NUT Carcinoma in Elderly Women With Special Morphology and Immunophenotype - a Diagnostic Pitfall

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Case Report

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Abstract

Introduction: NUT carcinoma is a rapidly progressing and rare neoplasm mainly affecting the younger patients and most of them express epithelial markers, but not neuroendocrine markers, with a survival time of about one year. Retrospective studies have shown that pathologists and clinicians do not have enough understanding of it due to the lack of typical clinical manifestations, imaging, and morphological features.

Case report: A 60-year-old female was diagnosed with repetitive epistaxis, nasal pain, and nasal congestion. Imaging examination showed obvious masses in the right nasal sinus and frontal sinus. Histopathologically with two types of tumor cells were first discovered: one was small fusiform cells with sparse cytoplasm and densely stained nuclei, and the other was large tumor cells with abundant cytoplasm, round or oval nuclei with obvious nucleoli, some cells with eosinophilic cytoplasm, obviously eccentric nuclei without nucleoli, resembling plasma cells. The sudden appearance of keratinization was another prominent feature. Immunohistochemical staining showed that P63 and NUT were diffusely positive, P40 and synaptophysin were focal positive, CK5/6 and cytokeratin AE1/AE3 were punctate positive in limited areas. Fluorescence in situ hybridization revealed *BRD4-NUT* translocation. The patient was still alive with 20 rounds of radiation after surgery without recurrence or metastasis after a 3-month follow-up.

Conclusion: we report for the first time the eldest and surviving case of NUT carcinoma originating in the nasal cavity with special morphological features and different immunohistochemical results among previous study cases with focal positive for synaptophysin and only few cells were punctate positive stained with AE1/AE3. NUT antibody should be performed in the undifferentiated or poorly differentiated malignancies, particularly those with cytoplasmic vacuolation of medium-sized cells and/or the abrupt keratinization, irrespective of patient age.

Introduction

NUT carcinoma (NC), was reported for the first time in 1991 with two cases of primary thymic carcinomas[1, 2]. The clinical behavior was extremely invasive, resulting in a very dismal prognosis, the median overall survival time was less than 7 months[3]. At present, there are no more than 30 cases of NC originating in nasal cavity in the world, which is attributed to insufficient understanding of this tumor by clinicians and pathologists, leading to missed diagnosis of some cases. In 2012, Of the 151 cases of primary paranasal sinus carcinoma, only 3 cases (2%) NCs were found [4]. In the same year, Evans et al. retrospectively analyzed 114 cases of mediastinal tumors and found 4 cases of NC (3.5%)[5]. In 2014, Gökmen-Polar et al.[6] screened 2 cases of NC (1.8%) out of 110 cases of thymic carcinomas. The earliest studies have shown that NC tends to occur in the midline position of children and adolescents. Today we introduce a case of NC in an elderly woman, who is by far the oldest patient in the nasal cavity. Meanwhile, 19 cases of the primary nasal cavity with perfect clinical data were summarized, and an update on the clinical, pathological, molecular characteristics, and targeted therapies of the NC were provided.

Case Presentation

A 60-year-old woman was treated for epistaxis and nasal pain. The main complaint was with 4 years history of recurrent epistaxis, recently nasal congestion, nasal bleeding, paroxysmal sneezing, and runny nose for 10 days. Allergic rhinitis was diagnosed in another hospital at the initial visit, but with no significant improvement after treatment. Ten days ago, there was no obvious cause of nasal pain to our University Hospital. Nasal endoscopic examination revealed a significant mass blocking the nostril in the right sinonasal tract extending from the lateral wall of the nasal cavity to the nasal vestibule. The lesion was soft and ulcer on the surface, light pink mucosa, and left deviation of the nasal septum. Cranial computed tomography showed the value was about 40HU, and the bone around the lesion was compressed and thinned. Magnetic resonance imaging showed an irregular heterogeneously enhancing soft tissue lesion, blocking the right nasal cavity and frontal sinus (longest diameter of 2.5cm x1.3cm, Fig. 1). Both T2W1 and T1W1 fat suppression sequences showed moderately high signal intensity. The surrounding bone compression and thinning with the nasal septum bended to the left, and the local boundary between the right inferior turbinate and the lesion was not clear. During the operation, irregularly shaped masses with surface ulcers were found in the right nasal cavity and frontal sinus, and biopsies were taken. Explored the boundary of the tumor, the root from the lateral

wall of the right sinonasal tract, the posterior end reached the middle turbinate, the lower part to the inferior turbinate, and the upper part to the olfactory fissure area. the lesion was ablated with low-temperature plasma, up to the olfactory fissure area at the top of the nose and back to the middle turbinate.

Histopathologically, it was a nest-like poorly differentiated change, composed of a dense arrangement of cells, accompanied by extensive necrosis. There were two types of tumor cells after resection: the one was small fusiform cells with sparse cytoplasm and densely stained nuclei, and the other was medium and large tumor cells in undifferentiated state, with abundant cytoplasm, round or oval nuclei with obvious 1–2 obvious nucleoli, the ratio of nucleus to the cytoplasm are high, some cells with eosinophilic cytoplasm, obviously eccentric nuclei without nucleoli, resembling plasma cells. In addition, a phenomenon that cannot be ignored was the sudden appearance of keratosis in the poorly differentiated area (Fig. 2).

The immunohistochemistry (IHC) results displayed P63 and NUT were diffusely positive, P40 and synaptophysin were focal positive, CK5/6 and cytokeratin AE1/AE3 were punctate positive in limited areas (Fig. 4). Chromogranin A, CD56, CD45RO, NKX2.2, P16, Vimentin, S-100, were all negative, the Ki-67 index was > 40%. The morphologic and immunophenotypic features indicated NC. The *BRD4-NUT* rearrangement was found through FISH test, and the patient was diagnosed as NC ultimately (Fig. 5). The patient survived after receiving twenty doses of radiotherapy 3 months postoperatively.

Discussion

NC is a highly aggressive and rare carcinoma. The molecular feature is characterized most commonly by the *BRD4-NUT* translocations, fusion of the NUT with BRD3 and NSD3 genes was found in approximately one-third of cases[7].

NC is probably underdiagnosed because it was originally found in the midline organ, so it is called "NUT midline cancer", another factor is the reports that NC occurs only in children or adolescents [7]. However, according to recent kinds of literature, it can occur in any site of patients of all ages[8–11]. A similar situation is also verified in Table 1: The median age of the disease was 42 years old, of which the youngest patient was 10 years old, and the oldest patient was 60 years old (our reported case). Unexpectedly, the most common site of NC is not the head and neck (only 1/3), but the lung (about 1/2). At present, most of the latest literature reports are primary lung NC[12, 13]. Moreover, an increasing number of non-midline positions have been reported, such as thymus, bladder[14], pancreas[8],salivary glands[15], lacrimal sac[16], thyroid[17], bone and soft tissue[18]. Malignant tumors in the nasal cavity are more common in head and neck tumors, accounting for about 50%, however, NC of the nasal cavity is very invasive and often invades the surrounding areas, among which frontal sinus and ethmoid sinus are the most commonly involved sites[19]. This clinical feature can remind clinicians as a diagnostic hint. So far, no predisposing factors of NC have been found, EB virus, human papillomavirus infection, and smoking were not associated with NC[11] [20].

Table 1
Clinicopathological characteristics of sinonasal tract NC

Author (year)	Sex/ Age	Site	keratinization	IHC	FISH testing	Treatment	Prognosis
Wei[39],2012	M/55	nasal cavity	Y	CK, P63, NUT (+)	N	C	Alive (40months after surgery)
	M/42	nasal cavity	Y	CK, P63, NUT (+)	<i>BRD4-NUT</i> rearrangement	CR	Dead (9months after surgery)
	F/59	nasal cavity	N	CK, P63, NUT (+)	N	C	Alive (12months after surgery)
	M/50	nasal cavity	N	CK, P63, NUT (+)	<i>BRD4-NUT</i> rearrangement	N	Dead (1months after surgery)
Bishop[4],2012	M/26	maxillary sinus	N	NUT, EMA, AE1/AE3(+); P63(-)	NA	C	Dead (8months after surgery)
	M/33	maxillary sinus	N	NUT, EMA, AE1/AE3, P63, CD34(+)	NA	C	Dead (11months after surgery)
	M/48	ethmoidal sinus	Y	NUT, EMA, AE1/AE3, P63(+)	NA	CR	Dead (16 months after surgery)
Suzuki[40],2014	F/18	right nasal cavity, and maxillary and ethmoidal sinus	N	Vimentin and NUT (+), CD138 and P63(focal) AE1/AE3 (spotty)	<i>BRD4-NUT</i> rearrangement	CR	Alive (12months after surgery)
Stirnweiss[41],2015	F/14	right anterior ethmoid and maxilla	NA	NA	<i>NUT</i> rearrangement	R	Dead (98days after surgery)
Edgar[42],2017	M/53	left sinonasal	Y	CK5/6, p16, p40, p63 and NUT (+)	<i>BRD4-NUT</i> rearrangement	CR	Dead (3 months after surgery)
Kakkar[16],2018	M/30	left sinonasal and orbital	Y	NUT and p40 (+); p16 and CD34(focal)	NA	NA	NA

Abbreviations: M male, F female, Y yes, N no, NA not available, C chemotherapy, CR chemoradiotherapy, R radiotherapy

Author (year)	Sex/ Age	Site	keratinization	IHC	FISH testing	Treatment	Prognosis
	F/31	left nasal cavity, ethmoid, sphenoid and maxillary sinuses	Y	NUT and p40 (+); p16 (focal)	NA	C	Dead (2 months after surgery)
	M/25	right sinonasal	Y	CK, NUT, and p40(+); p16 + (focal)	NA	NA	NA
	F/10	nasolacrimal duct	Y	CK, NUT, and p40(+); p16 + (focal)	NA	R	NA
	F/30	left nasal cavity	N	NUT and p40(+); p16 + (focal)	NA	NA	NA
Albrecht[43],2019	M/48	left sphenoid sinus	NA	NA	<i>BRD4-NUT</i> rearrangement	CR	Dead (6 months after surgery)
Pragya[44],2020	M/44	right sinonasal	N	CK, P63, NUT (+); Syn (focal)	NA	NA	NA
Crocetta[45],2021	F/56	right sinonasal	Y	CK, NUT (+)	NA	CR	Dead (6 months after surgery)
current case,2022	F/60	right sinonasal and Frontal sinus	Y	P63 and NUT (+), P40 and Syn(focal) CK and CK5/6 (spotty)	<i>BRD4-NUT</i> rearrangement	R	Alive (3months after surgery)
Abbreviations: M male, F female, Y yes, N no, NA not available, C chemotherapy, CR chemoradiotherapy, R radiotherapy							

Morphologically, the microscopic appearance of NC is not specific. Undifferentiated carcinoma or squamous cell carcinoma is often diagnosed for lack of typical morphological features. It is not difficult to diagnose NC, while, it is hard to think of the diagnosis of NC. Some of the diagnostic clues so far include the presence of spindle cells and neutrophil infiltration[4]. The appearance of sudden squamous differentiation within the lesion, especially in undifferentiated areas, was first reported as a clue to the diagnosis of histopathology by Stelow [9]. Subsequent literature has concluded that the frequency of squamous cell

differentiation can be as high as 50–82%, which may be helpful to the diagnosis of NC[4]. We identified and summarized the coexistence of two kinds of cells of shape and size for the first time: small fusiform cells with sparse cytoplasm and densely stained nuclei, and the other was medium and large tumor cells with sparse cytoplasm, round or oval nuclei with obvious nucleoli, some cells with eosinophilic cytoplasm, obviously eccentric nuclei without nucleoli, resembling plasma cells. As distinguished from other poorly differentiated carcinomas or sarcomas, such as olfactory neuroblastoma, lymphoma, undifferentiated carcinoma, and Ewing sarcoma. Those with cytoplasmic vacuolation of medium-sized cells and the abrupt keratinization, remind us to add NUT antibody, finally confirmed as NC. The reason for sharing this case is to remind pathologists the possibility of NC should be considered for small or medium-sized round or spindle cells with or without sudden keratosis in the malignant neoplasm of head and neck.

As an inexpensive and rapid method for NC diagnosis, NUT antibody is particularly important and has 100% specificity and 87% sensitivity to diagnosis[14]. But at present in China, most hospitals lack this antibody due to the lack of knowledge about this kind of tumor, which is also a major reason for misdiagnosis. Since it cannot be distinguished from other poorly differentiated or undifferentiated neoplasms, NUT is encouraged for use in any undifferentiated malignancy as it provides an economical and rapid approach.

Although NC shows no specific findings on general immunostaining, another recommended antibody is P63, which is more sensitive to NC diagnosis than P40. Matsuda et al. demonstrated that P40 was completely negative in 4 of 7 NCS (57%), and was only focal or patchy positive in the other 3 cases[21]. In our case, P63 was diffusely positive, whereas P40 showed only focal staining. Of course, it has also been reported that the expression of P40 and P63 is variable, or even negative simultaneously[22, 23]. Therefore, the use of P40 antibodies alone may lead to diagnostic traps and is not suitable for the identification of NC alone. Neuroendocrine markers, mainly synaptophysin and chromogranin, can be locally expressed in NC[24][25]. According to summary statistics (Table 2), merely two cases expressed synaptophysin (including our case). This finding, although uncommon, the same suggests that small biopsies expressing both neuroendocrine and epithelial markers should not be misdiagnosed as neuroendocrine carcinoma. Meanwhile, an epithelial marker for NC barely expressed was found in our case, which was different from that confirmed in most literatures (Table 2). As was the case in our study only spotty CK5/6 and cytokeratin AE1/AE3 staining. This situation, in particular, to alert all physicians the potential diagnostic pitfalls, as it is sometimes possible to misdiagnose sarcomas or lymphomas without expressing individual epithelial markers, which would be best to use the epithelial markers in combination. Rapid and accurate diagnosis is very necessary for NC patients with short survival time. NUT is strongly recommended as a specific diagnostic marker for NC.

Table 2
Summary of clinical characteristics of sinonasal tract NC

	Number of patients (%)
Median age (range)	42 (10 – 60)
Gender	
Male	11 (57.9%)
Female	8 (42.1%)
keratinization	10 (52.6%)
IHC	
NUT	17(89.4%)
AE1/AE3	12 (63.1%)
P63	11 (57.9%)
P40	7 (36.8%)
P16	6 (31.6%)
CK5/6	2 (10.5%)
Syn	2 (10.5%)
Treatment	
R	3 (15.7%)
CR	5 (26.3%)
C	5 (26.3%)
Molecular testing	7 (36.8%)
Median survival time (months)	6 (1 – 16)
Abbreviations: IHC immunohistochemistry, C chemotherapy, CR chemoradiotherapy, R radiotherapy	

Diagnosis, in this case, was eventually made by the FISH detected *BRD4-NUT* fusion. In 2004, French for the first time discovered the *BRD4-NUT* fusion gene at the molecular level[7]. At present, it is a relatively mature research surface that NC is molecularly characterized by the fusion between NUT gene with bromodomain and extra terminal (BET) family members [26]. Among them, *NUT* is most commonly rearranged with *BRD4* or *BRD3* [27], rare reports with regard to *NSSD3- NUT*, *ZNF532- NUT*, *WHSC1L1- NUT*, and *NAP1L4- NUT* fused carcinomas are also emerging [4, 28–31].

Surgical treatment is the main treatment for NC at present. Adjuvant therapy such as radiotherapy and chemotherapy benefit some patients, but the effect of long-term treatment is not satisfactory and the disease progresses rapidly. Nicole found that the patients who achieved remission were those who initially underwent surgery, emphasizing that the choice and sequence of initial treatment were critical to prognosis [10]. Previous literature showed that radiotherapy and complete tumor resection were the keys to improving overall survival, but chemotherapy had little effect on survival[3, 10]. Consistent with our reported case—although only three months follow-up, the patient was alive with twenty times of radiotherapy after complete surgical excision of the lesion. A few studies have found that PD-L1 positive NC patients have better survival, even without immunotherapy. This conclusion needs to be supported by more literature and studies[13].The pathogenesis of NC is speculated to be through NUT-mediated histone modification of the whole gene suite to change the expression of oncogene or tumor suppressor gene[32]. Through the deepening understanding of the mechanism of NC, targeted therapies targeting BET/BRD4 bromine domain inhibitors have been gradually carried out. Clinical trials have achieved preliminary results, with many patients benefiting from

prolonged survival and varying degrees of remission[33]. Recently, a bivalent inhibitor of BET protein BRD4 (AZD5153) was reported to be more effective than a monovalent inhibitor of BET protein[34].

The prognosis of NC was not satisfactory. Bauer et al. concluded that the survival time was only 6.7 months by summarizing the prognostic data of 54 cases of NC patients[3]. Some scholars believe that the prognosis of patients with translocation of *NUT* and other genes is better than that of patients with *BRD4-NUT* gene rearrangement[7]. The recent survival analysis of 124 cases of NC patients showed that the site of disease and the type of gene fusion had a great relationship with the prognosis of patients. Among them, tumors arising in the chest had the worst prognostic outcome, with a survival time of 4.4 months. Patients with BRD3-NUT fusion and NSD3-NUT fusion had a longer survival time than those with BRD4-NUT fusion, with a median time of 36.5 months for the former and 10 months for the latter[12].

The details about the clinical characteristics, IHC, and molecular testing of the nineteen primary sinonasal tract NC patients of clinical data more comprehensive are summarized in Table 1 and Table 2. The median age was 42 years old, with the youngest patient being 10 years old and the oldest patient 60 years old (our reported case). Male to female ratio of 1.38:1, a slight male predominance. Five patients accepted chemotherapy and radiotherapy separately, three patients accepted chemoradiotherapy after surgery. The median survival time was 6 months (range 1 – 16). IHC staining was observed for more than 50% expression NUT (89.4%), AE1/AE3(63.1%), P63(57.9%), approximately one-third expressed P40 and P16, and only two cases were expressed synaptophysin, including our reported case. *BRD4-NUT* fusion was rearrangement was present in six patients and *NUT* rearrangement was present in one patient.

Conclusion

We report a case of NC, which is unique in age, morphology, and immunohistochemical staining. This is the first case of sinonasal tract NC to show focal or only spotty positive for P40, synaptophysin, CK5/6, and AE1/AE3 is the oldest and alive patient with unique morphological features among previous study cases. The main objective is to expand the understanding of NC, and strongly recommended that the possibility of NC should be considered in small round cells or squamous differentiation tumors arising in the head and neck regardless of patient age, clinical presentation, and disease location or whether there is an expression of P63. It was confirmed again that immunohistochemical NUT antibodies greatly promoted the diagnosis of NC.

Abbreviations

NC: NUT carcinoma; IHC: immunohistochemistry; FISH: fluorescence in situ hybridization; BET: bromodomain and extra terminal; M: male; F: female; Y: yes; N: no; NA: not available; C: chemotherapy; CR: chemoradiotherapy; R: radiotherapy

Declarations

Authors' contributions

Xuejing Wei performed the manuscript writing and the literature collecting; Cheng -Ming collected the data; Xiaojing Teng and Yanning Zhang did the analysis and interpretation of data; Guangyong Chen supervised all studies and editing of the manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article. Declarations Ethics approval and consent to participate

Not applicable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests.

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Consent

The patient has signed informed consent

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Figures

Figure 1

Magnetic resonance imaging: showed irregular heterogeneously enhancing soft tissue lesion (**A** T2 imaging, coronal plane; **B** T1 imaging, coronal plane)

Figure 2

Morphological features of NC: A fusiform and irregular cells with sparse cytoplasm and densely stained nuclei (H&E stain, 200 × approximately). B Sudden appearance of keratosis (H&E stain, 400 × approximately). C. D High-power reveals medium and large tumor cells with sparse cytoplasm, round or oval nuclei with obvious nucleoli(C), some cells with eosinophilic cytoplasm, like plasma cells (D) (H&E stain, 400 × approximately).

Figure 3

Immunohistochemical stainings of the NC: Few tumor cells have a spotty expression of CK5/6 (A) and cytokeratin AE1/AE3 (B) (magnification $\times 200$). P40 (C) and synaptophysin (D) focal positive (magnification $\times 200$).

Figure 4

Immunohistochemical stainings of the NC: **A** P63 diffuse positive (magnification $\times 200$). **B** NUT nuclear immunostaining positive (magnification $\times 400$).

Figure 5

FISH results showed typical *BRD4-NUT* translocation: The red signal represents *BRD4* and the green signal represents *NUT*, yellow signal indicate fusions.