THE ROLE OF CARCINOEMBRYONIC ANTIGEN IN PREDICTING COLORECTAL CANCER IN RESOURCE POOR SETTING OF KWAZULU-NATAL, SOUTH AFRICA

By

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College of Health Sciences
University of KwaZulu-Natal
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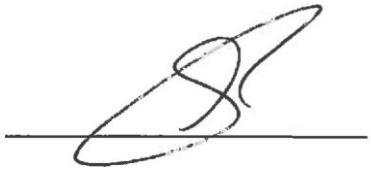
PREFACE

This study represents original work by the author and has not been submitted in any other form to another University. Where use was made of the work of others, it has been duly acknowledged in the text.

The research described in this dissertation was carried out in the Department of Surgery, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa under the supervision of Professor TE Madiba and Dr Z Moolla.



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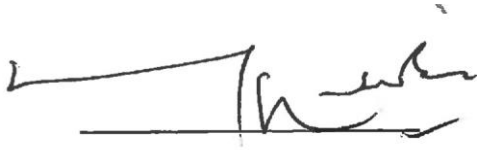

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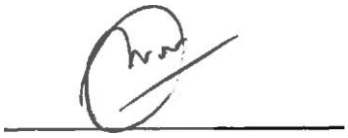

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Co-supervisor

DECLARATION

I, Yugan Dylan Naicker, declare that:

(i) The research reported in this thesis, except where otherwise indicated is my original work.

(ii) This thesis has not been submitted for any degree or examination at any other university.

(iii) This thesis does not contain other person's data, pictures, graphs or other information unless specifically acknowledged as being sourced from other persons.

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a) Their words have been rewritten but the general information attributed them has been referenced.

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DEDICATION

To my wonderful wife Devina, I love you.

ACKNOWLEDGEMENTS

- I am thankful to my supervisor at the Department of Surgery, University of KwaZulu-Natal, Professor TE Madiba, and my co-supervisor, Dr. Zaheer Moolla for their guidance, invaluable constructive criticism and advice during this research.
- I would like to express my gratitude to my parents Professor T Naicker (Anita) and Mr G Govender (Nelson), for their support throughout the course of my studies.
- Finally, I am indebted to my wife for her patience and support during the writing of this dissertation.

LIST OF ABBREVIATIONS

Abbreviation	Name
CRC	Colorectal cancer
Ca 19-9	Cancer antigen 19-9
CEA	Carcino-embryonic antigen
gFOBT	Guaiac faecal occult blood test
IALCH	Inkosi Albert Luthuli Central Hospital
SA	South Africa
S- CEA	Serum levels of Carcino-embryonic antigen
TPA	Tissue plasminogen activator
UICC	Union for International Cancer Control

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OVERVIEW OF THESIS

Colorectal cancer (CRC) is the third most common malignancy in the world with the global incidence of CRC projected to rise up to 60% by 2030. More than one million new cases are diagnosed per annum and 530 000 deaths are reported per year worldwide. Importantly the slow growth of CRC warrants early screening to reduce both the incidence and mortality of the disease. Screening should be to primarily detect CRC at a relatively early stage (stage 1 and 2). However, currently the most reliable diagnostic tool, colonoscopy, is not readily accessible in the resource deprived setting of KwaZulu-Natal, South Africa.

Carcinoembryonic antigen (CEA) is one of the most widely used tumour markers worldwide. It was first described in 1965 by Gold and Freedman. CEA is a glycoprotein with a molecular weight of 200 000 Daltons. It was initially identified and immunolocalized on both foetal colon and colon adenocarcinoma. CEA is also present in normal tissue at $\leq$3 ng/ml, which is a 60-fold lower concentration than in malignant tissue. Data on the role of CEA as a prognostic marker for CRC in sub-Saharan Africa is limited.

KwaZulu-Natal is a province of South Africa, with an estimated population of 12 million. Despite this large population, only 8 medical facilities offer colonoscopy to the public health care sector, with resultant huge delays in acquiring screening or diagnostic colonoscopies in this resource-deprived setting. CRC is the fourth most common cancer in South Africa (SA).

Given that there is currently no reliable tumour marker available for this pathology, coupled with a background of limited availability of diagnostic measures like colonoscopy, the value of CEA as a tumour marker for CRC in this resource-deprived setting should be further explored. It was therefore the aim of this study to determine whether serum CEA level in patients symptomatic for lower GI pathology correlates with the histological presence and severity of primary colorectal cancer in a large referral centre within KwaZulu-Natal and further, to explore the possibility of using the CEA level to fast-track patients to colonoscopy.

We present a retrospective analysis of prospectively collected clinical data investigating the demographic data, stage of CRC, and CEA level prior to chemotherapy, radiotherapy and/or definitive surgery. We conducted a retrospective case study on 380 patients who attended the Colorectal Clinic at the tertiary referral hospital, Inkosi Albert Luthuli Central Hospital, Durban, South Africa during the period of 2007-2016. Variables collected included race, age, and gender, pre-treatment CEA, and stage of CRC**.** GraphPad Prism 5.00 for Windows (GraphPad Software, San Diego, California, USA) was used for data analysis.

Similarities and differences between our findings and currently known data were observed. Despite the proposal of CEA as a tumour marker for CRC, we confirm its low sensitivity as a screening test for CRC. Moreover, in light of the poor access and long delays associated with colonoscopy in the public health care sector of South Africa, it is urgent that patients with suspected CRC are speedily evaluated via non-invasive techniques. We wondered if, in resource-poor settings, an elevated serum CEA could also serve as a test that serves as a

trigger for clinicians to perform colonoscopy in order to diagnose CRC. However, CEA has no diagnostic role due to its low sensitivity and specificity to CRC, and we have not shown any benefit of CEA levels as a risk assessment tool to be used to fast-track symptomatic patients for colonoscopy. We recommend therefore that less invasive approaches be promoted in resource-limited settings including the use of Guaiac faecal occult blood test (gFOBT).

CHAPTER ONE

BACKGROUND AND LITERATURE REVIEW

1.1 Introduction

Carcinoembryonic antigen (CEA) is one of the most widely used tumour markers worldwide. It was first described in 1965 by Gold and Freedman[1, 2]. Carcinoembryonic antigen was initially identified and immunolocalized on both healthy foetal colon and adenocarcinoma of the colon, but it is absent from healthy adult colon [2]. The term "carcinoembryonic antigen" was coined because it was initially only identified in cancer and embryonic colon.

The CEA molecule is an onco-developmental human tumour marker and bears the cluster differentiation designation, CD66[3]. It is a membrane surface glycoprotein that interacts with the microskeleton of the cell [4, 5]. Carcinoembryonic antigen is released from the cell surface, into the interstitial space from where it enters into the general circulation [6].

Colorectal cancer (CRC) is the third most common malignancy in the world [7]. More than one million new cases are diagnosed per annum, and 530 000 deaths are reported per year [7]. The slow growth of CRC warrants early screening as early identification would enable better management. A good biomarker for early identification of CRC has the potential to reduce both the incidence and mortality of the disease. However, currently the most reliable screening tool, colonoscopy, is not readily accessible in resource-deprived settings.

Kwa-Zulu Natal is a province of South Africa, with an estimated population of 12 million. Despite its large population, only 8 centres offer colonoscopy in the public health care sector, resulting in huge delays in acquiring screening/diagnostic colonoscopies. Perhaps CEA may have a larger role as a risk assessment tool for CRC development in these communities. Nonetheless,

the use of using CEA as a risk assessment tool to guide diagnostic investigation in symptomatic patients has limitations.

1.1 Factors affecting serum CEA levels in patients with CRC include:

- Tumour Stage: CEA levels are elevated with increased disease stage. This was shown in an early study in which the proportion of patients with increased CEA concentration (>5ug/l) were as follows: Dukes A disease 3%; Dukes B disease 25%; Dukes C disease 45%; and Dukes D disease 65% [8].
- Tumour Grade: Several studies have shown that well differentiated CRC's produce more CEA per gram of total protein than poorly differentiated tumours [9, 10]. Consequently, S-CEA tends to be higher in patients with well-differentiated tumours compared with those poorly differentiated tumours. This counter-intuitive finding may explain why some patients with advanced CRC do not have increased S-CEA values.
- Liver Status: The liver is the primary site of metabolism of CEA[11] . Carcinoembryonic antigen is initially taken up by the Kupffer cells, and modified by removing its sialic acid residues [12]. Thereafter, it is endocytosed by liver parenchymal cells, where it undergoes degradation. Therefore, in conditions where the liver function is impaired such as in certain benign liver diseases, serum CEA levels are elevated [12].
- Tumour Site: CRC's located at the left colon generally have increased CEA levels compared to those located on the right [8, 13].
- Smoking increases the CEA value by a factor of two in both males and females [14].

1.2 CEA as a marker for CRC

1.2.1 Screening

The World Health Organisation defines screening as the presumptive identification of unrecognized disease in an apparently healthy, asymptomatic population by means of tests, examinations or other procedures that can be applied rapidly and easily to the target population[15], the objective being to identify those at increased risk of having a disease or disorder [16, 17]. The non-specific presentation of an early stage CRC makes clinical detection difficult. The aim of screening should be to detect the disease in asymptomatic patients at a relatively early stage (Dukes A or B). CEA concentrations are, in general, more often raised in smokers than in non-smokers, and more frequently elevated in men than in woman [14]. Fletcher *et al* calculated that CEA has a sensitivity of 36% and specificity of 87% in screening for Dukes stages A or B (Appendix 5) in CRC [16]. The same study used an upper limit of normal for CEA (2.5ug/l) which rendered the positive predictive value of CEA to be unacceptably low and thus of little value as a screening test for CRC. This is also in agreement with recommendations of the National Institution of Health Consensus Development Conference of 1981 [18].

1.2.2 Diagnosis

Studies have demonstrated a lack of sensitivity and specificity for CEA that limit its application in diagnosis, especially in early disease [16]. With respect to CRC, although more than 80% of patients with advanced disease have elevated circulating CEA levels, the CEA assay alone should not be used as the sole diagnostic test for suspected cancer[19]. A sensitivity of 78% for Dukes stage B and 91% for Dukes stage C has been achieved using CEA levels in combination with Ca19-9 and tissue plasminogen activator (TPA) levels [20]. Regarding the specificity of CEA

in CRC, it must noted that CEA is increased in most types of advanced adenocarcinomas as well as in multiple benign disorders [19].

1.2.3 Assessing Prognosis

Multiple studies report that patients with high pre-operative levels of CEA have a worse prognosis than those with lower levels [21-22]. Several studies have investigated the prognostic impact of CEA in Dukes A or B disease and found that high pre-operative CEA levels correlate with poorer prognosis [23-28]. In another study, although CEA alone was not independently prognostic, when combined with CA 242, the elevation of both markers indicated a poorer prognosis in this group of patients[26]. The forgoing studies are producing conflicting results suggesting that CEA has limited use as a prognostic marker in CRC.

The post-operative use of CEA may also have prognostic value. Evidence suggests that high post-operative levels may also predict adverse outcome [29]. After an oncological resection of a CRC, CEA levels return to normal values within 4-6 weeks[29]. Failure of the CEA level to return to normal within 6 weeks post resection is frequently associated with early recurrent disease [29].

2.0 Problem Statement and Research question

Colorectal cancer (CRC) is the fourth most common malignancy in the South Africa[30]. The slow growth of CRC engenders a potential for earlier detection thereby potentially reducing both the incidence and mortality of the disease. However, currently the most reliable screening tool, colonoscopy, is not readily accessible in resource-deprived settings.

Due to the dire shortage of colonoscopy in KwaZulu-Natal, CEA may have value as a risk assessment tool for CRC development. However thus far, the role of CEA in screening and diagnosis remains unproven and its role on prognostication shows promise.

Therefore, the overall impact of this study is to determine whether serum CEA levels in patients symptomatic for lower GI pathology correlates with the histological presence of primary CRC, stage of CRC and to identify any link to patients' demographics. Also, we seek to determine whether CEA can be used as a surrogate biomarker to expedite colonoscopy and consequently confirmation of a colorectal cancer in resource poor settings.

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CHAPTER TWO

Manuscript entitled “The role of carcinoembryonic antigen in predicting colorectal cancer in resource poor setting of KwaZulu-Natal, South Africa”- for submission to DOHET approved *South African Journal of Surgery*

THE ROLE OF CARCINOEMBRYONIC ANTIGEN IN PREDICTING COLORECTAL CANCER IN RESOURCE POOR SETTING OF KWAZULU-NATAL, SOUTH AFRICA

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ABSTRACT

Background: Colorectal cancer (CRC) is the fourth most common malignancy in South Africa. Currently the most reliable screening tool, colonoscopy, is not readily accessible in resource-deprived settings of KwaZulu-Natal. The aim of this study was to determine whether serum CEA levels in patients symptomatic for lower GI pathology correlates with the histological presence and severity of primary colorectal cancer in a large referral centre within KwaZulu-Natal. Perhaps CEA may have a larger role as a marker for CRC development in these resource deprived communities.

Patients and Methods: This study was a retrospective analysis of prospectively collected clinical data of 380 patients with colorectal cancer attending a tertiary referral centre in KwaZulu-Natal. Patients were of various age groups, various population groups and both genders. Serum levels of CEA were analysed and stratified into < 5 µg/l and ≥ 5 µg/l. Data were analyzed using descriptive statistics and findings were compared with those from the existing literature.

Results: CEA levels were studied in 380 consecutive patients with known pre-treatment CEA levels. The mean CEA level of the study population was 170.0 ± 623.3 µg/l. The number of participants with a CEA level < 5µg/l was 151 (39.74%) whilst the majority 229 (60.26%) had a CEA level ≥ 5 µg/l. There was no significant correlation between CEA levels and gender ($p=0.8$) or age ($p=0.6$). CEA levels were highest in the Black African race group. Pairwise comparison demonstrated a statistically significant difference between the Black and Indian population groups ($p=0.02$). The current study demonstrates an upregulation of CEA as the stage of CRC progresses ($p<0.0001$).

Conclusion: There was no significant difference in CEA levels across age and gender. A positive correlation was noted between CEA level and stage of CRC. CEA levels were highest in the black race group. Low sensitivity of CEA as a screening test for CRC was confirmed.

INTRODUCTION

Colorectal cancer (CRC) is the third most common malignancy in the world (1). The global incidence of CRC is projected to rise up to 60% by 2030 (2, 3). More than one million new cases are diagnosed per annum, and 530000 deaths are reported per year. CRC evolves across four distinct carcinogenic conduits: the chromosomal instability pathway (4), the microsatellite instability pathway (4), the Cytosine-Phosphate-Guanine(CpG) methylator pathway-1 (5) and pathway-2 (6). Importantly the slow growth of CRC warrants early screening to reduce both the incidence of and mortality from the disease. The aim of screening should be to detect CRC at a relatively early stage (stage 1 and 2). However, currently the most reliable diagnostic tool, namely colonoscopy is not easily accessible in resource-deprived settings.

Carcinoembryonic antigen (CEA) is one of the most widely used tumour markers worldwide. It was first described in 1965 by Gold and Freedman (7, 8). CEA is a glycoprotein with a molecular weight of 200 000 (9). It was initially identified and immunolocalized on both fetal colon and colon adenocarcinoma (10). CEA has been found to be overexpressed in a wide variety of epithelial malignancies (11). The literature attributes the elevated CEA levels in CRC to tumour vascularity, necrosis, mitotic activity and differentiation (11). It is thus widely used clinically as both a blood and tissue tumour marker of epithelial malignancy, especially for tumours of the colon and rectum. CEA is also present in normal tissue at levels of $\leq$3 ng/ml, which is 60-fold lower concentration than that seen in malignant tissue (12).

Several studies have implicated high preoperative concentrations of CEA with adverse outcome in patients with Duke's B colorectal cancer (13). More recently, Su et al. (14) demonstrated an overall sensitivity of CEA for the detection of CRC at 37.0%; however, they also found levels to be directly related to stage namely 21.4%, 38.9%, and 41.7% for stages I-III, respectively. These are unacceptably low predictive values, hence the need to rely on colonoscopy as a gold standard. Data on the role of CEA as a prognostic marker for CRC in sub-Saharan Africa are limited (15).

KwaZulu-Natal is a province of South Africa, with an estimated population of 12 million. Despite this large population, only 8 medical facilities offer colonoscopy to the public health care sector, with resultant huge delays in acquiring screening or diagnostic colonoscopies in this resource-deprived setting. Currently, a reliable tumour marker for CRC is unavailable.

In light of the fact that CRC is the fourth most common cancer in South Africa (SA) (16), the aim of this study was to determine whether serum CEA levels in patients symptomatic for lower GI pathology correlate with the histological presence and severity of primary colorectal cancer in a large referral centre within KwaZulu-Natal. Perhaps CEA may have a larger role as a risk assessment tool for the development of CRC in these resource-deprived communities. However thus far, the value of CEA in screening, diagnosis, and prognosis in KwaZulu-Natal remains to be accurately defined.

MATERIALS AND METHODS

Study setting: This study was carried out in the Colorectal Clinic at Inkosi Albert Luthuli Central Hospital, the tertiary referral hospital in Durban, South Africa.

Study Population: The study population (n=380) consisted of patients diagnosed with colorectal cancer who were extracted from the on-going colorectal cancer database which is archived in the Gastrointestinal Cancer Research Centre of the University of KwaZulu-Natal. Patients who were tested for baseline CEA form the basis of this analysis. They included Indian, Black African, White and Coloured patients, as described by the South African Government. CEA levels were compared across various age groups and both genders with colorectal cancer. International Statistical Classification of Diseases and Related Health Problems (10th revision) (ICD-10) diagnosis codes were used to identify colorectal cancer.

Study design: This was a retrospective analysis of prospectively collected clinical data of patients with known baseline CEA levels. Serum was collected from patients with confirmed colorectal cancer but who have not yet had resectional surgery. The period of study was 2007 to 2016. The serum was immediately analysed for CEA levels using the enzyme-linked immunosorbent assay (method). A serum CEA >5 µg/l was considered elevated.

Colonoscopy was performed by gastroenterologists using an Olympus Medical Systems, Tokyo, Japan colonoscope. All participants received four litres of polyethylene glycol solution for bowel preparation. Biopsies were obtained and evaluated by state pathologists via histopathological examination. TNM and UICC staging (Appendix 6) are used in the colorectal cancer database. For the purpose of this paper UICC staging was used. The inclusion criteria for the study comprised patients with histologically confirmed colorectal cancer and staging.

Demographics, site of primary tumour, presence of metastatic disease and CEA level prior to chemotherapy, radiotherapy and/or definitive surgery were collated into a datasheet for statistical analysis.

Ethical considerations

This retrospective clinical study received institutional ethics approval (BE016/17). Additionally, approval was obtained from the National Department of Health and the Hospital manager.

Statistical Analysis

Nonparametric data are represented as median and interquartile range (IQR). GraphPad Prism 5.00 for Windows (GraphPad Software, San Diego, California, USA) was used for data analysis. The Fisher's Exact/Chi Square test was used for analysis. To determine statistical significance across all study groups a Kruskal-Wallis (Dunn's multiple comparison) or a Mann-Whitney U test was carried out. Spearman coefficients were used to correlate CEA levels with the patient demographic as well as the stage of CRC. A p value of < 0.05 was considered as statistically significant.

RESULTS

There was a total of 380 patients with colorectal cancer including 46 (12.11%) younger presenters (age $\leq$ 40 years) and 334 (87.89%) older presenters (age $\leq$ 40 years). The study population (n=380) consisted of Indian (47.6%), Black African (34.5%), White (14.7%) and Coloured (3.2%) patients with a histologically confirmed colorectal cancer. Two hundred and twelve (55.8%) participants were males and 168 (44.2%) were female (Male: Female ratio = 1: 0.79). The mean age of the study population was 57.7 ± 13.6 years.

CEA levels were stratified into < 5 µg/l and ≥ 5 µg/l and is outlined in Table 1. The mean CEA level of the study population was 170.0 ± 623.3 µg/l. The number of participants with a CEA level < 5 µg/l was 151 (39.74%) whilst 229 (60.26%) had a CEA level ≥ 5 µg/l.

Table 1: CEA Level µg/l based on race, age and gender

	Sample size (n)	CEA level*	p value
Race			
Indian	181	155.8 ± 612.9	p= 0.08 #
Black African	131	232.0 ± 742.9	
Coloured	12	98.98 ± 349.7	
White	56	39.66 ± 104.8	
Age			
< 40 Years	46	266.7 ± 736.3	p= 0.60
> 40 Years	334	157.5 ± 607.5	
Gender			
Female	168	219.2 ± 779.0	p= 0.80
Male	212	131.1 ± 462.7	

Legend

* *CEA level in Mean ± Standard deviation*

\# Pairwise comparisons of each race groups vs another one, as well as Black African vs all others (Mann-Whitney Test):

Between all 4 races	p=0.08
Black African vs Indian	p=0.04
Black African vs Coloured	p=0.06
Black African vs White	p=0.13
Black African vs all others	p=0.02
Indian vs Coloured	p=0.23
Indian vs White	p=0.93

CEA levels were evaluated across the study population and there was no significant difference between a CEA level ≥ 5 µg/l and gender (Kruskal-Wallis H = 272.8; p = 0.8; Table 1). CEA levels were higher in females (219.2 ± 77.9 µg/l) compared to males (131.1± 462.7 µg/l). However, this did not reach statistical significance.

CEA levels were highest in Black African patients (232.0 ± 742.9 µg/l) compared to other population groups (Table 1). The difference across the population groups did not reach statistical significance (p=0.08). However, pairwise comparison demonstrated a statistically significant difference between the Black African and Indian population groups (p=0.04). The difference in the CEA levels between the Black African group on the one hand and the other population groups combined on the other, was also statistically significant (p=0.02).

As shown in Figure 1, mean CEA levels declined with increasing age (p= 0.008). There was no significant difference in CEA levels between young presenters (age ≤ 40 years) and older presenters (age > 40years) (p = 0.87). This is well demonstrated in Table 2. Additionally, as the severity of CRC increased, CEA levels increased significantly across all age groups (Figure 2). Table 3 shows the staging in study patients. As shown in Figure 2, an elevated CEA level is associated with a higher stage of CRC (r^2 = 0.054; p < 0.0001). The odds ratio of having a CEA level ≥5µg in stage 4 CRC was 11.28 (CI=4.51 – 28.18; p<0.0001).

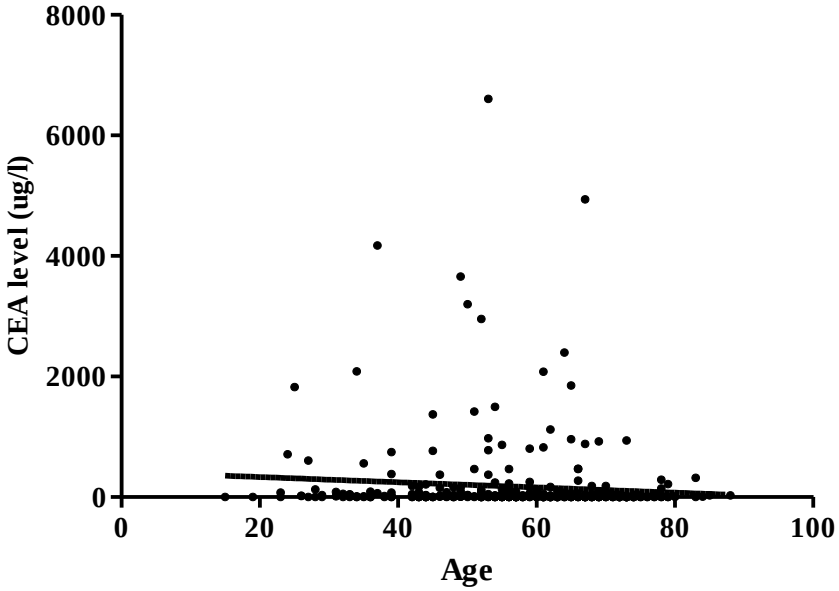

Figure 1: Correlation between the level of CEA (µg/l) and age (r^2 = 0.008; *p* = 0.008). ***Mean CEA levels declined as age increased***

Table 2: Contingency of CEA level (µg/l) in gender, age and race

Gender									
CEA Level (µg/l)	***Male (n = 212)***				***Female (168)***				***Fisher's Exact Test***
	N		%		N		%		
< 5	86		40.56		65		38.69		p = 0.75
>5	126		59.43		103		61.31		
Age									
CEA Level (µg/l)	***< 40 years (n=46)***				***> 40 years (n=334)***				***Fisher's Exact Test***
	N		%		N		%		
< 5	18		39.13		135		40.42		p = 0.87
>5	28		60.87		199		59.58		
Race									
CEA Level (µg/l)	***Indian (n = 181)***		***Black African (n = 131)***		***White (n = 56)***		***Coloured (n = 12)***		***Chi Square Test***
	N	%	n	%	n	%	N	%	X^2 = 2.90,3 *p* = 0.40
<5	75	41.44	45	34.35	25	44.64	6	50	
>5	106	58.57	86	65.65	31	55.36	6	50	

Table 3: Staging in 380 patients with Colorectal Cancer and known preoperative CEA levels

Stage	**n**	**%**
Stage I	24	9
Stage II	59	15.5
Stage III	58	15.3
Stage IV	143	37.6
Not staged	86	22.6

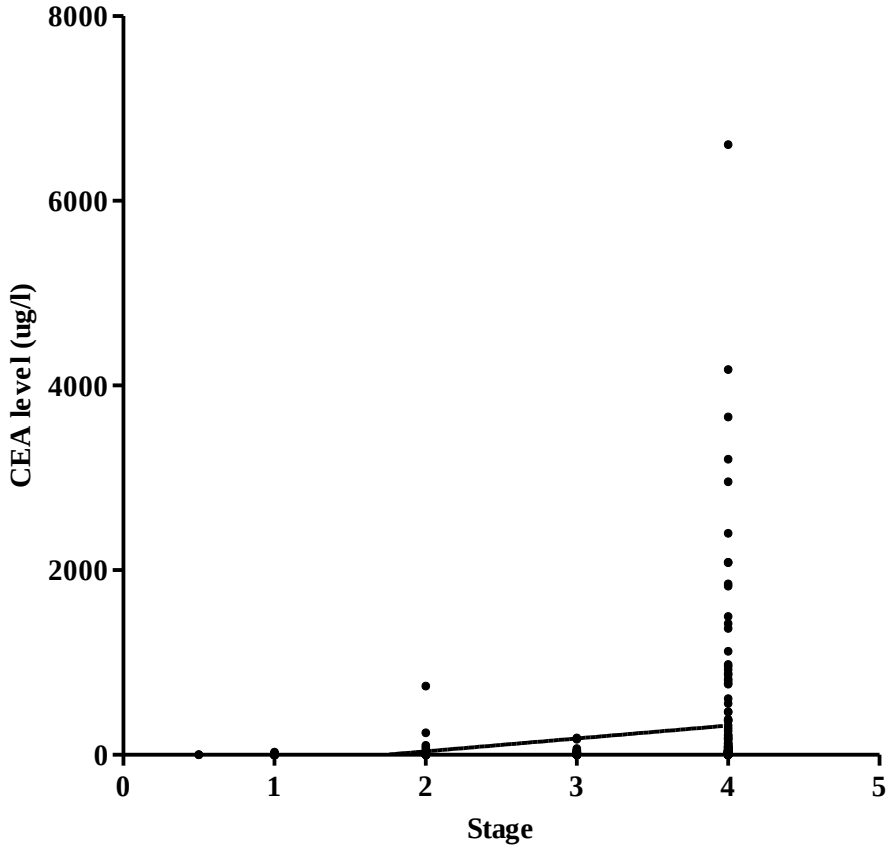

Figure 2: Correlation between the level of CEA (µg/l) and the stage of CRC (r^2 = 0.054; *p* < 0.0001). ***An elevated CEA level is associated with a higher stage of CRC.***

DISCUSSION

The current study demonstrates an upregulation of CEA as the stage of CRC progresses. Whereas age is an established risk factor for the development of CRC (17), we report in this study that there was no significant difference between the CEA levels and age. This is supported by a large Korean study, which showed serum CEA to be a vital risk factor for the development of advanced colorectal neoplasms in both young (< 50 years) and old adults (≥ 50 years) (17).

Internationally the incidence of CRC is reportedly higher in men than in women and strongly increases with age (18). In South Africa, the cumulative lifetime risk of developing CRC amongst all race groups is reported at 1.24 for males and 0.74 for females (16). This series demonstrated no significant difference in the CEA levels between males and female genders.

In the past, colorectal cancer was reported to be an uncommon disease in South African Blacks (20). Westernization of the rural Black African population of South Africa has resulted in an increase in dietary animal fat and protein (meat) intake (21), which are established risk factors for the development of CRC (22) and may explain the observed increase in the diagnosis of colorectal cancer among Black African patients. This study has shown that CEA levels were highest in Black African patients compared to other race groups. While it is tempting to postulate that this is related to the later stage at presentation of the disease in the Black African race group this needs to be validated by further research.

The current study reports a significant positive correlation between baseline CEA levels and the stage of CRC. The clinical implication of this finding is that CEA levels may be used as a prognostic marker for stage IV CRC although it does not appear to be a good marker for stage I,II and III disease. However, the clinical use of CEA as a prognostic marker for stage IV CRC is limited as management at this stage of disease would largely be palliative. These findings are corroborated by the findings of Kim et al which demonstrated that elevated CEA levels correlated well with advanced stages of CRC and a poorer clinical outcomes (17).

Zhang et al have previously postulated that the combined detection of serum CEA and CA 19-9 could play a complementary role in the diagnosis of CRC, and could significantly improve the sensitivity for the diagnosis of CRC (23). CA 19-9 might be a tumour biomarker in addition to CEA for CRC (23). Despite this proposal, we demonstrated in this series that only 60.26% of patients with confirmed CRC had an elevated CEA level. Other authors have also shown a very low sensitivity of this test and concluded that there is no role for CEA assessment as a screening tool for CRC (24). We concede that CEA has no diagnostic role due to its low sensitivity and specificity to CRC. Therefore, in light of the poor access and long delays associated with colonoscopy in the public health care sector of South Africa (25), it is crucial that one is able to better evaluate patients with suspected CRC via non-invasive techniques such as the stool guaiac occult blood test.

Limitations of the present study include a lack of quantitative stratification of groups into smoking and non-smoking sub-groups bearing in mind that CEA concentrations are affected by a

variety of factors including smoking and gender (26). Also, comparison of the White or Coloured race groups was not statistically significant due to the small sample sizes of these groups respectively. Furthermore a potential bias of the sampling technique is that this study only included patients who had a pre-operative level of CEA. Also, patients are often referred to the Colorectal unit after surgical resection with pre-cluded their inclusion in this study.

In conclusion this study reports no significant difference in CEA levels across age or gender. The CEA levels were found to be highest in the Black African race group and the present series confirms a low sensitivity of CEA as a diagnostic test for CRC. Finaly, this study does identify that CEA levels are higher with increased stage of CRC. The clinical implication of this finding is that CEA levels may be be a reasonably good marker for stage IV CRC although it has failed to demonstrate reliability as a risk assessment tool for stage I, II and III disease. Having said this, we concede that the clinical use of CEA as a marker even for stage IV CRC remains limited as management of this stage of disease would largely be palliative. We have therefore not shown the benefit of CEA levels as a risk assessment tool to be used to fast-track symptomatic patients for colonoscopy. For now it is worth exploring the possibility of fast-tracking patients with high CEA to radiological investigations, and, if extensive metastatic disease is found, then a possibility of colorectal cancer can be entertained and thus the need for urgent diagnostic colonoscopy avoided. We further recommend that less invasive approaches be promoted in resource-limited settings including the use of Guaiac faecal occult blood test (gFOBT).

Special attention to improve colorectal cancer screening in African countries are necessary to improve survival rates. Finally, the role of hereditary colon cancer in young Black Africans and its impact on survival remains largely unexplored.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

FUNDING

College of Health Sciences, University of KwaZulu-Natal provided funding for this project

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CHAPTER THREE

Appendix 1-Study protocol as submitted to the Postgraduate Office

The Role of Carcinoembryonic Antigen in predicting colorectal cancer in resource deprived areas

MMed protocol

YD Naicker

206501310

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Title of Study: THE ROLE OF CARCINOEMBRYONIC ANTIGEN IN PREDICTING COLORECTAL CANCER IN RESOURCE POOR SETTING OF KWAZULU-NATAL, SOUTH AFRICA

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Supervisor: Prof TE Madiba (Department of Surgery; Colorectal Surgery, IALCH)

Co-Supervisor: Dr Z Moolla (Department of Surgey; Fellow at Colorectal Surgery, IALCH)

1. ACRONYMS AND ABBREVATIONS

CEA: Carcinoembryonic enzyme assay

CRC: Colo-rectal cancer

Ca 19-9: Cancer antigen 19-9

TPA: Tissue plasminogen activator

IALCH: Inkosi Albert Luthuli Central Hospital

2. BACKGROUND AND PURPOSE OF STUDY

3. AIMS OF THE STUDY

Primary Aim

The aim of this study is to determine whether serum CEA level in patients symptomatic for lower GI pathology correlates with the histological presence of primary CRC.

Secondary Aim

- To correlate CEA levels with patient demographics
- To correlate CEA levels with stage of CRC

Specific objectives of study

- Can CEA be used as a surrogate biomarker to expedite colonoscopy and confirmation of a colorectal cancer in resource poor settings (where there is a shortage of endoscopists and long queues for colonoscopy).

4. LITERATURE REVIEW

4.0 Introduction

Carcinoembryonic antigen (CEA) is one of the most widely used tumour markers worldwide. It was first described in 1965 by Gold and Freedman[1, 2]. It was initially identified and

immunolocalized on both fetal colon and colon adenocarcinoma, but is absent from healthy adult colon [2]. The term CEA was coined because it was only identified in cancer and embryonic colon at the time.

The CEA molecule is an onco-developmental human tumour marker and bears the cluster differentiation designation, CD66[3]. It is a membrane surface glycoprotein immunoglobulin that interacts with the microskeleton of the cell [4, 5]. It is released from the cell surface, into the interstitial space and thereby enters into the general circulation[6].

Colorectal cancer (CRC) is the third most common malignancy in the world. More than one million new cases are diagnosed per annum, and 530000 deaths are reported per year [7]. It's slow growth means that early screening for CRC has the potential to reduce both the incidence and mortality of the disease. However, currently the most reliable screening tool, colonoscopy, lacks access in resource deprived settings.

Kwa-Zulu Natal is a province of South Africa, with an estimated population of 12 million. Despite its large population, only 8 centres offer colonoscopy in the public health care sector, resulting in huge delays in acquiring screening/diagnostic colonoscopies. Perhaps CEA may have a larger role as a risk assessment tool for CRC development in these communities. Thus far the role of CEA in screening, diagnosis, and prognostication remains to be adequately defined. Nonetheless, the use of using CEA as a risk assessment tool (to guide diagnostic investigation) in symptomatic patients has limitations.

4.1 Factors affecting serum CEA levels in patients with CR include:

- Tumour Stage: CEA levels are elevated with increased disease stage. This was shown in an early study in which the proportion of patients with increased CEA concentrations

(>5ug/l) were as follows: Dukes A disease 3%; Dukes B disease 25%; Dukes C disease 45%; and Dukes D disease 65% [8].

- Tumour Grade: Several studies have shown that well differentiated CRC's produce more CEA per gram of total protein than poorly differentiated tumours [9, 10].
- Liver Status: The liver is the primary site of metabolism of CEA. It is initially taken up by the kupffer cells, and modified. Thereafter, it is endocytosed by liver parenchymal cells, where undergoes degradation. Therefore, in conditions where the liver function is impaired such as in certain benign liver diseases, serum CEA levels are increased [11].
- Tumour Site: CRC's located at the left colon generally have increased CEA levels compared to those located on the right [8, 12].
- Smoking increases the CEA value by a factor of two in both males and females [13].

4.2 CEA as a marker for CRC

4.2.1 Screening

The non-specific presentation of an early stage CRC makes clinical detection difficult. Therefore, the aim of screening should be to detect the disease at a relatively early stage (Dukes A or B). CEA concentrations are, in general, more often raised in smokers than in non-smokers, and more frequently elevated in men than in woman [13]. Fletcher *et al* (1986) calculated that CEA has a sensitivity of 36% and specificity of 87% in screening for Dukes stages (A or B) in CRC. The latter study used an upper limit of normal for CEA (2.5ug/l) [14] which rendered the positive predictive value of CEA to be unacceptably low and thus of little value as a screening test for CRC (defined as procedures for detection of disease in asymptomatic individuals). These results

were corroborated by data from a large cohort, population based Framingham *et al* study, which examined CEA levels in serum samples from patients with newly detected CRC[15]. This is also in agreement with recommendations of the National Institution of Health Consensus Development Conference of 1981 [16].

4.2.2 Diagnosis

The lack of sensitivity and specificity limit the application of CEA in diagnosis, especially in early disease [14]. With respect to CRC, although >80% of patients with advanced disease have circulating CEA, the CEA assay alone should not be used as the sole diagnostic test for suspected cancer[17]. Regarding the specificity of CEA in CRC, it must be remembered that CEA is increased in most types of advanced adenocarcinomas as well as multiple benign disorders [17].

More recently, the antigen antibody reactions targeting CEA alone or in combination with other tumour markers, show higher sensitivities in the diagnosis of CRC. A sensitivity of 78% for Dukes stage B and 91% for Dukes stage C has been achieved using this technology in combination with Ca19-9 and TPA levels [18].

4.2.3 Assessing Prognosis

Multiple studies report that patients with high pre-operative levels of CEA have a worse prognostic outcome than those with lower levels [19-20]. Several studies have investigated the prognostic impact of CEA in Dukes A or B disease [21-26]. A predominance of studies report that high CEA levels can predict adverse outcome. However, in a study examining only a subset of

Dukes B stage, the predictor indictor value of CEA was negative [22]. In a similar study, although CEA alone was not prognostic in Dukes B patients, when combined with CA 242, the two markers together yielded significant prognostic information in this subgroup of patients[24]. Therefore, preoperative serum CEA levels can provide prognostic data in patients with Dukes A or B CRC. Carcinoembryonic antigen may thus be able to help identify the subset of patients with early CRC with aggressive disease who may benefit from adjuvant chemotherapy. There is however, currently no evidence to show the benefit of adjuvant chemotherapy based solely on pre-operative CEA levels.

The post-operative use of CEA may also have prognostic value. Evidence suggests that high post-operative levels may also predict adverse outcome [27]. After an oncological resection of a CRC, CEA levels returns to normal values within 4-6 weeks. Failure of the CEA to return to normal within 6 weeks post resection is frequently associated with early recurrent disease[27].

In light of the value of CEA as a screening tool for CRC, this study aims to determine whether serum CEA level correlates with the histological presence of primary CRC.

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5. STUDY SETTING

Inkosi Albert Luthuli Central Hospital (IALCH) -Colorectal clinic

6. STUDY DESIGN

Retrospective review of prospectively collected data

7. STUDY POPULATION

The study population consisted of patients referred to IALCH colorectal clinic between the period 2007-2013.

Inclusion Criteria: histologically confirmed presence of all stages of CRC

Exclusion criteria: absence of CRC

8. SAMPLE SIZE

Consulted institutional biostatistician.

9. SAMPLE COLLECTION

Blood was collected at venepuncture from patients attending the IALCH colorectal clinic between the period 2007-2013, a large central referral hospital in Durban, KwaZulu-Natal, South Africa.

10. STUDY PERIOD

Serum was collected from patients during the period 2007-2013.

11. METHODS AND TOOLS

Patient demographics, date of diagnosis and pre-treatment CEA values will be analysed. Demographics will include age, sex, race, site of primary tumour, presence of metastatic disease, CEA level prior chemotherapy/Radio-therapy or definitive surgery.

Serum CEA levels was analysed using the enzyme linked immunosorbent assay that identified CEA. A serum CEA >5ng/ml will be considered increased.

12. STATISTICAL ANALYSIS:

All statistical analysis was undertaken using GraphPad Prism version 5 (GraphPad software version 5, San Diego, Califonia, USA). To analyse non-normal data, we used non-parametric t-test (Mann-Whitney U). Spearman coefficients were used to evaluate correlations between

biomarkers. A p value of <0.05 was considered as statistically significant. Graphical data represented as median and interquartile range. BREC approved data-base study.

The data collected will be captured and subsequently analysed using the Statistical Package for Social Sciences (SPSS version 24). Descriptive statistics such as frequencies, percentages, means, medians, standard deviations and interquartile ranges will be used to summarize results. The CEA levels will be categorized to indicate whether they are elevated or not and further cross-tabulated with stage of cancer. The results will be presented in tables and graphically using bar graphs.

13. SELECTION BIAS

All patients who present with incisional hernia during the above specified study period may not be covered.

14. DATA ANALYSIS TECHNIQUES

Descriptive data analysis will be conducted using the Microsoft Excel generated spreadsheet.

15. LIMITATIONS TO THE STUDY

All patient's medical records may not be found, particularly if index abdominal surgery was done at other hospitals either than Addington hospital.

16. FUNDING

No funding is required

17. ETHICAL CONSIDERATIONS

Informed patient consent was obtained for collection of the samples and use for statistical analysis. The exclusive use of recorded data does not affect the clinical outcomes of the patients involved. The confidentiality of the patient will be maintained at all times.

18. INSTITUTIONAL ETHICAL REVIEW BOARD

The study protocol will be submitted to the Bio- medical Research and Ethical Committee for review.

19. PERMISSIONS

Approval has been obtained from the Inkosi Albert Luthuli Central Hospital and the Kwazulu Natal Department.

Appendix 2- DOHET approved journal guidelines for submission of manuscript

Submissions

- Online Submissions
- Author Guidelines
- Copyright Notice
- Privacy Statement

Online Submissions

Already have a Username/Password for South African Journal of Surgery?
GO TO LOGIN

Need a Username/Password?
GO TO REGISTRATION

Registration and login are required to submit items online and to check the status of current submissions.

Author Guidelines

Accepted manuscripts that are not in the correct format specified in these guidelines will be returned to the author(s) for correction, and will delay publication.

AUTHORSHIP

Named authors must consent to publication. Authorship should be based on substantial contribution to:

(i) conception, design, analysis and interpretation of data;

(ii) drafting or critical revision for important intellectual content; and

(iii) approval of the version to be published. These conditions must all be met (uniform requirements for manuscripts submitted to biomedical journals; refer to www.icmje.org).

CONFLICT OF INTEREST

Authors must declare all sources of support for the research and any association with a product or subject that may constitute conflict of interest.

RESEARCH ETHICS COMMITTEE APPROVAL

Provide evidence of Research Ethics Committee approval of the research where relevant.

PROTECTION OF PATIENT'S RIGHTS TO PRIVACY

Identifying information should not be published in written descriptions, photographs, and pedigrees unless the information is essential for scientific purposes and the patient (or parent or guardian) gives informed written consent for publication. The patient should be shown the manuscript to be published. Refer to www.icmje.org.

ETHNIC CLASSIFICATION References to ethnic classification must indicate the rationale for this.

MANUSCRIPTS Shorter items are more likely to be accepted for publication, owing to space constraints and reader preferences.

Original articles not exceeding 3 000 words, with up to 6 tables or illustrations, are usually observations or research of relevance to surgery. References should preferably be limited to no more than 15. Please provide a structured abstract not exceeding 250 words, with the following recommended headings: *Background, Objectives, Methods, Results,* and *Conclusion.*

Scientific letters/short reports, which include case reports, side effects of drugs and brief or negative research findings should preferably be 1500 words or less, with 1 table or illustration and no more than 6 references. Please provide an accompanying abstract not exceeding 150 words.

Editorials, Opinions, etc. should be about 1000 words and are welcome, but unless invited, will be subjected to the SAJS peer review process.

Review articles are rarely accepted unless invited.

Letters to the editor, for publication, should be about 400 words with only one illustration or table, and must include a correspondence address.

Obituaries should be about 400 words and may be accompanied by a photograph.

MANUSCRIPT PREPARATION Refer to articles in recent issues for the presentation of headings and subheadings. If in doubt, refer to 'uniform requirements' - www.icmje.org. Manuscripts must be provided in **UK English**.

Qualification, affiliation and contact details of ALL authors must be provided in the manuscript and in the online submission process.

Abbreviations should be spelt out when first used and thereafter used consistently, e.g. 'intravenous (IV)' or 'Department of Health (DoH)'.

Scientific measurements must be expressed in SI units except: blood pressure (mmHg) and haemoglobin (g/dl). Litres is denoted with a lowercase 'l' e.g. 'ml' for millilitres). Units should be preceded by a space (except for %), e.g. '40 kg' and '20 cm' but '50%'. Greater/smaller than signs (> and 40 years of age'. The same applies to ± and °, i.e. '35±6' and '19°C'.

Numbers should be written as grouped per thousand-units, i.e. 4 000, 22 160...

Quotes should be placed in single quotation marks: i.e. The respondent stated: '...' Round **brackets** (parentheses) should be used, as opposed to square brackets, which are reserved for denoting concentrations or insertions in direct quotes.

General formatting The manuscript must be in Microsoft Word or RTF document format. Text must be single-spaced, in 12-point Times New Roman font, and contain no unnecessary formatting (such as text in boxes, with the exception of Tables).

ILLUSTRATIONS AND TABLES If tables or illustrations submitted have been published elsewhere, the author(s) should provide consent to republication obtained from the copyright holder.

Tables may be embedded in the manuscript file or provided as '**supplementary files**'. They must be numbered in Arabic numerals (1,2,3...) and referred to consecutively in the text (e.g. 'Table 1'). Tables should be constructed carefully and simply for intelligible data representation. Unnecessarily complicated tables are strongly discouraged. Tables must be cell-based (i.e. not constructed with text boxes or tabs), and accompanied by a concise title and column headings. Footnotes must be indicated with consecutive use of the following symbols: * † ‡ § ¶ || then ** †† ‡‡ etc.

Figures must be numbered in Arabic numerals and referred to in the text e.g. '(Fig. 1)'. Figure legends: Fig. 1. 'Title...' All illustrations/figures/graphs must be of **high resolution/quality**: 300 dpi or more is preferable but images must not be resized to increase resolution. Unformatted and uncompressed images must be attached as '**supplementary files**' upon submission (not embedded in the accompanying manuscript). TIFF and PNG formats are preferable; JPEG and PDF formats are accepted, but authors must be wary of image compression. Illustrations and graphs prepared in Microsoft Powerpoint or Excel must be accompanied by the original workbook.

REFERENCES Authors must verify references from the original sources. *Only complete, correctly formatted reference lists will be accepted.* Reference lists must be generated manually and **not** with the use of reference manager software. Citations should be inserted in the text as superscript numbers between square brackets, e.g. These regulations are endorsed by the World Health Organization,[2] and others.[3,4-6] All references should be listed at the end of the article in numerical order of appearance in the **Vancouver style** (not alphabetical order). Approved abbreviations of journal titles must be used; see the List of Journals in Index Medicus. Names and initials of all authors should be given; if there are more than six authors, the first three names should be given followed by et al. First and last page, volume and issue numbers should be given. **Wherever possible, references must be accompanied by a digital object identifier (DOI) link and PubMed ID (PMID)/PubMed Central ID (PMCID).** Authors are encouraged to use the DOI lookup service offered by CrossRef.

Journal references: Price NC, Jacobs NN, Roberts DA, et al. Importance of asking about glaucoma. Stat Med 1998;289(1):350-355. [http://dx.doi.org/10.1000/hgjr.182] [PMID: 2764753]

Book references: Jeffcoate N. Principles of Gynaecology. 4th ed. London: Butterworth, 1975:96-101. *Chapter/section in a book:* Weinstein L, Swartz MN. Pathogenic Properties of Invading Microorganisms. In: Sodeman WA jun, Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: WB Saunders, 1974:457-472.

Internet references: World Health Organization. The World Health Report 2002 - Reducing Risks, Promoting Healthy Life. Geneva: World Health Organization, 2002. http://www.who.int/whr/2002 (accessed 16 January 2010).

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2. The submission has not been previously published, nor is it before another journal for consideration.
3. The text complies with the stylistic and bibliographic requirements in **Author Guidelines**.
4. The manuscript is in Microsoft Word or RTF document format. The text is single-spaced, in 12-point Times New Roman font, and contains no unnecessary formatting.
5. Illustrations/figures are high resolution/quality (not compressed) and in an acceptable format (preferably TIFF or PNG). These must be submitted as 'supplementary files' (not in the manuscript).
6. For illustrations/figures or tables that have been published elsewhere, the author has obtained written consent to republication from the copyright holder.
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8. An abstract has been included where applicable.
9. The research was approved by a Research Ethics Committee (if applicable)
10. Any conflict of interest (or competing interests) is indicated by the author(s).

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Appendix 3- Institutional Ethics Approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

25 August 2017

Dr YD Naicker (206501310)
Discipline of Surgery
School of Clinical Medicine
yugandylannaicker@gmail.com

Dear Dr Naicker

Protocol: The role of carcinoembryonic antigen in predicting colorectal cancer in resource deprived areas.
Degree: MMed **BREC reference number: BE016/17**

EXPEDITED APPROVAL

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 21 December 2016.

The study was provisionally approved pending appropriate responses to queries raised. Your response received on 07 August 2017 to BREC correspondence dated 16 February 2017 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given **full ethics approval** and may begin as from 25 August 2017.

This approval is valid for one year from **25 August 2017.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **RATIFIED** by a full Committee at its next meeting taking place on **10 October 2017.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor V Rambiritch
Deputy Chair: Biomedical Research Ethics Committee

cc supervisor: drzmolla@hotmail.co.za
cc postgraduate administrator: ianti...@ukzn.ac.za

Appendix 4- Department of Health Approval to conduct the study

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

Physical Address: 330 Langalibalele Street, Pietermaritzburg
Postal Address: Private Bag X9051
Tel: 033 395 2805/ 3189/ 3123 Fax: 033 394 3782
Email:
www.kznhealth.gov.za

DIRECTORATE:
Health Research & Knowledge Management

HRKM Ref: 271/17
NHRD Ref: KZ_2017RP0_386

Date: 3 August 2017
Dear Dr Y Naicker
UKZN

Approval of research

1. The research proposal titled **'The Role of Carcinoembryonic Antigen in predicting colorectal cancer in resource deprived areas'** was reviewed by the KwaZulu-Natal Department of Health.

   The proposal is hereby **approved** for research to be undertaken at Inkosi Albert Luthuli Central Hospital.

2. You are requested to take note of the following:
   a. Make the necessary arrangement with the identified facility before commencing with your research project.
   b. Provide an interim progress report and final report (electronic and hard copies) when your research is complete.

3. Your final report must be posted to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za

For any additional information please contact Mr X. Xaba on 033-395 2805.

Yours Sincerely

Dr E Lutge
Chairperson, Health Research Committee
Date: 07/08/17

Fighting Disease, Fighting Poverty, Giving Hope

Appendix 5: Duke's staging for Colorectal Cancer

- **Dukes A:** invasion into but not through the bowel wall
- **Dukes B:** invasion through the bowel wall but not involving lymph nodes
- **Dukes C:** involvement of lymph nodes
- **Dukes D:** widespread metastases

Appendix 6: UICC (Union for International Cancer Control) staging for Colorectal Cancer

Primary tumor (pT)

- **TX:** primary tumor cannot be assessed
- **T0:** no evidence of primary tumor
- **Tis:** carcinoma in situ, intramucosal carcinoma (involvement of lamina propria with no extension through muscularis mucosae)
- **T1:** tumor invades submucosa (through the muscularis mucosa but not into the muscularis propria)
- **T2:** tumor invades muscularis propria
- **T3:** tumor invades through the muscularis propria into the pericolorectal tissues
- **T4:**
    - **T4a:** tumor invades through the visceral peritoneum (including gross perforation of the bowel through tumor and continuous invasion of tumor through areas of inflammation to the surface of the visceral peritoneum)
    - **T4b:** tumor directly invades or adheres to other adjacent organs or structures

Regional lymph nodes (pN)

- **NX:** regional lymph nodes cannot be assessed
- **N0:** no regional lymph node metastasis
- **N1:** metastasis in 1 - 3 regional lymph nodes
    - **N1a:** metastasis in 1 regional lymph node
    - **N1b:** metastasis in 2 - 3 regional lymph nodes
    - **N1c:** no regional lymph nodes are positive but there are tumor deposits in the subserosa, mesentery or nonperitonealized pericolic or perirectal / mesorectal tissues
- **N2:** metastasis in 4 or more regional lymph nodes

- **N2a:** metastasis in 4 - 6 regional lymph nodes
- **N2b:** metastasis in 7 or more regional lymph nodes

Distant metastasis (pM)

- **M0:** no distant metastasis by imaging; no evidence of tumor in other sites or organs (this category is NOT assigned by pathologists)
- **M1:** distant metastasis
  - **M1a:** metastasis confined to 1 organ or site without peritoneal metastasis
  - **M1b:** metastasis to 2 or more sites or organs is identified without peritoneal metastasis
  - **M1c:** metastasis to the peritoneal surface is identified alone or with other site or organ metastases

Stage grouping

Stage 0:	Tis	N0	M0
Stage I:	T1 - T2	N0	M0
Stage IIA:	T3	N0	M0
Stage IIB:	T4a	N0	M0
Stage IIC:	T4b	N0	M0
Stage IIIA:	T1 - T2	N1 / N1c	M0
	T1	N2a	M0
Stage IIIB:	T3 - T4a	N1 / N1c	M0

	T2 - T3	N2a	M0
	T1 - T2	N2b	M0
Stage IIIC:	T4a	N2a	M0
	T3 - T4a	N2b	M0
	T4b	N1 - N2	M0
Stage IVA:	any T	any N	M1a
Stage IVB:	any T	any N	M1b
Stage IVC:	any T	any N	M1c