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Title

Thoracic NUT carcinoma: common pathological features despite diversity of clinical presentations

Author list

Amal FEKKAR¹, Camille EMPROU^{1,2}, Christine LEFEBVRE³, Gilbert FERRETTI^{4,5}, Olivier STEPHANOV¹, Daniel PISSALOUX⁶, Anne McLEER^{1,2}, Anne-Claire TOFFART^{2,7}, Sophie ROUSSEAUX², Saadi KHOCHBIN², Sylvie LANTUEJOUL^{5,6}, Séverine VALMARY-DEGANO^{1,2}

- (1) CHU Grenoble-Alpes, Department of pathology, 38043 Grenoble, France
- (2) Institute for Advanced Biosciences, CNRS UMR5309, Inserm U1209, Grenoble Alpes University, F-38000, Grenoble, France
- (3) CHU Grenoble-Alpes, Laboratory of hematology,38043 Grenoble, France
- (4) CHU Grenoble-Alpes, Department of radiology, 38043 Grenoble, France
- (5) University of Grenoble Alpes, F-38000, Grenoble, France
- (6) Centre Léon Bérard and CRCL, Department of biopathology, 28 rue Laënnec, 69373 Lyon, France
- (7) CHU Grenoble-Alpes, Department of Pneumology and Physiology, 38043 Grenoble, France

ABSTRACT

NUT carcinoma (NC), formerly known as NUT midline carcinoma, is a rare and very aggressive cancer. It is genetically defined by the presence of acquired chromosomal rearrangement of the *NUTM1* (NUclear protein in Testis Midline carcinoma family member 1) gene at chromosome 15q14 with a member of the *bromodomain-containing protein (BRD)* family gene, usually *BRD4*. Although primarily reported in the head and neck, and mediastinum locations of younger individuals, it is now established that NC arises in multiple sites in patients of all ages, with no gender predilection. NC is very likely to be underdiagnosed because of a lack of awareness of both clinicians and pathologists on the one hand, and of a nonspecific histological presentation on the other hand. As it is indistinguishable from other poorly differentiated carcinomas, pathologists should consider NC as a differential diagnosis of any poorly differentiated tumour. Diagnosis is now easily made by immunohistochemistry, using a highly sensitive and specific NUT monoclonal antibody. Despite chemo- or chemo-radiotherapy, the prognosis of this tumour remains very poor. We report here a series of 3 cases of NC with different clinical and pathological presentations in order to draw attention on some common morphological features that can help clinicians and pathologists to think about this rare entity.

Keywords

NUT, carcinoma, BRD, thorax

INTRODUCTION

NUT carcinoma (NC), formerly known as NUT midline carcinoma, is an aggressive, poorly differentiated subtype of carcinoma [1] defined by the presence of acquired chromosomal rearrangement of the *NUTM1* gene (NUclear protein in Testis Midline carcinoma family member 1) localized on chromosome 15q14, with other genes including *BRD4* and, to a lesser extent, *BRD3* [2]. NC is underdiagnosed, mainly because of a nonspecific morphology, and its true incidence is therefore unknown [3]. NC was first described in the 1990s and has been reported primarily in the head and neck, and mediastinum of young individuals [4]. Nevertheless, it is now established that NC arises in multiple sites and affects patients of all ages, with no gender predilection [5]. NC is usually locally invasive and widely metastatic at diagnosis [6]. Despite therapy, median survival time is as low as 6.7 months and the estimated 2-year progression free survival is only of 9% [6]. As NC resembles other poorly differentiated carcinomas, this diagnosis should be considered in case of poorly differentiated tumour [1] and verified by immunohistochemistry using a highly sensitive and specific NUT monoclonal antibody, which typically shows a speckled nuclear staining. Demonstration of a specific chromosomal rearrangement by molecular analyses such as RT-PCR or FISH [4] is no longer mandatory, even though FISH is commonly used to confirm the *NUMT1* rearrangement. With the aim of drawing attention to the common morphological features that can help pathologists to consider this rare diagnosis, we report 3 cases thoracic NC with diverse clinical presentations.

Case report 1

A 29-year-old female with no past history was admitted in 2010 for dyspnoea, haemoptysis and left supraclavicular pain. Thoracic, abdominal and pelvic computed tomography (CT) showed left mediastinal, left pulmonary and latero-uterine tumours with pleural and peritoneal

effusions. Biopsy of the latero-uterine mass revealed an undifferentiated homogeneous proliferation, made of small-to-medium round malignant cells arranged in diffuse sheets. Nuclei harbour a vesicular chromatin and prominent nucleoli. A high mitotic rate was noted as well as many neutrophils within the stroma. At immunohistochemical analysis, tumour cells stained positively for CD45, CD43 and CD138 and were negative for epithelial markers, suggesting a haematological proliferation. The patient underwent chemotherapy with cytarabine and mitoxantrone. In the absence of any response to treatment, radical resection of the latero-uterine tumour was performed, whose analysis confirmed initial findings (Figure 1). Tumour karyotype showed a t(15;19) translocation. Diagnosis of NC was therefore considered, and confirmed by immunohistochemistry with the NUT monoclonal antibody and by RT-PCR revealing a *BRD4-NUTM1* gene fusion. Despite chemotherapy with cisplatin and etoposide, the patient died within 2 weeks.

Case report 2

In 2018, a 19-year-old female presented with a 3 months history of neck pain with anorexia and weight loss. Contrast-enhanced CT showed a pathological fracture of C4 associated with a large pulmonary mass within the right lower lobe, extending to the inferior middle mediastinum (Figure 2) and many other lytic bone lesions. Microscopic examination of a biopsy performed on the left iliac wing showed an undifferentiated malignant tumour infiltrated by many neutrophils. The proliferation was composed of small to intermediate-sized cells with a monomorphic appearance and prominent nucleoli. Necrosis and high mitotic index were observed. Immunohistochemistry showed expression of cytokeratins, synaptophysin and epithelial membrane antigen by the tumour cells. In the absence of any clear diagnosis, NUT immunohistochemistry was performed, demonstrating a strong, speckled nuclear staining in >80% of tumour cells. FISH showed a rearrangement of the *NUTM1* gene in 80% of tumour

cells. Despite chemotherapy (cisplatin and etoposide) and antalgic radiotherapy, the patient died less than 2 months after diagnosis.

Case report 3

A 51-year-old non-smoking male with no remarkable medical history presented in 2019 with a lung mass in the right lower lobe. Histological analysis showed a proliferation of poorly differentiated small to intermediate tumour cells. Many neutrophils infiltrated the tumour (Figure 3). Immunohistochemistry was positive for p40 and negative for TTF1, CD56, chromogranin and synaptophysin. PD-L1 showed a weak membranous positivity in 5% of tumour cells. ROS1 and ALK were negative. Neutrophilic infiltration of this poorly differentiated tumour was suggestive of the diagnosis of NUT carcinoma. Diagnosis was confirmed by immunohistochemistry and FISH. Despite chemotherapy with cisplatin and navelbine, the patient developed brain metastases and passed away 5 months after diagnosis.

DISCUSSION

Diagnosis of NUT carcinoma (NC) is difficult because this tumour lacks specific features in terms of risk factors or clinicopathological presentation. This case series suggests that in the presence of a poorly differentiated thoracic tumour, NC should be considered, especially when neutrophils infiltrate the tumour.

NC is a tumour reported to arise in various sites, with a predilection for the head and neck and mediastinum [7]. NC corresponds to up to 18% of all poorly differentiated carcinomas of the upper aerodigestive tract, particularly the sinonasal region [1]. Neither tobacco smoking nor viruses play a pathogenic role [1]. NC morphology is that of a poorly differentiated carcinoma, with a distinctly monomorphic, clonal appearance, displaying a focal squamous differentiation with abrupt keratinization in 33-40% of cases [1]. Tumour cells demonstrate frequent mitoses

and single cell or geographic necrosis. The histologic features of NC are characteristic by the monomorphism of the cells, separation artefacts with a fried egg appearance with lack of nuclear moulding, the abrupt squamous differentiation and, notably, the presence of infiltrating neutrophils [1]. In our 3 cases, the neutrophilic infiltrate was prominent and represents an important feature pointing towards this diagnosis. NC expresses with few exceptions cytokeratins and is usually diffusely positive for p63 antibody. Although rare, neuroendocrine markers and even TTF1 may be found positive and should not be used to rule out the diagnosis of NC and to favour the diagnosis of SCLC [1]. NC shows a positive immunostaining with the anti-BRG1 antibody, which makes it possible to exclude a deficient SMARCA4 / BRG1 tumour which is part of a novel group of undifferentiated tumours described in the latest WHO classification [8]. In 2009, the C52B1 monoclonal antibody raised against the NUT protein was developed [1]. This antibody is highly specific, and does not stain other human tumours than NC [2]. To be regarded as positive, NUT immunostaining must harbour a typical nuclear punctate pattern in more than 50% of tumour cells [9]. Using FISH as a “gold standard”, immunohistochemistry has a sensitivity of 87%, a specificity of 100%, a negative predictive value of 99% and a positive predictive value of 100% [3]. FISH allows the detection of *NUTM1* rearrangements but is unable to identify the fusion partner of *NUTM1* [3]. For *NUMT1* fusion detection by RT-PCR, specific probes have to be used. Most of the time, only *BRD3*- or *BRD4*-*NUT* fusions are detected by RT-PCR [3]. RNA sequencing techniques allowing *NUTM1* rearrangement detection without the knowledge of the partners could be interesting to discovery of new or unusual fusions. Such an identification is relevant as a recent study indicates that *BRD3-NUT* variants could be associated with a better survival than *BRD4-NUT* [5]. To date, chemotherapy does not improve outcome of patients with NC [6]. However, a precise diagnosis remains critical since experimental studies have revealed the molecular basis of the oncogenesis induced by the BRD4-NUT fusion protein and identified new potential specific therapeutic

strategies. NUT is a testis-specific factor and the recent generation of a mouse model where this gene is invalidated has shown that NUT recruits p300 to induce a genome-wide histone hyperacetylation which occurs in late differentiating male germ cells before histone-to-protamine exchange [10]. In the case of BRD4-NUT fusion protein, NUT keeps its ability to recruit p300, leading to the creation of hyperacetylated chromatin domains (BRD4-NUT foci) [11]. Cellular CBP/p300 are sequestered in these foci, making them unavailable for the various cell signalling involving these enzymes such as p53 acetylation in response to genotoxic assaults [11]. Therefore, any therapeutic approach leading to the dispersion of these BRD4-NUT foci could be considered as a valid therapeutic solution. With this regard, small molecules directly targeting the BDR4 portion of BRD4-NUTM1, called bromodomain inhibitors, drive terminal differentiation of NC cells in vitro and could thus inhibit *BRD-NUTM1* oncogene expression [1,7]. Interestingly, among four patients with *BRD4-NUTM1* fusions treated with the OTX015/MK-8628 bromodomain inhibitor, two of them had a rapid response with tumour regression and symptomatic relief, and one had relative tumour stabilization [1]. In addition, a small molecule inhibitor of CBP/p300 has been newly developed, which leads to a clear dispersion of the BRD4-NUTM1 and p300 foci [11]. Based on the fact that the expression of *BRD4-NUTM1* is associated with globally decreased histone acetylation, histone deacetylase inhibitors (HDACi) could also represent promising targeted therapeutic agents by restoring chromatin acetylation and hence dispersing the oncogenic BRD4-NUT foci [1]. These promising treatments reinforce the importance to identify NC among other undifferentiated neoplasms especially thoracic SMARCA4-deficient undifferentiated tumours, which are poorly sensitive to cytotoxic chemotherapy regimen and which unfortunately do not have a targeted therapy [8].

In conclusion, our observation suggests to consider the diagnosis of NUT carcinoma in patients presenting with a poorly differentiated chest malignant tumour, especially in the presence of

neutrophilic infiltration. The availability of a highly specific monoclonal antibody makes the diagnosis easy to perform by immunohistochemistry. FISH remains the “gold standard” for diagnosis, but does not allow the identification of the fusion partner. RNA based assays such as RT-PCR or RNA sequencing can allow the detection of the fusions transcripts provided they suitably designed. Prognosis remains extremely poor, but targeted therapies seem promising in patients harbouring *BRD4-NUTM1* variants.

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Legends of figures.

Figure 1. (A) The right radical adnexectomy leads to a tumour resection showing a diffuse growth of poorly cohesive intermediate to large sized tumour cells with irregular vesicular nuclei and prominent nucleolus. Many neutrophils infiltrate the tumour. (B) NUT monoclonal antibody showing a speckled nuclear immunohistochemical staining. (C) R banded karyotype after 24h unstimulated culture performed on fresh ovarian tissue detected the specific translocation t(15;19)(q14;p13), associated with a translocation t(2;12)(p23;p13) and other structural abnormalities (16q, 20p). Red arrows: specific translocation t(15;19), small black arrows: associated cytogenetic abnormalities.

Figure 2. (A) Axial contrast-enhanced CT images: A1- large heterogeneous soft tissue mass in the right lower lobe extending in the inferior middle mediastinum (arrow). A2- Infiltrative poorly circumscribed mass in left retropharyngeal space (arrows) associated with osteolysis of the corpus of the 5th cervical vertebrae (arrow head) and extension within the cervical canal (curved arrow). (B) Morphological aspects (Hematoxylin-Eosin-Saffron stain): nested cohesive basophilic tumour cells with oval, hyperchromatic nuclei, a prominent nucleolus and small amounts of cytoplasm, with many neutrophils infiltrating the tumour. A giant osteoclastic cell (arrow). Original magnification: x200 (B1), x400 (B2). (C) Immunohistochemistry: C1- cytoplasmic expression of pancytokeratin AE1/AE3 antibody, x200. C2- cytoplasmic expression of synaptophysin, x200. C3- NUT monoclonal antibody showing a speckled nuclear immunohistochemical staining, x400.

Figure 3. A- Morphological aspects (Hematoxylin-Eosin-Saffron stain): diffuse growth of anisokaryotic cells with round nuclei, prominent nucleoli and scanty, clarified cytoplasm. Many

205 neutrophils infiltrate the tumour. x400. B- Immunohistochemistry: NUT monoclonal antibody
206 showing a speckled nuclear staining (immunoperoxidase, x400). C- Break apart Fluorescent In
207 Situ Hybridization (FISH) showing a NUTM1 gene rearrangement.
208

REFERENCES

- [1] C.A. French, NUT Carcinoma: Clinicopathologic features, pathogenesis, and treatment: NUT Carcinoma, *Pathol Int.* 68 (2018) 583–595. <https://doi.org/10.1111/pin.12727>.
- [2] H. Haack, L.A. Johnson, C.J. Fry, K. Crosby, R.D. Polakiewicz, E.B. Stelow, S.-M. Hong, B.E. Schwartz, M.J. Cameron, M.A. Rubin, M.C. Chang, J.C. Aster, C.A. French, Diagnosis of NUT Midline Carcinoma Using a NUT-specific Monoclonal Antibody:, *The American Journal of Surgical Pathology.* 33 (2009) 984–991. <https://doi.org/10.1097/PAS.0b013e318198d666>.
- [3] C.A. French, NUT midline carcinoma, *Cancer Genetics and Cytogenetics.* 203 (2010) 16–20. <https://doi.org/10.1016/j.cancergencyto.2010.06.007>.
- [4] A.G. Evans, C.A. French, M.J. Cameron, C.D.M. Fletcher, D.M. Jackman, C.S. Lathan, L.M. Sholl, Pathologic Characteristics of NUT Midline Carcinoma Arising in the Mediastinum:, *The American Journal of Surgical Pathology.* 36 (2012) 1222–1227. <https://doi.org/10.1097/PAS.0b013e318258f03b>.
- [5] N.G. Chau, C. Ma, K. Danga, H. Al-Sayegh, V. Nardi, R. Barrette, C.S. Lathan, S.G. DuBois, R.I. Haddad, G.I. Shapiro, S.E. Sallan, A. Dhar, J.J. Nelson, C.A. French, An Anatomical Site and Genetic-Based Prognostic Model for Patients With Nuclear Protein in Testis (NUT) Midline Carcinoma: Analysis of 124 Patients, *JNCI Cancer Spectrum.* 4 (2020) pkz094. <https://doi.org/10.1093/jncics/pkz094>.
- [6] D.E. Bauer, C.M. Mitchell, K.M. Strait, C.S. Lathan, E.B. Stelow, S.C. Lüer, S. Muhammed, A.G. Evans, L.M. Sholl, J. Rosai, E. Giraldi, R.P. Oakley, C. Rodriguez-Galindo, W.B. London, S.E. Sallan, J.E. Bradner, C.A. French, Clinicopathologic Features and Long-term Outcomes of NUT Midline Carcinoma, *Clin Cancer Res.* 18 (2012) 5773–5779. <https://doi.org/10.1158/1078-0432.CCR-12-1153>.

- 233 [7] L.M. Sholl, M. Nishino, S. Pokharel, M. Mino-Kenudson, C.A. French, P.A. Janne, C.
234 Lathan, Primary Pulmonary NUT Midline Carcinoma: Clinical, Radiographic, and Pathologic
235 Characterizations, *Journal of Thoracic Oncology*. 10 (2015) 951–959.
236 <https://doi.org/10.1097/JTO.0000000000000545>.
- 237 [8] D. Lokuhetty, V.A. White, I.A. Cree, Thoracic Tumors, WHO Classification of
238 Tumours, 5th Edition, Volume 5, IARC Lyon, 2021.
- 239 [9] J.A. Bishop, C.A. French, S.Z. Ali, Cytopathologic features of NUT midline carcinoma:
240 A series of 26 specimens from 13 patients: Cytopathology of NUT Midline Carcinoma, *Cancer*
241 *Cytopathology*. 124 (2016) 901–908. <https://doi.org/10.1002/cncy.21761>.
- 242 [10] H. Shiota, S. Barral, T. Buchou, M. Tan, Y. Couté, G. Charbonnier, N. Reynoird, F.
243 Boussouar, M. Gérard, M. Zhu, L. Bargier, D. Puthier, F. Chuffart, E. Bourova-Flin, S. Picaud,
244 P. Filippakopoulos, A. Goudarzi, Z. Ibrahim, D. Panne, S. Rousseaux, Y. Zhao, S. Khochbin,
245 Nut Directs p300-Dependent, Genome-Wide H4 Hyperacetylation in Male Germ Cells, *Cell*
246 *Reports*. 24 (2018) 3477–3487.e6. <https://doi.org/10.1016/j.celrep.2018.08.069>.
- 247 [11] N. Reynoird, B.E. Schwartz, M. Delvecchio, K. Sadoul, D. Meyers, C. Mukherjee, C.
248 Caron, H. Kimura, S. Rousseaux, P.A. Cole, D. Panne, C.A. French, S. Khochbin, Oncogenesis
249 by sequestration of CBP/p300 in transcriptionally inactive hyperacetylated chromatin domains,
250 *EMBO J*. 29 (2010) 2943–2952. <https://doi.org/10.1038/emboj.2010.176>.

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