Exam Q8 Research

Research in Personalized Healthcare

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Aantal vragen: 25

Maximum score: 115 pt

Vragen hebben deels betrekking op de abstracts en deels op de stof die tijdens de modules is behandeld. De abstracts worden een week van tevoren gedeeld via BrightSpace.

Download deze file en voeg je antwoorden toe. Als dat handiger is (bijvoorbeeld voor schetsen), kun je een foto maken en die invoegen in het document.

Denk eraan de file geregeld op te slaan!

Lever het document met de antwoorden in **voor 16:00** via Brightspace: 1920 Q8 Biomedical Evidence > Activities > Assignments > Take Home Exam Q8.
Mail het tevens als bijlage aan Joost.Hoenderop@radboudumc.nl.

(Studenten met special faciliteiten kunnen het tot 16:30 uploaden in een aparte inlevermodule)

Bij Technische problemen, mail Joost.Hoenderop@radboudumc.nl of bel 06 34 15 07 41.

Topic in Personalized Healthcare Research

1. In the preliminary evaluation of a biomarker the aim is to demonstrate that the levels of the biomarker are different between people with and without the outcome of interest. The outcome may be that a person is affected by a disease or not (diagnosis), that they may require treatment or not (prognosis), or even that they may respond to therapy or not (prediction). An appropriate design to evaluate the biomarker at this stage is a *case-control* study. Explain why. [8 pt]

2. Explain why measuring PFAS concentration in drinking water as an exposure is not a biomarker, but PFAS concentration as a exposure urine is a biomarker. [2 pt]

Infectious Disease and Global Health

Impact of social determinants on antiretroviral therapy access and outcomes entering the era of universal treatment for people living with HIV in Italy

Background
Social determinants are known to be a driving force of health inequalities, even in high income countries. Aim of our study was to determine if these factors can limit antiretroviral therapy (ART) access, outcome and retention in care of people living with HIV (PLHIV) in Italy.
Methods
All ART naïve HIV+ patients (pts) of Italian nationality enrolled in the ICONA Cohort from 2002 to 2016 were included. The association of socio-demographic characteristics (age, sex, risk factor for HIV infection, educational level, occupational status and residency area) with time to: ART initiation (from the first positive anti-HIV test), ART regimen discontinuation, and first HIV-RNA < 50 cp/mL, were evaluated by Cox regression analysis, Kaplan Meier method and log-rank test.
Results
A total of 8023 HIV+ pts (82% males, median age at first pos anti-HIV test 36 years, IQR: 29–44) were included: 6214 (77.5%) started ART during the study period. Women, people who inject drugs (PWID) and residents in Southern Italy presented the lowest levels of education and the highest rate of unemployment compared to other groups. Females, pts aged > 50 yrs., unemployed vs employed, and people with lower educational levels presented the lowest CD4 count at ART initiation compared to other groups. The overall median time to ART initiation was 0.6 years (yrs) (IQR 0.1–3.7), with a significant decrease over time [2002–2006 = 3.3 yrs. (0.2–9.4); 2007–2011 = 1.0 yrs. (0.1–3.9); 2012–2016 = 0.2 yrs. (0.1–2.1), $p < 0.001$]. By multivariate analysis, females ($p < 0.01$) and PWID ($p < 0.001$), presented a longer time to ART initiation, while older people ($p < 0.001$), people with higher educational levels ($p < 0.001$), unemployed ($p = 0.02$) and students ($p < 0.001$) were more likely to initiate ART. Moreover, PWID, unemployed vs stable employed, and pts. with lower educational levels showed a lower 1-year probability of achieving HIV-RNA suppression, while females, older patients, men who have sex with men (MSM), unemployed had higher 1-year risk of first-line ART discontinuation.
Conclusions
Despite median time to ART start decreased from 2002 to 2016, socio-demographic factors still contribute to disparities in ART initiation, outcome and durability.

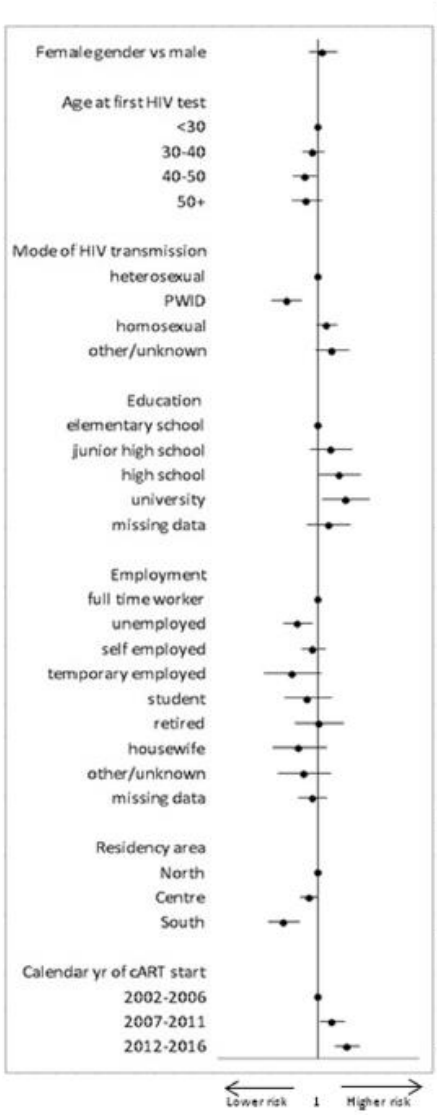
Also in high income countries, social determinants are known to be a driving force of health inequalities. These inequalities are also seen when focusing on HIV and access to care.

3. You are designing a prospective study to unravel the reasons for sociodemographic inequality in HIV care in the Netherlands. Which two key HIV related biological characteristics would you include in your longitudinal follow up of subjects that will be included and followed in your study. [2 pt]

4. Explain your answer to question 2. Why are these key variables reflecting the quality of HIV care ? [4 pt]

5. According the study presented above, which sociodemographic group(s) tend(s) to have an **advantage** with respect to access to cART ? [4 pt]

Study figure 3:



	HR	95% CI		p
Female gender vs male	1.03	0.94	1.13	0.518
Age at first HIV test				
<30	1.00			
30-40	0.97	0.90	1.04	0.342
40-50	0.91	0.84	0.99	0.033
50+	0.92	0.83	1.03	0.139
Mode of HIV transmission				
heterosexual	1.00			
PWID	0.81	0.73	0.89	0.000
homosexual	1.06	0.99	1.14	0.106
other/unknown	1.10	0.98	1.23	0.099
Education				
elementary school	1.00			
junior high school	1.10	0.95	1.26	0.214
high school	1.16	1.01	1.34	0.041
university	1.21	1.03	1.43	0.020
missing data	1.08	0.93	1.25	0.326
Employment				
full time worker	1.00			
unemployed	0.87	0.79	0.96	0.006
self employed	0.97	0.89	1.05	0.406
temporary employed	0.84	0.69	1.02	0.073
student	0.93	0.79	1.10	0.392
retired	1.01	0.85	1.19	0.914
housewife	0.88	0.73	1.05	0.163
other/unknown	0.91	0.76	1.09	0.299
missing data	0.96	0.88	1.06	0.453
Residency area				
North	1.00			
Centre	0.94	0.88	1.00	0.049
South	0.79	0.71	0.88	<0.001
Calendar yr of cART start				
2002-2006	1.00			
2007-2011	1.10	1.01	1.19	0.029
2012-2016	1.22	1.12	1.33	<0.001

Fig. 3 Multivariable model of factors associated with virological response (HIV RNA < 50) (adjusted for CD4 count, viral load, pregnancy status, smoking)

6. According figure 3, name at least five factors that appear to be significantly related to obtaining HIV viral suppression? Explain the mechanism behind the factor. [10 pt]

Alzheimer's Disease and Dementia

Case study

Mr B lives alone in an apartment. He has had Parkinson's Disease for 8 years and is in the moderate disease stage. Mr B liked to play billiard with his friends in a centre near his home. His daughter usually takes him there. Lately he is not so keen to go anymore. He feels sad about that, but he says he is not able to participate physically and socially in an adequate manner. The motor assessment the of the physiotherapist reveals he has freezing of gait when needing to initiate steps around the pool table, he does not strike the ball hard enough due to difficulty with timing the movement. He also speaks with a soft voice, especially when he is tired, because of his Parkinson's Disease. He feels self-conscious towards his friends. Moreover, he often feels fatigued and then can't be bothered to go. The occupational therapists discusses with him if this is the most relevant activity for him. After his confirmation she asks him how he is used to perform this activity and with whom at what time and where. She consequently discusses the planning of the activity in the week and how to ensure he has some rest before going to the centre to play. In collaboration with the physiotherapist, the occupational therapist explores with Mr B what are the best external cueing strategies to enhance the stepping and striking movements. A rocking movement before taking a step with focused attention was most effective. For the striking it helped him to count from 1-3 before hitting the ball. The OT trained the strategies with Mr B at the centre. Mr B informed will inform his friends and the workers of the centre about the strategies and the fact that he needs some more time, attention and adaptations for starting up his walking and when it is his turn with billiard playing.

7. What makes the intervention described in the case study a person-centred intervention? [4 pt]

8. Why is this intervention called "a complex intervention"? [4 pt]

9. What research framework should be used when developing and evaluating and implementing such an evidence-based psychosocial intervention? [4 pt]

10. Which research phases does this model describe? [8 pt]

Renal disorders

Missense mutation in the Kv1.1 voltage-gated potassium channel-encoding gene KCNA1 is linked to human autosomal dominant hypomagnesemia.

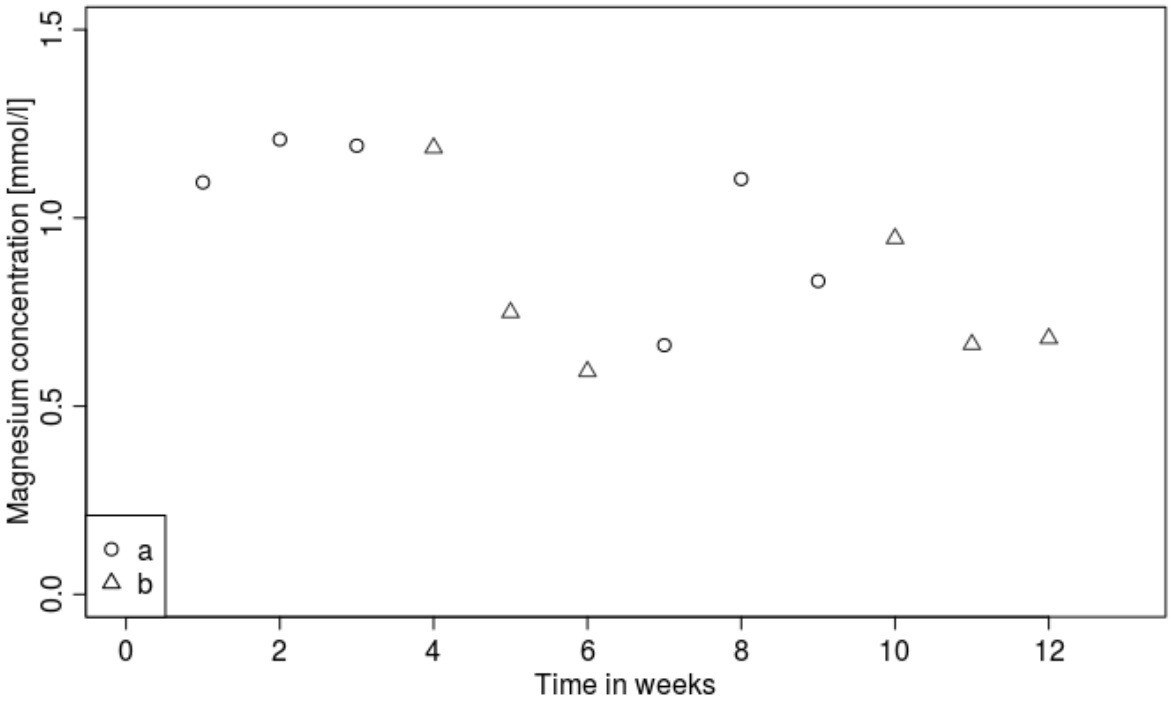
Primary hypomagnesemia is a heterogeneous group of disorders characterized by renal or intestinal magnesium (Mg^{2+}) wasting, resulting in tetany, cardiac arrhythmias, and seizures. The kidney plays an essential role in maintaining blood Mg^{2+} levels, with a prominent function for the Mg^{2+}-transporting channel transient receptor potential cation channel, subfamily M, member 6 (TRPM6) in the distal convoluted tubule (DCT). In the DCT, Mg^{2+} reabsorption is an active transport process primarily driven by the negative potential across the luminal membrane. Here, we studied a family with isolated autosomal dominant hypomagnesemia and used a positional cloning approach to identify an N255D mutation in KCNA1, a gene encoding the voltage-gated potassium (K^+) channel Kv1.1. Kv1.1was found to be expressed in the kidney, where it colocalized with TRPM6 along the luminal membrane of the DCT. Upon overexpression in a human kidney cell line, patch clamp analysis revealed that the KCNA1 N255D mutation resulted in a nonfunctional channel, with a dominant negative effect on wild-type Kv1.1 channel function. These data suggest that Kv1.1 is a renal K^+ channel that establishes a favorable luminal membrane potential in DCT cells to control TRPM6-mediated Mg^{2+} reabsorption.

11. The kidney plays an essential role in the Mg^{2+} balance. Which two nephron segments play an essential role and what is the most important segment with respect to the Mg^{2+} balance? [3 pt]

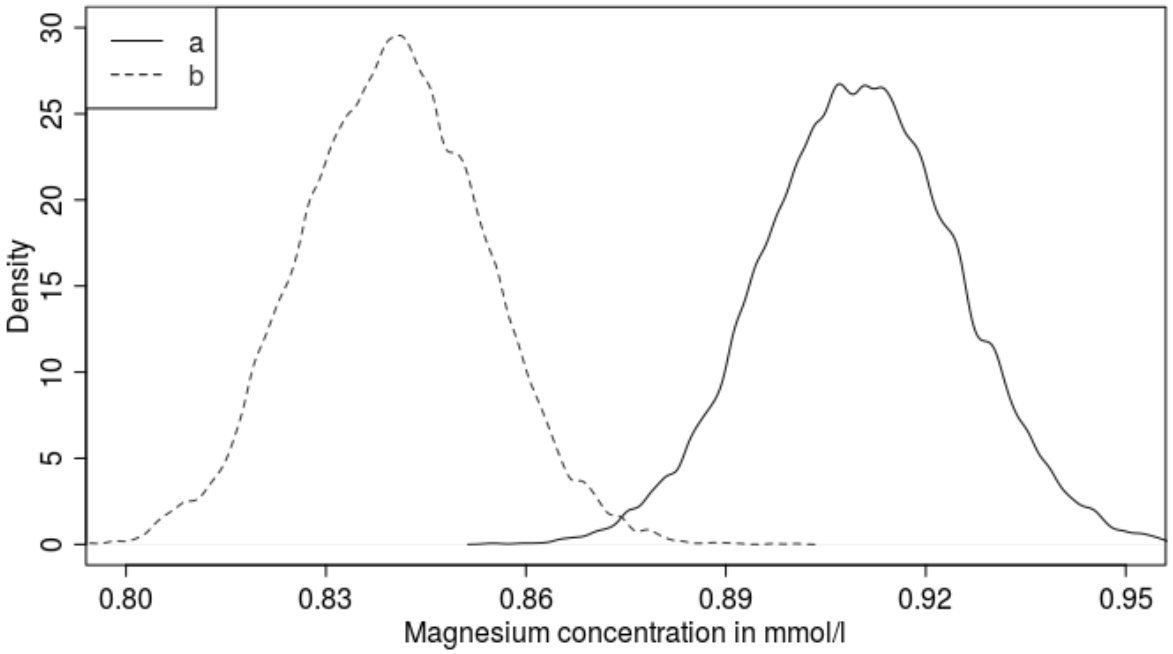
12. As stated in the abstract primary hypomagnesemia is a heterogeneous group of disorders characterized by renal or intestinal magnesium wasting. How can one conclude, by just looking at blood and urinary magnesium values, whether there less absorption in the intestine (intestinal cause) or whether there is reduced reabsorption in the kidney (renal cause)? [4 pt]

13. How could a loss of function mutation in the potassium Kv1.1 affect the magnesium homeostasis in this patient? Explain the mechanism and including the renal cell model with the involved transporters. [5 pt]

A physician and patient have decided to try magnesium supplementation to treat the renal magnesium wasting. In order to find the optimal treatment, they have performed an N-of-one study to compare two possible therapies. The therapies have been labeled '*a*' and '*b*' and were administered over four treatment periods of 3 weeks each.



14. What kind of bias can be observed from this figure? Explain how you have come to this conclusion. [2 pt]



15. The figure above shows the posterior distributions of serum potassium after respectively treatment *'a'* and *'b'.* We would like to know the probability that treatment *'a'* results in higher serum magnesium concentrations than treatment *'b'*. How should this be calculated? You can choose to explain in words, of to provide the calculation itself (e.g. in R code). [6 pt]

Nanomedicine

Targeted Therapy of Colon Cancer by Aptamer-Guided Holliday Junctions Loaded with Doxorubicin

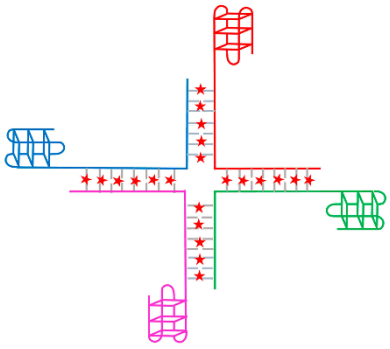
Purpose:
Chemotherapy is the primary treatment for advanced colon cancer, but its efficacy is often limited by severe toxicities. Targeted therapy in the form of selectively drug delivery system (SDDS) is an important strategy to reduce adverse effects. Here, we aim to design a novel SDDS with potential for practical application using biocompatible components and scalable production process, for targeted delivery of doxorubicin (Dox) to colon cancer cells.

Methods:
The SDDS was made of a self-assembled DNA nano-cross (Holliday junction, or HJ) functionalized by four AS1411 aptamers (Apt-HJ) and loaded with Dox.

Results:
Apt-HJ had an average size of 12.45 nm and a zeta potential of -11.6 mV. Compared with the monovalent AS1411 aptamer, the quadrivalent Apt-HJ showed stronger binding to target cancer cells (CT26). A complex of Apt-HJ and doxorubicin (Apt-HJ-Dox) was formed by intercalating Dox into the DNA structure of Apt-HJ, with each complex carrying approximately 17 Dox molecules. Confocal microscopy revealed that Apt-HJ-Dox selectively delivered Dox into CT26 colon cancer cells but not the control cells. Moreover, Apt-HJ-Dox achieved targeted killing of CT26 cancer cells in vitro and reduced the damage to control cells. Importantly, compared with free Dox, Apt-HJ-Dox significantly enhanced the antitumor efficacy in vivo without boosting the adverse effects.

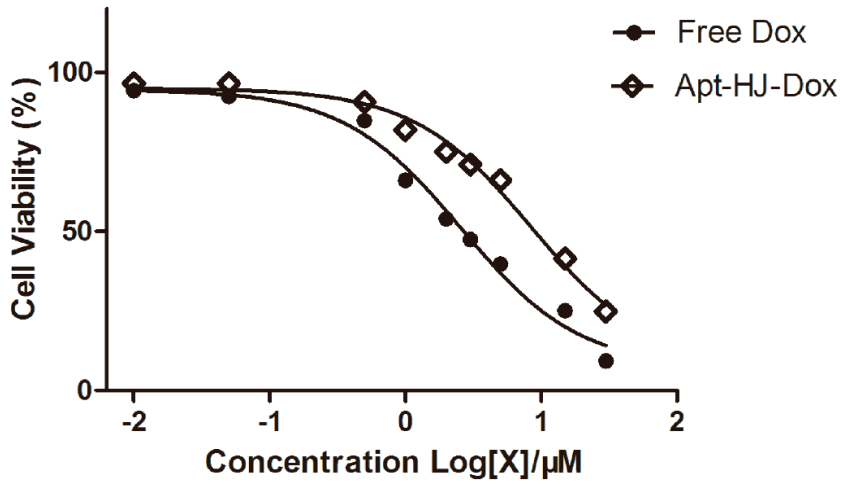
Conclusion:
These results suggest that Apt-HJ-Dox has application potential in targeted treatment of colon cancer.

16. On the right a schematic drawing of the Apt-HJ-Dox is shown. The outer segments represent the AS1411 aptamer and the red stars symbolize the Dox molecules that intercalate into GC basepairs. Aptamers are short, single-stranded oligonucleotides (DNA or RNA) that can form unique three-dimensional structures to serve as ligands that bind with target molecules.
Briefly describe, in a few sentences, what you can derive from the abstract about the generic characteristics of this target molecule for AS1411. [4 pt]

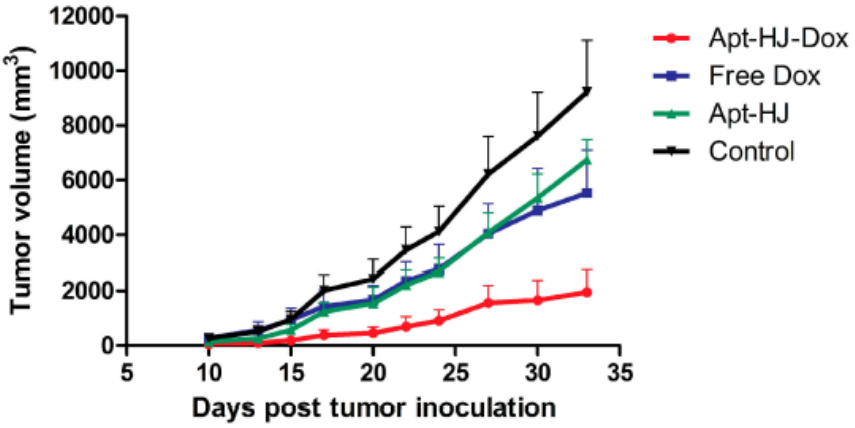


17. Provide two main reasons why the quadrivalent Apt-HJ is to be preferred over the monovalent AS1411 aptamer when it comes to targeted Dox delivery in vivo. Briefly explain your answers. [3+3 pt]

18. The authors compared the cytotoxicity generated by Apt-HJ-Dox or free Dox *in vitro*, both on CT26 colon cancer cells and on a control cell line. For which of the two cell lines (CT26 or Controls) is the result depicted on the right? Additionally (since it may take just a few clicks to google the answer), in a few sentences, provide the rationale that allows the deduction of the answer using just the graph and/or the abstract information. [1+5 pt]



19. The authors also investigated effects *in vivo*. Tumor-bearing BALB/c mice were administered with PBS (control), free Dox, or Apt-HJ with or whitout Dox, by daily intraperitoneal injection for 20 days (n=6 mice/group), and tumor volume was recorded (see graph). Clearly the Dox-carrying nanobody outperforms the free Dox.
    Provide a scientific rationale explaining why the aptamer-holiday junction particle without Dox is as efficient as the free Dox in this experiment. [4 pt]



Bioinformatics

A new coronavirus associated with human respiratory disease in China

Emerging infectious diseases, such as severe acute respiratory syndrome (SARS) and Zika virus disease, present a major threat to public health. Despite intense research efforts, how, when and where new diseases appear are still a source of considerable uncertainty. A severe respiratory disease was recently reported in Wuhan, Hubei province, China. As of 25 January 2020, at least 1,975 cases had been reported since the first patient was hospitalized on 12 December 2019. Epidemiological investigations have suggested that the outbreak was associated with a seafood market in Wuhan. Here we study a single patient who was a worker at the market and who was admitted to the Central Hospital of Wuhan on 26 December 2019 while experiencing a severe respiratory syndrome that included fever, dizziness and a cough. Metagenomic RNA sequencing of a sample of bronchoalveolar lavage fluid from the patient identified a new RNA virus strain from the family Coronaviridae, which is designated here 'WH-Human 1' coronavirus (and has also been referred to as '2019-nCoV'). Phylogenetic analysis of the complete viral genome (29,903 nucleotides) revealed that the virus was most closely related (89.1% nucleotide similarity) to a group of SARS-like coronaviruses (genus Betacoronavirus, subgenus Sarbecovirus) that had previously been found in bats in China. This outbreak highlights the ongoing ability of viral spill-over from animals to cause severe disease in humans.

20. The abstract describes the discovery of the virus that is now known as Cov-SARS-2. The authors write that this new virus has 89.1% nucleotide sequence similarity with a group of SARS-like coronaviruses. Such a level of identity could have been obtained from the comparison of the sequence with a database of virus genomes, using e.g. BAST. To determine whether the genomes are actually homologous another value that BLAST gives you is actually more relevant than sequence similarity. Which one is that, and what are the minimum and maximum scores that that value can obtain? [3 pt]

21. The abstract mentions DNA sequence similarity. In the course we have discussed protein sequence similarity, which takes into account that some amino acids are more similar to each other than others. There are multiple ways of determining how similar amino acids to each other. How can you determine how similar amino acids are to each other by comparing homologous protein sequences? [2 pt]

22. The SARS-virus and COVID-19 virus genomes are highly similar, but differences between the coding regions for the same protein in different virus strains have been found. Explain where in a protein we expect to find these differences and why? [5 pt]

23. Researchers have designed a virus ontology. The ontology has a hierarchical structure. Explain what is meant by a hierarchical structure of the ontology? Illustrate the hierarchical structure in the virus ontology using these five terms from the ontology: “Coronavirus”, “RNA virus”, “WH-Human 1 coronavirus”, “2019-nCoV” “SARS-Cov2”. You may use a drawing. [2 pt for a good explanation of the concept or hierarchy; 3pt for putting terms in the right order]

24. In the abstract, two different sources of the COVID-19 virus are mentioned: a seafood market and bats. Depending on the source, different measures for prevention of future outbreaks may be implemented. Suppose you aim to determine which of these sources as a potential cause of severe disease among humans gained most interest among the general population, with Google search terms as a proxy. How would you study this using Google Trends? [5 pt]

25. It has been found that the COVID-19 Spike protein interacts with the human ACE2-receptor to infiltrate cells. Some human individuals may possess genetic variants in ACE2 that result in ACE2 proteins with weaker affinity for SARS-Cov2 and therefore may be protected against virus entry through ACE2 binding.
    Individual A possesses this missense variant: ACE2 p.Lys353Arg
    Individual B possesses this missense variant: ACE2 p.His34Asp
    How would you analyze these two mutations in order to find out which individual is more susceptible for infection? Mention your steps and the tools you would use. [5 pt]