

IMMUNO HISTOCHEMICAL APPROACH TO THE DIAGNOSIS OF UNDIFFERENTIATED TUMOURS- AN INSTITUTIONAL STUDY

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ABSTRACT

BACKGROUND

Undifferentiated tumours form a heterogeneous group of tumours with little or no evidence of differentiation. The objective of our study is to identify the lineage of morphologically undifferentiated tumours by means of immunohistochemistry. It also highlights the application of appropriate panel of antibody available for particular lineage.

MATERIALS AND METHODS

This prospective study was carried out at SCB medical College and Hospital Cuttack in the Department of Pathology for a period of two years (September 2011 to August 2013).

Total 50 cases of undifferentiated tumours by light microscopy were included in the present study.

RESULTS

Out of 50 cases of undifferentiated tumours, 33 were assigned an epithelial lineage (carcinoma 66%) taking into consideration the cytokeratin positivity, 11 cases came out to be of lymphoid origin (22%) with LCA positivity, 1 case of sarcoma (2%) was diagnosed and 3 came out to be malignant melanomas (6%). 2 cases were left undifferentiated with all four broad lineage markers negative.

CONCLUSION

The study highlights the importance of immunohistochemistry in dealing with undifferentiated tumours. It helped in assigning a lineage to the undifferentiated tumour by studying tumour morphology and judicious use of Antibodies.

KEYWORDS

Immunohistochemistry, Antibodies, LCA-Leucocyte Common Antigen.

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BACKGROUND

Histopathology forms an integral and indispensable part in the field of tumor diagnosis. Differentiation refers to the extent to which neoplastic parenchymal cells resemble the corresponding normal parenchymal cells, both morphologically and functionally; lack of differentiation is called anaplasia.¹

Malignant neoplasms are characterized by a wide range of parenchymal cell differentiation, starting from tumours that show their origin, the well differentiated, to an end where the tumor cells enter a zone where their own identity becomes questionable, the undifferentiated end. These undifferentiated tumours have always put pathologist into a

dilemma. By definition, an undifferentiated neoplasm lacks morphologic features to unequivocally substantiate sarcoma, lymphoma, carcinoma or melanoma.²

Undifferentiated malignant neoplasms are a daunting diagnostic problem for anatomic pathologists, calling for a tour de force in morphological skill, clinicopathologic correlation, and application of adjunctive laboratory study.

The diagnostic acumen is important for accurate classification of neoplasms, especially with the availability of new and more specific therapeutic regimens. Owing to their importance in deciding the therapy, and also guiding the prognosis,³ it becomes imperative to find ways to reach their true identity. For example, the diagnosis of lymphoma for an undifferentiated predicts a better clinical outcome compared with that of carcinoma.⁴

Various methods have been employed till date to solve the intriguing mystery they pose to the pathologists by virtue of their silent histomorphology. They include application of special stains, immunofluorescence, immunohistochemistry, and now the molecular methods which explore them at their very roots, the genes.

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It won't perhaps be an exaggeration to say that immunohistochemistry has brought a 'Brown revolution' to histopathology laboratories helping to reach a specific histogenetic origin of histologically undifferentiated tumours.⁵ The value of immune-histochemical procedures for identification of the true nature of undifferentiated tumours has been proved by studies in which approximately 90% of tumours posing diagnostic difficulties by morphology could be accurately classified by exploiting immunohistochemistry.⁶

The most widely used approach to immune-histochemical evaluation of undifferentiated tumours is to first determine the broad category of neoplasia i.e. carcinoma, sarcoma, lymphoma or melanoma. An extensive array of antibodies is available to the surgical pathologist to facilitate characterization of undifferentiated tumours. In reality, each tumor requires an "individually constructed panel" composed of carefully selected antibodies that recognize all reasonable diagnostic possibilities in the context of the tumours' morphology, anatomic site, and clinical/radiologic findings. Most studies recommend a screening panel to demonstrate the expression of makers of major lineages (epithelial, mesenchymal, lymphoid, and melanocytic). Based on the result of the screening panel, a more detailed or specific panel is commonly followed to further subclassify the tumor or conform a particular diagnosis.

Aims and Objectives

The present study is designed to undertake the immunohistochemical evaluation of cases of undifferentiated tumors diagnosed at the department of pathology S.C.B. Medical College, Cuttack. It aims to highlight the role of immunohistochemistry in lineage assignment of undifferentiated tumours, and thereby help to guide their further management.

MATERIALS AND METHODS

This is a Prospective study carried out from the biopsies received in the Department of Pathology SCB MCH Cuttack for a period of 2 years (September 2011 to August 2013).

Total of 50 cases diagnosed as undifferentiated tumours on light microscopy were subjected to immunohistochemical evaluation using formalin fixed paraffin embedded tissue sections. For immunohistochemistry, Biogenex Ready to use mouse monoclonal antibodies (optimally diluted) were used. Results were interpreted in light of pattern of expression for the particular antibodies and their uniformity. A lineage was assigned according to the immunohistochemical finding.

All histologically diagnosed cases of undifferentiated tumours by light microscopy were included in the study, irrespective of age, sex and site of presentation.

- Cases diagnosed as undifferentiated tumours on light microscopy.
- Cases where 2 or more differential diagnoses arose as a result of anaplasia, e.g. carcinoma vs. lymphoma.

Tumours showing any evidence of broad lineage differentiation (even though specific diagnosis could not be made) were excluded from our study. The relevant clinical findings & investigation findings if any were noted down. Only those cases that showed no evidence of lineage differentiation were included. Cases which could be unequivocally diagnosed as belonging to a particular broad lineage of differentiation (epithelial, mesenchymal, hematopoietic or melanocytic) were excluded from the present study.

Tissues obtained at biopsy were fixed in formalin and subjected to routine paraffin embedding and Haematoxylin & Eosin (H & E) staining, ensuring adequate sections to examine the entire biopsy and immunohistochemical staining. These included incisional as well as excisional biopsies.

The undifferentiated tumours were assigned a morphological category, from one of the four: large polygonal cell, small cell, spindle cell and pleomorphic. This morphological category, along with the age and site, guided the application of the antibodies. (Miller 2008).

Principle of Immunohistochemistry

The basic principle, as with any other special staining method is a sharp localization of target components in the cell & tissue, based on a satisfactory signal-to-noise ratio. Amplifying the signal, while reducing non-specific background staining (noise), is the major strategy to achieve a satisfactory & practically useful result.

Antigen Retrieval (AR)

It is a method of unmasking antigenic sites in tissue sections by proteolytic enzyme digestion, heat mediated methods & mixed technique.

Major factors affecting AR- results are heating temperature, heating time, pH value, chemical composition & molarity of AR solution.

Other AR solutions are EDTA at pH-8, Tris-EDTA at pH-9.9/10. We used citrate buffer of volume 400-600 ml in a suitably sized microwave resistant plastic container containing 25 slides for two cycles of 10 mins each with 1 min interval at 750 watt.

Quality Control

Differences in tissue processing & technical procedure may produce variable results. Hence controls used as fresh autopsy/surgical specimens were processed in same manner as patient's sample.

Positive Tissue Control

It is used to indicate correctly prepared tissues & proper staining.

Negative Tissue Control

Negative control was used to verify the specificity of the labelling of the target Ag by primary Ab. Example-primary antibody was not added in the procedure.

After immunohistochemical categorization, the tumours were assigned a broad lineage category of differentiation, as carcinoma, lymphoma, sarcoma, and melanoma. These tumours were studied with regard to age distribution, sex distribution, site wise distribution and the broad morphological categories.

RESULTS

Results are categorised according to the morphology. Out of 50 cases, large polygonal cell category constituted the majority of cases, i.e. 42 out of 50 (84%). There were 7 cases of small cell (14%), 1 case of spindle cell (2%) and no case of pleomorphic (0%) category. All of these cases were subjected to immunohistochemistry taking into consideration the morphological category, possible differential diagnosis along with site, age and clinical presentation.

According to age distribution, cases ranged from 12 years to 70 years of age. Mean age of presentation was 45.86 years. Majority of cases belonged to 41-60 years age group, constituting 48% of all cases, followed by 16-40 years age group (28%).

Out of 50 cases, 29 were male (26 with large polygonal cell morphology, 3 with small cell morphology); while 21 were female (16 with large polygonal cell, 4 with small cell and 1 with spindle cell morphology).

Site	Number of Cases	Percentage (%)
Lymph node	11	22%
Nasopharynx	15	30%
Nasal cavity	06	12%
Gastrointestinal tract	09	18%
Others (tonsil, thigh, retroperitoneum)	09	18%

Table 1. Site Wise Distribution

Out of 50, 15 cases belonged to nasopharynx region (30%), 6 cases were from nasal cavity (12%), 11 from lymph node (22%), 9 cases from gastrointestinal tract (18%), 9 from other sites (18%). Out of 'others', there were 4 cases from oral cavity, and one case each from larynx, skull bone, tonsil, thigh and posterior mediastinum. The lymph node biopsies were from those cases that did not have any previous history of lymphoproliferative disorder or known primary malignancies.

Antibody	No. of Cases Applied in	No. of Cases Positive in	No. of Cases Negative in
Cytokeratin	50	34/50	16/50
CD 45	49	11/49	38/49
Vimentin	06	04/06	02/06
S100	06	03/06	03/06

Table 2. Application of Antibodies and Findings

Tumours are categorised after application of antibodies. Cytokeratin was positive in 34 cases of 50. Out of 34 positive, 33 were carcinomas, whereas 1 was synovial

sarcoma, which showed vimentin co-expression. Out of the 16 negative cases, 11 were lymphomas (showing LCA positivity), 3 were melanomas (vimentin and S100 positive) and 2 were the undiagnosed cases.

CD 45 (LCA) was applied in 49 cases, where lymphoma formed a differential diagnosis. It was positive in 11 cases, which were further worked out for lymphoma subtyping. Out of 38 negative cases, 33 were carcinomas (with CK positivity), 3 were melanomas (vimentin and S100 positive) and 2 were undiagnosed.

Vimentin was applied in 6 cases where differential diagnoses were sarcoma (1 case, result positive), malignant melanoma (amelanotic) (3 cases, result positive) and 2 cases (result negative), that were left undiagnosed following CK and CD 45 negativity.

S100 was applied in 6 cases. Out of these, 3 had malignant melanoma in differential diagnosis (result 2 positive, 1 negative); and 3 were worked out following negativity for CK and CD 45 (result 1 positive, 2 negative). Thus, 3 cases of malignant melanoma (amelanotic) were diagnosed, which were subsequently found out to be HMB 45 positive; one of the three was totally unsuspected on light microscopy.

Thus, the application of individual antibodies from a primary panel, was tailored as per individual case, highlighting the role of light microscopic presentation, leading to economic use of antibodies.

Diagnosis on immunohistochemistry	Number of cases (out of 50)	Percentage
Carcinoma	33	66%
Lymphoma	11	22%
Sarcoma	01	02%
Melanoma	03	06%
Undiagnosed	02	04%

Table 3. Lineage Assignment on the Basis of Immuno-histochemistry

Amongst 50 cases of undifferentiated tumours, 33 were assigned an epithelial lineage, taking into consideration the cytokeratin positivity; 11 cases came out to be of lymphoid origin with LCA positivity, 1 case of sarcoma was diagnosed, and 3 came out to be malignant melanomas. Two cases were left undifferentiated, with all four broad lineage markers negative.

A total of 50 cases of were studied during the period of September. The cases diagnosed as undifferentiated on light for study.

They were studied with regard to sex, site wise distribution, and divided into four as large polygonal cell, small cell, spindle cell and pleomorphic.

Differential diagnoses based on this approach were formed and immunohistochemical markers applied to derive a lineage of differentiation (epithelial, mesenchymal, hematopoietic, and melanocytic). This formed the basis of further categorization of the tumours, e.g. T cell B cell in case of lymphomas.

Out of 50 cases in present study, large polygonal cell category constituted the majority of cases, i.e 42 out of 50 (84%). There were 7 cases of small cell (14%), 1 case of spindle cell (2%) and no case of pleomorphic (0%) category.

Bianchini et al (2003) divided the undifferentiated tumours morphologically into round cell, epithelioid cell, spindle cells, myxoid pattern and pleomorphic, and found that round cell was the most prevalent pattern (51.2%). There were other authors who did not categorize the tumours morphologically and applied a complete panel of antibodies to all tumours. Among them are Vege DS et al (1994) and Bhagat et al (2013). We found the approach of categorizing undifferentiated tumours morphologically as useful, as advocated by Miller. This, along with the age and site of presentation can help to narrow down the differentials and hence guide the judicious application of antibodies in a limited resource set up, especially when patients cannot afford the cost of antibodies. This should be weighed against the urgency of report (taking into account the clinical condition of patient), as a step wise approach may relatively delay the diagnosis if the morphological differentials don't work out well on immunohistochemistry.



Figure 1 (H & E Stain 400X)

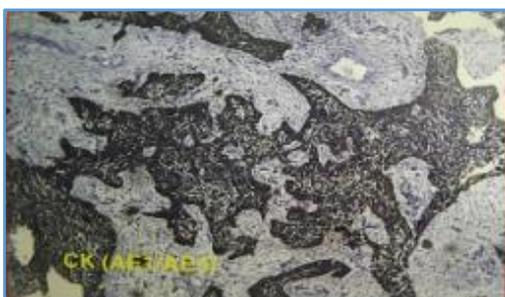


Figure 2. CK (AE1/AE3) Positivity 100X

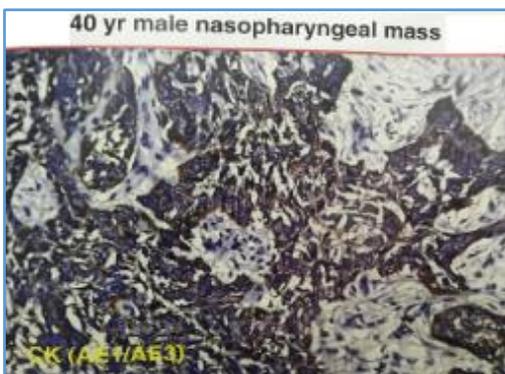


Figure 3. CK (AE1/AE3) Positivity 400X



Figure 4. CD 45 Negativity

DISCUSSION

Out of 50 cases of undifferentiated tumours, immunohistochemistry could categorize 48 tumours (96%) into broad lineage differentiation. 33 were assigned an epithelial lineage (66%), taking into consideration the cytokeratin positivity; 11 cases came out to be of lymphoid origin (22%) with LCA positivity, 1 case of sarcoma was diagnosed (2%), while 3 came out be malignant melanomas (6%).

Out of 50 cases, 2 were left undifferentiated, with all four broad lineage markers negative. In this regard, Bhagat et al could categorize 98.65%, Vege DS et al could categorize 85.5%,⁷ Coindre JM et al (1986) could categorize 90%, while Michie et al (1987) could arrive at a diagnosis in 86% of undifferentiated tumours, by immunohistochemistry. In the study by Bianchini et al, immunohistochemistry allowed a conclusive diagnosis for 60.5% of tumours,⁸ and was suggestive for 20.9%.

In the present study, the most crucial role of immunohistochemistry was to distinguish between carcinoma and lymphoma, followed by a differential of malignant melanoma in some cases. This related to the most frequent morphological category of large polygonal cell type. Dai YR (1989) also reported the problem of differentiating carcinoma and lymphoma, and approached it by ancillary methods. Gatter KC et al (1984) in their study preferred to divide the morphologically undifferentiated tumours as "unclassifiable", "probable carcinomas" or 'probable lymphomas', which were subsequently subjected to immunohistochemistry.

In our study, cases ranged from 12 years to 70 years age. Mean age of presentation was 45.86 years. Majority of cases belonged to 41 – 60 years age group, constituting 48% of all cases, followed by 16 – 40 years age group (28%) (Table 2).

Bhagat et al reported an age range from 2 to 70 years. Undifferentiated tumours were most prevalent in the 7th decade of life (34.9% of cases) in study by Bianchini WA et al. The difference could be attributed to geographical factors like race. Life expectancy etc.

Gender distribution shows 29 male and 21 female (out of 50). In Bhagat et al's study, there were 43 male and 31 female (out of total 74 cases). Undifferentiated tumours were twice as prevalent in men as in women, as per study by Bianchini WA et al. So, our study could relate to these

authors in the observation that undifferentiated tumours are more common in men than in women.

The most common site of presentation of undifferentiated tumours in our study was nasopharynx, followed by lymph node. Out of 50, 15 cases belonged to nasopharynx region (30%), 6 cases were from nasal cavity (12%), 11 from lymph node (22%), 9 cases from gastrointestinal tract (18%), 9 from other sites (18%). Out of 'others', there were 4 cases from oral cavity, and one case each from larynx, skull bone, tonsil, thigh and posterior mediastinum (TABLE-3).

According to Bianchini WA et al, the most frequent locations were lymph nodes (20.9%), Pharynx and neck (16.3%), paranasal sinus (14%), nose (11.6%).

In contrast, Bhagat et al reported bone/soft tissue as most frequent site, followed by gastrointestinal tract and lymph nodes. The difference could be attributed to the criteria for inclusion. In their study, cases reported as sarcomas in general were also included for further categorization into specific diagnoses; whereas we excluded the tumours that were morphologically confirmed as that of mesenchyme lineage, even if specific diagnosis could not be arrived at.

Cytokeratin was positive in 34 cases of 50. Out of 34 positive, 33 were carcinomas, whereas 1 was synovial sarcoma, which showed vimentin co-expression. Out of the 16 negative cases, 11 were lymphomas (showing LCA positivity), 3 were melanomas (vimentin and S100 Positive) and 2 were the undiagnosed cases. CD 45 (LCA) was applied in 49 cases, where lymphoma formed a differential diagnosis. It was positive in 11 cases, which were further worked out for lymphoma subtyping.

Out of 38 negative cases, 33 were carcinomas (with CK positivity), 3 were melanomas (vimentin and S100 positive) and 2 were undiagnosed. Vimentin was applied in 6 cases where differential diagnoses were sarcoma (1 case, result positive), malignant melanoma (amelanotic) (3 cases, result positive) and 2 cases (result negative), that were left undiagnosed following CK and CD 45 negativity. S100 was applied in 6 cases. Out of these, 3 had malignant melanoma in differential diagnosis (result 2 positive, 1 negative); and 3 were worked out following negativity for CK and CD 45 (result 1 positive, 2 negative). Thus, 3 cases of malignant melanoma (amelanotic) were diagnosed, which were subsequently found out to be HMB 45 positive; one of the three was totally unsuspected on light microscopy.

Thus, out of 50 cases of undifferentiated tumours, 33 were assigned an epithelial lineage (carcinomas 66%), taking into consideration the Cytokeratin positivity; 11 cases came out to be of lymphoid origin (22% lymphomas) with LCA positivity, 1 cases of sarcoma (2%) were diagnosed, and 3 came out be malignant melanomas (6%). Two cases were left undifferentiated, with all four broad lineage markers negative.^{9,10}

Bhagat et al also reported carcinoma as the most frequent diagnosis among undifferentiated tumours, making 36.5%, followed by lymphoma (24.32%). In the study conducted by Coindre JM et al, non-Hodgkin's lymphoma

was the most common result (57%), followed by Carcinomas (22%), melanomas (5%), sarcomas (7%) and others (2%) among tumours that could be classified by immunohistochemistry. Lymphoma was also the most common diagnosis in studies by Gatter KC et al (66%) =, and Vega DS et al (35.9%). Thus, different authors have got different results, which we think could be ascribed to the criteria for inclusion owing to the subjective variation in calling a tumor as undifferentiated. This aspect points to the need of having strict morphological criteria for diagnosing a tumor as undifferentiated which are not found to be defined.

Summary

Total of 50 cases diagnosed as undifferentiated tumours on light microscopy were subjected to immunohistochemical evaluation using formalin fixed paraffin embedded tissue sections. The main aim of the study was to categorize the tumours as per the broad lineage categories. Antibodies were selected from a primary panel of antibodies, consisting of Cytokeratin Cocktail (AE1/AE3), CD 45 (LCA), vimentin and S100. The panel was individualized as per the morphological category and differential diagnoses in histopathology. Results were interpreted in light of pattern of expression for the particular antibodies and their uniformity. A lineage was assigned according to the immunohistochemical finding.

Cases ranged from 12 years to 70 years age. Mean age of presentation was 45.86 years. Nasopharynx was the most common site. Out of 50 cases, large polygonal cell category constituted the majority of cases, i.e. 42 out of 50 (84%). There were 7 cases of small cell (14%), 1 case of spindle cell (2%) and no case of pleomorphic (0%) category.

The major differential diagnoses on light microscopy were carcinoma and lymphoma, followed by malignant melanoma.

Out of 50 cases of undifferentiated tumours, 33 were assigned an epithelial lineage, taking into consideration the cytokeratin positivity; 11 cases came out to be of lymphoid origin with LCA positivity, 1 case of sarcoma was diagnosed, and 3 came out to be malignant melanomas. Two cases were left undifferentiated, with all four broad lineage markers negative.

Thus, the study highlights the importance of immunohistochemistry in dealing with undifferentiated tumours. Though a panel approach is advocated, the application of complete panel is not feasible in limited resource setting, where patients cannot afford the cost of ancillary techniques. This necessitates a judicious yet appropriate use of antibodies. This study highlights the role of tumor morphology in guiding the application of appropriate antibodies, and thus making judicious use of antibodies.

CONCLUSION

Undifferentiated tumours form a diagnostic difficulty to histopathologists. Their identity is important in deciding the further management of the patient.

Immunohistochemistry forms an important tool in assessing these tumours. A systematic approach in the immunohistochemical evaluation is necessary in this regard.

Light microscopic differential diagnoses as per site and morphology, play an important role in selection of antibodies, and hence judicious use in a limited resource set up.

Some cases may be left undiagnosed in spite of application of broad lineage markers, necessitating application of more number of antibodies or molecular methods in such cases. Thus, the study highlights the importance of immunohistochemistry in dealing with undifferentiated tumours. Though a panel approach is advocated, the application of complete panel is not feasible in limited resource setting, where patients cannot afford the cost of ancillary techniques. This necessitates a judicious yet appropriate use of antibodies. This study highlights the role of tumor morphology in guiding the application of appropriate antibodies, and thus making judicious use of antibodies.

Limitations of the Study

This study was restricted to just broad lineage differentiation of undifferentiated neoplasms. Further work up to pin point a diagnosis could not be done due to limitation of markers. Aberrant expression of markers could not be studied, as a tailored panel was applied to individual cases, depending upon the light microscopic differentials.

Two cases could not be delineated. This could be ascribed to the limitation of markers, or extensive loss of differentiation in the neoplasms.

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