Multiplex Immunoassay for Assessing Risk Factors of Gastric Cancer

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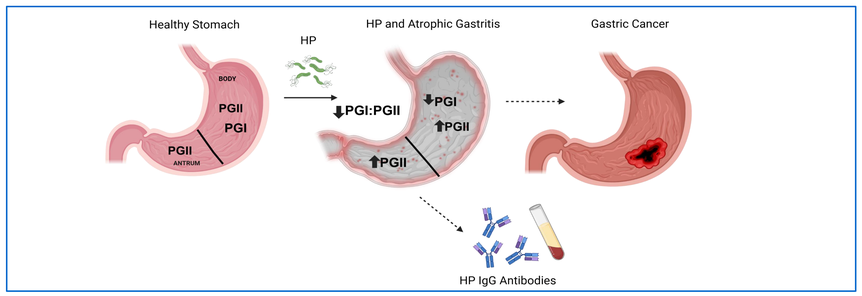
Background:

- Gastric Cancer (GC) is the 5th most common cancer worldwide. Incidence varies by region, being most common in E. Asia, followed by E. Europe and S./Central America, and migrants from those regions. Additional known risk factors include sex, age, smoking, alcohol, diet, and most importantly, history of Helicobacter pylori (HP) infection.
- While HP increases the risk for GC by 3-fold, not all HP strains have the same carcinogenic properties. CagA- and VacA-positive strains were reported to increase the risk particularly in Whites, while OMP- and/or HP0305-positive appear more informative in E. Asians. Some studies have shown potential utility of pepsinogen I/II ratio in screening gastric cancer and precancerous lesions. [2]



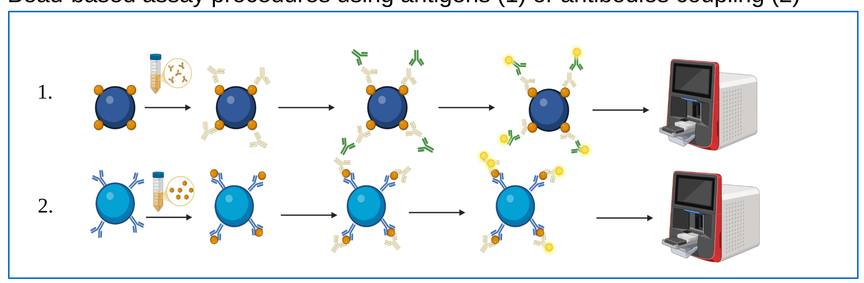
Aims:

- To develop a custom multiplex bead-based assay to test (Aim 1) serum antibodies of anti-HP strains HP0305, CagA, OMP, and VacA; (Aim 2) levels of PGI and PGII; and (Aim 3) determine past HP exposure and PGI/PGII ratios in N=275 plasma samples for an ongoing epidemiologic study of GC.

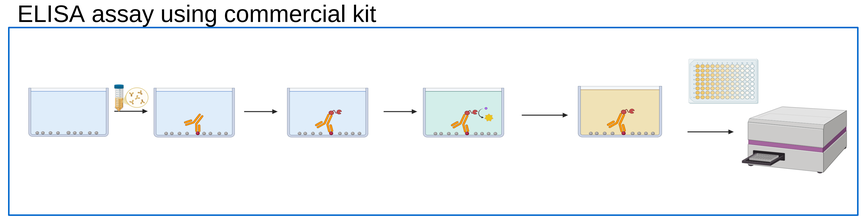
Methods:

- **Bead-based assays development:** Peptides (HP) or antibodies (PGI/II) for coating the beads were chosen based on antigenicity regions and specificity, respectively. Titrations were performed to determine optimal serum dilution and concentrations of peptides/antibodies. Specificity of reagents was tested by coating beads with antigens for PGI/II. Internal laboratory controls with known HP and PGI/II status, plus commercial controls were used.
- **Samples/Controls:** N=275 human plasma samples obtained through an ongoing Gastric Cancer study.
- **Kits**: GastroPanel Unified ELISA kit (BioHit Oyj), xMAP Antibody Coupling kit (Luminex)
- **Peptides & antibodies:** HP peptides: recombinant 166aa HP0305, 267 aa Cag, 226aa OMP; 209aa VacA. Coating & detection antibodies: Biotin-SP-conjugated AffiniPure Goat anti-human IgG; monoclonal and polyclonal mouse/rabbit atbs specific to PGI/II
- **Detection:** Bead-based: Fluorescent intensity (FI) was detected using Luminex xMAP Intelliflex Instrument. ELISA: Optical density (OD) was detected using SpectraMax 384+ instrument.
- **Analysis**: Custom: Median FI (MFI) ratio greater than that of the negative controls plus 3SD were deemed positive. To distinguish 'positivity' (+) from 'negativity' (-), for each bead-based HP analyte, cut-off values were calculated by multiplying 3x Standard Deviation (3SD) of the median fluorescent intensity (MFI) ratio of the negative controls. ELISA: per manufacturer's instructions, concentration (EIU) was then calculated using GraphPad Prism v10.1.2. +/- was determined using the manufacturer's recommendations.

Bead-based assay procedures using antigens (1) or antibodies coupling (2)



ELISA assay using commercial kit



Results:

HP custom 4-plex bead-based assay:

- The Tables below show MFI ratio cut-off values for each analyte and MFI ranges for samples.
  - N=210 (76.3%) of samples tested positive of at least 1 of the HP substrains.
  - Intraplate coefficient of variation for custom assay was ≤20%.

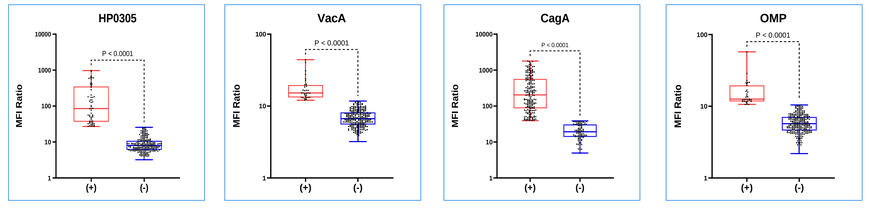
MFI cut-off values to distinguish +/- samples for each analyte

	HP0305	CagA	OMP	Partial VacA
NC MFI Avg.	3031.4	6242.1	1642.9	2403.4
Blank MFI Avg	128.2	174.1	182.2	222.7
(-) Ratio Avg.	23.65	35.85	9.02	10.79
Ratio SD	0.79	0.97	0.50	0.36
(-) Ratio+3SD	**26.0**	**38.8**	**10.5**	**11.9**

Per analyte MFI & MFI Ratios for N=275 samples diluted at 1:400 in custom 4-plex

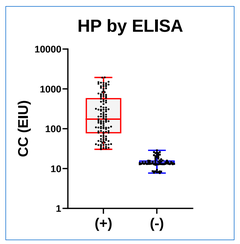
	HP0305	CagA	OMP	Partial VacA
	MFI			
Mean/Range	6432.9 (299.3 - 139697.2)	44394.4 (779.5 - 282622.4)	1080.3 (222.7 - 9467.6)	1340.1 (416.3 - 6993.8)
	MFI Ratio			
Mean/Range	52.4 (3.2 - 978.2)	66.9 (4.9 - 1777.8)	7.1 (2.2 - 57.3)	8.2(3.2 - 44.1)
Intra-plate %CV	≤20%			

Boxplots of positive/negative samples for HP0305, CagA, OMP, and VacA. Positivity determined based off the designated cut-off value



GastroPanel ELISA:

- For PGI, samples concentrations <30µg/L are indicative of atrophic gastritis per manufacturer's instructions.
  - N= 31 (11.3%) were below 30µg/L
- Concentrations >30EIU are positive for HP infection
  - N=150 (54.5%) were considered positive by kit.



Concentration (EIU) of HP tested by GastroPanel ELISA kit. N=275 samples were diluted at 1:400.

Sample values and inter/intra-plate coefficient of variation based on GastroPanel ELISA kit

	ELISA		
	PGI	**PGII**	**HP**
N samples tested	275		
	µg/mL		**EIU**
Mean	84.9	13.7	217.7
Range	0.7-284	2-56.9	7.5-1942.2
Intra-plate CV	3.20%	1.70%	2.51%
Inter-plate CV	3.56%	1.95%	3.71%

PGI and PGII bead-based assay:

- While the study is on-going, currently preliminary results for PGI/II antigen confirmation demonstrate strong binding of both monoclonal and polyclonal antibodies to their respective PG antigens, with minimal cross-detection between PGI and PGII.

Discussion/Conclusion:

- The custom bead-based assay detected additional HP-positivity compared to the ELISA kit.
- Use of the bead-based assay allowed for higher range/specificity of detection and can allow for additional incorporation of targets using the same amount of sample, consumables, and time.
- We have successfully coupled/confirmed beads with anti-PGI antibodies and have goals of expand it for inclusion of PG-II and other additional biomarkers.

Acknowledgements:

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References:

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5. Images created in https://BioRender.com