18 July 2024

To Australian Cystic Fibrosis Research Trust,

FINAL REPORT
Sponsor/Scheme: Cystic Fibrosis Australia/ACFRT Innovation Grant
Title: The use of non-invasive biomarkers as a screening tool and the role of gut inflammation and microbes in adult cystic fibrosis colorectal cancer
UNSW Ref: RG191510 "Adult-specific complication of gastrointestinal malignancies"

I would like to provide a report for the aforementioned study. As mentioned in our previous report, our recruitment was affected and temporarily halted due to COVID19 lockdowns. We have since completed all the anticipated recruitment. The team would like to share the following summary/abstract of the project.

Background: People with cystic fibrosis (pwCF) have an increased risk of colorectal cancer (CRC). While colonoscopy is the currently recommended screening test for pwCF, this study aimed to evaluate the role of immunochemical faecal occult blood test (iFOBT) and emerging CRC screening tests, faecal calprotectin (FC) and faecal tumour pyruvate kinase isoenzyme type M2 (TuM2-PK), in this population.

Methods: A prospective, observational study was conducted at Royal Prince Alfred Hospital, Sydney, Australia, from February 2019 to March 2023. Patients meeting screening criteria provided stool samples for iFOBT, FC, and TuM2-PK within 3 months of colonoscopy. Abnormal findings included pre-malignant (adenomatous polyps) and malignant ileocolonic lesions.

Results:
Participants
Among 49 participants, 12 (24%) had adenomatous polyps and 2 (4%) had ileocolonic malignancy. The characteristics of the 49 participants were as follows:
mean age 48 (SD 8) years; female 53%; F508del homozygous 45%; pancreatic insufficient 65%; cystic fibrosis-related diabetes 51%; post-solid

organ transplant 29%; mean FEV1 72 (SD 22) % predicted among non-transplant recipients.

Immunochemical faecal occult blood test (iFOBT)
None of the 41 participants with negative iFOBT had malignancy; 30 (73%) had no adenomatous or malignant pathology, while 11 (27%) had pre-malignant lesions. iFOBT for malignancy showed AUC=0.93 (95% CI: 0.86-1.00), p=0.044, sensitivity 100%, specificity 87%, PPV 25%, NPV 100%. For pre-malignant lesions, iFOBT showed AUC=0.53 (95% CI: 0.34-0.72), p=1.0, sensitivity 17%, specificity 73%, PPV 83%, NPV 86%.

Faecal calprotectin (FC)
FC for malignancy showed AUC=1.0 (95% CI: 0.0-1.0), p=0.019, and AUC=0.726 (95% CI: 0.56-0.90), p=0.086 for pre-malignant lesions. FC optimal cut-offs were >991 µg/g for malignancy (sensitivity, specificity, PPV, NPV: 100%) and <89 µg/g for pre-malignant lesions (sensitivity 63%, specificity 83%, PPV 63%, NPV 83%).

Faecal tumour pyruvate kinase isoenzyme type M2 (TuM2-PK)
TuM2-PK for malignancy showed AUC=0.829 (95% CI: 0.70-0.90), p=0.12, and AUC=0.607 (95% CI: 0.45-0.77), p=0.27 for pre-malignant lesions. TuM2-PK optimal cut-offs were >18.6 ul/L for malignancy (sensitivity 100%, specificity 87%, PPV 100%, NPV 87%) and <10.6 ul/L for pre-malignant lesions (sensitivity 43%, specificity 92%, PPV 43%, NPV 92%).

Novel screening scoring tool for CF
A scoring tool was developed leveraging FC and TuM2-PK cohort performance: Calprotectin either ≤ 100 or ≥ 1000 (2 points), TuM2-PK ≤ 8 (1 point), and TuM2-PK ≥ 18 (2 points). A colonoscopy is recommended if score ≥ 2. This scoring system was significantly associated with identification of pathology on colonoscopy. Fisher's exact test showed p=0.01934, 95% CI [1.162-440.428], OR=9.394, sensitivity 92.86%, specificity 42.86%, PPV 39.39%, NPV 93.75%, indicating high sensitivity and NPV for identifying patients without adenomatous polyps or CRC/ileocolonic malignancy.

Conclusion: The study indicates a high rate of abnormal colonoscopy findings in pwCF, highlighting the need for CRC screening. iFOBT is not effective for pre-malignancy detection but may exclude ileocolonic malignancy. FC and TuM2-PK could aid in clinical decisions regarding colonoscopy. Further validation in larger cohorts is necessary.

I would also like to update the ACFRT on the other impacts of this funding. The funding of this project by ACFRT has paved way for the following:

- Nicole Taylor, who is the Clinical Nurse Consultant (CNC) at the RPA CF clinic/unit has now commenced her PhD as a direct result of this

funding. Dr Sheila Shivam (Respiratory Physician, RPA), Dr Josie van Dorst (UNSW) and I are her supervisors.
- As part of this, we will explore the gut microbial profile in more detail.
- Developed international collaboration with Prof Jochen Mainz (Germany) to include his CF specific symptoms questionnaire to include symptoms as additional end-points to this project, considering the high burden of GI symptoms in our patient population.

Yours sincerely,

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