

report_CDKN2A__Oct9_2023.R

mg

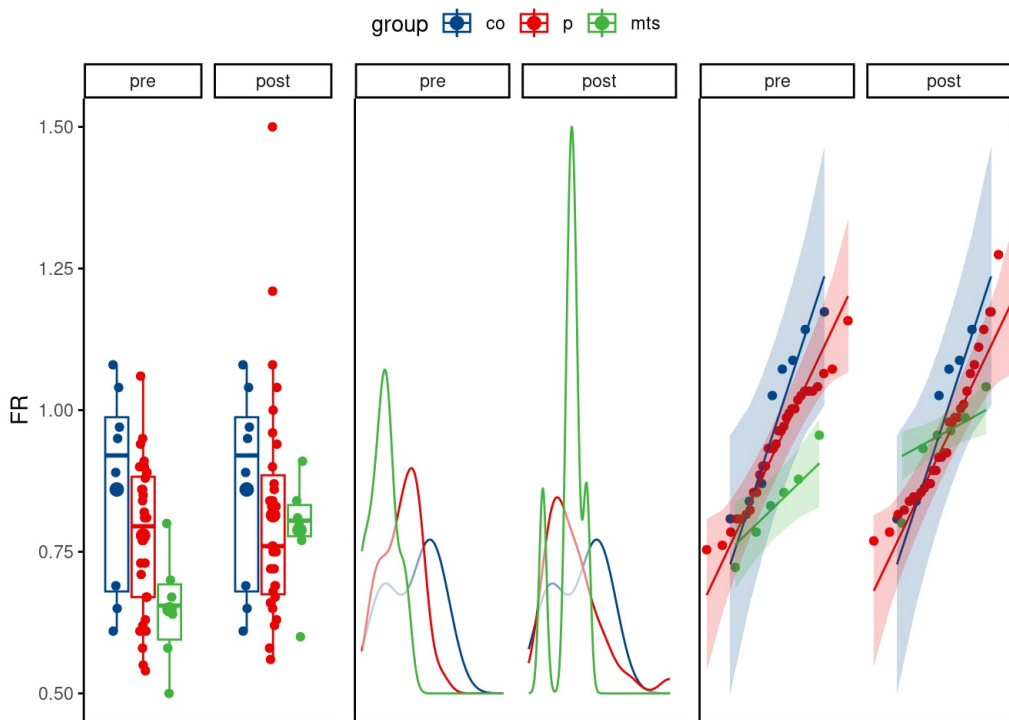
2023-10-09

```
#  
#  
# Oct 9 2023 Mo -- CDKN2A  
# Oct 5 2023 Th -- triplot; spaghetti; lin mixed model - dg, outlier,  
#                   emmeans, custom contrasts  
# Oct 4 2023 We -- CDK4 ddPCR CNV  
#  
#  
# Eva Lukacova  
#
```

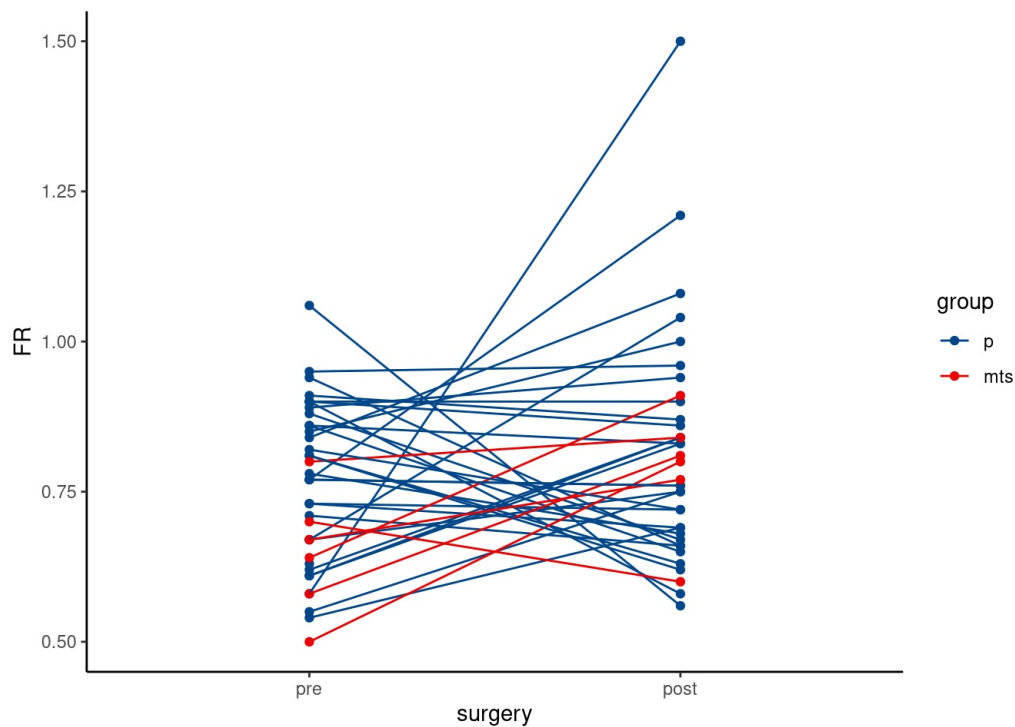
CDKN2A

EDA

Triplot (for FR by surgery and group)



Spaghetti plot for surgery (w group wo co)



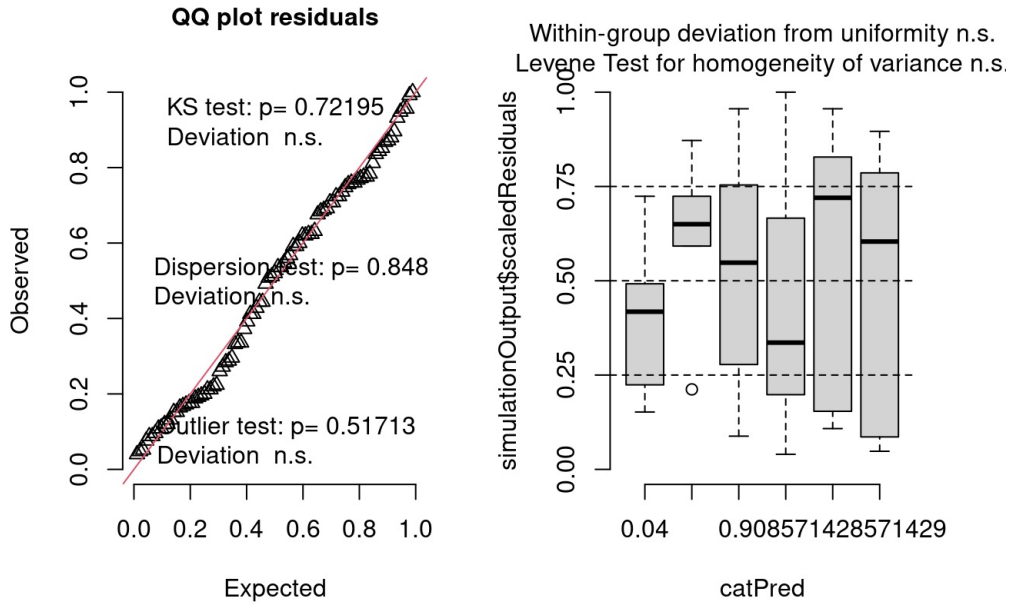
Linear mixed model (to fit Repeated measures ANOVA)

Model fit

<i>Predictors</i>	<i>Estimates</i>	FR		<i>p</i>
		<i>CI</i>		
(Intercept)	0.84	0.75 – 0.93		<0.001
group [p]	-0.06	-0.16 – 0.03		0.183
group [mts]	-0.14	-0.27 – -0.01		0.031
surgery [post]	0.04	-0.02 – 0.11		0.203
Random Effects				
σ^2	0.03			
τ_{00} id	0.00			
ICC	0.05			
N_{id}	46			
Observations	91			
Marginal R^2 / Conditional R^2	0.069 / 0.118			

Diagnostics

DHARMA residual



=> no outlier(s)

Estimated marginal means

by group (w FDR correction of pvals)

group	emmean	SE	df	lower.CL	upper.CL
co	0.860	0.042	42.28	0.775	0.945
p	0.797	0.021	42.96	0.754	0.839
mts	0.718	0.049	42.28	0.620	0.817

contrast	estimate	SE	df	t.ratio	p.value	lowerCL	upperCL
co - p	0.063	0.047	42.41	1.343	0.186	-0.054	0.181
co - mts	0.142	0.064	42.28	2.198	0.100	-0.019	0.302
p - mts	0.078	0.053	42.39	1.472	0.186	-0.054	0.211

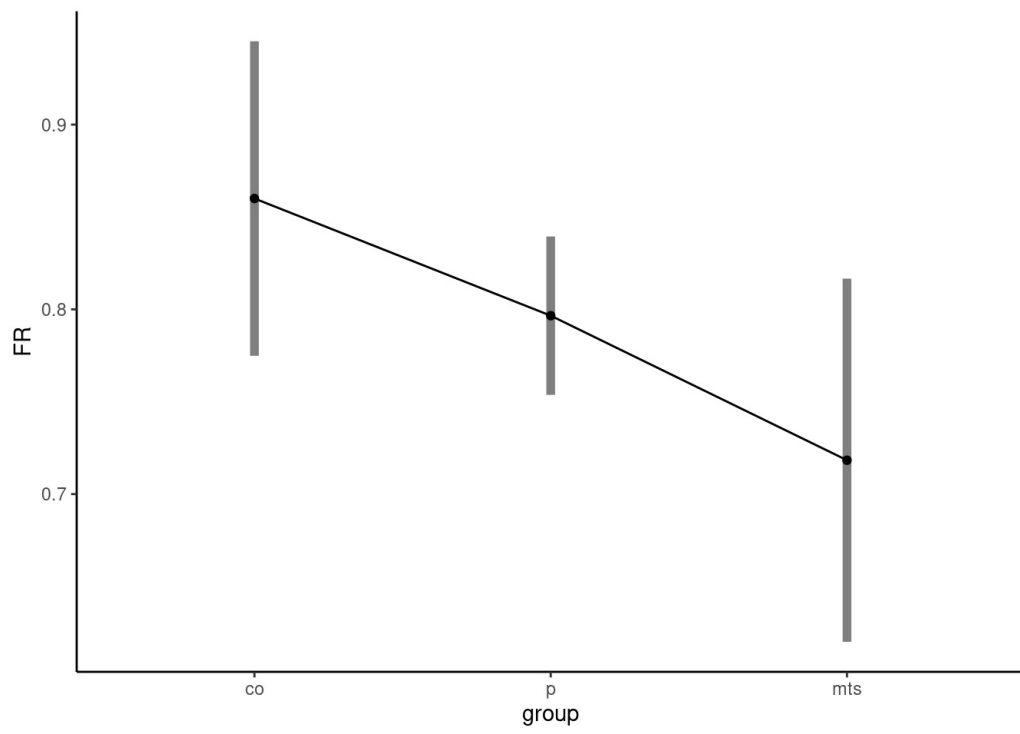
by surgery

surgery	emmean	SE	df	lower.CL	upper.CL
pre	0.770	0.028	79.00	0.714	0.826
post	0.813	0.028	79.13	0.757	0.869

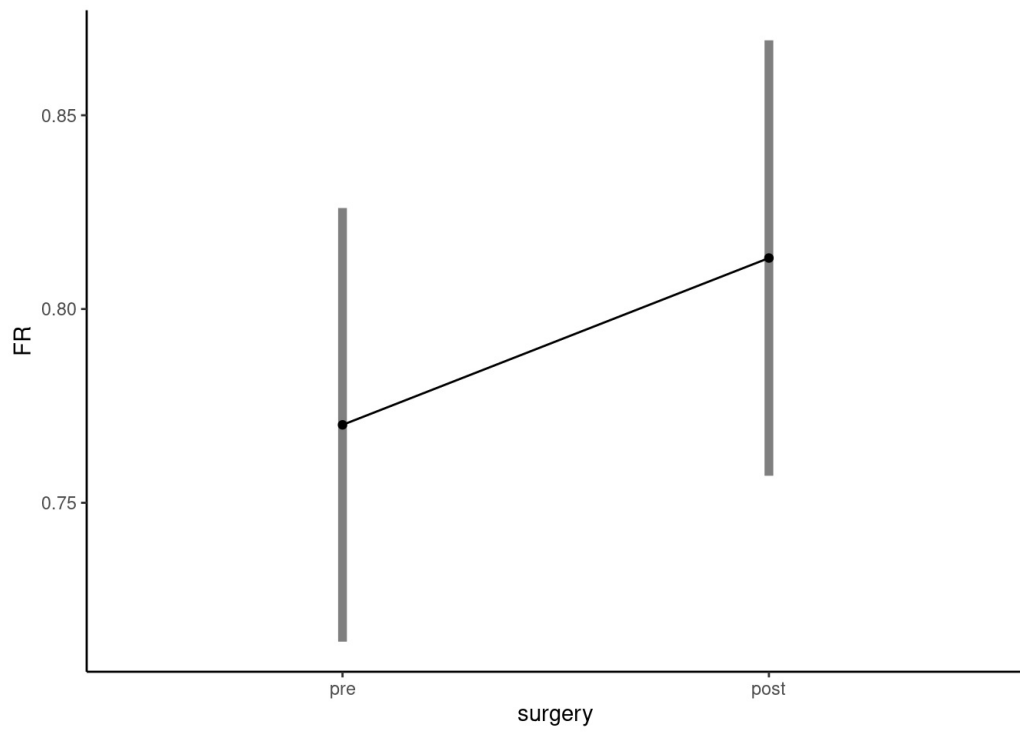
contrast	estimate	SE	df	t.ratio	p.value	lowerCL	upperCL
pre - post	-0.043	0.034	44.71	-1.282	0.206	-0.111	0.025

Marginal means plot

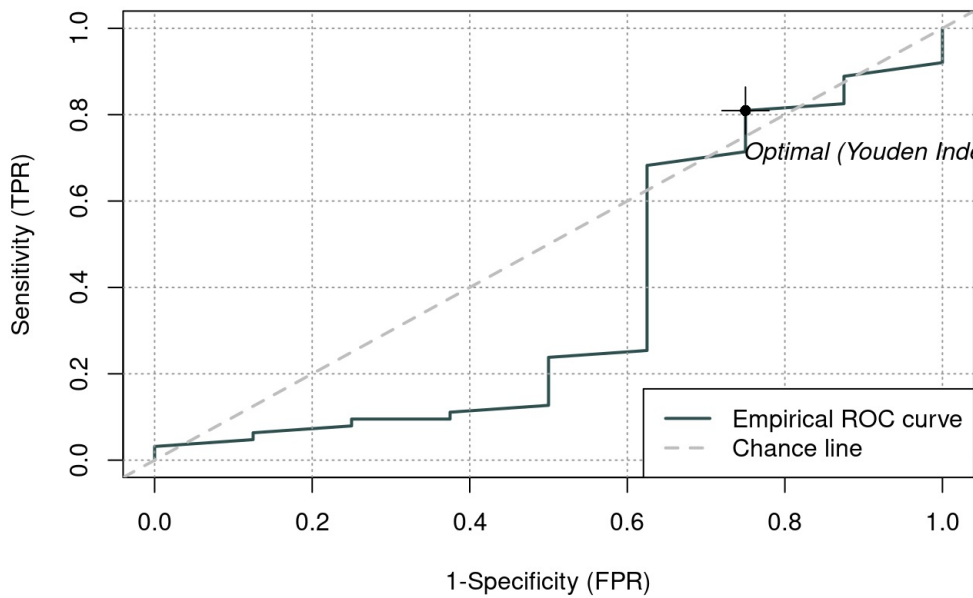
group



surgery



Empirical ROC

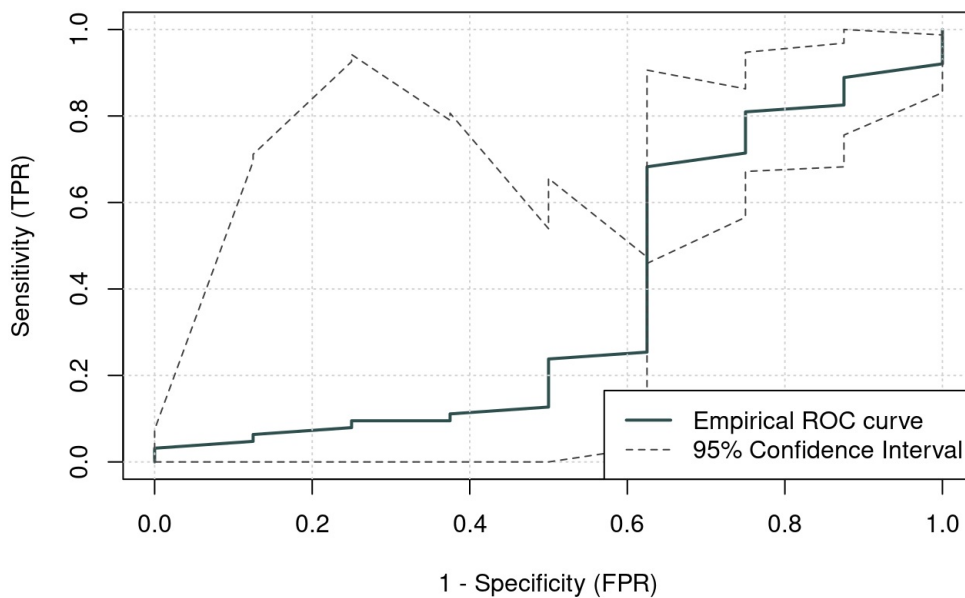


png
2

Youden index and cutoff

value	FPR	TPR	cutoff
0.05952	0.75000	0.80952	0.66000

95% ci for empirical ROC



png
2

Session info

```
-- Session info
setting value
version R version 4.0.5 (2021-03-31)
os      CentOS Stream 8
system  x86_64, linux-gnu
ui      X11
language (EN)
collate en_US.UTF-8
ctype   en_US.UTF-8
tz      Europe/Bratislava
date    2023-10-09
pandoc  3.1.1 @ /usr/lib/rstudio/resources/app/bin/quarto/bin/tools/ (via rmarkdown)
```

```
-- Packages
package * version  date (UTC) lib source
DescTools * 0.99.41  2021-04-10 [1] CRAN (R 4.0.5)
DHARMA   * 0.4.6    2022-09-08 [1] CRAN (R 4.0.5)
dplyr    * 1.1.2    2023-04-20 [1] CRAN (R 4.0.5)
emmeans  * 1.7.5    2022-06-22 [1] CRAN (R 4.0.5)
ggplot2  * 3.4.2    2023-04-03 [1] CRAN (R 4.0.5)
ggpubr   * 0.4.0.999 2021-07-17 [1] Github (kassambara/ggpubr@ac5a01f)
ggsci    * 3.0.0    2023-03-08 [1] CRAN (R 4.0.5)
kableExtra * 1.3.4    2021-02-20 [1] CRAN (R 4.0.5)
lme4     * 1.1-34   2023-07-04 [1] CRAN (R 4.0.5)
Matrix   * 1.6-0    2023-07-08 [1] CRAN (R 4.0.5)
ROCit    * 2.1.1    2020-06-14 [1] CRAN (R 4.0.5)
sjPlot   * 2.8.12   2022-12-13 [1] Github (strengjacke/sjPlot@4058580)
```

```
[1] /home/mg/R/x86_64-pc-linux-gnu-library/4.0
```

```
[2] /opt/R/4.0.5/lib/R/library
```

report_CDKN2B__Oct9_2023.R

mg

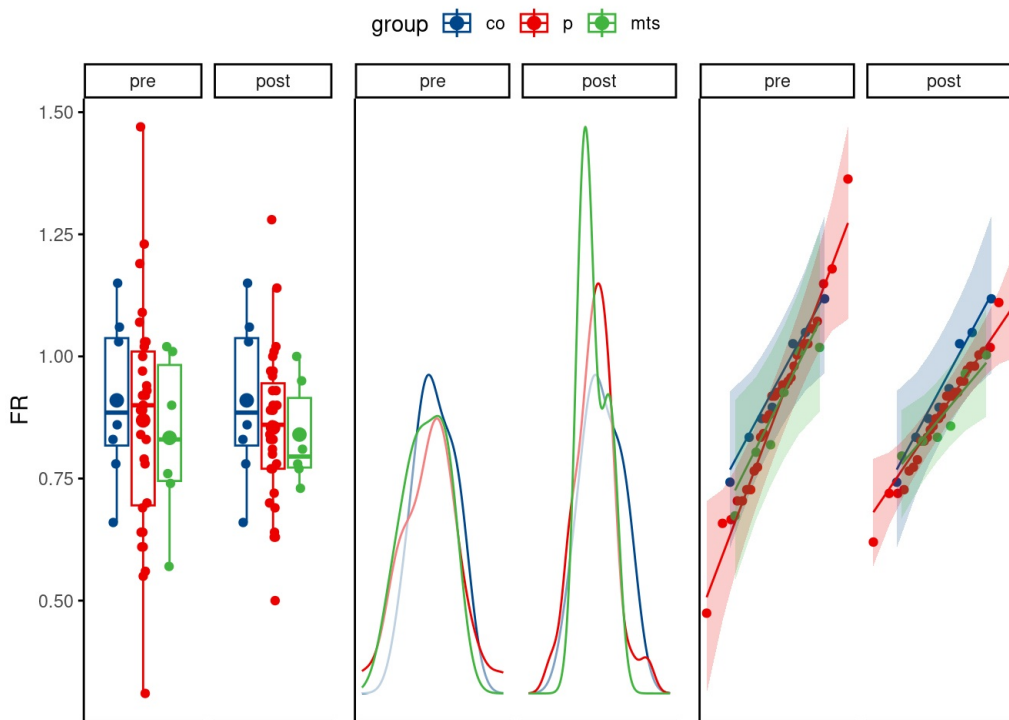
2023-10-09

```
#  
#  
# Oct 9 2023 Mo -- CDKN2B  
# Oct 5 2023 Th -- triplot; spaghetti; lin mixed model - dg, outlier,  
#                   emmeans, custom contrasts  
# Oct 4 2023 We -- CDK4 ddPCR CNV  
#  
#  
# Eva Lukacova  
#
```

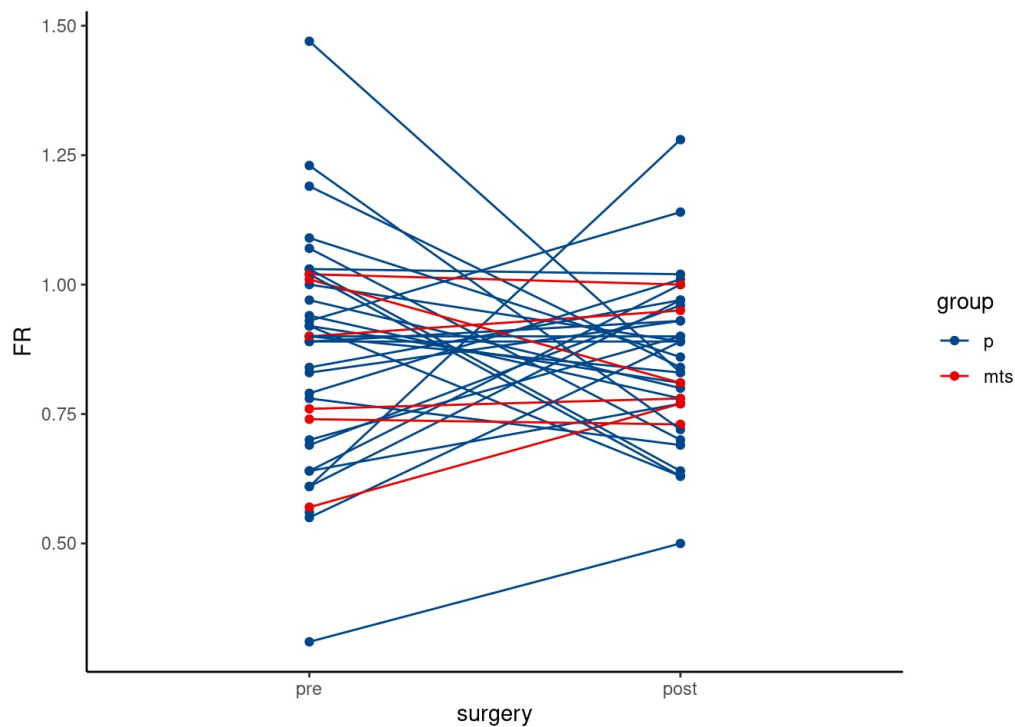
CDKN2B

EDA

Triplot (for FR by surgery and group)



Spaghetti plot for surgery (w group wo co)



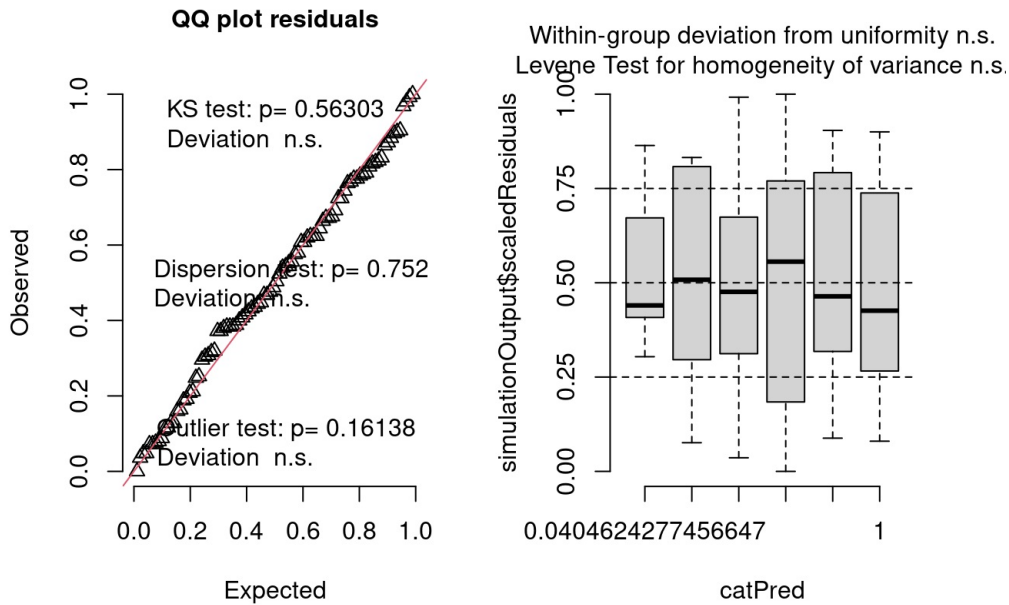
Linear mixed model (to fit Repeated measures ANOVA)

Model fit

<i>Predictors</i>	<i>Estimates</i>	FR		<i>p</i>
		<i>CI</i>		
(Intercept)	0.91	0.81 – 1.02		<0.001
group [p]	-0.05	-0.16 – 0.06		0.373
group [mts]	-0.07	-0.22 – 0.07		0.328
surgery [post]	-0.01	-0.08 – 0.06		0.792
Random Effects				
σ^2	0.03			
τ_{00} id	0.00			
ICC	0.10			
N_{id}	46			
Observations	90			
Marginal R^2 / Conditional R^2	0.014 / 0.115			

Diagnostics

DHARMA residual



=> no outlier(s)

Estimated marginal means

by group (w FDR correction of pvals)

group	emmean	SE	df	lower.CL	upper.CL
co	0.910	0.049	41.61	0.811	1.009
p	0.861	0.025	42.97	0.811	0.911
mts	0.837	0.056	41.61	0.723	0.950

contrast	estimate	SE	df	t.ratio	p.value	lowerCL	upperCL
co - p	0.049	0.055	41.88	0.896	0.563	-0.087	0.186
co - mts	0.073	0.075	41.61	0.983	0.563	-0.113	0.259
p - mts	0.024	0.062	41.82	0.395	0.695	-0.129	0.178

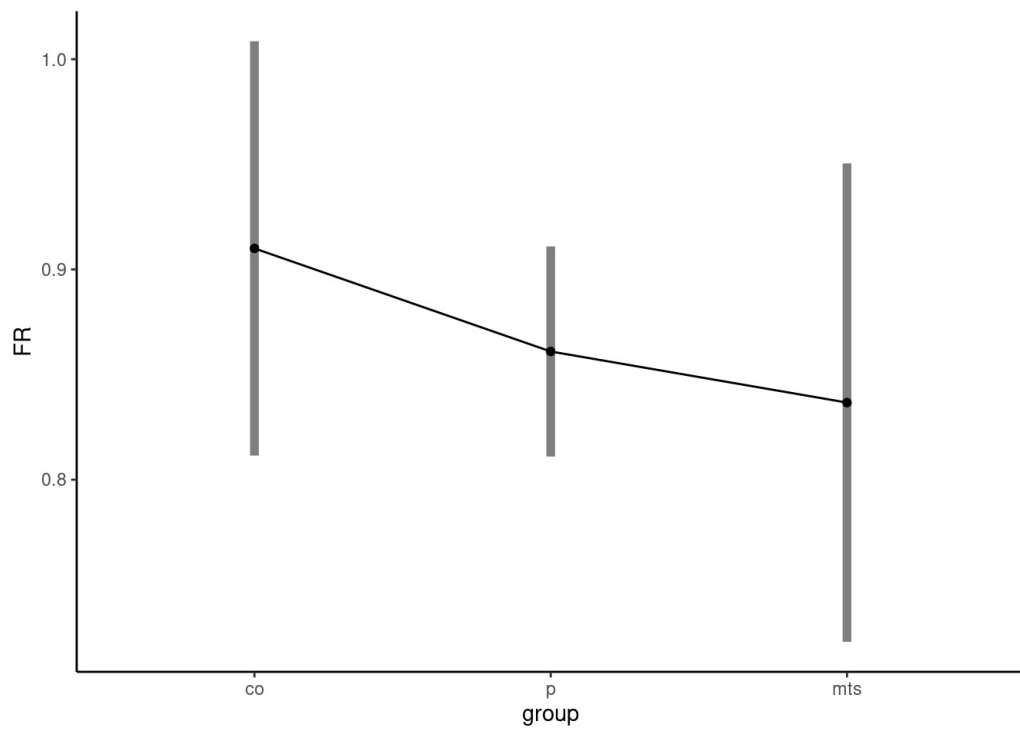
by surgery

surgery	emmean	SE	df	lower.CL	upper.CL
pre	0.874	0.032	76.17	0.81	0.938
post	0.864	0.032	76.17	0.80	0.928

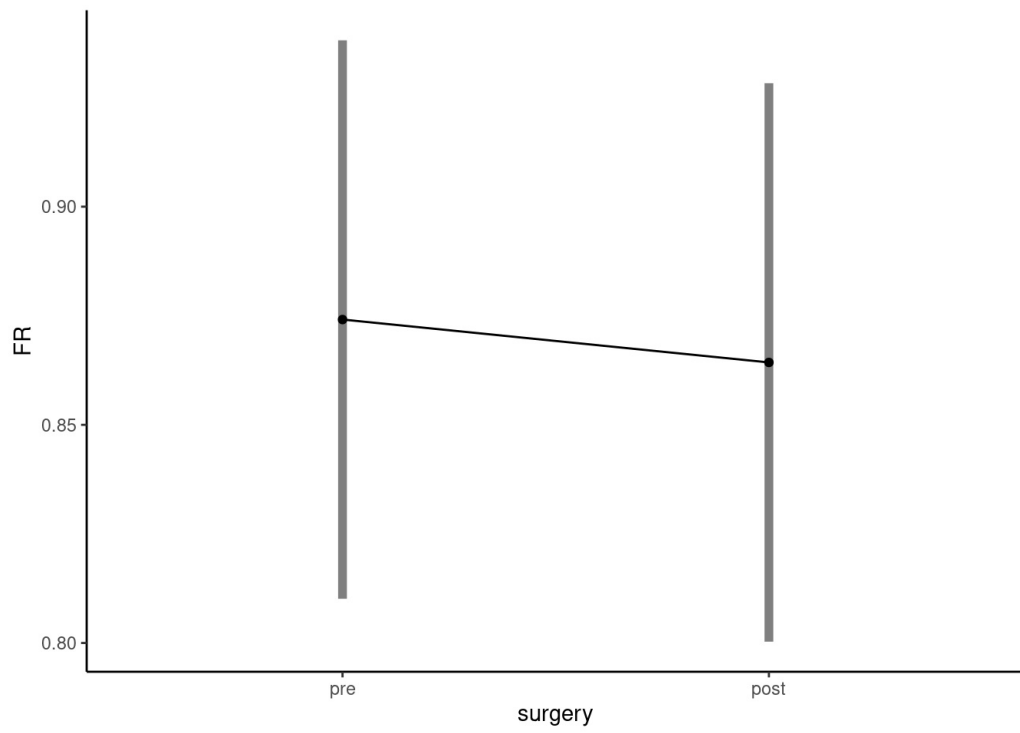
contrast	estimate	SE	df	t.ratio	p.value	lowerCL	upperCL
pre - post	0.01	0.037	44.37	0.264	0.793	-0.065	0.085

Marginal means plot

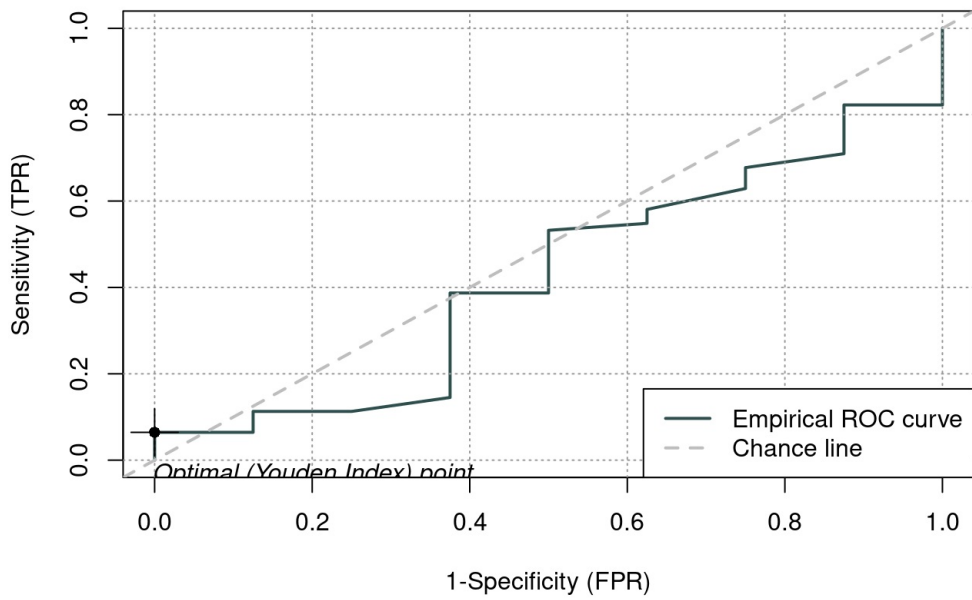
group



surgery



Empirical ROC

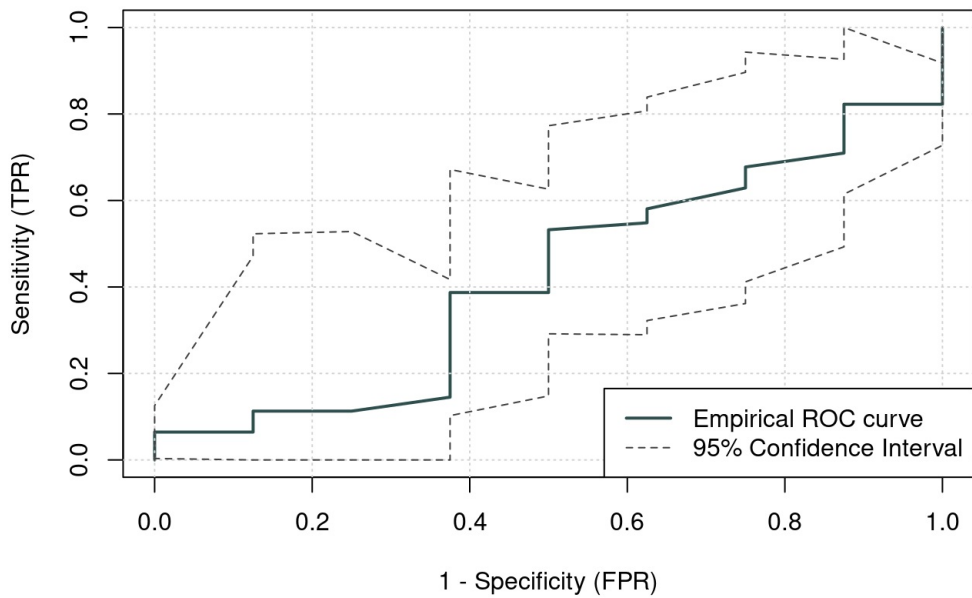


png
2

Youden index and cutoff

value	FPR	TPR	cutoff
0.06452	0.00000	0.06452	1.19000

95% ci for empirical ROC



png
2

Session info

```
-- Session info
setting value
version R version 4.0.5 (2021-03-31)
os      CentOS Stream 8
system  x86_64, linux-gnu
ui      X11
language (EN)
collate en_US.UTF-8
ctype   en_US.UTF-8
tz      Europe/Bratislava
date    2023-10-09
pandoc  3.1.1 @ /usr/lib/rstudio/resources/app/bin/quarto/bin/tools/ (via rmarkdown)
```

```
-- Packages
package * version  date (UTC) lib source
DescTools * 0.99.41  2021-04-10 [1] CRAN (R 4.0.5)
DHARMA   * 0.4.6    2022-09-08 [1] CRAN (R 4.0.5)
dplyr    * 1.1.2    2023-04-20 [1] CRAN (R 4.0.5)
emmeans  * 1.7.5    2022-06-22 [1] CRAN (R 4.0.5)
ggplot2  * 3.4.2    2023-04-03 [1] CRAN (R 4.0.5)
ggpubr   * 0.4.0.999 2021-07-17 [1] Github (kassambara/ggpubr@ac5a01f)
ggsci    * 3.0.0    2023-03-08 [1] CRAN (R 4.0.5)
kableExtra * 1.3.4    2021-02-20 [1] CRAN (R 4.0.5)
lme4     * 1.1-34   2023-07-04 [1] CRAN (R 4.0.5)
Matrix   * 1.6-0    2023-07-08 [1] CRAN (R 4.0.5)
ROCit    * 2.1.1    2020-06-14 [1] CRAN (R 4.0.5)
sjPlot   * 2.8.12   2022-12-13 [1] Github (strengjacke/sjPlot@4058580)
```

```
[1] /home/mg/R/x86_64-pc-linux-gnu-library/4.0
```

```
[2] /opt/R/4.0.5/lib/R/library
```

report_CDK4__Oct9_2023.R

mg

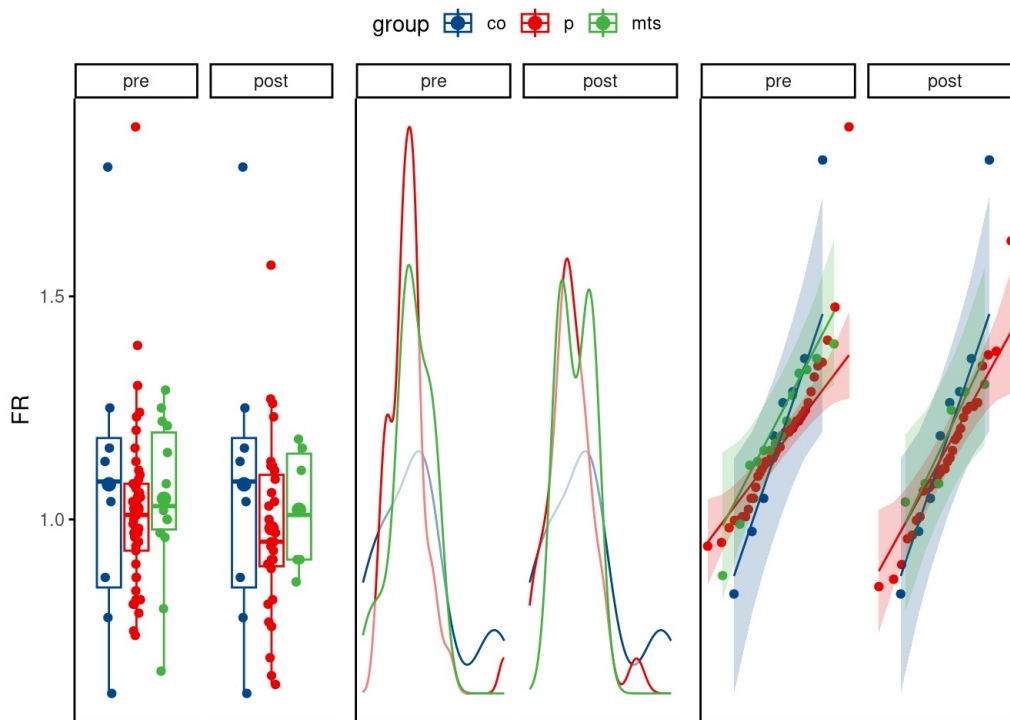
2023-10-09

```
#  
#  
# Oct 9 2023 Mo -- saving figs; txt  
# Oct 5 2023 Th -- triplot; spaghetti; lin mixed model - dg, outlier,  
#                   emmeans, custom contrasts  
# Oct 4 2023 We -- CDK4 ddPCR CNV  
#  
#  
# Eva Lukacova  
#
```

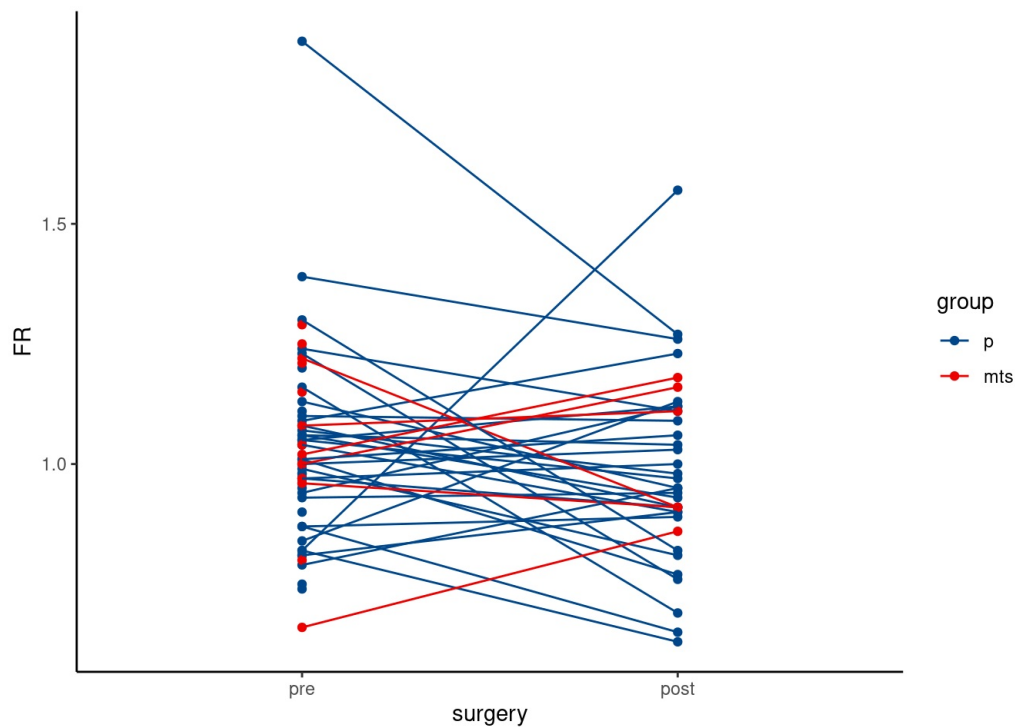
CDK4

EDA

Triplot (for FR by surgery and group)



Spaghetti plot for surgery (w group wo co)



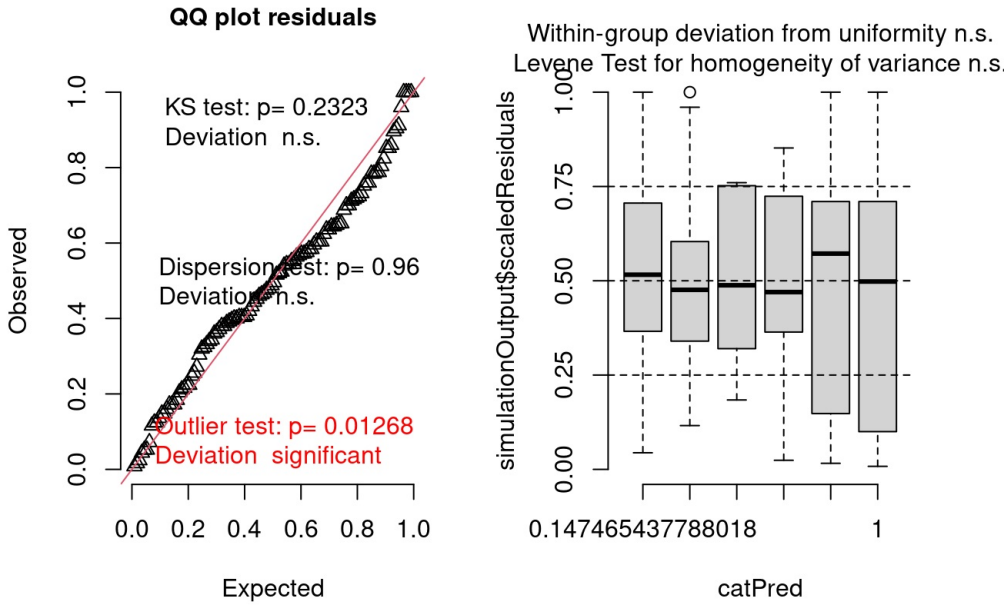
Linear mixed model (to fit Repeated measures ANOVA)

Model fit

<i>Predictors</i>	<i>Estimates</i>	FR		<i>p</i>
		<i>CI</i>		
(Intercept)	1.10	0.96 – 1.23		<0.001
group [p]	-0.08	-0.22 – 0.06		0.266
group [mts]	-0.04	-0.21 – 0.13		0.631
surgery [post]	-0.04	-0.10 – 0.02		0.214
Random Effects				
σ^2	0.02			
τ_{00} id	0.02			
ICC	0.49			
N_{id}	67			
Observations	112			
Marginal R^2 / Conditional R^2	0.025 / 0.506			

Diagnostics

DHARMa residual



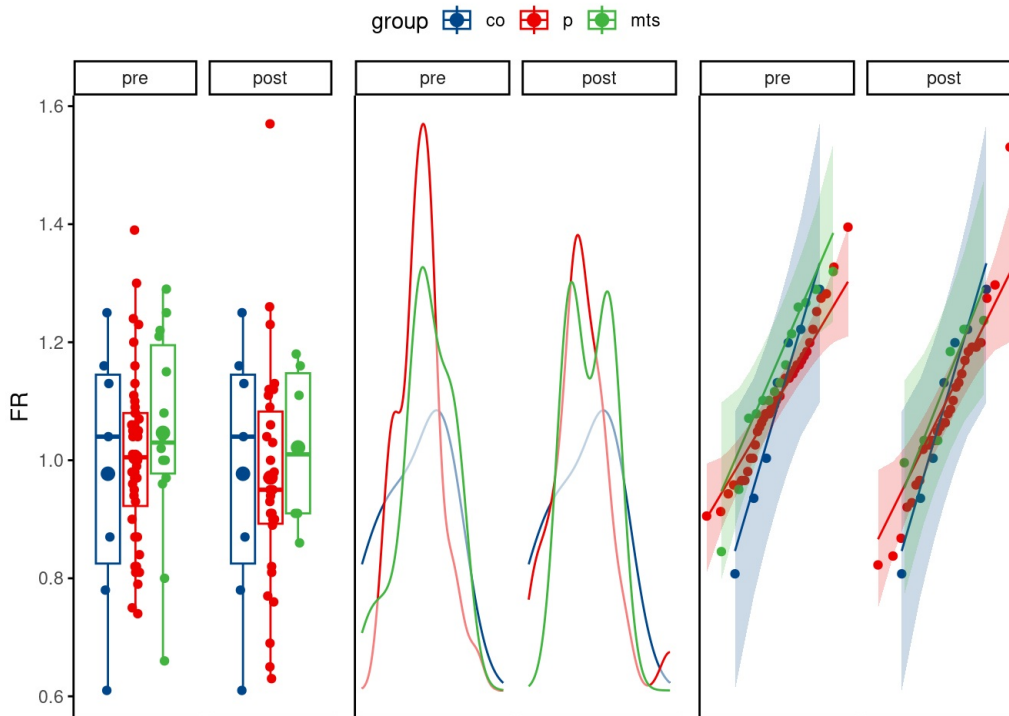
=> outlier(s)

one in co, one in p => search for them

p -> M7

co -> H5

=> remove them



=> rerun wo outliers

Model fit

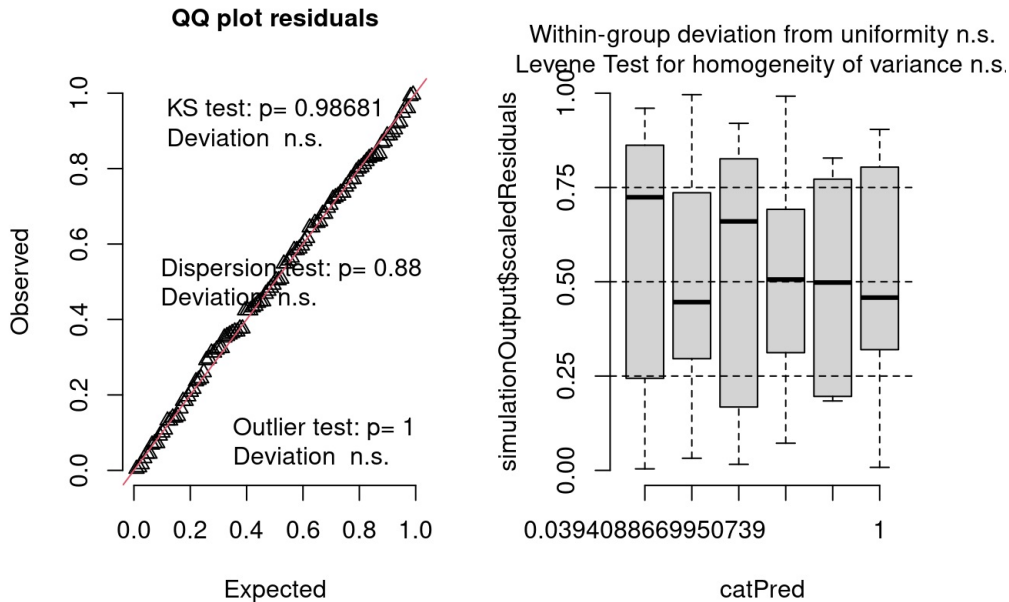
Predictors	Estimates	FR	
		CI	p
(Intercept)	0.99	0.88 – 1.10	<0.001
group [p]	0.01	-0.10 – 0.12	0.874
group [mts]	0.06	-0.07 – 0.19	0.374
surgery [post]	-0.03	-0.09 – 0.03	0.333

Random Effects

σ^2	0.02
$\tau_{00 \text{ id}}$	0.01
ICC	0.29
N_{id}	65
Observations	108
Marginal R^2 / Conditional R^2	0.023 / 0.309

Diagnostics

DHARMA residual



Estimated marginal means

by group (w FDR correction of pvals)

group	emmean	SE	df	lower.CL	upper.CL
co	0.977	0.053	48.88	0.871	1.083
p	0.986	0.023	59.52	0.941	1.032
mts	1.038	0.042	71.40	0.953	1.122

contrast	estimate	SE	df	t.ratio	p.value	lowerCL	upperCL
co - p	-0.009	0.057	50.40	-0.159	0.874	-0.151	0.133
co - mts	-0.060	0.068	56.85	-0.892	0.564	-0.227	0.107
p - mts	-0.051	0.048	67.74	-1.077	0.564	-0.168	0.066

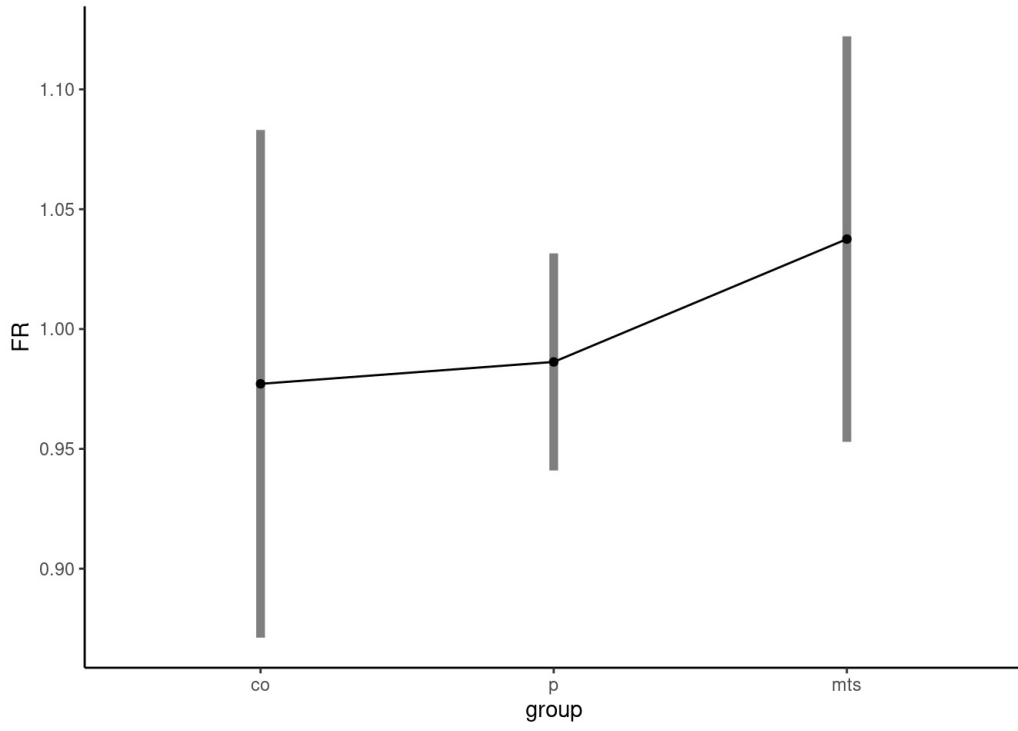
by surgery

surgery	emmean	SE	df	lower.CL	upper.CL
pre	1.015	0.026	82.95	0.963	1.067
post	0.986	0.030	96.69	0.926	1.045

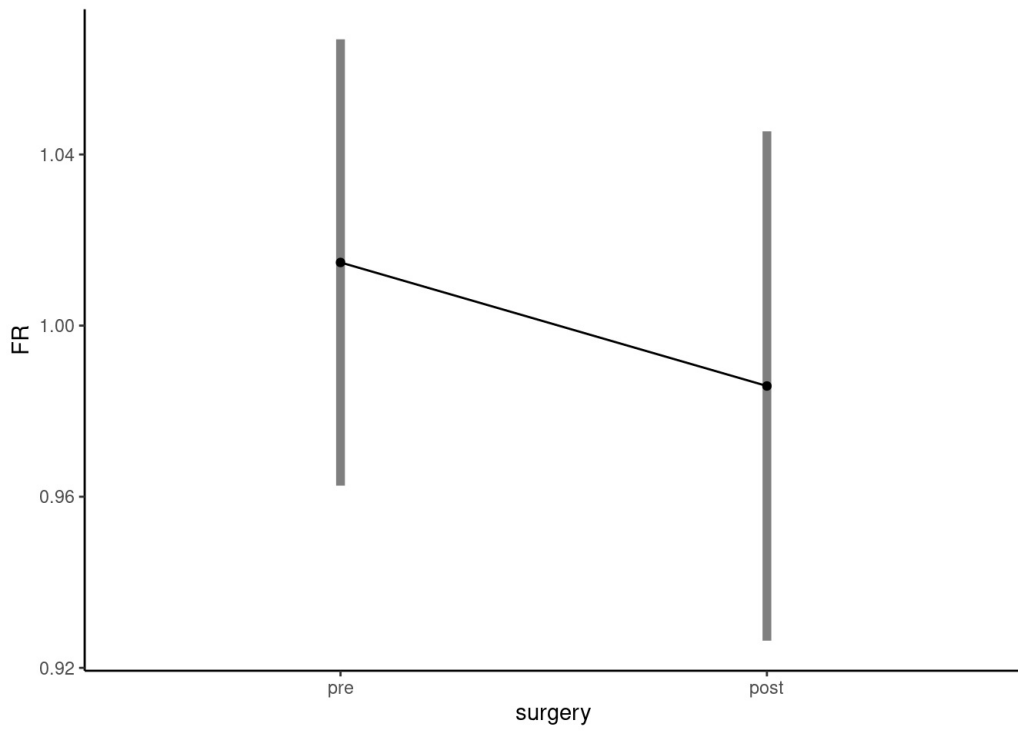
contrast	estimate	SE	df	t.ratio	p.value	lowerCL	upperCL
pre - post	0.029	0.03	53.06	0.968	0.337	-0.031	0.089

Marginal means plot

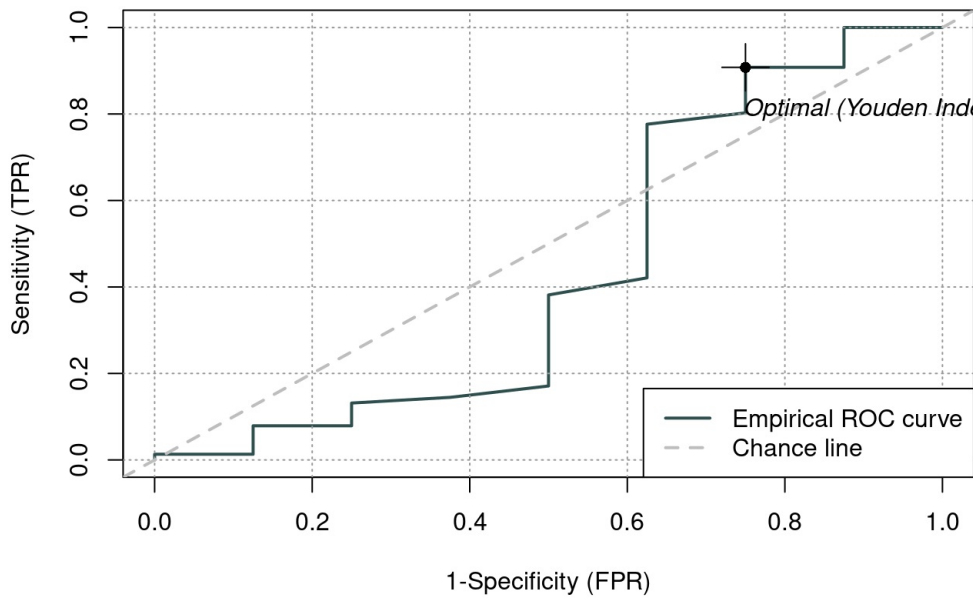
group



surgery



Empirical ROC

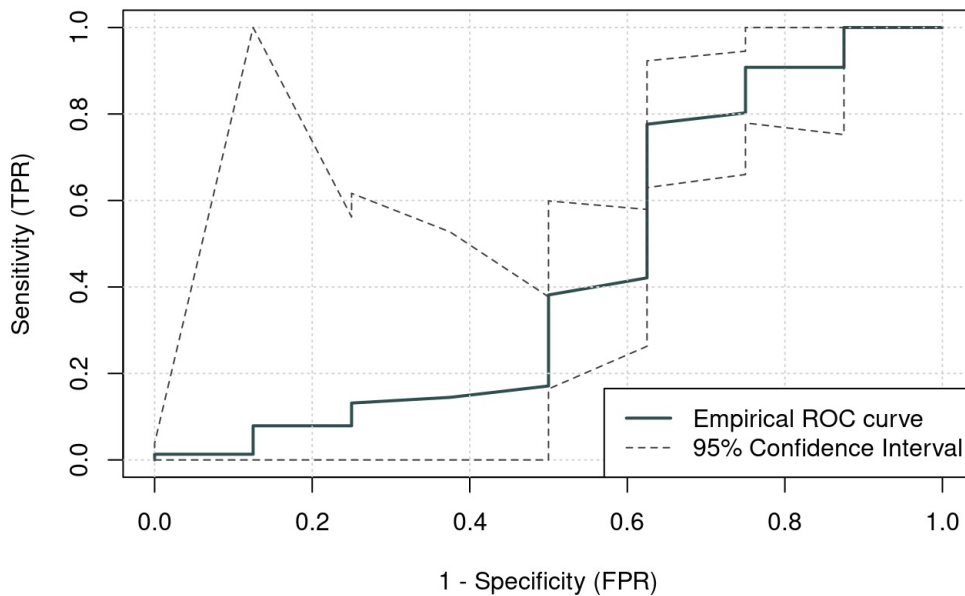


png
2

Youden index and cutoff

value	FPR	TPR	cutoff
0.1579	0.7500	0.9079	0.7900

95% ci for empirical ROC



png
2

Data analysis

Data were explored and analyzed in R [1], ver. 4.0.5, with the aid of libraries listed below at Session info.

Distribution of the Final Ratio (FR) was visualized by boxplot overlaid with swarmplot, density plot and Quantile-Quantile plot with the 95% confidence band constructed by bootstrap. Change of FR between pre- and post-surgery stages was visualized by the spaghetti plot.

Linear mixed model of the form $Fr \sim \text{group} + \text{surgery} + (1|id)$ was fitted to the data by REML method. There, group was a factor with three levels: control, primary, metastasis and surgery was a factor with two levels: pre, post. The fitted model was subjected to diagnostic analysis of residuals. If outliers were detected (two outlier for CDK4, none for CDKN2A and CDKN2B) then they were excluded from considerations and the model was refit and subjected to diagnostics. Effect size was quantified by the Adjusted R^2 .

The fitted linear mixed model was used to estimate the marginal means. Post-hoc pairwise comparisons were performed with the Benjamini-Hochberg correction of p-values in the case of the comparisons for group factor. The marginal means together with their 95% confidence interval were visualized by the marginal means plot.

[1] R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/> (<https://www.R-project.org/>).

Session info

```
— Session info —————
setting value
version R version 4.0.5 (2021-03-31)
os CentOS Stream 8
system x86_64, linux-gnu
ui X11
language (EN)
collate en_US.UTF-8
ctype en_US.UTF-8
tz Europe/Bratislava
date 2023-10-09
pandoc 3.1.1 @ /usr/lib/rstudio/resources/app/bin/quarto/bin/tools/ (via rmarkdown)

— Packages —————
package * version date (UTC) lib source
DescTools * 0.99.41 2021-04-10 [1] CRAN (R 4.0.5)
DHARMA * 0.4.6 2022-09-08 [1] CRAN (R 4.0.5)
dplyr * 1.1.2 2023-04-20 [1] CRAN (R 4.0.5)
emmeans * 1.7.5 2022-06-22 [1] CRAN (R 4.0.5)
ggplot2 * 3.4.2 2023-04-03 [1] CRAN (R 4.0.5)
ggpubr * 0.4.0.999 2021-07-17 [1] Github (kassambara/ggpubr@ac5a01f)
ggsci * 3.0.0 2023-03-08 [1] CRAN (R 4.0.5)
kableExtra * 1.3.4 2021-02-20 [1] CRAN (R 4.0.5)
lme4 * 1.1-34 2023-07-04 [1] CRAN (R 4.0.5)
Matrix * 1.6-0 2023-07-08 [1] CRAN (R 4.0.5)
ROCit * 2.1.1 2020-06-14 [1] CRAN (R 4.0.5)
sjPlot * 2.8.12 2022-12-13 [1] Github (strengjacke/sjPlot@4058580)

[1] /home/mg/R/x86_64-pc-linux-gnu-library/4.0
[2] /opt/R/4.0.5/lib/R/library
```