Radboud

Examination	.	**B1RES2 Research, tweede semester**
Date		June 14th 2019
Start		9:00 h

After finishing the exam, you can take this examination set along with you.
Please hand in the OTHER part (the answering form) to the supervisor.

You are allowed to use a calculator of the type Casio FX-82MS.

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

During the exam, you have access on a computer to these books:

- 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

GENERAL INSTRUCTIONS

- This exam consists of **4** open questions.
- The available time is **2** hours.
- Check if your examination set is complete.
- Please write *your name and student number* on each page of the answering form.
- Write your answers on the answering form in the open space below the questions. Read the questions carefully before phrasing your answers.
- Be concise and complete in your answers.
- If necessary you can also use the *backside* of the pages.
- Refrain from using abbreviations in your answers, and write legibly (illegible answers are considered incorrect).
- Please do not use a pencil.
- The use of audiovisual and technical devices is not allowed, unless it is mentioned explicitly elsewhere on this page. Any inappropriate use of such equipment is regarded as fraud.
- Except for the exam forms, some loose writing material, your student card, your table should be empty. No boxes or cases are allowed.
- **After finishing the exam, please hand the answering form to the supervisor. If you have comments about the questions we refer you to the hyperlink of the digital comment form that is included in your "studenten webdossier" below "toetsen".**

SUCCESS

ATTENTION !!

FIRST PUT YOUR NAME AND STUDENT NUMBER ON EVERY PAGE OF THE ANSWERING FORM!

Question 1 Q3: What we can learn from urine – dr. H. Pluk (15 points)

Comparison of associations of urine protein-creatinine ratio versus albumin-creatinine ratio with complications of CKD.
Fisher H, Hsu CY, Vittinghoff E, Lin F, Bansal N.

BACKGROUND: Urine albumin-creatinine ratio (ACR) and protein-creatinine ratio (PCR) are important markers of kidney damage and are used for prognosis in persons with chronic kidney disease (CKD). Despite how commonly these measurements are done in clinical practice, relatively few studies have directly compared the performance of these two measures.

SETTING & PARTICIPANTS: 131 participants with CKD in the Chronic Renal Insufficiency Cohort Study.

RESULTS: Mean estimated glomerular filtration rate (eGFR) was 43±13 (SD) mL/min/1.73 m^2 and median values for PCR and ACR were 140 and 46 mg/g, respectively. In continuous analyses, higher ACRs and PCRs were similar and both were associated with lower serum hemoglobin levels and higher potassium levels.

CONCLUSIONS: Routine measurement of PCR may provide similar information as ACR in managing immediate complications of CKD.

Adapted from Am J Kidney Dis. 2013, 1102-8.

a. It has been shown before that high total proteinuria and albuminuria are associated independently with adverse outcomes in patients with CKD. In this study, Fisher et al. measured and compared spot urine albumin-creatinine ratio (ACR) and protein-creatinine ratio (PCR) values. Explain why spot urine protein-creatinine ratio is a valid alternative for total protein measurements in 24 hrs urine. Include in your answer the reason why creatinine is chosen in this approach. (4 pt)

b. Albumin and protein are used as biomarkers for CKD. Name four general conditions/features of biomarkers that are required for a molecule/compound to be considered for use as a biomarker. (4 pt)

c. Fisher et al. found the following result: “As shown in Figure 1.1, ACR and PCR were highly correlated (Pearson coefficient 0.92)”. Use this information and the information given in the abstract to sketch Figure 1.1 below. Pay attention to axes labels and units. You do not need to complete the legend. 5 (pt)

Figure 1.1 Scatter plot of albumin-creatinine ratio versus protein-creatinine ratio.

d. Fisher et al. used the calibration curve in Figure 1.2 to determine the concentration of creatinine in urine. How can you determine if this calibration curve is valid? Name and briefly explain two points to consider. (2 pt)

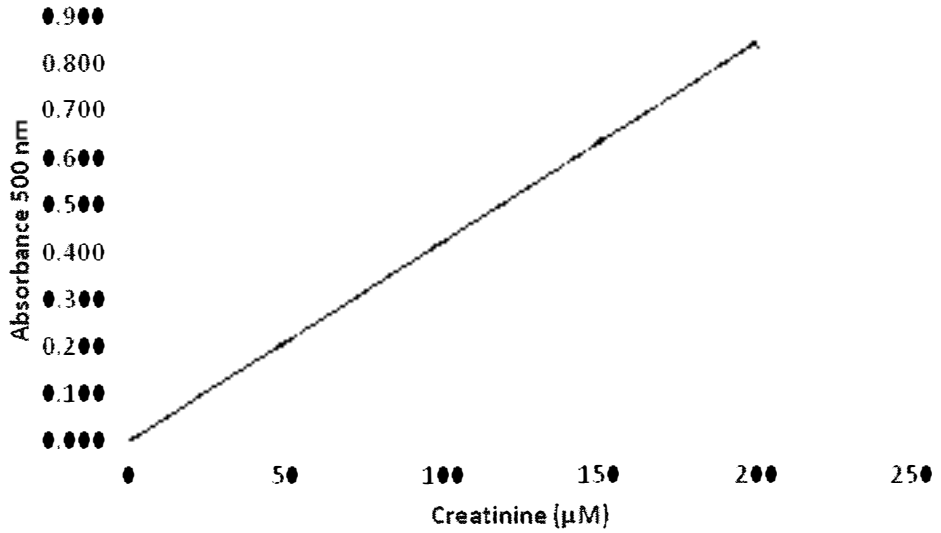
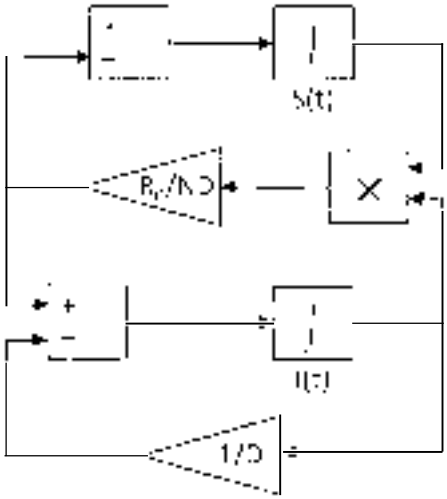


Figure 1.2 Calibration curve.

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

In the standard SIR model, people who recover from the disease are resistant, and stay resistant for the rest of their lives. Figure 2.1 shows the Simulink diagram of that model.



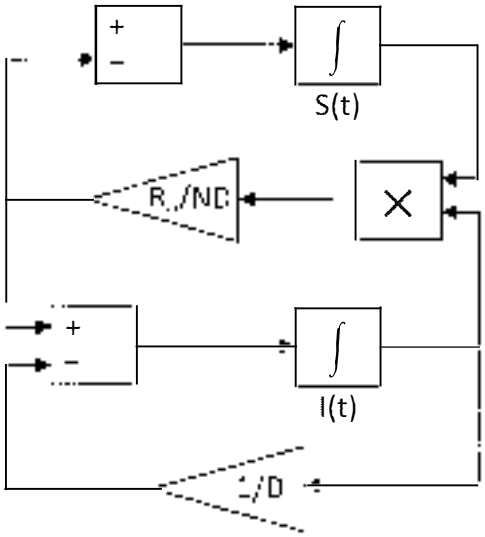
$S(t)$	number of susceptible people at time t
$I(t)$	number of infected people at time t
R_0	basic reproductive number
D	duration of infectiousness
N	population size

Figure 2.1 Simulink diagram of the standard SIR model.

For pertussis (whooping cough), resistant individuals lose their immunity after a few years. T is the average number of years people are immune after an infection.

a. Show that, in a model for pertussis, the differential equation for the number of resistant people $R(t)$ is $\frac{d}{dt}R(t) = \frac{1}{D}I(t) - \frac{1}{T}R(t)$ (5 pt).

b. Add, in the diagram below, an integrator block for $R(t)$ and extend the diagram to make it a model for pertussis. (5 pt).



For pertussis, the relevant parameters are:

R_0	5
D	5 days = 5/365 years
T	5 years

A simulation was performed using these parameter settings for a population size of 100000. The initial number of susceptible people was set to 20000, and the initial number of infected people at 1. Figure 2.2 shows the results.

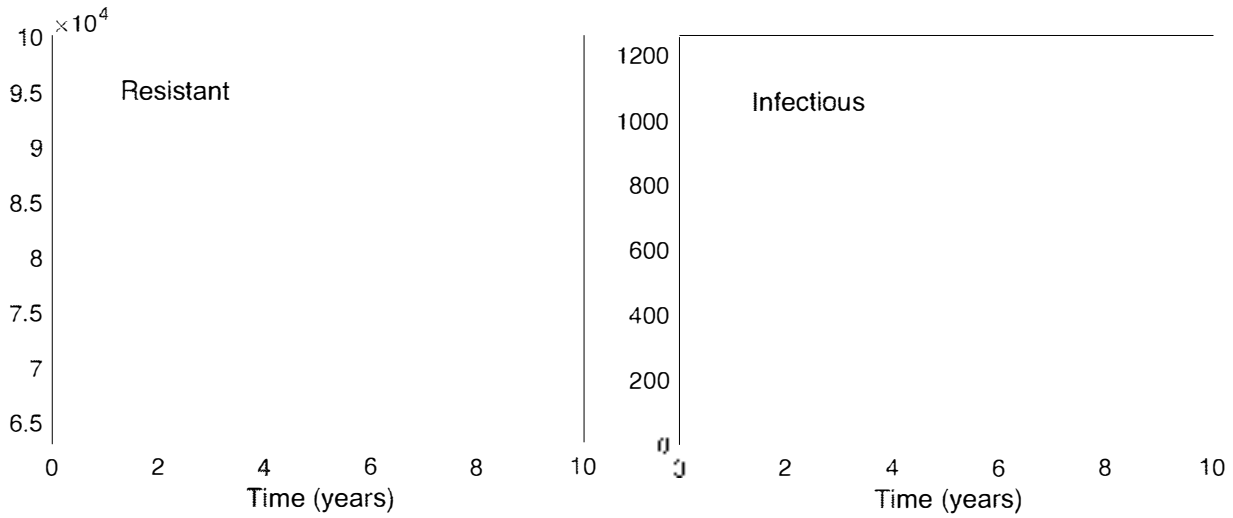


Figure 2.2 Simulation results: number of resistant (left) and infectious (right) people.

As you can see, there are repetitive outbreaks, but after some 10 years $R(t)$ and $I(t)$ reach stationary values that we will call R_{stat} and I_{stat}.

Figure 2.2 shows that $R_{\text{stat}} \approx 80000$ and $I_{\text{stat}} \approx 220$. Hence, the value of the ratio of the stationary values is

$$\frac{R_{\text{stat}}}{I_{\text{stat}}} \approx 360$$

c. Show that it follows from the differential equation in question a and the values provides for T and D that the ratio of the stationary values must be 365. (5 pt).

Question 3

Q4: Population research: Associations and causal relations – dr. F. de Vegt (25 points)

Use 'Barregard *et al. Blood cadmium levels and incident cardiovascular events during follow-up in a population-based cohort of Swedish Adults: The Malmö diet and cancer study– abstract and tables.*'

In this exam, a hazard ratio may be interpreted as a relative risk

a. What is the research question in the study of Barregard et al?
   In addition, specify the determinant, the outcome and the study population (4 pt).
   Research question:
   Determinant:
   Outcome:
   Study population:

b. See Table 3. Explain what the meaning is of the marked results mentioned in the last column:

   2.6 (1.0 – 6.9) (3 pts)

c. Explain why age is not likely to be a confounder in the research of Barregard et al. (3 pts)

d. What has been done in the research of Barregard et al. to control for confounders? (2 pts)

e. List two other ways how to control for confounders in general (2 pts)

f. What is information bias? Do you think information bias is likely in the research of Barregard et al.? Explain your answer. (3 pts)

Table 2 of the article from Barregard et al. shows the number of major adverse cardiac events per quartile class of blood cadmium. Assume that every person has the same follow-up time.

g. The percentage of people with a 'Major adverse cardiac event' for the 3th quartile is 10.4%. Calculate a 95% confidence interval for this percentage. (4 pts)

h. Which test can be used to see whether the percentages of people with a 'Major cardiac event' differ between the 4 quartile groups? (1 pts)

i. In Table 3 seven outcome parameters are analysed in several ways. The statistical section mentioned 'A two-tailed p-value < 0.05 was considered statistically significant'. What methodological problem did the authors not deal with? (3 pts)

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

Immunohistochemistry – 7 pts

A PhD student is researching T-cells in the spleen. He wants to visualize the T-cell receptor (TCR) and is questioning which label to use for his secondary antibody for immunohistochemistry (IHC).

a. Name an advantage and a disadvantage of using fluorescently labeled antibodies. (4 pts)

John has optimized his IHC protocol for spleens of mice, but also wants to verify his data in human spleen.

b. What are the consequences of changing to human spleen for his IHC staining protocol? Name and explain two changes the PhD student should make. (3 pts)

Elisa – 8 pts

Patients with Systemic Lupus Erythematosus (SLE) are characterized by auto-antibody production against nucleosomes, the basic building block of chromatin. It appears that the level of anti-nucleosome antibodies in the circulation of SLE patients is associated with disease activity. To monitor disease activity, you can measure the anti-nucleosome levels in the blood of SLE patients with an ELISA.

c. Which materials and equipment do you need in general to perform an ELISA in the laboratory? (4 pts)

d. In addition to general materials and equipment, what do you specifically need to be able to measure the level of anti-nucleosome antibodies in blood of SLE patients using an ELISA? (4 pts)

Flowcytometry – 5 pts

In order to determine whether a change in cytokine concentration has influenced the composition of the various lymphocyte subpopulations you like to use flow cytometry to investigate the 4 main lymphocyte populations.

e. Which antibody combination should be applied to determine each lymphocyte population separately? Describe the 4 main lymphocyte subsets with the relevant antibodies to identify them. (2 pts)

f. How should you perform the flow cytometry analysis to evaluate correctly these populations? Describe the different flow cytometry plots by mentioning their axes in the correct rank order, starting with FS/SS and the population that should be gated. (3 pts)

FOR QUESTION 3

Barregard *et al. Blood cadmium levels and incident cardiovascular events during follow-up in a population-based cohort of Swedish Adults: The Malmö diet and cancer study– abstract and tables*.

Environmental Health Perspectives 2016;124(5):594-600

Abstract

BACKGROUND: Cadmium exposure may increase the risk of cardiovascular disease. The only published longitudinal study on cadmium and incident cardiovascular disease was performed in American Indians with relatively high cadmium exposure.

OBJECTIVES: Our aim was to examine the association between blood cadmium at baseline and incident cardiovascular events in a population-based study of Swedish men and women with cadmium levels similar to those of most European and U.S.

METHODS: A Swedish population-based cohort (n = 6,103, age 46-67 years) was recruited between 1991 and 1994. After we excluded those with missing data on smoking, 4,819 participants remained. Acute coronary events, other major cardiac events, stroke, and cardiovascular mortality were followed until 2010. Associations with blood cadmium (estimated from cadmium in erythrocytes) were analyzed using Cox proportional hazards regression including potential confounders and important cardiovascular risk factors.

RESULTS: Hazard ratios for all cardiovascular end points were consistently increased for participants in the 4th blood cadmium quartile (median, 0.99 µg/L). In models that also included sex, smoking, waist circumference, education, physical activity, alcohol intake, serum triglycerides, HbA1c, and C-reactive protein, the hazard ratios comparing the highest and lowest quartiles of exposure were 1.8 (95% CI: 1.2, 2.7) for acute coronary events, and 1.9 (1.3, 2.9) for stroke. Hazard ratios in never-smokers were consistent with these estimates.

CONCLUSIONS: Blood cadmium in the highest quartile was associated with incident cardiovascular disease and mortality in our population-based samples of Swedish adults. The consistent results among never-smokers are important because smoking is a strong confounder. Our findings suggest that measures to reduce cadmium exposures are warranted, even in populations without unusual sources of exposure.

CITATION: Barregard L, Sallsten G, Fagerberg B, Borné Y, Persson M, Hedblad B, Engström G. 2016. Blood cadmium levels and incident cardiovascular events during follow-up in a population-based cohort of Swedish adults: the Malmö Diet and Cancer Study. Environ Health Perspect 124:594-600; http://dx.doi.org/10.1289/ehp.1509735.

Outcome and model	Quartiles of blood cadmium			
	1	2	3	4
Acute myocardial infarction				
Sample size (*n* = 4,745)	1,195	1,191	1,181	1,178
No. of events (*n* = 344)	75	77	79	113
Incidence/1,000 person-years	3.7	3.8	4.0	6.1
Model 1 (344 events)	1.0	1.1 (0.8, 1.5)	1.1 (0.8, 1.6)	1.9 (1.4, 2.5)
Model 2 (305 events)	1.0	1.2 (0.8, 1.6)	1.3 (0.9, 1.8)	1.7 (1.1, 2.7)
Model 3 (283 events)	1.0	1.0 (0.7, 1.5)	1.1 (0.8, 1.6)	1.8 (1.2, 2.8)
Major adverse cardiac event[b]				
Sample size (*n* = 4,726)	1,191	1,187	1,174	1,174
No. of events (*n* = 479)	96	193	122	158
Incidence/1,000 person-years	4.8	5.2	6.3	8.0
Model 1 (479 events)	1.0	1.2 (0.9, 1.5)	1.4 (1.1, 1.9)	2.1 (1.6, 2.7)
Model 2 (422 events)	1.0	1.2 (0.9, 1.7)	1.5 (1.1, 2.0)	1.9 (1.3, 2.7)
Model 3 (394 events)	1.0	1.1 (0.9, 1.5)	1.4 (1.0, 1.9)	1.9 (1.3, 2.8)
Any stroke				
Sample size (*n* = 4,583)	1,201	1,196	1,195	1,191
No. of events (*n* = 336)	76	71	74	115
Incidence/1,000 person-years	3.8	3.5	3.7	6.2
Model 1 (336 events)	1.0	0.9 (0.6, 1.2)	0.9 (0.7, 1.3)	1.8 (1.3, 2.4)
Model 2 (294 events)	1.0	0.9 (0.6, 1.3)	1.0 (0.7, 1.4)	1.9 (1.3, 2.9)
Model 3 (271 events)	1.0	0.8 (0.6, 1.2)	0.9 (0.7, 1.4)	2.1 (1.3, 3.2)
Ischemic stroke				
Sample size (*n* = 4,796)	1,202	1,201	1,196	1,197
No. of events (*n* = 278)	63	58	59	98

Table 3

Hazard ratios for incident first cardiovascular disease by quartiles of cadmium concentration in blood (time scale = age) in never-smokers.

Outcome	Quartiles of blood cadmium			
	1	2	3	4
Acute coronary event[a]				
Sample size (*n* = 1,782)	702	578	441	61
No. of events (*n* = 111)	47	31	26	7
HR (95% CI)	1.0	0.9 (0.6, 1.5)	1.0 (0.6, 1.6)	2.3 (1.0, 5.1)
Acute myocardial infarction				
Sample size (*n* = 1,782)	702	578	441	61
No. of events (*n* = 106)	44	31	24	7
HR (95% CI)	1.0	1.0 (0.6, 1.5)	1.0 (0.6, 1.6)	2.4 (1.1, 5.4)
Major adverse cardiac event[b]				
Sample size (*n* = 1,777)	700	576	440	61
No. of events (*n*= 136)	57	39	32	8
HR (95% CI)	1.0	1.0 (0.7, 1.5)	1.1 (0.7, 1.7)	2.2 (1.0, 4.6)
Any stroke				
Sample size (*n* = 1,785)	703	579	442	61
No. of events (*n* = 111)	41	32	31	7
HR (95% CI)	1.0	1.0 (0.6, 1.6)	1.2 (0.7, 2.0)	2.2 (1.0, 4.8)
Ischemic stroke				
Sample size (*n* = 1,789)	704	581	443	61
No. of events (*n* = 85)	32	25	22	6
HR (95% CI)	1.0	1.0 (0.6, 1.7)	1.1 (0.6, 2.0)	2.5 (1.0, 6.0)
All-cause mortality				
Sample size (*n* = 1,793)	705	582	445	61
No. of events (*n* = 238)	93	75	59	11
HR (95% CI)	1.0	1.0 (0.7, 1.3)	1.0 (0.7, 1.3)	1.3 (0.7, 2.4)