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| **A close up of a logo  Description automatically generated**  **UNIVERSITY OF TORONTO MISSISSAUGA**  **TERM TEST 3**  **BIO360H5, Winter 2023**  **Celia Hein**  **Duration – 100 min**  **Aids: Non-Programmable Calculator, Crib Sheet (3 sheets, double sided)**  *The University of Toronto Mississauga and you, as a student, share a commitment to academic integrity. You are reminded that you may be charged with an academic offence for possessing any unauthorized aids during the writing of an exam, including but not limited to any electronic devices with storage, such as cell phones, pagers, personal digital assistants (PDAs), iPods, and MP3 players. Unauthorized calculators and notes are also not permitted. Do not have any of these items in your possession in the area of your desk. Please turn the electronics off and put all unauthorized aids with your belongings at the front of the room before the examination begins. If any of these items are kept with you during the writing of your exam, you may be charged with an academic offence. A typical penalty may cause you to fail the course.*  *Please note, you* ***CANNOT*** *petition to* ***re-write*** *an examination once the exam has begun.* |

**TEST QUESTIONS**

1. This exam contains 18 problems with a total of **40 questions on pages 2 - 9**.

2.Pages 11 – 17 contain the description of a case study with three parts.

3. There is additional space for notes on pages 10 and 18.

4. Question point values add to a total of 50 points:

- Matching questions are worth 1 point each (28 points)

- Multiple choice questions are worth 2 points each (22 points).

5. Choose the single best answer for each question.

6. No marks are deducted for incorrect answers, so answer all questions.

7. Transfer all of your answers to the Scantron computer sheet. No answers on the test paper will be marked.

8. You must hand in both your Scantron computer sheet AND your test paper.

9. No questions will be answered during the test.

**SCANTRON computer sheet**

1. Follow the instructions on the sheet.

2. Use PENCIL and erase any changes completely.

3. Bubble your Form (A or B).

4. Write your student number in the boxes and bubble in the numbers in the correct columns.

5. Write your name, date, and course in the upper right in the spaces provided.

6. Do NOT write anything along the top or side of the Scantron sheet.

7. For each question bubble your answer during the time allowed.

**REFER TO CASE STUDY**

Familiarize yourself with the topic and case study description on page 8.

**Part 1: LN Particulate Accumulation and Age**

Refer to pages 8 - 9 to answer the questions in this section. The researchers saw that lung lymph nodes (LLNs) were black, while mesenteric (intestinal) lymph nodes (MLNs) were beige (Fig. 1). They believed the blackening of LLN tissues was caused by the accumulation of inhaled, carbon-based pollutant particulates, which they expected to increase with age.

**Topic: Study Design**

*2 marks* **1 Multiple choice:** What best describes the study type of Part 1? Select one answer and enter it under question 1 of the Scantron form.

(A) Laboratory experiment.

(B) Field experiment.

(C) Case-control study.

(D) Cross-sectional survey.

*2 marks* **2 Multiple choice:** Which of the following best describes the researchers’ sampling design in Part 1 of this study? Select one answer and enter it under question 2 of the Scantron form.

(A) Randomization.

(B) Blocking.

(C) Stratification.

(D) Simple Random Sample.

**Matching:** Refer to case study on page 8. For each of the statements listed below, indicate whether it is true (A) or false (B). Use questions 3 – 6 on the Scantron form, one question per statement.

*1 mark* **3** A The age of the donor is an observed, random variable.

*1 mark* **4** B The type of tissue is an observed, random variable.

*1 mark* **5** A percent particulate accumulation is an observed random variable

*1 mark* **6** B The level of replication of the analysis in Fig 2. was 17.

*2 marks* **7 Multiple choice:** Refer to Fig. 2. Assume the authors sampled lung and mesenteric LN tissue from each individual. Which of the following statements most accurate? Select the best answer and enter it under question 7 of the Scantron form.

(A) This is paired sampling, so the conditions of simple linear regression are not met.

(B) This is paired sampling, which reduces variance of the model.

(C) This is not paired sampling because LLNs and MLNs are modelled separately.

(D) This is not paired sampling because it was not a “before and after” study.

**Topic: Hypothesis Testing**

*2 marks* **8 Multiple choice:** Refer to Fig. 2. The authors noticed that particulate accumulation in in LLNs was noticeably higher for individuals with age > 40 years. If the authors were to perform a test using age categories ( < 40, 40 – 64, and ≥ 65 years) to explain mean particulate accumulation, what test should they use? Assume the data is normally distributed. Select the best answer and enter it under question 8 of the Scantron form.

(A) A Wilcoxon rank sum test.

(B) A Chi-square test.

(C) A *t*-test assuming unequal variances (two-sample *t*-test as taught in this course).

(D) A one-way ANOVA.

**Part 2: Macrophage Particulate Accumulation**

The researchers investigated several types of macrophages in LLN tissue to find out where the particulates accumulate. The researchers performed a two-way ANOVA using age category and macrophage type to explain the number of cells containing particulate per mm2.

**Topic: One-way ANOVA**

As we have not yet learned how to perform an ANOVA with two predictors, your instructor re-analyzed the data using a one-way ANOVA with the three groups shown in Fig. 3, where each group represents all age categories. The R output is shown in Fig. 11.

*2 marks* **9 Multiple choice:** Refer to Fig. 3 and 4. Are the assumptions and conditions for performing an ANOVA on these data met? Select the best answer and enter it under question 9 of the Scantron form.

(A) Yes, there is no major issue with the data.

(B) No, because the equal means assumption is not met.

(C) No, because the equal variances assumption is not met.

(D) No, because the data are not independent, they are paired.

**Matching:** Refer to Fig. 4 and 5. Assume the conditions for the ANOVA are met. For each of the statements listed below, indicate whether it is true (A) or false (B). Use questions 10 – 14 on the Scantron form, one question per statement.

*1 mark* **10** A The total sum of squares (SS) is 7403699.

*1 mark* **11** B A Bonferroni correction should be applied to the pairwise comparisons.

*1 mark* **12** A The total df is 53.

*1 mark* **13** B The overall effect size was medium.

*1 mark* **14** A The study design was balanced.

*2 marks* **15 Multiple choice:** Refer to Fig. 5 and 6. Assume all test conditions have been met. What is the best interpretation of these results? Select the best answer and enter it under question 15 of the Scantron form.

(A) The mean of CD68-CD169+ was significantly different from the other two groups.

(B) There was a significant difference between groups, but it cannot be determined which groups were different.

(C) The mean of CD68+CD169- was significantly higher than the other two groups.

(D) The mean of CD68+CD169+ was significantly lower than the other two groups.

**Topic: Regression Models**

**Matching:** Refer to Fig. 7 and the description of three regression models, A - D, in the table below. Note that the axis does not start at 0. For each of the data (sub)sets listed below, indicate the corresponding model (A, B, C or D). Use questions 16 – 19 on the Scantron form, one question per statement.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Model | Intercept | Slope | P-value for slope | R2 |
| Model A | -7.05 | 0.33 | 0.316 | 0.031 |
| Model B | 18.32 | 0.02 | 0.937 | < 0.001 |
| Model C | 24.70 | -0.14 | 0.600 | 0.02 |
| Model D | 44.95 | 0.20 | 0.59 | 0.02 |

*1 mark* **16** C CD68-CD169+ w/particulates.

*1 mark* **17** B CD68+CD169+ w/o particulates.

*1 mark* **18** D CD68-CD169+ w/o particulates

*1 mark* **19** A CD68+CD169+ w/particulates.

*2 marks* **20 Multiple choice:** Which of the following is the best option for dealing with outliers or influential points. Select one answer and enter it under question 20 of the Scantron form.

(A) If removing the point reduces the p-value, you should remove it.

(B) If removing the point increases the R2, you should remove it.

(C) You should present the analysis with and without the point.

(D) If the point has a Cook’s D score > 1, you should remove it.

**Part 3: Particulate Accumulation Impairs Immune Function**

The researchers investigated several immune markers (CD36, CD209, and CD80/86) associated with immune activation, phagocytosis, and phagocytotic capacity in both LLN and MLN tissue. They found that immune function of LLNs decreased with age, presumably because of particulate accumulation.

**Topic: Influential Points**

**Matching:** Refer to Fig. 8. Consider the labeled data points P1-P4 and their respective regression models. For each of the statements listed below, indicate whether it is true (A) or false (B). Use questions 21 – 24 on the Scantron form, one question per statement.

*1 mark* **21** A Point P1 has a larger predicted value than point P2.

*1 mark* **22** A Point P2 has a larger squared residual than point P1.

*1 mark* **23** B Point P3 has a large squared residual and high leverage.

*1 mark* **24** A Point P4 has a large squared residual and high leverage.

*2 marks* **25 Multiple choice:** Which of the following is the best option for dealing with outliers or influential points. Select one answer and enter it under question 25 of the Scantron form.

(A) If removing the point reduces the p-value, you should remove it.

(B) If removing the point increases the R2, you should remove it.

(C) You should present the analysis with and without the point.

(D) If the point has a Cook’s D score > 1, you should remove it.

**Topic: Residual Analysis**

The researchers fitted a simple regression model of each marker against age. Box 1 shows how they described the regression results in the published paper. They did not comment on residual analysis, hence your instructor re-analyzed the data in R to check the residuals.

**Box 1:** " expression of the key activation markers CD80 and CD86 [CD80/86] and the phagocytic marker CD36 decreased with age specifically in CD68+CD169− macrophages in LLNs but not in MLNs. In contrast, CD209 expression was not altered significantly with age in any macrophage subset at either site. These results show that […] the expression of functional markers specifically in the CD68+CD169− subset within LLNs decreases with age, suggesting that particulates may have specific effects on macrophage function.”

*2 marks* **26 Multiple choice:** Refer to Box 1, the statistics in Fig. 10, and the residual plots in Fig. 11 and 12. What is the appropriate interpretation of the regression results of marker CD209 for the lungs and the mesentery? Select one answer and enter it under question 26 of the Scantron form.

(A) Marker CD209 was *not* statistically significant in the lungs nor the mesentery, but the effect in both sites was large enough to be relevant.

(B) Marker CD209 was *not* statistically significant in the lungs nor the mesentery, and the effect was too small to be practically relevant in the mesentery only.

(C) Marker CD209 was *not* statistically significant in the lungs nor the mesentery, but there may be a considerable risk of a type II error, more data are needed to be sure.

(D) Marker CD209 was *not* statistically significant, and the effect in both sites was too small to be biologically relevant.

(E) The model should not be interpreted; it is not valid because at least one condition has been violated.

**Matching:** Refer to Figures 11 and 12. Compare the residual plots of the CD209 model in LLN and MLN tissues new model. For each of the statements listed below, indicate whether it is true (A) or false (B). Use questions 27 – 31 on the Scantron form, one question per statement.

*1 mark* **27** B The distribution of the residuals is less skewed in Fig. 11 than in Fig. 12.

*1 mark* **28** A The model in Fig. 12 fits better in terms of the thickening of the plot.

*1 mark* **39** B The model in Fig. 11 fits better in terms of the relationship being linear.

*1 mark* **30** A The model in Fig. 12 fits better in terms of influential points.

*1 mark* **31 A** Both models may benefit from a log-transformation of the data.

**Topic: Regression Interpretation**

**Matching:** Refer Figures 8 – 10. Assume all models met the conditions for linear regression. For each of the statements listed below, indicate whether it is true (A) or false (B). Use questions 32 – 37 on the Scantron form, one question per statement.

*1 mark* **32** A No models showed a statistically significant, positive association with age.

*1 mark* **33** B In all models the effect size was biologically relevant.

*1 mark* **34** A In the lungs, models CD36 and CD80/86 were statistically significant and had large effect sizes.

*1 mark* **35** B The LLN model for marker CD80/86 had a Pearson’s r of 0.25.

*1 mark* **36** A The LLN model for marker CD209 was not statistically significant, though it had a medium effect size.

*1 mark* **37** B Overall, these results suggest that particulate accumulation in the LLNs reduces expression of markers CD36 and CD80/86.

**Topic: Bonferroni Correction**

*2 marks* **38 Multiple choice:** A reviewer for the journal might have requested that the researchers use a Bonferroni correction to account for the number of tests performed with the age data. Refer to Fig. 10 and apply a Bonferroni correction (with *k* = 6). How would the Bonferroni correction affect the interpretation of the hypothesis tests? Select the best answer and enter it under question 34 of the Scantron form.

(A) The LNN model for CD209 would be statistically significant.

(B) The LLN models for CD36 and CD80/86 would not be statistically significant.

(C) The LLN model for CD36 would be statistically significant, but the LLN model for CD80/86 would not be statistically significant.

(D) There would be no change in interpretation for any model.

**Case study: air pollution reduces lung immune function**

People become more susceptible to various diseases as they age, particularly to diseases in the lungs. Ural et al. (2022) investigated the effects of lifelong exposure to air pollution on lung-lymph nodes and lung immune function.

Lymph nodes are small structures found throughout the body that contain important immune cells of various types. They are vital to the body’s immune response against cancers and infectious diseases. The researchers saw that some lymph nodes were black in color and others were beige. The lungs, unlike other organ systems, are continuously exposed to the environment and carbon-based air pollutants, such as those found in vehicle exhaust, heating emissions, and fires. The researchers found that lymph nodes in the lungs (LLNs) turned black with age, unlike intestinal, mesenteric lymph nodes (MLNs). They found that long-term exposure to air pollution resulted in the accumulation of inhaled particulates in CD68+CD169- macrophages (cells with the ability to “eat” foreign bodies through phagocytosis) in LLNs. They also found that macrophages with accumulated particulates had impaired immune function at several markers.

|  |  |
| --- | --- |
| Who: | * 84 deceased organ donors aged 11 – 93 years, with no history of smoking |
| What: | * **Tissue samples** (LLN, MLN): from lymph nodes in the lungs (LLN) and mesentery (MLN). * **Age** (years): of each organ donor, ranging from 11 - 93 * **Percent of LN containing particulates**: Particulate content in LNs, quantified by measuring the area (μm2) of each LN that contained black particulates * **Percent expression of immunity markers** (CD80/86, CD36, and CD209): associated with immune activation, phagocytosis, and phagocytic capacity * **Percent production** of arginase and cytokine -IL6+: positively associated with immune response |
| Why | * **Part 1:** To test the association of particulates to lymphatic region (LLN and MLN) and donor age * **Part 2:** To assess which types of macrophages accumulate inhaled particulates * **Part 3**: To test if macrophages with particulates have impaired immune function |

**Note:** The description, analyses and results presented here may differ from the original paper.

**Reference:**

* Ural, B.B., Caron, D.P., Dogra, P. *et al.* Inhaled particulate accumulation with age impairs immune function and architecture in human lung lymph nodes. *Nat Med* **28**, 2622–2632 (2022). https://doi.org/10.1038/s41591-022-02073-x

**Part 1: Lymph Nodes and Age**

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**Figure 1.** Photos of lung lymph node (LLN) tissue and mesenteric lymph node (MLN) tissue. a) Photos from several donors comparing tissues from both sites, and b) photos showing LLN tissue from donors ranging in age.

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**Figure 2.** Linear regression plot showing percent particulate accumulation per μm2 in LLN and MLN tissues from donors ranging in age.

**Part 2: Macrophage Particulate Accumulation**

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**Figure 3.** Boxplot of the three macrophage subsets summarizing the number of cells containing particulate per mm2.

Table

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**Figure 4.** Linear regression plot showing percent particulate accumulation per μm2 in LLN and MLN tissues from donors ranging in age.

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**Figure 5.** R output of one-way ANOVA comparing mean count of cells containing particulates per mm2 across three macrophage subsets.

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**Figure 6.** R output of Tukey tests of pairwise comparisons.

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**Figure 7.** Linear regression plots showing function percent arginase production and cytokine -IL6+ (functions of immune response) across donor ages from two types of macrophages, each with and without particulate accumulation.

**Part 3: Particulate Accumulation and Immune Function**

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**Figure 8.** Linear regression plots showing function percent expression of immunity markers in LLN tissue across a range of donor ages.

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**Figure 9.** Linear regression plots showing function percent expression of immunity markers in MLN tissue across a range of donor ages.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| LN Type | Marker | Intercept | Slope | P-value for slope | R2 |
| LLN | CD36 | 62.02 | -0.63 | < 0.001 | 0.55 |
| CD209 | 4.89 | 0.25 | 0.140 | 0.11 |
| CD80/86 | 26.95 | -0.30 | 0.021 | 0.25 |
| MLN | CD36 | 47.63 | -0.05 | 0.822 | < 0.01 |
| CD209 | 30.78 | -0.16 | 0.38 | 0.04 |
| CD80/86 | 36.28 | 0.10 | 0.64 | 0.01 |

**Figure 10.** Summary of linear regression models shown in Figures 8 and 9.

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**Figure 11.** Residual plots of linear regression model of marker CD209 in LLN tissue.

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**Figure 12.** Residual plots of linear regression model of marker CD209 in MLN tissue.

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