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Research

Five Year Request Pattern for Tumour markers in a Tertiary Centre in Nigeria

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Abstract

This study is a 5 year review of requests pattern for assay of four tumour markers: Prostate specific antigen, (PSA), Carcinoembryonic antigen (CEA), Human chorionic gonadotrophins (HCG) and Alpha feto protein (AFP). All plasma samples for the markers from February 1998 – December 2002 were processed weekly by immuno-radiometric assay method. Biodata from request form were collated and analyzed. A total of 4,249 requests were received for the period under review which showed an increase yearly from inception to the last year of review. Requests for PSA assay were the most common, 2,258 (53.1%). A total of 845 tests (19.89%) were recorded for AFP. This was followed by CEA, 745 tests (17.53%) and HCG, 401 (9.44%), indicating suspicion of malignancies of colorectal region and gestational trophoblastic diseases, respectively. There appears to be an increasing trend in the proportion of requests with values outside the reference range over the years in review. The implication of this request pattern is discussed.

Keywords: Tumour markers requests, prostrate specific antigen PSA, carcinoembryonic antigen CEA, human chorionic gonadotrophins HCG, alpha feto protein AFP

Introduction

A tumour marker is a substance that is present in or produced by a tumour or host in response to a tumour and can be used to differentiate a tumour based on measurement in blood or secretions (Chan & Stewart, 1999). Although there is no ideal tumour marker, the positive predictive value of these markers combined with their usefulness in monitoring treatment of specific cancers make their analysis worthwhile and very useful in patient care (Ablin, 1997). A useful approach to evaluating tumour markers is to do multiple tests for the same analyte or multiple markers for the same type of cancer. This is the receiver operating characteristic (ROC) curve. The ROC curve can be constructed by plotting sensitivity versus specificity or true positivity rate versus false positive rate.

Prostate specific antigen (PSA) is a glycoprotein containing 237 amino acids and a molecular weight of 28,340 daltons. (Belanger *et al*, 1995). It is secreted by ductal epithelium and has been demonstrated in the male and female periurethral gland, anal gland, apocrine sweat gland and some other glands. However, it is still the most useful tool available for the diagnosis and staging of prostate cancer as well as the most widely used laboratory test in oncology (Kardamakis, 1996).

Carcinoembryonic antigen (CEA) is an oncofeotal antigen produced in significant amount by many feotal cells and some cancers especially of the colorectal region but only in trace amount by normal adult cells. It is a glycoprotein of 180-200 KD (Gold and Freeman, 1965). Over 90% of primary colorectal cancers produce CEA (Adam & Morris, 1996). Elevated levels have also been demonstrated in about 50% of patients with metastatic breast cancer (Adedapo *et al*., 2000).

Alpha feto-protein (AFP) is produced by the

liver and gastrointestinal tract epithelium during gestation and falls after birth to very low levels.

Serum levels are elevated in about 70% of patients with hepatocellular cancer (Wallach, 1996) It is also elevated to a lesser extent in a variety of benign conditions especially inflammatory disease of the liver (Chan and Stewart, 1999).

Human chorionic gonadotrophin (HCG) is a marker, which is nearly exclusively produced by the trophoblastic epithelium of the placenta under normal circumstances. The levels also rise in pregnancy and are secreted into the blood by trophoblastic tumours as well as germ cell neoplasm of the testis and ovaries (Chan and Stewart, 1999). The usefulness of the marker lies in its sensitivity in monitoring the burden of secreting trophoblastic tumours. The β subunit is measured by radioimmunoassay to avoid cross reactivity with luteinizing hormone, which shares a similar α - subunit with HCG.

This study was carried out in the Radioimmunoassay Laboratory of the University College Hospital, Ibadan to show the pattern of request and the relative frequency of different cancers in this environment. The Laboratory is largely supported by the International Atomic Energy Agency, Vienna that provides equipment and tracers for PSA, CEA, AFP and β-HCG.

Materials and methods

The record of all the requests processed since the inception of the tumour markers laboratory in February 1998 were accessed and analyzed as shown in Table 1. Whole blood specimen in heparinised bottles were sent to the laboratory, where the plasma was separated from red cells and stored for weekly batch analysis using Radioimmunoassay (RIA) reagents and tracers supplied by Skybio Limited TytheFarm Wyboston Bedfordshire, U.K.

Table 1: A five-year request pattern for tumour markers

Year	PSA no(%)	AFP no(%)	CEA no(%)	HCG no(%)	Total no(%)
1998	169 (35)	139 (28.8)	102 (21.2)	72 (15)	482 (11.34)
1999	175 (32.2)	156 (28.7)	135 (24.9)	77 (14.25)	543 (12.78)
2000	368 (52.6)	131 (18.7)	113 (16.2)	87 (12.4)	699 (16.45)
2001	611 (59)	183 (17.6)	175 (16.9)	68 (6.6)	1037 (24.41)
2002	935 (62.7)	236 (15.8)	220 (14.9)	97 (6.5)	1491 (35.02)
Total	2258 (53.1)	845 (19.89)	745 (17.53)	401 (9.44)	4,249

PSA: Prostrate Specific Antigen, AFP: Alpha Feto Protein, CEA: Carcinoembryonic antigen, HCG: Human Chronic Gonadotrophin

Method of analysis

The Immunoradiometric method was employed to assay the level of the 4-tumour markers. 100µl of plasma was mixed vigorously with 200µl of assay buffer and a polystyrene bead in a 75 x 12mm plastic tube. The mixture was left for 2hrs and later washed twice with 2ml of wash buffer. 200µl of ^{125}I tracer was then added in a fume chamber and left on a rotator for 2 hrs. Washing was again carried out and counting was done on a gamma counter for 100secs. The standards were treated similarly. The samples and standards were processed in duplicates.

Principle of the assay

Immunoradiometric assay (IRMA) is a method capable of measuring the primary reaction between hapten or antigen and a single antibody. In IRMA the antibody is labeled with a radioactive isotope usually ^{125}I. It involves typical 'sandwich' or two sites IRMA. Antibody is first attached to a solid phase by passive adsorption or by covalent binding. Antigen from the sample is then allowed to react with the solid phase antibody, other protein is washed away and a labeled antibody is added which reacts with the bound antigen through a second and distinct antigenic determinant. The bound count is directly proportional to the concentration of the antigen.

The analysis used in this study is based on the reference values for this environment, PSA (0-4µg/L), CEA (0-8µg/L), AFP (0-10kU/L) and ßHCG (0-5mµ/L). All the results were analyzed using epi-info statistical package according to the indication for the requests and where such was not stated on the forms it was regarded as a sample for screening.

Table 2a-d: Frequency, range and mean of tumour markers

2a: PSA

	Freq	Mean µ/gL	Range
Carcinoma of the Prostate	1050	86.2	1-2746
Benign Prostatic Hyperplasia	981	31.4	0.1 – 915
Screening	207	27.8	1-1265
Others (Non-specific)	20	55.0	1– 273
Total	2258		

2b: AFP

Indication	Freq	Mean (kU/L)	Range
Chronic Liver Diseases (Cirrhosis, PLCC etc.)	487	240	1 – 3940
Other Malignancies	105	31	0.1 – 440
Screening	253	44.8	1. 880
Total	845		

Results

A total of 4,249 requests were received for the four tumour markers during the period under review, Table 1. The results showed that there is generally an increase in the total number of requests sent from the year 1998 – 2002. The table also showed that PSA was the most frequently requested marker of all the markers (53.1%) accounting for over half of the total request made over the entire period. The request ranged from 35% in 1998 to 59% in 2001 and almost 63% in 2002. The most common indication for this request was suspicion of carcinoma of the prostate (Table 2). The mean result for this indication was 86.2ug/L (range 1-2746). The age range for all requests for PSA is 40-90 years.

AFP was the second common marker requested (19.89%). The most common indication for this assay was suspicion of chronic liver disease including cirrhosis. The mean was 240kU/L (range 1-3940). The commonest indication for CEA was suspicion of malignancy of the colon and rectum. βHCG is the least requested marker. The request was exclusively from the female patients whose age ranged from 9->70years.The commonest indication being gestational trophoblastic disease (Table2).

2c: CEA

Indication	Freq	Mean (μg/L)	Range
Malignancies colon, rectum	414	148	1 – 1500
Chronic Liver diseases	125	32	5 – 182
Screening	172	40	0.1 – 500
Miscellaneous	34	12	0.1 – 560
Total	745		

2d: BHCG

Indication	Freq	Mean U/L	Range
Gestational trophoblastic diseases	299	697	0.5-8,864
Screening	72	307	0.2 – 5385
Others	30	773	4.5 – 3500
Total	401		

Table 3 shows the percentage of request within the reference interval while Table 4 shows a consistent decrease in the percentage of requests with values within the reference interval over the years under review except for CEA assay.

Table 3: The percentage of requests within the reference interval over period of study

Year	PSA (0-μg/L)	AFP (0-10kμ/l)	CEA (0-8μg/l)	HCG (0-5mU/l)
1998	95 (169)	118(139)	82(102)	43(72)
	56.2%	84.9%	80.4%	59.7%
1999	79(175)	128(156)	114(135)	42(77)
	54.9%	82.1%	84.4%	54.5%
2000	177(368)	105(131)	98(113)	43(87)
	48.1%	80.3%	86.7%	49.4%
2001	303(611)	136(183)	122(175)	33(68)
	49.6%	74.3%	69.7%	48.5%
2002	291(935)	169(236)	166(223)	32(97)
	31.1%	71.6%	74.4%	32.9%

Total number of requests for each year in parenthesis

Table 4: Percentage of results above the reference ranges

Year	PSA (0-μg/L)	AFP (0-0kμ/l)	CEA (0-8μg/l)	HCG (0-5mU/l)
1998	74 (169)	21 (133)	20 (102)	29 (72)
	43.8%	15.1%	19.6%	40.3%
1999	96 (175)	28 (156)	21 (135)	35 (77)
	45.1%	17.9%	15.6%	45.5%
2000	191 (368)	26 (131)	15 (113)	44 (87)
	51.9%	19.7%	13.7%	57.5%
2001	308 (611)	47 (183)	53 (175)	35 (68)
	50.4%	25.7%	30.3%	51.5%
2002	644 (935)	67 (236)	57 (221)	65 (97)
	68.9%	28.4%	25.4%	67.1%

Total number of requests for each year in parenthesis

DISCUSSION

The need to screen for any tumour in the human subject is of prime importance because cancers that are detected early often have better prognosis. The tumour marker-screening laboratory was established late in 1997 and started full operation in February 1998. Over the past 5 years, it could be seen that the awareness of the services rendered by this laboratory has grown from a total of 482 samples examined for all the range of tumour markers at inception in 1998 to 4,249 samples in the year 2002. This represents more than a-nine fold increase. This figure reflects the bulk of requests from a University Tertiary hospital located in the South Western part of Nigeria with catchment areas from Oyo, Osun, Ogun and Lagos states.

Of all the tumour markers requested, PSA was most commonly requested. The preponderance for PSA can be explained by the fact that prostate cancer is the most common cancer among Nigerian males from our cancer registry morbidity figures (Ogunbiyi and Shittu, 1999). More men are getting conscious of the increasing incidence of prostate cancer, hence the increase in the percentage of people consulting their Physicians for screening even in the absence of symptoms. PSA is currently the tumour marker of choice for prostatic carcinoma. Various indices of PSA have been developed in an attempt to refine its sensitivity and improve its clinical value.

This includes PSA velocity, free versus complex PSA, PSA index or PSA density. (Ogunbiyi and Shittu, 1999). The free to total PSA ration is lower in patients with prostate cancer than in those with elevated PSA level due to benign prostate hyperplasia (Wymenga & Mensink, 1999). CEA has been found to be elevated in a variety of cancers such as uterine (Juang et al 2000), lung (Lee *et al* 1991) breast (Molina *et al*, 1999), liver (Khalifa *et al*, 1999), gastrointestinal tract (Falcone *et al*, 1985), colorectal (Holubee *et al*, 2000), kidney (Kokocinska *et al*, 1996) and pancreatic cancer (Nazli et al 2000). It has also been found elevated in non-neoplastic conditions such as pleural effusions and others (Garcia-Pachon et al1997). Probably, the most useful application of this marker is post surgical prognostic indicator in the treatment of colorectal neoplasms.

Generally there has been an increase in the number of requests received for all tumour markers and this could be attributed to increased awareness of the provision of tumour marker services and or reliability of the results obtained from the requests. This study highlights the possibility of an increase in the number of patients with diagnosis of malignancy within the years of review. Although tests for tumour makers are known not to be specific or sensitive enough to be used for mass screening of cancers they are mainly used internationally to monitor patients' response to treatment and to check for recurrence of cancer. In some types of cancers, their level may reflect the extent or the stage of the disease and can be useful in predicting how well the disease will respond to treatment.

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