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ROLE OF IMMUNOHISTOCHEMISTRY IN DIAGNOSIS OF POORLY DIFFERENTIATED/ UNDIFFERENTIATED MALIGNANCY

Pathology

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ABSTRACT

Introduction: The term undifferentiated tumor has been used in reference to a heterogeneous group of tumors with little or no evidence of differentiation. In this review we apply the term undifferentiated to tumors lacking evidence of lineage differentiation on the basis of routine light microscopic morphology alone. IHC provides diagnostic guidance in approximately 90% of undifferentiated malignant tumours and malignancies of unknown origin. **Aims & Background:** To study the origin of primary or identify the identity of undifferentiated malignancy. To study the lineage of differentiation whether is carcinoma, sarcoma, lymphoma or neuroendocrine neoplasm. **Material and Methods:** A retrospective and prospective analysis of the histopathology and immunohistochemistry records and clinical case files of total 45 cases over the period of January 2024 to August 2025 were done. History was studied in detail in each case with respect to presenting complains of patients, site of lesion, age, and sex of patient distribution. Specimens were fixed in 10% neutral buffered formalin followed by routine paraffin processing by automated tissue processor. Manual staining was done by using hematoxylinand eosin (H&E) stain. Mounting was done with DPX (distyrene, plasticizer, and xylene). Stained slides were examined under a light microscope. Further IHC was done for all selected cases and final diagnosis was made on basis of detailed analysis of IHC marker positivity. The slides were examined and data generated from observations was used for statistical analysis. **Results:** In present study, a total 45 cases of poorly differentiated or undifferentiated malignancies were studied histologically, in which 17 patients were female (37.78%) and 28 patients were male (62.22%) with Male to Female ratio being 1.65:1.Majority of tumors showed peak occurrence in the age group of 3rd to 5th decades, with a mean age of presentation of 53.5 years. Our study showed the dominance of carcinomas, which accounted for 84.4% of all cases. Within the carcinoma category, adenocarcinoma and squamous cell carcinoma were the most common subtypes. **Conclusion:** Our study provides valuable insights into the distribution and characteristics of poorly differentiated and undifferentiated malignancies. The findings highlight the dominance of carcinomas, which accounted for 75.6% of all cases with adenocarcinoma and squamous cell carcinoma being the most common subtypes, followed by neuroendocrine neoplasms and sarcomas. The findings of this study reinforce the importance of an integrated approach involving both histopathology and immunohistochemical analysis to improve the accuracy of diagnoses and facilitate the development of tailored treatment plans.

KEYWORDS

Undifferentiated Malignancy, Poorly Differentiated Malignancy, Immunohistochemistry.

1. INTRODUCTION

The term undifferentiated tumor has been used in reference to a heterogeneous group of tumors with little or no evidence of differentiation.Some may link this terminology to morphologically undifferentiated neoplasms that cannot be otherwise classified, even with the application of immunohistochemistry. In our view, however, such tumors are extremely rare. For such reason, in this review we apply the term undifferentiated to tumors lacking evidence of lineage differentiation on the basis of routine light microscopic morphology alone.

An undifferentiated malignant tumor represents either a metastasis of unknown origin or a primary neoplasia without obvious cell line of differentiation. It should be noted that undifferentiated tumor generally implies a high-grade malignancy, frequently associated with pleomorphic to anaplastic appearance.[1]

Diagnosis is intended essentially to identify the subsets of primary of unknown origin tumors sensitive to specific treatment.However, the therapeutic choice, and several favourable subsets of primary of unknown origin tumors, warrants further histopathological characterization, which is often performed with immunohistochemistry (IHC) and, more recently, using molecular analyses [3].

IHC provides diagnostic guidance in approximately 90% of undifferentiated malignant tumours but usually at the end of a fastidious and expensive algorithm based on both morphology and IHC. However, identification of the primary site of origin may represent a difficult challenge for the pathologist when dealing with a small sample size along with increased generation of tumour-specific primary antibodies and the need for complementary molecular analysis.

For treatment purposes, it is crucial to determine whether an undifferentiated neoplasm is epithelial, mesenchymal, or hematopoietic. In general, the diagnosis of lymphoma for an undifferentiated tumor predicts a better clinical outcome compared with that of carcinoma.[5] The value of immunohistochemical procedures for identification of the true identity of undifferentiated tumors has been proved by studies in which approximately 90% of tumors posing diagnostic difficulties by morphology could be accurately classified by exploiting immunohistochemistry.[5-7]

Immunohistochemical dissection of undifferentiated tumors is also helped by categorizing them into small round blue cell tumors (SRCTs) or large cell tumors. The latter group is further divided into (1) carcinomatous tumors, (2) sarcomatous or sarcoma-like tumors, and (3) tumors with overlapping features. Each category entertains a broad list of entities from epithelial, mesenchymal, hematopoietic, or melanocytic lineage in the differential diagnosis.

2. MATERIALS AND METHODS

- **Study Design:** This is a prospective and retrospective analytical and comparative study conducted in the Department of Pathology.
- **Sample Size and Period of Data Collection:** Total 45 cases are reported as undifferentiated or poorly differentiated malignancy on light microscopy. Data collected from January 2024 to august 2025.

- **Inclusion Criteria**
  - Adequate sample size
  - Well preserved biopsy
  - Biopsy reported as undifferentiated or poorly differentiated malignancy.
- **Exclusion Criteria**
  - Inadequate sample size
  - Poorly preserved biopsy
  - Biopsy reported as carcinoma/sarcoma/lymphoma

Methodology

A retrospective and prospective study of the histopathology records and clinical case files of total 45 cases were done. History was studied in detail in each case with respect to presenting complaints, site, age, and sex distribution. Specimens were fixed in 10% neutral buffered formalin, tissue sections of 3 to 4 micrometre thickness were cut followed by routine paraffin processing. Staining was done by using hematoxylin and eosin (H&E) stain. Mounting was done with DPX (distyrene, plasticizer, and xylene). Stained slides were examined under a light microscope. Reporting was as undifferentiated or poorly differentiated malignancy. Also done the Immunohistochemical stain

for further confirmation of lesion and final diagnosis was made on basis of detailed analysis of IHC marker positivity. The slides were examined and data generated from observations was used for statistical analysis.

3. RESULTS

Total 45 cases of histologically diagnosed Poorly differentiated malignancy and undifferentiated malignancy were taken over a period of January 2024 to August 2025. The study showed there was a male predominance with 28 (62.22%) males and 17 (37.78%) females and the male to female ratio was 1.65:1. Majority of tumors showed peak occurrence in the age group of 3rd to 5th decades. The mean age of presentation was 53.5 years.

Table/ Figure 1: Age Wise Distribution

Age group	Number of cases	Percentage
21-30	3	6.7
31-40	9	20
41-50	9	20
51-60	8	17.8
61-70	9	20
71-80	6	13.3
81-90	1	2.2

The commonest site of occurrence in our study was lung and pleural cavity which constituted 15.7% of all cases, followed by Gastrointestinal tract (13.4%).

Table/Figure 2: Site Wise Distribution

Site	Number of Cases	Percentage
Oral Cavity	4	8.9
GIT	6	13.4
Brain	2	4.4
Endometrium	2	4.4
Cervix	3	6.7
Vaginal vault	1	2.2
Vulval mass	1	2.2
Liver and gall bladder	5	11.1
Lung and pleural cavity	7	15.7
Nasal cavity and PNS	5	11.1
Neck	5	11.1
Arm	1	2.2
Left iliac Bone	1	2.2
Axillary Lymph node	1	2.2
Inguinal region	1	2.2

Out of 45 cases, 23 cases were histologically diagnosed as Undifferentiated malignant tumor, while 21 cases as poorly differentiated malignancies. 1 case was diagnosed as metastatic poorly differentiated malignancy. These cases were further processed with relevant immunohistochemical markers. The most common panel which was used, constituted of the following markers- CK5/6, CK7, P63 Or P40, Pankeratin, vimentin, desmin, synaptophysin, S100, CD45 and TTF-1. The panel of immunohistochemical markers differed for each case.

After Immunohistochemistry, final diagnosis was given.

Table 3/Figure 3: Distribution of Tumors After Differentiation of Tumors with IHC.

Diagnosis	Number of cases	Percentage
1. Carcinoma	34	75.6%
Adenocarcinoma	11	
Squamous Cell Carcinoma	12	
Epithelial Myoepithelial Carcinoma	1	
Metatstatic poorly differentiated Carcinoma	3	
Metastatic Squamous cell Carcinoma	2	
Metastatic small Cell Carcinoma	1	
Sarcomatoid Carcinoma	1	
Mesothelioma	1	
Sinonasal Undifferentiated Carcinoma	1	
Olfactory Neuroblastoma	1	
2. Sarcoma	3	6.8
Pleomorphic Rhabdomyosarcoma	2	
Undifferentiated Stromal Sarcoma	1	
3. Non- Hodgkin Lymphoma	2	4.4
4. Neuroendocrine Neoplasm/Tumor	4	8.8
Neuroendocrine Neoplasm	3	
Mixed Neuroendocrine and Non-Neuroendocrine Neoplasm	1	
5. Inconclusive/ Differentiation not possible	2	4.4

Our study showed the dominance of Carcinomas, which accounted for 75.6% of all cases. Within the carcinoma category, adenocarcinoma and squamous cell carcinoma were the most common subtypes. Neuroendocrine Neoplasm/Tumor constituted 8.8% of all cases. The relatively low incidence of sarcomas (6.8%) was seen, Pleomorphic rhabdomyosarcoma was the most common subtype in this category. A small proportion of cases (4.4%) was diagnosed as Non-Hodgkin lymphoma. A small proportion of cases (4.4%) remained inconclusive or had differentiation not possible, emphasizing the need for advanced diagnostic techniques and expert pathological review.

4. DISCUSSION

Undifferentiated malignancies represent a heterogeneous group of tumors that lack clear histological evidence of differentiation. These tumors pose significant challenges in diagnosis and classification due to their diverse origins and the absence of characteristic features. They may arise from various primary sites and include carcinomas, sarcomas, lymphomas, neuroendocrine tumors, or metastatic diseases with an unknown origin.

If morphology is not sufficient, in particular for an undifferentiated neoplasm with no clear lineage differentiation, the first diagnostic IHC panel will include a few antibodies directed against epithelial antigens (broad spectrum anti pan-cytokeratin antibody cocktail, for example, AE1/AE3, KL1, pan-Cytokeratin Plus [AE1/AE3+ 8/18]), lymphoid antigens (CD45 or CD20 and CD3) , for sarcoma SMA, H-caldesmon, Desmin and melanocyte-differentiation antigens (S100 protein, SOX10) [2,8]. Vimentin is a nonspecific marker; however, a vimentin-negative tumour is unlikely to be a sarcoma (with the exception of alveolar soft part sarcoma), lymphoma, or melanoma [9].

No single antibody is fully sensitive and specific for a particular tumour; however, some antibodies are especially useful when used within small panels [9]. Over the past few years, novel antibodies have been developed, essentially those directed against transcription factors and improved the specificity of diagnosis, when used in combination with other more “classical” antibodies [9,10]. However, in approximately 5% of cases, IHC may not provide any definitive information and the final diagnosis of an undifferentiated malignant neoplasm is given without any reference to the tumour origin, despite all the investigations.

Precise identification of the tumor's lineage i.e. whether an undifferentiated neoplasm is epithelial, mesenchymal, or hematopoietic. and primary site is critical for determining the most appropriate treatment options, as therapeutic strategies vary widely based on the tumor's type. In general, the diagnosis of lymphoma for an undifferentiated tumor predicts a better clinical outcome compared with that of carcinoma. The value of immunohistochemical procedures for identification of the true identity of undifferentiated tumors has been proved by studies in which approximately 90% of tumors posing diagnostic difficulties by morphology could be accurately classified by exploiting immunohistochemistry.[1–3]

In this study, we analyzed 45 cases of poorly differentiated or undifferentiated malignancies, revealing a higher frequency in males (62.2%) compared to females (37.8%), with a male-to-female ratio of 1.65:1. This trend aligns with several previous studies, which have reported a male predominance in undifferentiated tumor cases. The peak incidence occurred in individuals aged 30-60 years, with a mean age of 53.5 years, which mirrors the common age range for a variety of cancers, particularly carcinomas.

Carcinomas accounted for the majority of the cases in our study (75.6%), with adenocarcinoma and squamous cell carcinoma being the predominant subtypes. These findings are consistent with the existing literature, which often shows carcinomas as the most frequent tumors in the undifferentiated category. For instance, studies by O'Neill et al. [11] reported that carcinomas, especially adenocarcinomas and squamous cell carcinomas, are the most commonly observed

neoplasms in cases of undifferentiated malignancy, with adenocarcinoma comprising approximately 40% of cases. Similarly, in our study, adenocarcinoma and squamous cell carcinoma were the most frequently encountered carcinoma subtypes.

The role of Immunohistochemistry (IHC) in diagnosing undifferentiated malignancies is crucial. In our study, IHC proved essential in categorizing the tumors, helping to distinguish between carcinomas, sarcomas, lymphomas, and neuroendocrine neoplasms. Markers such as CK5/6, CK7, P63, Vimentin, P40, and TTF-1 were indispensable in defining the tumor's origin. This finding is in accordance with reports by Padhy et al. [12], who observed that IHC can accurately identify the tumor lineage in 85-90% of undifferentiated cases. The combination of morphological examination and IHC is essential for accurate diagnosis, particularly when dealing with small biopsy samples or tumors with ambiguous histological features.

Table 4/Figure 4: Differential Diagnosis of Undifferentiated Malignant Neoplasms and their Markers: [17]

Malignant neoplasms	Markers
Carcinoma	CK, EMA
Melanoma	SOX10, S100, HMB45, Melan A, MITF
Sarcoma	Vimentin, Desmin, Actin, CD34, ERG, MyoD1, Myogenin, SMA, H-Caldesmon
Lymphoma	Cd45, CD3, CD20, CD19,CD79a
Neuroendocrine neoplasms	SYN, CHR, CD56, INSM1
Mesothelioma	CK, EMA, Calretinin, WT1, D2-40,CK5/6

In our study, sarcomas accounted for 6.8% of cases, which is relatively low compared to carcinomas. The most common sarcoma subtype observed was pleomorphic rhabdomyosarcoma, which is consistent with other studies. Zhao et al. [13] also reported a relatively low incidence of sarcomas in undifferentiated tumor cases, with rhabdomyosarcoma being among the more frequently identified subtypes. The lower frequency of sarcomas compared to carcinomas underscores the predominant role of epithelial neoplasms in undifferentiated malignancies.

Interestingly, a small number of cases (4.4%) in our study could not be definitively classified due to inconclusive differentiation. This observation highlights a significant diagnostic challenge, as some tumors, even with the aid of advanced IHC techniques, remain difficult to categorize. This problem is not uncommon, as Gallo et al. [14] and other studies have noted that undifferentiated tumors of unknown origin often present substantial diagnostic difficulties, especially when tissue samples are suboptimal or when the available panel of antibodies is insufficient for conclusive identification.

Neuroendocrine tumors (NETs) made up 8.8% of the cases in this study, which is in line with their expected frequency among undifferentiated malignancies. NETs, including small cell carcinomas and mixed neuroendocrine tumors, can be challenging to diagnose due to their heterogeneous presentation. The use of specific IHC markers such as synaptophysin, chromogranin, and CD56 was essential for identifying neuroendocrine features in these tumors. Our findings align with the work of Muenst et al. [15], who emphasized the importance of these markers in the accurate diagnosis of neuroendocrine tumors, as the management of these tumors differs significantly from that of carcinomas or sarcomas.

Non-Hodgkin lymphoma (NHL) was diagnosed in 4.4% of cases, further emphasizing the need to include lymphoma in the differential diagnosis of undifferentiated tumors. Lymphomas, particularly small round blue cell lymphomas, often resemble small cell carcinomas and other undifferentiated malignancies. The use of IHC markers like CD45, LCA, and PAX5 was crucial in confirming the diagnosis of lymphoma in these cases. Pan B cell markers(CD19,CD20) and Pan T cell markers(CD3, CD5) were use for further differentiation of lymphoma. This finding is consistent with other studies, which have documented the importance of immunophenotyping in distinguishing lymphomas from other undifferentiated tumors.Regarding the site distribution of undifferentiated malignancies, our study showed that the lung and pleural cavity were the most common sites of involvement (15.7%), followed by the gastrointestinal tract (13.4%). These findings are consistent with the general understanding that undifferentiated carcinomas often arise in organs with high epithelial turnover, such as the lungs and gastrointestinal system. The gastrointestinal tract is known to be a frequent site for both primary and metastatic undifferentiated tumors, as highlighted in studies by Tanaka et al. [16], which report similar site distribution patterns.

Here are some of the images from our study case.

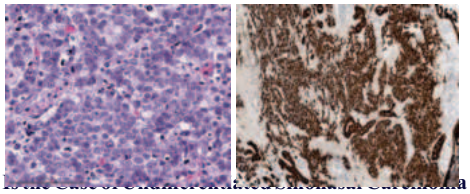

Fig.5.1

Above case(fig 5.1) is of undifferentiated sinonasal carcinoma which is reported as high grade undifferentiated malignancy on light microscopy and further immunohistochemistry was performed on same biopsy and its was positive for Pankeratin AE1/AE3, Cytokeratin 7 markers and negative for synaptophysin, CD99 and CD45.

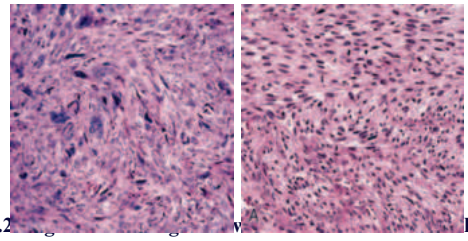

Fig. 5.2 lygonal Atypical Cells and Left Side Image Shows Tumor Cells can be Spindly and Arranged in Fascicles.

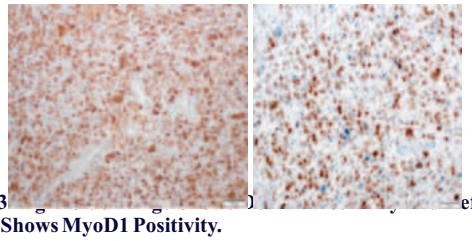

Fig. 5.3 eft Side Image Shows MyoD1 Positivity.

Fig no. 5.2 and fig no. 5.3 is the case of pleomorphic rhabdomyosarcoma, in which it is reported as undifferentiated tumour most likely sarcoma on light microscopy. Further immunohistochemistry was done and it is positive for desmin and myoD1 markers and negative for MDM2, S100 and HMB45.

5. CONCLUSION

Our study provides valuable insights into the distribution and characteristics of poorly differentiated and undifferentiated malignancies. The findings highlight the dominance of carcinomas, which accounted for 75.6% of all cases with adenocarcinoma and squamous cell carcinoma being the most common subtypes, followed by neuroendocrine neoplasms and sarcomas. While IHC was invaluable in establishing the tumor lineage in the majority of cases, some tumors remained undifferentiated, emphasizing the limitations of current diagnostic techniques. The findings of this study reinforce the importance of an integrated approach involving both histopathology and immunohistochemical analysis to improve the accuracy of diagnoses and facilitate the development of tailored treatment plans. The development of advanced diagnostic techniques, such as molecular diagnostics, may help improve the accuracy of diagnosis and treatment planning.

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