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Research Re-Exam Semester 2 – 2019-2020

July 10, 2020

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06 81 89 41 20.

The questions must be answered in English. If you cannot remember a specific English term, you may use the Dutch term.

Be precise in your answers. Adding correct but irrelevant information will not increase your score. Adding incorrect information, even if it is irrelevant, will lower your score.

During the exam, you may want to consult these books (see link in Brightspace):

- Baynes & Dominiczak: Medical Biochemistry
- Campbell: Statistics at square one
- Donders: Literature Measurement errors
- Fletcher: Clinical Epidemiology
- van Oosterom en Oostendorp: Medische Fysica
- Petrie and Sabin: Medical Statistics at a Glance
- Turnpenny: Emery's Elements of Medical Genetics
- Form with statistical formula’s

Name:

Student Number:

Question 1
Q3: What we can learn from urine – dr. S. Heemskerk (20 points)

Comparison of polycyclic aromatic hydrocarbon metabolite concentrations in urine of mothers and their newborns
K. Urbancova et al. *Science of the Total Environment,* **723** (2020) 138116

Polycyclic aromatic hydrocarbons (PAHs) are environmental contaminants produced during incomplete combustion of organic matter. Humans can be exposed to them via several pathways (inhalation, digestion, dermal exposure). The aim of this study was to assess the concentration of 11 monohydroxylated metabolites of PAHs (OH-PAHs) in 660 urine samples collected from mothers and their newborns residing in two localities of the Czech Republic — Most and Ceske Budejovice* — in 2016 and 2017.
After enzymatic hydrolysis, the target analytes were extracted from the spot urine samples using liquid-liquid extraction, with extraction solvent ethyl acetate and a clean-up step using dispersive solid-phase extraction with the Z-Sep sorbent. For identification and quantification, ultra-high performance liquid chromatography coupled with tandem mass spectrometry was ap-plied.
2-OH-NAP was the compound present in all of the measured samples and it was also the compound at the highest concentration in both mothers' and newborns' urine samples (median concentration 5.15 µg/g creatinine and 3.58 µg/g creatinine). The total concentrations of OH-PAHs in urine samples collected from mothers were 2 times higher compared to their children. The most contaminated samples were collected in Most in the period October 2016–March 2017 from both mothers (12.59 µg/g creatinine) and their newborns (8.29 µg/g creatinine).
The concentrations of OH-PAHs in urine samples, which were collected from both mothers and their newborns as presented in this study, are comparable with those found in our previous study between 2013 and 2014. In addition, they are slightly lower or comparable to other studies from Poland, USA, Germany, China, and Australia.

**The district of Ceske Budejovice in Southern Bohemia was selected as a control locality. The district of Most is one of the mining districts in Northern Bohemia, which was characterized by significant air pollution due to power plants and local heating emissions in the '70s and '80s, and use of brown coal with a high content of sulphur. The outcome of this load was seen in the shortening of life expectancy by two years in both males and females. This did not change in the period 1990–2017.*

A. Each mother filled in a questionnaire. Mention 5 variables you need to include in the questionnaire and explain why. (**5 points**)

B. Why do the authors measure urinary 2-OH-NAP concentration relative to creatinine? Explain your answer. (**4 points**)

The **validation** of the presented study was described as follows:

The method accuracy was controlled by using the Standard Reference Material (SRM) 3673. Limits of quantification (LOQs) were 0.01–0.025 ng/mL with recovery 77–113% and repeatability (RSD) 3–14%. For the two metabolites 6-OH-CHRY and 3-OH-BaP, which were not certified in the SRM 3673, the performance parameters were measured by the analysis of an artificially contaminated urine blank sample. The recovery for 6-OH-CHRY was 95% (RSD 13%, LOQ 0.01 ng/mL) and for 3-OH-BaP, it was 97% (RSD 16%, LOQ 0.9 ng/mL).
Background contamination by the target analytes was also monitored. A procedural blank sample (deionised water was used instead of urine) was prepared together with each batch of samples. The blank sample contained traces of 1-OH-NAP, 2-OH-NAP, 1-OH-PHEN, 2-OH-PHEN, 3-OH-PHEN and 1-OH-PYR (concentrations below 0.02 ng/mL urine). The concentration of contamination in the blank sample was subtracted from all samples prepared on the same day as the procedural blank sample.

C. Please read the text concerning the validation of the presented study. Explain whether the results are reliable and mention 3 parameters which you have used to determine this. **(4 points)**

The highest correlation between urine samples collected from mothers and their newborns was found for 2-OH-NAP, with the coefficient of determination (R squared) R2 = 0.79 in the urine samples collected in Ceske Budejovice from April to August 2017.

D. Please explain 2 factors to which the remaining 20 percent can be attributed to. **(4 points)**

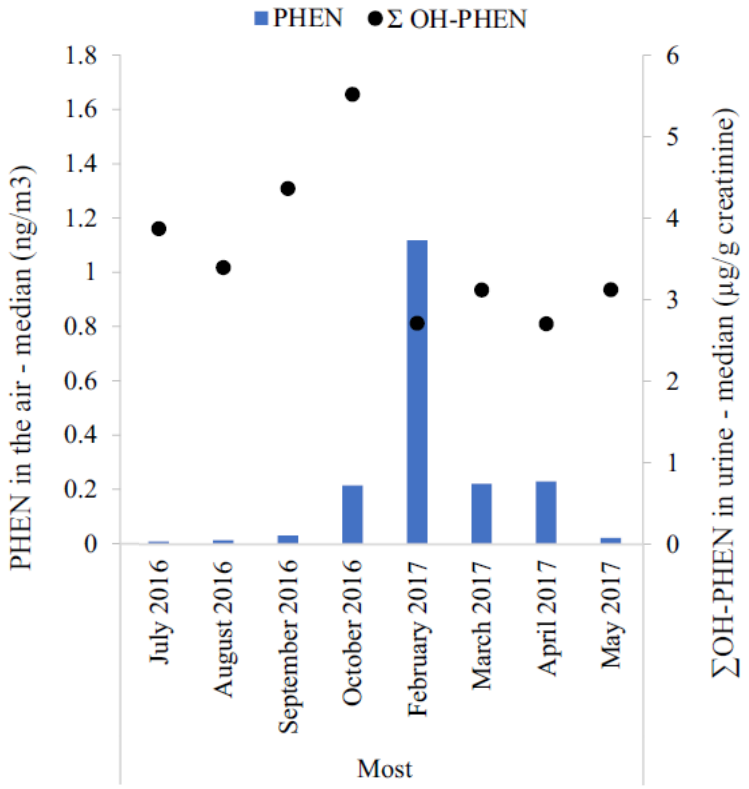


Fig. 2. Comparison of PHEN concentrations in the air and ∑OH-PHEN measured in urine.

E. What can be concluded ffrom Figure 2?
Use the word “correlation” in your answer.(**3 points)**

Question 2
Q4: Modelling Epidemic Outbreaks – dr. T. Oostendorp
(15 points)

The standard model that is used to predict the number of infectious people in an epidemic is the SIR model. In the future covid-25 pandemic, it will be important to estimate the number of people that will occupy beds in the intensive care (IC) units. In order to build this into a SIR model, a separate group of IC patients $P(t)$ is introduced, according to the diagram of figure 2.1.

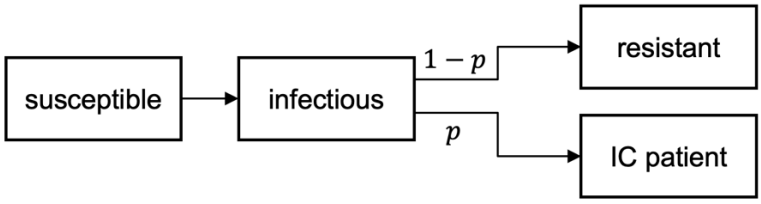


Figure 2.1 Diagram of SIR model for covid-25 including IC patients.

People that are infected stay infectious for D days. After that, the fraction p will move to the IC, and the rest will become resistant. Those that move to the IC will stay there for C days, after which they either die or recover. For covid-25, we assume that $D = 7$ days, $C = 21$ days and $p = 0.01$.

A. Explain that the differential equation for $P(t)$ is as stated below (4 pts).

$$\frac{d}{dt}P(t) = \frac{p}{D}I(t) - \frac{1}{C}P(t)$$

Note: there are two versions of question B; the first one is for students that did the module Modelling Epidemic Outbreaks in 2020 and learn R, the second one is for those that did it in the years before and learned Simulink.

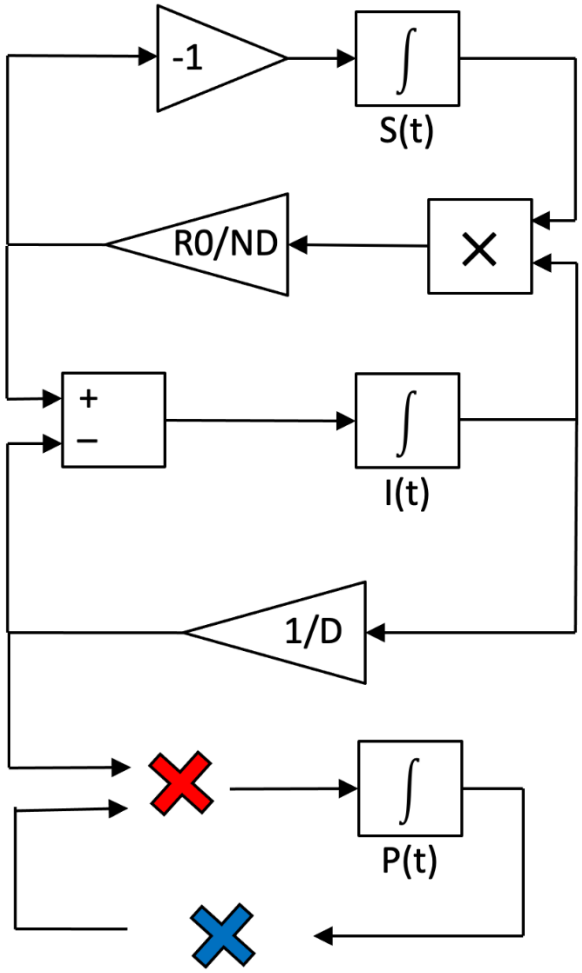
Version 1 (students in 2020; R):

B. Below is part of a program in R that implements a SIR model for covid-25. Add code in order to compute the number of IC patients. (5 pts)

```
SimDuration <- 100
N <- 100
R0 <- 2
D <- 7
C <- 21
p <- 0.01
S <- numeric(SimDuration)
I <- numeric(SimDuration)
P <- numeric(SimDuration)


P[1] <- 0
I[1] <- 0.1
S[1] <- N-I[1]


for (t in 1:SimDuration)
{

  newInfections   <- (R0/D) * (S[t]/N) * I[t]
  leaveInfections <- I[t]/D




  S[t+1] <- S[t] - newInfections
  I[t+1] <- I[t] + newInfections – leaveInfections




}
```

Version 2 (earlier students; Simulink):

B. Below is an unfinished Simulink diagram that implements the SIR model for covid-25, including the patients at the intensive care. What type of blocks should be inserted at the red cross and at the blue cross? Also, give the parameter value for the blue block. (5 pts)



Some policy-makers promote a strategy in which herd immunity is gradually achieved, while maintaining a constant number of patients in the IC.

C. What will be the ratio between the number of IC patients $P(t)$ and that of infectious people $I(t)$ in a situation where the number of IC patients is constant? (3 pts)

The purpose of increasing social distancing is to bring down the value of the basic reproductive number R_0. If R_0 is smaller than 1, the number new infections per day must decrease.

Figure 2.2 shows the result of a model study for vovid-25 in which at day 20 the value of R_0 drops instantaneously from 2 to 0.5 on day 20.

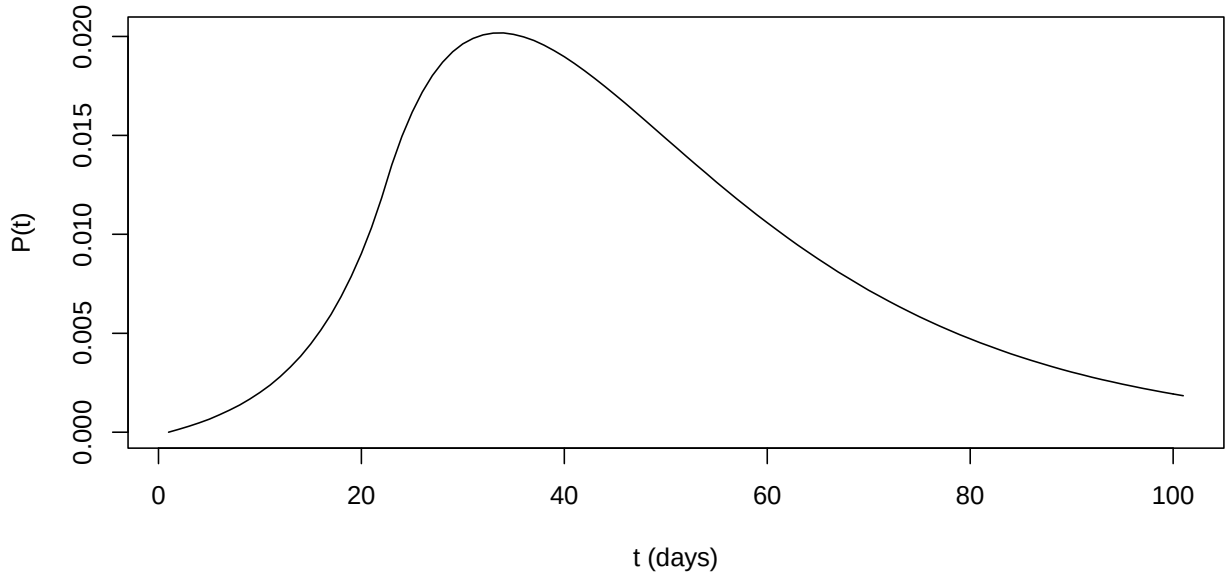


Figure 2.2 Prediction of number of IC patients if the basic reproductive number R_0 changes instantaneously from 2 to 0.5 on day 30.

D. Explain why, in this model, the number of IC patients still increases for some time after 20 days, even though from then on $R_0 < 1$ (3 pts)

Question 3

Q4: Population research: Associations and causal relations – dr. F. de Vegt (20 points)

Use ‘Dietary Habits and Physical Activity are Associated With the Risk of Breast Cancer Among Young Iranian Women: A Case-control Study on 1010 Premenopausal Women – abstract and tables. Mohammad Fararouei

A. What is the research question in the study of Fararouei et al?
In addition, specify the determinant, the outcome and the study population (2 pts).

Research question:

Determinant:

Outcome:

Study population:

B. The study design was a case-control study. Can the same research question also be studied in a randomised controlled trial (RCT)? Describe how a RCT looks like for this research question and explain whether this is feasible or not. (5 pts)

C. In a case-control study information bias may present. Describe what kind of information bias may be present in the study of Fararouei et al. and how likely this information bias is. (3 pts).

D. In Table 1 age was classified in 3 categories. If you had access to the exact ages of the women, how could you test for a difference in mean age between the cases and controls? (2 pts)

E. In Table 1 BMI was classified in 4 categories, ranging from ‘underweight’ to ‘obese’. Which test should be used to determine if the proportion of obesity is higher among cases compared to controls? (2 pts)

F. What is the interpretation of the result for smoking in Table 2? (3 pts)

G. See Table 2. The odds ratio (OR) for vigorous physical activity was 0.68. However, a stratified analyses for older and younger women showed that the OR was 1.09 for older women and 0.37 for younger women. How is this kind of difference in OR between subgroups called? Choose the right answer and explain the meaning of the term. (3 pts)

1) Confounding
2) Effect modification
3) Misclassification
4) Selection bias

Question 4
Q4: T- and B- cells in the lab – dr. E. Blaney Davidson
(20 points)

You are preparing a presentation about the differences between immunohistochemistry (IHC) and ELISA techniques. You want to explain how the involved antibodies bind their targets in a sandwich ELISA and how this is different from IHC.

A. Make a drawing including the antigen and the different antibodies in both techniques clearly visualizing the differences between the techniques. You are allowed to use text to guide the drawings. (5 pts)

B. After your presentation, one of your colleagues has a question and asks you to explain why you included an antigen retrieval step. Please explain why you use it and in which cases there is no need to use antigen retrieval (4 pts).

Patients with Corona due to COVID-19 infection display the presence of several cytokines in their blood, which is related to disease severity. IFN-γ is an important cytokine associated with a viral response. To measure the levels of IFN-γ in COVID-19 patients you will apply a direct sandwich ELISA (this means you have a combination of a capturing antibody and a labeled detecting antibody).

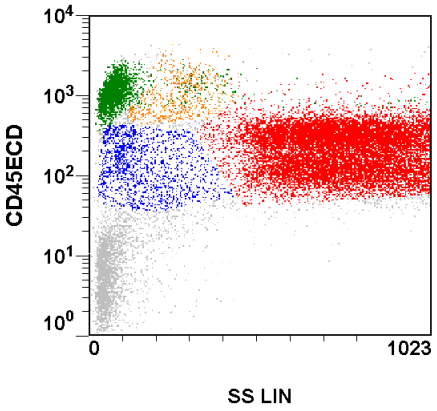
The following materials are available: standard ELISA plates, blocking buffers, washing buffers, substrate for horse radish peroxidase (HRP) and an ELISA plate reader.
In addition, the following antibodies, plasmas and antigens are available in the lab:

- HRP labeled Donkey (Ig) anti-human Ig
- Hamster(Ig) anti-rat Ig
- Mouse(Ig) directed at human IFN-γ
- Biotinylated Rat(Ig) directed at human IFN-γ
- Rat (Ig) anti-human Ig
- IFN-γ solution
- Plasma of COVID-19 patients
- Avidin-HRP conjugate

C. Write down the procedure to determine the level of IFN-γ in the circulation COVID-19 patients in a step-wise manner. In total there are 5 steps. Only indicate the sequence of steps regarding the application of antigen and antibodies that are required in this direct sandwich ELISA (so blocking and washing steps do not need to be indicated). (3 points)

D. Using this ELISA, how can you make an exact estimate about the absolute concentration of IFN-γ in the circulation? (1 point)

In order to determine whether the change in cytokine concentration as the result of a viral infection has influenced the composition of leukocyte subpopulations you like to use immunophenotyping using flowcytometry. You decide to use "CD45" and side scatter to investigate the four main leukocyte populations in bone marrow. The figure below is the population plot you made.



E. Which leukocyte populations can be found in the plot above? Mark their location by giving the color of the population in the plot. (3 points)

F. By using the combination of "CD45" and "SS" in the flow cytometrical determination of bone marrow you can differentiate between subpopulations of cells. Which information is provided by using this combination? Explain your answer. (4 points)

Abstract for question 1 – What we can learn from urine

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Dietary Habits and Physical Activity are Associated With the Risk of Breast Cancer Among Young Iranian Women: A Case-control Study on 1010 Premenopausal Women

MohammadFararouei[1]AqsaIqbal[2]ShahabRezaian[3]ZahraGheibi[1]AriaDianatinasab[4]SabaShakarami[5]MostafaDianatinasab[6]Clinical Breast Cancer, Volume 19, Issue 1, February 2019, Pages e127-e134

https://doi.org/10.1016/j.clbc.2018.10.011

Abstract

Background: Several studies conducted in developed countries introduced diet and physical inactivity as major risk factors for several types of cancers. However, the impact of diet and physical inactivity on the risk of breast cancer (BC) is understudied, and the limited findings are controversial. In addition, no or limited knowledge is available from the developing world.

Patients and methods: This case-control study was performed from November 2014 to March 2016 on 1010 young women aged 20 to 50 years who were newly diagnosed with BC (cases) or without cancer (controls). Data was obtained via a validated questionnaire and the global physical activity questionnaire (GPAQ2). Also, patients' medical and histopathology reports were reviewed.

Results: The results of multiple logistic regression suggested that, except for the common risk factors for BC (older marital age, family history of BC, smoking, and being a passive smoker), eating red meat (adjusted odds ratio [aOR] >8 portions/week [p/w] vs. 0-2 p/w, 1.15; 95% confidence interval [CI], 1.04-1.28); eating fish (aOR >8 p/w vs. 0-2 p/w, 1.55; 95% CI, 1.12-2.76), fruit consumption (aOR 0-4 p/w vs. >8 p/w, 1.96; 95% CI, 1.07-3.82), pickle consumption (aOR >8 p/w vs. 7-8 p/w, 1.46; 95% CI, 1.31-1.70), and intensity of physical activity (aOR light vs. vigorous, 1.68; 95% CI, 1.47-1.98) were directly associated with a higher risk of BC in young women.

Conclusion: Our study supported the hypothesis that unhealthy dietary habits and physical inactivity are risk factors for BC. We found that a healthy diet containing low fat and high fruits and vegetables with regular exercise are effective ways to reduce the risk of BC among young women.

Keywords: Diet; Epidemiology; Lifestyle; Physical activity; Risk factor.

Table 1. The Unadjusted Association of Sociodemographic Variables, Dietary Factors, and Physical Activity With Breast Cancer Development

(Modified table, not all variables listed)

Variables	New Cases N = 505, n (%)	Controls N = 505,N (%)	*P* Value
Age, y			.303
< 40	115 (22.8)	137 (27.1)	
40-45	171 (33.9)	169 (33.5)	
46-50	219 (43.4)	214 (42.4)	
Education			.018
Primary or illiterate	184 (36.4)	183 (36.2)	
Secondary	76 (15.1)	110 (21.8)	
High school	160 (31.7)	127 (25.2)	
College	85 (16.8)	85 (16.8)	
BMI, kg/m^2			.062
Underweight (<18.5)	11 (2.2)	10 (2.0)	
Normal (18.5-24.9)	149 (29.5)	151 (29.9)	
Overweight (25-29.9)	222 (43.9)	254 (50.3)	
Obese (≥30)	123 (24.4)	90 (17.8)	
Intensity of physical activity			.009
Light (<600 MET-minutes per week) and moderate (600-3000 MET-minutes per week) intensity	423 (83.8)	390 (77.2)	
Vigorous intensity (3000 MET-minutes per week)	82 (16.2)	115 (22.8)	
Smoking			< .001
Non-smoker	430 (85.1)	466 (92.3)	
Smoker	75 (14.9)	39 (7.7)	

Abbreviations: BMI = body mass index; MET = metabolic equivalent; p/w = portion per week.

Table 2. Adjusted Association Between The Study Variables And Breast Cancer Risk Among Young Women Using Multiple Regression

(Modified table, not all variables listed)

Variables	OR	95% CI		*P* Value
		Lower Limit	Upper Limit	
Education				
History of breast disease				
Never	1	–	–	–
Ever	4.71	2.67	8.34	.001
Age at first marriage, y				
> 30	1	–	–	–
< 18	0.35	0.18	0.65	.001
18-24	0.49	0.26	0.88	.021
25-30	0.91	0.46	1.80	.799
Not married	1.07	0.44	2.55	.881
Family history of breast cancer				
No	1	-	-	-
Second relative	1.52	0.88	2.64	.132
Close relative	3.41	1.93	6.01	< .001
Oral contraceptive usage				
Never	1	–	–	–
Ever	1.77	1.32	2.38	< .001
Smoking				
No	1	–	–	–
Yes	2.48	1.56	3.96	< .001
Passive smoker				
No	1	–	–	–
Yes	1.71	1.28	2.27	< .001
Intensity of physical activity[a]				
Light intensity	1	–	–	–
Vigorous intensity	0.68	0.47	0.98	.036

All study variables were included in the final model including: age, education, body mass index, occupation, age at first marriage, history of breast disease, family history of breast

cancer, oral contraceptive usage, intensity of physical activity, smoking, passive smoker, and dietary habit factors (high carbohydrate/sugar use, pickle use, fruit use, vegetable use, dairy use, and meat, fish, and chicken per week and dairy kind).

Abbreviations: CI = confidence interval; MET = metabolic equivalent; OR = odds ratio; p/w = portions per week.

a Light: < 600 MET-minutes per week; Vigorous intensity: 3000 MET-minutes per week; moderate intensity: 600-3000 MET-minutes per week.
b Any kind of fish.