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Core Needle Biopsy of Adrenal Neoplasm-A Study of Diagnostic Efficacy of Combination of A103 and AE1/AE3

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Abstract: *Incidence rate of metastatic carcinoma to adrenal cortex is close to that of primary neoplasm of the organ. Precise diagnosis of Primary Adrenocortical neoplasm and their differentiation from metastatic carcinoma is essential. Fine Needle Aspiration yields uncertain cellularity, whereas surgical specimen are associated with relatively high morbidity. Core Needle Biopsy is becoming rapidly popular mode for tissue diagnosis of the neoplasm of this region. Routine Hamatoxelene and Eosin stain does not always give precise diagnosis and help of immunohistochemistry is necessary more often than not .Though combination large array of antibody provide most specific diagnosis in FNA specimen, we tried to find combination of minimum number of immunomarkers in Core Needle Biopsy specimen to reach precise diagnosis. Formalin-fixed, paraffin-embedded cell blocks from 31 adrenal Core Needle Biopsy specimens were stained for AE1/AE3 and A103. Immunohistochemical results were analyzed in semiquantative manner. All 11 cases containing normal, hyperplastic, and neoplastic adrenal cortical cells showed Grade 2-4 positivity, while none of them revealed positive reaction to AE1/AE 3.In contrast, all metastatic carcinoma showed varying intensity positive reaction to AE1/AE3 and was uniformly negative for A103. We found combination of Immunomarkers (A103 and AE1/AE3) is very effective and sensitive and can be used frontline antibodies in Core Needle Biopsy in differentiating primary from metastatic neoplasm involving Adrenocortical region.*

Keywords*: Core-needle Biopsy, Adrenal, A103/AE1/AE3*

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I. Introduction

Incidence rate of Adrenal Neoplasm is less than neoplasm involving many other organs like Lung ,Liver, Large Intestinal tract. Peculiarity of Adrenocortical neoplasm is that almost 50% of such tumours are of metastatic origin, many of which share similar histomorphology with those of Primary neoplasm of the region . (1)With advent of Radio imaging technique ,Ultrasonography, CT and MRI Scan detect tiny Adrenocortical masses more readily.(2)Core Needle Biopsy of Adrenal mass is an useful tool for evaluation of such lesion. Adrenal mass are usually detected either while following investigative protocol of suspected neoplastic disease or during staging investigation of known metastatic malignancy. Benign, nonfunctioning, adrenocortical adenomas are common, reported to be present in 2% to 60% of the general population.(2,3) However, since the adrenal gland is known to be a common site of metastatic disease, it is necessary to determine clonal character of tumour cell and confirm that such a mass does not represent metastasis.(4)

It is difficult to differentiate, at times, between primary and metastatic neoplasm involving adrenocortical region even in Core Needle Biopsy while using routine Haematoxyelene and Eosin stain and necessitate use of immunohistochemical markers more often than not. In past, the immunohistochemical distinction between primary adrenal and extra-adrenal tumors on FNA and surgical specimens has been made with relatively small number of antibodies, none of which recognized antigens on adrenal cortical cells. Thus, using only routine Haematoxyelene and Eosin stains and a short panel of "supporting" immunohistochemical stains, a diagnosis of a primary adrenal tumor was essentially made by method of exclusion. Nowadays ,however ,a large panel of Immunohistochemical markers including the monoclonal antibody D-11, Adrenal 4 binding protein(Ad4BP),A103,Calretinin and Inhibin A, are routinely used in advanced resourceful countries to detect primary Adrenocortical neoplasm and differentiate them from metastatic malignancies. With limited resources in developing country like India, we cannot always have ready access to such large array of relatively expensive Immunohistochemical markers, many of which are not readily available. Against such reality, we tried to find relatively inexpensive, readily available antibody combinations of high sensitivity and specificity and which could be used as `Frontline` antibodies to differentiate primary from metastatic adrenocortical neoplasm. We have used combination of A103 and AE1/AE3 to that end.

II. Materials And Methods

Clinical ,Radiological data including copy of histopathology requisition slips and paraffin blocks of previously diagnosed cases of Adrenocortical neoplasm were collected from tertiary treatment center in Kolkata. Team of physician, surgeon and pathologist in Medical College, Kolkata went through the clinical and Radiological data including CT ,MRI and as well as all other investigative reports .

All of the sections represented antemortem Core Needle Biopsy material of confirmed diagnosed neoplasm involving Adrenocortical region. Though pathologist and Radiologists obviously attempted for biopsy of Neoplastic lesion involving Adrenal Cortex, we found 8 out of total 31 cases represented benign lesion including normal and hyperplasitic adrenocortical tissue. All adenomas and adrenocortical carcinomas were clinically non-functional.

5 micron sections from paraffin wax embedded blocks were cut and stained with Hematoxylene and Eosin stain. Other section of same thickness were mounted on aminopropyltriethoxysilane treated slides (Sigma, Poole, Dorset, UK) and dried overnight at 37 C. Endogenous peroxidase activity was blocked on incubation in 3% alcoholic hydrogen peroxide for 10 minutes. Immunohistochemical analysis was performed with the mouse monoclonal antibody, A103, at a dilution of 1:400 and with cytokeratins AE1/AE3 (Boehringer Mannheim, Indianapolis, IN) at a dilution of 1:1,000. Antigen retrieval was performed by microwaving the sections in a citrate buffer at a pH of 6.0 for 10 minutes followed by designated cooling period.

Localization was performed using biotinylatedantimouse immunoglobulin (1:200 dilution, (Dako, Copenhagen, Den mark) and peroxidase streptavidin biotin complex (Dako). Diaminobenzidine (Dako) was used as the chromagen. Sections were counterstained using Harris's haematoxylin. Negative controls, where the primary antiserum was omitted and replaced with mouse immunoglobulin (Dako), were performed in all cases. Positive controls were comprised of otherwise unremarkable ovaries .Following footsteps of Zhang P J.et al, Immunoreactivity was evaluated according to previously designated semi quantitative protocol (table 1).

Table 1

Semiquantative assay of Immunoreactivity of A103 and AE1/AE3 under light microscopy		
1.	Negative	Tumour cell cytoplasm did not show any Immunoreactivity
2.	Positive	Tumour cell cytoplasm show Immunoreactivity
a.	Grade 1	Less than 10% of Tumour cell cytoplasm show weak or strong intensity of Immunoreactivity
b.	Grade 2	11 -25% of Tumour cell cytoplasm show weak or strong intensity of Immunoreactivity
c.	Grade 3	26 -50% of Tumour cell cytoplasm show weak or strong intensity of Immunoreactivity
d.	Grade4	51% or more of Tumour cell cytoplasm show strong intensity of Immunoreactivity

Table 2

A103 and Cytokeratin AE1/AE3 Immunoreactivity in Primary Adrenal Gland lesion and neoplasm					
		Adrenocortical Benign/normal lesion	Adrenocortical Hyperplasia	Adrenocortical Adenoma	Adrenocortical Carcinoma
	Total(n=11)	(n=6)	(n=2)	(n=2)	(n=1)
A 103	Negative	Nil	Nil	Nil	Nil
	Positive	6	2	2	1
	Grade 1	x	x	x	x
	Grade 2	1	x	1	x
	Grade 3	1	1	x	x
	Grade 4	4	1	1	1
Cytokeratin AE1/AE3	Negative	6	2	2	1
	Positive	Nil	Nil	Nil	Nil
	Grade 1	x	x	x	x
	Grade 2	x	x	x	x
	Grade3	x	x	x	x
	Grade 4	x	x	x	x

Table 3

A103 and Cytokeratin AE1/AE3 Immunoreactivity in metastatic tumor to Adrenal Gland										
		Lung	Liver	Kidney	Large Intestinal Tract	Breast	Pancreas	Prostatic Adenocarcinoma	Malignant Meningioma	Gallbladder Adeno carcinoma
	Total(n-20)	(n=8)	(n=1)	(n=2)	(n=2)	(n=1)	(n=1)	(n=2)	(n=1)	(n=2)
A103	Negative	8	1	2	2	1	1	2	1	2
	Positive	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	Grade 1	x	x	x	x	x	x	x	x	x
	Grade 2	x	x	x	x	x	x	x	x	x
	Grade 3	x	x	x	x	x	x	x	x	x
	Grade 4	x	x	x	x	x	x	x	x	x
Cytokeratin AE1/AE3	Negative	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	Positive	8	1	2	2	1	1	2	1	2
	Grade 1	x	x	x	x	x	x	x	x	x
	Grade 2	x	x	x	x	1	1	2	1	2
	Grade 3	7	1	2	x	x	x	x	x	x
	Grade 4	1	x	x	2	x	x	x	x	x

III. Result

Table 2 and 3 summarizes results of immunohistochemical study of such primary and metastatic lesion. In all 11 confirmed cases of primary adrenocortical lesion in our study, significant positivity to A103 was noted. 8 of such 11 cases represented normal, hyperplastic and non-neoplstic tissue. 2 were adenomas whereas 1 was adrenocortical carcinoma. A103 reactivity was of Grade 2 to 4 (mostly grade 4) in all cases. Sole Adrenocortical carcinoma showed Grade 4immunoreactivity. Immunostaining was in a granular pattern within the cytoplasm of intact benign adrenal cortical cells as well as in Hyperplastic, Adenomatous and Malignant adrenocortical tumors. In cases in which cell membranes were disrupted, the antibody showed a netlike granular distribution pattern associated with scattered naked nuclei of adrenal cortical cells. None of such primary adrenocortical lesion revealed immunoreactivity to cytokeratins AE1/AE3.All of 20 confirmed cases of metastatic carcinoma, were found positive for cytokeratins AE1/AE3 albeit of varying Grades (Table 3).None of the cytokeratin-positive tumor cells was positive for A103; however, few A103- positive cells were scattered among the cytokeratin-positive cells in 4cases- apparently remnant of original Adrenocortical tissue amidst metastasis.

IV. Discussion

Saboorian et al. in a prospective study established that metastases represented 45.1% of 133 of studied adrenal neoplasm, whereas primary adrenal lesions represented the remaining portion 51.1% of the cases.(1) Owing to the almost equal incidence of primary adrenal and extra- adrenal neoplasm, one of the observations of this study was the importance of obtaining a tissue diagnosis of an adrenal lesion for patients with a history of an extra-adrenal malignant neoplasm. In addition, it was found that the majority of metastatic lesions were from the lung (81.7%) -predominantly adenocarcinoma, followed by those from the kidney (6.7%) and melanoma (6.7%).(1, 3)

Vazquez B.J et al. in their study described sites of primary tumor that metastasized into the adrenal glands in descending order of incidence rate were -Kidney (37%), Lung (15%), Colon (9%), Pancreas (8%), Neuroendocrine (4%), Leiomyosarcoma (4%), Liposarcoma (4%), Melanoma (4%), Liver (3%), Gastrointestinal tract (2%), Breast (1%). Lymphoma (1%), Ovary (1%), Thyroid (1%), Cholangiocarcinoma (1%), Endometrium (1%), and Tonsils (1%). (5)Nance KV.described importance of Immunohistochemistry as an essential tool for making precise diagnosis in differentiation between Primary and metastatic adrenocortical neoplasm. They opined along with immunohistochemistry , electron microscopy have had an important role in narrowing the differential diagnosis.(6)In such scenario, with goal to seek out suitable minimum number of Immunohistochemical-marker combination, we went through literature and found that In the past, the immunohistochemical distinction between primary adrenal and extra-adrenal tumors on FNA and surgical specimens has been made on a relatively small number of antibodies, none of which recognized antigens on adrenal cortical cells.it was essentially diagnosis made by exclusion of possible differential diagnosis.

Although the usefulness of A103 in diagnosis of Primary Adrenocortical neoplasm in surgical specimens of the adrenal gland has been reported since long, (7, 8) It was Coulie et al (9)first cloned Melan-A gene from human melanoma cell line SK-MEL-29. Concurrently, Kawakami and coworkers(10)independently

cloned the same gene and designated it as MART-1. The Melan-A recombinant protein subsequently was produced in Escherichia coli by Chen and coworkers (11)and used to generate mouse mono- clonal antibodies, one of which was A103 .It was found in subsequent studies that A103 immunoreactivity occurred in a limited number of other cell types, including adrenocortical cells, Leyding cells of the ovary and testis, Sertoli cells, theca and granulosa cells of the mature ovarian follicle, and luteal cells of the corpus luteum.(7)

Since then, several studies have been performed to test the reliability of A103 immunoreactivity in the assessment of adrenal gland tumors. Busam et al (7)reported that none of14 renal cell carcinomas (clear cell type) or 5 hepatocellular carcinomas was reactive for A103. Five Pheochromocytomas, 111 carcinomas, 40 germ cell tumors, and 33 miscellaneous nonmelanocyticepithelioid tumors also were negative. (7)In contrast, A103 reactivity was seen in 5 (100%) of 5 adrenocortical adenomas, 16 (100%) of 16 adrenocortical carcinomas, and 13 (100%) of 13 metastatic adrenocortical tumors.(4) The study by Renshaw and Granter (8)supported these findings when they reported A103 reactivity in 15 (68%) of 22 adrenocortical adenomas and 2 (50%) of 4 adrenocortical carcinomas and no reactivity in 43 renal tumors (including 33 renal cell carcinomas and 8 oncocytomas) or 25 hepatocellular carcinomas. Loy and coworkers reported 21 cases of Adrenocortical tumours were positive whereas none of 16 metastatic carcinomas from the lung, kidney, breast, liver and esophagus were positive.(12)Another study by Ghorab and colleague(13)found 31 0f 32 Adreenocortical neoplasm(21 adenoma,11 carcinomas) were A103 positive. With exception 1,all of 86 Renal Cell Carcinoma including 67 Clear cell variant of Renal Cell Carcinoma and 25 Hepatocellular carcinomas were negative to A103.

AE 1/AE3 also known to some as `Keratin cocktail or pankeratin in literature detects CK1 - 8, 10, 14 - 16 and 19, but does not detect CK17 or CK18. AE1/AE3 was reported consistently positive in Adenocarcinoma of Lung(14),Clear cell variant of Renal cell carcinoma , Adenocarcinoma of large intestinal tract, Breast, Pancreas, Prostate, Malignant Meningioma and Malignant Mesothelioma.(15-19)We chose to study the effect of combined expression of A103 and AE1/AE3 in such adrenal tumors.

We noted 100% positivity toA103 in all Primary Adrenocortical lesion, all of which and negativity to AE1/AE3 which is in concurrence to earlier study of Sandra JS.et al and Zhang PJ. et al.(20, 21) Cytokeratin (AE1/AE3) was positive in all cases of Metastatic Carcinoma to Adrenal Cortex in our study. All cases of metastatic Lung carcinoma were positive to AE/AE 3 where 87. 5% showed grade 3 and remaining 12.5 % showed grade 4 positivity. In contrast to the study of Sandra JS.et al. (20)we found varying degree of low intensity positivity in Metastatic Clear cell variant of Renal cell carcinoma and Hepatocellular carcinoma. Our finding is in concurrence with Ankur RS. et al. (22)

Presently combination of monoclonal antibodies including D11, adrenal 4 binding protein (Ad4BP),A103,calretinin, and inhibin A are used in developed countries to detect primary adrenocortical neoplasm.

D11 is a monoclonal antibody that is known to recognize several 59-kd proteins capable of binding apolipoprotein E. Primary Adrenocortical neoplasm showed nuclear positivity, though varying degree of cytoplasmic positivity was noted in100% of Hepatocellular carcinomas,60% of lung carcinomas and occasional Renal Cell Carcinoma.(23)Calretinin,a calcium binding protein is typically used in nural ,mesothelilial and ovarian sex cord stromal tumour. Researchers found its expression in 73% of Adrenocorticaltumours (24) which was confirmed by Zhang et al. They found and all 16 of 16(100%) and 11 of 12 (92%) Adrenocortical adenoma and carcinoma, respectively were calretinin positive. (21)The nuclear adrenal 4 binding protein (Ad4BP), also known as steroid factor-1, is a transcription factor that regulates steroidogenic cytochrome P-450 gene expression. The presence of Ad4BP mRNA has been reported in all steroidogenic cells except for gonadotropin-producing cells in the pituitary.(25) Ad4BP has been reported in 100% of adrenal cortical carcinomas ,whereas no cases of Renal cell carcinomas Hepatocellular carcinoma exhibited positivity. Fetsch et al (26) reported the value of inhibinA in discriminating between adrenocortical lesions and metastatic renal cell carcinomas. They found that positive reaction in 22 (100%) of 22 adrenocortical tumors. None of 23 metastatic renal cell carcinomas was reactive for inhibin A.

Renshaw and Granter (8) compared the reactivity of A103 and inhibin A in 24 adrenocortical neoplasms, 43 renal tumors, and 25 hepatocellular carcinomas. They concluded that both antibodies were useful for the immunohistochemical diagnosis of adrenocortical neoplasms; however, A103 was marginally more specific, and inhibinA slightly more sensitive. (8)

It is established that combination of monoclonal antibodies including D11, adrenal 4 binding protein (Ad4BP),A103,Calretinin, andInhibin A is most effective in diagnosis of primaty adrenocortical neoplasm and thereby differeciating them from metastatic tumours in FNA and surgical specimens. However combination of Immunomarkers (A103 and AE1/AE3) is very effective and sensitive and can be used `Vanguard` antibodies in Core Needle Biopsy to the same purpose.

Images

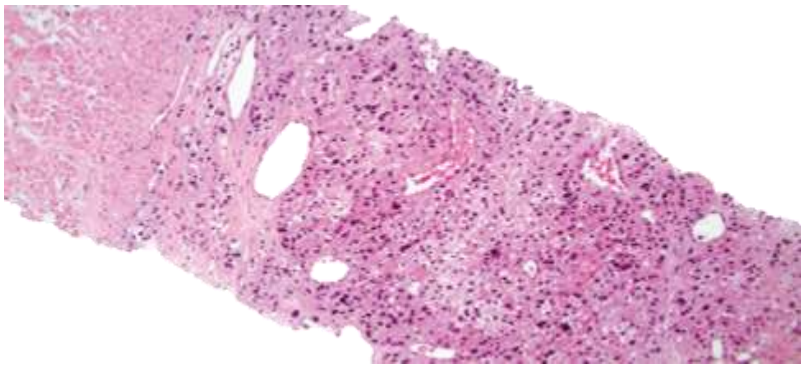

Image 1:. Adrenocortical Carcinoma- Core Needle Biopsy -Scanner View

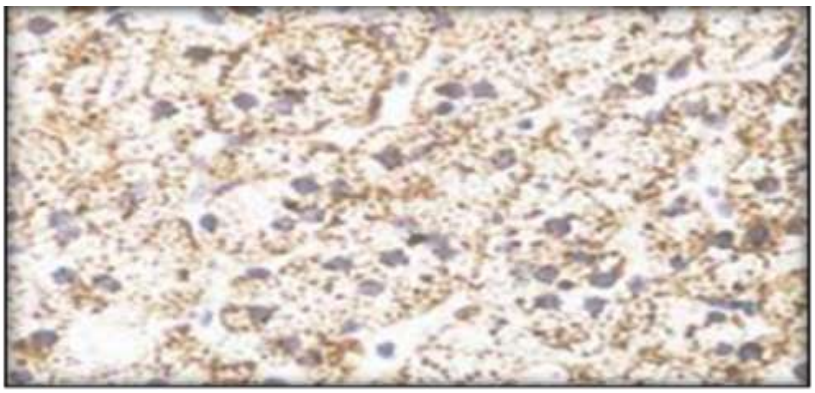

Image 2: Adrenocortical Adenoma with Cytoplasmic Granular positivity to A 103 (x 400)

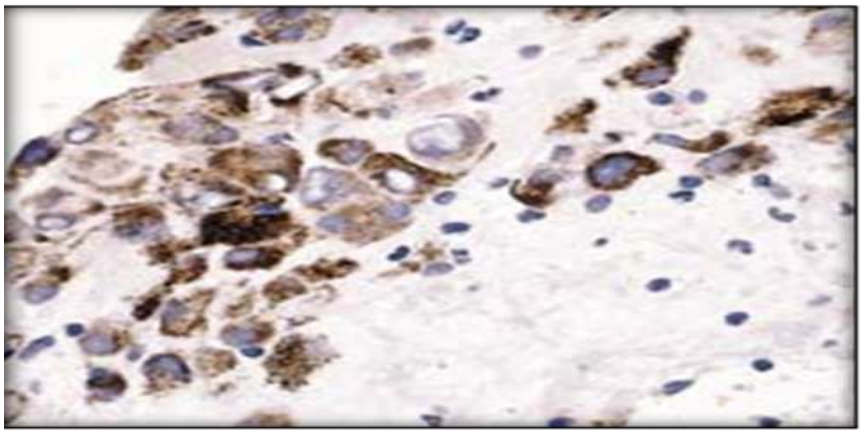

Image 3:. Adrenocortical Carcinoma with Grade 4 positive reaction to A103 (x 400)

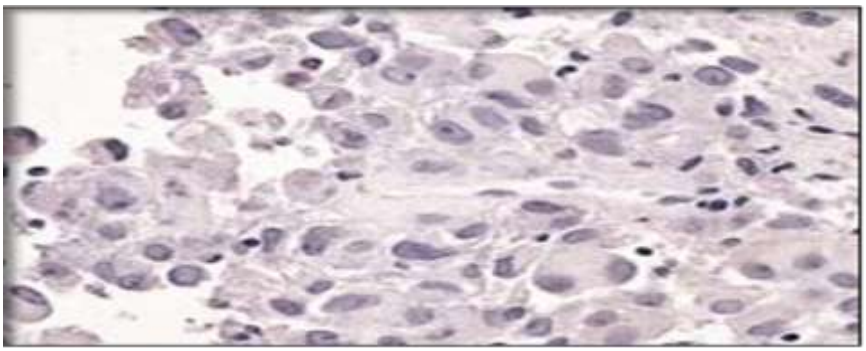

Image 4:. Adrenocortical Carcinoma with negative reaction to AE1/AE3 (x 400)

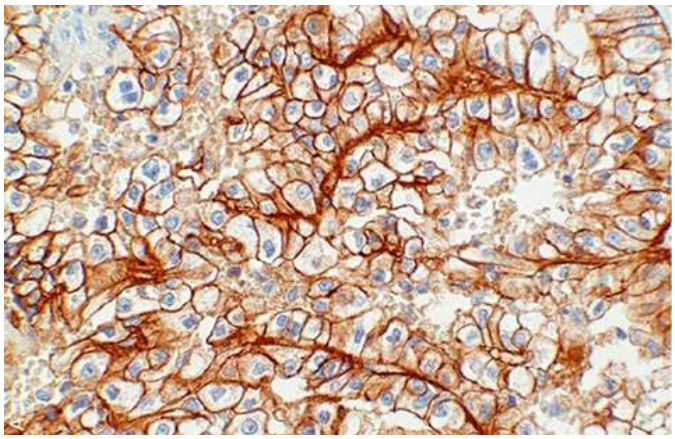

Image 5: Metastatic Colonic Adenocarcinoma with positive reaction to AE1/AE3 (x400)

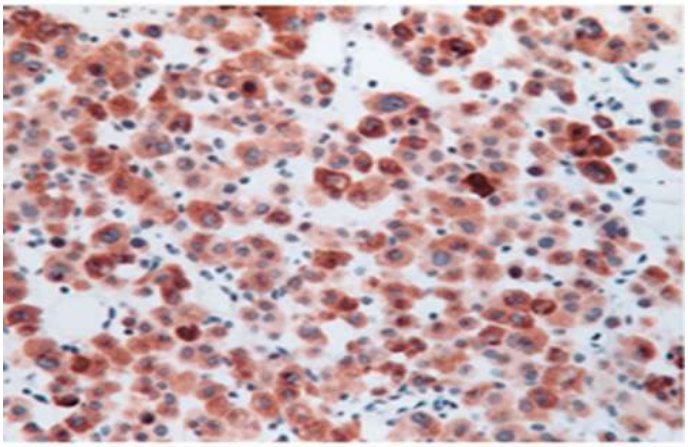

Image 6: Metastatic Lung Adenocarcinoma with positive reaction to AE1/AE3 (x400)

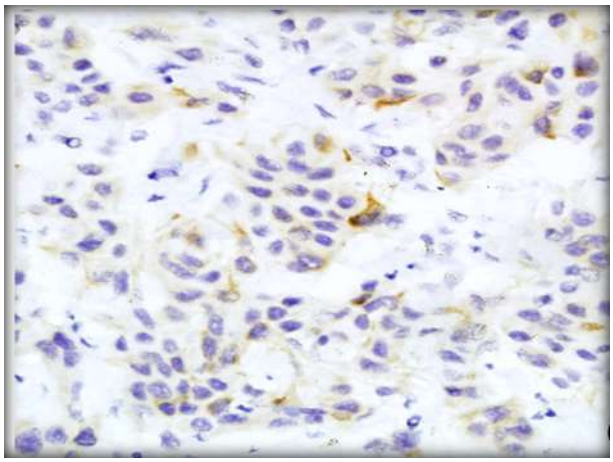

Image 7: Metastatic Prostatic Adenocarcinoma with Grade 1 -2 positive reaction to AE1/AE3 (x400)

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