BLOOD BIOMARKERS FOR PREDICTION OF PARKINSON'S DISEASE IN *GBA1* POSITIVE INDIVIDUALS

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BACKGROUND

Mutations in the glucocerebrosidase gene (*GBA1*), which encodes the enzyme glucocerebrosidase (GCase), constitute the most significant genetic risk factors for Parkinson's Disease (PD).

AIMS

To quantify the GCase enzymatic activity as well as aggregated (**Asyn$_a$**) and monomeric (**Asyn$_m$**) asynuclein levels in peripheral blood mononuclear cells (PBMCs) from PD patients with and without *GBA1* mutations (GBA-PD and nonGBA-PD), non-manifesting GBA1 carriers (GBA-nonPD) and healthy controls (HC).

METHODS

GCase activity was measured fluorometrically, while **Asyn$_m$** and **Asyn$_a$** were measured by ELISA assay in **PBMCs**.

STATISTICAL ANALYSIS:

- **ROC curve analysis** assessed the accuracy (AUC) of each biomarker and their respective ratios.
- **Logistic regression** evaluated the blood biomarkers association with PD status.
- **Predicted probabilities** assessed the distribution of GBA-nonPD individuals relative to healthy controls. Data were analyzed in relation to the best threshold calculated comparing HC and PD groups (Youden index); the binary outcome variable was defined as PD = 1 and non-PD (including HC and GBA-nonPD) = 0.

RESULTS

BIOCHEMICAL PARAMETERS OF THE STUDY COHORT

➢ GCase activity was lower in *GBA1* positive individuals. Asyn$_a$ was higher in both GBA-PD and nonGBA-PD than HC.

	HC (n=56)	GBA-nonPD (n=8)	GBA-PD (n=50)	nonGBA-PD (n=72)	p-value (post-hoc)
Sex (M:F)	21; 35	6; 2	28; 22	49; 23	**0.004**
Age (yrs)	59.6 ± 11.2	59.8 ± 14.6	59 ± 9.6	66.3 ± 8.7	**<0.001** (nonGBA-PD>HC,GBA-PD)
GCase activity (nmol/h/mg)	8.1 ± 2.01	5.7 ± 2.6	6.1 ± 1.6	9.05 ± 2.3	**<0.001** (HC>GBA-nonPD, GBA-PD); (nonGBA-PD>GBAnon-PD, GBA-PD)
Asyn$_m$ (ng/mg)	356.4 ± 140.5	326.9 ± 73.6	347.8 ± 124.0	339.2 ± 134.9	ns
Asyn$_a$ (ng/mg)	1.1 ± 0.7	1.8 ± 0.8	1.8 ± 0.9	2.2 ± 1.1	**<0.001** (HC<GBA-PD, nonGBA-PD)

Table 1. Demographic and biochemical parameters of the study cohort. *Data are presented as mean ± SD. Group comparison performed with Kruskal-Wallis; Chi-square test was used for gender variable.*

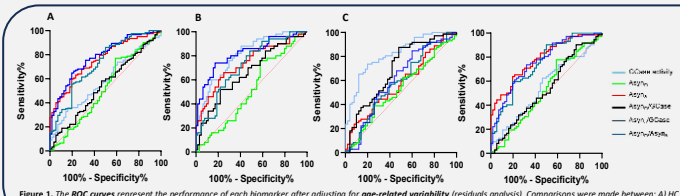

Figure 1. *The **ROC curves** represent the performance of each biomarker after adjusting for **age-related variability** (residuals analysis). Comparisons were made between: A) HC and the total PD cohort(PDtot), including iPD and GBA-PD; B) HC and GBA-PD; C) GBA-PD and noGBA-PD; D) HC vs nonGBA-PD.*

	HC vs PD tot	HC vs GBA-PD	GBA-PD vs nonGBA-PD	HC vs nonGBA-PD
GCase activity	0.6	**0.8**	**0.8**	0.6
Asyn$_m$	0.5	0.5	0.5	0.5
Asyn$_a$	**0.8**	**0.7**	0.6	**0.8**
Asyn$_m$/GCase	0.5	**0.7**	**0.7**	0.5
Asyn$_a$/GCase	**0.8**	**0.8**	0.6	**0.8**
Asyn$_m$/Asyn$_a$	**0.7**	**0.7**	0.6	**0.7**

Table 4. *Logistic regression results. Biomarkers with statistically significant association with PD status highlighted in bold; non-PD: including HC and GBA-nonPD.*

➢ **Asyn$_a$**, **Asyn$_a$/GCase** were the **most accurate parameters** for distinguishing **HC** from **PD tot** as well as **nonGBA-PD** (AUC=0.80).

➢ In the **GBA-PD** group, **GCase activity** and **Asyn$_a$/GCase** ratio showed the highest discriminatory power (AUC=0.80).

DISTRIBUTION OF PD STATUS PROBABILITIES ACROSS GROUPS

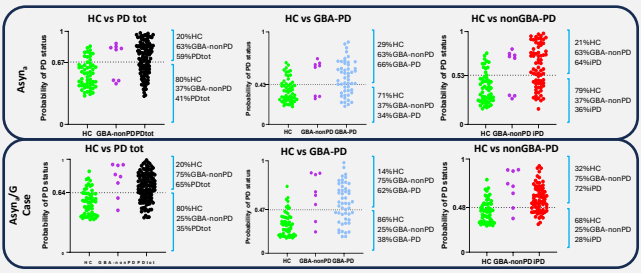

Figure 2. *The graphs show the **distribution of probabilities for each group** to belong to the **PD category**, according to different biochemical association levels (below threshold = low association, above threshold = high association). Probability values were derived using **logistic regression**, where the binary variable (**Healthy = 0, PD = 1**) was modeled based on the biomarkers.*

➢ **Asyn$_a$** (p< 0.001) and **Asyn$_a$/GCase** ratio (p<0.001) were significantly associated with PD status.

➢ Among **GBA-nonPD** individuals, five subjects (63%) based on **Asyn$_a$** and six individuals (75%) for **Asyn$_a$/GCase** were consistently identified as highly associated with PD status.

	p-value nonPD vs PD tot	p-value nonPD vs GBA-PD	p-value nonPD vs nonGBA-PD
Gcase activity	n.s.	**<0.001**	**0.04**
Asyn$_m$	n.s.	n.s.	n.s.
Asyn$_a$	**<0.001**	**<0.001**	**<0.001**
Asyn$_m$/Gcase	n.s.	**0.02**	n.s.
Asyn$_a$/Gcase	**<0.001**	**<0.001**	**<0.001**
Asyn$_m$/Asyn$_a$	**<0.001**	**0.006**	**<0.001**

Table 4. *Logistic regression results. Biomarkers with statistically significant association with PD status highlighted in bold; non-PD: including HC and GBA-nonPD.*

CONCLUSIONS

These findings support the potential of **Asyn$_a$** and **Asyn$_a$/GCase** ratio as promising biomarkers to discriminate the PD population from HC and to identify individuals potentially at risk of PD. This approach may represent an important step towards the validation of PD peripheral biomarkers in a clinical perspective.