**Protocol: Comparison of Carcinoembryonic Antigen Levels Between Portal and Peripheral Blood in Patients With Appendiceal or Colorectal Adenocarcinoma**

Version Number: 1.31

Date of Protocol: 7/02/2023

SYNOPSIS

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**Summary**

Study title: Comparison of Carcinoembryonic Antigen Levels Between Portal and Peripheral Blood in Patients With Appendiceal or Colorectal Adenocarcinoma

Protocol version: 1.31

Objectives Primary objective: Our research intends to clarify the mechanism by which CEA is released into peripheral blood in patients with appendiceal or right sided colon adenocarcinoma by examining for the correlation between CEA levels in peripheral and portal blood of these patients.

Secondary objectives: To correlate the median CEA levels in the peripheral and portal vein samples across immunohistochemical and histopathological (i.e., differentiation, node metastasis, lymphovascular invasion, etc) variables of the primary appendiceal or right sided colon adenocarcinoma.

Study design Prospective cohort

Planned sample size Based on the Tabuchi et al, study, mean CEA level for portal vein samples were 26.6 ± 6.4 ng/mL and 8.1 ± 1.9 ng/mL for periperhal vein samples.1 Using these values, an enrollment ratio of 1, alpha value of 0.05, power set to 90%, with a continuous endpoint, as a two independent sample study, the estimated total sample size is 6 patients2

Selection criteria All patients with appendix or right sided colon adenocarcinoma who undergo surgical treatment to completely remove or reduce their cancers under our Peritonectomy Unit (Prof David Morris) at St George Hospital will be eligible.

Study procedure Patients undergoing cytoreductive surgery with the Peritonectomy Unit for appendiceal and/or right sided colon cancer at St George Hospital would be considered for surgery (especially those with a pre-operatively elevated CEA i.e., > 5 microg/L). Once under a general anaesthetic for their operation and immediately after laparotomy or laparoscopy, peripheral and portal blood specimens would be collected from all included patients. Peripheral blood would be taken from an antecubital venipuncture. This would only be 5 to 10mls of blood. Portal blood would be collected from the ileocaecal vein by inserting a venous catheter (open or laparoscopically), prior to resection of this segment of bowel as previously would be discussed with the patient and consented for (N.B. this would routinely be done as part of the peritonectomy that the patient would already be undergoing as part of their cancer treatment surgery). This would be 10mls of blood in total. This would be in line with the methods and materials section of the Tabuchi et al paper referenced below.

Statistical considerations Sample size calculation – Please see sample size calculation above; this is calculated off a previous study1

Analysis plan – Serum CEA levels will be compared between the peripheral and portal venous samples with a Mann-U-Whitney test performed of the median values.

Duration of the Study 2 years – Essentially long enough to get planned sample size (n = 6) or significant results.

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# BACKGROUND

## Disease Background\*

Background. Differences between appendiceal and colorectal adenocarcinoma have been published extensively, most recently regarding gene expression between high grade appendiceal cancer and colorectal cancer.1 We recently noted within our unit and institution a significant disparity in the proportion of liver metastases between appendiceal and colorectal adenocarcinomas (3.1% and 24%, respectively). In a recent paper by Lee et al, carcinoembryonic antigen’s role in production of liver metastases in colorectal cancer has been well described.2 As an extrapolation of this, a theory has been proposed by the first author regarding the natural progression of appendiceal cancer. To provide support for this theory, a systematic review and meta-analysis was performed to prove that a significantly higher proportion of patients have an elevated CEA in appendiceal as opposed to colorectal adenocarcinoma.

Methods. A systematic review and meta-analysis was conducted in accordance with the PRISMA guidelines involving Medline (PubMed), EMBASE (Ovid), Cochrane Central Register of Controlled Trials (CENTRAL), Cumulative Index to Nursing and Allied Health Literature (CINAHL), Clinicaltrials.gov, Web of Science and Google Scholar databases.3 All studies that involved patients with appendiceal and colorectal adenocarcinoma were eligible for inclusion. Data were analysed by grouping appendiceal and colorectal adenocarcinoma in separate meta-analyses and then comparing their weight proportions of elevated CEA using MedCalc Statistical Software.4

Results. 1928 articles were initially identified with 137 articles included in the final synthesis after systematic exclusions were made. Ninety-three articles were included in the final meta-analysis. The overall weighted proportion of elevated CEA for appendiceal and colorectal adenocarcinoma were 56% (95% CI: 47% to 65%) and 42% (95% CI: 38% to 46%), respectively.

Discussion. In conclusion, appendiceal cancer appears to have a higher proportion of elevated CEA when compared to colorectal adenocarcinoma. The proposed theory for the natural progression of appendiceal cancer and the resultant disparity in liver metastases between appendiceal and colorectal adenocarcinoma are supported by the findings in this study’s meta-analyses. However, future research should focus on comparing portal vein and peripheral vein CEA in patients with appendiceal cancer to further support this proposed theory.

## Rationale for Performing the Study\*

The usefulness of serial carcinoembryonic antigen (CEA) determinations in staging, detection, prognosis, and monitoring of therapy in patients with colorectal cancer has been reported frequently during past the 50 years. However, its utility in patients with appendiceal cancer is limited.

Our research intends to clarify the mechanism by which CEA is released into peripheral blood in patients with appendiceal and/or right sided colon cancer by examining for the correlation between CEA levels in peripheral and portal blood of these patients.

At St George Hospital, patients with appendix and/or right sided colon cancer may undergo surgical treatment to completely remove or reduce their cancers under our Peritonectomy Unit (Prof David Morris). With patient's consent, we wish to take samples of venous blood from a patients arm and a patient's bowel (prior to the segment of bowel that is already planned to be removed, is removed) to test for levels of a common tumour marker (usually used in colorectal cancer patients) called carcinoembryonic antigen (CEA). We expect that the CEA levels in the venous blood from the patients bowel would be significantly lower than the peripheral venous samples.

This would allow us to explain a theory that we've developed regarding progression of disease in appendix cancer versus how it progresses in colorectal cancer. This would allow them for treatment options to be further explored for patients with colorectal cancer (and appendix cancer) such that potentially patients with appendix and / or colorectal cancer may have reduced spread of their disease to their liver (and lungs). Feasibility sample size calculations require us to have anywhere between 24 to 50 patients to get potentially significant results.

We believe this would be of very low risk for additional injury as to the patient as they have already been consented patients and the intervention is on a segment of bowel that has already been earmarked for removal as part of the consent for the peritonectomy the patient would already be undergoing under our unit.

# STUDY OBJECTIVES\*

## Primary Objective\*

Our research intends to clarify the mechanism by which CEA is released into peripheral blood in patients with appendiceal cancer by examining for the correlation between CEA levels in peripheral and portal blood of these patients. We have recently published a hypothesis paper in the British Journal of Surgery and wish to repeat the study by Tabuchi et al, but in patients with appendiceal adenocarcinoma to support this theory.1, 7 This would aim to compare CEA levels between peripheral and portal vein serum samples in patients with appendiceal and/or right sided colon adenocarcinoma at time of their index peritonectomy. Our study hypothesis would be that there would not be a significant difference between the peripheral and portal vein CEA samples in these patients with appendiceal adenocarcinoma. However there would be an expected significant difference between the peripheral and portal vein CEA samples in patients with right sided colon adenocarcinoma.

## Secondary objectives

To correlate the median CEA levels in the peripheral and portal vein samples across immunohistochemical and histopathological (i.e., differentiation, node metastasis, lymphovascular invasion, etc) variables of the primary appendiceal and right sided colon cancer.

# STUDY Design\*

## Design\*

Prospective cohort

## Study Groups

Patients with appendiceal or right sided colon cancer (suspected or confirmed) undergoing cytoreductive surgery under the Peritonectomy Unit at St George Hospital

## number of participants\*

Based on the Tabuchi et al, study, mean CEA level for portal vein samples were 26.6 ± 6.4 ng/mL and 8.1 ± 1.9 ng/mL for peripheral vein samples.1 Using these values, an enrollment ratio of 1, alpha value of 0.05, power set to 90%, with a continuous endpoint, as a two independent sample study, the estimated total sample size is 6 patients.2

## number of centres

Single site – St George Hospital; expected number of participants: 6 eligible patients

## duration

* Start date: ASAP; End date: 2 years or long enough to recruit 6 eligible patients or until consistently significant results have been found.
* Expected time frame for the recruitment phase of the study: 2 years

# Participant section

## Inclusion Criteria\*

\* Sex: All

\* Age range: 18 years and above

\* Disease status: Appendiceal or right sided colon cancer (suspected or confirmed)

\* Willingness to give written informed consent and willingness to participate to and comply with the study.

\* Pre-operative carcino-embryonic antigen (CEA) level > 5 microg/L

## Exclusion Criteria\*

\* Participants who cannot provide informed consent.

\* Patients who have had a previous peritonectomy (including those who have previously undergone a right hemicolectomy)..

# STUDY Outline\*

## Study Flow Chart

Diagram of the study design

Identification of potential participants

Screening / consent

Enrolment

Intervention:venous sampling of patients undergoing cytoreductive surgery (aim: within 30 days of initial visit)

## Investigation plan\*

|  |  |  |
| --- | --- | --- |
| List Interventions | Enrolment Visit | Post-operative / Final study Visit |
| Informed Consent | ✓ |  |
| Inclusion / Exclusion criteria | ✓ |  |
| Physical examination | ✓ | ✓ |
| Adverse Event & Serious Adverse Event Assessment |  | ✓ |

Measurement of serum tumour markers are routinely performed as per the peritonectomy work-up checklist (as attached supplemental to the HREA), this is usually from a peripheral venepuncture. However, taking venous samples from mesenteric / portal venous samples immediately after laparotomy (or laparoscopy) but before surgical resection is not routine. However, a right hemicolectomy is routinely performed as part of a peritonectomy for patients undergoing cytoreductive surgery for appendiceal and/or right sided colon cancer, so this blood would be being sampled from a vein that is already earmarked for surgical resection as part of the normal process of the operation that the patient would be perioperatively appropriately consented for. Hence, we believe this would cause negligible if at all any further harm to the patient than what the usual risks (and benefits) the operation would normally be for the patient.

Regarding the blood sampling, five ml of blood will be taken from a vein in the patient’s arm (this will be from an intravenous cannula as part of your general anaesthetic such that there will be no additional venepuncture burden) as well as another 5 ml of blood from the ileocolic / appendicular vein during the initial stages of your peritonectomy surgery (total number of blood tests per patient: 2; total volume: 10ml; NB: 5-10mL is equivalent to 1-2 teaspoons). The blood sampling would take less than one minute during surgery.

## Study Procedure Risks\*

Very low risk of injury to the surgeon performing the blood sampling and as noted in section 5.2 of this protocol that there would also be very low risk of having the interventions noted within this study be of harm to the patient as the segment of bowel that venous blood is to be sampled from is already earmarked for surgical resection within the bounds of the preoperatively consented operative procedure that the patient has consented to.

## Recruitment and Screening\*

All patients undergoing cytoreductive surgery with the Peritonectomy Unit for appendiceal and/or right sided colon cancer at St George Hospital would be considered for surgery (especially those with a pre-operatively elevated CEA i.e., > 5 microg/L) when seen by Prof David Morris or Dr Nima Ahmadi in their relevant clinics at St George Hospital. Entry into the study will be completely voluntary and done without bias or coercion. Potential participants will be screened as part of their triaging and referral process already in place at the relevant clinics as stated above.

## Informed Consent Process\*

Patients will be consented regarding the purpose of the study and the methods and materials that will be utilised regarding this study and the intervention. This will be recorded on the consent form included in the HREA. Informed consent will be done by the team’s Fellow General Surgeon, Dr Mohammed Breakeit whom is a study team member that is not the treating clinician. This will ensure an ‘arm’s length’ principal is adhered to and unequal relationship avoided. The patient will have until the day of surgery to consider participating in this study. The participant essentially then will have from the time the decision is made to recommend the patient undergo a peritonectomy (in agreement with the patient and the treating Surgeon) to the day of surgery to consider participation in this study (i.e., the participant will have up to roughly 30 days to consider participation).

## Enrolment Procedure\*

The participant will be enrolled into the study after the informed consent process has been completed and the participant has met all inclusion criteria and none of the exclusion criteria. The participant will receive a study enrolment number, and this will be documented on all study documents.

## Randomisation Procedure

Not applicable

# SAFETY\*

## Adverse Event Reporting\*

Defined as any untoward medical occurrence in a participant which does not necessarily have a causal relationship with the study intervention or an adverse event can therefore be any unfavorable or unintended sign, symptom or condition and/or an observation that may or may not be related to the study intervention.

## Serious Adverse Event Reporting

Defined as any untoward medical occurrence that results in the following: death, is life-threatening, requires inpatient hospitalisation or prolongation of existing hospitalisation, persistent or significant disability/incapacity, condition requiring unnecessary medical or surgical intervention.

## Data Safety and Monitoring Board

## membership and responsibilities

Not applicable

## Early Termination

The only reasons this study would be terminated early would be 1) if there were technical difficulties with obtaining the mesenteric / portal venous samples, or 2) if there were found to be significantly higher serum CEA levels in the portal venous samples as opposed to the peripheral samples (as this would be contrary to the hypothesis – also this would only be a relative contraindication to terminating the study as these results may still be worthwhile publishing). Patients already involved with the study will be informed of this early termination by the Principal Investigator should this happen. A final study report will be compiled on the available results and correspondence sent to the HREC.

# BLINDING AND UNBLINDING

This study is not intended to be blinded in any way.

# STATISTICAL CONSIDERATIONS\*

Sample Size or Power Calculation\*

Based on the Tabuchi et al, study, mean CEA level for portal vein samples were 26.6 ± 6.4 ng/mL and 8.1 ± 1.9 ng/mL for periperhal vein samples.1 Using these values, an enrollment ratio of 1, alpha value of 0.05, power set to 90%, with a continuous endpoint, as a two independent sample study, the estimated total sample size is 6 patients2

Statistical Analysis Plan\*

Serum CEA levels will be compared between the peripheral and portal venous samples with a Mann-U-Whitney test performed of the median values.

# STORAGE AND ARCHIVING OF STUDY DOCUMENTS\*

This research project will require the use of an electronic database using Microsoft Access. Microsoft Access will be password-protected and with restricted access to the study investigators. Identifying personal/health information and the linking master file will be stored separately with restricted access as per above. Hard copies (e.g., consent forms) will be stored with the study documents. This will be analysed using the statistical software, SPSS. The research data will be stored for a minimum of 5 years upon completion of the research project. Once the study documents and data are no longer required, they will be securely destroyed as per NSW Health policies and handling procedures.

# REFERENCES\*

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