Transforming growth factor β-induced protein ig-h3 as potential novel plasma biomarker of endometriosis

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INTRODUCTION

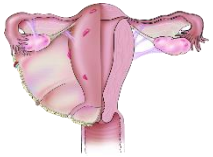

Figure 1. Schematic drawing of areas often involved in endometriosis. Alimi et al. 2018

Endometriosis is a common **female gynaecological disorder** that **affects around 190 million women worldwide**. Since symptoms of endometriosis are highly heterogeneous, patients are often misdiagnosed. **Earlier diagnosis and treatment of patients could be achieved with discovery of non-invasive diagnostic biomarkers for endometriosis.**

AIM OF THE STUDY is to **identify novel biomarker candidates for endometriosis using antibody microarray platform and ELISA assays.**

PATIENTS AND METHODS

Patients with primary infertility were included in the study and were characterized as **controls (absence of endometriosis) or cases (presence of endometriosis)** after laparoscopy surgery and histological analysis. **Peritoneal fluid (PF) and plasma samples** of the selected patients were collected following a strict SOP and used for further analysis. The study was divided into two phases: discovery and validation phase. In discovery phase, the **PF samples** of 12 patients **were analysed** in a dual-colour approach on eight **scioDiscover antibody microarrays** (Sciomics®) targeting **1360 different proteins.** Three differential proteins **(COMP, TGFBI and AGT)** were selected for validation on PF samples while two **(COMP, TGFBI)** were further validated on plasma samples.

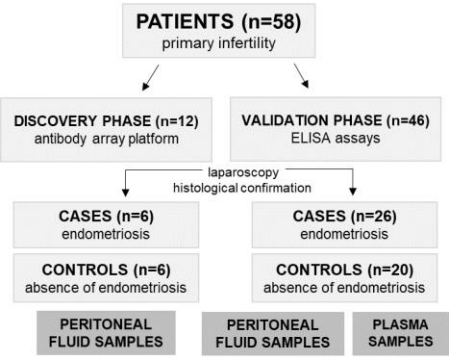

Figure 2. Study design scheme.

Validation phase was conducted on 46 patients (n=20 controls, n=26 cases) using **commercially available ELISA assays. The ROC curve and AUC calculation** were based on the test sample predictions of each respective split tested with linear support vector machine **(SVM) classification model .**

RESULTS

Antibody microarrays identified **16 proteins** that had significantly higher levels **in the PF samples of cases *versus* controls** (Figure 3.) The validation on PF samples using **ELISA assays confirmed these results for three of the proteins (COMP, TGFBI and AGT).** However, ROC analysis showed very **good diagnostic potential only for COMP and TGFBI (AUC=0.78 and 0.84)** (Figure 4.). **This was confirmed by additional classification model based on a linear SVM using all three proteins** (data published in V. Janša et al. Scientific Reports 2021).

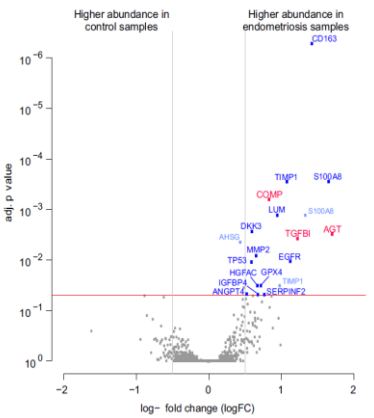

Figure 3. Volcano plot of the protein array data. Proteins with positive logFC had higher levels in the peritoneal fluid of the cases *versus* controls; and vice versa for proteins with negative logFC.

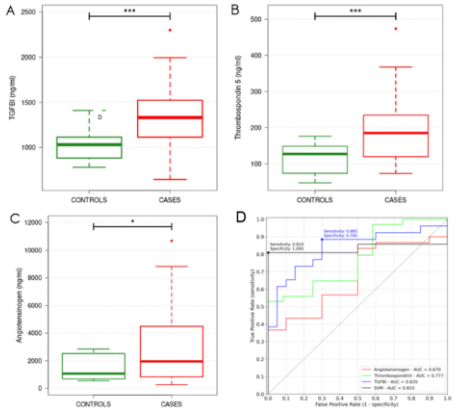

Figure 4. Validation of (A) TGFBI, (B) COMP and (C) AGT levels in peritoneal fluid of endometriosis patients and control patients. (D) ROC curves assessing the diagnostic profiles of all three proteins.

The **validation of COMP and TGFBI protein was repeated** in the same cohort of patients **using plasma samples.** Only **TGFBI was confirmed to be significantly higher (p=0.0007)**, while there were no differences in levels of COMP (p=0.8839) in plasma samples of cases *versus* controls. **ROC analysis** revealed that **TGFBI has good diagnostic characteristics (AUC=0.76)** (Figure 5.)

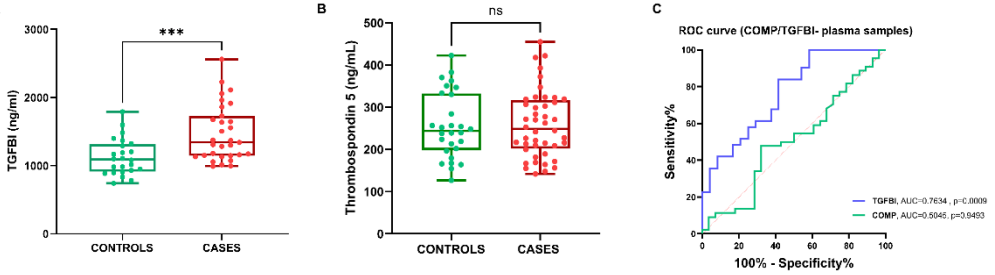

Figure 5. Validation of (A) TGFBI and (B) COMP levels in plasma samples of endometriosis patients and control patients. (C) ROC curves assessing the diagnostic profiles of two proteins.

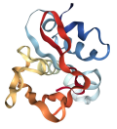

Figure 6. Structure of TGFBI protein.

CONCLUSION

This study **identified several biomarker candidates of endometriosis in PF samples** using antibody microarrays. This led to validation studies on peritoneal fluid and **plasma samples and finally revealed TGFBI as potential plasma biomarker of endometriosis.** Further validation of TGFBI in higher number of patients to confirm its diagnostic potential in clinics is currently ongoing.