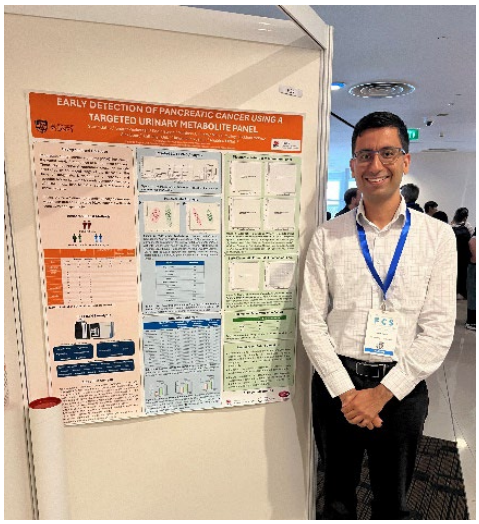
Name: Sumit Sahni
Position & Affiliation: Senior Research Fellow, Kolling Institute, The University of Sydney
Full Reference: **Sumit Sahni**, Bharat Venkatesh, Sooin Byeon, Matthew J. McKay, Mark P. Molloy, Siobhan McKay, Alex Boue, Anthony J Gill, Jaswinder S Samra, Anubhav Mittal. *Early detection of pancreatic cancer using a targeted urinary metabolite panel.*
Conference/Meeting Name: Frontiers in Cancer Science 2024
Location: Singapore
Dates:13-15 November 2024
Presentation Type: Poster



Presenting my poster during poster session at the conference.

I attended Frontiers in Cancer Science 2024 conference in Singapore, which is one of the premier cancer conferences organised in the Asia Pacific region. More than 600 delegates attended the conference over 3 days, with a number of international speakers from all over the world (e.g., USA, Belgium, Australia, etc.). The conference was themed to bring together cancer researchers with complementary knowledge and expertise from across the globe for the exchange of ideas and information. Some of the key invited speakers included Prof Douglas Green, Prof Katy Rezvani, Prof Sarah-Maria Fendt, Prof Zemin Zhang, Prof Vinod Balachandran, Prof Jeffrey Miller, Prof Luc Morris.

One of the key areas where the conference ended up converging was on different cancer immunotherapies and immunomodulatory approaches. Some key talks on NK cell based therapies, adaptive T cell therapies, cancer vaccines and also microbiome based immune modulation were major highlights of the conference. Apart from that, cancer metabolism and its emerging role as a therapeutic vulnerability in cancer was also another big area of discussion.

I gained number of new ideas for my own research at USYD. Although conference was not directly focused on pancreatic cancer, which is my area of research interest, there were number of interesting complementary research talks (e.g., in cancer metabolism) which are very useful for our research. I met Prof Vinod Balachandran who was pioneered mRNA vaccine therapy in pancreatic cancer patients, and learning from his experience and approach was fascinating. We are now planning to invite him to Australia from the International Association of Pancreatology Meeting in 2025, which I am co-organising.

Some of the new methodologies which were used by researchers in their talks were fascinating (e.g., MALDI-MS based metabolomics) and will be very interesting for us to incorporate in our research. We will look into implementing such techniques in our research projects in near future.

Some of the keynote speakers demonstrated how their initial discovery research has been finally translated to clinic, e.g., initial identification of neo-antigens in pancreatic cancer patients who had immune hot tumours (<10% of patients), led to idea of developing vaccines against neoantigens in PC patients who have immune cold tumours (90% of patients). This has now undergone Phase I trials with promising outcomes and Phase II trials are now underway. Similar, NK cell based therapies in AML patients and development of allogenic products (instead of autologous) has brought down the cost

and time of these therapies tremendously and will benefit patients. These stories with clinical translation outcomes will be very inspiring for SCP membership.

This was my first international conference since COVID pandemic and was a very good experience. Meeting researchers from different cancer research areas was a personal highlight for me.

EARLY DETECTION OF PANCREATIC CANCER USING A TARGETED URINARY METABOLITE PANEL

Sumit Sahni,[1] Bharat Venkatesh,[1] Sooin Byeon,[1] Matthew J. McKay,[1] Mark P. Molloy,[1] Siobhan McKay,[2] Alex Boue,[2] Anthony J Gill, [1,2] Jaswinder S Samra,[2] Anubhav Mittal [1,2]

[1]University of Sydney, Sydney, New South Wales, Australia

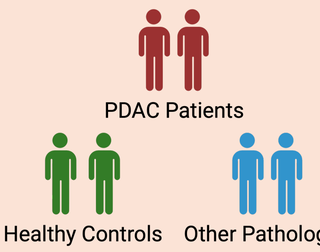
[2]Upper GI Surgical Unit, Royal North Shore Hospital and North Shore Private Hospital, New South Wales, Australia

Background and Objective

Pancreatic ductal adenocarcinoma (PDAC) has one of the worst survival outcomes of all common cancers. These dismal survival outcomes could be majorly attributed to advanced stage of the disease at the time of diagnosis. Development of sensitive and specific biomarker tests for early detection/diagnosis of PDAC could lead to better survival outcomes for these patients.

In this study, we aimed to identify urinary biomarkers which can detect early stage PDAC patients with high sensitivity and specificity.

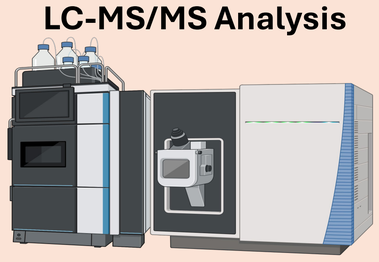
Research Plan & Methods



		PDAC		Healthy Controls		Other Malignant	Other Benign
		Discovery	Validation	Discovery	Validation		
Sex	Male	36	18	23	19	25	27
	Female	16	14	22	10	5	7
Median age (years)		65.7	66	64.2	61.3		
Stage	IA	1	-				
	IB	2	5				
	IIA	6	3				
	IIB	17	8				
	III	4	6				
	IV	5	2				
Lymph Node	Y	20	15				
	N	8	8				
	ND	24	9				
Grade	1	-	-				
	2	24	14				
	3	4	8				
	NA	24	9				

Table 1: ***Patient and Other Cohorts Characteristics***

LC-MS/MS Analysis



Previous Metabolite Panel

Glycolic acid, Myoinositol, Trigonelline, Hydroxyacetone, Hippurate, Creatinine

Metabolite From Literature Search

TMAO, Acylcarnitine, Taurine, Hypoxanthine

Amino Acids Panel

Serine, Glutamine, Arginine, Glycine, Aspartate, Glutamate, Methionine, Threonine, Alanine, Proline, Cysteine, Lysine, Tyrosine, Valine, Phenylalanine, Isoleucine, Leucine, Histidine, Tryptophan, Asparagine

Statistical Analysis

- Unsupervised Principal Component Analysis (PCA) and supervised Partial Least Square – Discriminant Analysis (PLS-DA) was preformed to determine group separation and variables of importance. Metabolites with Variable Importance in the Projection (VIP) score >1.0 considered for biomarker analysis.
- Univariate Area Under Receiver Operating Characteristic (AUROC) curve analysis was used to determine diagnostic ability of metabolites. Analytes chosen based on diagnostic ability.
- Multivariate AUROC analysis of selected metabolite panel was performed to assess its diagnostic ability.
- Metabolite biomarker panel's sensitivity and specificity was assessed in an independent validation cohort.

Targeted LC-MS/MS Analysis

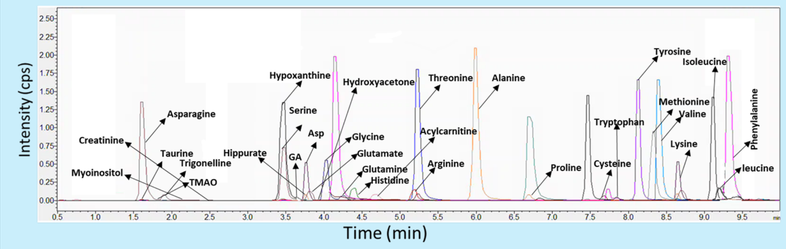


Figure 1: ***Representative LC-MS/MS spectra for urinary metabolite identification.***

Multivariate Analysis

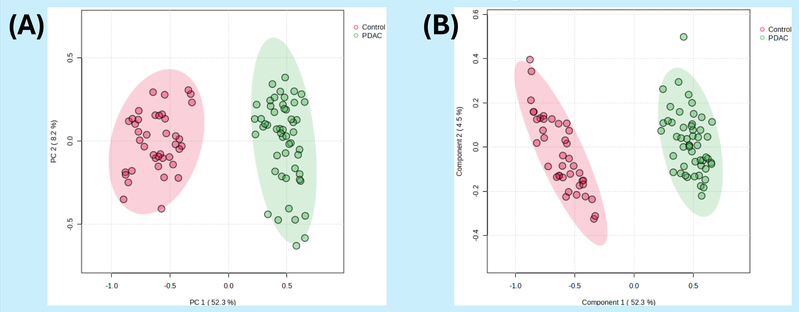


Figure 2: ***Multivariate Analysis.*** ***(A)*** *Unsupervised Principal Component Analysis (PCA) Analysis. Score plot between principal components derived from the urinary metabolite profile of PDAC patients (green) and healthy controls (green).* ***(B)*** *Supervised Principal Least Square – Discriminant Analysis (PLS-DA) Analysis. Score plot between components derived from the urinary metabolite profile of PDAC patients (green) and healthy controls (green).*

Name	VIP Score
Hypoxanthine	2.33
Cysteine	1.92
Valine	1.56
Leucine	1.28
Hydroxyacetone	1.27
Trigonelline	1.23
Glycine	1.22
Arginine	1.18
Taurine	1.05
Phenylalanine	1.02

Table 2: ***Top identified metabolites from PLS-DA multivariate model.*** *VIP score was used to determine the importance of individual metabolites in the diagnostic multivariate model.*

Univariate Analysis

	PDAC vs Healthy Controls		PDAC vs Benign Pancreatic Pathologies		PDAC vs Other Malignant Pathologies		PDAC vs All Other Pancreatic Pathologies	
Metabolite	AUC	P-value	AUC	P-value	AUC	P-value	AUC	P-value
Hypoxanthine	1.0	<0.0001	0.834	<0.0001	0.745	<0.0001	0.792	<0.0001
Valine	1.0	<0.0001	0.649	<0.0001	0.684	<0.0001	0.666	<0.0001
Leucine	0.994	<0.0001	0.853	<0.0001	0.854	<0.0001	0.853	<0.0001
Hydroxyacetone	0.994	<0.0001	0.713	<0.0001	0.713	<0.0001	0.672	<0.0001
Cysteine	0.993	<0.0001	0.684	<0.0001	0.704	<0.0001	0.693	<0.0001
Phenylalanine	0.989	<0.0001	0.893	<0.0001	0.879	<0.0001	0.886	<0.0001
Arginine	0.981	<0.0001	0.584	<0.0001	0.566	<0.0001	0.576	<0.0001
Glycine	0.963	<0.0001	0.782	<0.0001	0.728	<0.0001	0.757	<0.0001
Trigonelline	0.904	<0.0001	0.87	<0.0001	0.941	<0.0001	0.903	<0.0001
Taurine	0.89	<0.0001	0.682	<0.0001	0.543	<0.0001	0.617	<0.0001

Table 3: ***Univariate AUROC for PDAC diagnosis against*** ***(i)*** *Healthy Controls;* ***(ii)*** *Benign Pancreatic Pathologies;* ***(iii)*** *Other Malignant Pancreatic Pathologies; and* ***(iv)*** *All Other Pancreatic Pathologies.*

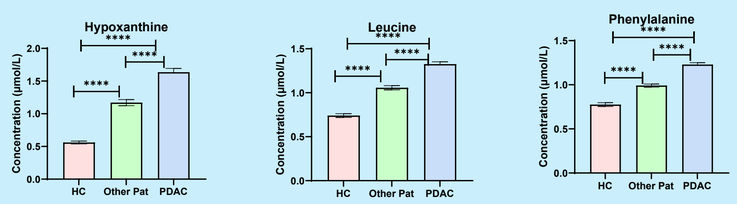


Figure 3: ***Levels of selected metabolites.*** *The levels of metabolites were compared between healthy controls (HC), patients with other pancreatic pathologies (Other Pat); and PDAC patients.* ***(A)*** *Hypoxanthine;* ***(B)*** *Leucine; and* ***(C)*** *Phenylalanine. ****p < 0.0001 compared to HC or Other Pat, as shown.*

Diagnostic Potential of Biomarker Panel

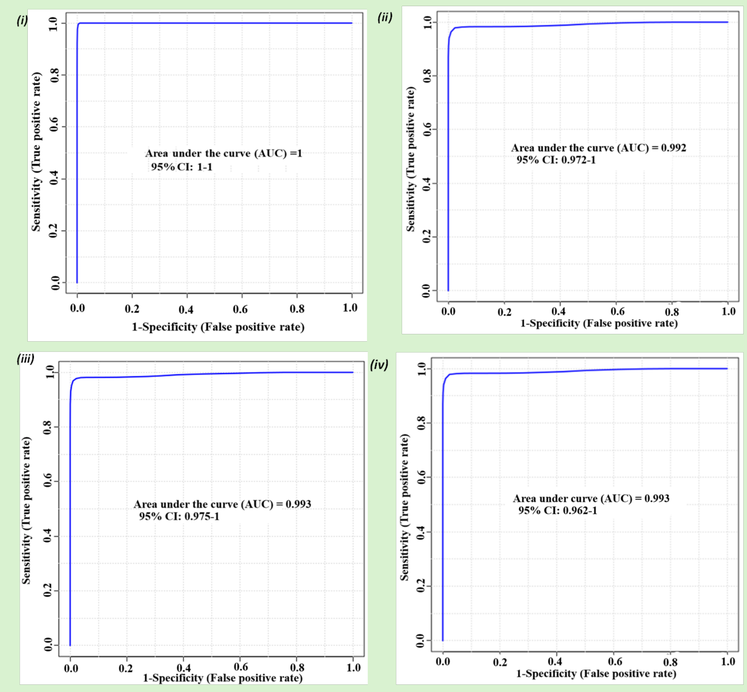


Figure 4: ***Diagnostic Potential of Urinary Metabolite Panel. AUROC curves for multivariate models for PDAC diagnosis against:*** ***(i)****. Healthy Controls;* ***(ii)*** *Benign Pancreatic Pathologies;* ***(iii)*** *Patients with Other Malignant Pancreatic Pathologies; and* ***(iv)*** *All Other Pancreatic Pathologies. Models were developed based on PLS-DA for three-metabolite panel of Hypoxanthine, Leucine and Phenylalanine.*

Early Diagnostic Potential of Biomarker Panel

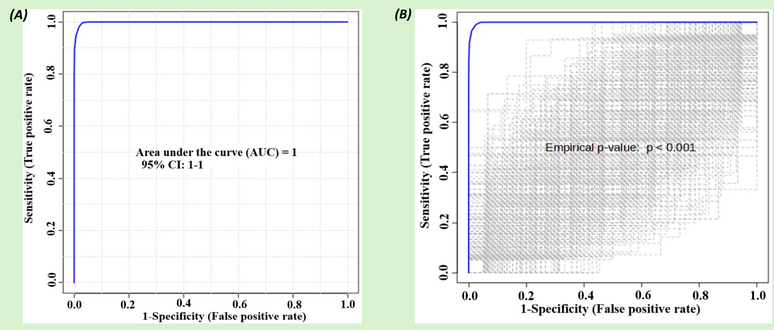


Figure 5: ***Diagnostic Potential of Metabolite Biomarker Panel for Early-Stage PDAC patients.*** ***(A)*** *AUROC model for metabolite panel (Hypoxanthine, Leucine and Phenylalanine) to diagnose early stages (I and II) PDAC patients compared to healthy controls.* ***(B)*** *Permutation test for AUROC model developed in (A).*

Biomarker Panel Validation Cohort

	Predicted Outcomes	
	PDAC	HC
PDAC	32	4
HC	0	30

Table 4: ***Predictive ability of the biomarker panel in an independent validation cohort.***

Summary and Future Directions

- Multivariate and univariate statistical analysis were performed to identify a metabolite signature (Hypoxanthine, Leucine and Phenylalanine) which can diagnose PDAC with excellent sensitivity and selectivity.
- Identified metabolic biomarkers showed high predictive ability in an independent validation cohort.
- Future validation in longitudinal pre-diagnostic specimens is required to determine the ability of this biomarker panel in identifying patients in asymptomatic high-risk populations.

Acknowledgements

Proudly supported by Cancer Institute NSW

Sydney Cancer Partners

PanKind – The Australian Pancreatic Cancer Foundation