

A Novel Microbe-Host Biomarker Panel for Early Detection of Ovarian Cancer in Routinely Collected Pap Test Clinical Samples

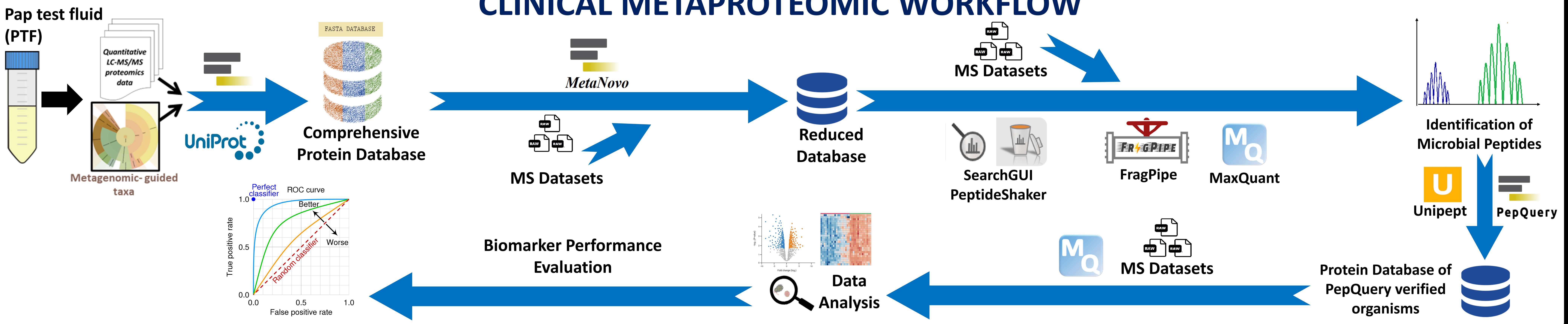
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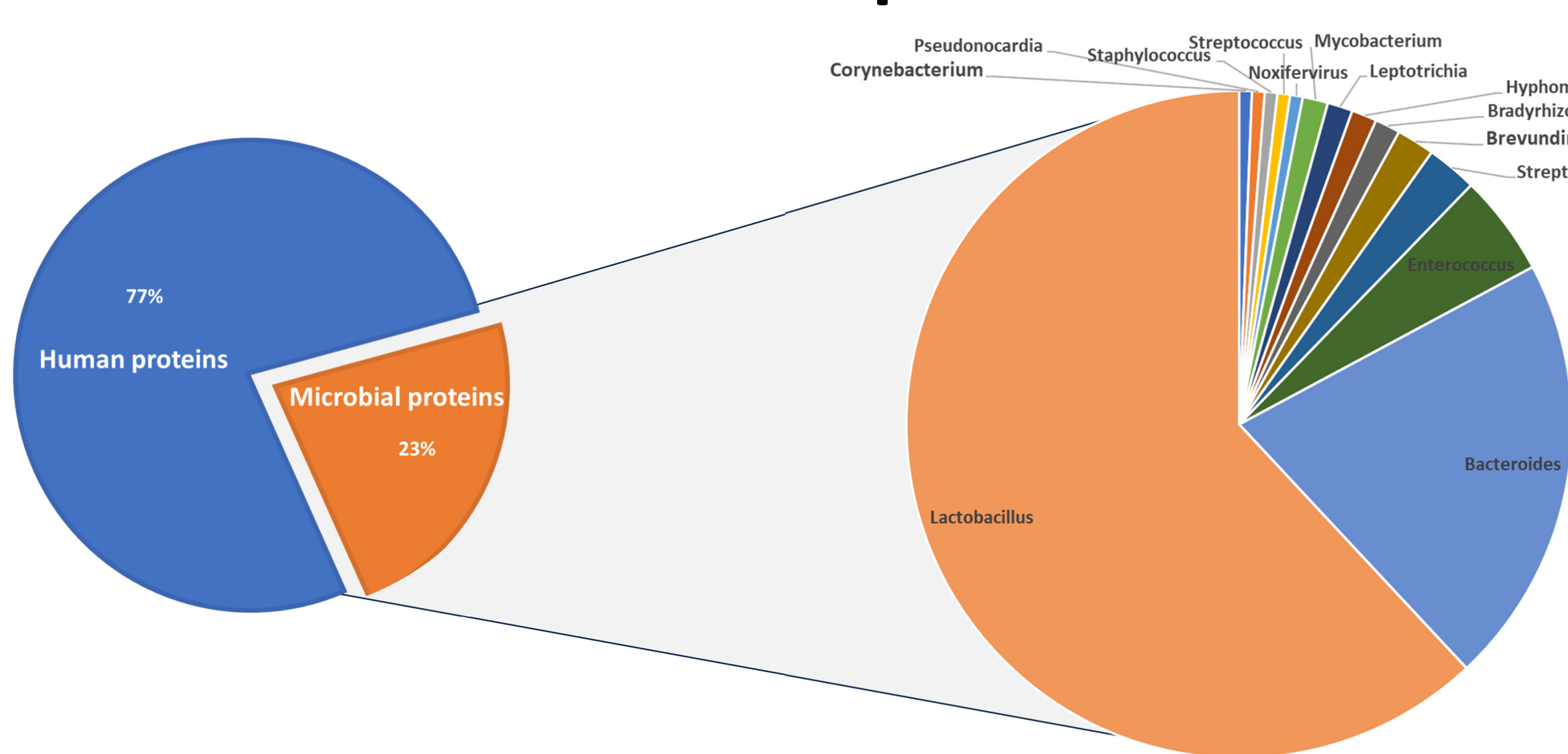
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Background. Reliable methods for early detection of ovarian cancer (OvCa) are still lacking, contributing to poor survival outcomes. Changes in the female reproductive tract microbiome, which disrupt the homeostasis between host (human) cells and bacterial microorganisms, have been associated with OvCa development. These findings have led many experts to suggest that bacteria-derived molecules, such as microbiome-encoded proteins, making up the metaproteome, may hold a key to developing more effective tools for early detection and screening of OvCa. Following this rationale, we have determined that residual fluid from liquid Papanicolaou test (Pap test fluid, PTF) samples are a rich source of microbiome and human proteins. Furthermore, a pilot study analyzing quantitative mass spectrometry (MS)-based proteomics data from PTF samples revealed that microbial and human protein signatures differ between women with the most commonly diagnosed OvCa subtype (high-grade serous ovarian cancer, HGSOC), and those women without cancer. A combined microbe and human peptide panel from this pilot study shows the potential for improved discrimination between OvCa and non-OvCa PTF samples which could transform early detection.

CLINICAL METAPROTEOMIC WORKFLOW

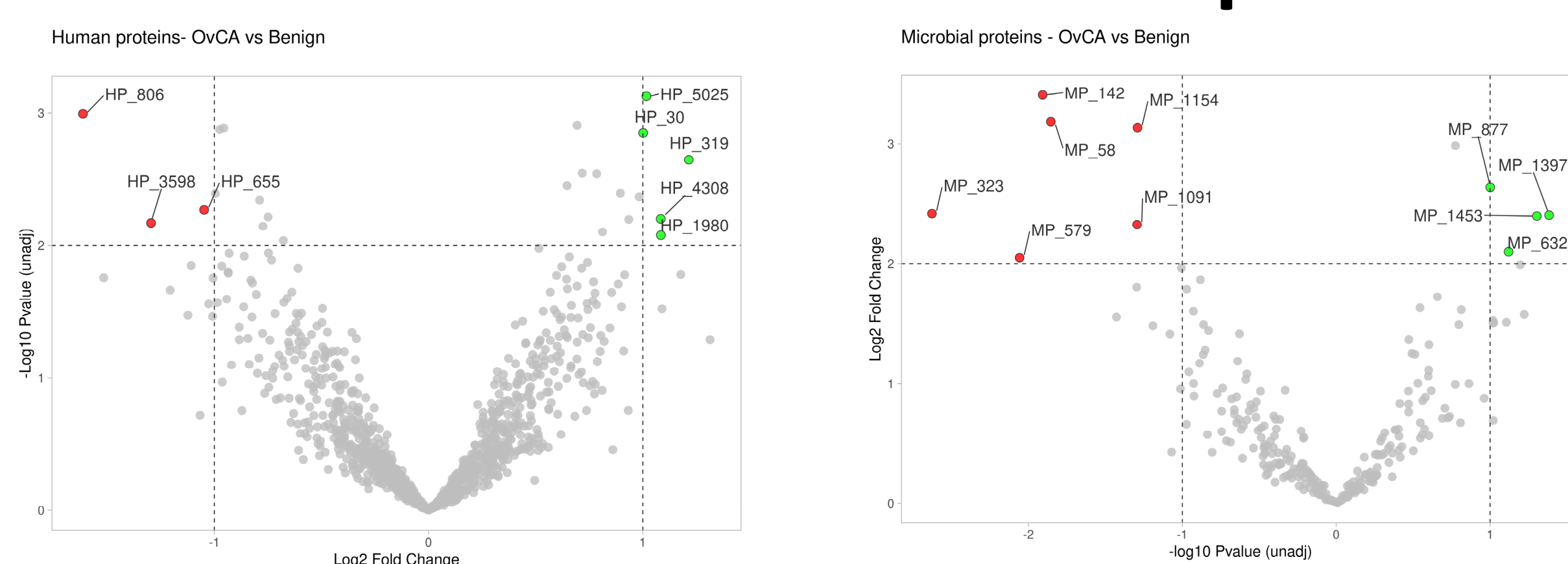


A) PTF samples are a rich source of microbial and human proteins



Analysis of 40 PTF samples from OvCa, benign conditions and healthy women detects many microbial proteins albeit at a smaller proportion to the human host. Microbial proteins map to many known bacteria of the female reproductive tract.

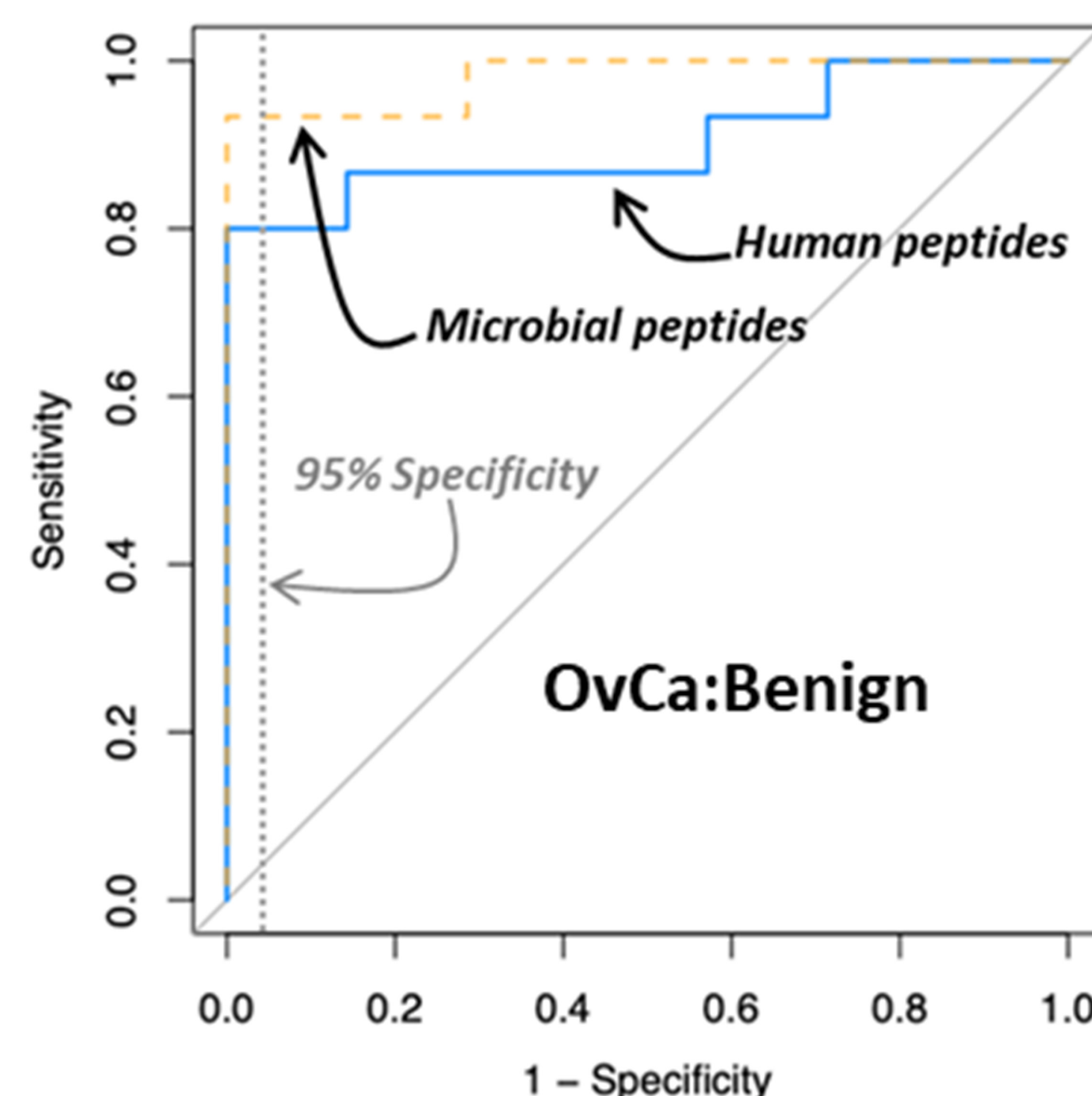
B) PTF samples contain differentially abundant microbial and human proteins



Abundance ratios of human and microbial proteins compared between PTF samples from women with benign gynecological conditions and OvCa.

RESULTS

C) Microbial proteins offer improved detection of OvCa compared to human proteins alone

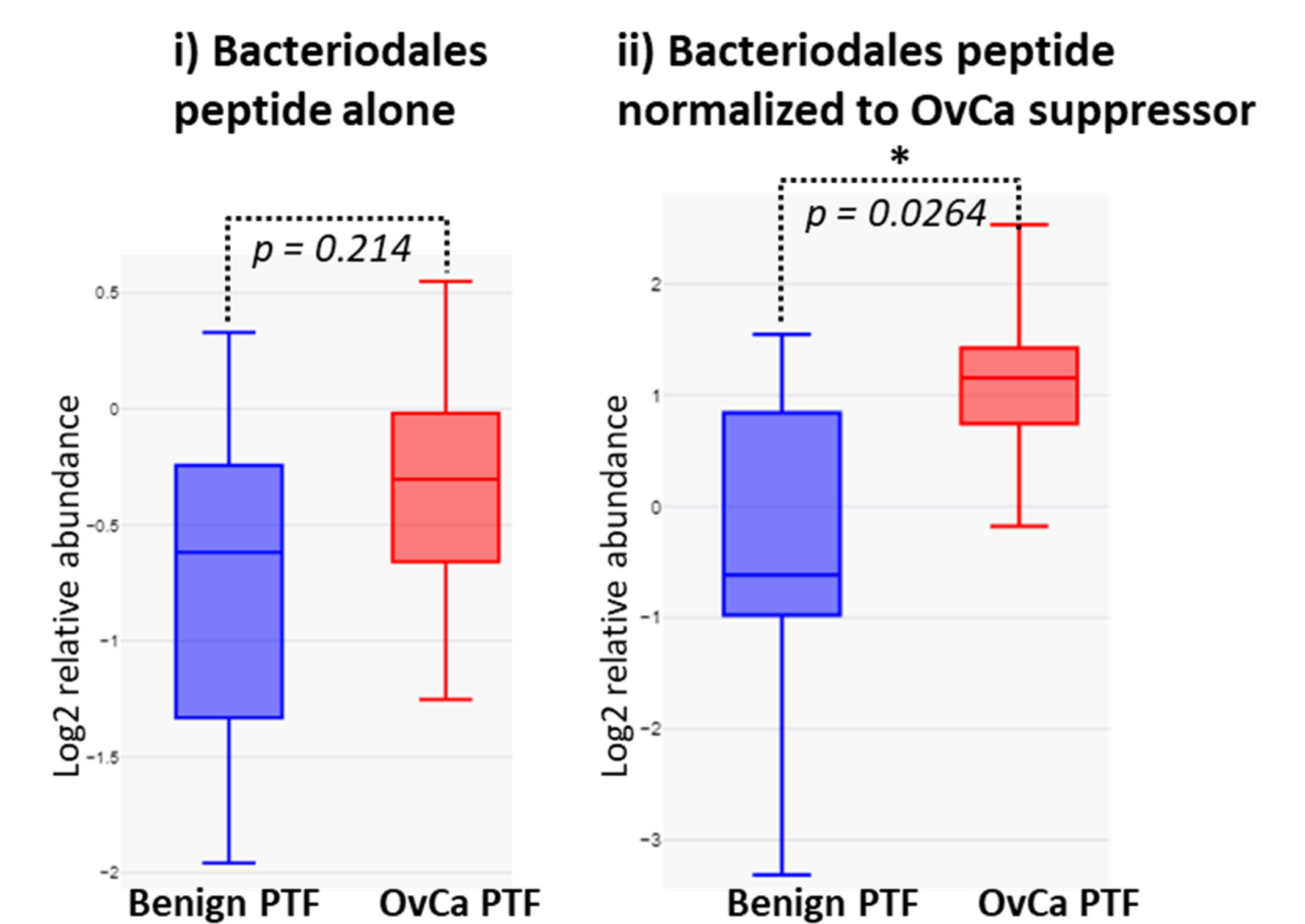


The receiver operator characteristic (ROC) curve shows that a panel of differentially abundant peptides from microbial proteins outperforms a panel of human peptides.

ACKNOWLEDGMENTS

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D) Combining microbial and human peptide markers can improve OvCa detection



A peptide from the order Bacteriales with non-statistically significant differential abundance between benign and OvCa samples (**panel i**) shows improved performance when normalized to a human OvCa suppressor protein (**panel ii**).

CONCLUSIONS AND FUTURE DIRECTIONS

- PTF samples are a rich source of microbial and human proteins that show differential abundance between OvCa and non-OvCa samples.
- Peptides from microbial proteins show promise as OvCa biomarkers alone, with potentially improved performance when combined with human proteins associated with OvCa.
- Next steps include *verification* in a second, expanded quantitative proteomics dataset from PTF samples, followed by *validation* of high-performing biomarker candidates using targeted MS-based assays.