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# Hypotheses on Need of Functional Receptor System for SARSCoV-2, Not Just ACE2, for Infectivity and on Binding of SARSCoV-2 in Blood Mainly with Serum ACE2

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## Abstract

The aim of this paper was to put forward and justify - basing on the literature - two hypotheses:

A. SARSCov-2 viruses bind [are blocked] in the blood of people infected with

   1. Soluble ACE2 [plasma ACE2], and

   2. With a membrane of a small fraction of erythrocytes; blocking viruses in such a way would lead to significant weakening of the pandemic

B. Not every ACE2 molecule in first of all the epithelial or endothelial cell membranes [and not just t his molecule] is a functional receptor for SARSCoV-2; a functional receptor must not only recognize the virus and bind it, but „draw it inside the cell", which is therefore the target cell; only people who have such functional receptors are susceptible to infection.

Fully functional SARSCoV-2 receptor: a: Must recognize receptor binding domain [RBD] in virus spike proteins. b: Through the TMPRSS-2 protease molecules present in it, appropriately located next to the enzymatic ACE2 fragment, should cut the spike protein of sucked virus in the appropriate place. c: It should also contain furin and cathepsin L [the latter in endosomes] to facilitate additional cleavage in the virus, leading to fusion and release of viral genetic material. d: Must have appropriate and properly distributed lipid microdomains rich in cholesterol [112] and sphingolipids, without which it is impossible to fuse the membranę with the viral envelope, and thus endocytosis.

These microdomains, the so-called rafts, seem to be a carrier of the quasiallosteric effect, which clearly changes the spatial structure of the sites in the cell membrane, enabling fusion.

In people with fully functional viral receptors, the expression of the correct variants of the ACE2, TMPRSS-2, furin and cathepsin L-genes must be very efficient. Also this expression, but also probably the virus binding itself [with the fusion] requires certain conditions [epigenetic].

Non-functional receptors - resulting from the expression of mutants of the above-mentioned genes [with different polymorphisms] either do not recognize the virus at all [despite the correct ability to generate Ang1-7 and participate in the transport of amino acids or recognize it, but the effect is weak or, despite the correct recognizing, they are not capable of fusion.

Final conclusion Y: SARSCoV-2 viruses bind to 1. The ACE2 molecules circulating - in quite significant amounts - in serum [hypothesis A1]. 2. Erythrocytes' membranes using so-called hemagglutinin esterase occuring in the virus envelope, acting on sialic acid of erythrocytes' glycans; it is possible that the virus attaches itself particularly to the membrane of erythrocytes already attacked by free radicals [ROS] [hypothesis A2].

Final conclusion Z: It is quite justified to assume that the level of fully functional SARSCoV-2 receptors grows with age whereas the level of serum ACE2 being the main component of the viruses' block conversely - it decreases with age

***Keywords:*** *ACE2; Covid19; soluble ACE2; hemagglutinin; sheddase [ADAM17]; TMPRSS-2 [priming enzymes]; rafts; functional receptors of SARSCoV-2*

ACE2 [angiotensin converting enzyme 2] was found to be Covid19 receptor [also SARS and NL63, but not MERS] [1]. The viruses, so Covid19 are so dangerous because we, let virus in owing the receptors. They "suck the virus inside the cell". And their formation is encoded in our genes.

It is not reported at all what are COVID-19 target cells! Are they cells of all mucous membranes or may be bronchi or alveoli or all endothelial and epithelial cells? If all cells exerting ACE2 activity are the target cells for Covid19? Or if every membrane ACE2 is enough to be a functional Covid19 receptor?

I am assuming that there is a certain group of people who will not become infected even in contact with an infected person, i.e. with his/her breath and secretions. Why? Because these people do not have functional receptors for the SARS CoV-2 virus. Another words, they are not susceptible. I believe that if everyone had such receptors, the pandemic would spread so much that, in fact, probably more than half of people would be seriously ill, and finally over 15% of the population would die. Let us consider the fact that the receptor for SARS CoV-2 is angiotensin converting enzyme 2, present in the cell membranes of many epithelial cells, not only in the lungs, not only in the respiratory tract, but basically in all blood and lymph vessels, in the heart, kidneys, in virtually all membranes of the entire digestive system, but essentially the entire body with greater or lesser density [2-4]. Quite a lot of ACE2 exists in blood serum [3,4].

If every ACE2 molecule was really a receptor, it would have to be absolutely tragic: much, much worse than it is even in places with extremely high infection rates like, say, the United States, or Spain, or earlier in Wuhan.

## Aim of the Study

The aims of the study are to put forward and justify - basing on the literature - two hypotheses:

A. SARSCov-2 viruses bind [are blocked] in the blood of people infected with

    1. Soluble ACE2 [plasma ACE2], and

    2. With a membrane of a small fraction of erythrocytes; blocking viruses in such a way would lead to significant weakening of the pandemic - in some way turning it into a hidden pandemic.

B. Not every ACE2 and not just molecule in first of all the epithelial or endothelial cell membrane is a functional receptor for SARSCoV-2; a functional receptor must not only recognize the virus and bind it, but „draw it inside the cell", which is therefore the target cell; only people who have such functional receptors are susceptible to infection.

So, an additional objective is to consider if the level of such functional receptors for SARSCoV-2 increases with age thus explaining that the infectivity of people over the age of 70 is particularly high.

### Hypothesis A1

The SARSCoV-2 virus, with its S [spike] protein binds to the receptor being the ACE2 enzyme. So it is possible that viruses that get into the blood [and anyway most viruses from the cells get into the bloodstream and/or lymph or generally in extracellular space] quite strongly bind to the ACE2 molecules circulating - in quite significant amounts - in serum.

### Hypothesis A2

SARSCoV-2 viruses bind to erythrocytes' membranes using so-called hemagglutinin esterase [5,6] occuring in the virus envelope, acting on sialic acid of erythrocytes' glycans.

In case both hypotheses are true most of viruses is for a long time excluded from the „chain of pathogenicity and infectiveness", because: a. viruses are bound, b. they stopped to be multiplied. c. they hardly can infect anyone. But what's more: in the initial period after infection [which is extremely easy] the number of viruses in blood is much smaller than the number of potentally blocking objects including circulating ACE2 molecules, which means that they most possibly are completely blocked. Despite the fact that in parallel the number of viruses arrives as a result of multiplication by cells - in the lungs [and/or elsewhere].

And this phenomenon can, in my opinion, be the cause of the „hidden pandemic". But maybe we should rather talk about an alleviated phase. A more detailed discussion of this phase of the pandemic will be the subject of a separate paper on the non-epidemiological model of the spread of the pandemic.

## Potential viral blocks in the blood: plasma ACE2 [a shedding]. Erythrocyte membrane

There is a special enzyme called sheddase [ADAM 17] that detaches the outer domains [ectodomains] from membrane-bound proteins and they simply pass out into the plasma [7]. ADAM enzymes (a disintegrin and metalloproteinase) are $Zn^{2+}$ dependent, multi-domain cell surface proteins which belong to the adamalysin protein family. They are closely related to other metalloenzymes, even snake venom metalloproteinases (SVMP). There are a whole series of such enzymes in the human body depending on the presence of phosphatidylserine on the surface of the cell membrane] [8-10] disintegrins ADAM, which detache from its entire enzyme its extracellular domain, so-called ectodomain [7].This is the case with ACE2 [7,11].

It is not certain whether only ADAM17 action is a source of relatively large amounts of ACE2 in serum able to bind to the virus. If sheddase activity were increased, more ACE2 would be found in the serum and less in the membranes, and therefore the fewer membrane virus receptors there would be.In my opinion the shedding of ACE2 from the membrane might be slightly more effective in younger people than the older ones. Just take into consideration that ADAM 17 and similar enzymes are crucial for the liberation of very important bioregulators deciding on the regulation of growth and immunity [7].

Ectodomain shedding of proteins may be regulated by several stimuli, including protein kinase C (PKC), calcium ionophores, G-protein-coupled receptors, and mitogen-activated protein kinases Murphy (2009) [7,11-13].

Some researchers claim that such ectodomains [ie serum ACE2] with blood flow to the kidneys and are excreted in the urine [form of „microalbuminuria"] [9,14]. But it turns out that in sheddase-free cell lines, the virus is better bound [SARS-work by Jia., *et al*. 2009 [15]]. But this is no evidence, because even if sheddase was present [but of course not cut off the ectodomain yet] a virus would be tied up. Jia., *et al*. studied the origin of „soluble" ACE2 in the upper human airways and suggest that this form of ACE2 is needed to modify inflammatory processes [15].

It turned out [Bennion., *et al*. 2016 [16]] that in patients with ischemic stroke serum ACE2 activity is much lower than in healthy people, but the sheddase activity in both groups was the same. Maybe some plasma proteases which level increase during ischemic stroke, cut soluble ACE2;the other explanation is the existence of low-molecular inhibitor of ACE2 [14].

By the way-if we concern shedding, surface remodeling by secreted lysosomal enzymes might lead to endocytosis-mediated plasma membrane repair [17].

Xiao., *et al*. [2016] [18] have shown that the formation of „soluble" ACE2 in the proximal tubules by sheddase [ADAM17 i.e. metallopeptidase being identical to tumor necrosis factor-α-converting enzyme: TACE] is particularly high in [diabetic] hyperglycemia, but is also absolutely dependent on the activity of membrane protein kinase C δ [PKCδ] one of the major regulatory pathways. Most probably due to high glucose levels - the passage of PKCδ from the cytosol into the membrane results in activation of sheddase. But very strange ADAM-17-mediated shedding of ACE2 does not take place in pancreatic islets of male mice-experimental diabetes model [19].

Roca-Ho., *et al*. [2017] [20] believe that the increase in the amount of ACE2 in diabetes [mouse model] occurs especially in the pancreas, liver and serum and that this increase in serum ACE2 is something like the hypercompensation of the renin-angiotensin-aldosterone system. Braz., *et al*. [2020] [21] found that the plasma ACE/ACE2 ratio was higher and the angiotensin II to Ang 1-7 ratio was lower in patients with rheumatoid arthritis. This suggests the lack of correlation of the soluble form concentration and its enzymatic activity exists.

Regardless of the amount and origin of the soluble ACE2 it is undoubtedly enzymatically active, and therefore produces angiotensin 1-7, very beneficial for our body, for the lungs, heart, kidneys and, in general, for the entire human mineral and water management [21-23]. However, the inhibitory effect of a small molecule ACE2 inhibitor in plasma [probably of a peptide] [17] should be taken into account.

Such an inhibitor is very common; hence it has been reported that the enzymatic activity was not parallel to the amount of ACE2 protein [24].

But regardless of this, such serum ACE2 will obviously bind viruses. It turned out that soluble ACE2 partially inhibits the entry of the virus into target cells [25]. But this is the best confirmation of my hypothesis B! Because why soluble [serum] ACE2 inhibits the entry of the virus? Because it competes with the right membrane receptor for the virus-like competitive inhibition ! But regardless of this, such serum ACE2 will obviously bind viruses.

Battle., *et al*. [4] came to the conclusion that ACE2 serum could be used in therapy It has been suggested that probable way of fighting the CoV could be the injection of ACE-2 which will result in the preventing the interaction of the CoV to off-infected cells [26]. Lei., *et al*. [2020] [27,28] generated a novel recombinant protein by connecting the extracellular domain of human ACE2 to the Fc region of the human immunoglobulin IgG [however, ACE2 mutant with low catalytic activity was also used in the study]. Fusion protein has high affinity binding to the receptor-binding domain (RBD) of SARS-CoV and 2019-nCoV [27]. Moreover, fusion proteins potently neutralized SARS--CoV and 2019-nCoV *in vitro*. So there is a possibility that plasma would act favourably, not only as a possible source of antibodies, but also above all this so-called soluble ACE2, which would bind the virus. And since it would not be related to cell membranes, it would not enter cells, there would be no virus multiplication there.

If only plasma ACE2 binds virus-? I think not. SARS [an „older cousin" of the current virus] has the protein hemagglutinin esterase [5], which has an affinity for certain complex carbohydrates [sialic acid] in the erythrocyte membrane, ergo predestines viruses to bind to erythrocytes. Quite recently Kim [6] found that SARS-CoV-2 contained hemagglutinin-esterase(HE) as well. SARS-CoV-2 hemagglutinin--esterase (HE) acts as the classical glycan-binding lectin and receptor-degrading enzyme. It acts as the classical glycan-binding lectin and receptor-degrading enzyme. Besides purified viral S glycoprotein [not necessarily HE] can agglutinate chicken erythrocytes [29], indicating that the major hemagglutinating factor might be the spike protein which acts as the major sialic acid binding protein. O-acetylated SAs interact with the lectin-like spike glycoprotein of SARS CoV-2 for the initial attachment of viruses to enter into the host cells.

Let us take into account that spike proteins of the viral envelope are quite densely glycosylated, which, even according to some researchers, changes the possibilities of binding the virus to the receptor and antibodies [30]. Inhibition of glucosidases in cell endoplasmic reticulum was found to impair the entry of viruses via a post-receptor-binding mechanism ie altering the glycan processing of ACE2 [30].

Would it be beneficial for the organism. On the one hand, yes, because it would block viruses, and it is known that the virus would not enter the erythrocytes, and even if it did, it would not be possible to duplicate it, because we know that mature mammalian erythrocytes do not have the genetic apparatus.

But of course, it could have a negative effect on haemoglobin binding oxygen anyway. Besides, it was claimed [31] that beyond the classical pulmonary immune-inflammation view, the occurrence of an oxygen-deprived blood disease, with iron metabolism dysregulation, might happen during Covid19. Also prominent erythrocyte aggregates obstructing the lumen of renal capillaries might prove binding of SARSCoV-2 with erythrocytes [32].

However, it is possible that the virus attaches itself particularly to the membrane of erythrocytes already attacked by free radicals. Such erythrocytes would be destined to be destroyed anyway, and in turn could bind the virus, thus block it. If even a small percentage of erythrocytes dead [and renewed] per day bind viruses, it would be a significant „block enhancement".

### Towards hypothesis B. More about why the percentage of functional receptors increases with age

According to Aguiar., *et al*. [2020] [33] incidentally, indicating a very low expression of ACE-2 in the lungs and suggesting the possibility of other - especially in the lungs - types of receptors and co-receptors for SARS CoV-2 than ACE2 [although they still consider it?! - this enzyme is the „decisive receptor"]. They consider ADAM 17, which is the very sheddase that detaches ACE2 from membranes, to be one of these important additional but important receptors for SARS CoV-2. This would be a contradiction to my previous considerations: because the more of this sheddase, the better [assuming the concept of Aguiar., *et al*. [33]] the binding of the virus could be, not worse, as I think. But according to other authors [12,13,34] the proteolytic cleavage - facilitating the fusion of the cell membrane with the viral lipid envelope, to the cytoplasm, or rather to the endosomes of the target cell [priming] - occurs under the influence of other proteases [TMPRSS2, furin, cathepsin L], not just ADAM17.

How can we know what the infectivity limits are? Just look for data on how many ACE2-producing cells there are in the human body. There were studies which showed that in a single human body, there are about 1.3 or 1.6 x $10^9$ such cells, i.e. about 1% of all epithelial and endothelial cells [35-40], i.e. those that border with the lumen of blood vessels, lymph vessels and the digestive tract, that is, they are „on the membrane".

Epithelial cells account for about 7%, or $10^{12}$. Studies on the expression of the ACE2 gene, both at the RNA and protein levels, have shown that the highest expression of potential SARS-COVID-2 receptors is in the small intestine. This is a frightening amount, much greater than elsewhere in the body [35,39,41]. There is also a lot in the testes [could this be one of the reasons why men may be sensitive to COVID-19?; the other one is the localization of gene for ACE2 on chromosome X [42].

Then we have a group of organs and tissues, which have quite a lot of ACE2. These are: kidney, heart, thyroid, adipose tissue, salivary glands. Much less of them exists in the lungs, liver and adrenal glands and the least ACE 2 is found in nerves, muscles [striated], brain and spleen [39].

In summary, the number of cells containing ACE2 is approximately 1.5 - 2 x $10^9$ in a single human body. However, if we multiply that potentially by a million recipients, this will give us numbers of the order of $10^{15}$, maybe $10^{16}$.

So if we know how many ACE2 cells we have in a human body, we get a ceiling because more viruses cannot stick. It is not the shedding [that eventually diminishes with age] the reason enough that with age we have more and more functional receptors for SARS CoV-2. Shedding affects [in my opinion] only fractions of a promille, while apparent differences in infectivity depending on age are clearly enormous. I assumed [separate paper!] that the % of functional receptors decreases with age exponentially [prepared for separate publication]. If, for the oldest group, the mean content of target cells containing fully functional SARS CoV-2 receptors is 80% of all cells having ACE2, this is 40% for group II, 20% for group III and for the youngest [group IV] on average 10% [from 1.65 x $10^9$ for one person; probably for the 0 - 5 years old group it may be even 10 times less].

There are reports that the level of ACE2, a potential SARSCoV-2 receptor, is higher in the elderly, but in the small intestine [41], the richest reservoir of ACE-2 in the human body. This fact could support my idea that the level of viral receptors increases with age, therefore it is justified to assume the highest "ceiling" for the virus, i.e. a higher maximum number of SARSCoV-2 binding sites in the oldest group. The authors [41] did not investigate the level of ACE-2 in other tissues. However, increased expression of the ACE2 gene in one tissue makes it probable, although not absolutely certain, the possibility of increased expression in other tissues.

The matter is complicated by two problems:

1. Whether the level of serum ACE2, is parallel to its level in cells, or more precisely in the cell membrane [3,5,16,43,44]. Skarstein-Kolberg [2020] [45] claims that: a. The direction of action, ACE and ACE2 levels are opposite. b. Since the serum ACE level is higher in children, the ACE2 level is probably lower. c. It is not known whether lower infectivity of children - than adults -- with Covid19 is associated with low levels of membrane-associated ACE2. d. It is unknown whether there is an inverse correlation of serum ACE2 levels and ACE2 expression in the lungs and other tissues. The above statement a does not have to be true regarding the levels of ACE and ACE2. Statement b seems to be absolutely unsupported.

2. The role of ACE2 in the course of Covid19 is, or rather may be, dual. On one hand, it is a potential virus receptor, and on the other hand, it is very beneficial for the body - and therefore protects the lungs and heart during disease caused by the virus, because it produces Ang1-7, which has a beneficial effect on blood pressure, vasoconstriction, but also on kidney function; especially since ACE2 simultaneously destroys angiotensin II [Ang 1-8], which has an opposite, detrimental effect. A number of authors are therefore of the opinion that the beneficial effects of membrane ACE2 outweighs possible negative effects [46-51].

The same discussion is about the favourable [protective] or unfavourable role of serum ACE2 and thus ADAM17 shedding and sheddase [12,52-54]. Especially that some researchers consider serum ACE2 to be an indicator of ACE2 gene expression. The activity of serum ACE2, so production of Ang1-7, is not good measure of amount of serum ACE2, which still might bind SARSCoV-2, as there exists small molecular compound, most probably short peptide, being strong ACE2 inhibitor [24].

Fagyas., *et al*. [55] reported about a much higher content of plasma ACE2 in people from the oldest group [75+] with aortic stenosis. But activity of serum ACE2 was four times higher for the group with aortic stenosis compared to the activity in healthy people, but also those suffering from hypertension 75+ but without stenosis.

Mind you, my be it is out of the scope. Not all individuals exposed to HIV become infected [as those famous African sex workers. Su., *et al*. [55] found that the IRF1 [Interferon regulatory factor-1] promoter in HIV-resistant people was primed by increased basal histone deacetylase-2 binding, independently of transcription regulators, STAT1 and nuclear factor-κB/p65, implicating an epigenetic silencing mechanism. These data suggest that transitory IRF1 responsiveness in HIV-R may be one of the key contributors to the altered susceptibility to HIV infection during the early stages of primary HIV infection.

## Summarizing of arguments for hypothesis B

In general, the SARS CoV-2 functional receptor cannot be the same as any membrane-bound ACE2 molecule [with its amino acid transporter domain. This is because of discussed below.

**Modifications and structural aspects:** ACE2 being transported to the membrane [trafficking] can also undergo post-translation modification [30]. However only glycosylation was reported. But this is crucial for virus entry. ER glucosidases I and II sequentially located in endoplasmic reticulum trim the three terminal glucose moieties on the N-linked glycans attached to nascent glycoproteins. These reactions are the first steps of N-linked glycan processing of ACE2 molecule. Chan., *et al*. [57] used deep mutagenesis to identify ACE2 mutants that bind more tightly to the spike protein and combined mutations to further increase binding affinity.

Besides postrantranscription modification might take place, especially the splicing stage. The ACE2 gene is located on chromosome X and contains 18 exons, many of which resemble the corresponding exons in the ACE gene [57]. The complete cDNA encodes a 805 amino acid protein, which resembles a chimera composed of a single ACE-like catalytic ectodomain fused to the transmembrane protein, collectrin which plays a role in regulation of renal amino acid transport and has been implicated in insulin exocytosis and β-cell proliferation [57]. The collectrin domain might be important for some kind of virus penetration, while the other routes do not need this part of molecule [58].

ACE2 is a chaperone for aminoacids' transporter domain, which is not involved in dimerization, suggesting that ACE2 may be a homodimer even in the absence of collectrin domain. Cleavage of the C-terminal segment, especially residues 697 to 716 of ACE2 by proteases,

such as transmembrane protease serine 2 (TMPRSS2), enhances the S protein-driven viral entry. However the presence of collectrin domain may block the access of TMPRSS2 to the cutting site on ACE2 [59].

**Need of priming enzymes:** The functional viral receptor may consist quite a big fragment of entire membrane of the target cell, in any case not just the membrane ACE2 itself; it may include the protease TMPRSS2 [25,61-66] and furin, and possibly as co-receptors the CD147, and GRP78 protein, cathepsins L and B [66], and even. ADAM17 sheddase [39]. But it turned out that a similar phenomenon - called „priming of virus" - can occur under the influence of trypsin, elastase and some other proteolytic enzymes [60], but especially lysosomal protease: cathepsin L [25]. But do these cathepsin L work inside or extracellularly? Certainly, both [because there are known cases of both endocytosis and exocytosis of such enzymes]. Kominami., *et al*. [67] proved that about 30% of cathepsin L of macrophages undergoes exocytosis and passes into serum. Similar situation was shown within fibroblasts.

The cleavage of S1-S2 subunits to expose S2 for fusion to cell membrane via host proteases including cathepsins, cell surface transmembrane serine (TMPRSS) protease, furin (expressed highly in lungs) [68,61] trypsin and factor Xa [69] were described. It is suggested that blocking TMPRSS2 may be a key strategy for treating patients [25]]. It was hypothesized that the additional furin site present at S1/S2 may also allow SARS-CoV-2 to spread more efficiently than other SARS-like-CoVs [70].

It is interesting that as a result of auto lysis [or autoactivation] the extracellular catalytic domain of TMPRSS2 may be detached from the rest of the enzyme and go to plasma [71,72]. But again: is this a form of „irretrievable disposal" of this protein [on the way to urine] or to make it work „elsewhere" and destroy viruses before they connect to ACE2?! Well, the truncated form of TMPRSS2 containing the catalytic domain shed into supernatants, displays only a minor enzymatic activity [63]. But it is not sure if such a situation takes place in whole body.. Both SARS-CoV-2 receptor ACE2 and TMPRSS2 are primarily expressed in bronchial transient secretory cells [64]. Very limited -both in range and localization [only special line of cells]-amount of ACE2 was found in the most most vulnerable to virus attack pneumocytes AT2 in lungs [73].

Interestingly, this enzyme [TMPRSS2] is „upregulated" by androgens [74] and is involved in the etiogenesis of prostate cancer. This may be one of the reasons men are more likely to suffer from Covid 19 than women; compare [42].

Here we should remind that SARS-CoV-2 utilizes two pathways of penetration [25]. If the plasma membrane-route proteases [mostly TMPRSS2] are available, the virus can fuse via an "early pathway" at the plasma membrane, but if not, the virus can fuse via a "late pathway" at the endosomal membrane [together with ACE-2].

Thus, the activation of virus fusion varies, depending on the protease in the local environment, what confirms virus flexibility. The „late pathway" might be clathhrin dependent [then the cytoplasmic tail of ACE2 is not required] [59] and independent [75]. The last manner was shown to be dependent on lipid neighbourhood [compare bit later].

## The overwhelming role of structure of ACE2 neighbourhood

Perhaps for membrane fusion with the viral lipid envelope and endocytosis, microdomains in the cell membrane structure rich in sphingolipids and cholesterol are needed. Wang., *et al*. [75] showed already in 2008 that removal of cholesterol from the membrane inhibited the binding of the SARS pseudovirus. Moreover, cholesterol- and sphingolipid-rich lipid raft microdomains in the plasma membrane, which have been shown to act as platforms for many physiological signaling pathways, were shown to be involved in virus entry. Wang., *et al*. [76] recently showed that loading cells with cholesterol from blood serum using the cholesterol transport protein apolipoprotein E (apoE) enhances the entry of pseudotyped SARS-CoV-2 and the infectivity of the virion: with high cholesterol there was almost twice the total number of endocytic entry points. Cholesterol seems to traffic ACE2 to the endocytic entry site where SARS-CoV-2 presumably docks. What is more in the target cells cholesterol optimally positions furin for priming SARS-CoV-2. Depletion of cellular cholesterol with methyl-beta-cyclodextrin (MβCD) blocked almost all viral entry.

But Park and Gallagher in 2017 [77] proved that there are fusion-inhibiting peptides which bind to spike proteins, interfere with refolding and prevent infection. The most interesting was that cholesterol or palmitate adducts increased antiviral potencies up to 1000-fold. Antiviral effects were evident after S proteolytic cleavage and unlike lipid-free peptides, the lipopeptides suppressed CoV S protein-directed virus entry taking place within endosomes [„late phase"] of virus internalization [78]. So it looks like both infectivity and therapy clues might apply the same mechanisms:here an evident and strong dependence of virus penetration on the lipid neighbourhood.

The membrane fusion reaction is dependent on the lipid composition of the viral and/or cellular membranes (in both of them, sphingolipids and cholesterol molecules tend to pack together and form microdomains called "lipid rafts" floating within the „sea" of phospholipids); viral transmembrane receptors seem to be concentrated within rafts and serve as „hotspots" for viral entry; cholesterol in rafts promotes fusion by reducing the energy needed to form fusion intermediates [78]. Studies on the influence of cholesterol on SARS-CoV infectivity make it plausible that ACE2 is a raft protein. By disrupting raft formation, ACE2 is no longer concentrated in microdomains and this reduced receptor availability lessens SARS-CoV docking.

Lipid rafts are specialized dynamic nanoscale regions, 10 and 200 nm in size, and consisting of sphingolipids, glycosphingolipids, cholesterol and glycosyl phosphatidylinositol GPI]-linked proteins[78A ]. They perform many tasks including, cell signalling, membrane trafficking, cell polarity regulation, polarized secretion, epithelial cells' transcellular transport, endocytosis and autophagy [78B] Their protein conformation determines whether lipid rafts are categorized as "planar", composed mainly of flotillin proteins, or "caveolae", enriched in caveolin proteins both mediating lipid raft signal transduction [78 A].

The S2 subunit of SARSCoV-2 contains the fusion peptide (FP) [79] which is the functional fusogenic element of the S protein. The FP is a short segment (15 - 25 amino acids), conserved across the Corona viral family composed of mostly hydrophobic residues. In the presence of $Ca^{2+}$, the FPs are able to induce greater membrane ordering, indicating that $Ca^{2+}$ may promote fusion by stabilizing a structure of the FP that affects local structure of lipid environment in rafts;it was shown that SARS has two points of binding $Ca^{2+}$ within FP [not just one as in MERS] [also Millett and Whitaker [79]].

Wang., *et al*. (2020) [111] found that cholesterol concomitantly traffics ACE2 to the endocytic entry site where SARS-CoV-2 presumably docks to efficiently exploit entry into the cell. Furthermore, in cells producing virus, cholesterol optimally positions furin for priming SARS-CoV-2, producing a more infectious virion with improved binding to the ACE2 receptor. *In vivo*, age and high fat diet induces cholesterol loading by up to 40% and trafficking of ACE2 to endocytic entry sites in lung tissue from mice. They [111] propose COVID19 severity is partially based on tissue cholesterol level and the sensitivity of ACE2 and furin to cholesterol. Molecules that reduce cholesterol or disrupt ACE2 localization with viral entry points or furin localization in the producer cells, may reduce the severity of COVID19 especially in obese patients.

## The decisive role of point mutations [polymorphism]and thr impact of age

There must be and there are point mutations [polymorphisms] in the ACE2 gene which determine phenotypic differences; and so it turned out that in serum ACE2 there were 64% of CC and CT mutants, but there was no TT. But this may mean that a small point mutation may decide whether or not a given ACE2 molecule is a SARS COV 2 receptor, as it was suggested by Ciaglia., *et al* [80]. She believes that the low infectivity and low incidence of Covid19 in children may be caused not only by the immune system including the famous cross resistance as a result of numerous vaccinations in children [81], but precisely polymorphisms in the ACE2 gene and their expression. Mind you, they (and some other authors [82]) found that ACE2 levels in children were higher than in adults, therefore in the strong opposition to infectivity and morbidity being observed.

However, it has to be emphasized that many authors evaluate ACE2 presence just in serum [„circulating ACE2"] not in the tissues [as membrane bound and/or free cytoplasmic protein although quite often only expression of ACE gene on the level of transcription is presented].Such an approach is not correct as may mislead us because we are devoid of information about the true level of a potential virus receptor. So, some authors claim there is more ACE2 in older than in younger humans [or experimental animals –mice, sheep], while the

others are of the opposite opinion. Lechien., *et al*. [83] reported that expression of ACE2 and TMPRSS2 evaluated in murine models may increase with age.

ACE2 was predominantly expressed in alveolar epithelium, bronchiolar epithelium, endothelium and smooth muscle cells of pulmonary vessels with similar content [84], whereas no obvious signal was detected in the bronchiolar smooth muscle cells. ACE2 expression is dramatically reduced with aging. in both genders: young-adult vs. old (by 78% in male and 67% in female, respectively.

Robinson., *et al*. [66] found that aged cardiomyocytes were double positive for ACE2 and TMPRSS2, critical for viral entry. Schuler., *et al*. [85] found that expression of SARS-CoV-2 spike protein primer TMPRSS2 was highest in ciliated cells and type I alveolar epithelial cells (AT1), and TMPRSS2 expression was increased with aging in mice and humans.. Fernández-Atucha., *et al*. [86] found slightly biggerACE2 serum activity in older [> 55 years old] group than in an younger, but it was signficant only for women.On the contrary, young Chinese females showed significantly higher ACE2 activity than aged females [87].

Recently, a retrospective study analyzed clinical data from 2135 pediatric cases with COVID-19 and found that over 90% of these pediatric patients were asymptomatic, mild, or moderate cases [88]. Although there currently lacks an explanation to this phenomenon, the authors speculated a potential implication of ACE2 [88]. So we can only speculate either.

Stawiski., *et al*. [89] quite recently identified natural ACE2 variants that are predicted to alter the virus-host interaction and thereby potentially alter host susceptibility. In particular, human ACE2 variants S19P, I21V, E23K, K26R, T27A, N64K, T92I, Q102P and H378R are predicted to increase susceptibility. Interestingly, Jia., *et al*. [2009] [15] found that a point mutation in the ACE2 ectodomain, L584A, markedly attenuated shedding of ACE2 with ADAM17.

Sieńko., *et al*. [90] report that some single nucleotide polymorphisms (SNPs) of ACE2 might be a risk factor of COVID-19 infection. It looks like genotypes affect ACE2 structure, even its serum concentration. Both COVID-19 morbidity and mortality might depend on ACE 2 allele frequency [90].

Devaux., *et al*. [91] reported that evidently humans are not equal with respect to the expression levels of the cellular ACE2. They review the most recent evidence that ACE2 expression and/or polymorphism could influence both the susceptibility of people to SARS-CoV-2 infection and the outcome of the COVID-19 disease.

Li., *et al*. [92] showed the differences in distribution and allele frequency [AF] of expression quantitative trait loci for ACE2 in different populations, indicating the diversity of ACE2 expression pattern in populations. Well, they did not find direct evidence supporting the existence of coronavirus S-protein binding-resistant ACE2 mutants in different populations. But the East Asian populations have much higher AFs in the variants associated with higher ACE2 expression in tissues, which may suggest different susceptibility or response to 2019-nCoV/SARS-CoV-2 from different populations under the similar conditions.

In the coding sequences of these genes Vargas-Alarcon., *et al*. [93] detected one probably-damaging polymorphism located in the TMPRSS2 gene (rs12329760) that produces a change of amino acid. Besides, polymorphisms with possible functional consequences were detected in the non-coding sequences of genes: three in ACE2, seventeen in TMPRSS2, six for cathepsin L. These polymorphisms concern binding sites for transcription factors and microRNAs.

Rare variants namely point mutation (Leu351Val) and especially(Pro389His-thus changing the chargé!), predicted to interfere with SARS-CoV-2 spike protein binding, were also observed [94]. Besides, Benetti., *et al*. [94] found polymorphism (Asn720Asp), which lies in a residue located close to the cleavage sequence of TMPRSS2, which likely affects the cleavage-dependent virion intake.This substitution is represented in the Italian and European populations but is extremely rare in the Asian population. They also show that two rare variants, namely, c.1051C>G p.(Leu351Val) and c.1166C>A p.(Pro389His) predicted to cause conformational changes impacting interaction with viral receptor binding domain [94].

I am of the opinion that for ACE2 to become a candidate to be the functional receptor of the virus, just a small point mutation in a gene for ACE2 is necessary [although even then it might be not enough to be the functional receptor], which will cause ACE2 to change enough to recognize the virus [SARSCoV-2 and SARS but not MERS [1,59] and participate in its penetration. Of course such a quite incidental mutation might have been appeared much, much earlier and quite recently SARSCoV-2 found its target cell because by chance had fit to such a mutated membrane ACE2. In my opinion it is quite probable that the expression of such a mutated gene has been blocked -in utero-in all tissues or in all except one line of cells, perhaps not the alveoli, in the majority of newborn human beings. In turn some epigenetic factors [maybe from food or our intestinal microbiota] may unblock the expression of indeed functional receptors of SARSCoV-2 in older people, leading to increased infectivity.

But the other explanation is possible. Maybe usual membrane ACE-2 molecule is not at all adapted to being a virus receptor - or rather in spite it might bind the virus it does not contribute effectively to the cascade of events leading to the internalization/endocytosis of the virus. Then, the ability to be a fully functional SARSCoV-2 receptor manifests itself only as a result of a point mutation in the ACE2 gene. Such a mutation in normal cells [non-stem and non-germline] - „an acquired change" in the Lamarck sense - would not be hereditary.

So, I think that: only the limited number of people have a functional COVID-19 receptor and only in these persons "the disease will possibly develop"!!

The spike protein of SARS-CoV-2 contains a cleavage site for the protease furin that is absent from SARS-CoV .Cantuti-Castelvetri et al. [ 94 A]recently showed that neuropilin-1 (NRP1), which is known to bind furin-cleaved substrates, potentiates SARS-CoV-2 infectivity. NRP1 is abundantly expressed in the respiratory and olfactory epithelium, with highest expression in endothelial and epithelial cells. Cleavage of spikes with furin generates a polybasic Arg-Arg-Ala-Arg carboxyl-terminal sequence on S1, which that binds to cell surface neuropilin-1 (NRP1) as the receptor. It was shown with x-ray crystallography and biochemical approaches by Daly *et al*. 2020 [94B ]].

## Crucial role of epigenetic events

There have been significant epigenetic effects on ACE2 expression [95,96] as well as regulation of ACE2 by sirtuin -NAD+ dependent deacetylase SIRT1 (silent information regulator T1) [97]; if by sirtuin then by diet-nutriepigenetic effects [vitamin PP and resveratrol-the bioregulator of sirtuin [98], also by glycosylation [np.96] [the role of diabetes].

The participation of sirtuin 1 in the viral entry and spread processes is justified as it was found that sirtuin1 upregulation diminishes upregulation of ADAM17 sheddase if even not downregulating it [99]. No one has explicitly articulated it, but there is an overwhelming impression that activation or upregulation of ADAM 17 by a SARSCoV-2 virion -passed into the cell through clathrin-dependent endocytosis [58] - and thus detachment of ACE2 from the target cell membrane is yet another human defense mechanism against viral multiplication.

The concept of down and upregulation is often abused, and it is hardly ever explained whether it concerns the level of gene expression [and at what stage], endo/exocytosis or trafficking.In any case a certain decrease in activity or downregulation of sirtuin could induce increased shedding of ACE2 from membranes into the plasmaIn older people, zinc ion malabsorption and NAD+ deficits [100] might cause a decrease in sirtuin activity and an increase in ADAM17 and ACE2 shedding activity. This also would cause the release of proinflammatory TNF alpha [46] into the plasma, which would in turn cause a decrease in evident nonspecific immunity, but in turn this [another protective feedback] would cause sirtuin activation again [101] and shedding decrease -in the elderly, as I previously assumed.

But additionally, in turn, a very important way of regulating metabolism with calmodulin, and therefore membrane kinases dependent on it, inhibits the activity of ADAM17 [102] contrary to protein kinase pathway activated with phorbol ester. So the cross-talking of these two very important regulation pathways might be an explanation of eventual slight activation of shedding process in young versus old people.

Well, also the deficits of vitamin D [101] and melatonin in elderly people might hinder the struggle of the elderly with Covid19, because the two substances mentioned here counteract the negative effects of angiotensin II [Ang 1-8] - destroyed by ACE2 - on inflammatory processes, oxidative shock and apoptosis.

Paizis., *et al*. [2005] [102] reported an increase in ACE2 expression in chronic liver injury, and Burrell., *et al*. [2005] [105] the same increase during myocardial infarction. So all the time is question if good or bad sites of ACE2 action prevail. Benefits of ACE2 related to its involvement in intestinal digestion of short peptides, and thus the absorption of neutral amino acids, is emphasized by Samavati., *et al* [104].

Behl., *et al*. [2020] [105] emphasize the better situation of young people [including children] against infection with SARSCoV-2, despite the fact that the level of ACE2 expression is elevated in them.

Xie., *et al*. [84] found that: 1. ACE2 -in rats was predominantly expressed in alveolar epithelium, bronchiolar epithelium, endothelium and smooth muscle cells of pulmonary vessels with similar content, but not in in the bronchiolar smooth muscle cells. 2. ACE2 expression is dramatically reduced with aging in both genders. Hu., *et al*. [2020] [106] report the lack of dependence of ACE2 expression and infectivity both in women and elderly people.

Uhal., *et al*. [2006] [107] showed that expression of ACE2 is clearly dependent on the cell cycle and its expression diminishes almost to zero in the mitotically active cells e.g. during the lung fibrosis. But one can imagine that during embryonal growth and in the stem cells it does either. Mind you, ACE2 is not at all necessary in the lung as Ang1-7 might be formed due the prolylendopeptidase [43].

Besides, angiotensin II, so ACE, might cause downregulation of ACE2 with its internalization and even proteolysis in lysosomes. Ziegler., *et al*. [108] discovered that ACE2 is a human interferon-stimulated gene both *in vitro* using airway epithelial cells and *in vivo*.

The more data on the different ways of regulation [including hormonal one] and influence on gene expression and polymorphisms, on the membrane itself and shedding [and exocytosis and endocytosis- in general - the movement of protein molecules], more possibilities of post-transcription and/or postranslation modification of ACE2, the more likely it is that a sharp distinction should be made between the functional SARS CoV-2 receptor [not only recognizing the virus, but „drawing it" inside the cell]and ACE2 itself, e.g. both that in serum, capable [?] of hydrolyzing angiotensin II [thus forming Ang 1-7], but also for binding the virus, and ACE-2 particles in the membrane [forming Ang1-7 but also transporting amino acids] but finally unable to draw virus into the cell.

Very recently Chen., *et al*. [109] emphasized that they are on the contrary to the expectation that ACE2 is the sole receptor for the virus, instead They remind that expression of ACE2 is high in Asian females and young people ie those who are known to be less susceptible, and even less inflicted by fatal outcome, while it is low in males, further decreases with age, so those who are most susceptible to serious diseases; it would suggest that even a negative correlation between ACE2 expression and COVID-19 severity and fatality might take place.

I agree with Chen., *et al*. [108] that there is no reason to believe that only membrane ACE2 is a functional SARSCov-2 receptor. On the other hand, I do not see any justification for the belief that the level of ACE2 expression is inversely correlated with the „density of functional coronavirus receptors. Let us take into account 1. the lack of parallelism of ACE2 expression and concentration, and even less the enzymatic activity of the ACE2 serum [inhibitors, hormonal and epigenetic effects]. 2. huge differences in ACE2 expression in adjacent and even phenotypically close areas [as well as the dependence of expression on the life phase of cells]. 3. previously unknown differences in the ACE2 and TMPRSS2 genes and their expression in different populations [race, nationality]. 4. last but not least very large differences in research results and the methodology used

## [An annex] about possible decrease of ADAM17 sheddase with an increase of age

Hartl et al. [110 ]using whole-genome sequencing identified a single rare nonsynonymous variant in ADAM17 co-segregating with an autosomal-dominant pattern of late-onset Alzheimer disease(AD) in one family. Subsequent genotyping identified five variant carriers among AD patients only. The mutation inhibits pro-protein cleavage and the formation of the active enzyme, thus leading to loss-of-function of ADAM17 alpha-secretase. Further,Hartl *et al*. [110]found a strong negative correlation between ADAM17 and APP gene expression in human brain and present in vitro evidence that ADAM17 negatively controls the expression of APP. Morancho *et al*. [2015][111] stu-

died cellular senescence ie a terminal cell proliferation arrest that can be triggered by oncogenes. One of the traits of oncogene-induced senescence (OIS) is the so-called senescence-associated secretory phenotype or senescence secretome.They found that these proteins are regulated transcriptionally and, in addition, that their shedding is limited by the protease ADAM17. The activity of the sheddase is constrained, at least in part, by the accumulation of cellular cholesterol.

Cleavage and release (shedding) of membrane proteins is a critical regulatory step in many normal and pathological processes. Evidence suggests that the antiaging transmembrane protein Klotho (KL) is shed from the cell surface by proteolytic cleavage[Ci-Di Chen et al. [2007][112].The author found that, cotransfection of KL with ADAM17[ADAM10 either] enhances KL cleavage, whereas cotransfection of KL with small interference RNAs specific to ADAM17 inhibits KL secretion. These results indicate that KL shedding is mediated mainly by ADAM17- and 10] in KL-transfected cells. The effect of insulin [112 ] is abolished when ADAM10 or ADAM17 are silenced. Insulin enhances KL shedding without increasing ADAM17 [and 10] mRNA and protein levels, suggesting that it acts by stimulating their proteolytic activities.

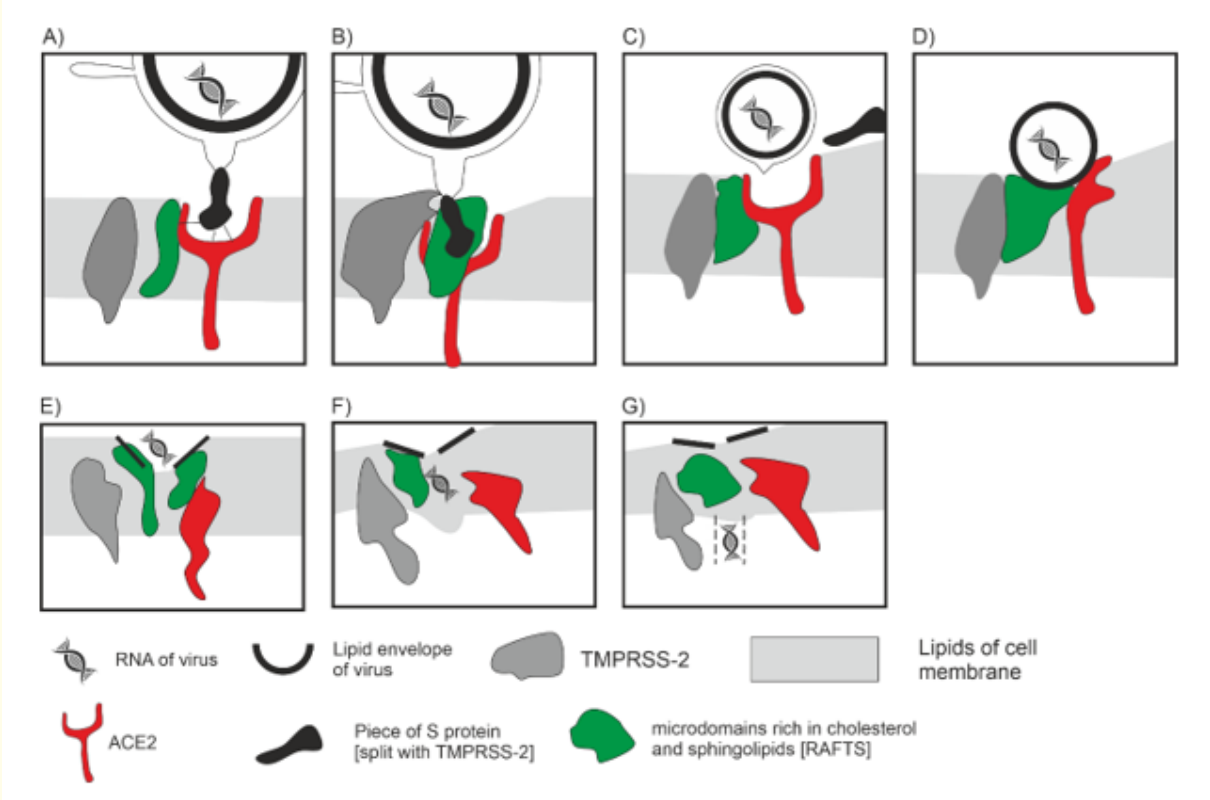


***Figure 1:*** *Subsequent phases of processes taking place in the contact between SARSCoV-2 virus and its functional receptor [containing suitable membranę ACE-2 molecule]*

Bandsma et al. [2015][113] emphasize that ADAM 17 metallopeptidase is responsible for processing large numbers of proteins. They described a family with a homozygous mutation in ADAM17 confirming the existence of a syndrome. with severe diarrhea, skin rash, and recurrent sepsis, eventually leading to her death at the age of 10 months. Interestingly, the patient developed unexplained systolic

hypertension and nonspecific hepatitis with apoptosis. This report provides evidence for an important role of ADAM17 in human immunological response and underscores its multiorgan involvement.

Multiple studies have indicated that ADAM17 is involved in hepatocyte apoptosis. In hypoxia-exposed liver cell lines, downregulation of ADAM17 led to upregulation of caspase-3 [114], a critical regulator of apoptosis. Bandsma et al. [113 ] found high caspase-3 expression in hepatocytes in the affected patient. It has been shown that ADAM17 inhibition stimulates caspase-1 and that caspase-1 is strongly involved in cell death receptor FAS-mediated apoptosis [115]. Finally, trials with inhibitors of ADAM17 have shown indications of liver toxicity [116].

Lorenzen et al. [2017][117] emphasize the rolr of so called redox regulation depending on the enzymatically controlled production and decay of redox active molecules. NADPH oxidases, superoxide dismutases, nitric oxide synthases, and others produce the redox active molecules superoxide, hydrogen peroxide, nitric oxide, and hydrogen sulfide. These react with target proteins inducing spatiotemporal modifications of cysteine residues within different signaling cascades. Thioredoxin family proteins are key regulators of the redox state of proteins. They regulate the formation and removal of oxidative modifications by specific thiol reduction and oxidation. All of these redox enzymes affect inflammatory processes and the innate and adaptive immune response.The transmembrane protein ADAM17 releases proinflammatory mediators, such as TNFα, and is itself regulated by a thiol switch.. Interestingly, cytokines can be expressed as cytosolic or membrane-bound “precursors” and are activated and released by redox-regulated, proteolytical cleavage via cytosolic multiprotein complexes called inflammasomes or specific proteases such as ADAM17 [118-122].

Moreover, ADAM17 cleaves members of the EGFR ligand family, which are essential for their function as growth factor and tissue regeneration [123-125]. Dysregulation of this pathway has been linked to inflammatory diseases, for example, cystic fibrosis and chronic inflammatory airway disease [126–130]

Energy homeostasis involves central nervous system integration of afferent inputs that coordinately regulate food intake and energy expenditure[Gelling et al. [2008][131 ] The authors report that adult homozygous TNFα converting enzyme (TACE){and TACE is just ADAM17 sheddase}-deficient mice exhibit one of the most dramatic examples of hypermetabolism yet reported in a rodent system These findings clarly prove an important role for ADAM17 in an energy homeostasis.

The results of the studies by the authors of the above-cited papers do not explicite say that with age the activity of ADAM17 decreases and downregulation of expression of sheddase occurs, which would be a direct confirmation of my assumptions about a lower level of blocking sites in the blood of infected people in old age. However the papers mentioned here emphasize the reduction of the level of [gene expression] ADAM 17 in the case of diseases evidently more common in seniors and generally more frequent as the age increases - from birth to old age. These include Alzheimer’s disease, senescence triggered with oncogenes, aging thus smaller level of an antiaging protein / s, imunity deficits, increased apoptosis, diabetes, airway disease / s, dysregulation of an energy homeostasis. It’s all tightly makes it more probable the assumption made by me about the decrease in ADAM17 sheddase activity with age, i.e. with a lower content of the ACE2 protein, i.e. its enzymatically active ectodomain.

## Final Conclusion

Summarizing [hypothesis B] ,the functional receptor of SARSCoV-2 is not just membrane bound ACE2 itself but:

1. Quite a big fragment of whole membrane of target cell/s with a complete set containing ACE2 and TMPRSS2 optionally ,but besides furin and endosomal compartment’s cathepsins and first of all some microdomains [„rafts”] of membrane rich in cholesterol and sphingolipids.

2. Membrane bound protein being result of expression of some special single gene polymorphism/s in ACE2 gene[an/or in the genes for priming enzymes] and perhaps besides some posttranscription and posttranslation modification/s.

3. Protein/s binding and „sucking in" the virus but only if there is a coincidence of some epigenetic or even nutriepigenetic events.

Final conclusion X: All three above mentioned possibilities do not exclude each other and even it is possible they might play in concert in this dangerous scenario.

Final conclusion Y: SARSCoV-2 viruses bind to 1/ the ACE2 molecules circulating - in quite significant amounts - in serum[hypothesis A1] 2/erythrocytes' membranes using so-called hemagglutinin esterase occuring in the virus envelope, acting on sialic acid of erythrocytes' glycans ; it is possible that the virus attaches itself particularly to the membrane of erythrocytes already attacked by free radicals[ROS] [hypothesis A2] .

Final conclusion Z: It is quite justified to assume that the level of fully functional SARSCoV-2 receptors grows with age whereas the level of serum ACE2 being the main component of the viruses' block conversely - it decreases with age.

Fully functional SARSCoV-2 receptor: a / must recognize receptor binding domain [RBD] in virus spike proteins b / through the TMPRSS-2 protease molecules present in it, appropriately located next to the enzymatic ACE2 fragment, should cut the spike protein of sucked virus in the appropriate place c / it should also contain furin and cathepsin L [the latter in endosomes] to facilitate additional cleavage in the virus, leading to fusion and release of viral genetic material d / must have appropriate and properly distributed lipid microdomains rich in cholesterol[109A] and sphingolipids, without which it is impossible to fuse the membrane with the viral envelope, and thus endocytosis.

These microdomains, the so-called rafts, seem to be a carrier of the quasiallosteric effect, which clearly changes the spatial structure of the sites in the cell membrane, enabling fusion.

In people with fully functional viral receptors, the expression of the correct variants of the ACE2, TMPRSS-2, furin and cathepsin L-genes must be very efficient. Also this expression, but and probably the virus binding itself [with the fusion!] requires certain conditions [epigenetic and local].

Non-functional receptors - resulting from the expression of mutants of the above-mentioned genes [with different polymorphisms] either do not recognize the virus at all [despite the correct ability to generate Ang1-7 and participate in the transport of amino acids] or recognize it, but the effect is weak or, despite the correct recognizing, they are not capable of fusion. All the above reasoning is visualized in the fig.1.

Step A: The S1 fragment of the viral spike [marked as black], more precisely its receptor binding domain [RBD] binds to the enzymatic domain of the membrane ACE2 [in its current configuration] - marked in red.

Step B: The RBD-ACE2 interaction induces: a / obvious weakening of the specific peptide bond between the S1 and S2 fragments [shown on the figure as bending of the S1 fragment] and b / energetic interaction [understood as adopting an energetically more durable structure] on the surrounding microdomains [rafts]- /marked with green/, which is transferred to the main priming TMPRSS2 enzyme [marked with dark gray-purple]; there was no such an interaction in stage A

Step C: The spatial structure of TMPRSS2 is then changed so that it binds the viral spike fragment S1 and a proteolytic cleavage occurs between S1 and S2 and the S1 fragment breaks off. This induces further changes in the spatial structure of rafts, TMPRSS2 and ACE2.

{ According to Duzgunes and Konopka[109 X ]the insertion of the fusion peptide into the host cell membrane leads to the conformational change of the now separate S2 domain of S, resulting in the formation of the 6-helix bundle and the close approach of the two membranes. The hydrophobic interactions between the fusion peptide and the transmembrane domains of the S protein leads to membrane destabilization and fusion}.

Step D: Elements of the cell membrane, in fact belonging to the functional receptor, namely the corresponding raft /s [microdomains] move towards the virus [its spike element S2, but right next to the lipid envelope]. The structural-functional linkage of rafts with TMPRSS2 and thus with ACE2 disappears. It is possible that the interaction with glycans from the spine surface of the virus plays a role at this stage and in the preceding stages[109B ].

Step E: The virus lipid envelope breaks as a result of fusion with rafts [lipid-lipid interactions] - a virion, hence viral RNA, protrudes into the envelope; the fragments of the viral lipid envelope[marked b;ack] henceforth become elements of the cell membrane.

Step F: The RNA of the virion slides through the lipid layer of the cell membrane towards the cytoplasm [alternatively endosomal tubule lumen]. Possibly a lipid-RNA interaction takes place here due to weak bonds of sphingosine, lecithin and cholesterol residues with purine and pyrimidine bases of RNA [as if the lipid raft domain plays the role of RNA carrier];some proteins being integral parts of rafts [ 78A] might also play important role in this and earlier steps.

Step G: The cell membrane closes, the viral RNA is in the cytosol [and / or endosomes- marked vertical dotted lines], TMPRSS2 and membrane ACE2 move via rafts to the surface [extracellular space light]. However, most likely it will not come back to step A, at most membrane ACE2 will be able to bind angiotensin 1-9 and possibly amino acids, but rather not virus anymore.

1. If there are nonfunctional receptors of SARS CoV-2 : the spatial structure of the extracellular [transmembrane] domain of ACE2 may be so altered [as a result of the expression of certain polymorphisms] that the RBD binding of the viral spine is completely absent and stage A is not achieved at all [this does not, however, interfere with the binding and hydrolysis of angiotensin 1-9].

2. tep A takes place, but due to: mutation/s in TMPRSS2 [possibly also furin] they do not bind to the spike of the virus or / and such a change in the local raft domain structure that it is not able to transfer interactions from ACE2 to TMPRSS2; then there is no stage B, and thus stage C

3. Inadequate structure of rafts [including their proteins as a result of mutation/s of their genes] may also exclude / make it extremely difficult to bind the lipid envelope of the virus with the lipid structure of the cell membrane within the potentially functional receptor - and it doesn't come to stage D, so I E and F

4. When the whole virion enters the endosomes, the deficit [mutation] of cathepsin L may hinder the release of the virus, or rather its RNA. It is also possible that a functional membrane receptor SARSCoV-2 must contain neuropilin-1 [NRF-1] [94A,94B], especially in some cells [first those completely devoid of membrane ACE2, such as astrocytes [ 109 C]].

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