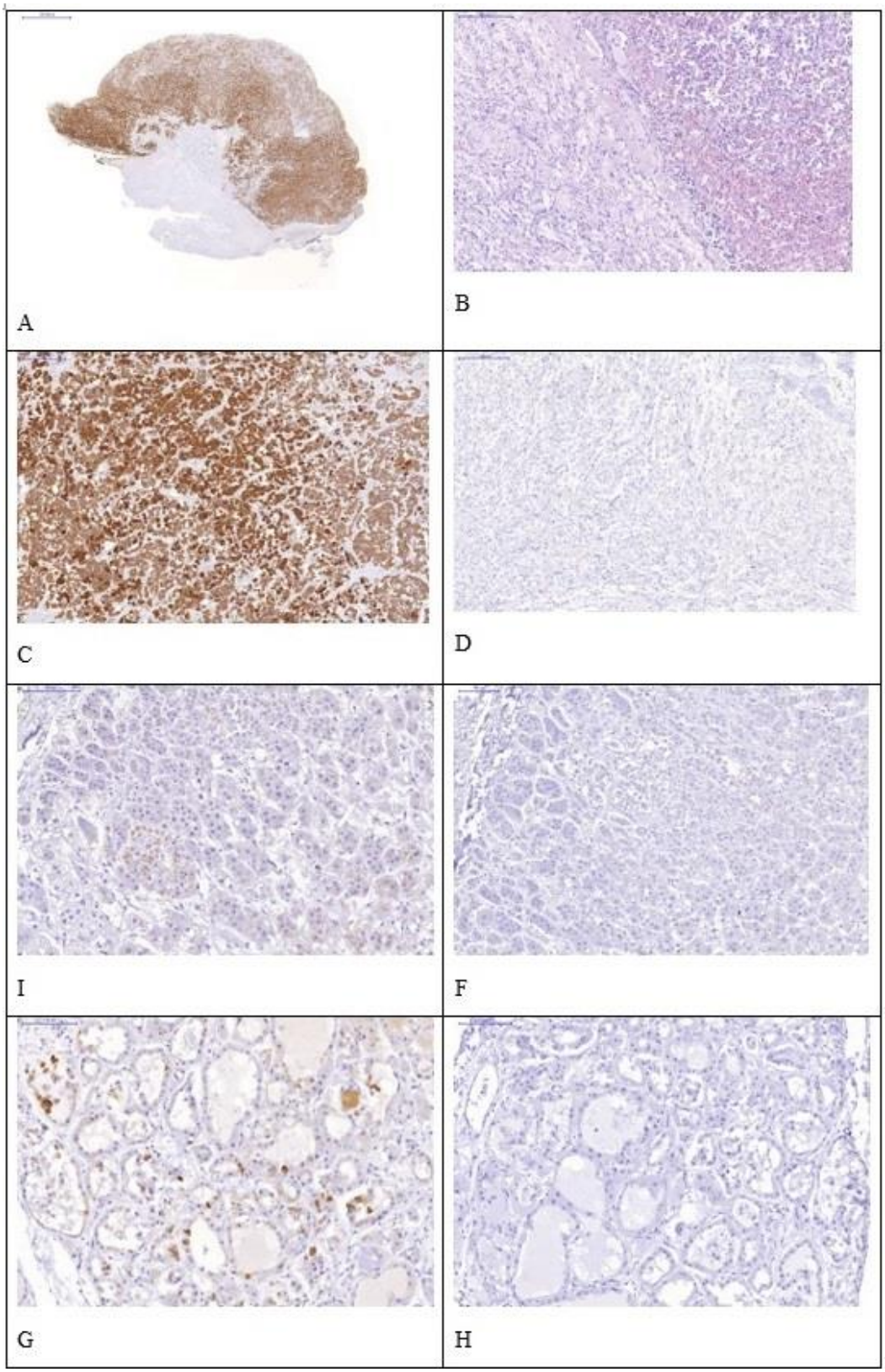

Figure 2. Changes in some endocrine organs of patients who died from COVID-19 infection
Uneven expression of SARS-Cov2 spike antigen by pituitary cells (A, B): abundant expression by adenohypophysis cells (C) and no expression by neurohypophysis cells (D);
Focal expression of the SARS-Cov2 spike antigen by a group of degeneratively altered *Caspasae3-negative* adrenal parenchymal cells , arrows (I, F);
Expression of SARS-Cov2 spike antigen by *Caspasae3-negative* thyroid follicle cells (G, H).
A, C, D, I, G—immunohistochemistry (IHH), rabbit polyclonal anti-SARS-CoV-2 Spike (GeneTex, USA), DAB; F, H—IHH, mouse monoclonal anti-Caspasae3 (clone 3CSP03, Diagnostic BioSystems, USA); B - H&E.
The length of the scale segment A - 2000 μm, B, C, D - 200 μm; I, F, G, H - 100 μm.

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At the same time, manifestations of virus-induced transformation of parenchymal cells in endocrine organs infected with SARS-CoV2, were sharply manifested. Obvious infiltration of the stroma and parenchyma of the endocrine organs with lymphocytes was observed, which commonly is associated with a cell-mediated immune mechanisms of their lesion (Fig_3).

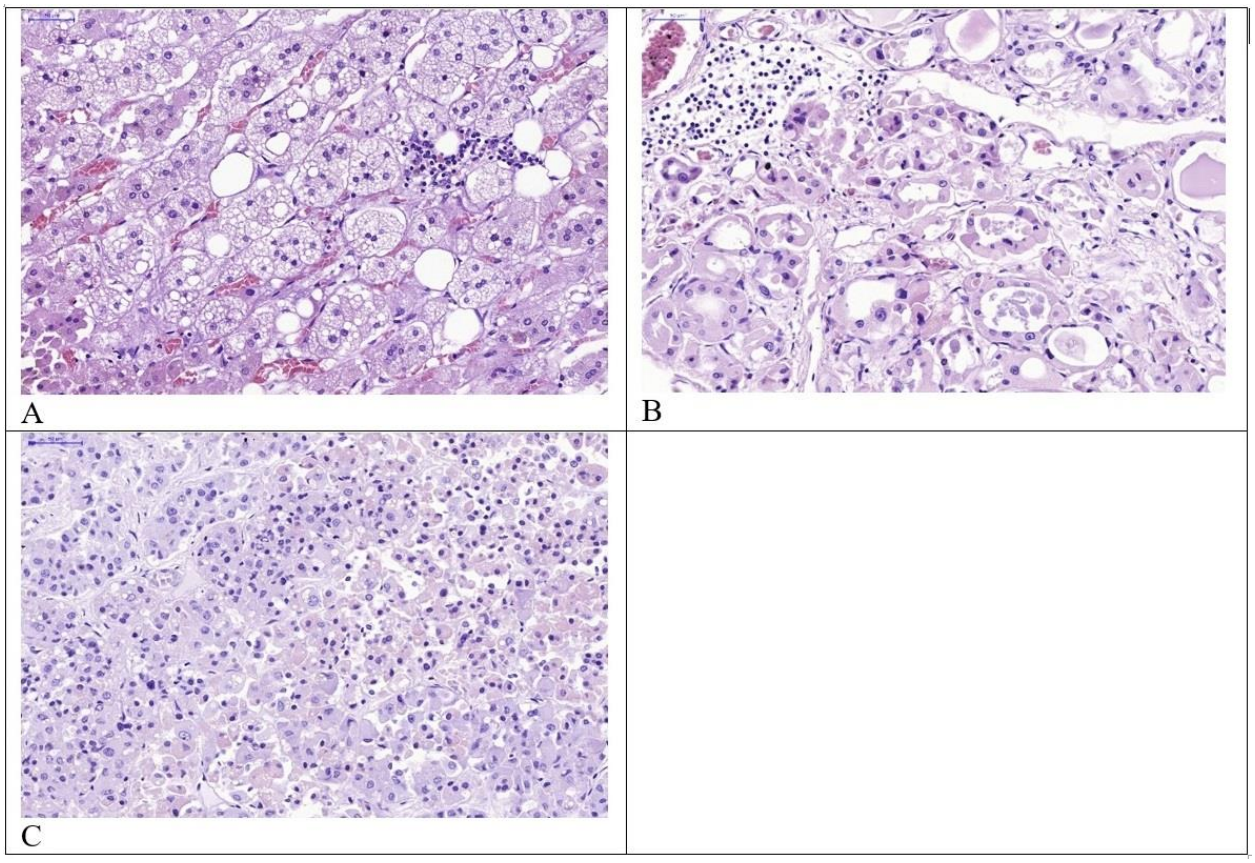

Figure 3. Infiltration by lymphocytes in stroma and parenchyma of some endocrine organs in victims of lethal COVID-19
(A - adrenal gland, B - thyroid gland, C - pituitary gland) A, B, C - H&E. Scale bar length: A, B, C - 50 μm.)

DISCUSSION

Although pathogenesis of COVID-19 sequela is still far from entire comprehension, it is generally accepted that antiviral protection is activated as a result of viral PARPs recognition by various variants of body pattern-recognizing receptors (PRRs): Toll-like receptors (TLRs), RIG-I-like receptors (RLR) and the cytoplasmic proteins family receptor involved in caspase activation (NALP), in ensemble causing involvement of both paleo- and neo-immunity components and resulting in the development of inflammation [21, 27-28].

The overcoming of antiviral defense by viruses is associated with the evolutionary developed strategies of pathogens to influence the immune system so that allows the virus to ensure its sufficiently effective reproduction. To understand the mechanisms for achieving this goal, searches are underway for the mimicking amino acid sequences in viral proteins and in human immune system proteins suggesting that it is precisely homologous amino acid sequences in viral proteins that can unbalance immune regulatory mechanisms and cause a wide variety of immunopathological consequences: from immunosuppression to a cytokine storm.

Currently, there are four major criteria for identifying molecular mimicry as a provoking mechanism of autoimmune diseases [29]: 1) “similarity between the host epitope and the epitope of a microorganism or

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environmental agent", 2) "detection of antibodies or T-cells that cross-react with both epitopes in patients with autoimmune disease", 3) "epidemiological link between the exposure to an environmental agent or microbe and the development of autoimmune disease", and 4) "reproducibility of autoimmunity in an animal model following sensitization with appropriate epitopes either after infection with a microbe or exposure to an environmental agent". To date, to our knowledge, only the first and third criteria are satisfied for the post-COVID syndrome or COVID-19 vaccine-associated side effects.

At the same time there are some concerns regarding the molecular mimicry concept itself. First of all, humans are challenged with multiple infections throughout their lives, including infections with the pathogens whose antigens cross-react with the human ones, but not all infected humans develop autoimmune diseases. For example, Kanduc et al. [30] demonstrates that pentapeptides of 30 common viruses are disseminated throughout practically the entire human proteome, and each viral pentapeptide is repeated almost more than 10 times. This massive viral-human peptide overlapping calls under doubt the possibility of the direct causal association between the virus–host sharing of amino acid sequences and the incitement to autoimmune reactions. Indeed, autoimmune diseases should theoretically approach a 100% real incidence according to Kanduc et al. [30], since the 30 viruses they examined practically are more or less disseminated throughout the entire mankind. Moreover, two years later Trost et al. [31]examined 40 pathogenic and nonpathogenic bacterial proteomes for the amino acid sequence similarity to human proteome and reported that one third of human proteins shares at least one nonapeptide with someone of these bacteria. Then the authors detailed the bacterial peptide overlapping with human proteome at the penta-, hexa-, hepta- and octapeptide levels using exact peptide matching analysis and demonstrated that virtually every human protein contains a bacterial pentapeptide or hexapeptide motif [32].

Besides that, although for a long time it was believed that T-cell receptors recognize sequential determinants only, several recent lines of evidence have demonstrated that the T cell cross-reactivity analyses could not rely on sequence similarity alone [33]. It was shown that individual T-cell receptors could recognize different peptide/MHC complexes that do not show strong sequence homology, and it was suggested that structural criteria rather than primary sequence might be critical for the T-cell receptor recognition.

In spite of all the above mentioned doubts, the fact of molecular mimicry displayed by the immunoreactive epitopes of SARS-CoV-2 proteins with marker autoantigens of common human endocrinopathies maybe of some prognostic significance. Indeed, 17-alpha-hydroxylase is an autoimmunity target in diseases involving steroid-producing cells, especially in Addison's disease, autoimmune polyglandular syndrome type I, and premature hypogonadism [34-35-36]. Carboxypeptidase E is a target of autoimmunity in late-onset (latent) autoimmune diabetes of adults with some diagnostic value to distinguish it from diabetes mellitus type 2 [37]. The same is true for receptor-type protein-tyrosine-phosphatases which are targeted in autoimmune diabetes mellitus [38]. Finally, the cytotoxic T-lymphocyte 4 antigen (CTLA-4) is expressed in pituitary, and antibodies against this target induce autoimmune hypophysitis [39]. The involvement of adenohypophysis and adrenals in antigen mimicry and in immunopathological inflammation during severe COVID-19, which we have demonstrated, seems to be very important for untoward lethal course of disease, because it apparently may jeopardize the appropriate defensive stress-related mechanisms protecting from systemic hypercytokinemia and related hemodynamic shock [16]. In our studies most pathogenic among human Coronaviruses (SARS-CoV and MERS-CoV) possessed with greatest number of epitopes shared with human endocrinocytes. SP of various Coronaviruses shared greatest number of epitopes with endocrine autoantigens, compared to their MP or NP. Thyroid autoantigens were most active in "sharing" their epitopes with highly pathogenic Coronaviruses, which corresponds to growing number of cases of autoimmune thyroid diseases cases described during and after COVID-19 [40] and even few of them – after anti-COVID-19 vaccination [41]. At the same time seasonal Coronaviruses quite often shared their immunodominant pentapeptides with several autoantigens of pancreatic islet β-cells. Before current pandemic they rarely attact attention as potential viral diabetogens, although in veterinary medicine insulin dependent diabetes due to seasonal coronavirus was described in a foal [42]. Interestingly, neurohypophysis was free from any histological signs of involvement in severe COVID-19, as well as its autoantigen, rabfillin-3A, displayed only one pentapeptide shared and with one seasonal human coronavirus only. These data may be related to recently discovered fact of protective role in COVID-19 played by oxytocin secreted by neurohypophysis [43].

Finally, we consider bioinformatics analysis to be an essential step in the preliminary evaluation of the risks and autoimmunity spectrum in COVID-19 complications, including the post-COVID-19 syndrome. Similar point of

view recently was emphasized by other researchers [44] Additionally, it may be useful in epitope selection for elaboration of the safest anti-COVID-19 vaccines. S [45].

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