Post-Acute Sequelae of Long COVID-19

(PASC)

SomaScan® Proteomics Analysis
Tracking the impact on ~7000 plasma proteins in PASC
19 August 2022

Blood samples from PASC Patients evaluated to identify opportunities for the diagnosis, prognosis and better treatment of Neurological PASC

antisense THERAPEUTICS

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Antisense Therapeutics SomaScan® Neurological Long COVID-19 Findings

- World first study to assess up to 7,000 plasma proteins in Long COVID-19 patients
- Collaboration with Feinberg School of Medicine NorthWestern University Chicago and NorthWestern Medicine Neuro-Covid Clinic[1]
- Retained blood samples from 92 subjects (48 patients with neurological symptoms) tested
  - Neurological Post-Acute Sequelae of COVID-19 (PASC) i.e NPASC
  - Most patient samples had been previously studied to characterize certain blood immune cells changes [1]
- Samples were successfully tested in the US by SomaLogic®, a leading proteomics group, for protein changes in the blood
- First results after statistical analysis using Somalogic's® DataViz program reported herein
  - Investigation has elucidated novel blood markers as potential diagnostic and therapeutic targets
  - Provisional patent applications have been filed in the United States (US) to seek protection for these new inventions
  - Diagnostic
    - a combination of 3 markers identifies all 48 NPASC subjects i.e high sensitivity
    - These 3 markers & another 2 differentiate 42/44 subjects without NPASC i.e high specificity
  - Therapeutic
    - 15 markers potentially amenable to treatment by currently available drugs and other therapeutic approaches on the market including with ATL1102, an immunomodulatory drug active in Relapsing MS and DMD[2]
  - The specific targets have not been identified as they are trade secrets

1. Visvabharathy L, et al " Neuro-COVID long-haulers exhibit broad dysfunction in T cell memory generation and responses to vaccination." https://www.medrxiv.org/content/10.1101/2021.08.08.21261763v3
2. https://www.antisense.com.au/wp-content/uploads/2021/09/WMS-ATL1102-DMD-PROTEOMICS-Poster-Submission-Final_1.2.pdf

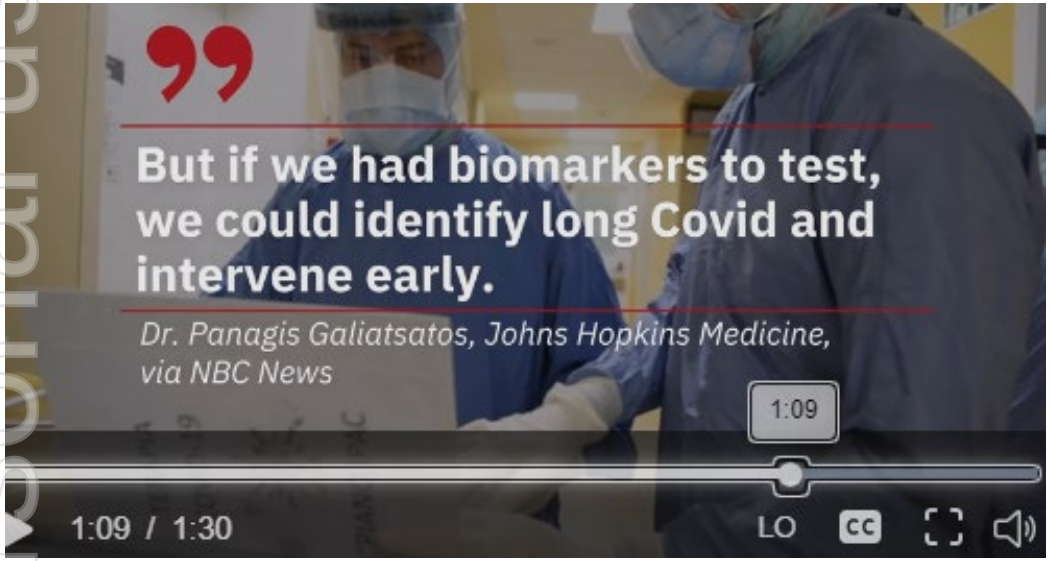
Of the 94.7 million people in the US diagnosed as infected and surviving COVID-19[1], approximately 82 million (87%) people are non-hospitalized and 45% of non-hospitalized patients[2] have developed some manifestation of Long COVID-19 syndrome which suggests ***more than 24 million people are afflicted by the condition to some extent***

The main neurological symptom is brain fog (defined with the established memory tests conducted) and reported in 81% ***suggesting an impact on nearly 20 million people in the US***

1. https://coronavirus.jhu.edu/map.html and https://www.worldometers.info/coronavirus/country/us/ as of 16 August 2022, 94.7 million people in the USA were diagnosed with COVID-19, 1.063 million deaths have been recorded, and 89.9 million people have recovered.

2. Estiri H et al "Evolving phenotypes of non-hospitalized patients that indicated long COVID". BMC Medicine (2021) 19: 249 https://doi.org/10.1186/s12916-021-02115-0

Acknowledged need for diagnostic and therapeutic approaches



https://www.msn.com/en-us/health/medical/brain-fog-and-other-long-covid-symptoms-can-last-more-than-a-year-according-to-study/vi-AA10gBis

Timeline of post-acute COVID19 infection

https://www.nature.com/articles/s41591-021-01283-z
From Nalbandian et al Nature Medicine 27, 601-615 (2021)

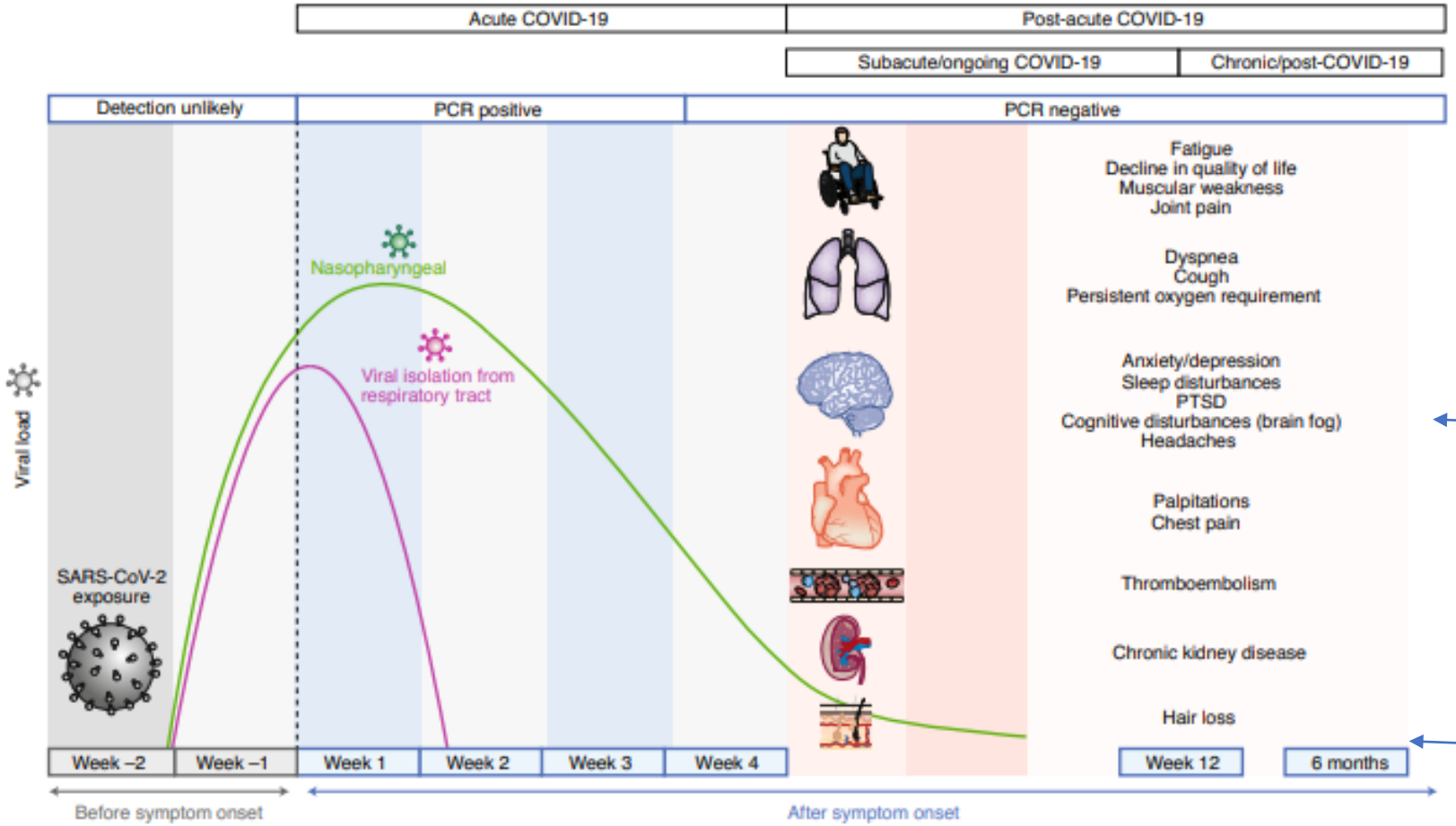


Fig. 1 | Timeline of post-acute COVID-19. Acute COVID-19 usually lasts until 4 weeks from the onset of symptoms, beyond which replication-competent SARS-CoV-2 has not been isolated. Post-acute COVID-19 is defined as persistent symptoms and/or delayed or long-term complications beyond 4 weeks from the onset of symptoms. The common symptoms observed in post-acute COVID-19 are summarized.

Neurological Post-Acute Sequelae of COVID-19 (PASC)
NPASC
(can include fatigue)

The WHO definition of PASC is at >12 weeks post acute COVID-19 symptoms

Methods- Samples

- Blood samples from 92 subjects (48 patients with Neurological-PASC) were sent to SomaLogic®
- Plasma samples were tested using the SomaScan® assay, a large scale, aptamer-based assay
- The normalized relative fluorescence units (nRFU) of over 7000 proteins was determined for the following patient groups:
  - Group 1&2 48 Patients affected by Neurological-PASC (NP*)
    (47 samples came from 1 site (Group 1), and 1* came from another site (Group 2)
  - Group 3 20 Patients who have recovered from COVID-19 with no symptoms
    Convalescent controls, of PASC (CC)
  - Group 4: 24 Healthy controls subjects (never had SARS-CoV-2 infection) (HC)

    - Of the above 92 samples 42 samples in G1, 17 samples in G3 and all 24 samples in G4 i.e 83 samples had blood immune cells changes studied and characterized in a prior study[1]

1 Visvabharathy L, et al " Neuro-COVID long-haulers exhibit broad dysfunction in T cell memory generation and responses to vaccination." https://www.medrxiv.org/content/10.1101/2021.08.08.21261763v3

Methods –Statistics Dataviz

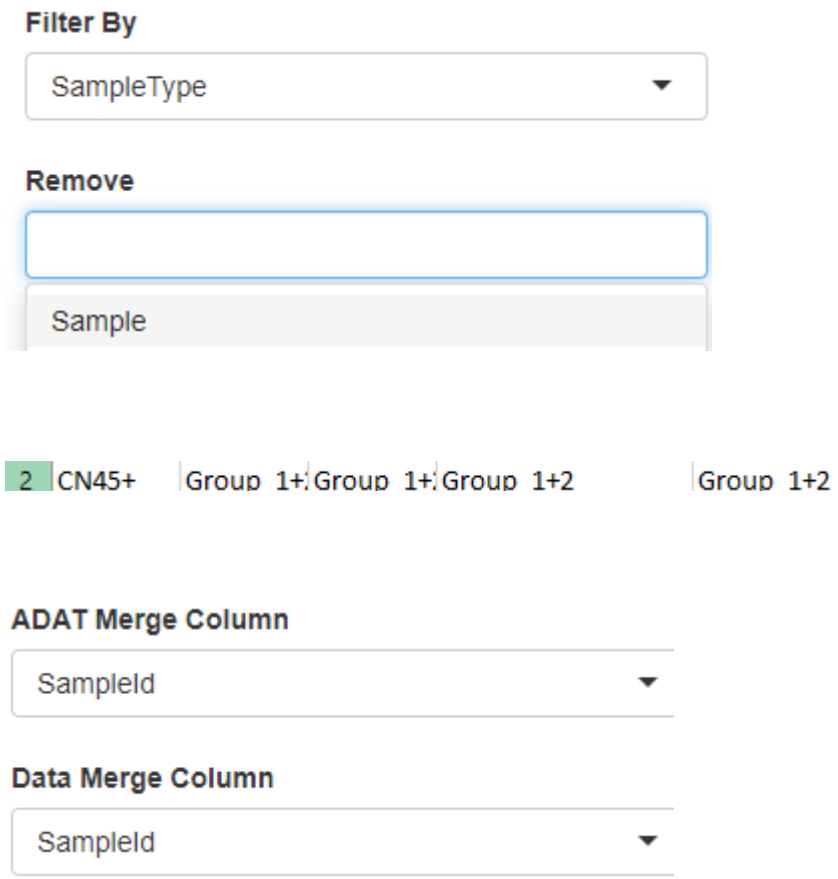
Somalogic® provided an ADAT file of data that was uploaded to their Dataviz program after logging in and starting the statistical tests.

The file is then filtered by “sample type” and the Buffer, QC and Calibrator samples are removed leaving only “Sample” -

A .csv file was created that gives each sample the correct identifier for its group.

This file was then uploaded to Dataviz under the “Merge Data header”.

The .csv file and the filtered ADAT file are then merged to provide Dataviz and the group identifiers for statistical tests



Plasma from one subject with NPASC from a different site was put into a separate group 2 and their data combined with 47 subjects’ data in group 1 (NP), and compared to group 3(CC), and group 4 (HC) controls

Methods-Statistics (T test and U test)

Parametric and Non-parametric tests to identify protein markers of interest

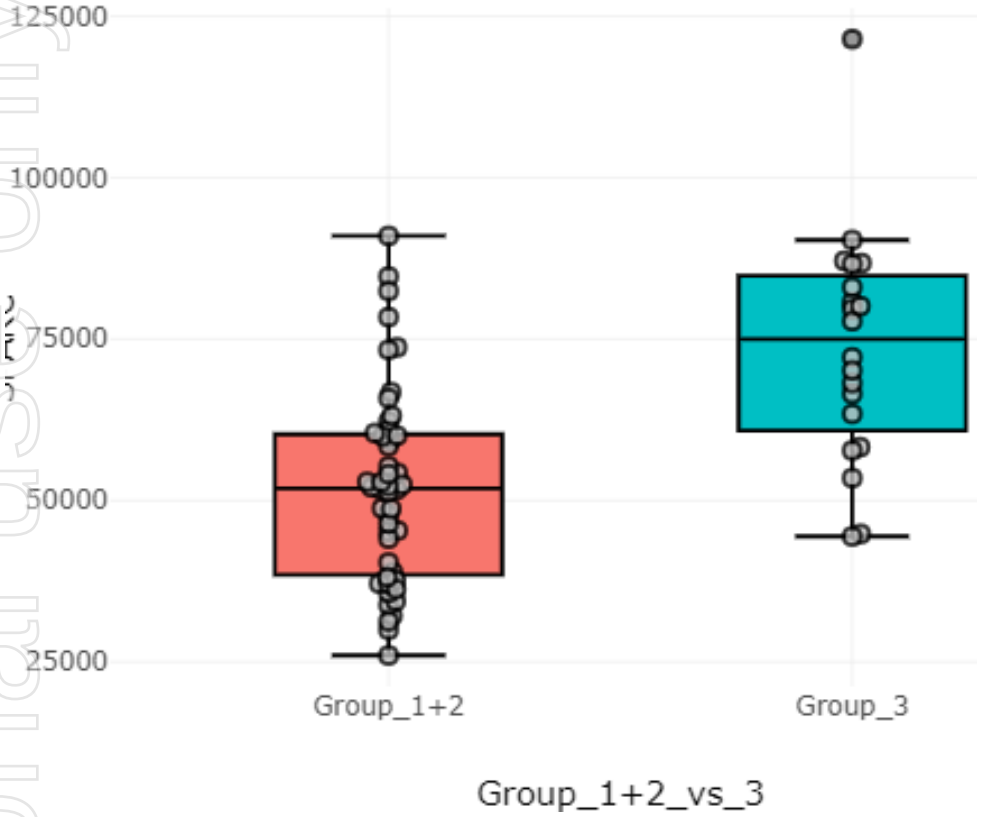
- T test and U test comparing
  - Group 1 to 3,
  - Groups 1 to 4,
  - Groups 1 to 3+4,
  - Groups 3 vs 4

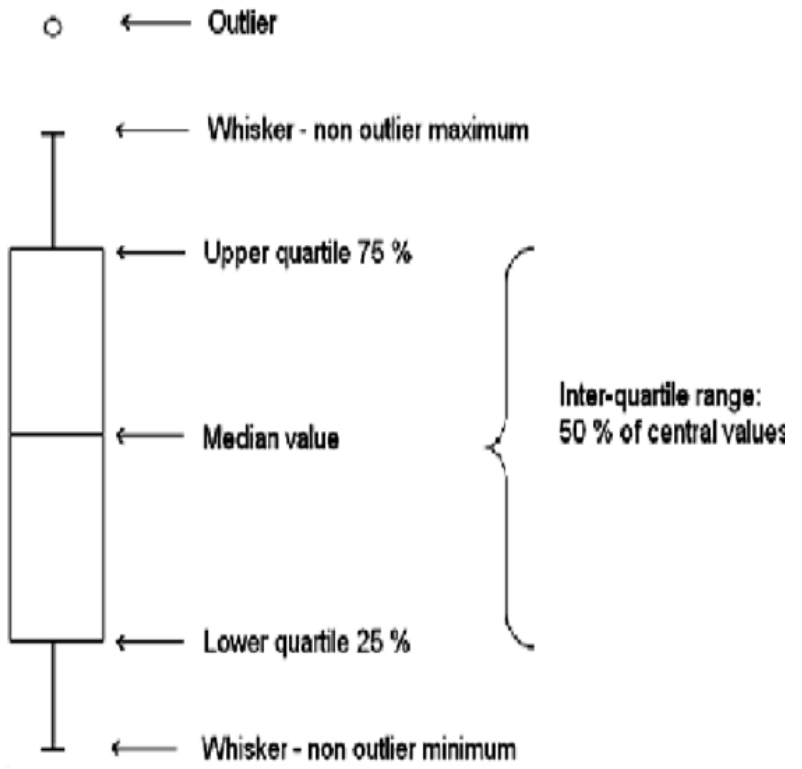
  was conducted with
  - p-values adjusted using Benjamini-Hochberg false discovery rate (FDR)
  - Bonferroni statistical tests,
  - using Somalogic's ® Dataviz software

		1 vs 3	1 vs 4	1 vs 3+4	3 vs 4
T-TEST	Bonferroni < 0.05	1	30	45	0
	FDR< 0.0005	0	11	22	0
	FDR <0.01	0	184	440	0
	FDR < 0.03	0			0
	FDR <0.05	25	891	1556	0
	FDR < 0.075	65	1242		0
		1 vs 3	1 vs 4	1 vs 3+4	3 vs 4
U-TEST	Bonferroni < 0.05	2	30	45	0
	FDR< 0.0005	0	4	0	0
	FDR <0.01	0	213	493	0
	FDR <0.03	10			
	FDR <0.05	53	1052	1957	0
	FDR < 0.075	344			0

Group 3 vs 4 had no different proteins via FDR or Bonferroni adjusted p values (but had some using standard p values)

Results Bonferroni T test < 0.05 (G1 vs G3)





Data Viz
Box plots shows Outliers,
The upper whisker also known as the upper fence,

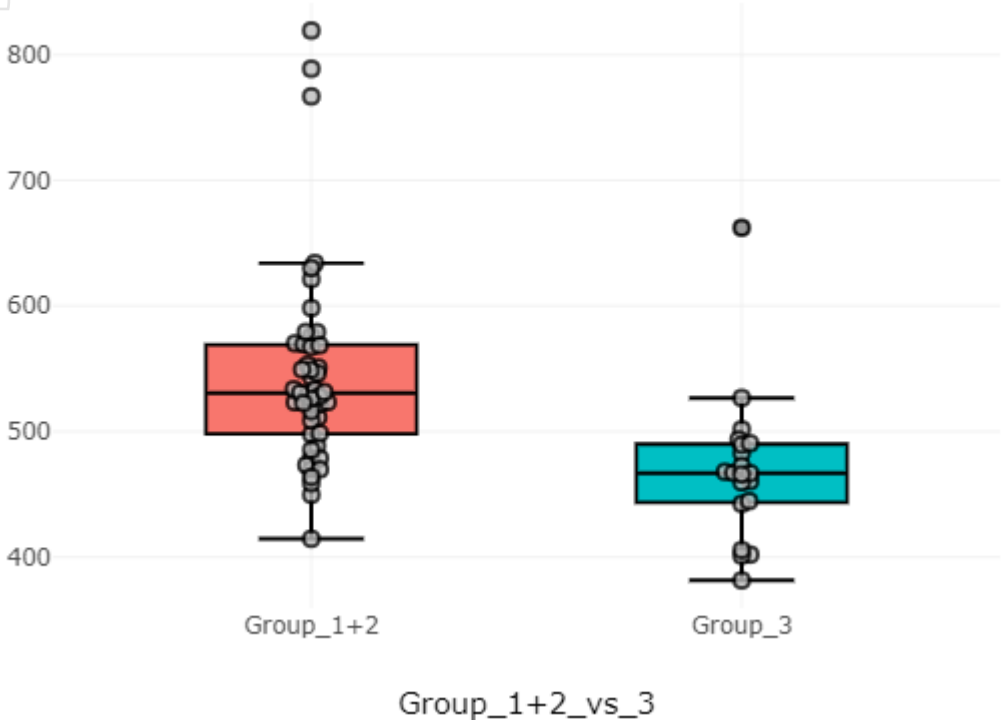
Interquartile range,

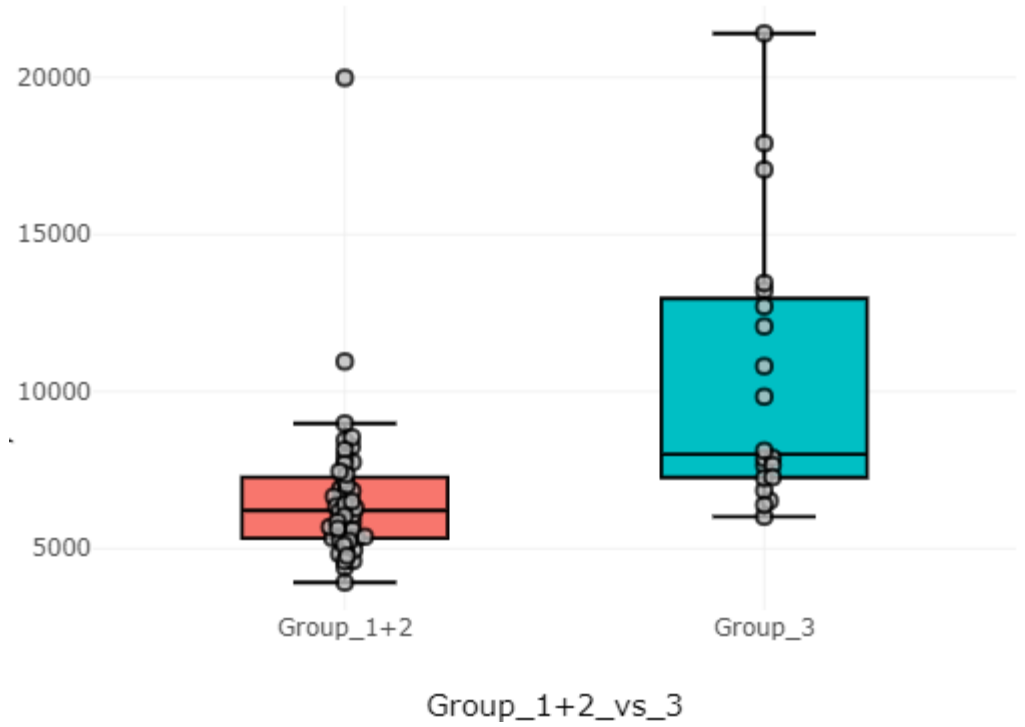
lower whisker also known as the minimum (or lower fence)

- 1 Target 'S' was identified in the parametric T –test with Bonferroni of <0.035
- The median % change in Group 1+2 NP patients was -31% versus the convalescent control Group 3

Results Bonferroni U test < 0.05 (G1 vs G3)

- 2 targets were identified in the non-parametric U –test, Bonferroni statistical analysis





Target ‘M’ Q3 also detected in the FDR<0.02 stats
13% increase in the median in G1 vs G3

Target ‘G’ also detected in the FDR<0.02 stats
22% decrease in median in G1 vs G3

- The second target is a diagnostic marker when used in combination with others

Methods- Statistics (ANOVA and Kruskalis Wallis)

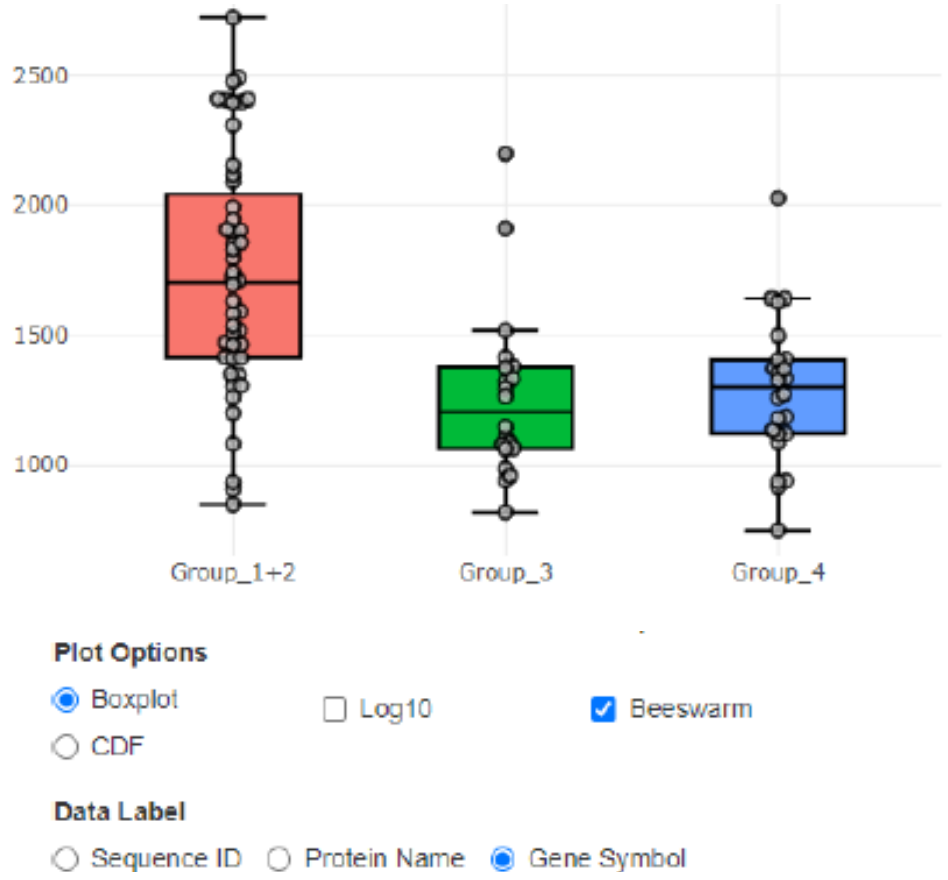
- ANOVA and Kruskalis Wallis (KW) tests were conducted comparing Group 1, 3 and 4 with
  - p-values adjusted using Benjamini-Hochberg false discovery rate (FDR) and
  - Bonferroni statistical tests, was used to identify proteins of interest,
  - using Somalogic's ® Dataviz software
- Plasma proteins with Bonferroni of <0.05 were identified
- Plasma proteins with a false discovery rate FDR of (<0.02) were identified.
- We computed the median % change in Group 1 versus convalescent and healthy control patients for the proteins of interest.
- These proteins were inserted into Genetrail3 to identify linked pathways

Results - Number of hits with ANOVA and KW

		1v 3 v4
ANOVA	Bonferroni < 0.05	31
	FDR< 0.0005	2
	FDR <0.01	149
	FDR <0.02	314
	FDR <0.05	778
	FDR < 0.075	1128
Kruskalis Wallis		
	Bonferroni < 0.05	9
	FDR< 0.0005	0
	FDR <0.01	118
	FDR <0.02	284
	FDR <0.05	905
	FDR < 0.075	1411

Target and Pathway Analysis was done on the ANOVA Bonferroni 31 markers plus 2 of 9 markers unique to KW

A marker of peripheral nerve damage in Autoimmune disease



- One of the 33 targets “G” identified was a marker of peripheral nerve damage in an autoimmune disease
- The Median levels of G was significantly elevated 29% in the Neuro PASC patients group 1+2, vs G3, G4
- Modulation of this target suggests there is peripheral neurological damage in these NP patients

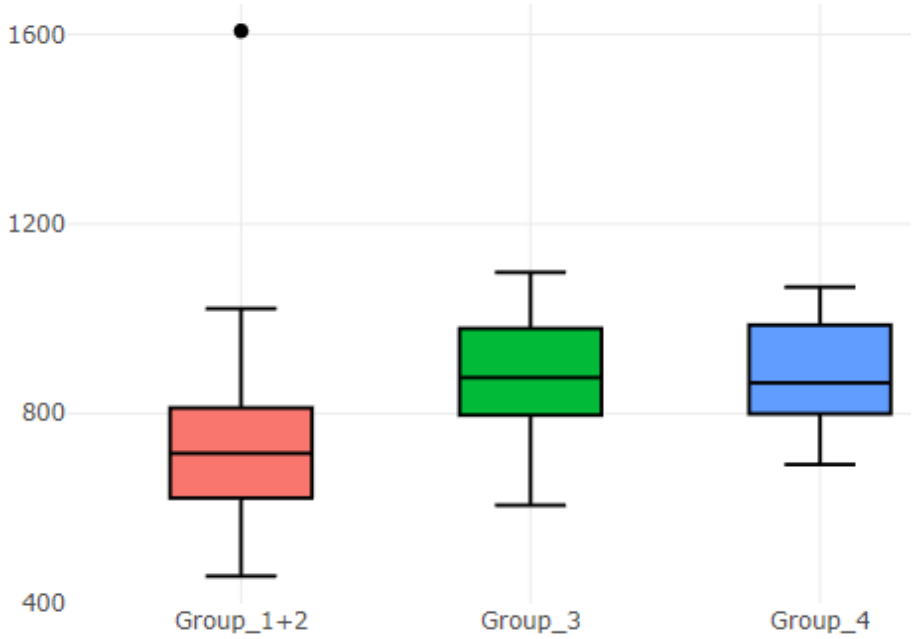
Results-ANOVA Bonferonni Pathway Analysis

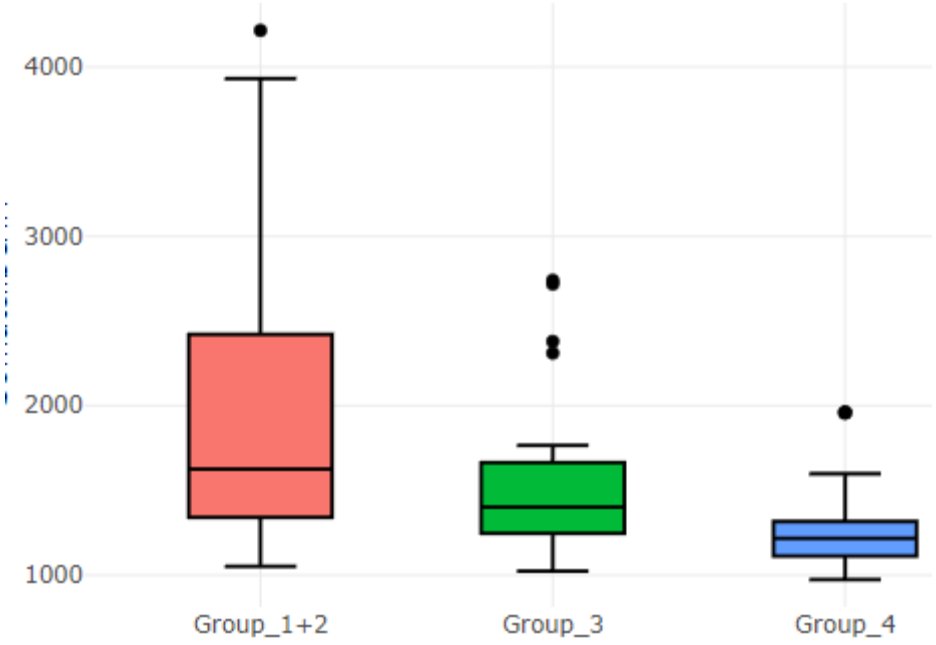
- Pathways were identified in inflammation and apoptosis

Pathway Analysis (Column K)					
WikiPathways (4/4)	Inflammatory Response Pathway (IFR)	Apoptosis/ Apoptosis Modulation and Signaling (APO)	Signaling Pathway		
KEGG (5/9) HITS)	C	Apoptosis	Signaling pathways	Signaling Pathway	C
Reactome (2/2)	C	Receptor binding			

(C) Pathway details not provided to maintain confidentiality

Results- Kruskalis Wallis Bonferroni < 0.05





- The 2nd target marker elevated, based on published data on the protein, we theorize indicates the body's attempt to repair neurological damage in NPASC

Results-ANOVA & KW FDR &Pathway Analysis

		1v 3 v4
ANOVA	*Bonferroni < 0.052	31
	FDR< 0.0005	2
	FDR <0.01	149
	FDR <0.02	314
	FDR <0.05	778
	FDR < 0.075	1128
Kruskalis Wallis		
	*Bonferroni < 0.052	9
	FDR< 0.0005	0
	FDR <0.01	118
	FDR <0.02	284
	FDR <0.05	905
	FDR < 0.075	1411

- Pathway analysis of those markers with an FDR < 0.02 identified pathways involving
  - Platelets
  - Inflammation and Fibrosis
  - Integrins
  - Antigen presentation
  - Viral pathways

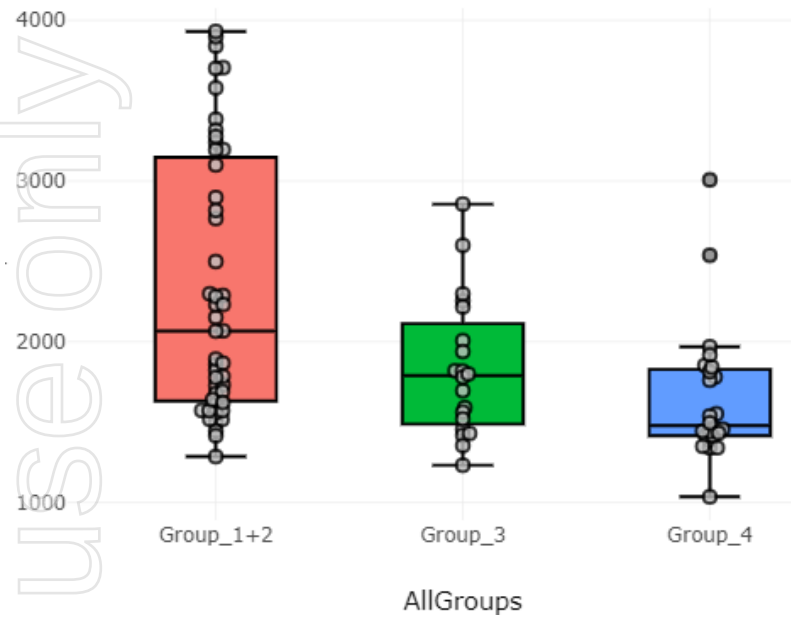
- FDR < 0.02 signifies a false discovery rate of less than 1 in 50

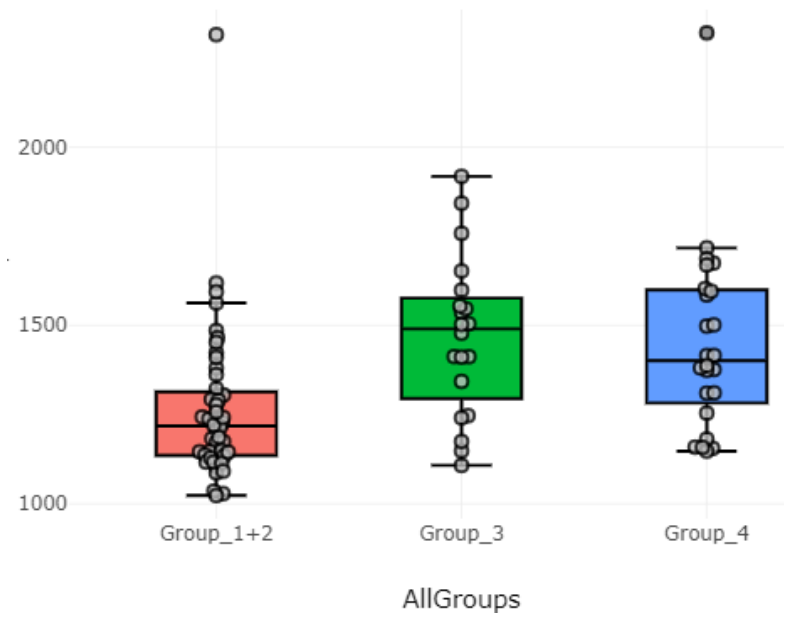
Results - Targets with approved drugs- therapy (ANOVA FDR <0.02)

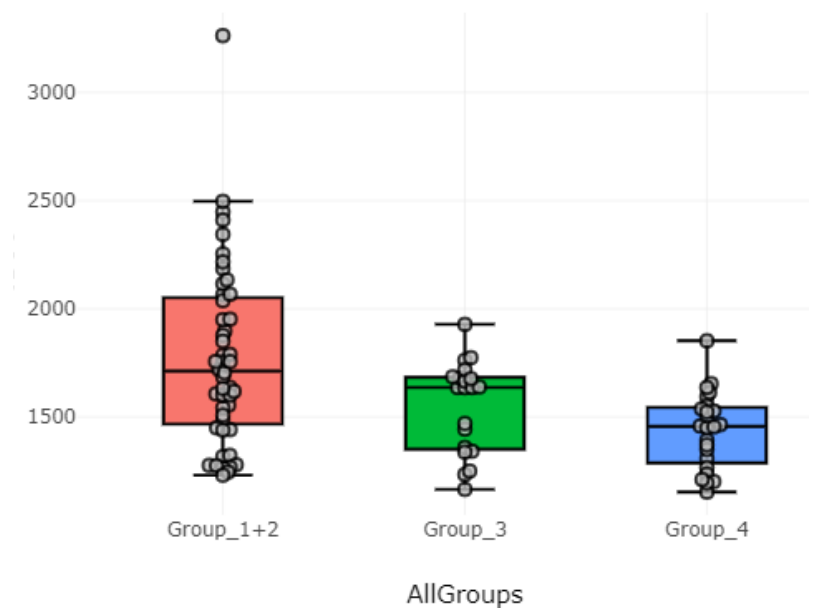
Target	FDR	% change NP
1.	0.007	+28%
2.	0.0071	-22%
3.	0.01	+15%
4.	0.011	+21%
5.	0.011	-25%
6.	0.011	+18%
7.	0.0088	-29%
8.	0.014	+29%
9.	0.015	-34%
10.	0.017	-48%
11.	0.018	-15%
12.	0.01	+22%

- The 314 targets in the ANOVA FDR < 0.02 were looked for in a database of targets to which there are drugs approved
- 12 targets were identified as potentially amenable to treatment by currently available drugs or therapies on the market
- This table shows ANOVA FDR levels & the % change in the median of 12 targets vs convalescent and healthy control
- One of the targets is also known as having the potential to be significantly modulated by ATL1102 in DMD patients and therefore suggestive of its therapeutic potential in the Long COVID-19

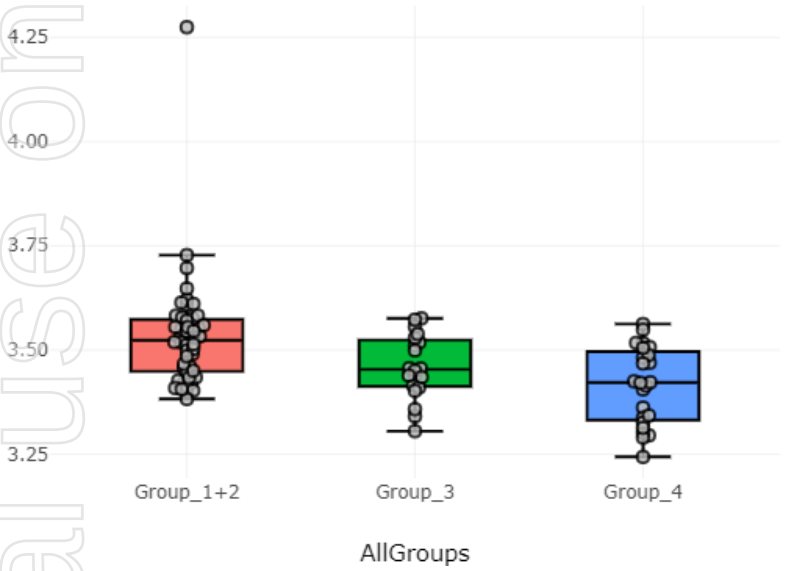
Box Plots of Targets 1-3 of 12 with approved drugs- therapy

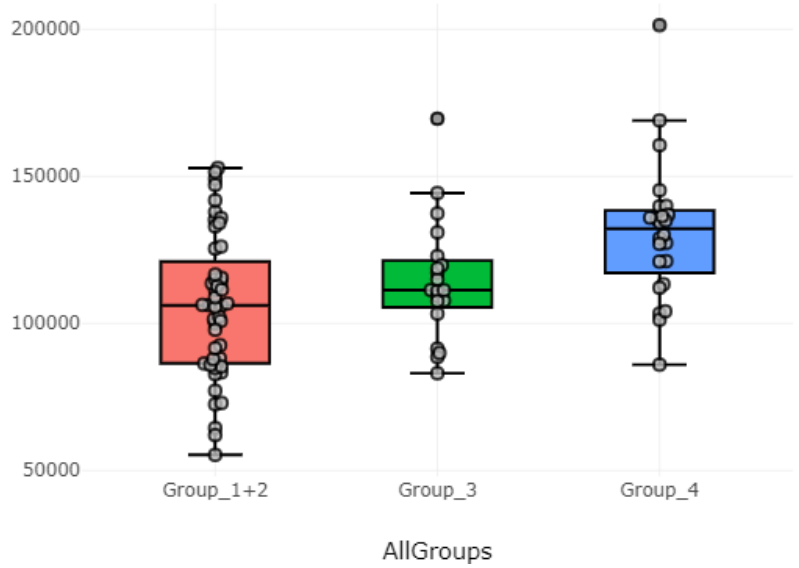


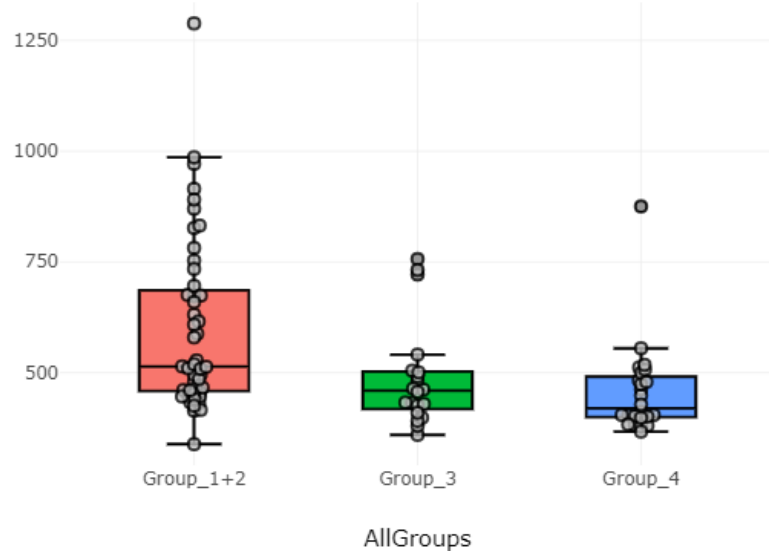




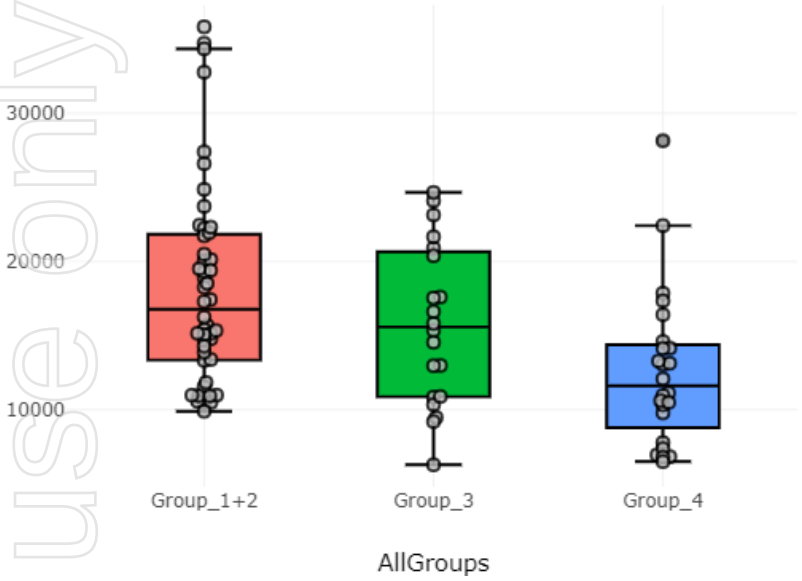
Box Plots of Targets 4-6 of 12 with approved drugs- therapy

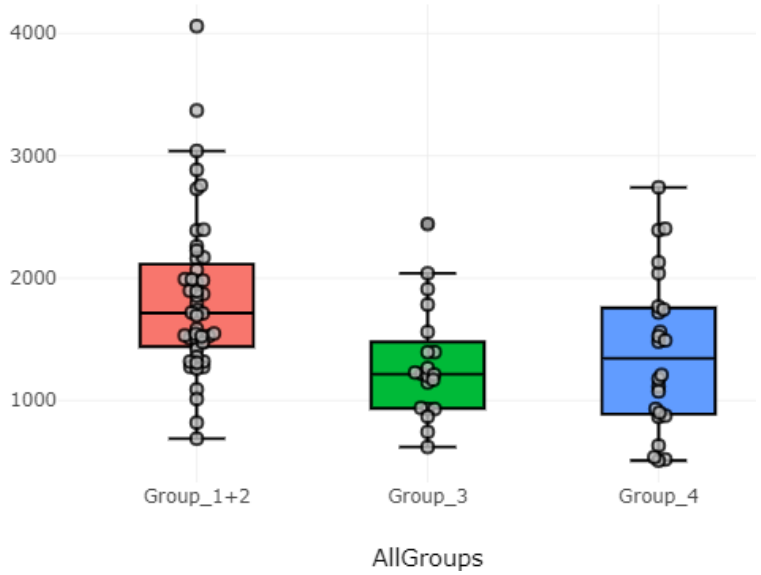


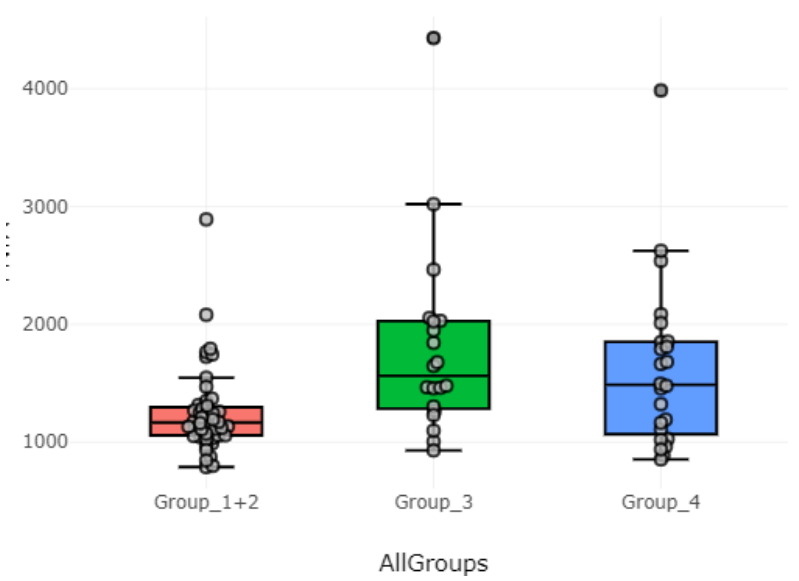




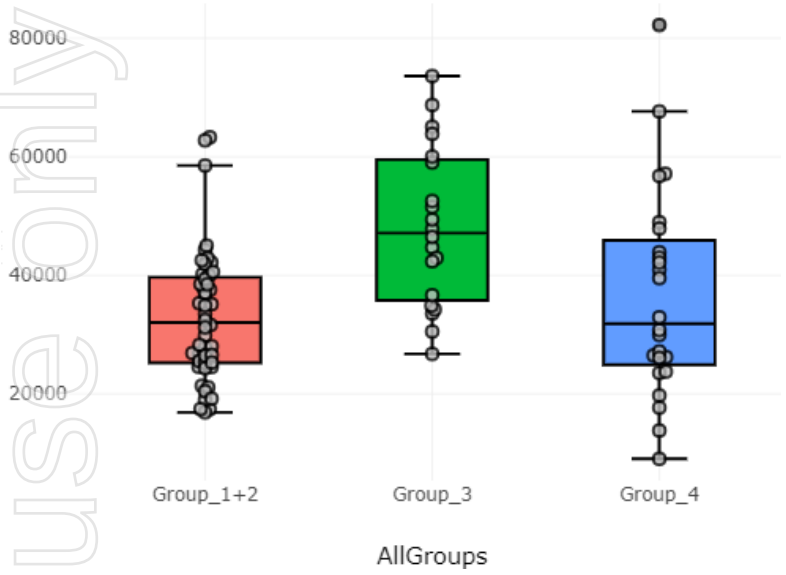
Box Plots of Targets 7-9 of 12 with approved drugs- therapy

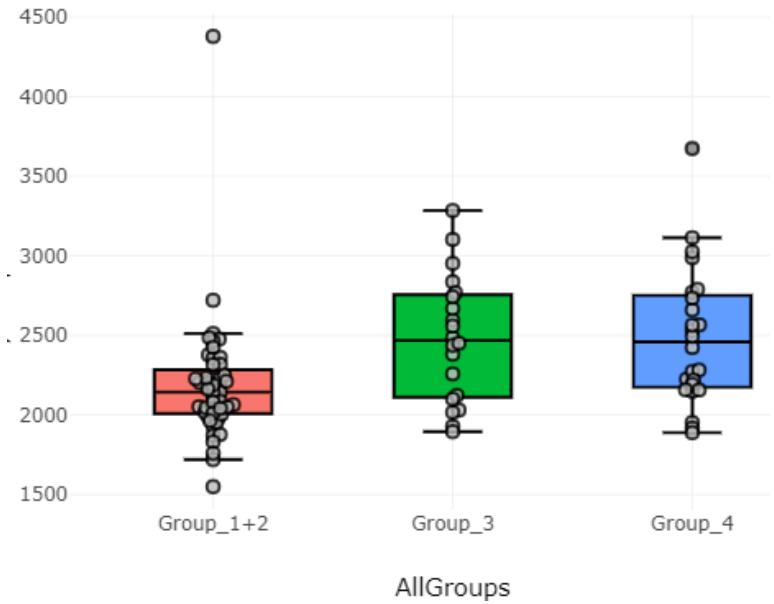


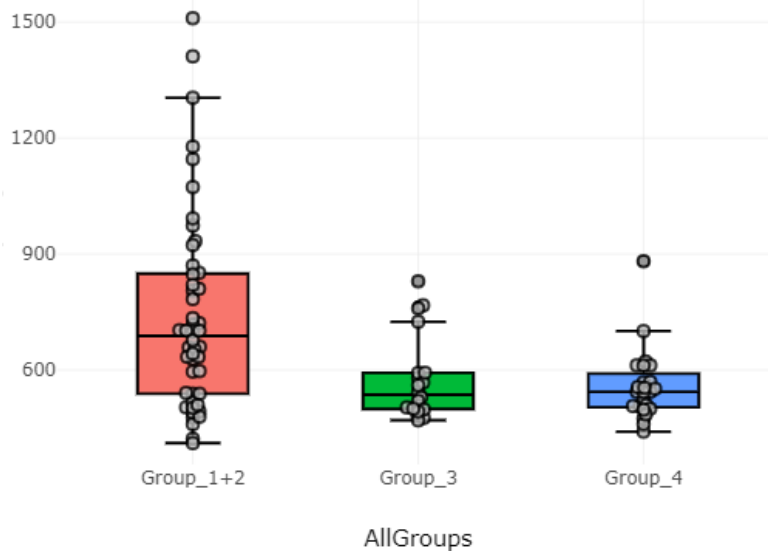




Box Plots of Targets 10-12 of 12 with approved drugs- therapy



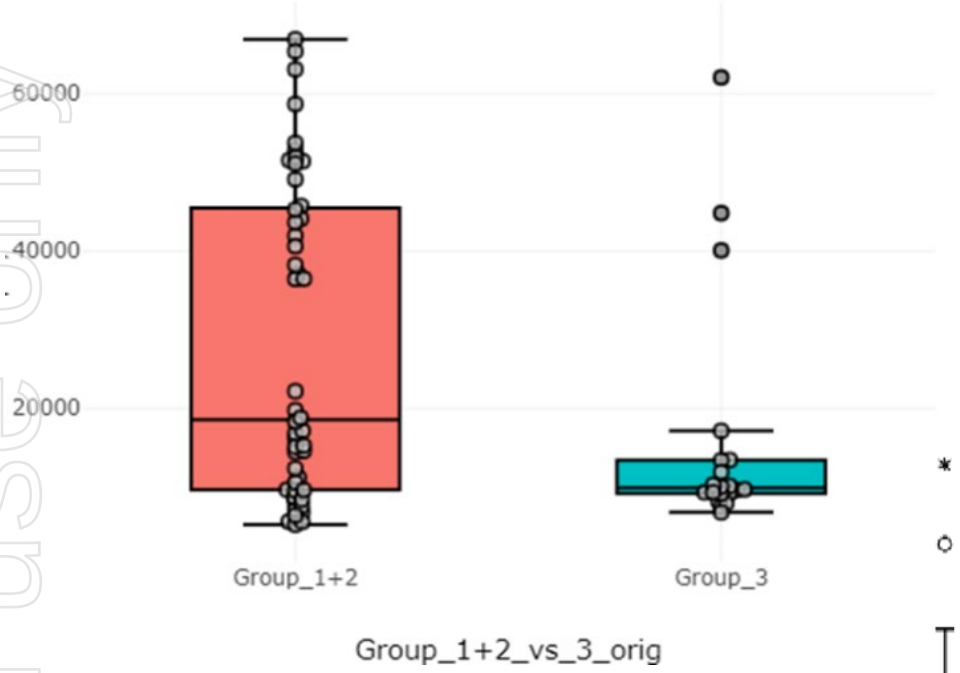




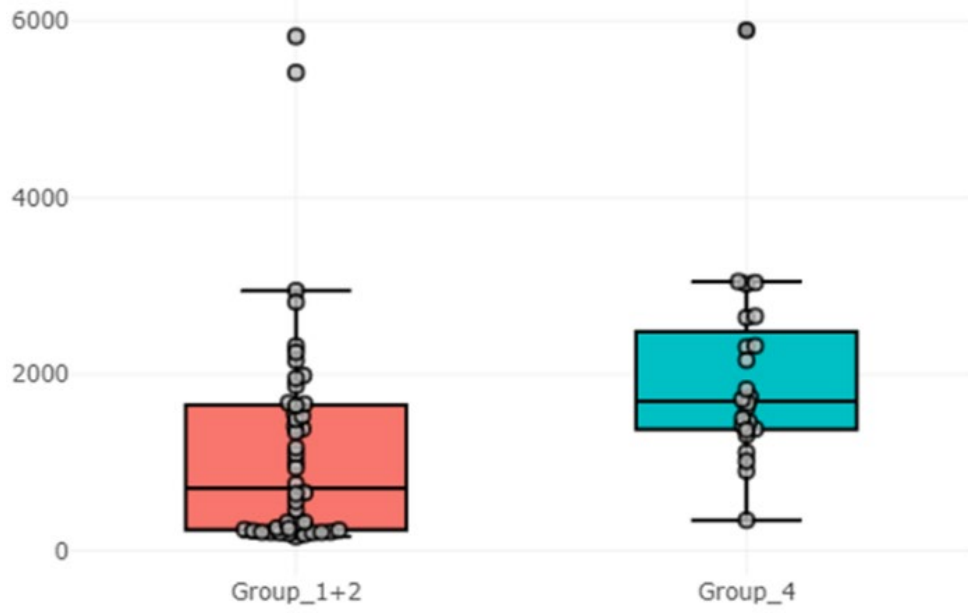
Potential Diagnostic Markers –Combination of 3-5 markers

- A combination of 3 markers identifies all 48/48 NPASC subjects
  - i.e providing high sensitivity for detection of subjects with NPASC
- The combination of 3 markers plus another 2 markers differentiates 42/44 subjects without NPASC
  - i.e providing relatively high specificity differentiating those subjects who are convalescent or healthy controls from NPASC subjects
- Further analysis and validation of these markers and other markers is to be conducted

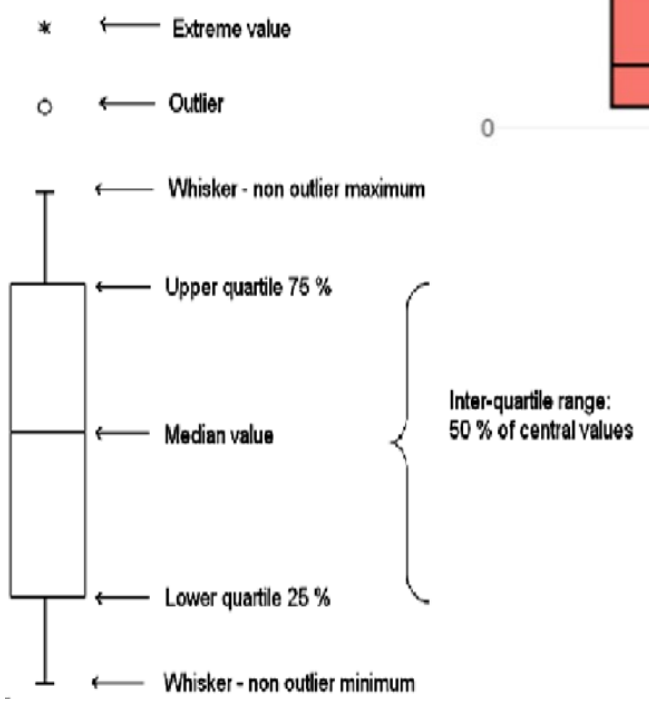
Potential Diagnostic Markers – Combination of 1 and 2



Group_1+2_vs_3_orig

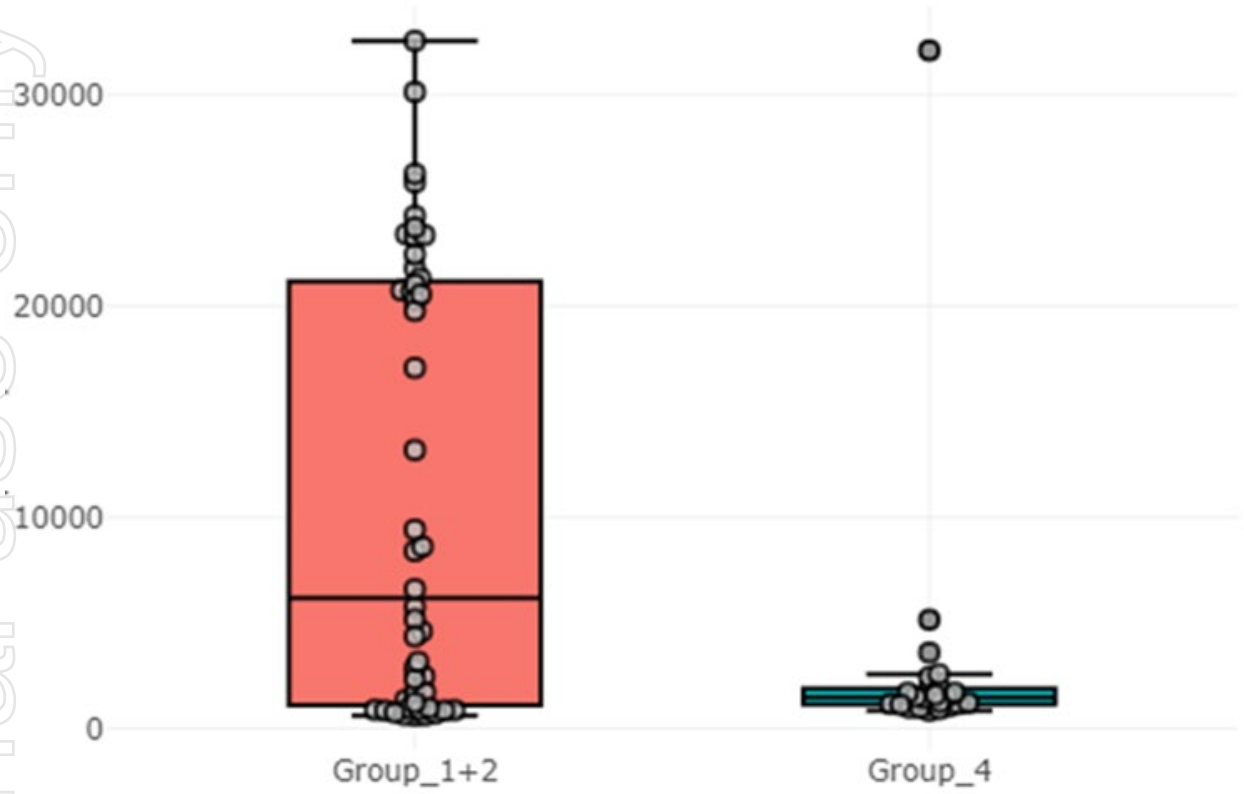


Group_1+2_vs_4

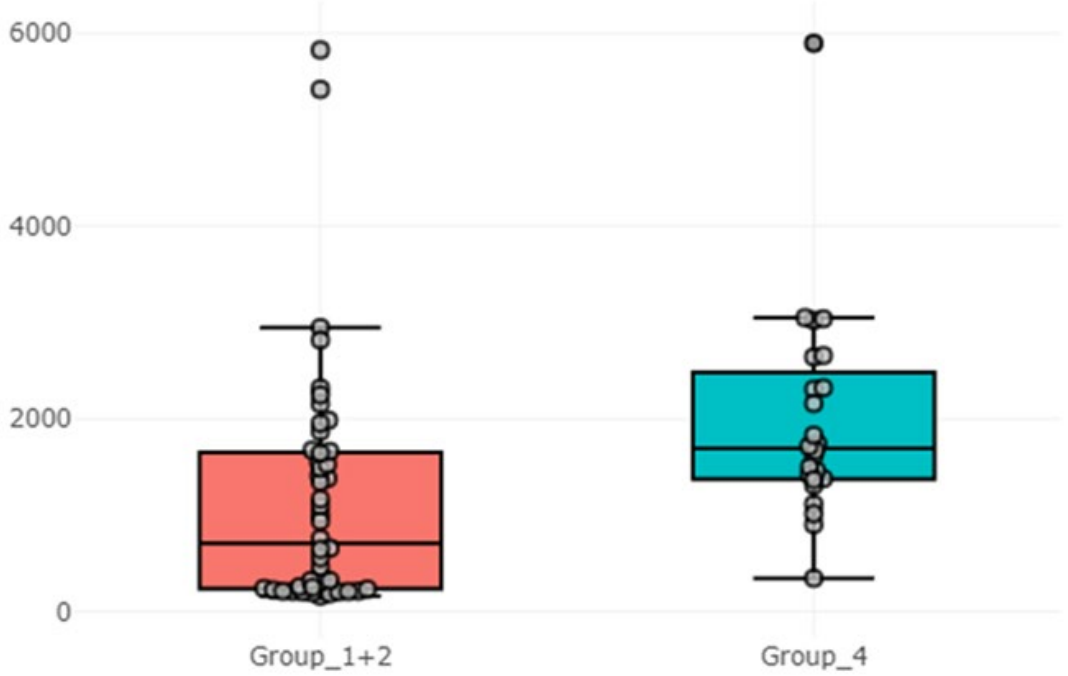


The upper whisker interquartile range, lower whisker, outliers and extreme values for each of the 48 subjects in G1+2, 20 subjects in G2, and 24 subjects in G4 are shown

Potential Diagnostic Markers - Combination of 3 and 2

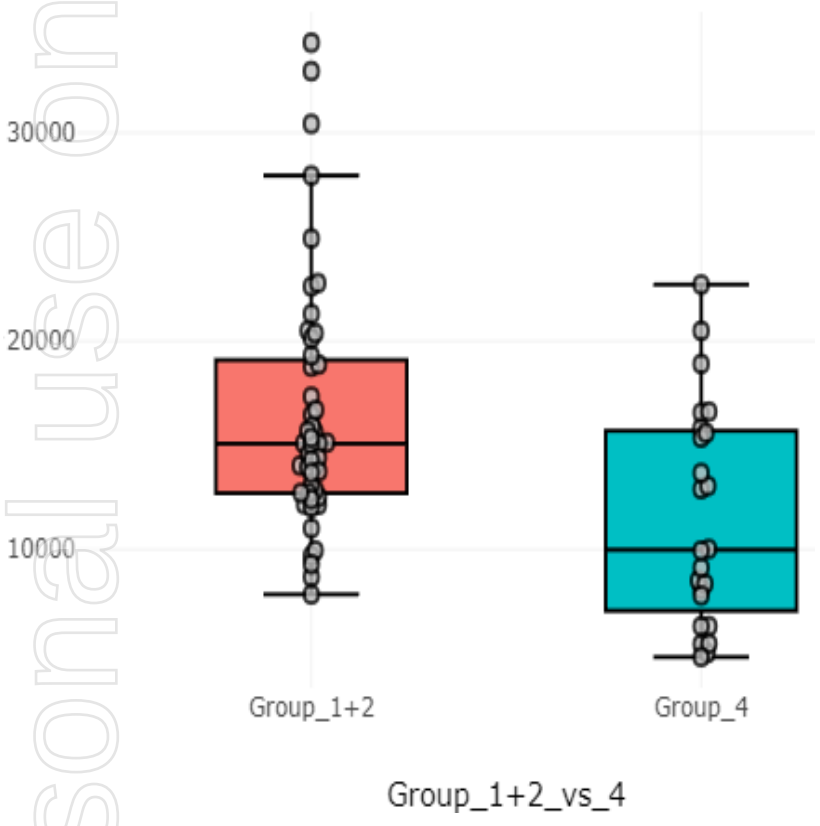


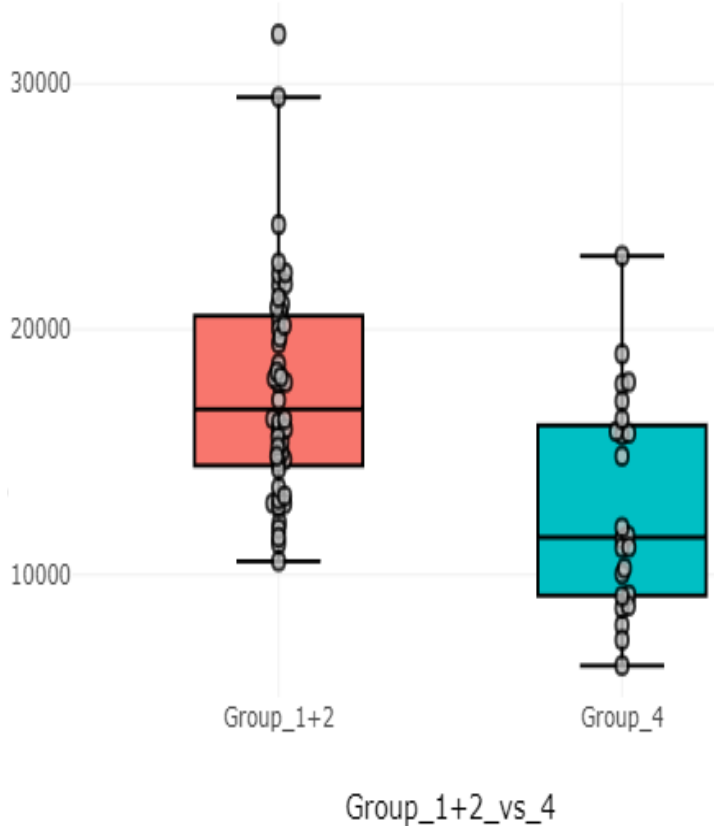
Group_1+2_vs_4

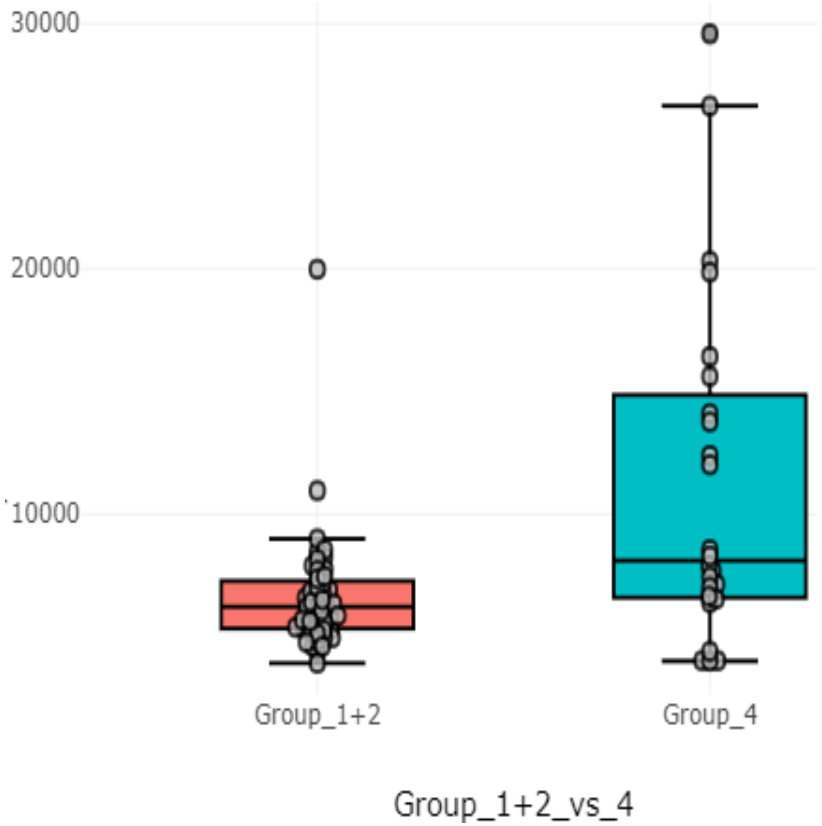


Group_1+2_vs_4

Potential – Backup Diagnostic Markers differentiate HC/CC







3 US Provisional patent applications filed

- Diagnostics provisional patent application titled “Biomarkers and Uses thereof”
- Therapeutic provisional patent application titled “ Methods for treating neurological post-acute sequelae of COVID-19 (NPASC)”
- Diagnostic and Therapeutics provisional patent application titled“ Methods for diagnosing and treating neurological post-acute sequelae of COVID-19 (NPASC)”

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&

2. Department of Microbiology-Immunology, Feinberg School of Medicine, Northwestern University, Chicago IL 60611 USAR Kennedy

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SOMALOGIC® FOR SOMASCAN® ASSAY

Statistician

Robert Kirk DeLisle

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