# MAD – Data Analysis & Biostatistics in R

James R. Hunter, Ph.D.

DIPA, EPM, UNIFESP

4 de setembro de 2020

## Section 1

## Introduction to Course

# What is Our Objective?

- Learn practical data analysis
  - Run a study from beginning to end
- Use R Language
- Learn (or refresh) basic biostatistics

#### Professor James Hunter

- Professor Afiliado, DIPA
- PhD., Retrovirology Laboratory, DIPA, UNIFESP
- Post-Doc, HIV Cure Project
- Prior career em business consulting and urban planning
  - Consulting & University Teaching
- Focus on Statistics and Quantitative Methods since 1973
- Work with R since 2010

#### Contact with the Professor

- email: jameshunterbr@gmail.com
- Twitter: @jimhunterbr
- cel: 11-9-5327-5656
- Office Hours:
  - ► Thurs. 14h 16.30h
  - ▶ EP2, Rua Pedro de Toledo 669, 6th Andar Fundos

# Philosophy

- The only way to learn a computer language is to write it
- The more code you write, the easier it will be
- Solve practical problems with R code

## Don't Panic...



## Questions

- Ask a lot of questions
- If you have a doubt, some of your classmates have it as well
- There are NO dumb questions

# Carl Sagan on Dumb Questions

- Astrophysicist who wrote and hosted the original Cosmos
- Book: The Demon-Haunted World: Science as a Candle in the Dark There are naive questions, tedious questions, ill-phrased ques-

tions, questions put after inadequate self-criticism. But every question is a cry to understand the world. **There is no such thing as a dumb question**.

## Always a Second Point of View







## Resource for Questions – Piazza

- New cloud site for class questions
- Sign up: https://piazza.com/unifesp.br/fall2020/infectomad1
- Questions to prof or to each other

# English??

- Why are we doing this course in English?
  - If we are in a Brazilian university?
  - ▶ If the prof speaks Portuguese? (Sim, ele fala)
- The language of science is English
  - ► For better or worse it's reality
  - Publications, even Brazilian ones English
- Like programming, the best way to perfect your English is to use it
- Course is about data analysis, not English
  - Any question can be in Portuguese
  - ▶ All submissions can be in Portuguese
- If I speak too quickly, let me know even during the class.

#### How Much Math Do I Need?

- What you learned in secondary education is enough
- No calculus
- Sums  $(\Sigma)$ , logarithms and exponents
- Equation for a straight line

$$y = b_0 + b_1 x$$

## Section 2

# Information and Knowledge

"We are drowning in information, but we are starved for knowledge". – John Naisbitt<sup>1</sup>

<sup>&</sup>lt;sup>1</sup>Although most frequently ascribed to futurologist John Naisbitt, this quote has many fathers and mothers. Taken here from Danielle Navarro, **Learning statistics with R: A tutorial for psychology students and other beginners**, 2020, http://compcogscisydney.org/learning-statistics-with-r

# Why Do We Need Statistics and Data Analysis?

- Can we see the things we study? NO
  - Viruses, bacteria, cells, nucleotides, proteins
- Machines that produce the genomic data we study are probabilistic
  - Term "calling bases" suggestion of error
- Natural process of cellular or viral replication error prone
- Human responses to disease, drugs, treatments
  - High level of uncertainty and variability
  - Natural differences between people

## Statistics Helps Find Underlying Truths

- Develop set of rules to process the information we receive
  - Script
- Form conclusions that others can understand, agree or disagree
- As post-grad students, you need to be able conduct basic analyses
  - More advanced models and methods need a specialist

## Necessary Skill for All Scientists

- Understand the statistics you read in papers and books
- Separate important from unimportant
- Separate true from false
- "Call Bullshit"<sup>2</sup> when you are being deceived
- Result: we need probabilistic means to find underlying truths

<sup>&</sup>lt;sup>2</sup>CT Bergstrom & JD West, **Calling bullshit: the art of skepticism in a data-driven world**, New York: Random House, 2020.

## Four Pillars of MAD

- Basic concepts of biostatistics
- Organization, cleaning and practial analysis of data
- Computational and programming tools that support data manipulation and analysis
- Workflow necessary to execute projects

## Work for the Course

- Group Project
  - Group of 2 4 people
  - Project based on your data
    - ★ Data for a thesis, dissertation
    - ★ Data for a project in laboratory
  - If you need data, we can find some together
  - Start with messy data (ie, real data)
    - ★ Clean and analyze it
  - Projects topics and data sources decided before 1 October
- Homework Assignments
  - 3 Assignments
    - ★ Can be worked on in groups
- Participation
  - Questions/Comments/Piazza

#### Course Submissions

- All homeworks and projects must be submitted in pdf format prepared as an R Markdown document.
  - I will not accept raw program files nor raw R Markdown documents.
  - You have to get your programs to work.
- We will talk about R Markdown later today.
- All submissions emailed to Prof. Hunter

#### Section 3

## R As Data Manipulation and Analysis Tool

## CRAN: The Comprehensive R Archive Network

- An educational NGO that is the owner of the mother code for R
- Official source for copies of the base software and packages

R is a system for statistical computation and graphics. It consists of a language plus a run-time environment with graphics, a debugger, access to certain system functions, and the ability to run programs stored in script files.

## History of R

- Based on a statistical programming language ("S")
  - S developed by Bell Labs in 1976
  - Still exists as commercial product
- R developed by Ross Ihaka and Robert Gentleman in 1995 in New Zealand
- Active community of developers and users
- More than 16,000 additional packages available in CRAN's repository
  - Many useful for biological analysis
  - ▶ Bioconductor another 2,000 packages
  - Many others scattered around various sources

# Virtues of R for Data Analysis

- Analyze via programs vs. clicking buttons
  - Control the sequence and options of operations in your analysis
- Programs will keep doing the same thing every day
  - No surprises because you clicked a button that changed your analysis
  - Only call for those options you understand
- Keeping a record of how you got the answer
  - Not just a record of the the answer
- FREE No cost, ever!
  - ▶ No stupidly expensive "student" version
  - Don't need "cracked" copies of software

# Reproducibility Crisis

- Being able to reproduce analyses over time and in different labs
- Most articles cannot be reproduced
- Nature's Reproducibility Checklist Workflows based on point-and-click interfaces, such as Excel, are not reproducible. Enshrine your computations and data manipulation in code.<sup>3</sup>
- R and Python trumps Excel, Graphpad and friends

<sup>&</sup>lt;sup>3</sup>Perkel. Challenge to Scientists Nature 584, no. 7822 (2020).

#### Is R Hard to Learn?

- If you have never programmed before, all computer languages are hard at first
- R much easier than most
- Initial Steps
  - Specify vectors and data frames
  - Execute statistical and mathematical functions
- Today you will be writing code!
- R gets hard when you start to write your own procedures
  - When you can't find them in the packages

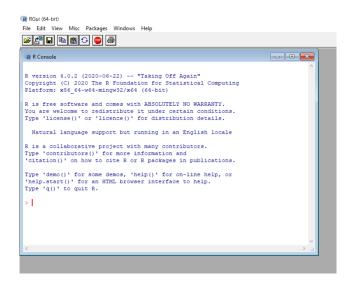
#### What You Need to Commit to

- Invest time in the course between classes
- Install the software (R and RStudio) on your laptops
- Read the material that suggested here and in my book
- Try out one of the basic R courses on the internet (recommended)
  - Get a second approach to the same material

## RStudio - Sophisticated Communication with R

- Integrated Development Environment ("IDE") for R
- Available since 2010
- Home of the Tidyverse
- Where you will do your R work
- Also FREE

# R Has a (Useless) Graphic User Interface ("GUI")



# R & Python

- Python another very popular language
  - Based on similar concepts to R
  - High-level interpreted language
- Launched in 1991
  - Guido van Rossum of the Netherlands
  - ► Name comes from English comedy group, "Monty Python's Flying Circus"
  - Not the snake species.
- Weaker than R in statistics
  - Need commands from various modules to do basic stat operations
    - Numpy, Pandas

## Section 4

## Course Resources

#### Course Files and Materials

- Stored on GitHub in course repository
  - Data for exercises and lectures
  - Chapters of my text
  - Other files of interest
- https://github.com/jameshunterbr/MAD-Infecto-2020

# Key Readings

- MAD Data Analysis & Biostatistics in R by Prof. Hunter
  - A text on the subject of the course in preparation
  - Will provide more detail of what I cover in classes {MAD -Materia de Analise de Dados}
- Statistics texts
  - Diez, Barr & Cetinkaya-Rundel, OpenIntro Statistics 4
  - Navarro, D. Learning statistics with R: A tutorial for psychology students and other beginners
- Basic R Books
  - Wickham & Grolemund, R for Data Science
  - Ismay & Kim, Statistical Inference via Data Science: A moderndive into R and the Tidyverse
  - Irizzary, Introduction to Data Science

#### RStudio Cheat Sheets

Series of 1 and 2 page summaries for a number of key packages of R functions



#### Online Courses

- edX Harvard courses on R in data science taught by Irizzary
- Coursera Johns Hopkins courses on R and R in biomedical applications
- Utrecht University (Netherlands) Introduction to R and data
- Coursera Duke University sequence of R courses by Cetinkaya-Rundel

All excellent

#### Sites about R

- R Bloggers (https://www.r-bloggers.com/)
- Tidyverse (https://www.tidyverse.org/learn/)
- Stack Overflow (https://stackoverflow.com/questions/tagged/r)
- Twitter (#rstats)

## R and RStudio Help Systems

- Very complete
- Every function (command) has a help screen
- Written by geeks for geeks
  - Explanations sometimes opaque
  - Especially error messages
- Last resort: copy error message and Google it
  - Someone, somewhere has not understood the same thing that troubles you

## Section 5

# Installing the Software

#### Install R

- Found on following site:
  - https://cran.r-project.org/



# From Initial Screen (Windows)

- Olick on the link "Download R for Windows"
- On the next screen, click on "base"
  - Mac skips this step



#### Real Installation

Click on Download R 4.0.2 for Windows



• Site will download the program on your computer

#### Installation of RStudio

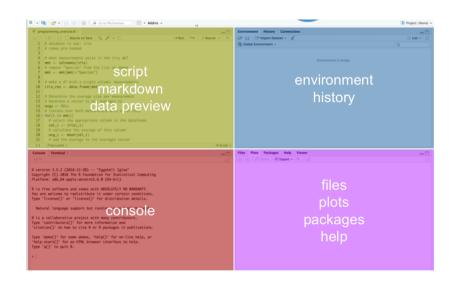
- Go to site: https://www.rstudio.com/products/rstudio/download/
- Scroll down to a big, blue button: "Download RStudio for Windows"
  - Gives version number and size of program



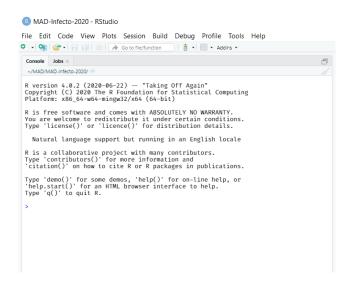
#### Start RStudio

- On Desktop (or through menus), double click on the icon for RStudio
  - Not the icon for R
- RStudio will open
  - R will automatically open within RStudio

#### RStudio Screen



## RStudio Console at Startup – Ready to Rock!



## Section 6

# Your First Program

## Load Packages

- Most important packages that extend base R
- We will use most during the course
- Simple script

## What Script Does - Line 1

- Line 1: assignment of set of packages to the name packages
  - ▶ Uses <- to make the assignment
- Set of packages combined into vector of package names
  - ▶ Function c() creates a multi-element vector
  - ▶ c() combine or concatenate
  - vector one dimensional matrix
- Elements of packages strings of class character
  - Enclosed in quotation marks ("")
- Result of Line 1



## Note: Assignment Operators

- Principal assignment operator: <-</li>
- Discourage use of =
  - You will confuse it with logical equals ==
    - ★ Guaranteed! We all do it

## What Script Does - Line 2

- Installs the packages
  - Goes out to CRAN mirror site
  - Downloads and installs packages
  - Many of the packages have dependencies so will install more packages
  - Dependencies: other packages needed for functions of calling package

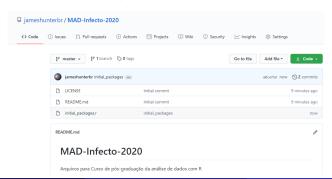
## Use of Scripts vs. Use of Console

- Write your commands in a script in R Markdown rather than Console
  - You can save your work
- Console is where commands are executed
  - Saving your history from Console more complicated

## Where Do I Find Script?

#### GitHub

- Public face of version control system called git
- Maintain a clear record of changes to scripts
  - ⋆ On Computer
  - ★ In remote repository
- GitHub repository for course
  - https://github.com/jameshunterbr/MAD-Infecto-2020



## How Do I Download Script?

- Click on script name: "initial\_packages.r"
  - Text file will appear
- Click on Raw button
- Right-click on file and save it to your R directory
  - ▶ Use "Save Page (As) . . . " command on pop-up menu

## Execute "initial\_packages.r"

- Files tab of lower right pane of RStudio
  - ▶ Click on initial-packages.r
- Script will open in upper left pane
- Click on Source button in program menu bar
- Follow progress in Console

```
| Decorate land | Decorate | Dec
```

## Section 7

# Basic Operations in R

#### Use R as a Calculator

```
5 + 5
## [1] 10
36 * 2500000
## [1] 90000000
5876/35.44320
## [1] 165.7864
2^25
       # exponent
## [1] 33554432
25 * (12 + 27)
## [1] 975
```

## Math Functions in R

Function	What It Does
abs(x)	absolute value of x
sqrt(x)	square root of x
log(x)	natural (Naperian) logarithm of $x$
exp(x)	natural exponent of x
log10(x)	logarithm base $10 \text{ of } x$
round(x, n)	round x to n decimal places
	·

## More Math Functions

		90		Functions					
■ \ <b>V</b> /		33	36	-17	Tal		ж		10.0
	10	ч.		100		l oil	ч.	Lul	

log(x)	Natural log.	sum(x)	Sum.
exp(x)	Exponential.	mean(x)	Mean.
max(x)	Largest element.	median(x)	Median.
min(x)	Smallest element.	quantile(x)	Percentage quantiles.
round(x, n)	Round to n decimal places.	rank(x)	Rank of elements.
signif(x, n)	Round to n significant figures.	var(x)	The variance.
cor(x, y)	Correlation.	sd(x)	The standard deviation.

## Functions at Work

```
abs(-287)
## [1] 287
sqrt(9849)
## [1] 99.24213
log(377898)
## [1] 12.84238
exp(12.84238)
## [1] 377898.2
log10(377898)
## [1] 5.577375
round(exp(12.84238), 0)
   [1] 377898
```

# Note about log() and exp()

- In example above, exponent of 12.84238 is 377898.2, not 377898
- R reports 5 decimal places on the screen
  - Internally, it is 12.8423795969182 (13 decimal places)
- We know that  $log(x) = e^x$
- We haven't broken any (major) mathematical laws.

```
x <- 377898
y <- log(x) # calculate the log of x and assign it to y
y
## [1] 12.84238
exp(y)
## [1] 377898</pre>
```

#### Comments

- Line 2 of the script above has a comment after it
- Comments start with a hashtag #
  - Everything after it on a line is not interpreted
- Comments remind us what we have done and why we did it
- Very important
- Use frequently

# Order of Calculation (*PEMDAS*)

Operation	Symbol	Example	PEMDAS
parentheses	()	5 * (7 + 2) = 45	P
exponents	^	$5^2 = 25$	E
multiplication	*	5 * 7 = 35	M
division	/	25/5 = 5	D
addition	+	5 + 7 = 12	Α
subtraction	-	5 - 7 = -2	S

- If you remove the parentheses from 5 \* (7 + 2)?
- $\bullet$  5 \* 7 + 2 = 37
- Remember: rules of mathematics don't change because we are using a computer

## Assignment

- (name of object) <- (definition of object)
- definition = the values that are the content of the object

## Assignment – Styles

These work

```
x <- 6
x <- "Hi!"
```

These work but are not recommended

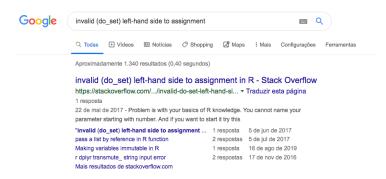
```
x = 6
```

• This produces an error (cannot start a command with a number)

```
> 6 = x
Error in 6 = x : invalid (do_set) left-hand side to assignment
> |
```

# What Do You Do When You See a Strange Error Message?

#### Google It



## Section 8

## Assignment – Variable Names

## Assignment – Variable Names

- Primary rules of R
- Must contain only letters (either upper or lower case), numbers or symbols . or \_.
- Must start name with a letter

#### Variable Names – Corollaries

- Should not include spaces.
  - "Snake case" overcomes this restriction
    - ★ Connecting words with underscore "\_"
- R reserved words cannot be used for variable names
  - ▶ Examples: TRUE, FALSE, if, else, for, function
- Variable names case sensitive
  - Variable and variable are 2 different names
  - Same for x and X

#### Cases in R



#### More on Variable Names

- Make them clear and informative
  - x, although popular, is useless as a name

```
## 1st version
peso <- 55 ## Person weighs 55 kg.
## 2nd version
peso kg <- 55 ## Clearer
## 3rd version, can convert to pounds
peso lb <- peso kg * 2.2
peso lb
```

## [1] 121

#### Variable Names – Last Shot

- Make a data dictionary
  - Record of what your variable names are, what kind of data they are and range of values
- Try to keep names as short as possible
- Camel case as alternative to snake case
- If you surround your variable name with single quote (') or backtick ('), spaces ok
  - viral load illegal
  - 'viral load' legal
  - But, don't use this

## Section 9

## Go to Presentation 2