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# **Title of the article: Exacerbation of skin lesions after local steroid therapy - tinea incognita**

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**A short running title:** Skin lesions after local steroid therapy

**Key words:** Tinea; Trichophyton; hydroxycorticosteroids

## **Exacerbation of skin lesions after local steroid therapy - tinea incognita**

### **Abstract**

Tinea incognita is a dermatophytic infection which has lost its typical clinical appearance because of improper use of steroids. We report a case of tinea incognita caused by *Trichophyton rubrum* under topical corticosteroid therapy. The skin lesion were on the abdomen and thighs. Use of corticosteroids was ceased and oral itraconazole and terbinafinum cream therapy was initiated to continue for four weeks. Following treatment, total clinical and mycological cure was established.

It was concluded that tinea incognito which was not a rare clinical entity, could be presented in various clinical forms and usually resulted from the wrong treatment modalities. Atypical erythematous plaques should be investigated in terms of presence of fungi and treated accordingly to establish total clinical and mycological cure.

**Key words:** Tinea; Trichophyton; hydroxycorticosteroids

## **Introduction**

Tinea incognito (TI) has first been described in 1968. TI is a dermatophytic infection in which topical or systemic steroids have modified the clinical appearance of the mycosis and mimicking other skin diseases <sup>(1)</sup>.

TI may mimic a number of other dermatological conditions, including lupus erythematosus, contact dermatitis, neurodermatitis, atopic dermatitis, rosacea, seborrheic dermatitis, psoriasis, and eczema, but also erythema migrans and the diagnosis is frequently missed or delayed <sup>(2)</sup>.

According to the original description of Ive & Marks, the scaly appearance may be retained and bizarrely altered by local steroids. Large extension of the lesions can occur.

We report a case of pustular dermatosis with an annular rash-tinea incognito caused by *Trichophyton rubrum* under topical corticosteroid therapy.

## **Case Report**

A 24-years-old woman suffered from an itchy skin lesion on her abdomen and thighs.

Physical examination revealed the multiple, concentric erythematous rings, pale center with non characteristic scaling with squamous and pustules of (different sizes) on abdomen and thighs area (Figures 1, 2). The patient had used two high-potency topical steroids suggested by dermatologists for 2 months. Direct microscopic examination of the skin scrapings with 10% potassium hydroxide preparation revealed fungal elements and *Trichophyton rubrum* was isolated in culture. Use of corticosteroids was ceased and itraconazole (200 mg per day orally) and terbinafinum cream therapy was initiated to continue for four weeks. Following treatment, total clinical and mycological cure was established (Figure 3). There has been no recurrence after 8 months of follow-up.



## Discussion

The incidence of tinea incognito (TI) appears to have increased over recent years<sup>(3,4)</sup>; easier access to topical steroids by patients and hasty use of steroids by physicians (e.g. without confirmation of mycological examination - «uncertain» skin lesions).

Tinea incognito mimics various other dermatological conditions<sup>(2,5)</sup>.

In Korean study the most common clinical manifestations were eczema-like lesion, psoriasis-like, and lupus erythematosus-like lesion<sup>(2)</sup>. Besides more than half (59.3%) patients were previously treated by non-dermatologists or self-treated.

In some cases, tinea incognito may retain the scaly appearance of tinea but be modified by local steroids so that it becomes extensive and bizarrely shaped as happened in our patient<sup>(5,6)</sup>.

The study by Ansar A et al the authors demonstrated that the most common type of infection was tinea corporis (32.1%), the prevalence of the other tinea in decreasing order was as follows: tinea faciei (26.8%), tinea cruris (14.3%), tinea manuum (12.5%), tinea pedis (8.9%), and tinea capitis (5.4%)<sup>(7)</sup>.

In different studies and cases were isolated different pathogens of skin lesions in the course of TI (*Trichophyton verrucosum*, *T. mentagrophytes*, *T. rubrum*, *Epidermophyton floccosum*, *Microsporum canis*, *T. violaceum*, *T. schoenleinii*, *T. erinacei*).

Among isolated strains, *Trichophyton rubrum* was the most frequently detected<sup>(2)</sup>.

*Trichophyton rubrum* is an anthropophilic dermatophyte that is distributed worldwide. Infections with this fungus are mostly confined to the keratinized epithelial layer of the skin and are characterized by superficial, scaly eruptions. However, if systemic or local immunity is compromised, inflammatory lesions may develop as happened in our patient.

Topical corticosteroids have provided a significant advance in the treatment of many dermatologic diseases since their development in the 1950s. These benefits are largely a result of the drugs' anti-inflammatory and antimetabolic actions. However, the advent of higher-potency formulations has also increased the likelihood of iatrogenic side effects<sup>(8,9)</sup>.

Administration of potent topical steroids before microbiological diagnosis is one of the biggest problems in the management of fungal infections. It has been demonstrated that potent topical steroids can increase the numbers of hyphae present on the surface of the skin in fungal infections and change the appearance of the lesions<sup>(10)</sup>.

Once recognized, tinea incognito usually requires systemic treatment with oral antifungal agents. In our case therapy with oral itraconazole and topical terbinafinum for 4 weeks was successful in clearing the fungal infection.

## Conclusion

It was concluded that tinea incognito which was not a rare clinical entity, could be presented in various clinical forms and usually resulted from the wrong treatment modalities. Thus atypical erythematous plaques should be investigated in terms of presence of fungi and treated accordingly to establish total clinical and mycological cure.

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Figure 1



Figure 2



Figure 3

**Legend of Figures:**

- Figure 1: Multiple, concentric erythematous rings, pale center with non characteristic scaling with squamous and pustules of (different sizes) on abdomen area;
- Figure 2: Concentric erythematous rings, pale center with scaling and squamous and pustules of (different sizes) thighs area;
- Figure 3: 4 weeks after treatment of Itraconazole (200 mg p.o) and topical terbinafinum.

# Unusual evolution of an anaplastic meningioma.

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## Introduction:

Meningiomas comprise 13 to 19 % of primary intracranial neoplasms <sup>(1,2,3,4)</sup> and their incidence is second only to gliomas. They are generally benign; malignant subtypes, although long recognized <sup>(5)</sup> are not always easy to define. Anaplastic meningioma constitute 1.0 % to 2.8 %<sup>(6,7)</sup>. Atypical and anaplastic meningioma seems to be more prevalent in men<sup>(8)</sup>. Despite their malignancy, extracranial metastases of malignant meningiomas are rare with an estimated incidence of 1–5 out of 1000 cases.<sup>(9)</sup>

Only 7 % involve vertebrae and just a few cases have been published.<sup>(10)</sup>

Hereby, we report a male patient with an anaplastic meningioma which was diagnosed in 2010 operated several times, with a metastasis in the vertebral column, with a gastric adenocarcinoma and peritoneal carcinomatosis.

## Case report:

A 57 years old patient during his holidays in Italy, had sudden strong headache, nausea, vomiting. The patient had a CT scan and a MRI of the brain. The doctors in Italy thought it was a glioblastoma which has bled. The patient was put on dexamethasone. He was transferred to Luxembourg after 2 weeks. He was operated in Luxembourg on 17.9.2010 (figure1). The histopathological result showed he had a malignant anaplastic meningioma (WHO III).

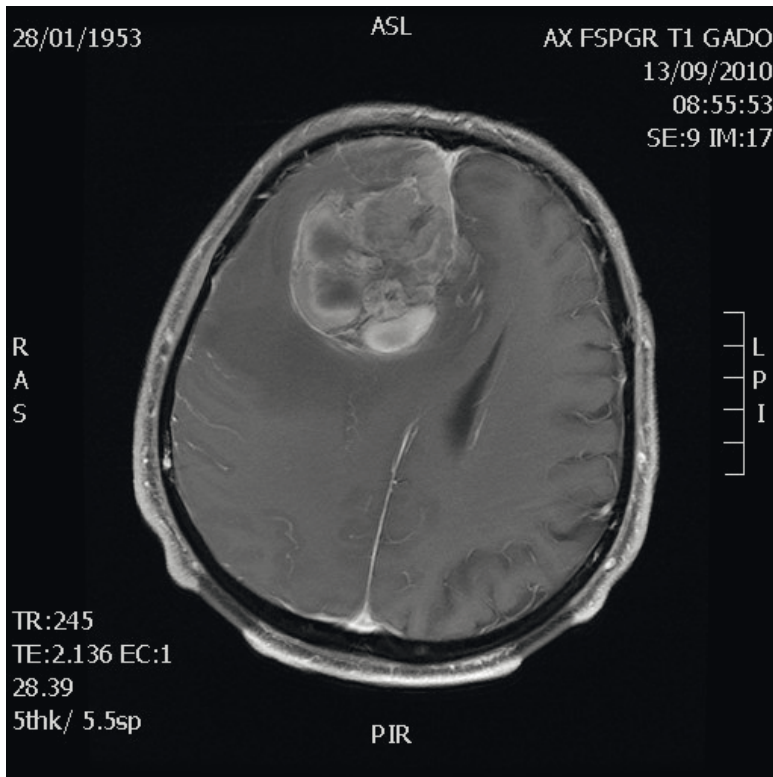


Figure 1: shows: T1 with gadolinium: massive extra axiale lesion taking contrast.

He underwent radiotherapy with 60Gy, he had a massive pulmonary embolism during the radiotherapy treatment. He did his first epileptic seizure in February 2011. On a control of 31 May 2011 the scanner showed a local relapse (figure 2). The patient was operated again on 15.6.2011 and this time the surgery was radical. The patient began a chemotherapy with Hydroxyurea after the second surgery.

He was operated a 3rd time of the brain tumor on 27.6.2012 it was a new location (figure3). The patient was the operated 4th time on 6.3.2013 of brain tumour of a local recidive in the frontal region. He had a grand mal epileptic fit and back pain. He had MRI of the vertebral column he had 2 fractures on D3 and D7. In the CT of the thorax which was being done to control the pulmonary embolism he had before they discovered a Gastric adenocarcinoma on the small curve of the stomach. He had a PET Scan on 3.4.2013 which showed a hypermetabolique tumor in the stomach and 2 suspicious localisations in the dorsal spine.

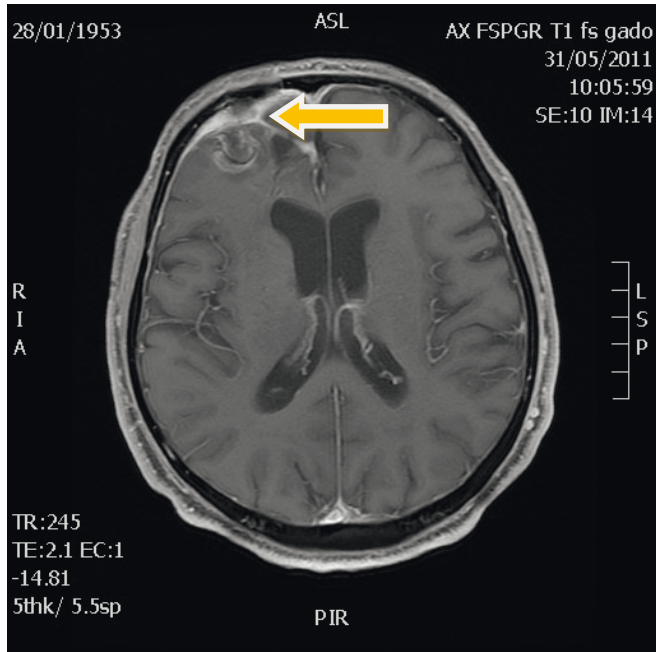


Figure 2: shows: T1 with gadolinium: a recurrence of the tumor from the original site. The yellow arrow shows the site.

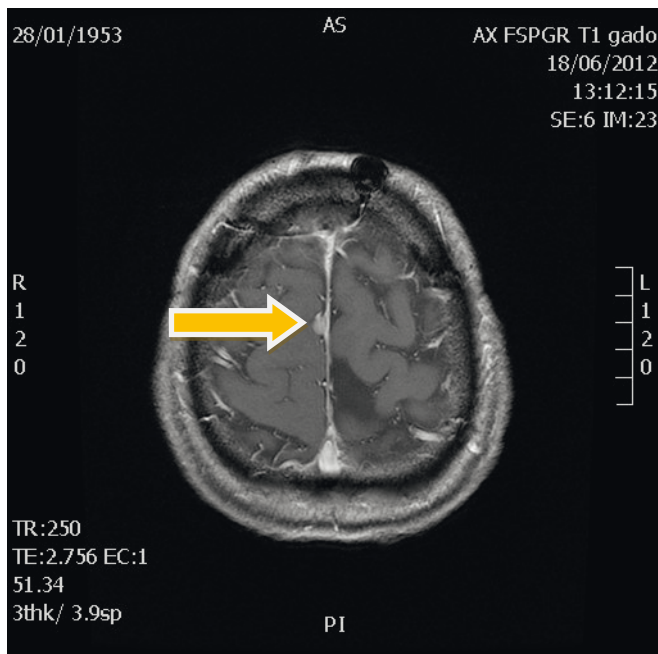


Figure 3: T1 with gadolinium: the yellow arrow shows a new tumor.

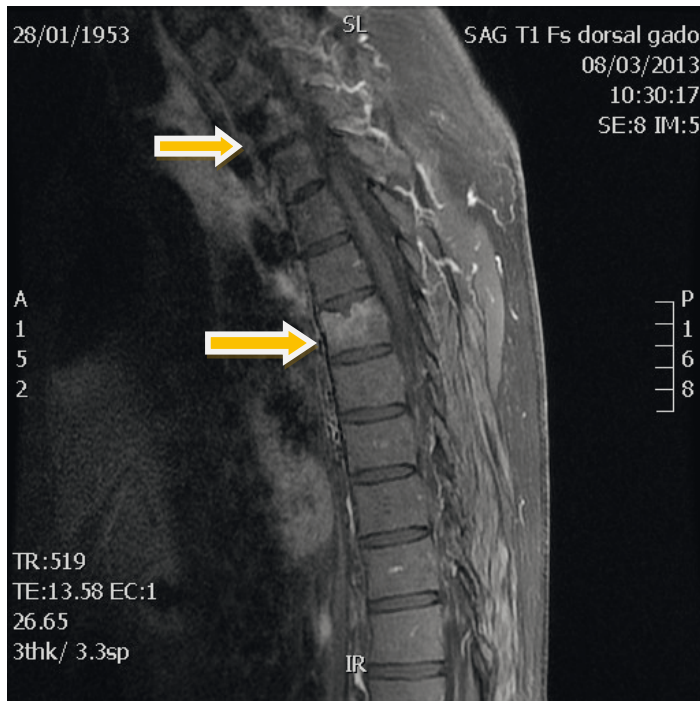
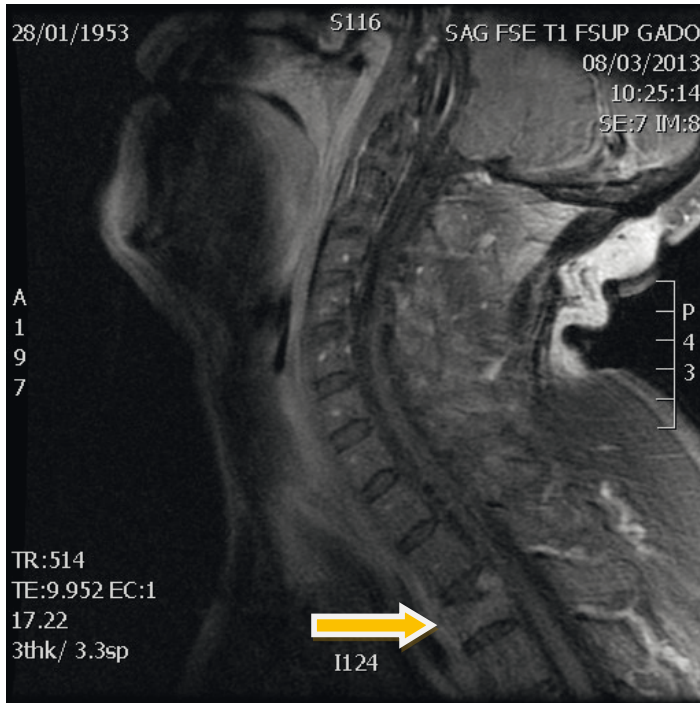


Figure 4 & 5: T1 plus gadolinium: shows tumoral infiltration of D3 and D7.



He had a biopsy of the vertebral body of D3 (figure 4 & 5). The biopsy revealed an atypical meningioma. The D7 biopsy was normal and he had a vertebroplasty. The images were sent to Munich for Cyberknife surgery but one day before the surgery they made a CT – Scan of the dorsal region (D3) and saw the tumour had invaded the local areas around D3 and it was in contact with the oesophagus and spinal cord. They postponed the surgery.

He eventually had a corpectomy of D3 and a ventral stabilisation from D2-D4 with decompression of the oesophagus and spinal cord (figure 6). He was irradiated with 44 Gy postoperatively.

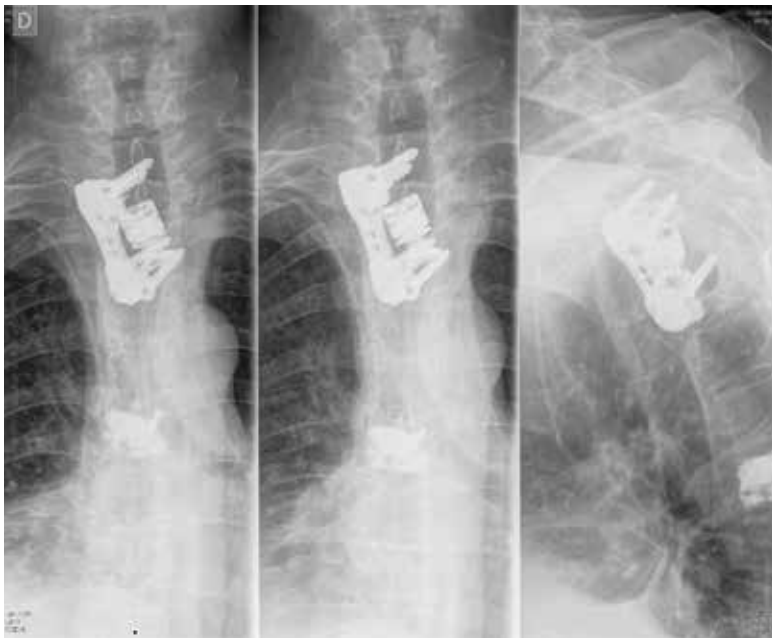


Figure 6: shows postoperative control ventral stabilisation from D2-D4.

For the gastric adenocarcinoma he began a chemotherapy treatment with Docetaxel, Cisplatinium, 5 Fluorouracil. On 21.1.2014 he a gastrectomy (95 % removed) and the general surgeon also discovered a peritoneal carcinomatosis which was done by laparoscopy. The biopsy for the stomach revealed an adenocarcinomatosis mildly differentiated and 5 metastasis in the ganglions (T4, N2, M0).

The patient died in the summer of 2014 on a palliative care unit. After 4 brain surgeries and a spinal cord decompression. There was never any neurological deficiency.

## **Discussion:**

The present case with anaplastic meningioma, metastasis, a gastric adenocarcinoma and peritoneal carcinomatosis is remarkable for several reasons.

Metastases are rare even for an anaplastic meningiomas with vertebral metastasis, gastric adenocarcinoma and peritoneal carcinomatosis. To our knowledge no such case has been published.

The lungs are the most common site for seeding, but lesions have been seen in the liver, bone, skin and subcutaneous tissue <sup>(11, 12)</sup>.

The tumor are commonly reported in elderly patients with a peak incidence in the seventh decade of life. In a study done on 936 patients that had meningioma 12 % of the men versus 4 % of the women had anaplastic meningioma <sup>(13)</sup>. In Children 7-16 % of meningiomas are malignant and the prevalence of malignancy decreases with age.<sup>(14)</sup>

Atypical and malignant meningiomas differ from benign lesions according to several histological features such as increased mitotic activity, increased cell density, presence of nuclear pleomorphism, loss of architectural cell disposition, existence of tumoral necrosis and brain invasion, papillary transformation and carcinoma, melanoma.

Imagistically making differential diagnosis can be very difficult. Several studies have shown that distinction between benign and anaplastic or atypical meningioma is not particularly reliable with magnetic resonance imaging (MRI) either<sup>(15,16)</sup>.

Magnetic resonance spectroscopy is another tool we can use. A number of studies have shown that alanine, glutamine/glutamate and choline levels are elevated in meningiomas<sup>(17,18)</sup>. Shino et al<sup>(19)</sup> suggested that higher grade meningiomas have increased lactate and choline/creatine ratios and can also have a methylene signal. We did not do the magnetic resonance spectroscopy.

The chemotherapy treatment with Hydroxyurea is debatable because Mason et al <sup>(20)</sup> used the drug in a group of patients who had one anaplastic, 3 atypical and 16 benign meningiomas. All tumors were recurrent or unresectable. The authors reported no change in 12 patients`tumors and clinical improvement in 2. The anaplastic tumor progressed. Hydroxyurea may work on benign meningiomas whereas on anaplastic meningioma may not.

The latency period for the vertebral metastasis in our case is 2 years . In the literature the latency period between diagnosis of intracranial tumor and appearance of the metastasis ranges from a few months to >20 years <sup>(21)</sup>.

Currently, to our knowledge, no consensus guidelines exist for the treatment of metastasizing meningiomas<sup>(22)</sup>.

The treatment options available for metastatic disease of the spine include medical therapy, surgery and radiation. In our case we did a biopsy and decompression and a ventral stabilization even though the patient did not have any neurological deficiency.

Concerning the gastric carcinoma the patient complained about dyspepsia in his past medical history in the literature we can see that 50 % of the patients may have nonspecific gastrointestinal complaints (23). In the patient case lack of early pathognomic symptoms delayed the diagnosis.

To conclude we can say although vertebral metastasis is very low, people known with meningiomas and complaining of back pain should be further investigated.

The patient was known to have dysphagia but he did not lose weight or anorexia. Its difficult to put a diagnosis just with physical examination at the early stages of a gastric carcinoma.

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# Unusual location of neurenteric cyst which bled in patient with multiple sclerosis.

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## Abstract:

Neurenteric cysts are seldom seen intracranially and most of the case report are about the posterior fossa. Approximately 141 cases have been reported so far and Neurenteric cyst is a rare developmental cyst of the CNS (Central Nervous System), such as a Rathke cleft and colloid cyst lined by columnar epithelium of presumed endodermal origin. We report a case of histologically proven case of neurenteric cyst. The cyst had bled then it shrank and it grew again. We should also mention that the patient had multiple sclerosis. It is difficult to make a proper diagnosis just from images. Complete surgical excision remains the gold standard.

**Keywords:** Neurenteric cyst, Multiple sclerosis, bleeding, regrowth, localisation.

## Introduction:

Neurenteric (NE) cysts, also called enterogenous cysts, are rare benign endodermal lesions of the central nervous system (CNS). They are approximately 3 times more common in the spine compared with the brain.<sup>(1)</sup>

### Intracranial

NE cysts are uncommon small cysts often located in the posterior fossa in midline or occasionally in cerebellopontine angle cistern<sup>(2,3)</sup>.

We report here a large extra axial frontal right 42.3 mm X 47.5 mm cyst with mass effect and which has bled, regrown in size and with sedimentation at the bottom of it. We could not find in the literature a similar case report.

## Case Report:

A 47 years old female patient which was known with multiple sclerosis, an extra axial right frontal cyst and diabetes type 1. The cyst is known from the year 2000 and the patient has been suffering from chronic headache from then. The patient did not have any neurological deficiency. She had a general weakness due to the multiple sclerosis in the left leg.

The patient had 3 MRIs which were done in 2000, 2003 and 2014. The MRI which was done in 2000 the neuroradiologist described an arachnoidian cyst in their report (Fig 1).

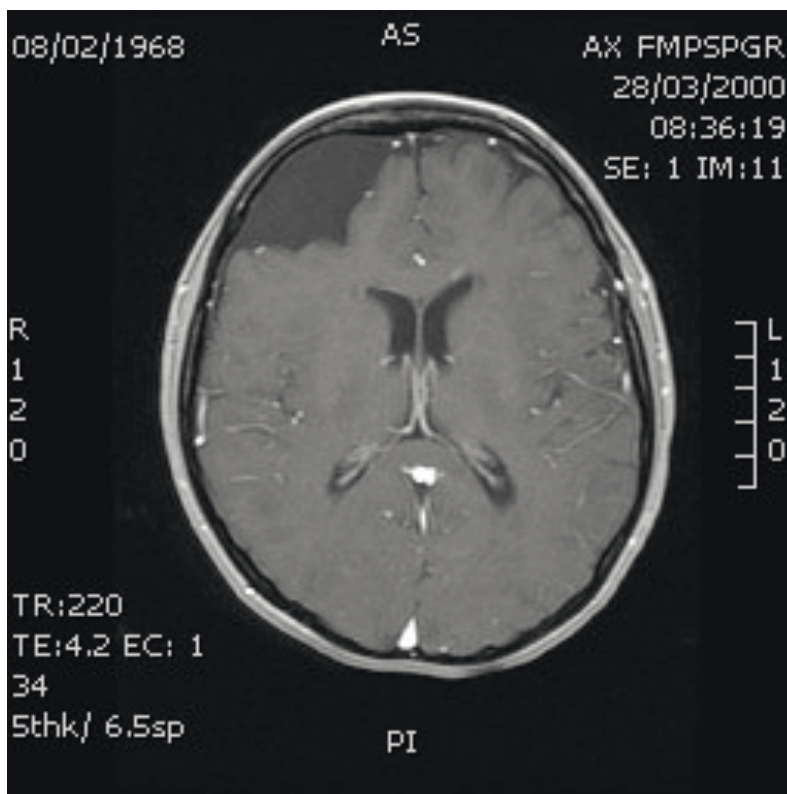


Figure 1: MRI of 03/00-contrast enhanced axial T1 weighted image shows extraparenchymal right mass (arrow) with CSF like signal intensity.

Then, she had a new MRI on 12 September 2003 in this MRI the neuroradiologist describes that the cyst has bled and it became smaller in size (Fig 2).



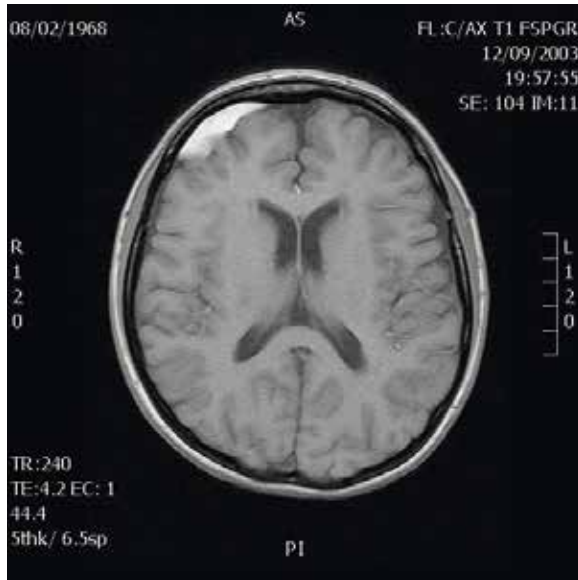


Figure 2: Axial T1 weighted image which shows a high signal intensity compatible with hemorrhage inside the lesion and a decrease in the size of the lesion.

The last MRI done on 8/5/2014 (Fig 3) showed axial T1 weighted images with a heterogenous signal and an expansion of the cyst.



Figure 3: Axial T1 weighted images shows a heterogenous signal of the lesion corresponding to fluid with high protein content and hemorrhagic components in the bottom part.

After talking with the patient, we decided to operate the cyst, with the help of neuronavigation and we used Gliolan®. Our initial suspicion was a low grade astrocytoma or an epidermoid cyst.

Intraoperatively, it looked less like a low grade astrocytoma or epidermoid cyst as suspected at first.

The histopathological results showed from the cytological point of view:

1. No cell material was useful. No tumour cell.

The immunohistochemistry confirmed that it was a neurenteric cyst.

## **Discussion:**

Neurenteric cysts, along with Rathke cleft and colloid cysts, are endodermally derived lesions of the CNS. First described by Puusep in 1934, NE cysts have had several names, including enterogenous cyst, enteric cyst, endodermal cyst, gastroenterogenous cyst, gastrocytoma, intestinoma, and archenteric cyst.<sup>(4-6)</sup>

Though they can occur along the entire neuraxis, they are approximately three times more common in the spine compared to the brain. These lesions are most frequently encountered in the lower cervical and upper thoracic spine.<sup>(7-9)</sup> Intracranial endodermal cysts are even rarer. When this occurs, the cysts are frequently located in the posterior fossa<sup>(10,11)</sup>. This case illustrates a solitary endodermal cyst that occurred as an extra-axial mass localized over the frontal lobe. The supratentorial localization of this lesion illustrates the need for consideration of the pathogenesis of this entity as well as its diagnostic differentiation from other cystic abnormalities in this region. A number of hypotheses have been postulated regarding the pathogenesis of endodermal cysts of the central nervous system. The predominant proposal has been that these cysts arise because of faulty separation of the ectodermally derived spinal cord and the endodermally derived foregut during closure of the neurenteric canal in the third week of embryonic life. These cysts are usually located in the posterior mediastinum but also occur rarely in the central nervous system, of which the spinal canal is the primary location<sup>(12, 13)</sup>.

Our case report is unique because in our knowledge there are no other cases which the neurenteric cyst where the cyst bled and regrew again causing a mass effect. We should also mention that our patient has Multiple Sclerosis. In the literature, there are only 2 cases where the cyst bled spontaneously<sup>(14,15)</sup>.

## **Conclusion:**

Supratentorial neurenteric cysts are rare. Neurenteric cysts should be included in the differential diagnosis when we have a well delimited cyst. The histopathological report was important for a correct diagnosis.

## Conflicts of Interest / Disclosures

The authors declare that they have no financial or other conflicts of interest in relation to this research and its publication.

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# Immune checkpoint inhibitor- related fatal myositis and myocarditis

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Myocarditis and myositis are rare, but now well-recognised irAE (immune related adverse events) of immune checkpoint inhibitors, both in combination and monotherapy.

Since the true incidence and definition of these irAEs is still unclear and data sharing <sup>(1)</sup> can provide insight, we would like to report a fatal, pathologically-proven myositis/myocarditis case after one dose of Pembrolizumab.

## Case report

A 66-year-old non-smoker was diagnosed in 2013 with loco-regionally advanced adeno-carcinoma of lepidic predominant histology of the right lung with right cervical lymph node metastasis. No mutations of EGFR, ERBB2, BRAF, RAS or Alk-rearrangement were detected.

Six cycles of chemotherapy with Cisplatin and Pemetrexed and four cycles of Pemetrexed were administered. Radiation with 66 Gy followed. Radiation-induced lung injury developed in the right apex. Complete response was maintained until the end of 2017, when bilateral micro-nodules developed, and bronchoalveolar lavage confirmed relapse of adenocarcinoma.

The patient had no history of cardiovascular disease, diabetes or hypertension: ECG was normal and he did not take any medication. Immunotherapy with Pembrolizumab was administered at a standard dosage of 2 mg/kg.

Three weeks later he was admitted to hospital because of pain and weakness in his legs and elevated liver tests. He reported one episode of dark urine.

Laboratory tests showed elevated ALT 340 U/l (reference range: <41 U/l ); AST 624 U/l (reference range <38 U/l ); CK 4105 U/l (reference range <190 U/l ); CK-MB 161 ng/ml (reference range < 4,9 ng/ml); Myoglobin 5046 ng/ml (reference range 28-72 ng/ml); Troponin T 1,8 ng/ml (reference range < 0,014 ng/ml); Aldolase 130 U/l, (reference range <7,6 U/l ); anti-acetylcholine receptor and myositis associated antibodies were negative.

He received i.v. methylprednisolone (1mg/kg) with a short-lived clinical improvement. ECG first showed a transient prolongation of QT and later a new left anterior hemiblock. Echocardiogram showed a hypertrophic cardiomyopathy with normal EF and no wall motion abnormalities. Echogram of the liver was normal. Despite improvement of laboratory tests, muscle weakness worsened, and he developed pitting edema. Plasmapheresis was started, during which respiratory arrest occurred. Cardiopulmonary resuscitation was successful, and the patient was transferred to the intensive care unit. The cardiac situation could be stabilized, but subsequently several attempts of extubation failed due to respiratory muscle failure and life support had to be withdrawn.

The pathology report confirmed severe myositis with dissociation and necrosis of muscle fibres in all the examined biopsies with inflammatory infiltration of CD3, CD8 positive T-cells and CD68 positive macrophages. No signs of eosinophilic granuloma, giant cells or vasculitis were seen. Similar changes were found in the myocardial biopsies, but to a lesser extent and in a patchy distribution. No signs of myocardial infarction were noted. Examination of the lungs confirmed bilateral adenocarcinoma, with marked necrosis in some areas.

Myocarditis and myositis are rare, but potentially life-threatening irAE of ICI. Cases of myocarditis are increasingly reported, as published in the Lancet earlier this year: Moslehi et al. identified 101 cases of myocarditis, of which 42% had concomitant myositis or myasthenia gravis <sup>(2)</sup>. Anquetil et al. identified 180 cases of myositis, of which 16% had evidence of myocarditis and 15% of myasthenia gravis <sup>(3)</sup>.

Mohsin et al. reported a case series of 6 patients with ICI-related myositis <sup>(4)</sup>. Touat et al. retrospectively analysed 10 patients with ICI-related myositis and found remarkably homogenous clinicopathological features; five of the patients showed evidence of myocarditis <sup>(5)</sup>.

Our patient's case shares many of the clinical and pathological features of the published case reports and series: no apparent risk factors, early onset, combination of myositis and myocarditis and the muscular and myocardial inflammatory infiltration with CD3/CD8 positive T-cells and CD68 macrophages.

Treatment recommendations include high-dose steroids, plasmapheresis, IVIG, mycophenolate, tacrolimus, Infliximab and Antithymocyte Globulin <sup>(6)</sup>.

Since the use of ICI is increasing and advancing into the adjuvant setting, identification of risk factors, tools for early diagnosis and appropriate treatment of this rare and serious irAE are needed <sup>(1)</sup>.

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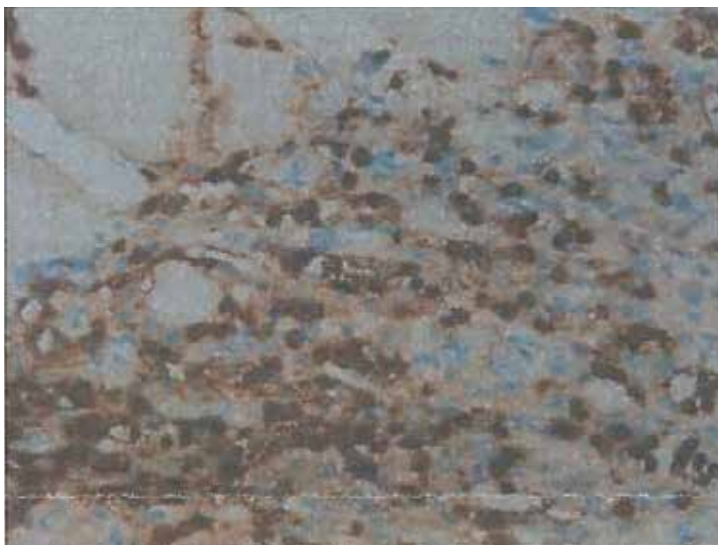


Photo: muscle tissue with diffuse CD3 positive, lymphocyte infiltration





# Une histoire de pièges diagnostiques

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## **Abstract:**

University education gives us the tools to draw up a list of differential diagnoses; practice teaches us to reason quickly and get to the point. This article presents a case of cervical adenocarcinoma whose diagnosis was delayed by a clinical suggestive of a genital infection, proved by the complementary examinations. The admitted objective of this work is to reinforce the idea that one diagnosis may hide another and that it is important to remain vigilant in unusual situations so as not to exclude any serious pathology from our investigations.

L'enseignement universitaire nous donne les outils nécessaires à dresser une liste de diagnostics différentiels ; la pratique nous apprend à raisonner rapidement et à aller à l'essentiel. Cet article présente un cas d'adénocarcinome cervical dont le diagnostic a été retardé par un tableau clinique évocateur d'une infection génitale, avérée par les examens complémentaires. L'objectif avoué de ce travail est de renforcer l'idée qu'un diagnostic peut en cacher un autre et qu'il importe de rester vigilant dans les situations peu communes afin de n'exclure aucune pathologie grave de nos investigations.

**Key words:** delayed diagnosis, cervical adenocarcinoma, atypical genital infection

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## **Introduction :**

Il nous arrive à tous, occasionnellement, de partir sur une mauvaise piste, de prendre la mauvaise direction dans notre démarche médicale, et d'ainsi retarder la prise en charge optimale du patient. Une part de ces diagnostics tardifs est imputable au formatage intellectuel des professionnels de la santé. Malgré un enseignement universitaire encourageant l'exhaustivité en matière de diagnostic différentiel, ainsi que la hiérarchisation des pathologies selon leur fréquence et leur gravité, nous sommes soumis par la suite à un risque d'automatisation et de raccourcis dans nos liens de causalité. Malheureusement, un prurit ne correspond pas systématiquement à une mycose, de même qu'une leucorrhée pathologique n'équivaut pas d'emblée à une infection gynécologique.

## **Présentation du cas**

Madame P, 30 ans, se présente aux urgences pour des douleurs vaginales ainsi que des leucorrhées purulentes malodorantes.

Elle nous expose les antécédents suivants :

- Gynéco-obstétricaux : G6P3 ; contraception par Mithra Sert 380© inséré en 2016 ; frottis cervico-vaginal ne présentant aucune anomalie, réalisé 3 semaines auparavant lors d'un examen gynécologique complet sans particularité.
- Assuétudes : tabagisme 5-10cig/j
- Familiaux : cancer du sein chez la sœur, à l'âge de 28 ans, sœur mutée BRCA 1 mais bilan génétique négatif chez la patiente

Lors de ce premier contact, la patiente décrit une douleur en majoration depuis quelques jours, des saignements importants sans rapport avec les cycles et l'apparition d'un œdème pubien s'étendant à la racine de la cuisse gauche, sans état pyrétique.

L'examen clinique révèle un col érythémateux et confirme les plaintes précédemment citées.

Le stérilet est retiré, une recherche Chlamydia/Gonocoque et un prélèvement bactériologique sont réalisés, ainsi qu'un bilan sanguin, qui se veut rassurant.

La patiente quitte le service avec pour traitement amoxicilline/acide clavulanique 875 + métronidazole et acide tranexamique.

Une semaine s'écoule et la patiente revient. Les saignements et l'odeur ont disparu, mais les leucorrhées et l'œdème persistent. Les résultats des écouvillons sont à présent connus : absence de Chlamydia/Gonocoque mais présence d'un Gardnerella et d'un Streptocoque du groupe A. Le traitement reçu correspond à l'antibiogramme.

L'examen au spéculum montre un col œdématié sans saignement actif ni trouble du relief, mais un saignement important réapparaît dès le retrait du spéculum. Le toucher vaginal est douloureux. Une échographie est réalisée, dévoilant une paroi cervicale postérieure épaissie à 4 cm, vascularisée (figure 1).

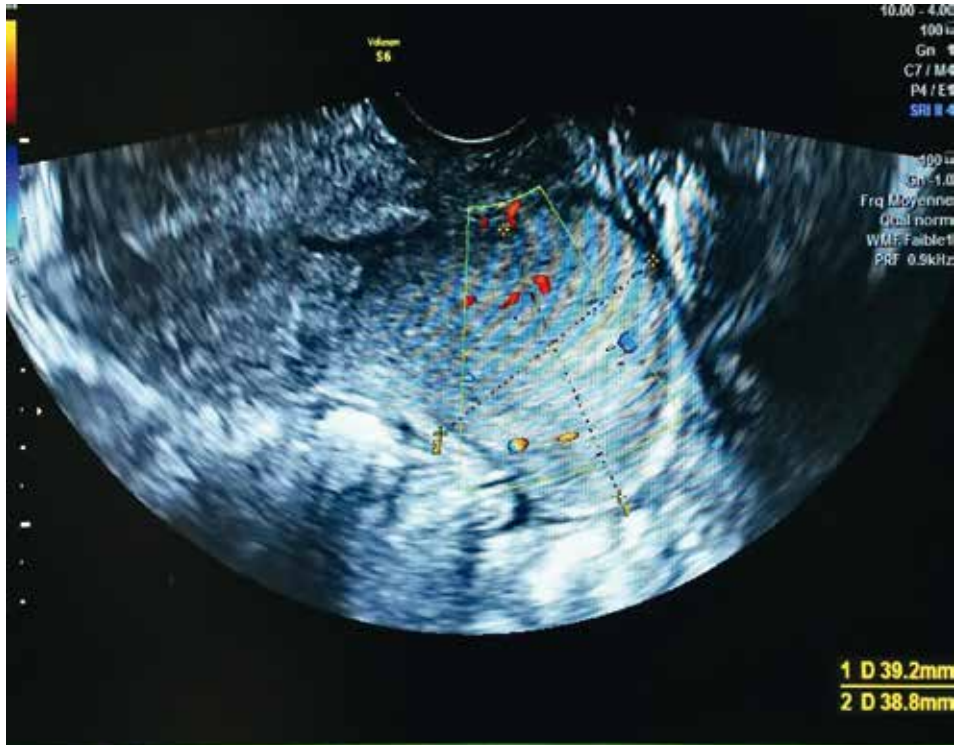


Figure 1

Une échographie des tissus mous confirme le lymphœdème, exclut une thrombose et nous rassure quant à l'intégrité des ganglions.

Un bilan sanguin sans particularité et des prélèvements vaginaux itératifs démontrant une flore vaginale normalisée complètent le bilan.

Vu le saignement apparu en cours d'examen, la biopsie de col n'est pas réalisée. Nous demandons à la patiente de se représenter 48h plus tard.

Lors de cette visite de contrôle, Madame P nous signale l'apparition de douleurs lombaires en coups de poignard. Une décision d'hospitalisation est prise.

L'examen colposcopique met en évidence une volumineuse masse cervicale d'allure glandulaire, jusqu'alors internalisée (figures 2 et 3), saignant facilement au contact (figures 4 et 5). Des biopsies sont réalisées et le diagnostic d'adénocarcinome moyennement à peu différencié infiltrant de l'endocol est posé.

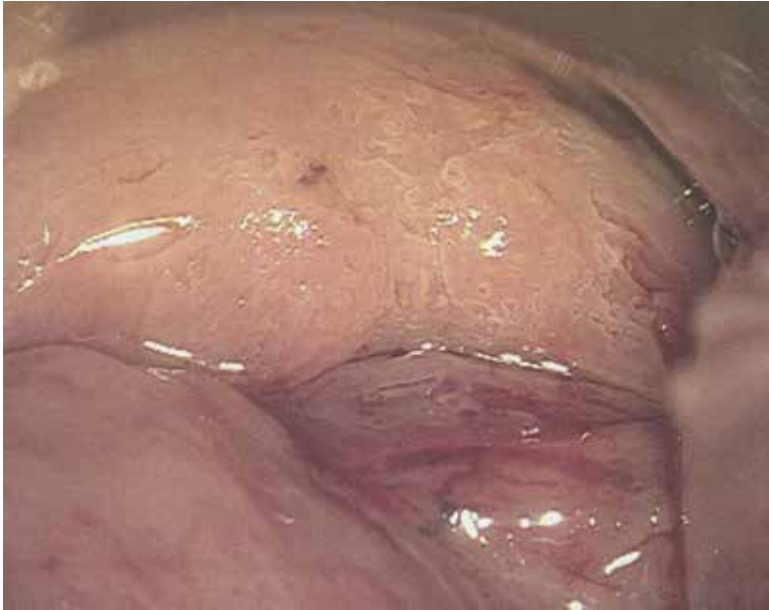


Figure 2



Figure 3

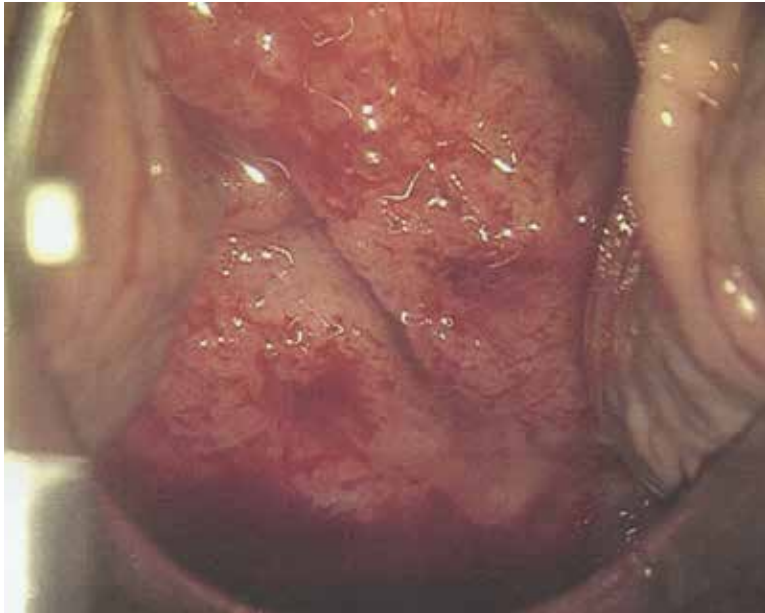


Figure 4

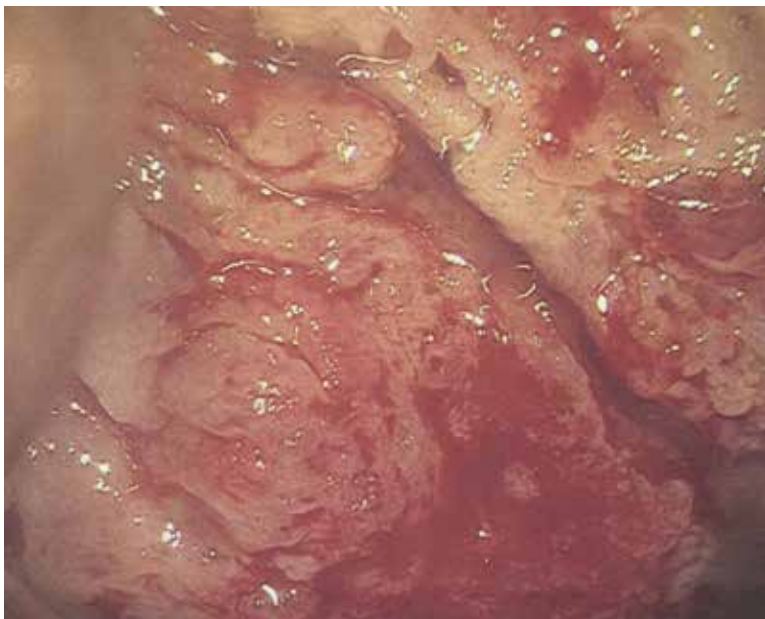


Figure 5

Un bilan par scanner abdomino-pelvien et IRM apportent des précisions concernant une masse supérieure à 5 cm, entreprenant endocol, exocol et isthme utérin, et infiltrant le fond vaginal et le paramètre gauche, sans atteinte urétérale. De multiples ganglions sont atteints jusqu'au niveau para-aortique. Un

Pet-Scanner accable le tableau par la mise en évidence de ganglions positifs en sous- et sus-rénal, tronc cœliaque et mésentériques supérieurs. Les ganglions sus-claviculaires gauche sont également suspects.

Un traitement prophylactique par enoxaparine 40mg est initié et la patiente est adressée en oncologie afin de débiter une chimiothérapie première. Malheureusement, la patiente succombera à sa maladie 4 mois après sa première visite aux urgences.

### **Discussion du cas**

L'adénocarcinome ne représente que 15-25 % des tumeurs retrouvées au niveau du col utérin, mais il touche des patientes essentiellement jeunes et l'évolution des lésions pré-invasives vers l'invasion est plus courte (moins de 5 années sont nécessaires pour passer d'un adénocarcinome in situ à un cancer invasif) et le pronostic est plus sombre<sup>(1)</sup>. Son dépistage est donc un problème majeur de santé publique, mais celui-ci reste complexe. Cela s'explique par le fait que l'outil standard de dépistage qu'est la cytologie n'est pas optimal pour les lésions de type glandulaire. Les cellules glandulaires sont plus difficilement échantillonnées de par leur localisation endocervicale, et quand elles le sont, l'interprétation des atypies est plus ardue. En effet, les cellules glandulaires, plus petites que les cellules malpighiennes, s'amassent habituellement en placards denses, avec une détection d'anomalie nucléaire rendue plus difficile, et les adénocarcinomes sont souvent bien différenciés, avec une structure plus proche du tissu cylindrique sain<sup>(2, 3)</sup>. Ces facteurs résultent en un taux de faux négatifs encore supérieur à celui retrouvé en cytologie pour les lésions épidermoïdes<sup>(4)</sup>, cependant, la probabilité de développer un adénocarcinome, même de stade 1A, dans les 3 ans suivant une cytologie normale était faible<sup>(5)</sup>.

Chez la patiente présentée dans cet article, outre le piège du faux négatif cytologique et le jeune âge de la patiente, nous avons été confrontés à une évolution fulgurante de la masse cervicale, initialement enfouie, avec un aspect d'un jour à l'autre totalement modifié par l'ulcération secondaire de la tumeur. Le frottis normal datant de 3 semaines aurait-il pu orienter le diagnostic si la cytologie avait été combinée à une recherche HPV ? De manière générale, la recherche HPV permet de déceler davantage de dysplasies de haut grade que la cytologie en milieu liquide, et diminue de ce fait l'incidence de cancer cervicaux<sup>(6)</sup>. Ce n'est malheureusement pas sans inconvénients, car si la sensibilité du test HPV est excellente (96,1% versus 53% pour la cytologie)<sup>(7)</sup>, sa spécificité est moindre (90,7% versus 96,3% pour la cytologie)<sup>(8)</sup>, ce qui occasionne un surdiagnostic et un surtraitement de lésions qui auraient régressé spontanément en dehors de toute prise en charge. La présence d'HPV 18 est impliquée dans 50% des cas d'adénocarcinomes, alors qu'il n'est présent que pour 15% des carcinomes épidermoïdes<sup>(1)</sup>. Dans les faits, il n'est actuellement recommandé ni de demander

une recherche HPV lorsqu'une cytologie est négative, ni de le dépister pour le triage des lésions glandulaires de type AGC. En effet, ces dernières sont associées à des lésions précancéreuses et cancéreuses dans 30% des cas, une investigation complémentaire par colposcopie, curetage de l'endocol et biopsies endométriales est alors vivement conseillée quelque soit le résultat d'une éventuelle recherche d'HPV.

Par ailleurs, dans le cas présenté, le diagnostic oncologique a été retardé par une infection avérée peu courante et potentiellement gravissime. Le portage vaginal de Streptocoque du groupe A (GAS), ou *Streptococcus pyogenes*, est décrit essentiellement durant la grossesse et dans le post-partum mais reste rare (0.03% de colonisation génitale chez la femme enceinte)<sup>(9)</sup>. Une hypothèse de l'origine de cette colonisation est la proximité de l'anus, mais il est communément admis qu'un système immunitaire affaibli et un état cancéreux sont des facteurs de risque d'infection par le GAS<sup>(10, 11)</sup>, tandis que les lésions cutanées et muqueuses sont des portes d'entrée vers une bactériémie<sup>(12)</sup>. La symptomatologie d'une infection génitale par le GAS comporte des douleurs pelviennes aiguës, des leucorrhées purulentes abondantes d'apparition rapide, un œdème labial, un possible prurit, une dysurie d'irritation muqueuse et cutanée, ...<sup>(9)</sup> Plusieurs agressines sont responsables de la pathogénicité du *Streptococcus pyogenes*, qui peut parfois conduire à des situations désastreuses telles que l'évolution vers une fasciite nécrosante ou un syndrome de choc toxique streptococcique<sup>(13)</sup>. Le traitement du GAS est simple et universel : la pénicilline, aucune résistance n'ayant été décrite.

## **Conclusion**

Le cas présenté nous rappelle l'importance de dresser, ne serait-ce que mentalement, un diagnostic différentiel, afin de n'exclure aucune possibilité. Même si la pratique et l'expérience nous confortent dans des décisions standards, essayons de préserver cet aspect plus scolaire de la médecine et d'éviter de prendre des raccourcis parfois hasardeux pour le devenir de nos patients. Il reste cependant primordial de hiérarchiser notre cheminement et de ne pas se perdre en démarches inutiles.

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# **An experimental study of erythrocyte membrane in rats against the background of energy drink oral intake by fluorescent probe technique**

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## **Abstract.**

**Aim.** The aim of our research was to assess the state of red blood cell (RBC) membranes in rats orally administered caffeinated energy drinks during two months using the fluorescent probe O1O (2-(2'-OH-phenyl)-5-phenyl-1,3-oxazole).

**Materials and methods.** Fluorescent probe O1O (2-(2'-OH-phenyl)-5-phenyl-1,3-oxazole), which locates in the area of glycerol backbones, carbonyl groups

of phospholipids, and alkyl chains of phospholipids (near carbonyl groups) in the bilayer, was used to assess the state of RBC membranes in suspensions prepared from whole blood samples obtained from ten female adult WAG rats orally administered a caffeinated energy drink at a dose of 12 ml/kg during two months and ten controls.

**Results.** Energy drink consumption was associated with a higher fluorescence intensity of the phototautomer form of the probe O1O (the fluorescence maximum is at a wavelength of 480 nm) in erythrocyte suspensions compared with the control animals. The observed change in the fluorescence of the probe is attributed to the increase in the viscosity of the probe environment in the membrane.

**Conclusion.** It was revealed using the fluorescent probe O1O that the long-term oral administration of caffeinated energy drinks to rats caused the increased membrane viscosity (i.e. reduced fluidity) in RBCs.

**Keywords:** caffeinated energy drinks, fluorescent probe, rats, cell membrane

Caffeinated energy drinks have become extremely popular among adolescents and young adults worldwide. They are believed to improve physical performance, endurance, and cognitive processes <sup>(1,2)</sup>. The effects of energy drinks on mental and physical performance are mediated by the high amount of caffeine. Some energy drinks may contain up to 550 mg per a can or a small bottle, whereas the caffeine toxicity threshold is considered to be 400 mg/day for adults <sup>(1,3)</sup>. Taking into account the fact that caffeinated energy drinks are primarily consumed by teenagers for whom the caffeine daily intake should be limited to 100 mg only, the problem of their consumption by children and adolescents seems to be of huge importance. In addition to caffeine, the list of energy drink constituents includes: glucuronolactone, taurine, L-carnitine, group B vitamins, etc.<sup>(1,2)</sup>.

The impact of caffeinated energy drinks as a whole and their ingredients in particular on the health of laboratory animals has been actively studied. It has been reported about numerous adverse effects of energy drinks <sup>(1,2)</sup>. However, there is no information on the influence of energy drink intake on the state of erythrocyte membranes.

**The aim of our research** was to study the state of erythrocyte membranes in rats after the two-month-long oral intake of caffeinated energy drinks using fluorescent probe O1O (2-(2'-OH-phenyl)-5-phenyl-1,3-oxazole).

## Materials and methods.

### 1. Description of laboratory animals used in this study and groups.

According to the conditions of our experiment, twenty female adult WAG rats weighing 180-200 g were randomly divided into control and experimental groups. The rats from the experimental group were administered a caffeinated energy drink of a famous brand daily (except for weekends) once at a dose of 12 ml/kg of body weight for 60 days ( $n = 10$ ). The control group consisted of 10 intact animals.

### 2. Preparation of RBC suspensions and description of the fluorescent probe.

The suspension of erythrocytes was obtained from blood of animals using saline physiological solution. Fluorescent probe O1O was dissolved in acetonitrile to the initial concentration of  $1 \times 10^{-4}$  mol/L. Twenty microliters of the probe was added to the RBC suspensions. The final content of each probe in RBC suspensions reached  $1 \times 10^{-6}$  mol/L. The lipid-to-probe molar ratio was 1000:1. The fluorescence of probe O1O (2-(2'-OH-phenyl)-5-phenyl-1,3-oxazole) was measured in RBC suspensions of both groups using fluorescence spectrometer "Lumina" (Thermo Fisher Scientific). The measurement was carried out after 1 hour after the addition of the probe to RBC suspensions. The fluorescence spectra of probes were measured in the range of 360-600 nm with monochromator slit width of excitation and fluorescence 5 and 5 nm, respectively, and the excitation wavelength of 330 nm. The fluorescent probe O1O was chosen for our research, since its fluorescent characteristics depend on the physico-chemical properties of its microenvironment: the proton-donor ability, the polarity and viscosity of the microenvironment<sup>(4-9)</sup>.

### 3. Bioethics.

All experimental procedures were performed in accordance with provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986). The experiment was approved by the Committee of Ethics and Bioethics of Kharkiv National Medical University.

### 4. Statistical analysis.

Experimental data were processed using the "GraphPad Prism 5" software. Mann-Whitney U test was used to compare the numerical values of two independent groups. Data are represented as median and interquartile range. Differences between groups were considered statistically significant at  $p < 0.05$ .

**Results.** It is well-known that the excited state proton transfer (ESIPT) reaction occurs when the ortho-hydroxy 2,5-diaryl-1,3-oxazole is in the excited state<sup>(4-9)</sup>: hydroxyl group in the ortho-position of the lateral benzene ring acts as proton

donor, whereas the nitrogen atom of oxazole ring acts as proton acceptor. As a result of this reaction, phototautomer form (T\*) is formed. It is fluorescent in significantly longer wavelengths compared with the initial (or so-called “normal”) form (N\*) [4-9]: the fluorescence maximum of the phototautomer form of O1O is at 480 nm, while the corresponding maximum for the normal form is at 375 nm.

Table 1

**Fluorescence intensity of probe O1O in RBC membranes  
of animals against the background  
of two-month-long consumption of energy drinks  
(median [interquartile 25%; 75%])**

Groups of animals	Fluorescence intensity, a.u.		
	Probe O1O		
	375 nm	480 nm	$I_{480}/I_{375}$
Control (n=10)	16 [14; 19]	1276 [1201; 1338]	80 [72; 90]
Oral intake of caffeinated energy drinks (n=10)	18 [15; 20] p>0.05	2456 [2367; 2596] p<0.001	136 [120; 158] p<0.01

The presence of two-band fluorescence provides us with the opportunity to perform ratiometric measurement, i.e. to use the ratio of the phototautomer form and the initial form fluorescence intensities ( $I_{T^*}/I_{N^*}$ ) as a parameter for assessment of the physical and chemical properties of the microenvironment.

The probe O1O locates in the area of glycerol backbones of phospholipids (closer to the center of the lipid bilayer), in the area of carbonyl groups of phospholipids and in the area of hydrocarbon chains of phospholipids (near carbonyl groups of phospholipids).

It was found that the oral consumption of energy drinks was accompanied by an increase in the fluorescence intensity of the phototautomer form of the probe (the emission maximum is at 480 nm) and, thus, by a higher value of the  $I_{480}/I_{375}$  ratio (Table 1). The mentioned changes in the fluorescence parameters of the probe point to the increase in the viscosity of the probe environment <sup>(7)</sup>.

Thus, fluorescent probe O1O allowed us to reveal that the prolonged oral administration of caffeine-containing energy beverages to rats is accompanied by the increased membrane viscosity (i.e. reduced fluidity) of RBCs.

## **Discussion.**

Maintenance of membrane fluidity in RBCs is absolutely necessary for viability of these cells<sup>(10)</sup>. It provides the proper interaction of ligands with receptors embedded in cell membranes and affects the action of ion channels and transporters<sup>(11)</sup>. Under normal conditions, the membrane fluidity and viscosity, which is the reciprocal of fluidity, depend on multiple factors, including structure of cell membranes and lipids involved in their formation, as well as on fatty acid composition of membrane-associated phospholipids, etc.<sup>(11)</sup>. It has also been reported that lipid peroxidation and oxidative stress affect the membrane fluidity as a whole and of RBCs in particular, decreasing it and therefore increasing the membrane viscosity<sup>(12, 13)</sup>. Lipid peroxidation is primarily associated with the influence of free radicals on polyunsaturated fatty acids diminishing their amount in phospholipid bilayer and therefore increasing the relative percentage of saturated fatty acids, which results in the increased membrane viscosity and decreased fluidity<sup>(14)</sup>.

It is worth noting that the consumption of energy drinks results in the activation of lipid peroxidation processes and oxidative damage to liver and brain cells<sup>(15)</sup>. Al-Basher GI et al reported that even perinatal exposure to caffeinated energy drinks induces lipid peroxidation and therefore promotes the development of oxidative stress<sup>(16)</sup>. Moreover, it is known that a higher rate of lipid peroxidation contributes to hemolysis due to the affected integrity of RBC membranes. Munteanu C et al demonstrated that the red blood cell count is reduced in rats orally administered caffeinated energy drinks. The authors attributed this decrease to the intensified destruction of RBCs<sup>(17)</sup>. Thus, there is strong evidence that the oral long-term consumption of caffeinated energy drinks is associated with the development of oxidative stress.

Our findings that suggest the association between the two-month-long consumption of caffeinated energy drinks and the increase in the viscosity of the lipid bilayer (i.e. with the decrease in the membrane fluidity), evidenced by the changes in the fluorescence of probe O1O, in RBCs of rats may be due to the activation of lipid peroxidation and the development of oxidative stress.

It is worth mentioning that the state of membrane affects oxygen diffusion capacity and thus respiratory function of RBCs<sup>(14)</sup>. Thus, the energy drink consumption-associated increase in RBC membrane viscosity found in this study may affect oxygen transport efficiency and contribute to a decrease in tissue oxygenation and the development of hypoxia.

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