Instituto Nacional de Salud Pública

ELEMENT
Early Life Exposure in Mexico to Environmental Toxicants Project

BONE LEAD

ELEMENT C123

Historical measurements of bone lead, description original variables and reanalysis for patella and tibia of each cohort

EQUIPMENT USED (K-XRF2 and K-XRF4)

K-XRF2 and K-XRF4 are the equipment to detect bone lead (tibia and patella), through the fluorescent K-ray with radioactive CADMIUM109 spectroscopy technique. [1]

- **K-XRF2**

At the beginning (**1993**), tibia and patella bone measurements were performed using only the KXRF2. This equipment performed one measurement in **each patella for 30 minutes and in right tibia for 30 minutes**, for a **total duration of 90 minutes**. To obtain a summary of patella results, a weighted average was calculated.

The limitation with this equipment was that given that it was parallel to the floor, it was not able to obtain a 90 degrees angle with the patella.

- **K-XRF4**

In **2000**, the new equipment KXRF4 was introduced. Its features included the possibility to obtain a 90 degrees angle with the patella. Since then, each equipment was specific for each area, **KXRF2 for the two tibias, and KXRF4 for the two patellas**.

1. Hu H, Milder FL, Burger DE. The use of K X-ray fluorescence for measuring lead burden in epidemiological studies: high and low lead burdens and measurement uncertainty. *Environ Health Perspect*. 1991;94:107-110. doi:10.1289/ehp.94-1567946

KXRF EQUIPMENT AND SOFTWARE

- **Equipment failure**

Both equipments operated in parallel from the January 4, 2000 to August 16, 2011. During this time, there were time windows in which one of the machines stopped working due to software or hardware failure. For this reason, for some participants there is only data for one region and not for both.

- **Software change**

On **June 12, 2000** there was an **update computers and software** for both equipment; this lead to discrepancy in the results and therefore **a subsample of measurements were reanalyzed**. The change responded to the **objective of reaching higher sensitivity in the analyses, to counteract the error introduced by the technician** at the time of placing the instrument or movement by the participant. **In 2002**, there was **another update** in the software that was used until the end of the analyses in 2011.

- **Measurement reanalysis**

The first reanalysis was conducted between June 1998- December 1999 before the first updated. The second was conducted 6 months after the update of software and computer (between June 2000 until October 22, 2002).

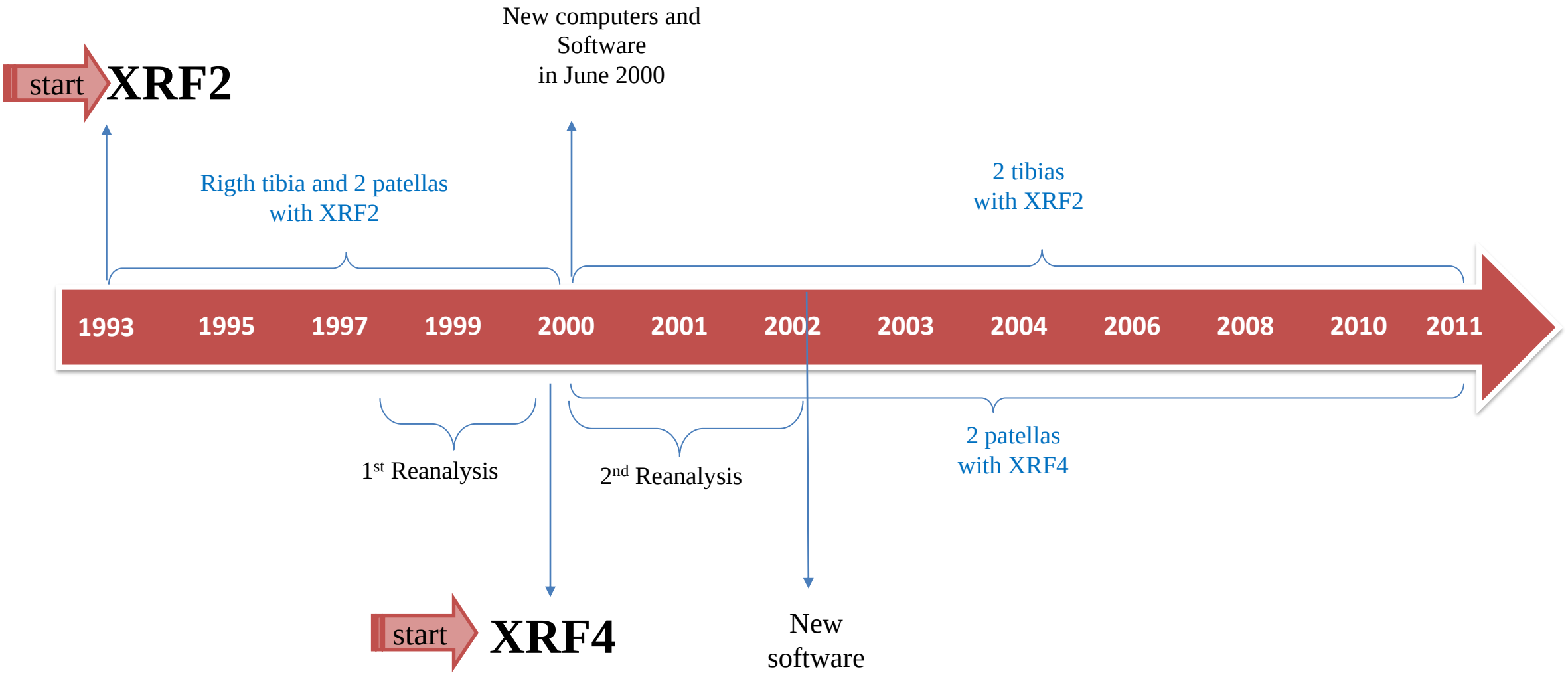
- **Imprecision in measurements**

The measurements in bone allowed for an imprecision of maximum 10.0 μg in tibia and 15.0 μg in patella of lead per gram of bone mineral.

- **Negative measurements**

Negative values are sometimes produced when true values are below the detection limit of the instrument.

DATES IN WHICH THE INSTRUMENTS XRF2 AND XRF4 WERE USED



- There were some missing measurements when the equipment failed. This is registered.

Number of observations for both, original variable and reanalyses

Variable	n			n by Cohort							
	Original	Reanalysis	%	Original				Reanalysis			
				C1	PL	BI	SF	C1	PL	BI	SF
Patella_1	3481	1223	35.1	1106	852	489	1034	0	606	291	326
Patella_2	2507	1225	48.9	133	850	489	1035	0	605	294	326
Patella	**3483**	**1230**	35.3	**1106**	**852**	**489**	**1036**	**0**	**608**	**294**	**328**
Tibia_1	2871	778	27.1	972	803	474	622	0	447	196	135
Tibia_2	1179	138	11.7	0	280	279	620	0	4	0	134
Tibia	**2874**	**779**	27.1	**972**	**805**	**474**	**623**	**0**	**447**	**196**	**136**

- Patella_1: right patella
- Patella_2: left patella
- Tibia_1: right tibia
- Tibia_2: left tibia
- Patella: The weighted-average by the inverse of the imprecision (using negative values)
- Tibia : The weighted-average by the inverse of the imprecision (using negative values)
- % percentage of measurements that were re-analyzed

The weighted-average by the inverse of the imprecision

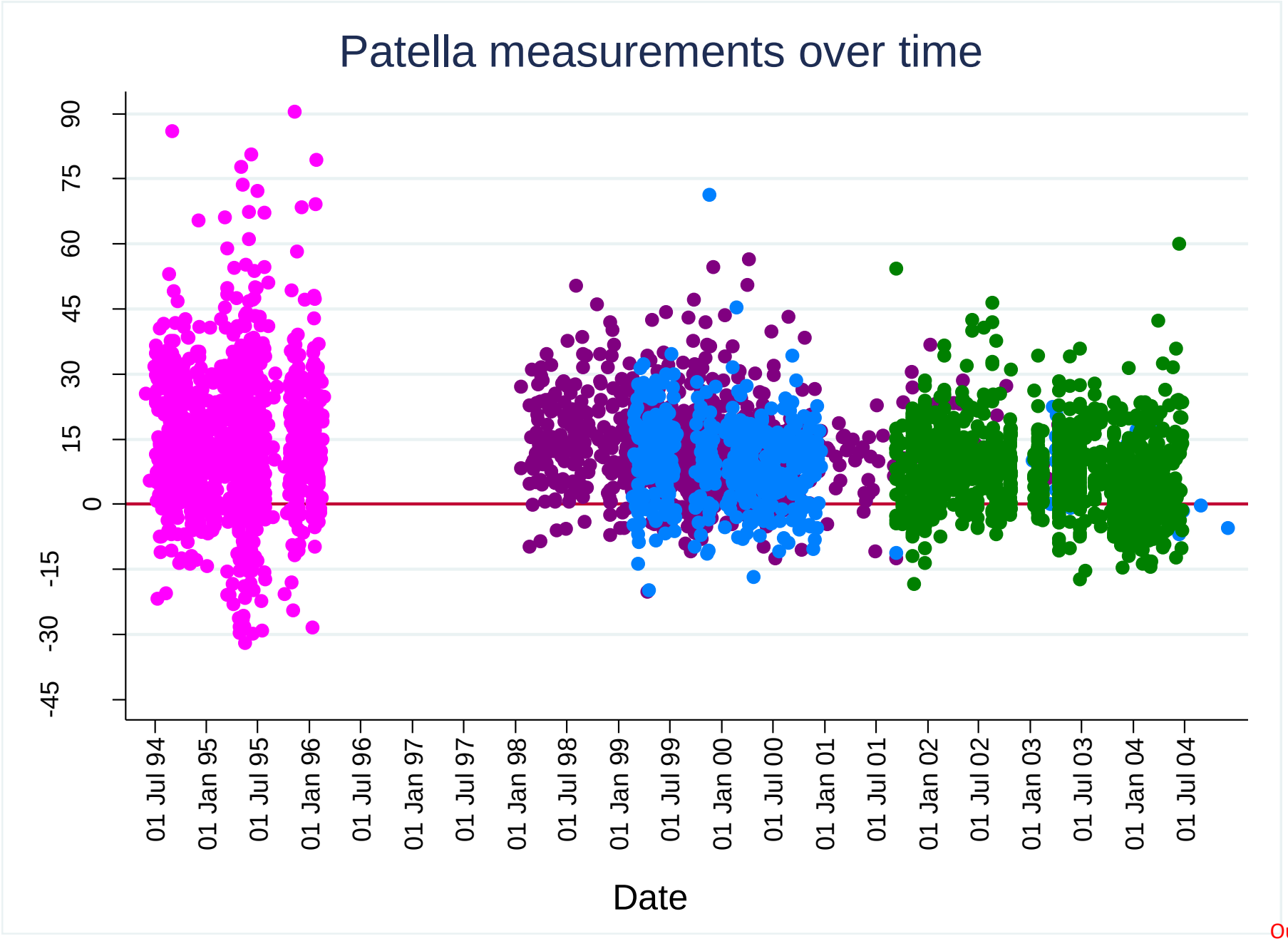
The two estimates for bone lead measurement (one for each leg) were computed, average, and weighted by the inverse of the proportion of the measurement error corresponding to each determination. [2] Formula:

$$Rotula = \frac{rotula_1\left(\frac{1}{improt_1}\right) + rotula_2\left(\frac{1}{improt_2}\right)}{\frac{1}{improt_1} + \frac{1}{improt_2}}$$

$$Tibia = \frac{Tibia_1\left(\frac{1}{imptib_1}\right) + Tibia_2\left(\frac{1}{imptib_2}\right)}{\frac{1}{imptib_1} + \frac{1}{imptib_2}}$$

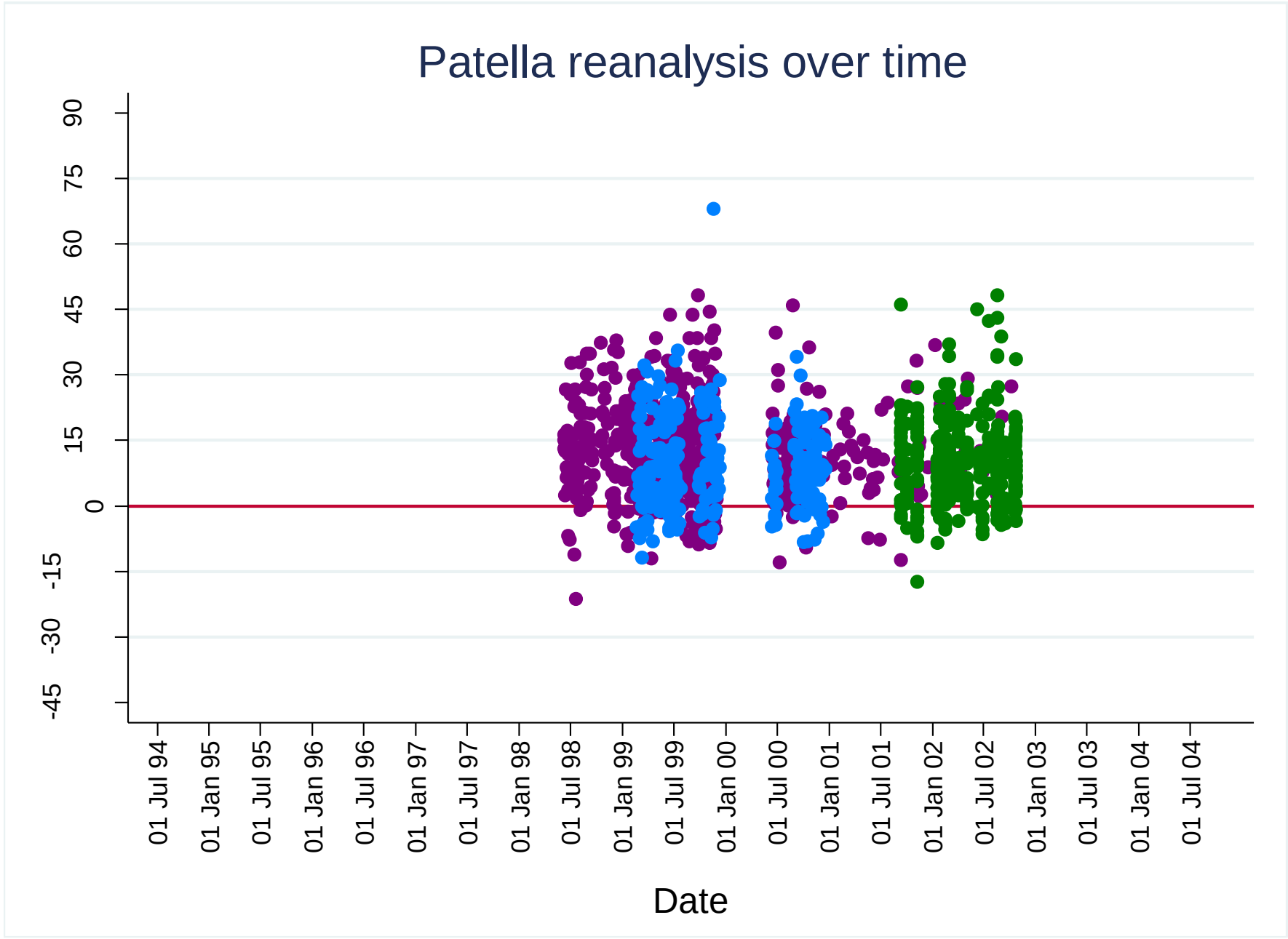
- Improt1: imprecision right patella
- Improt2: imprecision left patella
- Imptib1: imprecision right tibia
- Imptib2: imprecision left tibia
- The historical average considered negative measurements

- [2] Téllez-Rojo, Martha M et al. “Impact of Bone Lead and Bone Resorption on Plasma and Whole Blood Lead Levels during Pregnancy.” *American Journal of Epidemiology*,160 7 (2004), Pages 668–678

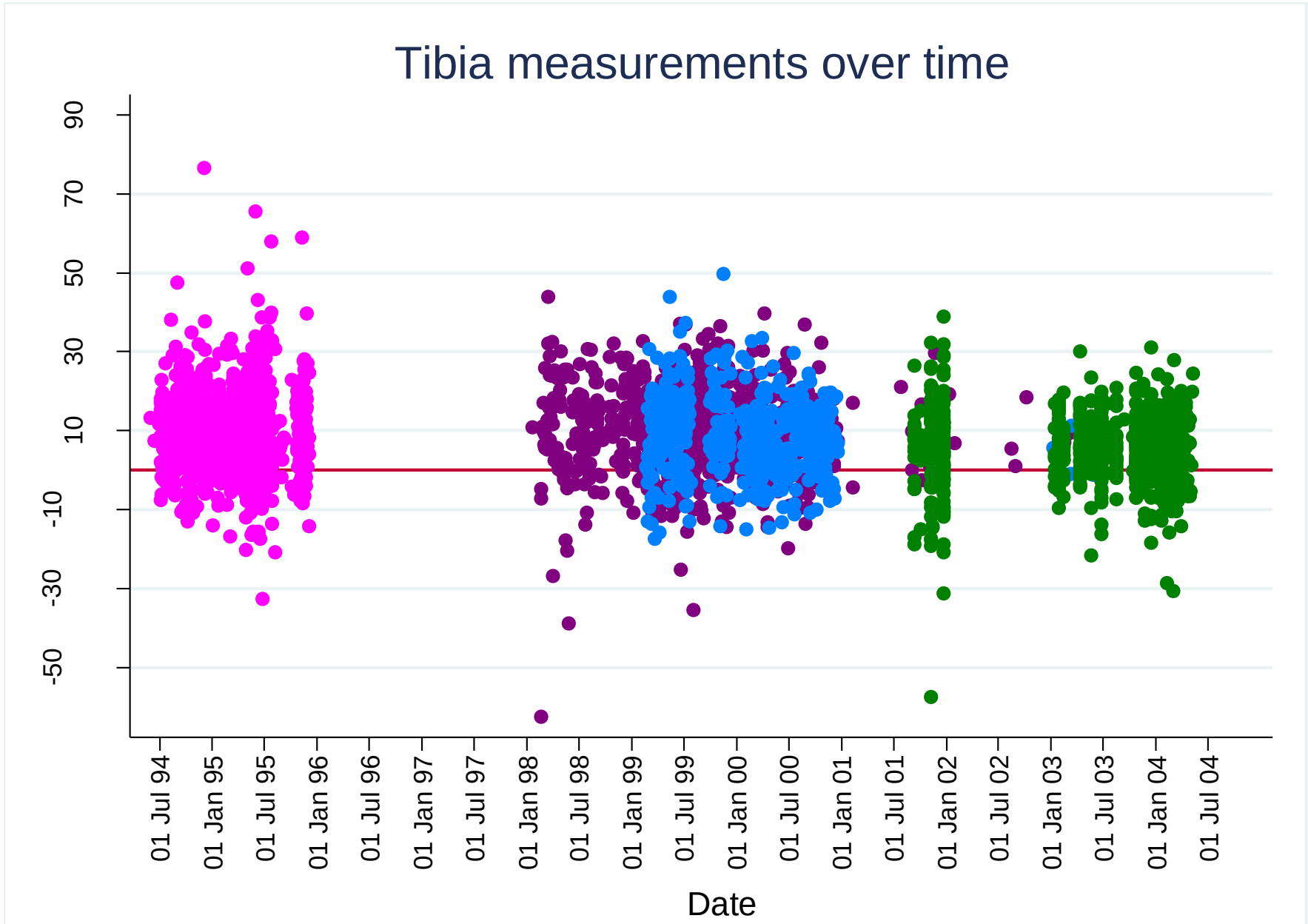


Cohort	Patella
C1	1106
PL	852
BI	489
SF	1036
Total	3483

Outlier not shown in the graph: C1 and Folio=31689
patella1=388.15 , imprecision=118.14

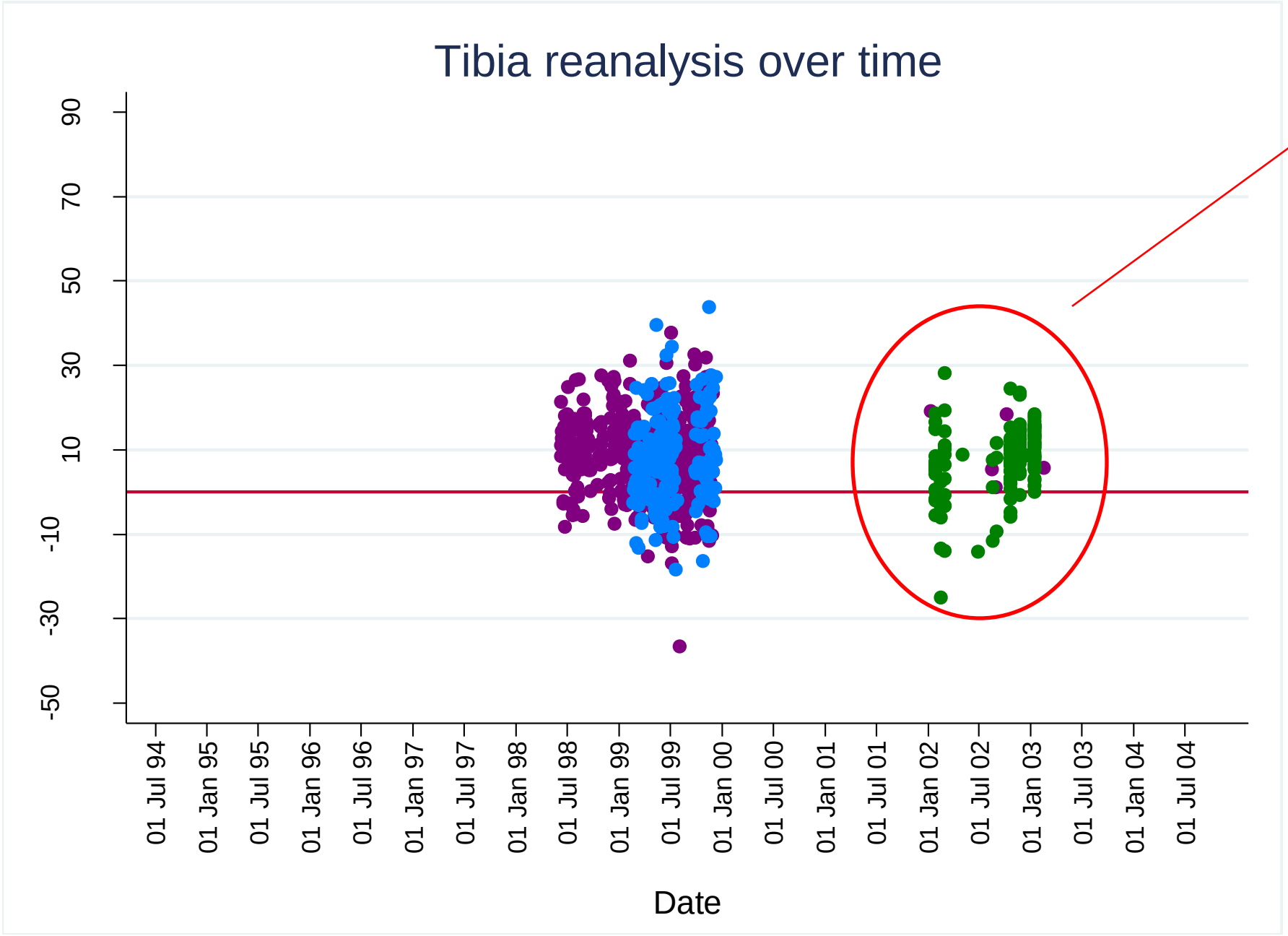


Cohort	Patella
C1	0
PL	608
BI	294
SF	328
Total	1230



Cohort	Tibia
C1	972
PL	805
BI	474
SF	623
Total	2874

Outlier not shown in the graph: PL and Folio=81
patella1=-439.9 , imprecision=544.9



These measurements only have reanalysis

Cohort	Tibia
C1	0
PL	447
BI	196
SF	136
Total	779

Comparative statistics including reanalysis of bone lead measurements from 1994-2004

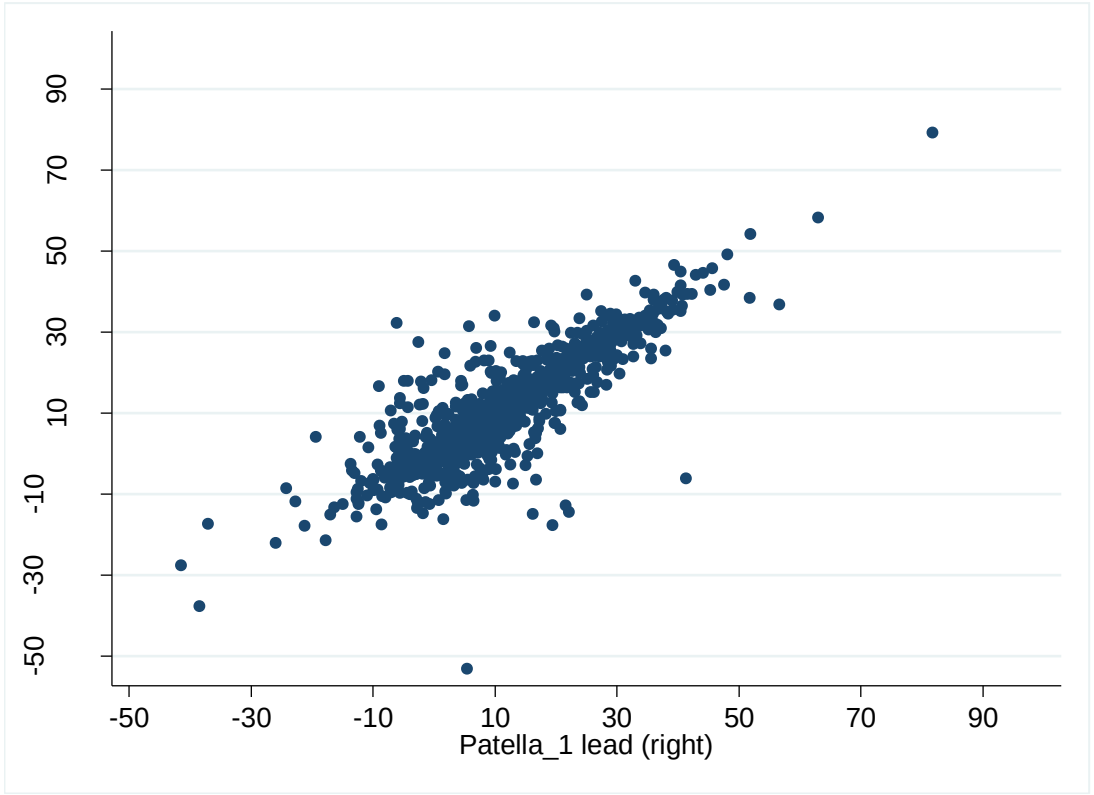
Site	XRF	Bone lead measurement (mean)		T-test for difference original and reanalysis	Imprecision		n		Correlation coefficient between original and reanalysis
		Original	Reanalysis		Original	Reanalysis	Original	Reanalysis	
Patella_1	2	14.5 (17.8)	11.9(13.8)	0.001	9.8(4.0)	9.0(2.1)	1,825	640	0.8326*
	4	8.5 (10.6)	10.1 (10.0)	0.002	6.1(1.4)	5.7(1.2)	1,653	582	0.9322*
Patella_2	2	13.2(13.5)	12.1(13.0)	0.127	9.1(2.3)	8.9(2.1)	854	640	0.8535*
	4	8.6(11.1)	10.0(10.9)	0.009	6.3(1.4)	5.9(1.4)	1,653	584	0.9465*
Patella	2	14.3 (16.8)	12.0(10.8)	**0.001**			1,825	642	0.8813*
	4	8.6(9.4)	10.0(9.3)	**0.001**			1,653	587	0.9598*
Tibia_1	2	9.9(10.8)	8.3(9.8)	0.006	6.7(1.7)	6.9(1.3)	1,689	636	0.8306*
	4	6.2(11.9)	8.3(9.4)	0.042	7.5(2.2)	5.9(1.8)	1179	141	0.9731*
Tibia_2	2	-	-		-	-	-	-	
	4	6.4 (11.3)	7.9 (10.0)	0.123	7.7(2.4)	6.0(1.8)	1,177	138	Original and reanalysis do not overlap
Tibia	2	9.9(10.9)	8.3(9.8)	**0.000**			1,689	636	
	4	6.2 (9.2)	8.0 (8.0)	**0.033**			1,182	142	

- No significant differences were found between the original measurements and the reanalysis (Patella and Tibia).
- The differences between equipment 2 and 4 if they significantly different.

*p<0.000

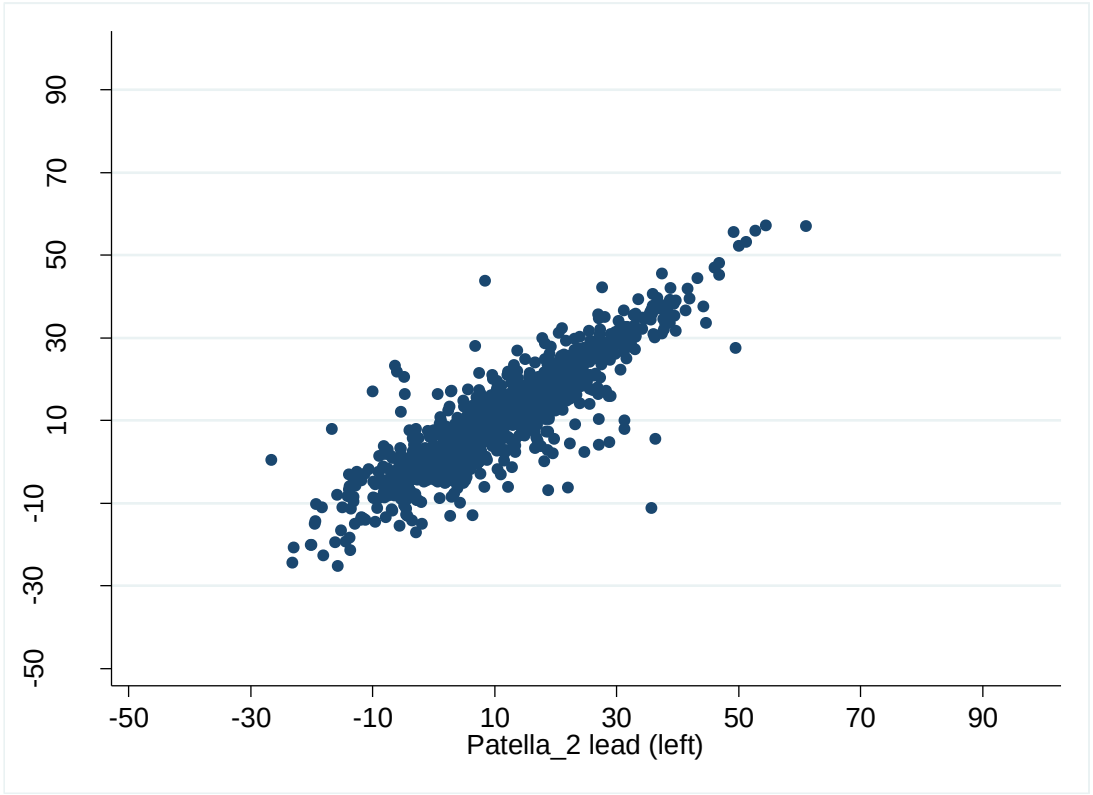
Scatter plot between **patella** measurements (original analysis and reanalysis)

Rigth



β_0 1.27 95%CI[0.80,1.74]
β_1 0.87 95%CI[0.84,0.91]

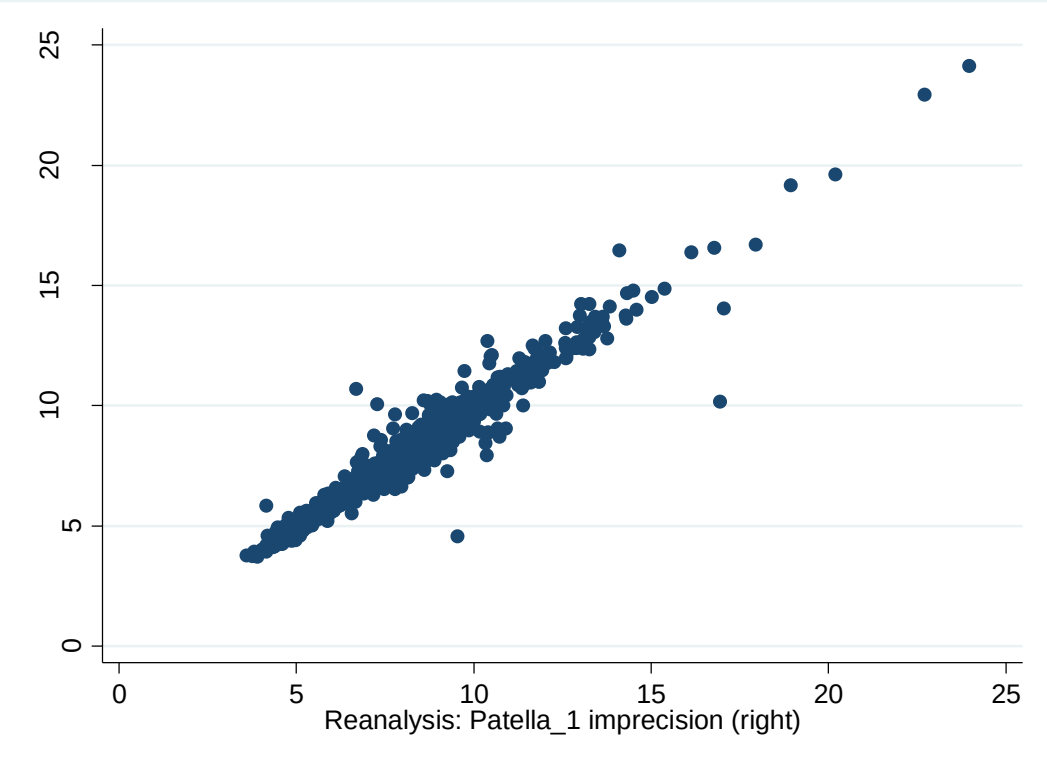
Left



β_0 1.15 95%CI[0.73,1.58]
β_1 0.88 95%CI[0.86,0.91]

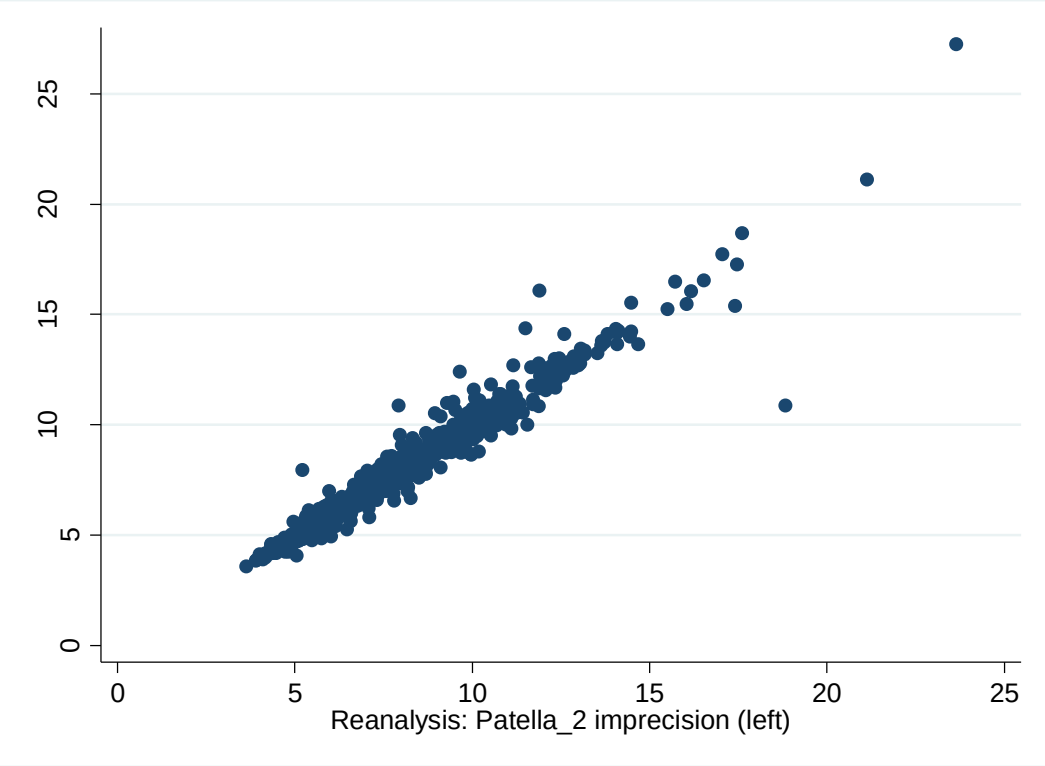
Scatter plot between **patella imprecisions** (original analysis and reanalysis)

Rigth



β_0 0.21 95%CI[0.13,0.31]
β_1 0.98 95%CI[0.96,0.99]

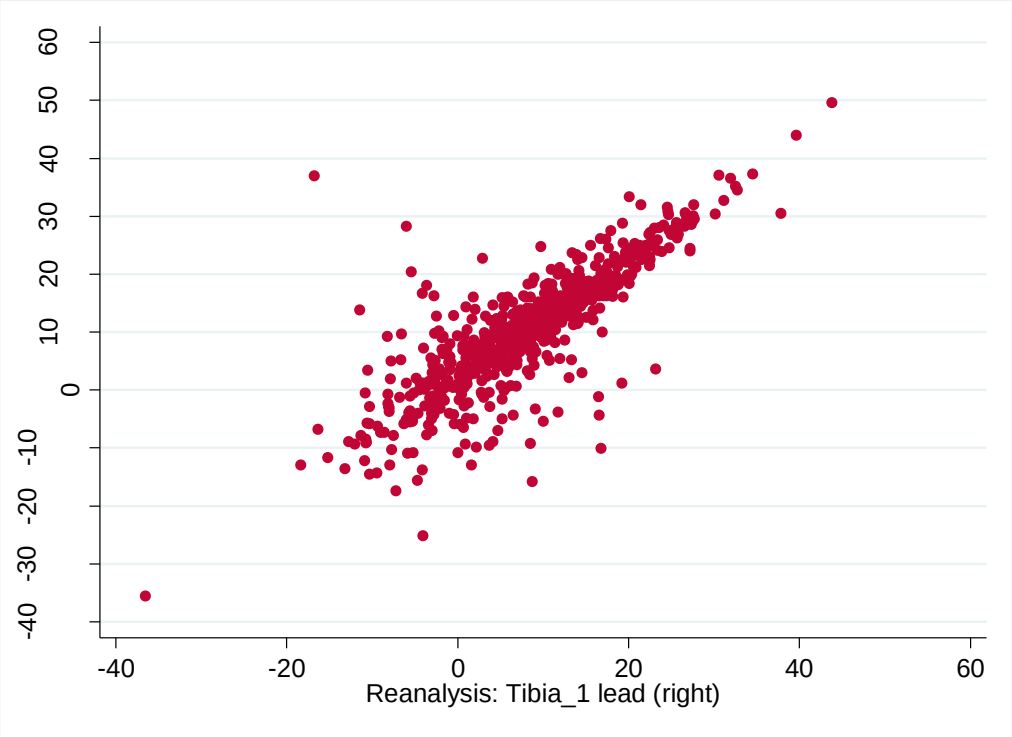
Left



β_0 0.36 95%CI[0.27,0.44]
β_1 0.96 95%CI[0.95,0.97]

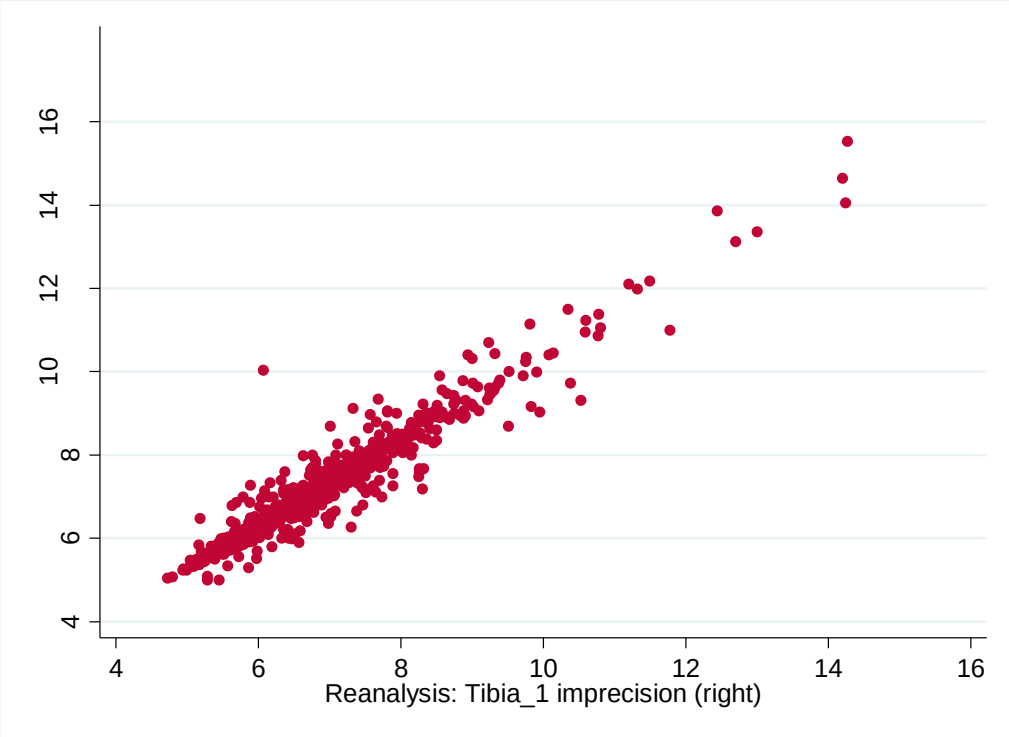
Scatter plot between **tibia rigth** (original analyses and re-analysis)

Tibia measurements



$$tibia1_r = 0.759 \;\; tibia1 + 0.31$$

imprecisions



$$imptib1_r = 0.912 \;\; imptib1 + 0.31$$

CONCLUSION

- Differences between original variables and reanalyzes were not statistically significant.

- There was no statistical difference in the point estimate between the observed measurement and the reanalysis. Imprecision was smaller, but didn’t reach statistical significance.

- Original measurements and imprecisions are highly correlated.

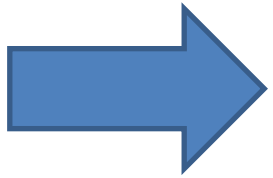
Based on these analyses and in order to increase the sample size, our recommendation is:

- In cases where there are 2 measurements available for each subject (original and reanalysis), use the reanalyzed measurement.

A proposed procedure to generate new variables that include old and reanalyzed bone lead measurements is described, along with a suggestion for analysis of negative measurements and calculation of averages

Proposed procedure

Original	Reanalysis
rotula1	rotula1_r
improt1	improt1_r
rotula2	rotula2_r
improt2	improt2_r
tibia1	tibia1_r
imptib1	imptib1_r
tibia2	tibia2_r
imptib2	imptib2_r



Step 1: Generation of new variables _u resembles union of observed and reanalyzed measurements

rotula1_u = rotula1

improt1_u= improt1

rotula2_u = rotula2

improt2_u= improt2

tibia1_u = tibia1

imptib1_u= imptib1

tibia2_u = tibia2

imptib2_u= imptib2

Replacement

SUGGESTION:

1. New variables were generated rotula1_u, rotula2_u, tibia1_u and, tibia2_u (_u resembles union original and reanalysis). These generated variables are the same as the originals.

2. For the variables *rotula1_u, rotula2_u, tibia1_u and, tibia2_u* that **had reanalysis**, the measurement was replaced for the reanalyzed measurement, including its imprecision.

3. We suggest that researchers using the variables *rotula1_u, rotula2_u, tibia1_u and, tibia2_u* replace **negative measurements** with random negative values with a uniform distribution between 0 and the lower detection limit of the instrument. [2][3]

- [2] Téllez-Rojo, Martha M et al. "Impact of Bone Lead and Bone Resorption on Plasma and Whole Blood Lead Levels during Pregnancy." *American Journal of Epidemiology*,160 7 (2004), Pages 668–678
- [3] Téllez-Rojo, Martha M et al. "Impact of breastfeeding on the mobilization of lead from bone." *American journal of epidemiology* 155 5 (2002): 420-8 .

Negative measurements for each variable

- The following table shows the total measurements for each variable, the negative measurements must be replaced with random numbers in a uniform distribution from 0 and the lower detection limit of the instrument.
- The random numbers must be generated by each researcher.

Variable	Total measurements	Negative measurements
rotula1_u	3,482	568
rotula2_u	2,508	413
tibia1_u	3,007	578
tibia2_u	1,312	356

Step 3: Calculation of patella and tibia averages

Once all positive measurements are obtained, is calculated the weighted average with the inverse imprecision for patella (with the variables *rotula1_u, rotula2_u*) and for tibia (with the variables *tibia1_u and tibia2_u*) with their respective imprecisions.

$$rotula\_u = \frac{rotula1\_u\left(\frac{1}{improt1\_u}\right) + rotula2\_u\left(\frac{1}{improt2\_u}\right)}{\frac{1}{improt1\_u} + \frac{1}{improt2\_u}}$$

$$tibia\_u = \frac{tibia1\_u\left(\frac{1}{imptib1\_u}\right) + tibia2\_u\left(\frac{1}{imptib2\_u}\right)}{\frac{1}{imptib1\_u} + \frac{1}{imptib2\_u}}$$

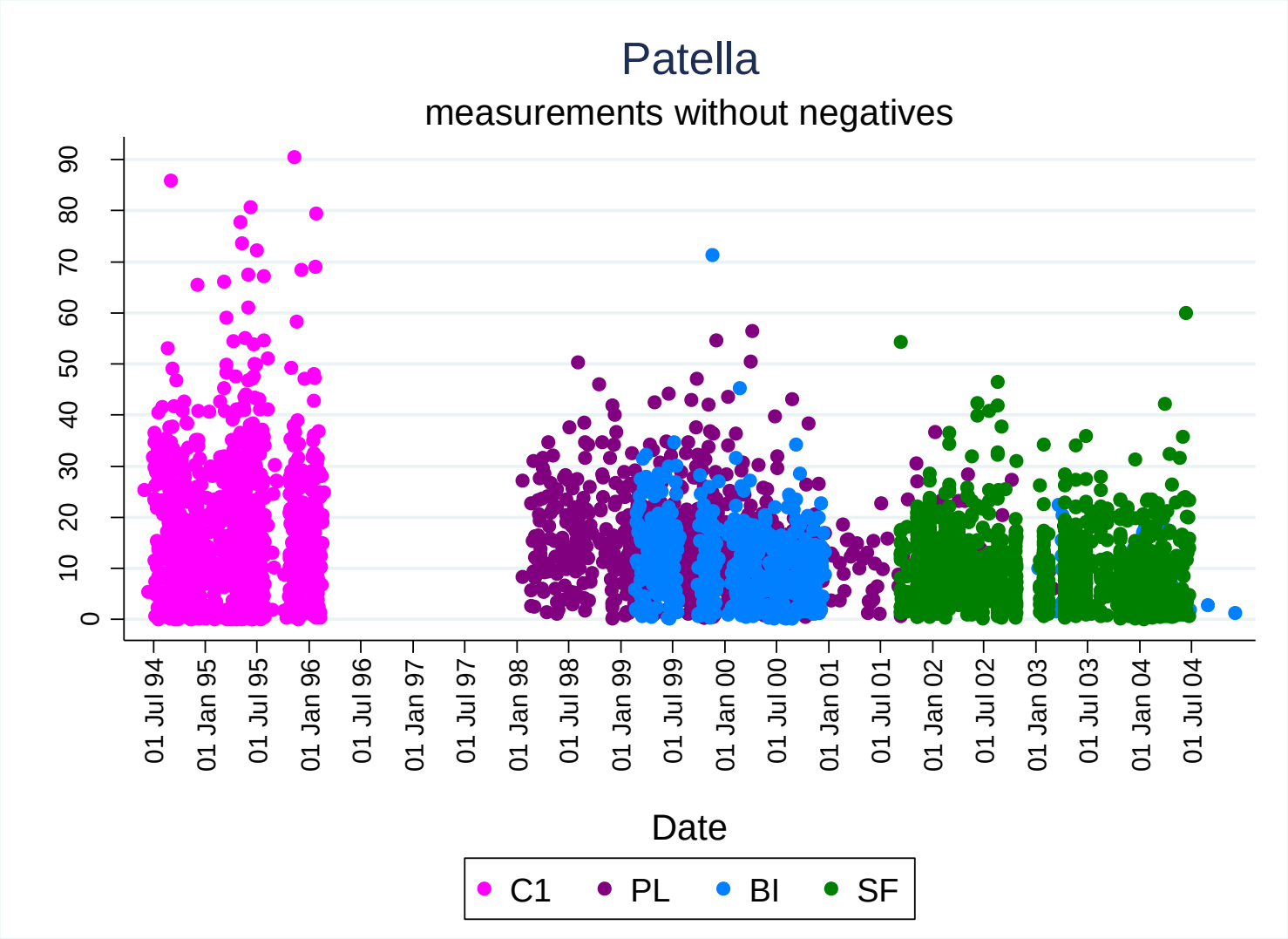
When there is only one measurement available (right or left), the average is the same as the available measurement.

Total of observations with the generated variables

Cohort	rotul1_u	rotul2_u	tibia1_u	tibia2_u
C1	1106	133	972	0
PL	852	850	803	280
BI	490	490	475	279
SF	1034	1035	757	753
Total	3482	2508	3007	1312

By cohort and months postpartum	rotula1_u	rotula2_u	tibia1_u	tibia2_u
C1- 1	626	0	626	0
C1-7	480	133	346	0
PL-1	206	205	197	21
PL-4	222	221	211	38
PL-7	207	207	192	81
PL-12	217	217	203	140
BI-1	490	490	475	279
SF-1	416	415	309	306
SF-3	381	382	241	240
SF-7	6	6	3	3
SF-12	231	232	204	205
Total	3482	2508	3007	1312

Distribution of how the patella average would look like without negative values



Distribution of how the tibia average would look like without negative values