

NUT Carcinoma: A Clinical, Morphological and Immunohistochemical Mimicker—The Role of RNA Sequencing in the Diagnostic Procedure

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Abstract

Background: NUT carcinoma is a highly aggressive and rare subset of squamous cell carcinoma with grim prognosis. It is under-recognized by both pathologists and oncologists. Recognition is challenging due to its rareness and the fact that its clinical and laboratory features as well as morphological and immunohistochemical characteristics may mimic other malignancies. **Case presentation:** An interesting case of NUT carcinoma in a 47-year-old male with a large tumor mass in the inferior part of the mediastinum and left lung and increased levels of serum alpha fetoprotein (AFP) is described. Immunohistochemical analysis of both the primary tumor in a bronchoscopy specimen and an excisional biopsy of a subcutaneous metastasis showed positivity for AFP and leukocyte common antigen (LCA) that were misleading and resulted in diagnostic pitfalls of mediastinal germ cell tumor (clinically) and hematomatous neoplasm (pathologic report). Immunohistochemical demonstration of NUT protein expression revealed the proper diagnosis, which was further confirmed by RNA sequencing revealing a *BRD4-NUTM1* gene fusion. **Conclusions:** Since NUT carcinoma can show a wide spectrum of histological and immunophenotypic features and can clinically mimic other tumors, use of RNA sequencing with identification of specific *NUTM1* fusion partner could be crucial when there are discrepant clinical and histopathological findings. As well, since the category of so-called *NUTM1*-rearranged neoplasms is rapidly expanding, identification of *NUTM1* fusion partner may be essential for the appropriate clinical management.

Keywords

NUT carcinoma, immunohistochemistry, alpha-fetoprotein, leukocyte common antigen, RNA sequencing

Background

NUT carcinoma, also named NUT midline carcinoma, is a highly aggressive and rare subset of squamous cell carcinoma. Its recognition is very challenging and demanding due to the following reasons: 1. it is rare and most pathologists and oncologists are not familiar with this malignancy, 2. its clinical and laboratory features may mimic other malignancies, 3. morphological and immunohistochemical analysis may be misleading if the diagnosis is not suspected, and 4. it is defined by the presence of a *NUTM1* fusion.¹ Most commonly, *NUTM1* is fused with either the *BRD4* or *BRD3* gene, and less commonly with *NSD3* or other genes.¹ Since bromodomain and extraterminal domain inhibitor therapies have been developed and are currently used in clinical trials, identification of *NUTM1* fusion partner may be essential for the appropriate clinical management.¹

Case Report

The patient was a previously healthy 47-year-old male, non-smoker, with an unremarkable medical history. He

came to medical attention because of progressive dry cough lasting for a few weeks, progressive dyspnea, sharp pain under the left costal margin and weight loss of 10 kg over the one month. He was referred to hospital where laboratory results showed leukocytosis ($14 \times 10^9/L$) with neutrophilia (84%), increased CRP (112 mg/L) and increased LDH (6.15 U/L; normal range <4 U/L) levels. Massive bilateral serohemorrhagic pleural effusion was found and evacuated (1700 ml from the left and 1800ml from the right pleural space). Computed tomography of the thorax showed a partially necrotic mediastinal/left lung mass, measuring 12 cm in the largest diameter which was radiologically suggestive of primary lung

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carcinoma. To clarify the diagnosis, bronchoscopy with biopsy was performed. Histologic analysis showed a poorly differentiated neoplasm that was immunohistochemically negative for keratins CK AE1-AE3 and CKMNF116, EMA, TTF-1 and SALL4, but strongly and diffusely positive for LCA. A hematolymphoid neoplasm was suggested as the most likely diagnosis and patient was transferred to our institution for further evaluation and treatment. Additional laboratory work-up showed increased serum AFP (6389 kIU/L, normal <5.8 kIU/L). ¹⁸F-FDG PET CT showed increased uptake with SUV 13 in mediastinal/left lung tumor, multiple metastases in mediastinal and peripheral lymph nodes, bones and subcutaneous tissue as well as diffusely increased uptake in pleura on both sides. Radiological and laboratory findings suggested a mediastinal non-seminomatous germ cell tumor (NS-GCT). Since this clinical diagnosis was highly discrepant with the primary pathologic report, the oncologist decided to repeat the biopsy.

A subcutaneous metastasis from the left shoulder was excised and sent for pathological evaluation. Hematoxylin and eosin-stained sections showed a neoplasm consisting of undifferentiated, large vesicular cells with conspicuous, solitary nucleoli and poorly demarcated cytoplasm (Figure 1a). Foci of abrupt keratinization were present (Figure 1a arrows). A wide immunohistochemical panel was applied (Table 1). Tumor cells were positive for LCA (Figure 1b), CK AE1-AE3, CK MNF116 (Figure 1c), EMA, p63 and TTF-1 (Figure 1d), focally positive for CK5, CK7, CK18, CD138, AFP (Figure 1e), p40 and negative for CD34, CD20, CD3, CD56, SALL4, CD117, Oct4, PLAP, CD30, CD163, Napsin A. Immunohistochemical staining for NUT protein (Figure 1f) showed the characteristic speckled nuclear reaction and, finally, a diagnosis of NUT carcinoma was rendered. For molecular analysis, we performed RNA sequencing using the OncoPrint™ Childhood Cancer Research Assay. Interestingly, we detected a *BRD4-NUTM1* gene fusion in 82585 reads. On RNA level, exon 14 of *BRD4* was fused to exon 2 of *NUTM1*, resulting in an in-frame fusion transcript containing many of the coding regions of both partner genes, including the BRD and ET domains of *BRD4* and AD1 domain of *NUTM1*.² The slides from the bronchoscopic biopsy were reviewed. The morphology of the tumor cells was identical to those in metastatic lesion but there were no morphological signs of squamous differentiation.

The patient received two cycles of chemotherapy according to the CAP protocol (cyclophosphamide, doxorubicin, platinol) but showed progressive disease and a fast deterioration of the performance status. Unfortunately, he succumbed less than 2 months after the diagnosis.

Discussion

NUT carcinoma is a poorly differentiated subtype of squamous cell carcinoma, commonly associated with a

rearrangement of the *NUTM1* gene with various partner genes, such as *BRD4*, *BRD3* and *NSD3*.¹ In our case we detected a *BRD4-NUTM1* fusion. Indeed, *BRD4-NUTM1* are the most frequent rearrangements in NUT carcinoma and have been shown to be present in up to 80% of NC.¹ The breakpoint seen in our case is common, which frequently involves exon 2 from *NUTM1* and exons 11 to 15 from *BRD4*.³ Fusion oncoproteins cause upregulation of *MYC* and hence autonomous tumor growth. NUT carcinoma is an aggressive malignancy, and a recent analysis of a large patient cohort identified three prognostic groups based on tumor location and presence and type of fusion.⁴ Interestingly, at least a subset of patients may benefit from the use of BET inhibitors, possibly in combination with other agents.⁵ Tumors with *NUTM1-BRD4* fusion could be sensitive to BET inhibitors.⁵ NUT carcinoma is a relatively under-recognized entity and is usually not suspected by both pathologists and oncologists. It may be misdiagnosed if it is not considered in the differential diagnosis and specifically looked for. For example, in the study by Evans et al⁶, a re-evaluation of 114 cases of undifferentiated mediastinal malignancies with immunohistochemical staining and FISH for NUT lead to the diagnosis of NUT carcinoma in 3.5% of the cases.

The diagnosis of NUT carcinoma can be very challenging for the following reasons:

1. Clinical presentation may mimic other neoplasms. In our case, a mediastinal tumor with elevation of AFP suggested a mediastinal NS-GCT. Principal tumors that secrete AFP are yolk sac tumor, hepatoblastoma, and hepatocellular carcinoma, and only rarely some other tumors like lung or gastrointestinal carcinomas, Sertoli-Leydig cell tumor or Wilms tumor. It can also be seen in chronic liver disease, such as cirrhosis and in acute hepatitis. Although NUT carcinoma only rarely produces AFP, a few reports have been published in the recent literature⁷ describing patients with NUT carcinoma with similar clinical presentation and elevated levels of serum AFP. In some cases, immunohistochemical staining of the tumor tissue for AFP was done and was negative.
2. By morphology it is an undifferentiated neoplasm which may or may not show foci of abrupt keratinization. Abrupt keratinization has been described to be characteristic for NUT carcinoma but is not always present and could very often be missing in small specimens harvested from endoscopic, aspirational or core needle procedures.
3. Immunohistochemical analysis yields frequently also ambiguous results. In our case the primary tumor from the bronchoscopy specimen showed an intense and strong positivity for LCA while there was no keratin expression. Keratin and LCA expression are in general mutually exclusive in practice of

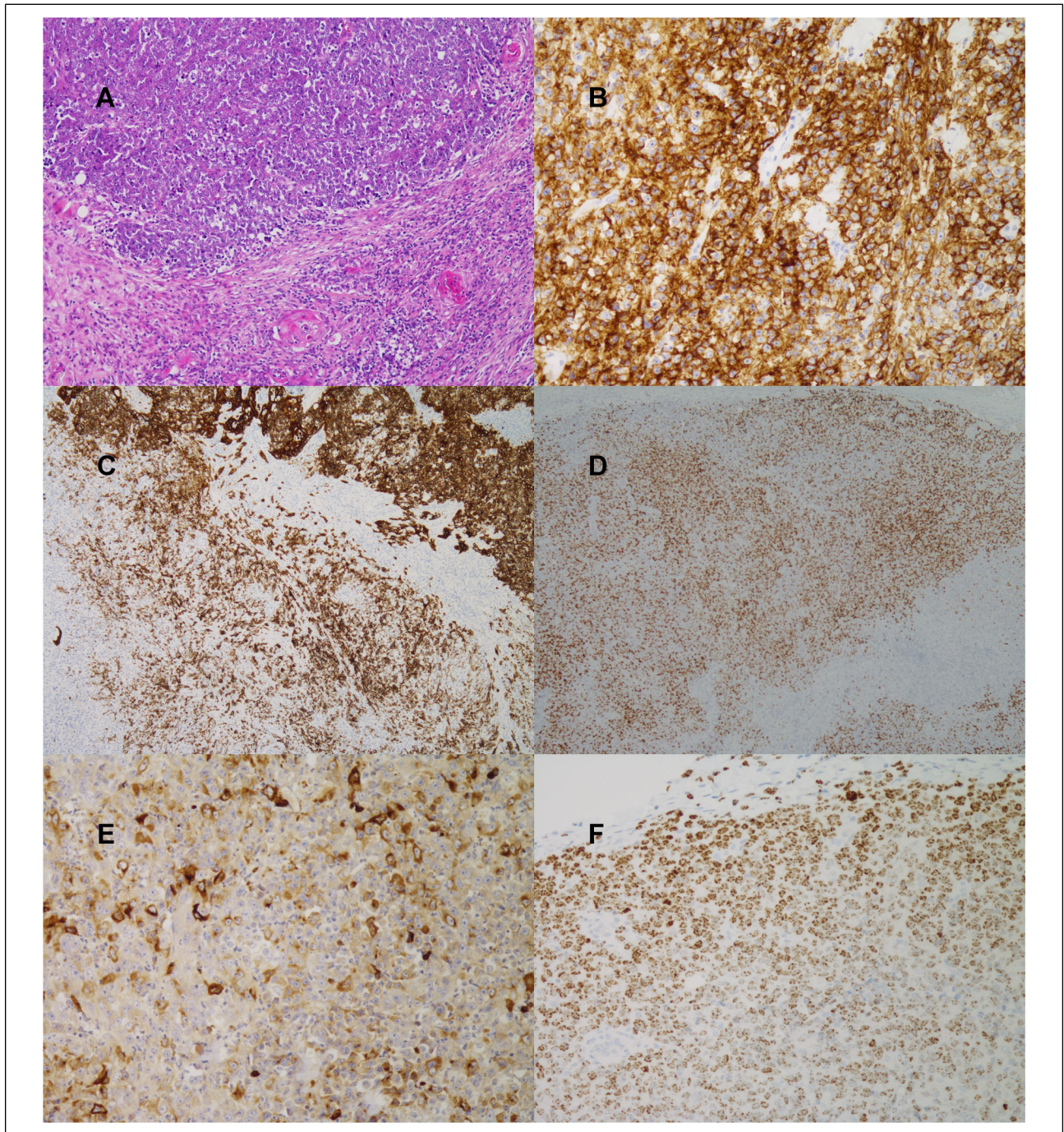


Figure 1. (A) H&E, 10 \times : poorly differentiated neoplasm with focus of abrupt keratinization (marked by arrows) (B) LCA immunohistochemistry, 20 \times (C) CK MNF116 immunohistochemistry, 5 \times (D) TTF-I immunohistochemistry, 5 \times (E) AFP immunohistochemistry, 20 \times (F) NUT immunohistochemistry, 20 \times : characteristic, speckled pattern of staining.

diagnostic surgical pathology and differential diagnosis between poorly differentiated/undifferentiated carcinoma and hematolymphoid neoplasm is greatly aided by immunohistochemical staining for the two markers with rare exceptions such as in small subset of otherwise characteristic B- and

T-cell lymphomas, but not in acute leukemias.⁸ LCA is considered highly specific for hematological neoplasms and is a commonly used marker of hematopoietic cells except erythrocytes and thrombocytes. Reports of aberrant expression of LCA in non-hematological neoplasms are more or less

Table 1. Immunohistochemistry.

Antibody	Manufacturer	Dilution
LCA	DAKO	1:400
CK AE1-AE3	DAKO	1:75
CK MNF116	DAKO	1:200
EMA	DAKO	1:50
p63	DAKO	1:50
TTF-1	Leica	1:50
CK5	Leica	1:50
CK7	DAKO	1:200
CK18	DAKO	1:200
CD138	DAKO	1:25
AFP	CellMarque	1:200
p40	Zytomed	1:200
CD34	DAKO	1:25
CD20	DAKO	1:50
CD3	DAKO	1:50
CD56	CellMarque	1:100
SALL4	CellMarque	1:25
CD117	CellMarque	1:200
Oct4	CellMarque	1:25
PLAP	DAKO	1:150
CD30	CellMarque	1:50
CD163	CellMarque	1:200
Napsin A	CellMarque	1:400
NUT	Cell Signaling Technologies	1:100

anecdotal consisting of very rare cases of undifferentiated and neuroendocrine carcinoma⁹⁻¹¹, one seminoma and one rhabdomyosarcoma.¹² On the basis of the extremely aggressive clinical behavior, tumor location (midline) and microscopic description of such undifferentiated/poorly differentiated neoplasm, we could presume that at least a few of them^{9,11} may represent NUT carcinoma, an entity non-existent in the time of publication of those reports.

Second point regarding immunohistochemistry of note in our case is tumor positivity for AFP. In some of the cases of NUT carcinoma with elevated levels of serum AFP that were immunohistochemically stained for AFP⁷, the tumor tissue itself was immunohistochemically negative for AFP staining. On the basis of that finding, D'Ambrosio et al⁷ concluded that negative AFP staining could exclude the diagnosis of mediastinal NS-GCT. Our conclusion is that immunohistochemical positivity for AFP cannot serve as a reliable marker of NS-GCT even if clinical presentation together with laboratory findings suggests that diagnosis, as we have shown in our case.

As well, strong and diffuse expression of TTF-1 is highly unusual in NUT carcinoma. So far, it was described only in a few cases.¹³⁻¹⁵ This may be

important potential source of misdiagnosis especially in tumors that on morphology lack squamous differentiation or are immunohistochemically negative for its markers such as p40 or CK 5/6.¹⁵ p40 or CK 5/6 negative tumor with TTF-1 expression could be erroneously diagnosed as lung adenocarcinoma.

4. A definitive diagnosis of NUT carcinoma can basically be made by NUT immunohistochemistry and/or *NUTM1* FISH in characteristic clinical context. Immunohistochemical staining has 100% specificity and 87% sensitivity in detection of NUT protein expression and is considered the first line approach to NUT carcinoma diagnosis.¹⁶ However, due to the use of next generation sequencing methods, *NUTM1* rearrangement with different fusion partners have been found in diverse array of other neoplasms that could show immunohistochemical positivity for NUT protein. For example, a *NUTM1* rearranged acute lymphoblastic leukemia (ALL) shows speckled nuclear positivity for NUT protein and since NUT carcinoma in our case showed diffuse LCA positivity, *NUTM1* rearranged ALL should be considered in the differential diagnosis. *NUTM1*-rearranged ALL is characteristically a disease of infant and pediatric population with many different fusion genes reported, none of them belonging to the BET family.¹ It seems reasonable to perform next generation sequencing for identification *NUTM1* fusion partner in order to confirm a diagnosis of NUT carcinoma in malignancy with positive NUT immunohistochemistry/*NUTM1* FISH especially if there are discrepant clinical and/or histopathological findings.³

To the best of our knowledge, this is the first case of NUT carcinoma showing aberrant expression of LCA described in the literature so far. Therefore, we suggest that all poorly differentiated/undifferentiated tumors with aggressive clinical picture, especially those arising in the midline should be tested for NUT protein and/or NUT gene translocation since morphology of NUT carcinoma is non-specific, particularly in small biopsies and its immunohistochemical profile can be very wide and misleading. Since the category of the *NUTM1* – rearranged neoplasms is rapidly expanding and new targeted drugs such as BET inhibitors are developing, identification of *NUTM1* fusion partner is essential for appropriate diagnosis and adequate clinical management.

Declaration of Conflicting Interests

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Ethical Approval

This study was approved by the Ethical board of Institute of Oncology Ljubljana, Slovenia, ERIDNPVO-0032/2020

Informed Consent

Signed consent for publication was obtained from patient's spouse since he passed away.

Trial Registration

Not applicable, because this article does not contain any clinical trials.

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