CLINICAL, RADIOLOGICAL, HISTOPATHOLOGICAL AND IHC PROFILE OF PATIENTS PRESENTING WITH CERVICAL LYMPHADENOPATHY DUE TO AN UNKNOWN PRIMARY

Dr. Akanksha Rajpoot¹, Dr. Veena L Karanth², Dr Chitra Y Bhat³*

¹ Senior Resident, Department of General Surgery, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, India. rajpoot.akanksha@yahoo.in

² Professor, Department of General Surgery, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, India. karanthkvl@yahoo.co.in

³ Assistant Professor, Department of General Surgery, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, India. chitrabhatkmc@gmail.com

*Corresponding Author: Dr Chitra Y Bhat, Assistant Professor, Department of General Surgery, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, India. chitrabhatkmc@gmail.com

Abstract

Background and Objective: Carcinomas of unknown primary (CUP) are histologically defined as the presence of a metastasis without detection of the primary tumour. It is a heterogeneous condition, wherein the type of the tumour, its extension and the treatment vary widely. Methodology: Data was collected for all patients presenting in high volume centre of Kasturba Medical College, Manipal between June, 2014 to June, 2019 with cervical lymphadenopathy as per ICD codes C77, C80 and C80.9. Results: Of the 122 cases, 74 cases (60.7 %) were found to be adenopathies of unknown primary origin. Out of these 74 cases, 24 cases were examined immunohistochemically, and commonly used panel of antibodies was found to be represented by CK 5/6, CK7, CK19, CK20, TTF1, p40, p63, CDX-2, PAX-8, EMA, SMA, LCA, Napsin and Synaptophysin. The most common histopathological diagnosis noted amongst CUP cases was Squamous cell carcinoma, followed by Metastatic poorly differentiated carcinoma and Adenocarcinoma. Correlation between the final histopathological diagnosis and IHC evaluation in the study population could not be established, possibly due to attrition and the financial burden bearing upon the patient. Conclusion: Hence, clinicians need to proceed with the most relevant imaging and the newly evolving investigations accordingly, as per individual presentation. Evolution of IHC, molecular targets and DNA microarrays will help us not only to better understand the pathogenesis of metastases but also to provide targeted therapy for this disease entity, improving the quality of life of the patients afterwards.

Keywords: Carcinoma of unknown primary, Metastasis of Unknown Origin, Cervical lymphadenopathy, Immunohistochemistry
**Introduction:**

**Carcinomas of unknown primary (CUP)** are histologically defined as the presence of a metastasis without detection of the primary tumour. It is a heterogeneous condition, wherein the type of the tumor, its extension and the treatment vary widely. CUP comprises of 3–5% of all cancers and affects both male and female population equally. The three most important characteristics of CUP are early dissemination, aggressiveness, and unpredictable metastatic pattern. The hypothesis behind the pathogenesis of CUP is believed to be spontaneous involution of primary site after seedling of metastases. The criteria for the diagnosis of CUP includes, biopsy proven metastatic neoplasm, with unknown anatomic origin of the primary and without any evidence of primary site of tumor origin on performing the following investigations which include Complete Blood Profile, Chest Radiograph, CT Abdomen and pelvis, Mammography and PSA. The criteria excludes malignancies like lymphoma, metastatic melanoma, and metastatic sarcoma as the natural history is well understood and stage- and histologic type-based treatments are available for these diseases. Most CUP cases are limited to epithelial and undifferentiated cancers. The most common histopathological subtype seen is squamous cell carcinoma. Due to the heterogeneous nature of this disease, the work up and treatment varies from patient to patient, hence it is difficult to compare different data from literature. Usually malignancies from head and neck and primary lymphomas are known to cause malignant lymphadenopathy of the neck. Occasionally, metastases from unknown sites can also present as cervical lymphadenopathy. As tumor from remote sites has a propensity to metastasize to neck, hence this study was attempted with an endeavor to study such cases in our hospital set up and understand the need for a definite approach in diagnosis of these patients.

**Materials and methods**

**Type of study** : Single center, observational, retrospective study

**Period of study** : June, 2017 - June, 2019

**Sample size** : 122 cases.

**Data collection** : Data of all patients presenting to ENT/GENERAL SURGERY/ ONCOSURGERY department with cervical lymphadenopathy as per ICD codes C77 (stands for cervical lymphadenopathy, C80 and C80.9 (stands for carcinoma of unknown primary) was collected, a total of 122 cases. Cases presenting with cervical lymphadenopathy due to non-malignant causes (4) and known primary malignancy, proven through preliminary investigations were excluded. Demographic & investigative data was compiled & transferred to an Excel sheet. Clinical and investigative work-up (imaging, biopsy, IHC) that helped in reaching the diagnosis was correlated.

**INCLUSION CRITERIA:**

Patient presenting with cervical lymphadenopathy with unknown primary, after evaluation done through USG+FNAC/Biopsy, Panendoscopy, CECT whole body/ PET scan. Above 18 yearsold.

**EXCLUSION CRITERIA:**

Patients presenting with cervical lymphadenopathy due to non-malignant causes, known primary malignancy or were diagnosed with a primary during the time of investigations
Observation & Results

122 patients presenting with cervical lymphadenopathy with an unknown primary were enrolled in this study. Amongst 122 cases, Primary site of tumor was not found in 74 cases (otherwise called as “CUP”) while 48 cases were excluded from the study as a primary malignancy could be diagnosed after a detailed evaluation. After applying the exclusion criteria to the study population, final sample size of this study was 74. The patients presenting with cervical lymphadenopathy of unknown origin were mostly in the age group 61-70 years (43.2%). Mean age was found to be 57 years. The Male: Female ratio was found to be 4:1 (58 males and 16 females). Most common presentation was found to be of unilateral lymphadenopathy – 62 cases (left side - 33 cases and right side - 29 cases). Majority of the study population was nil premorbid (40 cases; 54.1 %) and had a normal general physical examination (61 cases; 82.4 %). Most commonly involved group of lymph nodes were Jugulodigastric group of lymph nodes (II-IV), while least commonly involved were level I and VII.

Figure 1. Topography of the lesion involving different levels of cervical lymph nodes in the study population.

38 patients underwent USG Neck, while 36 patients underwent other investigative modalities for evaluation like CT Neck/ CECT whole body/ PET Scan (33 cases) and Pan endoscopy (3 cases). FNAC was performed in 50 cases which showed 31 cases of SCC, 8 cases of Adenocarcinoma, 10 cases of Metastatic carcinoma and 1 case s/o suspicious for malignancy (further investigations could not be done due to psychiatric illness).

Table 1. Final histopathological diagnosis in the study population
Immunohistochemistry evaluation of the biopsied sample was done in 24 out of 74 cases (32.4 %). Most commonly used IHC Panel in our hospital (Department of Pathology, KMC Manipal) comprised of the following IHC markers: P16, CK5/6, P40, P63, CK20, CK7. The baseline cost of the small panel (basic panel) of 5 markers in our hospital was Rs. 4000, while that of a large panel (advanced panel) of 8 markers was Rs. 8000. Primary tumor site was suggested to be ruled out from aerodigestive tract, urogenital system & hepatobiliary system, hence a definitive anatomic origin of the tumor could not be deciphered. Hence, the P value between the final HPE diagnosis and IHC was not considered significant (P=0.065). The diagnostic work-up for the 74 cases also included the following investigations, depending on the symptomatology and histopathological reports.

- CECT whole body – 11
- PET CT – 23
- PET CT + Panendoscopy – 1
- Pan endoscopy + Biopsy – 23
- Panendoscopy + Bone scan – 1
- Bone marrow biopsy and aspiration – 1
- Tuberculosis work-up (sputum AFB +/- Bronchoscopy) – 3
- UGI/ VDS + DNE – 3
- NIL Investigations – 8 cases

The cases which did not undergo any investigations (8 cases) for further evaluation after final histopathological diagnosis were due to attrition at the patient’s end, and hence were included in the study and designated as CUP. Multimodality treatment was used for treatment of such cases, which includes chemotherapy, radiotherapy, surgery (Modified Radical Neck dissection) or a combination of the above mentioned modalities. It was noted that majority of these patients were lost to follow up, after the initial diagnosis or after the initiation of treatment plan.

Table 2. Different modalities of treatment given in the study population.
Also we tried to look as to how many of these patients underwent IHC evaluation before initiation of therapy, and it was found that majority of the patients who were lost to follow up did not undergo an IHC evaluation, and hence remained undiagnosed and designated as CUP. Amongst excluded cases (N=48), most commonly found primaries were Lymphoma, Lung and Head and Neck malignancies (including thyroid). Other sites of primary found were carcinoma of Stomach, Ovary & Neuroendocrine tumor.

Table 3. Most frequently encountered primary malignancies in the excluded population.

<table>
<thead>
<tr>
<th>FINAL DIAGNOSIS</th>
<th>NUMBER OF CASES</th>
<th>IHC POSITIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoma</td>
<td>15</td>
<td>CD3/CD20/CD15/CD30/BCL6/BCL2</td>
</tr>
<tr>
<td>Ca Lung</td>
<td>7</td>
<td>CK5/6/P63/CK7/TTF/NAPSNIN</td>
</tr>
<tr>
<td>Ca Thyroid</td>
<td>2</td>
<td>TTF/CK7/CK20</td>
</tr>
<tr>
<td>Ca Ovary</td>
<td>1</td>
<td>CK7/WT1/ER</td>
</tr>
<tr>
<td>Neuroendocrine Tumor</td>
<td>1</td>
<td>SYNAPTOPHYSIN/CK/CD56</td>
</tr>
</tbody>
</table>

Discussion

This study analyzed 122 case files; out of which 74 cases with cervical adenopathy of unknown primary origin which presented to our hospital were studied in a span of last 5 years. There was an increased incidence noted in the age group of 61–70 years; with the mean age of 57 years. In our study, CUP was predominantly diagnosed in males (78.4 %), with a male to female ratio of 4:1. A similar study on 71 patients with CUP reported a male/female ratio of 1.7/1, with most of the patients aged between 51 and 70 years ⁵. As for the topography of lesions, the involvement of the jugular-carotid group of lymph nodes (Level II-IV) was found to be predominant. This was found similar to another study “Histopathological and immunohistochemical study of laterocervical lymph node metastases of unknown primary origin” by Ramona et al⁶, conducted at the University of Craiova, Craiova, Romania. The reported median interval between the first presentation and diagnosis in our study was found to be approximately 1 month. According to the review article “Diagnosis & management of neck metastases from an unknown primary” by Calabrese et al, Italy⁷; median interval was
reported to be 3 months. Most frequently found histopathological diagnosis in our study was squamous cell carcinoma, which was consistent with the findings observed in a review article “Diagnosis and management of neck metastases from an unknown primary”, published by Calabrese et al., Department of Head and Neck, European Institute of Oncology, Milan, Italy. A prevalence of N2 (multiple, ipsilateral, >3 and <6 cm) cases was seen in our study with a range of nodal size varying between 2-8 cm. This was again noted to be similar to the study “Diagnosis and management of neck metastases from an unknown primary” published by Calabrese et al., Department of Head and Neck, European Institute of Oncology, Milan, Italy. Studies suggest that the extent of workup in CUP remains a challenge and should be based on the clinical presentation, pathology, and the patient’s ability to tolerate therapy. The studies showed that an effective algorithm requires: Thorough medical history or physical examination, laboratory tests, including complete blood count, blood chemistry, routine chest X-ray, diagnostic lymph node biopsy, Panendoscopies with systematic biopsies of suspicious areas, computed tomography (CT) scan of the abdomen and pelvis/PET-CT. According to the article “Ultrasound of malignant cervical lymph nodes” by Ahuja et al., study conducted at Department of Diagnostic Radiology and Organ Imaging, The Chinese University of Hong Kong, Princeof Wales Hospital, ShaTin, New Territories, Hong Kong SAR, China; the combination of USG and FNAC (guided) has a high Specificity rate of 93%. However, when needle biopsy techniques fail, open incisional or excisional biopsy is necessary. In our study, 38 patients who underwent USG neck, also underwent FNAC (guided or non-guided) and were classified as either a C4 or C5 lesion with a definitive histopathological diagnosis. According to review article “Diagnosis & management of neck metastases from an unknown primary” by Calabrese et al., ideally, biopsies should be performed after PET scan, since it allows for sampling of the areas suspected in PET. Avoids false positive PET-scans at biopsy site. Kwee and Kwee analyzed the efficacy of CT and FDG-PET scan in their study “Combined FDG-PET/CT for the detection of unknown primary tumors: Systematic review and meta-analysis” conducted at the Department of Radiology, University Medical Centre Utrecht, Utrecht. A meta-analysis of 11 studies comprising 433 patients was done, most with cervical primaries and reported an overall primary tumor detection rate of 37%, pooled sensitivity of 84%, and specificity of 84% for these modalities in detecting unknown primary tumors. In our study, CECT/ PET scan done in 14 out of 48 (excluded) cases gave a clue to the suspicious primary site (lungs, tongue, tonsil, hypopharynx, mandible, thyroid, ovary), which on further histopathological evaluation, was confirmative and positive in all 14 cases. However amongst the included cases (N=74), 14 cases showed suspicious lesions in the lung, pyriform fossa and buccal mucosa on imaging, which on further evaluation were found to be metastatic deposits. Similar results were noted in the study “Metastatic Carcinoma of the Neck of Unknown Primary Origin – An Evolution and Efficacy of the Modern Workup” conducted at The Ohio State University, Columbus, Ohio by Joshua et al., published in 2009. The study enrolled 183 patients who underwent preoperative imaging in the form of CT, MRI, PET and/or PET-CT fusion scan, which identified suspicious primary tumor site location in 69 patients. Amongst these 69 patients, subsequent directed biopsies of these sites revealed positive results in 42 cases. IHC evaluation was done in 24 of 74 included cases after biopsy, however, not definitive but differential diagnosis was provided to plan on targeted treatment. The additional IHC markers done for further evaluation, suggested to consider primary from various possible anatomical locations, most commonly encountered being aerodigestive, hepatobiliary system and pancreas. IHC evaluation done in 26 out of 48 excluded cases, however noted a change in the histopathological diagnosis based on specific morphology and were found to be confirmative for the suspicious primary site. For example, increased TTF-1 and Napsin expression was seen in lung carcinoma, CD marker expression in lymphoma, WT-1 and ER expression in ovarian malignancy and Synaptophysin expression in neuroendocrine tumors, which lead to the definitive diagnosis and hence targeted treatment.
could be planned afterwards. Roh and Hong in their study “Utility of thyroid transcription factor-1 and cytokeratin 20 in identifying the origin of metastatic carcinomas of cervical lymph nodes” conducted at the Department of Pathology and Institute of Medical Science, Dong-A University College of Medicine, Busan, Korea analyzed the expression of TTF-1 and CK20 in 68 patients with CUP. They found that 29 patients had lung carcinoma as the primary tumor, 3 patients had the primary tumor in colorectal region, and primary tumors in other sites occurred in rest of the patients. TTF-1 expression was recognized in 69% of patients with metastatic lung cancer, but not in patients with metastatic gastrointestinal carcinomas, while CK20 expression was detected in 69% of metastatic gastrointestinal carcinomas but not in metastatic lung carcinomas.

**Conclusions**

CUP is a heterogeneous group of malignancy which presents with metastatic disease with probably an involuted primary site of tumor origin. The limitations currently faced in diagnosing and treating an unknown primary cancer remains a challenge in comparison with other malignancies, as there is no fixed diagnostic protocol for this disease. Correlation between the final histopathological diagnosis and IHC evaluation in the study population could not be established, possibly due to attrition. However, it was noted majority of the cases in the excluded group underwent IHC evaluation and hence were able to reach a primary site of tumor origin, thus aiding in diagnosis and planning targeted treatment. Imaging such as CECT whole body and PET scan with Panendoscopy and directed biopsies offers the greatest likelihood of successfully identifying an occult primary location in patients presenting cervical adenopathy. Last but not the least, a simple and cost-effective diagnostic algorithm with incorporation of IHC and appropriate imaging with directed biopsies can help us to reach the diagnosis, and provide targeted therapy for CUP.

**Acknowledgements**

My sincere thanks to Dr. Veena L Karanth, Professor, Department of General Surgery, Kasturba Medical College, Manipal and our institution, Kasturba Medical College, Manipal University, Manipal.

**Declarations**

Funding: Nil

Conflict of interest: No conflicts

Ethical approval: Obtained

**References**


