Research Square

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Neutrophil to Lymphocyte Ratio: a useful predictor of amputation in patients with Necrotizing fasciitis: Diagnostic accuracy study

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Research Article

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Abstract

Purpose: Necrotizing fasciitis is the rapidly progressive inflammation of fascia, with necrosis of subcutaneous tissues and subsequent high morbidity and even mortality. Neutrophil to Lymphocyte Ratio, Systemic Immune-inflammation Index and Platelet to Lymphocyte Ratio are recently being used as severity indicators in inflammatory conditions. This study attempted to find the usefulness of these three ratios as predictors of amputation in necrotizing fasciitis.

Methods: This was a cross sectional study in a tertiary level teaching institution in South India, from January 2018 until January 2019. 175 patients with clinical diagnosis of Necrotizing Fasciitis were studied and data analysed to find out the diagnostic accuracy of relevant study parameters.

Results: 13 out of the 175 patients had to undergo amputation. Diabetes mellitus, infection with *Klebsiella* and *E.coli,* low serum albumin and high blood glucose were found to be the most common factors associated with amputation. Neutrophil to Lymphocyte Ratio had acceptable sensitivity and very good specificity. Neutrophil to Lymphocyte Ratio also had an area under curve of 0.726. Multivariate logistic regression revealed Neutrophil to Lymphocyte Ratio to be the primary hazard factor with an Odds Ratio of 9.64.

Conclusion: Neutrophil to Lymphocyte Ratio and to some extent Platelet to Lymphocyte Ratio and Systemic Immune-inflammation Index are good predictors of amputation in patients with Necrotizing fasciitis. These simple and easily available laboratory tests can be included in the diagnostic panel for these patients and to titrate the treatment.

Introduction

Necrotizing fasciitis (NF) refers to a rare but severe form of infection which is characterised by rapidly spreading inflammation along the fascial planes as well as varying degrees of necrosis extending to the skin, subcutaneous tissues, and superficial fascia [1]. Because of its rapidity of spread and aggressive nature of infection, delayed diagnosis or treatment for NF usually contributes to varying extent of prolonged morbidity and very high mortality. The diagnosis is confirmed during surgical treatment itself; that is, by visual inspection of the necrotic fascia while doing fasciotomy. However, the need for surgical management and subsequent morbidity and other after-effects warrants the need for laboratory investigations which can act as clues for diagnosing NF.

Neutrophil to Lymphocyte Ratio (NLR) and Platelet to Lymphocyte Ratio (PLR) are two values; calculated from the peripheral neutrophil and lymphocyte counts and the peripheral platelet and lymphocyte counts respectively. The Systemic Immune-inflammation Index (SII) on the other hand integrates peripheral lymphocyte count, neutrophil count and platelet count into one indicator. All of these ratios can indicate the balance between the patient's inflammatory and immune status. The NLR, PLR and SII are low-cost measures which can be easily calculated from frequently used laboratory parameters and are also easily repeatable.

NLR is frequently employed in various conditions, such as to predict outcomes in cardiovascular patients, in-hospital mortality in septic patients, and also poorer prognosis and higher ICU admissions in acute pancreatitis [2–5]. NLR and to some extent PLR are used in cardiovascular diseases, oncology, diabetes, rheumatology etc for evaluating the inflammatory state and assessing the prognosis [6–14]. It is postulated that high NLR is indicative of an imbalance in the inflammatory response. Inflammatory factors which are related to infection, like interleukin-6(IL-6), interleukin-8(IL-8), and granulocyte colony-stimulating factor(GMCSF) could potentially stimulate neutrophil production [15]. On the other hand, systemic inflammation promotes lymphocyte apoptosis, attenuates cellular immunity, suppresses CD4 + cells, and stimulates CD8 + suppressor T-lymphocytes [16]. Recent studies have found that NLR levels were higher in patients with severe Covid-19 disease and were suggested as measures to confer a prognostic value amidst the pandemic [17–20].

It was in 2014 that Hu et al developed an indicator called the Systemic Immune-inflammation Index(SII) to predict the prognosis in hepatocellular carcinoma patients after curative resection [21]. This ratio incorporated the neutrophil, lymphocyte and platelet counts into a single index so as to reflect the significance of all three. Their hypothesis was that a high SII score(which was set at 330×109 cells/L) indicates poor outcome in these patients and found it to be an able predictor for 1-year survival and even tumour differentiation. There is some recently published evidence that also suggests that SII is an appropriate independent prognostic indicator for many cancer patients [22–25]. Studies in acute limb ischemia patients also show that these ratios can predict limb loss and death and can be used as a guide for preoperative stratification of risk and management of these patients [26, 27].

Because of the limited support from published literature these ratios are seldom used in decision making in various clinical situations, Also, their role is not yet studied in the setting of Necrotizing fasciitis. With this background, this study was formulated to assess the value of preoperative inflammatory biomarkers, namely the NLR, PLR and the SII, for predicting the 30-day death or amputation in patients admitted with a diagnosis of Necrotizing fasciitis.

Materials And Methods

This study was carried out at a high volume tertiary level teaching institute. The primary objective of the study was to assess the predictive accuracy of NLR, PLR and SII in predicting amputation among patients admitted to the General Surgical wards with Necrotizing Fasciitis. The current research was designed as a retrospective diagnostic test evaluation study and carried out for a period of 1 year from 1st January 2018 to 1st January 2019. 175 patients who were already part of the cohort of Necrotizing fasciitis were included in this research. Being a retrospective analysis of data, specific patient consent was not sought for as they had already given consent for secondary analysis of data.

As per the earlier study protocol, visual confirmation of necrosed fascia was done by a senior Consultant Surgeon. As part of the present study, patients' data was collected from their medical records to analyse the study variables. Consecutive patients admitted with a suspected diagnosis of Necrotizing fasciitis

and subsequently confirmed while doing fasciotomy were selected as the study subjects. Adult patients aged above 18 years were included. We excluded patients with potential risk of confounders for NLR and PLR analysis: necrotizing fasciitis secondary to trauma, any cardiovascular or cerebro-vascular event in the preceding six months, known active infections, known renal replacement therapy, active malignancies, hematopoietic disorders, use of anti-inflammatory medication within the preceding three months, hepatic failure, history of splenectomy or recent radiation.

Sensitivity of the reference test, that is confirmation of necrotizing fasciitis by visual assessment during fasciotomy was set as 100. The demographic characteristics, other medical records, preoperative laboratory data and operation records were analysed retrospectively. The laboratory parameters were checked for all patients and the values entered into data sheets. The data was analysed to calculate the three ratios for all the patients. NLR was calculated from values at admission by dividing the level of peripheral blood neutrophils (N) to lymphocytes (L) per litre. PLR was calculated from values at admission by dividing the value of peripheral blood platelets (P) to lymphocytes (L) per litre. The SII was calculated from these values according to the equation: SII = P x N/L. Mean normal values for the three ratios was selected as per available literature evidence : for NLR, the value was set at 1.76, for PLR, the cut-off was set at 120 and for SII, the value was set as 459 [28].

The study is reported as per the the STAndards for the Reporting of Diagnostic accuracy studies(STARD) criteria and the pertinent checklist is included [29]. Continuous variables are presented as mean with Standard Deviation(SD) and categorical variables are presented as frequencies with percentages. The Pearson's $\chi 2$ (Chi-squared) test was used for comparing the differences between the variables. The Mann-Whitney U test was applied for non-parametric data analysis. From the data, true positives, true negatives, false positives and false negatives were calculated and then 2 x 2 tables were constructed to determine sensitivity, specificity, positive predictive value and negative predictive values. Any correlation between the three ratios was tested by Pearson's correlation test. The predictive values of NLR, NLR and SII for the primary endpoint were also evaluated by calculating the area under the curve (AUC) from the receiver operating characteristic (ROC) curves. The Youden index was then calculated to determine optimal cut-off values for each ratio. Statistical analysis was carried out with Microsoft Office Excel and easyROC software version 1.3.1 [30]. Significance is reported wherever p is less than 0.05.

Results

Among the study group, the mean age was 58.27 years +/- 13.79. The youngest patient was 22 years old and the oldest was 94. There were 124 males (70.86%) and 51 females (29.14%) in the study group, with nobody from the third gender. 123 patients had some form of significant comorbidity. Diabetes mellitus was the commonest co-morbidity, 94 patients having this condition (53.71%). 56 patients had a history of cigarette smoking while 58 had a history of alcohol abuse. The mean duration since onset of symptoms was found to be 8.59 +/- 7.79 days. 89 patients out of the 175 had a positive isolate on culture. *Pseudomonas* was seen in 16 patients, *Klebsiella* in 11, *Staphylococcus* in 7, *E.coli* in 7, *Acinetobacter* in 5, *Enterococcus* in 5, MRSA in 4, *Streptococcus* in 3 and *Proteus* in 2. Mixed bacterial

growth was seen in 25 patients. 9 patients had to be admitted in ICU. 45 patients developed MODS. The mean duration of hospital stay was 16.35 +/- 14.56 days. The stay duration varied from 1 to 74 days. 40 patients expired while in the hospital.

13 patients had to undergo amputation. Diabetes mellitus was found to be a strong association with amputation [Table 1]. Culture positivity with *Klebsiella* and *E.coli* species were also found to be associated with amputation. Among the laboratory values, blood glucose levels and albumin levels were also found to be associated with amputation. However, there was no association between amputation and other factors including gender, other comorbidities, cigarette smoking, and alcohol abuse.

The mean value of NLR was 1.5 +/- 0.33. The lowest value was 1.0 while the highest was 2.7. Out of the 175, 38 patients had an elevated NLR value. PLR had a mean value of 46.24 +/- 46.27. The lowest value was 2.89 while the highest was 309.72. Among the 175, 12 patients had an elevated PLR. The mean value of SII was 385.4 +/- 229.83. The lowest value was 41.5 while the highest was 1238.49. Among the patients, 54 had elevated SII. NLR and PLR had a very weak positive correlation with a Pearson correlation coefficient of 0.0089 only (p value 0.91). NLR and SII had a weak positive Pearson correlation with a correlation coefficient of 0.2571 (p value 0.00059). PLR had a strong correlation with SII, with a Pearson coefficient of 0.6224 (p value < 0.00001).

NLR had a sensitivity of 46.15%, specificity of 80.25%, positive likelihood ratio of 2.34, negative likelihood ratio of 0.67, positive predictive value of 15.79%, negative predictive value of 94.89% and overall accuracy of 77.71%. For PLR, the sensitivity was 0%, specificity was 92.59%, positive likelihood ratio was 0, negative likelihood ratio was 1.08, positive predictive value was 0, negative predictive value was 92.02% and the overall accuracy was 85.71%. SII was found to have a sensitivity of 38.46%, specificity of 69.75%, positive likelihood ratio of 1.27, negative likelihood ratio of 0.88, positive predictive value of 9.26%, negative predictive value of 93.39% and overall accuracy of 67.43%.

The Area Under Curve for NLR was calculated to be 0.726 while that for PLR was calculated to be 0.520 and that for SII was calculated to be 0.544 [Table 2]. The optimal cut-off values for achieving maximum sensitivity and specificity for NLR was calculated to be 1.45 while that for PLR was calculated to be 32.53 and for SII it was found to be 249.772. Multivariate logistic regression revealed NLR to be the primary hazard factor with an Odds Ratio of 9.64. The model was found to be a good fit with a Chi-square value of 18.8929 (p value 0.0003) [Table 3]. The regression equation was found to be T1= 6.3040 + 2.2666 NLR + 0.008570 Glucose - 0.7464 Albumin.

Discussion

As per the results of this study, Neutrophil-to-lymphocyte ratio was found to be a strong predictor of the need for amputation. In the study conducted by Tasoglu et al, a preoperative NLR value of more than 5.2 was detected to be a stand alone predictive factor of amputation in patients with acute limb ischemia [26]. In the study by Demirtas et al, NLR was found to correlate well with severity in peripheral arterial disease [33]. Yepici et al evaluated the need for amputation in patients with diabetic foot infection and

found NLR to be a strong predictor [34]. Dinc et al showed that a higher NLR translated to higher mortality in patients who had to undergo amputation [35]. Spark et al also found that higher NLR level is associated with greater mortality in patients presenting with chronic critical limb ischemia [36].

Neutrophilic infiltrate can cause endothelial damage by releasing various inflammatory mediators and proteolytic enzymes. On the contrary lymphocytes can modify the effect of neutrophils and they also have an anti-atherosclerotic role. Endothelial damage is reported to cause worse outcomes in diabetic wounds. [37]. Lymphocyte activity can augment the collateral circulation and thus could be associated with increased possibility of limb salvage. Lymphocytes also have a modulatory effect on the inflammatory response and augment rate of tissue repair by stimulating production of interleukin-10 [38]. The protective activity of lymphocytes might explain the lower NLR and PLR in those patients with limb ischemia who improve.

The current study also demonstrated the usefulness of PLR and SII as diagnostic markers. In the study by Erdogan et al, NLR and PLR were found to reduce in patients with acute limb ischemia who improve with medical treatment [39]. Saskin et al in their study reported that increased NLR and PLR are found to be associated with extremity amputation in acute arterial occlusions [27]. Gary et al demonstrated that elevated NLR (with a cut off 3.95) and elevated PLR (with a cut off 150) were associated with very high risk for critical limb ischemia in patients with peripheral arterial disease [31, 32]. In the research by Chen et al, it was found that although PLR is an independent risk factor for higher mortality, NLR was considerably more sensitive and also a better marker on the ROC curve [40].

Naturally, higher platelet counts are associated with increased platelet activity [41]. Literature shows that increased platelet activity potentially reflects exaggerated release of inflammatory mediators which in turn promotes destructive inflammatory processes [42]. Higher levels of platelets represent increased thrombosis and also accelerated release of mediators which enhance atherosclerosis and inflammation. It is proven that platelet hyperactivity along with thrombosis plays the major role in the patho-physiology of atherogenesis and thus indirectly contributes to illness and death [43]. Moreover, inflammatory mediators can induce thrombocytosis by stimulating megakaryocytes and therefore, increased levels of platelet may be indicative of pro-thrombotic activity and ongoing inflammatory processes [44].

However, literature search revealed some studies which found a limited role for NLR and PLR also. In a study on hand osteomyelitis, Wyman et al found C-reactive protein(CRP) to be the most sensitive marker and a CRP value greater than 100 mg/L was found strongly associated with sepsis. Total leucocyte count, NLR and also PLR were found to be of limited usefulness in this series [45].

To summarise, when compared with other laboratory parameters used to predict as prognostic markers in inflammation like erythrocyte sedimentation rate, interleukin-6, C-reactive protein and D-dimer levels, NLR is a useful and more practical marker for clinical application. Due to its very low cost and being easily obtained in routine blood tests without any need for special assay equipment, NLR has the potential to be a simple, near real-time, accessible, as well as cost-effective biomarker, especially in the setting of healthcare facilities with limited medical resources.

This study has some limitations. First, being a single centre study with a relatively limited number of subjects, potential presence of selection bias is there. However, we did attempt to reduce such a bias by applying strict inclusion as well as exclusion criteria. Also, because of the retrospective study design, it was impossible to standardise the process or timing of blood collection and hence, there was a potential for disparity between patients. Also, it is worth mentioning that a comparison between the prognostic significance of NLR, PLR and SII and other markers of inflammation or ischaemia- injury like interleukin 6, C-reactive protein, myoglobin or creatinine kinase, would have added additional value.

Conclusion

This is the first study that attempted to investigate the role of Neutrophil to Lymphocyte Ratio, Platelet to Lymphocyte Ratio and Systemic Immune-inflammation Index in predicting amputation among patients with Necrotizing fasciitis. We found that increased Neutrophil to Lymphocyte Ratio and to some extent Platelet to Lymphocyte Ratio and Systemic Immune-inflammation Index are reliable predictive biomarkers of amputation. Our study findings support calculating Neutrophil to Lymphocyte Ratio to perform early stratification of risk in clinical settings. Further prospective studies can be modelled on our findings to establish the role of these ratios. Considering the high complication rates in necrotizing fasciitis, we propose using Neutrophil to Lymphocyte Ratio to predict chances for amputation as well as to prognosticate so that patients with elevated values may be offered more intensive or longer duration of therapy with an aim to control the risk factors aggressively.

Declarations

Competing Interests: None

Acknowledgements: None

Authors' Contributions

Study conception and design: PP, IPY

Acquisition of data: PP, IPY

Analysis and Interpretation of data: MCM, IPY

Drafting of manuscript: PP, VS, MCM

Critical revision of manuscript: VS, IPY

Compliance with Ethical Standards

Declaration of interest statement

- Disclosure of potential conflicts of interest: The authors report there are no competing interests to declare
- Research involving Human Participants and/or Animals: The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki. This research study was conducted retrospectively from data obtained for clinical purposes.
- Informed consent: Not taken separately as the study was a retrospective analysis of data

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Tables

Table 1

General Characteristics of 175 patients with Necrotizing fasciitis

Variable	Total	Amputation	No Amputation	Value	p value
	n=175	n=13	n=162		
Age, n(%)	58.27 +/- 13.79	58.54 +/- 12.01	58.25 +/- 13.96	-0.1109	0.9124
Gender, n(%)					
Male	124(70.86)	7(53.85)	117(66.86)	1.97	1.6
Female	51(29.14)	6(46.15)	45(25.71)		
Habits, n(%)					
Smoking	56(32)	3(23.07)	53(32.72)	0.5139	0.473
Alcohol	58(33.14)	3(23.07)	55(33.95)	0.6422	0.4229
Comorbidity, n(%)					
Diabetes	94(53.71)	12(92.31)	82(50.62)	8.4131	0.003*
Coronary disease	20(11.42)	2(15.38)	18(11.11)	0.2171	0.6412
Hypertension	48(27.42)	5(38.46)	43(26.54)	0.8588	0.3541
Renal disease	18(10.29)	1(7.69)	17(10.49)	0.1024	0.749
Culture result					
Pseudomonas	16(9.14)	2(15.38)	14(8.64)	0.6586	0.417
Klebsiella	11(6.28)	3(23.07)	8(4.94)	8.7766	0.003*
E.coli	7(4)	2(15.38)	5(3.09)	4.7399	0.0295*
Lab values					
Haemoglobin(g/dl)	10.69 +/- 2.11	10.4 +/- 1.67	10.71 +/- 2.14	0.7112	0.4777
Platelet(lakhs/microl)	2.6 +/- 1.46	2.52 +/- 1.43	2.61 +/- 1.46	0.2816	0.7795
Leucocyte(thousands/microl)	19.24 +/- 8.28	18962.31 +/- 6093.59	19260.31 +/- 8450.35	-0.0853	0.9283
Urea(mg/dl)	78.79 +/- 62.77	67.08 +/- 39.62	79.73 +/- 64.25	0.0597	0.952
Creatinine(mg/dl)	2.21 +/- 2.07	1.57 +/- 0.81	2.26 +/- 2.14	0.4609	0.6455

Albumin(g/dl)	2.49 +/- 0.52	2.24 +/- 0.41	2.51 +/- 0.53	2.1167	0.034*
Blood Glucose(mg/dl)	181.89 +/- 115.65	291.23 +/- 142.25	173.11 +/- 109.1	-2.8876	0.0038*
Study ratios					
NLR	1.5 +/- 0.33	1.67 +/- 0.26	1.48 +/- 0.33	-2.7027	0.0069*
PLR	46.24 +/- 46.27	39.29 +/- 21.69	46.79 +/- 47.69	-0.2361	0.8103
SII	385.4 +/- 229.83	429.63 +/- 272.89	381.84 +/- 226.64	-0.5263	0.5961

Data are presented as mean +/- standard deviation (SD) or the absolute number.

NLR - neutrophil to lymphocyte ratio, PLR - neutrophil to lymphocyte ratio, SII - Systemic Immune-inflammation Index . *statistically significant association

Table 2

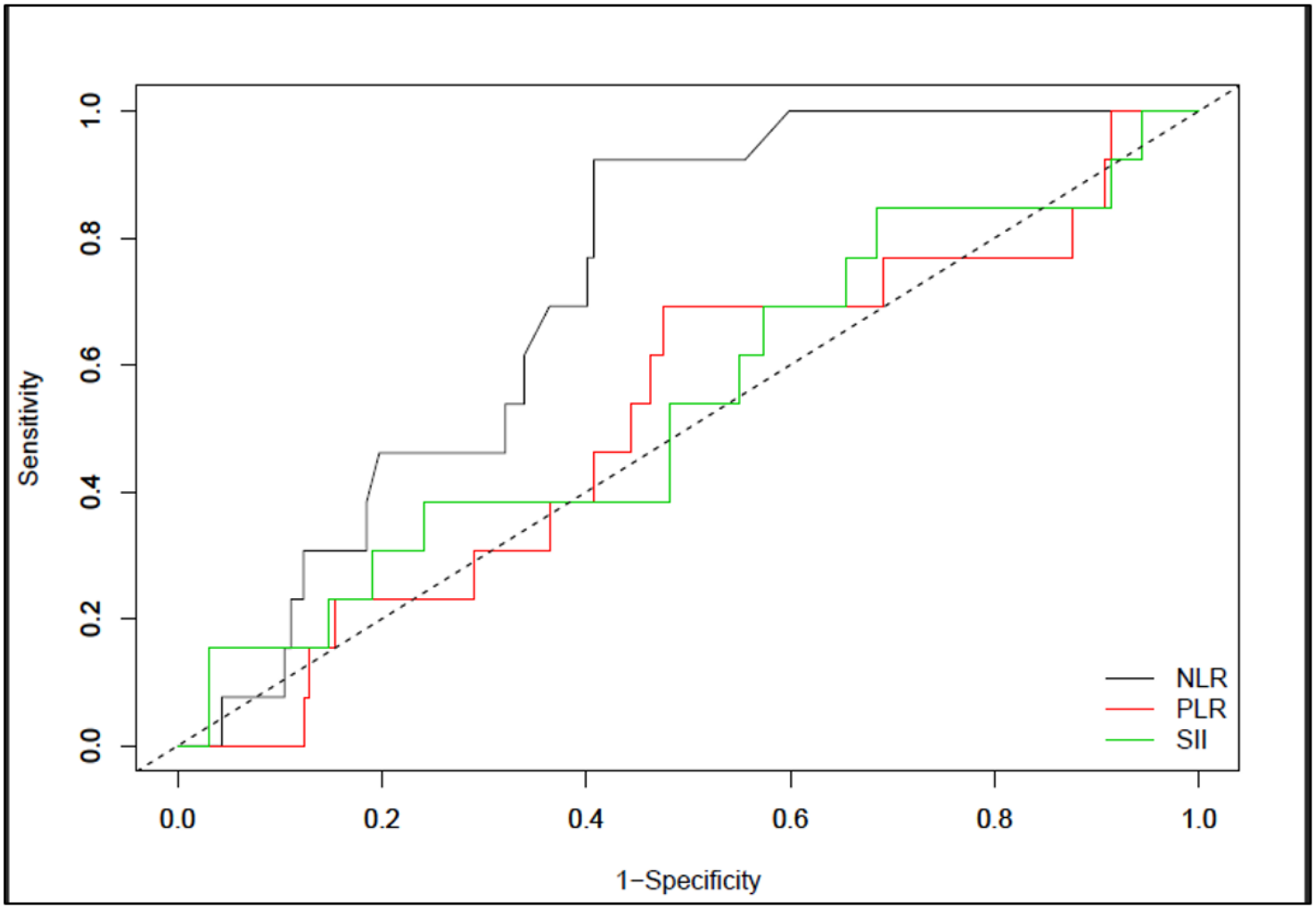
Multivariate logistic regression data

Variable	OR	p value	95% Confidence Intervals
NLR	9.6463	0.016	(1.5246,61.0350)
Glucose	1.0086	0.005	(1.0037,1.0135)
Albumin	0.4741	0.21	(0.1476,1.5227)

NLR - neutrophil to lymphocyte ratio

Table 3

ROC curve depicting Area Under Curve for the three ratios



NLR - neutrophil to lymphocyte ratio, PLR - neutrophil to lymphocyte ratio, SII - Systemic Immune-inflammation Index

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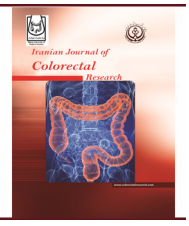

Cytokeratin Fragment 21.1 (CYFRA 21.1) is a Useful Tumor Marker in Colonic Adenocarcinoma: A Cross-sectional Study

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Abstract

Background: Colorectal carcinoma is rising worldwide, representing a major cause of cancer-related mortality and morbidity. Carcinoembryonic antigen (CEA) is an established tumor marker for colorectal cancer, with uses in screening, pre-treatment staging, post-therapeutic monitoring, and recurrence detection. However, multiple factors affect CEA, including smoking and benign gastrointestinal diseases. Hence, there is a need to investigate alternative tumor markers like cytokeratin fragment 21.1 (CYFRA 21.1).
Methods: This cross-sectional study aimed to determine if the combination of CYFRA 21.1 and CEA is superior to CEA alone as a diagnostic marker in colonic cancer. From June 2016 to December 2019, 69 consecutive patients with a histologically-confirmed diagnosis of colonic adenocarcinoma were studied. The serum levels of both tumor markers were analyzed before starting any definite treatment. The sensitivity and positive predictive values for both tumor markers were calculated. The correlation between tumor markers was tested using Pearson's correlation. The correlation between the TNM stage and tumor markers was tested using Spearman's Rho test.
Results: Forty-one patients had elevated CEA, while 33 patients had elevated CYFRA 21.1. CEA and CYFRA 21.1 mildly positively correlated with each other, with an R-value of 0.2598 (P=0.031). Spearman's correlation with the clinical stage of cancer was found to be 0.50834 for CEA (P<0.005) and 0.59828 for CYFRA 21.1 (P<0.005). The sensitivity of CEA was 59.42%, while that of CYFRA 21.1 was 47.83%. The combination of both had a sensitivity of 75.36%.
Conclusion: The combination of CYFRA 21.1 and CEA was more effective in picking up cases of colonic cancer than CEA alone. Both CYFRA 21.1 and CEA correlated well with the stage of the disease. Combining these biomarkers has great potential to evolve as a diagnostic aid in colonic cancer.

Keywords: Adenocarcinoma, Colon cancer, Tumor markers, CYFRA 21.1, Carcinoembryonic antigen

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Introduction

Colorectal cancer (CRC) is inarguably a major global health burden. The tumor often progresses to metastasis and is sometimes incurable, often with a lengthy disease process. The natural history proceeds from an easily curable pre-malignant stage through an early, localized, mostly treatable malignant stage. The overall survival of CRC is quite good when compared with other cancers. However,

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local recurrence is relatively common, even after radical curative surgery (1). The survival rates remain more favorable when the cancer is detected earlier: the stage-wise rates being 93%, 77%, 48%, and 7% at five years for diagnosis at stages I to IV, respectively (2). For this reason, early detection is crucial in improving these patients' disease-free periods and survival.

The fecal occult blood test (FOBT) remains one of the simplest methods for screening CRC. Results from several trials have reported a reduction in mortality to 15–33% while using FOBT-based screening (3). However, the potential benefits of FOBT are compromised by the limited sensitivity (13–50%) of detection in asymptomatic cohorts and the poor uptake (4, 5). Flexible sigmoidoscopy (FS) is the other test for first-line screening, complemented by colonoscopy when positive. FS offers better sensitivity over FOBT, picking up as much as 70–80% of advanced neoplasms of the colon and rectum (6). Though FS has proven to be efficacious for screening, it cannot be used to detect 40% of colonic tumors, which develop in the proximal segments (7, 8). The gold standard of screening is a colonoscopy, advocated only for high-risk groups in the United Kingdom but often employed for detecting sporadic cancers in the United States (9, 10). However, the compliance rates for these invasive tests remain on the lower side (11, 12).

Carcinoembryonic antigen (CEA) is the most common colorectal tumor marker. CEA is the one recommended by the American Society of Clinical Oncology and the National Academy of Clinical Biochemistry for assessing prognosis, monitoring response to treatment, and detecting metastases and recurrence (13, 14). CEA was first described by Gold and Freedman in 1965 when they identified it as an antigen that was detectable in both fetal colon and colonic adenocarcinoma but was absent from the healthy adult colon (15). Because of being present only in cancers and embryonic tissues, the protein was given the name CEA. Though the CEA is a cost-effective indicator of CRC, false positive elevation is frequently reported in smokers (16, 17). In addition to the above, several benign gastrointestinal diseases like ulcerative colitis, viral hepatitis, alcohol-related cirrhosis, and cryptogenic or biliary cirrhosis can potentially cause an increase in CEA levels (18).

Cytokeratin 19 is a kind of cytokeratin comprised of keratin and intermediate filaments of epithelial cells (19, 20). Circulating cytokeratin fragment 21.1 (CYFRA 21.1) is a biological tumor indicator reflecting fragments of cytokeratin 19. CYFRA 21.1 has already been proven to be a reliable biomarker in various malignancies, particularly that of the head, neck, and lungs (20, 21). The diagnostic performance of CYFRA 21.1 for CRC has been evaluated in some studies. However, its potential as a screening marker has not been previously assessed. Since CYFRA 21.1 is less vulnerable to factors like age, gender, and smoking history, CYFRA 21.1 may be better than CEA as a marker in the initial diagnosis and staging of CRC (22).

The search continues to find serum tumor markers other than or better than CEA for diagnosing CRC. An ideal biomarker would allow for easy diagnosis when the cancer is in its early stages, even before it starts its spread to other organs. It could ideally help clinicians to carry out patient stratification and to make optimal decisions about treatments. Furthermore, it can act as a predictor of overall outcomes and tumor recurrence. This concept formed the basis for this prospective single-center study, where we attempted to test the diagnostic efficacy of CYFRA 21.1 in combination with CEA.

The primary objective of this study was to find if a combination of CYFRA 21.1 and CEA is superior to CEA alone as a diagnostic marker in patients with CRC. The secondary objective was to find the correlation between CYFRA 21.1 and the cancer stage in these patients.

Patients and Methods

The current study had a prospective cross-sectional design and was carried out for a period of three years, from 1st June 2016 to 31st December 2019, at the General Surgery and Oncology wards of Government Medical College Trivandrum, Kerala, India.

The inclusion criteria deemed eligible adult patients aged 12 years or older, with histology-confirmed adenocarcinoma of the colon or rectum, admitted to our wards for any definitive treatment. The exclusion criteria ruled out patients with previously diagnosed cancers of any site to avoid interference with the values of the tumor markers. Patients with any previous treatment for the current cancer were also excluded.

Approval from Institutional Review Committee and clearance from Human Ethics Committee (IEC No. 02/09/2016/MCT dated 26/03/2016) were obtained before commencing the study. Blood samples were collected before the start of definitive surgery or chemotherapy. The subjects were briefed about the study procedure in detail, and informed consent and signatures were obtained before the data and sample collection.

The sample size for the study was estimated using the recommended formula for sample size estimation in diagnostic test studies, wherein the sensitivity of the new test and established test were taken from reference studies (22). With an acceptable power of 80% and an alpha error of 5%, the sample size was calculated at 69, which was set as the study sample size. CEA was measured using a solid-phase, two-site chemiluminescent enzyme immunometric assay, while CYFRA 21.1 was measured with a commercially available enzyme-linked immunosorbent assay kit. The normal range

of CEA was taken as below 5 µg/l, with a greater cut-off of 7 µg/l among smokers. The normal range of CYFRA 21.1 was set as below 1.96 ng/ml. Histopathological confirmation of cancer was considered the gold standard reference.

Statistical Analysis

As part of the data collection, a data gathering checklist was created to record the subjects' clinical details. These included presenting features, clinical examination findings, and relevant investigation results. Both tumor markers' sensitivity and positive predictive value (PPV) were calculated. The correlation between the tumor markers was tested using Pearson's correlation test. The correlation between the TNM stage of colonic cancer and tumor markers was tested using Spearman's Rho test. Statistical analysis was carried out with the help of Microsoft Office Excel and EpiInfo software (CDC, Atlanta). Data are reported as arithmetic means±the standard deviation and frequencies with the percentage in parentheses. Significance was considered at P<0.05.

Results

We studied 69 patients with colon cancer. The mean age was 60.33±10.99 years, with a maximum of 80 and a minimum of 38 years. There were 37 males (53.62%) and 32 females (46.38%). Eighteen patients had primary cancer in the rectum (26.09%), 18 in the sigmoid colon (26.09%), 9 in the cecum (13.04%), 7 in the transverse colon (10.14%), 6 in the ascending colon (8.69%), 5 in the splenic flexure (7.25%), 4 in the descending colon (5.79%), and 2 in the hepatic flexure (2.89%).

The mean values of CEA and CYFRA 21.1 were 27.71±62.85) mg/l and 10.45±20.08 ng/ml, respectively. Among the 69 patients, 41 had elevated CEA, while 33 had elevated CYFRA 21.1. Fifty-two patients were positive for either marker (Figure 1). The sensitivity of CEA was calculated at 59.42% (CI 46.92% to 71.09%), and that of CYFRA 21.1 was 47.83% (CI 35.65% to 60.20%). CEA had a PPV of 3.03%, while CYFRA 21.1 had a PPV of 2.46%. The combination of CEA and CYFRA 21.1 had a sensitivity of 75.36% (CI 63.51% to 84.95%), with a PPV of 3.82% (Table 1).

When Pearson's correlation was tested, the correlation between CEA and CYFRA 21.1 was calculated at 0.2598, indicating a weak positive correlation (P=0.031). Pearson's correlation between age and CEA was negative, with an R-value of -0.2613, while that for age and CYFRA 21.1 was also negative, with an R-value of -0.261. Sixteen patients presented in stage 1 of cancer (23.19%), 28 in stage 2 (40.58%), 19 in stage 3 (27.54%), and 6 in stage 4 (8.69%). CYFRA 21.1 correlated better with the disease stage than CEA (Figure 2). Spearman's correlation was tested between CEA and cancer stage; the rho value was 0.50834, which was significant (P<0.005). For CYFRA 21.1, the rho value was 0.59828, which was also significant (P<0.005). ANOVA revealed a significant relationship between the cancer stage and each marker, with an f-ratio value of 27.17364 (P<0.00001) for CYFRA 21.1 and 3.01865 for CEA (P=0.036038). The two-tailed test of the difference between the two markers was significant at a P-value of 0.0316 for a t-value of 2.1722.

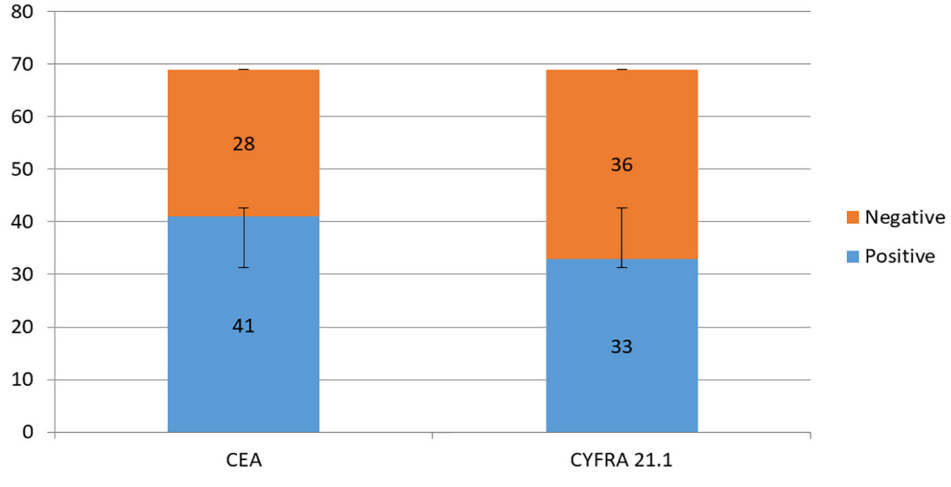

Figure 1: Distribution of positivity of the tumor markers.

Table 1: Performance characteristics* of CEA and CYFRA 21.1

	Sensitivity	Positive predictive value
CEA	59.42%	3.03%
CYFRA 21.1	47.83%	2.46%
CEA+CYFRA 21.1	75.36%	3.82%

*Specificity and negative predictive value could not be determined considering the study design. CEA: Carcinoembryonic antigen CYFRA 21.1 : cytokeratin fragment 21.1

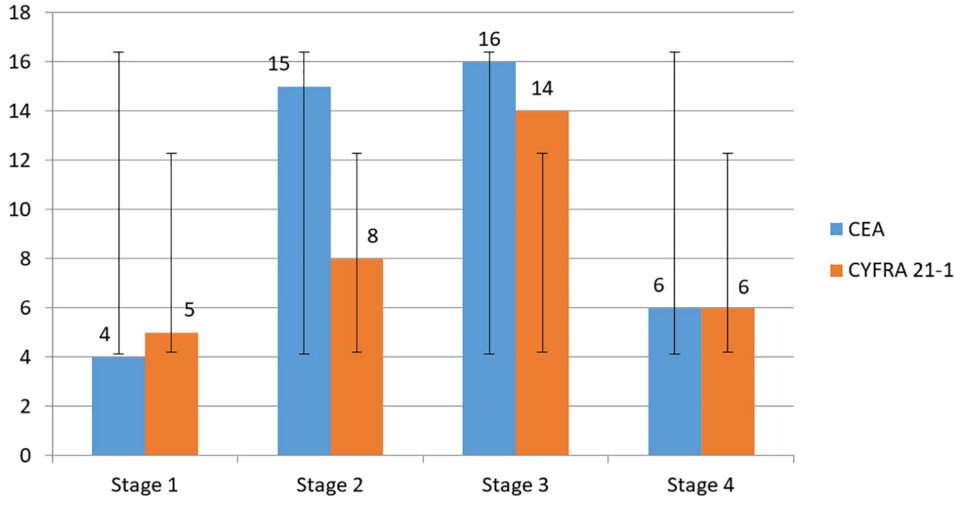

Figure 2: Stagewise distribution of the tumor markers. Spearman correlation for CEA: 0.50834, P<0.005; Spearman correlation for CYFRA 21.1: 0.59828, P<0.005; Significant difference between markers in the two groups: t-value=2.1722, P=0.0316

Discussion

In colorectal cancer, CEA has been in application universally, right from the start of pre-treatment staging to assessment of recurrence and response to chemotherapy regimens. CEA is a practical tool for suspecting metastasis or relapse and a predictive marker of worse prognosis when high preoperative levels do not reduce to normal ranges after resection (15). However, the high false-positive results and the lower sensitivity of CEA in the pre-treatment evaluation setting reflect that stand-alone CEA might be an unsuitable agent for population screening (23).

Most of the available literature supports our study findings. In a study, at a threshold of 5 ng/ml, the sensitivity of CEA for detecting CRC up to 1 and 4 years before the clinical presentation was 25% and 13%, respectively, at a specificity of 95% (24). At a threshold of 2.5 ng/ml for CEA, sensitivity for CEA and CYFRA 21.1 were 57.5% and 38.4%, respectively, with a specificity of 81% and 83.5%. CYFRA 21.1 and CA 125 were found to have no utility as screening markers and also did not add to the performance of CEA when employed in combination. Some studies suggest that only some subsets of CRC produce an elevation in serum CEA levels, which is specific to the malignant phenotype (25). Rising levels of CEA have been detected to be much more frequent in late-stage tumors (26). However, CEA levels often do not correlate with tumor grade, as suggested by previous studies (27).

Serum CEA has also been found to have very limited sensitivity for screening when used in asymptomatic people. In a study on 46 preclinical cases (29 were of early stage/17 were of an advanced stage), testing for CEA provided a lead time of up to two years in only 30% of future CRCs when a cut-off threshold was used that correctly identified 99% of the controls (28). In another research, elevated CEA levels provided a lead time of up to 7 months only in 19% of the 32 preclinical cases studied (17 were of early stage/15 were of an advanced stage) (29). However, both these studies involved the analysis of single cross-sectional design sample units and were limited to a maximum lead time of two years.

Though CEA is superior to the guaiac FOBT, it does appear to be inferior to Cologuard®. Cologuard is a fecal test that combines hemoglobin protein, seven KRAS gene point mutations, NDRG4 and BMP3 gene promoter hypermethylation, and b-actin DNA as a normalization marker (3, 4). CEA is also inferior to Epi proColon® (plasma SEPT9 DNA methylation), which has been evaluated in large prospective trials, and also the fecal immunochemical test, which is a more precise version of the FOBT for detecting hemoglobin (30-33). It is also important to note that the administration of 5-fluorouracil-based therapy can cause significant transient increases in CEA levels even if there is no disease progression. In research by Moertel et al., among the 99 subjects who developed liver toxicity while on chemotherapy, 19 patients had a false-positive increase in CEA levels. Their CEA values ranged from 5.1 to as high as 34 mg/l and subsequently returned to normal after cessation of chemotherapy (34).

Gawel et al. tested endoscopy trial specimens for a panel of biomarkers including CYFRA 21.1, alpha-fetoprotein, carbohydrate antigen (CA) 19-9, and CEA, and were able to develop an accurate algorithm for predicting high-risk adenomas as well as colorectal cancers with (35). In the study by Lim et al., CYFRA 21.1 showed significant diagnostic performance as well as great step-wise comparative potential when differentiating patients with colonic adenomas from benign controls (36).

There are also some studies that reveal findings different from ours. One study found that CYFRA 21.1 (cut-off≥1.13 ng/ml) had a sensitivity of 47% when compared with 37% for CEA (cut-off≥3.05 ng/ml) and 32.6% for CA 19.9 (cut-off≥23.1 ng/ml) when used in the initial staging work-up of primary CRC (26). When different cut-off values were used, CYFRA 21.1 showed a higher sensitivity for pre-treatment detection than CEA and CA 19.9

in colorectal adenocarcinoma in this study. The authors also noted a mildly significant correlative relationship between Dukes' stages and all three tumor markers (P<0.01). The areas under the receiver operating characteristic curves (AUC) for CYFRA 21.1, CEA, and CA 19-9 were 0.81±0.03, 0.74±0.03, and 0.62±0.04, respectively, when used for discriminating CRC from benign colorectal conditions. In addition to the above, CYFRA 21.1 was the most sensitive tumor marker among the three for detecting recurrent CRC at all cut-off values.

Xu et al. demonstrated that cytokeratin 19 mRNA was detectable in 41.9% of patients with CRC and in 3.3 % of controls (37). This sensitivity was evidently higher than the CEA mRNA detection rate (35.8% of CRC patients and 3.3% of controls). Holdenrieder et al. also showed that CYFRA 21.1 levels were predominantly higher in patients with CRC when compared with healthy controls (P<0.001) and also other benign gastrointestinal diseases (P=0.01), and even showed significant stage dependency (P=0.01) (38). Dressen et al. evaluated the diagnostic performance of a multiplex immunoassay panel that included CEA, CA 19-9, and CYFRA 21.1, revealing that a combination of CA 19-9 and CEA had the best diagnostic performance with a higher AUC (39). In their study, CEA showed the best performance as a single marker. In a study by Thomas et al., CYFRA 21.1 and CA 125 had no utility as screening markers and did not enhance the performance of CEA when used in combination (24).

The present study is not without its own set of limitations. Since we included only confirmed cases, we could not find the AUC for each tumor marker. Due to the study design, no follow-up was done to analyze prognosis or survival. Last, the sample size was comparatively small, which, along with the study's single-center setting, could mitigate the validity and generalizability of the study results.

Conclusion

A combination of CEA and CYFRA 21.1 can pick up more colon cancer cases than either of them alone. The poor sensitivity of CEA and CYFRA 21.1 make either of them useless as stand-alone screening tools for colon cancer. Both CEA and CYFRA 21.1 correlate well with the stage of cancer at presentation. In conclusion, CYFRA 21.1 holds great promise as a tumor marker in combination with CEA in colon cancer. The findings of this study demand the need for further large-scale trials to assess the potential of CYFRA 21.1. The results from our study could act as the groundwork for building and subsequently assessing longitudinal algorithms for CRC screening. Also, there is a potential for combining other promising new biomarkers with CEA to add to its performance.

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Authors' Contribution

Study concept and design: Dr Meer Chisthi.M, Dr Krishnakumar KG; Acquisition of data: Dr Meer Chisthi.M, Dr Krishnakumar KG; Analysis and interpretation of data: Dr Meer Chisthi.M, Dr Viswanathan KV; Drafting of the manuscript: Dr Meer Chisthi.M, Dr Viswanathan KV; Critical revision of the manuscript for important intellectual content: Dr Meer Chisthi.M, Dr Viswanathan KV; Statistical analysis: Dr Meer Chisthi.M, Dr Krishnakumar KG; Administrative, technical, and material support: Dr Krishnakumar KG, Dr Viswanathan KV; Study supervision: Dr Krishnakumar KG, Dr Viswanathan KV

Conflict of interest: None declared.

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Coexistence of Histologically Proven Chronic Lymphocytic Thyroiditis with Other Thyroid Disorders: A Retrospective Study

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Abstract

Background Hashimoto's thyroiditis (HT) is the commonest autoimmune thyroid pathology. It has been reported in increased numbers recently, probably due to the increase in autoimmune diseases across many parts of the world. It is sometimes found associated with other diseases as well as other diseases of the thyroid. There is an unproven association of this condition with thyroid cancer, particularly papillary thyroid carcinoma (PTC).

Methods This was a retrospective study performed over a period of 5 years. The objectives of this study were to find out the prevalence of histopathologically proven HT in surgically resected thyroid glands for various indications and its association with other thyroid disorders, especially thyroid malignancies. Total 4,630 patients who underwent thyroidectomy during the study period and met the criteria for inclusion were considered for analysis.

Results Histopathologically proven features of HT were present in 1,295 (28%) of the cases. Among these, 445 (34.36%) had only HT while 850 (65.66%) had HT along with other thyroid diseases. The most common disease associated with HT was multinodular goiter (44.2%), followed by PTC (15.2%). Patients with HT exhibited a higher rate of papillary cancer (16.7%) compared with patients without this pathology (13.8%). Statistically significant association between papillary cancer and HT was found among the female patients.

Conclusion The prevalence of HT in patients undergoing thyroidectomy is high in the studied population. A statistically significant association exists between papillary thyroid cancer and thyroiditis among female patients. This could form the basis for further research along these lines.

Keywords
- Hashimoto's thyroiditis
- papillary thyroid cancer
- multinodular goiter
- thyroid

Hashimoto's thyroiditis (HT) was first described by Hakaru Hashimoto, a Japanese surgeon and pathologist in 1912. It is the most common autoimmune thyroid disease and the commonest cause of hypothyroidism.[1] The disease occurs in 0.3 to 1.5 per 1,000 individuals worldwide and is found to be more common in females with gender preponderance of 5 to 20 times.[2] The pathophysiological hallmark of HT is diffuse lymphocyte infiltration of thyroid follicles resulting in

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glandular destruction, fibrosis, and parenchymal atrophy, subsequently causing thyroid dysfunction and occasional development of goiter.[3] Though 90% of patients with HT have high antithyroid peroxidase and antithyroglobulin antibody titers, histological diagnosis is considered more accurate.[4] Sonographic findings of diffuse HT include decreased echogenicity, heterogenecity, hypervascularity, and presence of hypoechoic micronodules with an echogenic rim.[5]

In the past, thyroiditis was considered as an uncommon disease incidentally diagnosed by the presence of lymphocytic infiltration in thyroid follicles on histopathology examination. Recently, increased number of thyroiditis has been reported. This could parallel the steady rise in frequency of other autoimmune disorders, mostly in the West and North of the world as compared with the East and South, probably due to modified environmental triggers.[6–8] Thyroiditis has been associated with other autoimmune diseases like Type 1 diabetes mellitus, multiple sclerosis, rheumatoid arthritis, celiac disease, vitiligo, and chronic urticaria.[8–11] The association of HT with thyroid cancer, in particular papillary thyroid carcinoma (PTC), was first described by Dailey et al in 1955.[12] This report underlined a significantly high prevalence of thyroid cancer in HT compared with the general population. Although several publications did support these findings,[13,14] the investigations by Crile[15] in population-based studies of patients with HT challenged this association. Another similar study done by Holm et al[16] in 829 patients added strong support to Crile's[16] findings. Later, Jankovic et al[4] did a systematic literature review in 2013 and concluded that population-based fine needle aspiration studies did not find a statistically significant correlation between HT and PTC. The objective of this study is to find out the prevalence of histopathologically proven HT among patients who underwent thyroidectomy for various indications in our institution and the association of HT with other thyroid diseases, especially thyroid malignancies.

Materials and Methods

All patients aged 12 years or above, who underwent thyroidectomy for various indications from 2011 to 2015 in the Department of General Surgery, in Government Medical College, Trivandrum, Kerala, India, were included in this retrospective study. The main indications for thyroidectomy were benign thyroid disease with pressure symptoms or cosmetic purposes; suspicious nodule(s) in the thyroid by clinical examination, imaging, or fine needle aspiration cytology (FNAC); and proven thyroid malignancy. All patients had undergone FNAC before surgery except in thyrotoxicosis. Those patients who underwent isthmusectomy alone for relieving pressure symptoms and thyroidectomy for underlying parathyroid disease were excluded. HT was defined histologically by the presence of diffuse lymphocytic infiltrates, lymphoid follicles with reactive germinal centers, Hurthle cell change of the follicular epithelial cells, parenchymal atrophy, and fibrosis.

From the hospital registry, data were abstracted by the residents who were given adequate training about the data abstraction protocol based on a pretested and standardized data abstraction form. We had ascertained the feasibility and availability of information needed for the data abstraction form by a preliminary review of three randomly selected sample case records. We abstracted relevant data of all thyroidectomy cases from 2011 to 2015. Descriptive statistics are reported in mean and standard deviation or median and interquartile range for continuous variables, and in absolute numbers and percentages for categorical variables. Chi-square test with appropriate correction, if needed, was used to find out the association between variables considered under the objective. All statistical analyses were implemented in R statistical software version 3.2.0. The level of significance was set at a p-value of 0.05.

Results

Total 4,631 patients who underwent thyroidectomy in our department for both benign and malignant diseases of the thyroid during the study period and met the criteria for inclusion were considered for analysis. Among this, histopathology report of one patient could not be traced and hence excluded. Age of the patients while undergoing thyroidectomy ranged from 12 to 82 years and the median age was 42 (34.51) years. Among those, 60 patients were below the age of 18 years. In these 60 patients, the indications included abnormal cytology, pressure effects, and cosmesis also. None of these 60 patients were found to suffer from any familial thyroid disorders. Postoperative histology revealed papillary cancer as the predominant finding in these patients, 22 (36.7%), followed by benign multinodular goiter (MNG), 15 (25%), lymphocytic thyroiditis, 15 (25%), cellular nodule, 4 (6.7%), and papillary cancer in a background of MNG, 4 (6.7%). The preoperative cytology results are displayed in ►**Table 1**.

Most of them were female 4,075 (88%) with a female-to-male ratio of 7:1. The mean age of the patients who presented with thyroiditis was 41.2 ± 11.8 years, whereas in patients without thyroiditis, the age was 43.3 ± 12.2 years. Even though thyroiditis and nonthyroiditis patients showed

Table 1 Cytology distribution of all study patients

Multinodular goiter (MNG)	70.8%
Lymphocytic thyroiditis	9.8%
Papillary carcinoma	7.4%
Nondiagnostic	5%
Follicular neoplasm	4.6%
MNG with thyroiditis	3.2%
Papillary carcinoma with thyroiditis	0.7%
Hurthle cell neoplasm	0.5%
Normal thyroid cells	0.27%
Medullary carcinoma	0.2%

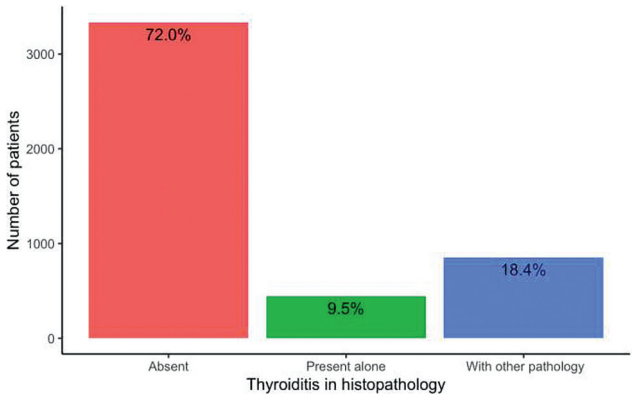

Fig. 1 Bar graph showing the distribution of thyroiditis in histopathology.

female predominance, it was more so with thyroiditis patients. Among thyroiditis patients, females constituted 93.3% (female-to-male ratio, 14:1), and in nonthyroiditis patients, females constituted 85.9% (female-to-male ratio, 6:1). Out of the 4,630 thyroidectomies, 508 had undergone hemithyroidectomy, 29 had subtotal thyroidectomy, 87 had near-total thyroidectomy, and 4,006 had total thyroidectomy. So, the most frequent thyroid surgery performed was total thyroidectomy (86.5%).

In this study of 4,630 patients, histopathologically proven features of HT were present in 1,295 (28%) patients, of which 445 (34.36%) had only HT while 850 (65.64%) had HT along with other thyroid diseases. These 445 patients underwent surgery because of pressure effects. The frequency distribution of thyroiditis in thyroidectomy specimens is given in ►**Fig. 1**. The most common disease associated with HT was MNG (44.2%), followed by PTC (15.2%), while 1.5% patients had both MNG and PTC. Other diseases in the decreasing order of frequency were cellular nodule, follicular carcinoma, follicular adenoma, medullary carcinoma, other malignancy, and Hurthle cell carcinoma. Altogether, HT coexists with PTC in 216 (16.7%) cases, with other malignancies in 18 (1.38%) cases, and with other benign thyroid diseases in 616 (47.6%) cases (►**Table 2**). Moreover, patients with HT exhibited a higher rate of PTC compared with patients without HT, irrespective of their gender (16.7% vs 13.8%, $p = 0.013$). Association between thyroiditis and papillary thyroid cancer is depicted in ►**Fig. 2**. Male patients who underwent thyroidectomy harbored PTC more often than females irrespective of their thyroiditis status. When 23% of male HT patients and 20.2% of males without HT suffered from PTC, only 16.2% and 12.8% of females in the HT and without HT group, respectively, had PTC. Statistically significant association exists between PTC and thyroiditis in female patients ($p = 0.003$) whereas it is lacking in males ($p = 0.56$). The occurrence of PTC in HT patients and without HT patients with respect to gender is given in ►**Table 3**.

Table 2 Hashimoto's thyroiditis' coexistence with other thyroid pathologies

Thyroid disorder	Number (%)
Multinodular goiter (MNG)	573 (44.2)
Hashimoto's thyroiditis only	445 (34.4)
Papillary carcinoma	197 (15.2)
Cellular nodule	32 (2.47)
MNG with papillary carcinoma	19 (1.47)
Follicular carcinoma	11 (0.85)
Follicular adenoma	11 (0.85)
Medullary carcinoma	4 (0.31)
Hodgkin's lymphoma	1 (0.08)
Hurthle cell carcinoma	1 (0.08)
Secondary from unknown primary	1 (0.08)
Total	1,295 (100)

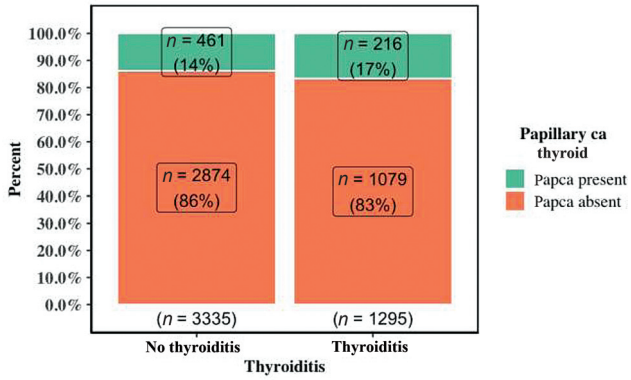

Fig. 2 Grouped bar graph showing association between thyroiditis and papillary thyroid cancer.

Table 3 Papillary cancer co-occurrence in patients with and without Hashimoto's thyroiditis

Sex	Thyroiditis present ($n = 1,295$)		Thyroiditis absent ($n = 3,395$)		p-Value
	Total no.	PTC (%)	Total no.	PTC (%)	
Female	1,208	196 (16.2)	2,866	366 (12.8)	0.003*
Male	87	20 (23)	469	95 (20.2)	0.56
Total	1,295	216 (16.7)	3,335	461 (13.8)	

Abbreviation: PTC, papillary thyroid carcinoma.

Discussion

Since the initial description by Dailey et al[12] in 1955, the association between HT and thyroid malignancy remains controversial, some studies suggesting a positive correlation while others strongly contradicting this. Some studies even postulate a cause and effect relationship between the two. The inflammatory response seen in HT stimulates the malignant transformation of follicular cells through reactive oxygen mediated DNA damage. The conflicting report seen in the literature may be due to differences in study design, selection bias, and ethnic and geographical variations. In this study, we attempted to find out the prevalence of HT in thyroidectomy in the Indian population and whether there exists any relationship between HT and PTC.

The prevalence of HT in this study was 28%; of this, 18.4% had HT along with other thyroid diseases and 9.5% had only HT. A study done in Korea by Yoon et al[17] reported a prevalence of 28.7% HT among PTC patients. But similar studies done by Repplinger et al[18] and Siriweera and Ratnatunga[19] in thyroidectomy patients showed prevalence of HT as 18% and 6.51%, respectively, based on final pathology. We focused our study on the distribution of various other thyroid disorders in the subgroup of patients with pathologically proven HT. Among this, 65.64% had associated thyroid pathologies, either benign or malignant. Overall, benign disease of the thyroid was more frequently associated with HT (47.6%) than malignancy (18%) and among the malignancies, PTC formed an overwhelming majority. Since the association between HT and PTC has been widely disputed in the literatures and both these diseases are common in our setting, we further explored the relationship. Out of the 1,295 patients with HT, 16.7% had coexistent PTC and it was 12 times more common than other thyroid malignancy. This could also be because PTC is the commonest type among all thyroid malignancies. If stratified by gender, females were more frequently affected by thyroid cancer than males, with female-to-male ratio of 9.6:1. There is a statistically significant association between PTC and thyroiditis in female patients ($p = 0.003$) whereas it is lacking in male patients. This may be because of the small sample size of male patients or due to the fact that males with thyroid nodules are often advised thyroidectomy with a lower threshold.

Resende de Paiva et al[20] conducted a large systematic review and meta-analysis involving 64,628 subjects to find out the association between HT and thyroid cancer. Among the HT patients, most of the patients were women (78.7%). PTC was seen in 9.03%, follicular carcinoma in 1.26%, medullary carcinoma in 1.62%, anaplastic carcinoma in 0.49%, and thyroid lymphoma in 0.37%. He concluded that an association exists between HT and PTC as well as HT and thyroid lymphoma, but no association was found between HT and other thyroid malignancies. In all subtypes of thyroid cancer, females were more often affected than males with ratios ranging from 1.5:1 to 4.8:1. These findings go well with our study. Daniel Repplinger et al[18] found that PTC occurred in 29% of patients with HT and 23% of patients without HT. Though PTC was the most common malignancy in patients with or without HT, it was significantly less common in non-HT group (94% vs 76%, $p = 0.001$). A histopathology study assessing the prevalence and severity of thyroiditis among surgically resected thyroid tumors found a significantly higher rate of lymphocytic infiltrate in PTC.[21] Most of the thyroidectomy specimen studies reported a positive correlation between HT and PTC.[3,22–24] However, FNAC studies done in the outpatient setting did not find a statistically significant association between the presence of HT and PTC. For example, Matesa-Anić et al[25] analyzed FNAC of 10,508 patients and found that the prevalence of PTC in patients with HT was 1.9% and patients without HT was 2.7%. A similar observation was seen in other FNAC studies.[16,26]

To date, the causative relationship between HT and PTC is not clearly established, though there are some proposed mechanisms in the literature. Wirtschafter et al[27] and Arif et al[28] in two different studies demonstrated expression of the RET/PTC1 and RET/PTC3 oncogenes in HT. Further study by Unger et al[29] found the expression of p63 in HT patients with PTC. Burstein et al[30] hypothesized that both HT and PTC are initiated by pluripotent p63-positive stem cell remnants. Another hypothesis states that elevated levels of thyroid stimulating hormone found in HT patients with hypothyroidism stimulate follicular epithelial proliferation, leading to PTC.[15,31,32]

Our study was on a large series of patients who underwent thyroidectomy for various indications mentioned above, over a span of 5 years. The limitation of this study is that this is a hospital-based retrospective study comprising thyroidectomy patients alone, hence subjected to selection bias. Also, we could not find complete information about these patients, including their thyroid hormone status and thyroid antibodies status.

But population-based studies in the literature with FNAC for diagnosing HT and coexistent PTC have met with several problems. The presence of HT in a patient can be confirmed only by histopathology examination of the entire gland. Focal HT and small PTC can be missed by FNAC due to sampling error. Furthermore, follicular cell changes associated with HT can be mistaken for thyroid neoplasm. In addition, several studies did not have a control group. A prospective study in a community or outpatient setting with clinical, imaging, antithyroid antibodies, and ultrasound-guided FNAC as a diagnostic tool for HT would probably address this issue. In the future, a prospective histopathology study of thyroid specimens obtained from a large number of subjects is required to establish the association between HT and PTC conclusively.

Conclusion

The prevalence of HT in patients undergoing thyroidectomy is high in Kerala state in India. Benign diseases of the thyroid are more frequently associated with HT than malignancy. A statistically significant association exists between papillary cancer and thyroiditis in female patients. We recommend that all patients with HT undergo periodic thyroid evaluation to exclude the development of papillary cancer. We also

recommend further research to elucidate the association between thyroiditis and thyroid malignancy.

Conflicts of Interest

None to state.

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Diagnostic Study

RIPASA and air scoring systems are superior to alvarado scoring in acute appendicitis: Diagnostic accuracy study

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ABSTRACT

Background: Acute appendicitis remains difficult-to-diagnose in spite of being a common acute abdominal condition. Early and correct diagnosis is essential either to proceed with early appendectomy or conservative approach so that complications and negative explorations can be minimised. Scoring systems can help in quick diagnosis and decision making. Though the Alvarado scoring is the widely used system, differences in diagnostic accuracy have been observed when it is applied to varied populations.

Materials and methods: The objective was to find the predictive accuracy of Modified Alvarado score, Appendicitis Inflammatory Response score and Raja Isteri Pengiran Anak Saleha Appendicitis score, in a diagnostic test evaluation study. From first January 2018 to first January 2019, 107 consecutive patients admitted with a diagnosis of suspected appendicitis were assessed with these scores. Sensitivity, specificity, positive and negative predictive value, positive and negative likelihood ratio and area under curve were determined for each.

Results: Negative appendicectomy rate was 15.89%. Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy were 64.44%, 58.82%, 89.23%, 23.81% and 63.55% respectively for Modified Alvarado; 97.78%, 29.41%, 88%, 71.43% and 86.92% respectively for Appendicitis Inflammatory Response; 87.78%, 76.47%, 95.18%, 54.17% and 85.98% respectively for Raja Isteri Pengiran Anak Saleha Appendicitis. Area under the curve was 0.726797 for Modified Alvarado, 0.946732 for Appendicitis Inflammatory Response and 0.910131 for Raja Isteri Pengiran Anak Saleha Appendicitis.

Conclusion: Appendicitis Inflammatory Response score probably is superior to Alvarado in the paediatric population because the variables scored are easy to apply to children, while Alvarado requires children to identify subjective symptoms which may not always be accurate. Appendicitis Inflammatory Response and Raja Isteri Pengiran Anak Saleha Appendicitis are better diagnostic scoring system for acute appendicitis than Modified Alvarado. Also, both these scores can be easily calculated by complete history, detailed clinical examination and basic laboratory investigations.

1. Introduction

Acute appendicitis is the one of the commonest reasons for emergency admission to general surgical wards. Acute appendicitis is still a difficult diagnostic entity and the management often involves complex decision making as it involves surgical exploration which utilises technical, financial and human resources. A quick and correct diagnosis of acute appendicitis with subsequent early appendicectomy can avoid complications arising from perforation. Though radiological examinations including Ultrasound and Computed Tomography(CT) scan can further aid in making a definite diagnosis and have been reported to have high sensitivity and specificity, it will inflate the cost to the patient and also the reporting time may further delay emergency appendicectomy. Another worry is regarding the harmful effects of radiation involved in CT scan.

Negative explorations can lead on to longer length of stay in hospital, higher costs and added morbidity and mortality as well. It is accepted that not all cases of appendicitis need to be treated surgically, especially those cases involving catarrhal appendicitis [1]. Unnecessary appendicectomies also should be avoided to avoid potential complications such

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as ileus (found in 1.2% of cases), incisional hernias (found in 0.68% of cases) and increased cost to the patient [2]. Hence it is beyond doubt that a quick and easy method to diagnose appendicitis in the clinical setting can be of great use to clinicians. With this purpose in mind, various scoring systems have been developed to aid in the clinical diagnosis of acute appendicitis.

Alvarado scoring system, which was first described in 1986, has remained the most popular scoring system in acute appendicitis for many decades. The scoring system remains popular as this scoring system has been proven to have very good sensitivity and specificity [3,4]. The Modified Alvarado Scoring System(MASS) is the system widely used globally. The Appendicitis inflammatory response (AIR) score is a newer scoring system used in suspected appendicitis, first reported in 2008. In previous studies, AIR scoring system has been found to outperform Alvarado scoring system as AIR score utilises more objective symptoms while Alvarado takes more subjective symptoms [5,6]. Also, many studies have independently shown the importance of C-reactive protein (CRP) in the assessment of patients with appendicitis [7,8]. The AIR score has incorporated CRP also as a variable whereas the Alvarado does not.

The Raja Isteri Pengiran Anak Saleha Appendicitis (RIPASA) score is another new diagnostic scoring system developed in 2008 at the Department of Surgery, Raja Isteri Pengiran Anak Saleha Hospital, Darussalam, Brunei. This scoring system, which was initially designed for use exclusively with the Asian population, is broader and simpler and consists of seventeen items with an additional parameter [9]. It has several parameters that are absent in the Alvarado score, such as age, gender and duration of symptoms prior to presentation, which were shown to affect the sensitivity and specificity of Alvarado scoring system in diagnosing acute appendicitis [10].

The three scoring systems, though different in having different maximum scores, have some overlapping parameters [Table 1]. To reiterate the facts, it goes without doubt that any scoring system which can improve over the Alvarado scoring system can turn out to be useful in diagnosing acute appendicitis and thus find generalised acceptance. This study aims to compare the predictive accuracy of AIR score and RIPASA as well as the widely used MASS in diagnosing acute appendicitis by comparing them with the gold standard of histopathologically confirmed appendicitis.

2. Materials and Methods

The primary objective of the study was to estimate the predictive accuracy of Alvarado score and AIR score and RIPASA score against the reference standard of histopathology in patients undergoing emergency appendicectomy at the General Surgical wards of our institution. The current study was designed as a prospective diagnostic test evaluation and carried out for a period of 1 year from January 1, 2018 to January 1, 2019.

Patients undergoing emergency appendicectomy for suspected appendicitis, defined as acute (lasting less than 4 days) non traumatic right iliac fossa pain consistent with a diagnosis of appendicitis (pain associated with nausea, anorexia, vomiting and fever along with clinical signs as tenderness and rebound tenderness in right iliac fossa, with or without ultrasound findings suggestive of appendicitis), were taken as the study subjects. Pregnant females, patients with a right iliac fossa mass, patients with a previous history of urolithiasis or pelvic inflammatory disease, and children below 12 years of age were excluded from the study. Institutional Review committee as well as Ethics committee clearance was obtained before commencing the study. The patients were briefed about the study and signed informed consent obtained before blood sample collection.

Sample size was estimated using standardised formula for sample size estimation in diagnostic test studies, where, sensitivity of the new tests was taken from reference studies [11,12]. Sensitivity of the reference test, that is histopathology was set as 100. With a power of 80% and alpha error of 5%, sample size was calculated for Alvarado, AIR and RIPASA scores separately and the highest value among the three, of 107, was taken as the study sample size.

A score of 7 was taken as high probability of acute appendicitis for

Table 1
Comparison of variables used in scoring systems used in appendicitis.

MASS		AIR			RIPASA	
Features	Score	Features		Score	Features	Score
					Patients:	
					Female	0.5
					Male	1.0
					Age <39.9 years	1.0
					Age >40 years	0.5
Symptoms		**Symptoms**			**Symptoms**	
Migration of pain	1				Pain Migration to RIF	0.5
Anorexia	1				Anorexia	1.0
Nausea	1	Vomiting		1	Nausea & Vomiting	1.0
		RIF pain		1	RIF pain	0.5
					Duration of Symptoms <48 h.	1.0
					Duration of Symptoms >48 h.	0.5
Signs		**Signs**			**Signs**	
Tenderness RLQ	2				Tenderness RIF	1.0
Rebound tenderness	1	Rebound tenderness	Light	1	Rebound tenderness	1.0
		Rebound tenderness	Moderate	2		
		Rebound tenderness	Strong	3		
Elevated temperature	1	Temperature 38.5^0 C or more		1	Fever >37 °C < 39 °C	1.0
					Guarding	2.0
					Rovsing Sign	2.0
Investigation		**Investigation**			**Investigation**	
Leucocytosis	2	White Cell Count (10^9/l)	10–14.9	1	Raised WBC	1.0
		White Cell Count (10^9/l)	15 or more	2		
		Proportion of PMNs (%)	70–84	1		
		Proportion of PMNs (%)	85 or more	2		
		C- reactive protein (mg/l)	10–49	1		
		C- reactive protein (mg/l)	50 or more	2		
					Negative Urine Analysis	1.0
Total score	9	Total score		12	Total score	16.5

Alvarado scoring system and a score of 5 for AIR and 7.5 for RIPASA, as per available literature. All the scores were done only for the study purpose and did not affect management. A detailed questionnaire was made to include the patients' clinical details including presenting symptoms, examination findings and other investigation results. The patients were monitored from the day of admission until discharge from the hospital. Daily follow-up included the monitoring of vital signs and systemic examination. Histopathology findings on the operated cases were collected and correlated with the scores.

The study is reported in line with the STAndards for the Reporting of Diagnostic accuracy studies(STARD) criteria and the checklist included [13]. Data are reported as mean, standard deviation (SD), median (range) or percentage. True positives, true negatives, false positives and false negatives were found out and 2×2 tables constructed to determine the sensitivity, specificity, positive and negative predictive values. The correlation between the three scores was tested with Pearson's correlation. The area under the receiver operating characteristic (ROC) curves was used to examine the performance characteristics of the scoring systems individually. The optimal cut off values for attaining maximum sensitivity and specificity were also calculated for the three systems. Statistical analysis was done with Microsoft Office Excel, MedCalc version 19.2(MedCalc Software Ltd) and easyROC software ver 1.3.1 [14]. Significance is reported wherever $p < 0.05$.

3. Results

In the study, there were 60 males(56%) and 47 females(44%) and there were no third gender patients. The mean age of the patients was 25.89(±1.41). The youngest patient was 13 and the oldest was 70 years old. The overall negative appendectomy rate was 15.89%(17 patients). The Alvarado scores ranged from 4 to 9 with a mean value of 7.33(±2.12). The AIR scores ranged from 5 to 11 with a mean value of 8.53(±2.83). The RIPASA scores ranged from 5 to 12 with a mean value of 8.91(±2.83).

The RIPASA and Alvarado scores were found to be strongly correlated positively, with a Pearson's coefficient of 0.77. The RIPASA and AIR scores were found to be weakly correlated positively, with a Pearson's coefficient of 0.66. The AIR and Alvarado scores were found to be have very weak correlation, with a Pearson's coefficient of 0.54.

Alvarado score was found to have a sensitivity of 64.44%, specificity of 58.82%, positive likelihood ratio of 1.57, negative likelihood ratio of 0.6, positive predictive value of 89.23%, negative predictive value of 23.81% and overall accuracy of 63.55%. The Youden index was calculated to be 0.365. AIR score was found to have a sensitivity of 97.78%, specificity of 29.41%, positive likelihood ratio of 1.39, negative likelihood ratio of 0.08, positive predictive value of 88.00%, negative predictive value of 71.43% and overall accuracy of 86.92%. Youden index was calculated to be 0.8678. RIPASA score was found to have a sensitivity of 87.78%, specificity of 76.47%, positive likelihood ratio of 3.73, negative likelihood ratio of 0.16, positive predictive value of 95.18%, negative predictive value of 54.17% and overall accuracy of 85.98%. Youden index was calculated to be 0.709.

The ROC curves were assessed and AUC was estimated. For Alvarado, the AUC was 0.726797, while the AUC for AIR was 0.946732 and the AUC for RIPASA was 0.910131 [Table 2, Fig. 1]. The optimal cut off for achieving maximum sensitivity and specificity for MASS was calculated to be 8, that for AIR was calculated to be 8 and that for RIPASA to be 7.5 [Table 3].

Table 2
Comparison of calculated diagnostic values for scoring systems.

Calculated Value	MASS Cut off 7	AIR Cut off 5	RIPASA Cut off 7.5
Sensitivity	64.44%	97.78%	94.4%
Specificity	58.82%	29.41%	76.5%
Positive Likelihood Ratio	1.57	1.39	4.01
Negative Likelihood Ratio	0.6	0.08	0.07
Positive Predictive Value	89.23%	88%	95.5%
Negative Predictive Value	23.81%	71.43%	72.2%
Area Under Curve	0.72679	0.94673	0.91013

4. Discussion

The overall negative appendectomy rate in the study was 15.89%, which was comparable and lower than those of similar studies [15,16]. A study performed in 2005 in the Netherlands found that approximately 15% of the patients underwent a negative appendectomy, and the number was found to be similar to another large Swedish study [17]. The negative appendectomy rate was as low as 13% in another large volume North American study [18]. However, studies by Rathod et al. [16] and Chong et al. [19] documented higher negative exploration rates of 22.9% and 20.69%, respectively. Large population based studies have suggested that the rate of negative appendicectomies is remaining stable (15–20%) and has not declined for the past 15 years despite the increasing availability of newer tests [20].

Alvarado scoring system has been the most popular scoring system in acute appendicitis for a long time, due to its claimed high sensitivity and specificity [3,4]. The caveat is that the diagnostic efficacy of Alvarado has been well proven in western population only whereas it showed relatively less specificity and sensitivity when applied to oriental populations [21,22]. As per the findings in this study, Alvarado score was found to have medium sensitivity and specificity only. In a study by Memon et al. in Indian population, the sensitivity and the specificity of the Alvarado scoring system were found to be 93.5% and 80.6%, respectively [23]. However, evaluation of Alvarado in a study conducted by Schneider et al. on paediatric population revealed a PPV of 58% only [24]. A systematic review showed that the Alvarado score accurately predicts appendicitis and performs well as a 'rule out' criterion for decision making for observation or admission, due to its high sensitivity [25]. However, the review also found that the Alvarado score cannot be used to 'rule in' a diagnosis of appendicitis, without proper surgical assessment and further diagnostic testing. The World Society of Emergency Surgeons'(WSES) Jerusalem guidelines in 2015 also stated that the Alvarado score (with cut-off score < 5) is sufficiently sensitive to exclude acute appendicitis but is not sufficiently specific in diagnosing acute appendicitis [26].

The current findings on AIR score of very high sensitivity and low specificity are in line with similar studies. In the study by Scott et al., an AIR score of 5 or more demonstrated high sensitivities for intermediate and high risk patients with appendicitis (90%) and also for patients with advanced appendicitis (98%) [27]. In another study, the AIR score has shown far better results than the Alvarado score [17]. The AIR score probably works better in the paediatric population than the Alvarado score because the variables scored are easy to apply to children. The Alvarado score requires children to identify nausea, anorexia, and migration of pain, which may not always be accurate. Probably this is why the Alvarado score compares better to the AIR score in the adolescent age group, because this age group closely mimics the cohort on which the Alvarado score was first designed. Di Saverio et al. suggested that the combination of AIR and Alvarado scores might significantly reduce the risk of over-diagnosing acute appendicitis and thus give a reliable diagnostic performance, thus enable the treating surgeons to avoid the routine use of CT [28].

The study results found high sensitivity as well as specificity for RIPASA. These are comparable with the study done by Chong et al. [19]. In that study, the RIPASA score at a cut-off threshold total score of 7.5 was found to be a better diagnostic scoring system than Alvarado score for the diagnosis of appendicitis. Rathod et al. obtained a sensitivity of 82.61% and a specificity of 88.89% with the RIPASA score, as well as a PPV of 96.61%, an NPV of 57.14% and a diagnostic accuracy of 83.91% [16]. Nanjundaiah et al. also showed better efficacy for RIPASA over Alvarado in their study [29]. Another study showed a sensitivity level of 81% for the Alvarado system when the cut-off value was set at 6.5, and a

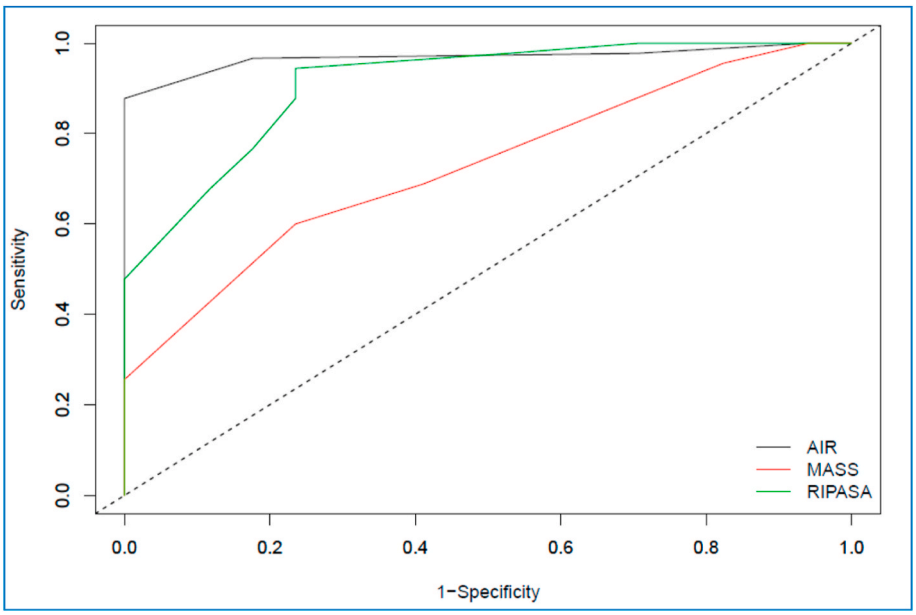

Fig. 1. Area Under Curve plots for scoring systems.

Table 3
Optimal Cut off values for maximum sensitivity and specificity for scoring systems.

Calculated Value	MASS Cut off 8	AIR Cut off 8	RIPASA Cut off 7.5
Sensitivity	60.0%	87.8%	94.4%
Specificity	76.5%	100%	76.5%
Positive Likelihood Ratio	2.550	Inf	4.01
Negative Likelihood Ratio	0.523	0.122	0.07
Positive Predictive Value	93.1%	100%	95.5%
Negative Predictive Value	26.5%	60.7%	72.2%
Area Under Curve	0.7268	0.96895	0.91013

sensitivity level of 83.1% for the RIPASA system when the cut-off value was set at 10.25 [12]. On the other hand, there are some studies in which RIPASA score was able to show no advantages over the modified Alvarado score in suspected acute appendicitis [30].

Ohmann and Eskelinen are few of the other scoring systems used for diagnosis of appendicitis in various centres. There are also reports on other diagnostic markers for appendicitis. For instance, a study, based on the results of univariate analyses, found some blood cell surface markers to be useful in the prediction of acute appendicitis namely HLADR + CD19, α/β TCR, and CD3/RA [31]. As per the results of another study, three factors, namely, body temperature ≥37.4 °C, C-reactive protein ≥4.7 mg/dl, and fluid collection surrounding the appendix on CT scan, have been found to be useful in predicting cases of complicated appendicitis preoperatively and facilitate decisions regarding emergency appendicectomy [32].

To summarise, the area under the ROC curve for the RIPASA and AIR scoring systems was significantly larger than it was with the Alvarado system. The RIPASA and AIR scores are fast and are definitely better in categorizing patients with suspected appendicitis and reduce the need for diagnostic imaging. Overall, a higher sensitivity, NPV and PLR and a lower NLR indicate that the RIPASA score and AIR scores are much better diagnostic tools than Alvarado score for diagnosing acute appendicitis in Asian population. The specificity of MASS can be improved significantly with only a minor drop in sensitivity if the cut off is raised to 7.5. However, the overall diagnostic accuracy would remain the same. In the case of AIR, specificity can be hiked to 100% with a slight gain in diagnostic accuracy if the cut off is raised to 8, albeit with a significant drop in sensitivity. For RIPASA, the ideal cut off remains the same at 7.5.

This study is not without its own drawbacks. First, the clinical diagnosis of acute appendicitis in the sample population was based on the clinical judgment of the surgical resident and registrar on duty which could have subjective variations. In addition, patients may have difficulty in defining the time of onset of symptoms. Also, different diagnostic modalities (abdominal ultrasonography) used in selected patients in the department could have affected the negative appendectomy rates detected in the study. Last, the sample size is comparatively small, which could attenuate the significance of the associations.

5. Conclusions

An ideal scoring system should work as a tool that speeds up as well as enhances the accuracy of decision-making, and at the same time saves up on the need for expensive or potentially harmful investigations. The Appendicitis Inflammatory Response score probably works better in the paediatric population than the Alvarado score because the variables scored are easy to apply to children. The Alvarado score requires children to identify nausea, anorexia, and migration of pain, which may not always be accurate. To conclude, this study validates that the Appendicitis Inflammatory Response score and Raja Isteri Pengiran Anak Saleha Appendicitis score have high discriminating powers and outperform the Modified Alvarado score. They could aid in selecting patients who require timely surgery or those who require further evaluation. Both these scores have the potential to turn out into scoring systems of choice if future research can substantiate our study findings.

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Declaration of competing interest

None.

Ethical approval

Research studies involving patients require ethical approval. Please state whether approval has been given, name the relevant ethics committee and the state the reference number for their judgement.

Yes.

Human Ethics Committee, Medical College, Thiruvananthapuram.

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CRediT authorship contribution statement

Meer M. Chisthi: Conceptualization, Formal analysis, Writing - original draft. **Anilkumar Surendran:** Conceptualization, Methodology, Writing - review & editing. **Jiju Therumpurathu Narayanan:** Formal analysis, Investigation, Visualization.

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Krishnakumar G. Kuttanchettiyar[1], Viswanathan Kollengode V[2], and Meer M. Chisthi[2]

Abstract

Background: Pancreatic cancer is a challenging disease, often requiring invasive procedures for diagnosis. Reliable tumour markers are essential for ensuring early detection and better patient outcomes. Although Carbohydrate Antigen 19-9 is the most commonly used marker, it is marred by low predictive accuracy and high false positivity. Carcino Embryonic Antigen also has limited practical use. A novel antigen, Cytokeratin fragment 21-1, is gaining significance for its diagnostic value in various tumours.

Materials and Methods: This prospective study aimed to evaluate the potential of Cytokeratin fragment 21-1 in comparison with Carbohydrate Antigen 19-9 and Carcino Embryonic Antigen in diagnosing pancreatic cancer. From January 2016 to December 2019, 45 patients with confirmed pancreatic ductal adenocarcinoma were included in this cross-sectional study.

Results: Carbohydrate Antigen 19-9 was raised in 22 patients, Carcino Embryonic Antigen was elevated in 17, and Cytokeratin fragment 21-1 was elevated in 30 cases. Carbohydrate Antigen 19-9 was found to be elevated in the presence of jaundice. Both Carbohydrate Antigen 19-9 and Cytokeratin fragment 21-1 had good correlation with stage of cancer, while Carcino Embryonic Antigen had very minimal correlation.

Conclusion: In this study, Cytokeratin fragment 21-1 was elevated in a higher number of cases than Carbohydrate Antigen 19-9 and Carcino Embryonic Antigen. Both Cytokeratin fragment 21-1 and Carbohydrate Antigen 19-9 correlated well with cancer stage. Also Cytokeratin fragment 21-1 was not affected by jaundice, unlike Carbohydrate Antigen 19-9. Therefore, Cytokeratin fragment 21-1 has the potential to be an effective individual tumour marker in pancreatic cancer.

Keywords

carbohydrate antigen 19-9, carcino embryonic antigen, cytokeratin fragment 21-1, pancreatic ductal adenocarcinoma, tumour marker

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Introduction

Pancreatic tumours stand out from other gastrointestinal malignancies due to their heightened likelihood of surgical inoperability and considerably low survival rates. Prompt detection is of utmost importance, as surgical intervention remains the sole curative measure for pancreatic cancer. Nonetheless, a mere 10%–30% of patients with pancreatic tumours are eligible for curative surgery, and out of those, only

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half are able to undergo R0 resection.[1] Even those patients who are able to undergo R0 resection with additional adjuvant treatment achieve a 5-year survival rate of approximately 25% only.[2] Those diagnosed with unresectable stage III and IV carcinomas are ineligible for any potentially curative treatment, leaving them with a dismal median survival period of around 5-12 months only.[3]

The non-specific nature of early-stage symptoms, coupled with the high biological aggressiveness of pancreatic malignancies, contributes to the significant delay in diagnosis and increased mortality. Due to the deep-seated location of the organ, obtaining tissue for definitive diagnosis is often challenging. Therefore, improving methods for early detection is crucial to increase the number of resectable carcinomas and to enhance patient outcomes. Various imaging modalities for pancreatic cancer include endoscopic ultrasound (EUS), computed tomography (CT), endoscopic retrograde cholangiopancreatography (ERCP), magnetic resonance imaging (MRI), and magnetic resonance cholangio-pancreatography (MRCP). However, many of these are prohibitively expensive or come with drawbacks such as radiation exposure and infrequent usability. Apart from the abovementioned methods, invasive methods like explorative laparoscopy and laparotomy may sometimes be needed for diagnosis and staging.

The commonest serum marker used in pancreatic cancer is the sialylated Lewis blood group antigen known as Carbohydrate Antigen 19-9 (CA 19-9).[4] It has been shown that CA 19-9 levels after surgery or radiation treatment directly affect the prognosis of pancreatic cancer cases.[5,6] The National Comprehensive Cancer Network (NCCN) recommendations continue to advocate the use of CA 19-9 as the biomarker in routine clinical practice.[7] However, inflammation, cholangitis, and biliary obstruction are common complications in advanced pancreatic cancer patients, and these factors can significantly affect CA 19-9 levels. Furthermore, CA 19-9 serum levels are significantly increased in both benign and malignant biliary diseases, such as choledocholithiasis, gall bladder cancer, and cholangiocarcinoma. Elevated CA 19-9 serum levels in 10%–50% of cases are found in benign or precursor pancreatic lesions such as acute or chronic pancreatitis, intraductal pancreatic mucinous neoplasm (IPMN), and pancreatic intraepithelial neoplasia (PANIN).[8-10] Even other benign conditions such as ovarian cysts, heart failure, Hashimoto's thyroiditis, rheumatoid arthritis, and diverticulitis have been associated with elevated serum CA 19-9 levels.[11,12]

Only approximately 40% of patients diagnosed with early stage I pancreatic cancer experience an increase in CA 19-9 levels.[13] CA 19-9 blood levels are unable to detect early or small tumours or precancerous lesions in 10%–15% of patients who have a higher risk of pancreatic cancer, such as those with hereditary pancreatitis, a family history of the disease, or Peutz-Jeghers syndrome.[14] Due to the fact that CA 19-9 production requires the presence of sialylated Lewis (Le) a blood group antigen, individuals with a Lea-b-phenotype (absence of Lewis antigen glycosyl-transferase) are unable to synthesize CA 19-9. This leads to a false-negative result when testing for CA 19-9 in these individuals (approximately 5%–10% of the population).[15] Additionally, due to its poor positive predictive value (PPV) of around .9% only, CA 19-9 cannot be used as an appropriate screening test in asymptomatic patients.[12,16]

CEA is a glycoprotein with established roles in predicting time to progression and overall survival in colorectal and lung cancer, as well as in detecting tumour recurrence. However, its use in pancreatic cancer diagnosis is not as evident, and although some data have been published, the diagnostic relationship is limited.[17]

Cytokeratin Fragment 21-1 also known as CYFRA 21-1 is a soluble structural protein fragment of cytokeratin 19 (CK19), an acid type cytokeratin, with a molecular weight of 40,000d.[18] Cytokeratin 19 (CK19) is a protein found in the intermediate filament proteins that are necessary for maintaining epithelial cell integrity. This antigen is recognized by 2 mouse monoclonal antibodies, KS 19-1 and BM 19-21, which were developed against the MCF-7 cell line. Normal and proliferative epithelium both express CYFRA 21-1, and monoclonal antibodies can be used to identify specific epitopes on cytokeratin 19 to characterize it.

There are various postulates explaining the possible effects of K19 in malignant tissues. It has been demonstrated that the release of CK19 fragment is closely related to the mRNA expression for CK19, and there is a possibility that the genomic change of CK19 DNA thus down-regulates the expression of mRNA for CK19.[19] The levels of CYFRA 21-1 are also found to be increased significantly in TNF-alpha-treated cells whilst displaying higher percentage of apoptosis, granular-like aggregation of CK19, as well as elevated activity of caspase-3 than non-treated cells.[20] Also, the levels of CYFRA 21-1 are found to decrease significantly when caspase-3 was inhibited in the TNF-alpha-treated cells. Hence, the release of CYFRA 21-1 could be reflective of cellular apoptosis during the process of tumour growth.

CYFRA 21-1 is already being developed as a tumour marker for squamous cell carcinoma, adenocarcinoma, and large cell carcinomas of non-small cell lung cancer (NSCLC). In addition to lung cancer, CYFRA 21-1 has also shown promise as a useful marker for monitoring tumour recurrence and predicting overall prognosis in various malignancies including those of the liver, cervical, oesophageal, breast, gastric, and bladder cancer.[21,22]

An ideal biomarker for cancer would allow for early detection and assist in treatment decisions, as well as predict overall outcomes and assess recurrence. However, finding such a biomarker can be challenging, and multiple biomarkers may need to be used in combination to achieve the desired level of accuracy. The search for new biomarkers for pancreatic cancer is ongoing and formed the basis of this study also.

Materials and Methods

The primary objective of the study was to compare the diagnostic efficacy of CYFRA 21-1 and CA 19-9 in patients with pancreatic cancer, while the secondary objective was to assess the correlation between the values of these markers and the stage of the cancer. The study was conducted using a cross-sectional design.

This study was conducted prospectively over a period of 3 years, from January 1, 2016, to December 31, 2019. Before beginning the study, permissions from the Institutional Review Committee and the Ethics Committee were obtained. The study was approved by the Human Ethics Committee, xxxx, vide IEC order no. 04/18/2015/MCT dated 31/07/2015. Adult cases with pancreatic ductal adenocarcinoma, as determined by cytology or histology, were eligible to be chosen as participants. To prevent interference with tumour marker readings, patients with any previously diagnosed malignancies or other causes of jaundice were removed from the study. The subjects were instructed on the study protocol, and written informed consent with signature was obtained from them for the blood collection. Before the initiation of definitive surgery or chemotherapy, blood samples were taken. There was no change in the management of the patients, and they underwent appropriate treatment based on multi-disciplinary tumour board decisions.

The sample size was calculated using a standardized formula for sample size estimates in diagnostic test studies,[23] with the sensitivity of the novel and established tests derived from reference studies. The study was designed with a sample size of 45, aiming for a power of 80% and an alpha error of 5%. Consecutive patients meeting the selection criteria were included. The levels of CEA and CA 19-9 were measured using a solid-phase, two-site chemiluminescent enzyme immunometric assay, while commercially available enzyme-linked immunosorbent assay kits were used to determine the levels of CYFRA 21-1. The normal range for CA 19-9 was set as below 37 U/mL, while for CYFRA 21-1 it was set below 1.96 ng/mL. The normal range of CEA was set at below 3 micrograms per litre, with a higher cutoff of 7 among smokers. Clinical staging was based on the AJCC staging system.

A comprehensive questionnaire was used to record clinical information for each case. Pearson's correlation coefficient was used to assess the correlation between the 3 tumour markers themselves and the correlation between age and tumour marker levels. Spearman's Rho test was used to examine the relationship between cancer stage and the tumour markers. The association between jaundice and the markers as well as between gender and markers was evaluated using the Chi-square test. Statistical analysis was conducted using Microsoft Office Excel and Epi Info software (CDC) version 6. Data are reported as arithmetic means (AM) with standard deviation (SD) or percentages, and statistical significance was considered whenever *P*-values were less than .05. All patient details are presented de-identified. We have followed relevant Equator guidelines, and the study is reported according to the STROBE checklist.[24]

Results

In this study, we examined 45 patients with pancreatic cancer. The study group was composed of 26 males and 19 females. The study group had a mean age of 59.73 years, with a range extending from 38 to 86 years old. The standard deviation of age was 10.24 years, indicating a significant variation in age within the study group. The participants were distributed across all stages, with 6 patients in stage 1, 19 in stage 2, 15 in stage 3, and 5 in stage 4. At the time of presentation, all patients except 9 presented with jaundice, which is a common symptom of pancreatic cancer (Table 1).

Among the 45 patients, 22 had elevated levels of CA 19-9, 17 had elevated levels of CEA, and 30 had elevated levels of CYFRA 21-1 (Chart 1). The mean values of CA 19-9, CEA, and CYFRA 21-1 were 940.62 (+/−2597.34) U/mL, 7.41 (+/−8.93) mcg/L, and 12.24 (+/−17.11) ng/mL, respectively, indicating that these tumour markers were present in measurable quantities in the pancreatic cancer patients under study. The range of values for these markers varied significantly, with CA 19-9 having the widest range of 2597.34 U/mL and CEA having the narrowest range of 8.93 mcg/L. It is interesting to note that while all patients had pancreatic cancer, the levels of tumour markers varied significantly, which could suggest that tumour markers may be influenced by the biological characteristics of individual patients.

We analysed the relationships between the 3 tumour markers. Pearson's correlation coefficient revealed a small negative relationship between CEA and CA 19-9, with an R-value of −.0179. In contrast, CEA and CYFRA 21-1 showed a weak positive association, with a correlation coefficient of .2555. CA 19-9 and CYFRA 21-1 showed a poor correlation, with an R-value of .2731. These findings suggest that the different tumour markers may have distinct biological implications in pancreatic cancer.

We analysed the relationship between gender and tumour marker positivity. For CA 19-9, the Chi-square statistic was

Table 1. Demographic Distribution of the Study Participants.

Demographic Characteristics		Frequency	Percentage
Gender	Male	26	57.78
	Female	19	42.22
Age group	31-40	1	2.22%
	41-50	7	15.55%
	51-60	12	26.67%
	61-70	16	35.56%
	71-80	8	17.78%
	81-90	1	2.22%
AJCC stage	I	6	13.33%
	II	19	42.22%
	III	15	33.33%
	IV	5	11.11%
Jaundice	Present	36	80%
	Absent	9	20%

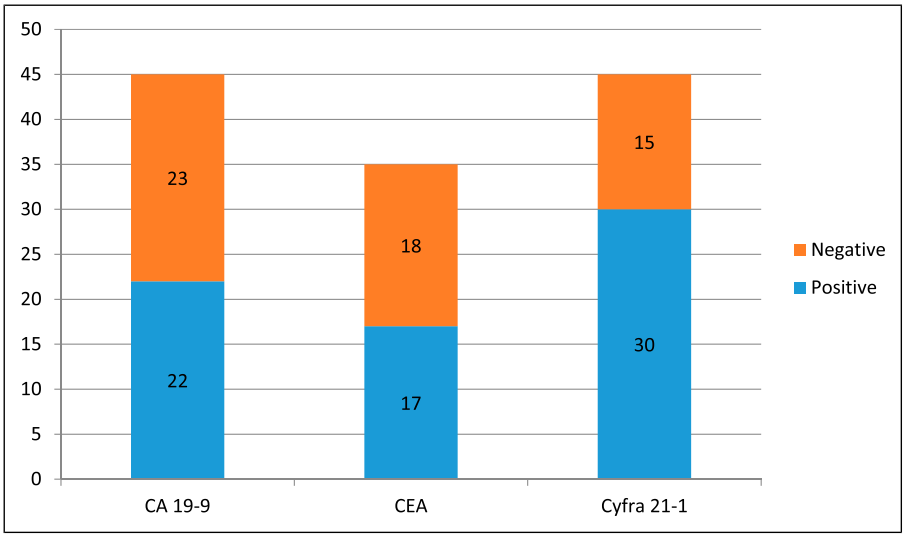

Chart 1. Distribution of positivity of the 3 tumour markers among study participants.

.3429, with a *P*-value of .558185. In the case of CEA, the Chi-square statistic was .8229, the *P*-value being .364346. For CYFRA 21-1, the Chi-square statistic was found to be .3302, with a *P*-value of .565534. None of the markers had any significant association with respect to gender.

We checked for any relationship between age and tumour marker levels with Pearson's correlation. In the case of CA 19-9, the R-value was .2125, with a *P*-value of .147043. For CEA, the R-value was found to be −.0181, the *P*-value being .982. For CYFRA 21-1, the R-value was found to be −.0019, with a *P*-value of .994618. None of the tumour markers had any significant correlation with the patients' age.

We also analysed the relationship between the tumour markers and jaundice by using the Chi-square test. We found a significant link between jaundice and CA 19-9, with a Chi-square statistic of 7.7816 and a significant *P*-value of .05. However, there was no link between CEA and jaundice, with a Chi-square statistic of 2.7011 and a *P*-value of .10028. Similarly, there was no relationship between CYFRA 21-1 and jaundice, with a Chi-square statistic of 1.6071 and a *P*-value of 2.0489.

To investigate the relationship between tumour markers and cancer stage, we used Spearman's correlation. We found a significant positive correlation between CA 19-9 and cancer stage, with a Rho value of .52348 and a *P*-value of .00022. In contrast, there was no significant correlation between CEA and cancer stage, with a Rho value of .02249 and a *P*-value of .8834. Interestingly, we found a significant positive correlation between CYFRA 21-1 and cancer stage, with a Rho value of .51269 and a *P*-value of .0032.

These results indicate that tumour markers may have distinct associations with different stages of pancreatic cancer. CA 19-9 was found to be negative in all AJCC stage 1 patients, whereas CYFRA 21-1 was shown to be positive in all stages (Chart 2). Lastly, we observed that all 3 tumour markers had lower values in stage 4 patients, which is surprising since this stage is generally considered the most advanced and severe.

Discussion

Our study, which included a sample of 45 patients diagnosed with pancreatic cancer, yielded some anticipated results as well as some unexpected ones concerning CA 19-9 and CYFRA 21-1. Our findings indicate that CYFRA 21-1 is more closely associated with pancreatic cancer than CA 19-9 while both CA 19-9 and CYFRA 21-1 showed a good correlation with the stage of pancreatic cancer. Also we observed that elevated levels of CA 19-9 were significantly linked to jaundice, a common symptom of pancreatic cancer.

Several studies have found a correlation between pre-treatment CYFRA 21-1 levels and overall survival, and as a potential marker for predicting treatment responsiveness to chemotherapy.[25] A comparison study of the diagnostic efficacy of CEA, CA 19-9, and CYFRA 21-1 found that the combination of all 3 markers resulted in the best outcomes, with the highest number of instances correctly identified.[26] Another study on pancreatic cancer indicated that CYFRA 21-1 demonstrates better sensitivity and predictive values than CA 19-9.[27] In this trial involving 59 patients, pre-treatment levels of CYFRA 21-1 showed a significant correlation with overall survival, regardless of the imposed cutoff (at a mean value of 4.9 ng/mL). Along with the presence of mutKRAS ctDNA, higher levels of CYFRA 21-1, CA 19-9, and CEA before the initiation of first-line chemotherapy have been significantly correlated to adverse overall survival.[28] Lee assessed multiple tumour markers among patients with primary pancreatic malignancy and those with benign pancreatic cysts and found CA 19-9 to have good diagnostic performance.[29] Based on 2 cutoff values, CYFRA 21-1 (≥2.0 and 1.83 ng/mL) was found to have an acceptable sensitivity of

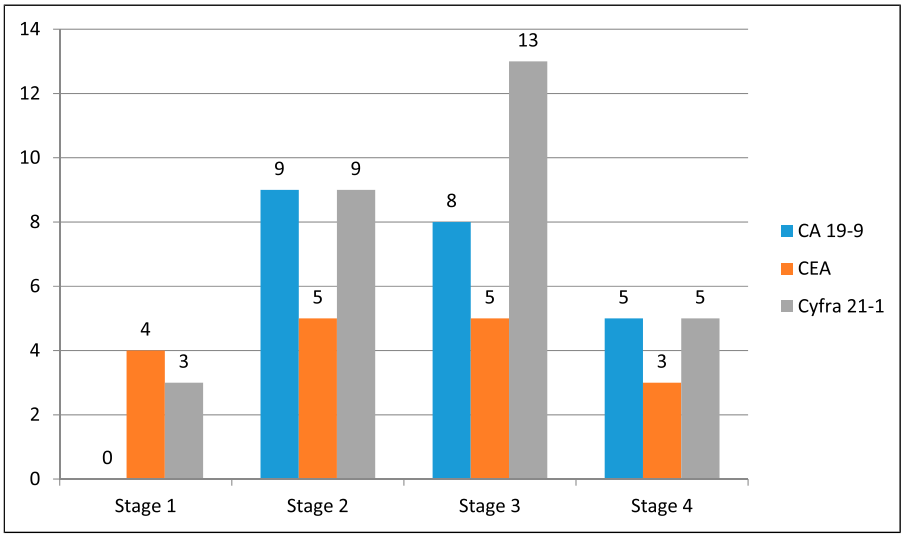

Chart 2. TNM stage-wise distribution of the 3 tumour markers.

80.4% and 82.3%, respectively, and was also much more significant than other tumour markers. A weak significant relationship was found between fluorodeoxyglucose (FDG) uptake by the tumour and CYFRA 21-1 or CA 19-9. Additionally, by multivariate analyses, both CA 125 and CYFRA 21-1 levels demonstrated independent prognostic significance for predicting overall survival.

CA 19-9 cannot be solely relied upon as a screening indicator in asymptomatic patients, but it can aid in differentiating between benign pancreatitis and pancreatic cancer to a certain extent. Studies by Liao et al, Groblewska et al, Banfi et al, and Jiang et al have reported excellent diagnostic performance in distinguishing between carcinoma and control groups, with high sensitivity and specificity rates. For instance, Liao et al found superior diagnostic performance in differentiating between 58 carcinomas and 102 controls, with a sensitivity rate of 81% and a specificity rate of 91%.[30] Similarly, Groblewska et al reported a sensitivity rate of 74% and a specificity rate of 100% in a study involving 62 carcinomas and 65 controls.[31] Banfi et al and Jiang et al also obtained high sensitivity rates of 79% and 85%, respectively, and a specificity rate of 100% in their studies involving carcinomas and control groups.[32,33]

Although the correlation is not strong, there is a link between serum CA 19-9 and tumour load, resectability, and overall survival. Patients with postoperative CA 19-9 levels exceeding 180 U/mL had a notably lower survival rate than those with lower values.[34] In patients with stage II–III cancer, where the accurate extent of disease spread may be challenging to determine before surgery, preoperative serum levels of CA 19-9 are considered clinically relevant in evaluating prognosis.[35] Independent predictors of survival in patients include low preoperative serum CA 19-9 levels, a significant postoperative decrease in levels, and stand-alone levels of less than 200 U/mL.[36]

In an interesting study, increasing the CA 19-9 serum cutoff to above 100 U/ml or 1000 U/ml for the purpose of identifying pancreatic cancer resulted in a higher specificity of 98% and 99.8%, respectively.[37] However, this was accompanied by a decrease in sensitivity, which declined to 68% and 41%, respectively. According to other studies, CA 19-9 levels have been found to increase in approximately 40% of Stage I pancreatic tumours.[38,39] In only approximately 33% of patients, CA 19-9 shows a modest increase in localized tumours (T1 and T2) with values greater than 120 U/mL.[40] While some researchers suggest that CA 19-9 levels above 300 U/mL should be carefully assessed for the presence of malignancy, others propose adjusting CA 19-9 values by incorporating C-reactive protein or bilirubin to enhance diagnostic accuracy.[41,42]

CEA has been validated as a useful tumour marker for staging and prognosis in colon cancer, but it is not an effective biomarker in pancreatic cancer. The main problem with CEA in pancreatic cancer is its very low sensitivity, which ranges from 25% to 56%, for distinguishing between carcinoma and controls (in spite of a high specificity of 82% to 100%).[43-46]

There are several limitations to this research. Firstly, it was conducted in a single centre which may limit the generalizability of the findings to other settings. Secondly, the study design did not allow for follow-up to assess prognosis and survival. Additionally, because only confirmed cases were included, it was not possible to calculate the sensitivity and specificity values and area under the curve for each marker. Moreover, the study did not include information on the patients' Lewis status, which could have impacted CA 19-9 levels. Finally, the sample size was relatively small, which may have reduced the statistical power of the analysis.

Conclusions

An ideal tumour marker should be able to provide both diagnostic and prognostic information, as well as the ability to stratify patients. Both CYFRA 21-1 and CA 19-9 correlate with the stage of pancreatic cancer and therefore provide information on the patient's prognosis. However, CA 19-9's low sensitivity makes it unsuitable as a screening tool for pancreatic cancer. Furthermore, CA 19-9 is limited by its false-negative results in Lewis-negative individuals and false-positive results in cases of obstructive jaundice and other benign diseases. In contrast, CYFRA 21-1 is more strongly associated with pancreatic cancer and is not impacted by jaundice. Therefore, CYFRA 21-1 shows significant promise as a tumour marker in pancreatic malignancies. Future studies may aim to address the limitations of this study and further investigate the potential of CYFRA 21-1 as a tumour marker in pancreatic cancer using high-quality sample cohorts and standardized multiplex formats.

Appendix

Abbreviations

CA 19-9	Carbohydrate Antigen 19-9
CEA	Carcino Embryonic Antigen
CYFRA 21-1	Cytokeratin fragment 21-1.

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Author Contributions

Krishnakumar G Kuttanchettiyar: Conception and design of the work and acquisition of data for the work; Viswanathan Kollengode V: Drafting the work and reviewing it critically for important intellectual content; Meer M Chisthi: Analysis and interpretation of data for the work and final approval of the version to be published.

Declaration of Conflicting Interests

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Ethical Statement

Ethical Approval

The study was approved by the Human Ethics Committee, Government Medical College, Trivandrum, Kerala, vide IEC order no. 04/18/2015/MCT dated 31/07/2015.

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Supplemental Material

Supplemental material for this article is available online.

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