

A white ECG (heart rate) line graphic is positioned horizontally across the middle of the page. The line starts with a sharp peak on the left, followed by a dip, then a series of smaller peaks and troughs, and ends with a sharp peak on the right. The word 'PULSE' is written in large, white, sans-serif capital letters, centered over the middle of the ECG line.

# PULSE

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**Dear Friends,**

Osler, one of the fathers of modern medicine stated in 1920 , "Always note and record the unusual... Publish it. Save it on a permanent record as a short concise note. Such communications are always of value". And that is precisely what we endeavor to do with our journal Pulse. Resolving clinical dilemmas, giving diagnostic solutions, improving patient outcomes are often achieved by investigations, clinical studies which are noted and published. Valuable hitherto unknown information often gets presented in case reports. These then make life simple, though I will not dare to say simpler.

Case in point is the findings presented by Dr. Nalini Bansal and her colleagues from Fortis Escort Heart Institute, New Delhi and is the first ever case reported of the presentation of gall bladder carcinoma with PAS positive hyaline globule masquerading as liver mass. Given the paucity of available clinical data and lack of literature, they represent a clinical challenge in daily practice and should be kept as one of the differential diagnosis of liver mass.

The Medical Case Reports section this time comprises of a wide range of topics including an atypical case of subacute combined degeneration of spinal cord, erythrophagocytosis in bone marrow in the diagnosis of typhoid, rare cases of primary retroperitoneal cavernous hemangioma and intraoral malignant melanoma in a young male and *Penicillium marneffeii* infection in an HIV positive patient.

We also have our salient activities like New Test Releases and Publications which include the latest updates from the last 6 months. We would be obliged to receive your contributions and suggestions for the various sections of our next issue of Pulse.

I also take this opportunity to thank all the contributors of the case reports, quizzes, and publications, and to the entire editorial team and support staff. Hope you will like this issue and will come forward to make it better with your suggestions and scientific contributions.

Warm regards,

**Dr. B. R. Das**

## Small Cell Type Undifferentiated Carcinoma of Gallbladder with PAS Positive Hyaline Globule Masquerading as Liver Mass

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### Introduction

Small cell type undifferentiated carcinomas of the gallbladder are unusual neoplasm. Most of these tumors are diagnosed on radiological imaging as a mass in gall bladder. Their presentation as liver mass and presence of PAS positive, hyaline globules on microscopic examination has never been reported. We herein report the first such case of gall bladder carcinoma and review the literature of small cell variant of undifferentiated carcinoma (UC).

### Case Report

A 41-year-old male was admitted in our hospital with diagnosis of obstructive jaundice. Patient had complaint of myalgia, lethargy, weight loss and upper abdominal pain since two months. He also had features of cholestasis like clay colored stools and itching for two month. There was no history of fever, projectile vomiting, hematemesis and black colored stools. Patient was a diagnosed case of type 2 diabetes mellitus (on oral hypoglycemic) and hemorrhoids.

On general examination patient had yellow discoloration of bulbar conjunctiva and skin. His vitals were stable. Mild tenderness was present over the right hypochondrium and epigastric region. There was no other remarkable finding on general and systemic examination.

Patient's laboratory data on admission were suggestive of deranged liver function test with raised total bilirubin (19.47 mg/dl; Normal range: 0-1.2 mg/dl), high alkaline phosphatase (315 U/L; Normal range: 40-129 U/L), mildly elevated SGOT (167 U/L; Normal range: 4-38U/L) and SGPT (130 U/L; Normal range: 16-63). Tumour markers, including carcinoembryonic antigen (CEA) (3.0ng/ml; Normal range: 0-5ng/ml), alpha-fetoprotein (6.2 ng/ml; Normal range: 0-7ng/ml) and cancer antigen CA 19.9 (7U/ml; Normal range: 0-37 U/ml) were within normal ranges. Serological test for hepatitis B and C were negative.

Radiological imaging was suggestive of enlarged liver. Almost entire right lobe of liver and segment IVB showed a large, well defined space occupying lesion measuring 14.4(AP)x 13(CC)x 12(TR) cm in size [Figure 1a, b]. This lesion shows inhomogenous peripheral enhancement in early and late arterial phases. There were vessels streaking

centripetally into area of central scarring with progressive filling of contrast in portal, hepatic and delayed phases. The mass was found compressing the liver hilum and causing minimal intra hepatic biliary radical dilatation. Gall bladder was normal in size and wall thickness [Figure 1b] with no intraluminal filling defect/enhancing mass seen. No significant hepatoduodenal/ mesenteric/ pre-paraaortic or retroperitoneal lymphadenopathy seen in abdomen. Chest X ray was normal.

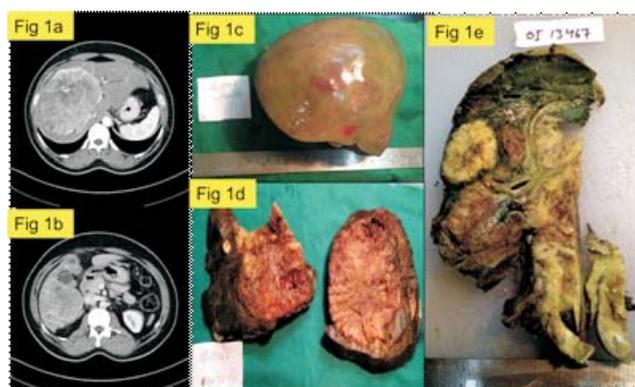


Figure 1: 1a: Contrast enhanced CT scan shows normal looking gall bladder with heterogeneous mass in segment 5 & 6; 1b: CECT shows intensely enhancing huge vascular mass lesion occupying almost entire right lobe of the liver; 1c: Gross showing large liver mass; 1d: Gross cut surface showing whitish large liver mass; 1e: Gross of liver with gall bladder showing infiltrative mass from gall bladder directly infiltrating into liver

On the basis of clinical assessment, laboratory result and radiological imaging a provisional diagnosis of large right side space occupying lesion of the liver (?Hepatocellular carcinoma/?Giant hemangioma/?FNH [Focal nodular hyperplasia]/?) compressing common hepatic duct, causing intrahepatic biliary radical dilatation were made.

In view of adequate left liver remnant decision was taken for surgery with intention of doing right trisegmentectomy. Intraoperative findings were large highly vascular right sided liver mass with normal appearing gall bladder. Gall bladder was found densely adherent to liver. No extrahepatic disease was appreciated. No regional lymph node metastasis was identified. The patient underwent right trisectionectomy with hepaticojejunostomy. Post operative recovery was uneventful with improvement in bilirubin level.

Serial slicing of the resected specimen showed a soft hemorrhagic mass of size 12 x 10 cm with a small satellite nodule of size 2 x 2cm. Gall bladder was found adherent to liver bed with focal thickening of wall and seen in continuation with the tumour mass but no mass identified in lumen [Figure 1 c, d, e].

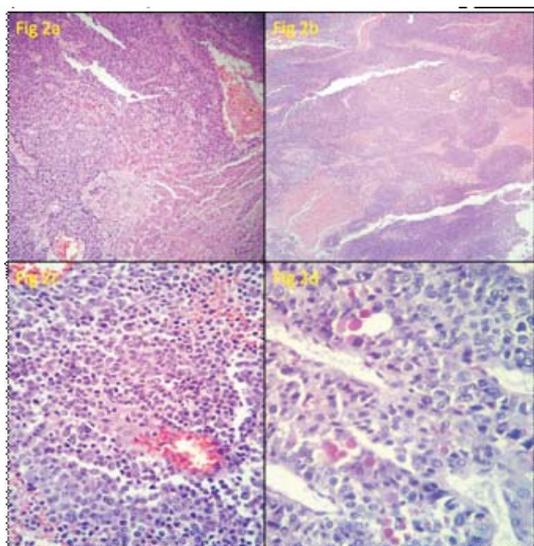


Figure 2: Microscopic examination of tumour mass

2a: Diffuse sheets of round tumour cells infiltrating hepatocytes (H&Ex10); 2b: Diffuse sheets of tumour cells (H&Ex4); 2c: Small round tumour cells (H&Ex40); 2d: PAS Positive diastase resistant globules in cytoplasm (H&Ex40)

On microscopic examination tumour mass showed un-differentiated malignant neoplasm composed of cells having round to oval nuclei, prominent nucleoli and scanty cytoplasm. No definite cell pattern was identified with these neoplastic cells (Figure 2a, b, c). Section from gall bladder showed that the tumour was arising from the lining epithelium and infiltrating through the muscularis. This tumour was seen directly infiltrating the adherent liver tissue and forming a mass of un-differentiated malignant cells (Figure 3a-d).

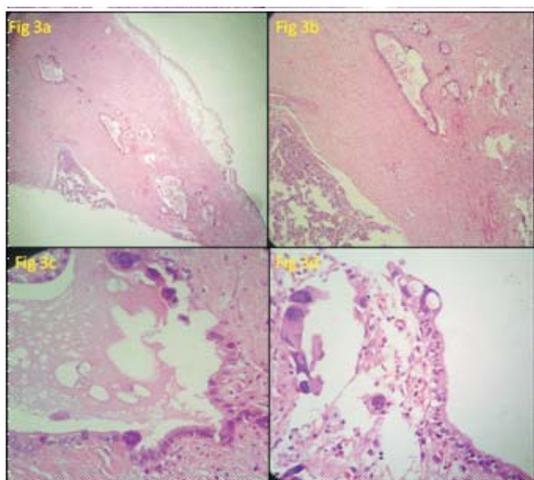


Figure 3: Microscopic section from gall bladder

3a: Tumour seen arising from gall bladder (H&Ex4); 3b: Malignant glands and cell nest (H&Ex10); 3c: Glands lined by malignant cells (H&Ex40); 3d: Tumour cells arising from lining epithelium (H&Ex40)

Focal area within the tumour mass showed presence of PAS positive, diastase-resistant, eosinophilic, hyaline globules within the neoplastic cell (Figure 2d).

On immunohistochemistry, tumour cells were diffusely pan CK positive; negative for viamentin, CD34, chromogranin, LCA (leucocyte common antigen), AFP alpha fetoprotein), and BCL2 negative with weak positivity for CK-19 (Figure 4a-d).

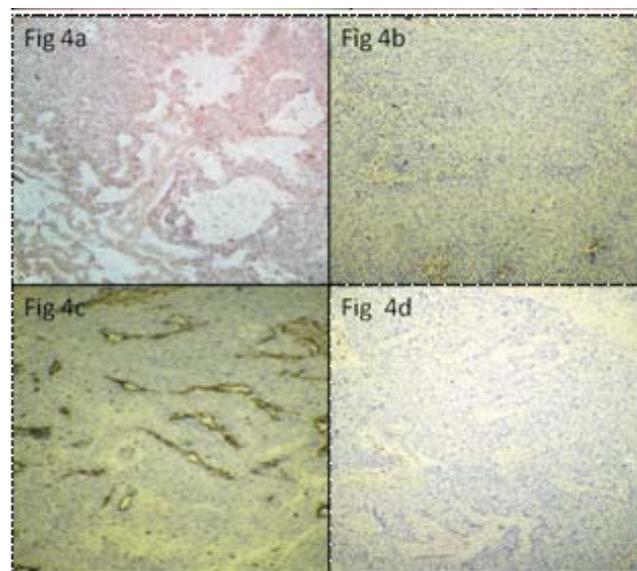


Figure 4: Immunohistochemistry

4a: IHC PanCK positive in tumour cells (H&Ex10); 4b: IHC LCA Negative in tumour cells (H&Ex10); 4c: IHC Viamentin Negative in tumour cells (H&Ex10); 4d: IHC Chromogranin negative in tumour cells (H&Ex10)

So a final diagnosis of UC originating from gall bladder and infiltrating into liver and forming a liver mass was made.

Postoperative patient was advised for chemotherapy. However, patient refused to take chemotherapy and went to alternative medicine practitioner. A follow up imaging done for pain abdomen nine month later revealed multiple liver metastasis. After this, patient condition deteriorated rapidly. Patient succumbed to multiple liver and brain metastasis at 12 month following surgery.

**Discussion**

UC represents high grade malignant neoplasm which lack differentiation and are composed wholly or partially of undifferentiated cells that retain feature indicative of epithelial origin only on immunohistochemical or ultrastructural ground. They represent grade IV tumour in AJCC (American Joint Committee on Cancer) grading system (1). They are also called anaplastic carcinoma. These tumours are clinically aggressive and usually fatal.

UC are reported in various organs like sinonasal tract, thyroid, lung etc and are very rarely reported in gall bladder with only few small case series and case reports (2, 3, 4, 5,

8, 10) The reported incidence of UC in gall bladder varies from 1.6 to 10.9% (2). Most cases are seen in the age group of 44-88 years, with a male to female ratio of 1:25 (3).

UC present clinically with right upper quadrant pain, anorexia, weight loss and very rarely with hemobilia (2, 4, 5). Tumour markers like CEA and CA19-9 remain usually normal in UC (2). On ultrasound most cases are seen as heterogenous intraluminal mass with a medium size of 5 cm.

Grossly most cases are seen as large mass varying in size from 1.3 cm to 10 cm, presenting as large intra-luminal polypoid mass within the gall bladder lumen. Few cases present as infiltrative mass (3). The polypoidal mass have rounded smooth contour and are not papillary as seen in papillary neoplasm (2).

Microscopically WHO has classified four variants of UC (6):

- a. Spindle and Giant cell type: This is the most common type of UC. They are composed of variable proportion of spindle cells, polygonal cells and giant cells (7, 8).
- b. Osteoclastic giant cell type: Morphologically resemble giant cell tumour of bone. They are composed of multinucleated osteoclastic giant cells and mononuclear cells.
- c. Small cell type: Composed of mainly sheets of round cells with prominent nucleoli.
- d. Nodular and Lobular type: They are composed of nodules or lobules of neoplastic cells.

Immunohistochemical staining is essential for definite categorization and includes positive staining of tumour cells for epithelial markers like panCK, EMA and negative staining for LCA, viamentin, bcl-2, S-100 and chromogranin.

The origin of this tumour is uncertain. They are two school of thoughts, one group believe that UC arises from dedifferentiation of pre existing well differentiated tumour while other believe that they originate as already aggressive undifferentiated cancer.

Treatment comprises of surgical excision with or without chemotherapy.

Prognosis of these cases is poor with a mortality of around 81% within a year (3).

Small cell UC are still rarer type of UC with only nine cases reported in English literature till date (Table 1). Most of these cases are seen in females (6/9) in the age group of 44-75 years. Grossly these cases are seen as intraluminal protruding mass in the gall bladder.

S.N.	AUTH OR	YEA R	JOURNAL	CASES	AGE/SEX	FOLLOWUP	MACROSCOPIC
1.	Guoera 1	1988	Cancer	1	44/M	Los: (Follow up)	Protruded
				2	75/F	Died (9month)	Unknown
				3	75/F	Died (9month)	Protruded
				4	56/F	Died (2month)	Protruded
				5	68/F	Live (59Month)	Protruded
				6	50/F	Autopsy	Protruded
				7	69/F	Autopsy	Infiltrating
				8	73/F	Autopsy	Protruded
2.	Parketa 1	2014	Zoumal of hepatobiliary pancreas		46/F	Died (7-8month)	5cm tumour
3.	Our case	2015			41/M	Died (712month)	Liver mass

Table 1: Cases of small cell UC of gall bladder

We herein report the first case of small cell UC presenting as liver mass. There is only a single case report of spindle cell type UC presenting as liver mass (9). Presentation of UC of gall bladder as a liver mass is rare and should be kept as one of the differential diagnosis of liver mass on imaging. All surgeons must understand the natural history, biology and imaging feature of this variant of gall bladder cancer for its early diagnosis which is vital for the survival of the patient.

Microscopically these cases show predominance of small cell population. Guo et al has reported focal PAS stain positivity in two cases of small cell UC. However no intracytoplasmic globules were described previously. In our case there was presence of focally intracytoplasmic PAS positive diastase resistant hyaline globules. So differential diagnosis of embryonal sarcoma was thought for; however immunohistochemical stains for same (viamentin & bcl-2) were negative.

Pathology of UC was an independent prognostic factor for poor survival in gall bladder cancer as per Park et al. All patients of small cell variant of undifferentiated gall bladder carcinoma have died within a year of diagnosis except one.

No case with similar morphological finding has been described previously in literature.

The rarity of small cell variant of UC of gall bladder pose significant limitations to the interpretation of our report. In view of scant literature definite guideline for surgery and chemotherapy still needs to be defined.

### Conclusion

UC of the gallbladder presenting as liver mass with eosinophilic intracytoplasmic globules are extremely rare neoplasm. Given the paucity of available clinical data and lack of literature they represent a clinical challenge in daily practice.

Careful analysis of histological and immunological features is also required, in addition to clinical and radiological criteria for its prognostic significance. Early diagnosis and surgical intervention with better understanding of tumor biology may offer improved prognosis and survival in this rare cancer.

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## An Atypical Case of Subacute Combined Degeneration of Spinal Cord

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### Summary

Vitamin B12 deficiency is an important nutritional disorder causing neurological manifestations of myelopathy, neuropathy and dementia. Subacute combined degeneration (SCD) is a rare neurological complication of vitamin B12 deficiency, characterized by demyelination of the dorsal and lateral spinal cord. Herein, we describe one case report, a 55 yrs old woman who presented with SCD, related to reduced intake of vitamin B12. The patient presented with motor symptoms, a definite sensory level and bladder dysfunction. She has pigmentation over knuckles of both hands and beefy tongue. After treatment with intramuscular B12 injections (injection Hydroxy Cobalamin 1000 $\mu$ g IM weekly for 1 month then continued monthly), the patient showed improvement of her symptoms. Abnormalities of the spinal cord as seen on MRI resolved in three months. In conclusion, SCD due to reduced intake of vitamin B12 is a reversible condition, when detected and treated early. We review the literature regarding these rarely reported features of vitamin B12 deficiency, and discuss aspects of management of this reversible condition. We emphasize the importance of awareness of autonomic disturbances in B12 deficient individuals.

### Background

This is an atypical case because:

- Presence of definite sensory level and band like sensation which is uncommon in cases of sub acute combined degeneration of spinal cord
- Early loss of pain, touch, temperature
- Early bladder and bowel involvement
- The patient is a non vegetarian

### Case Presentation

A 55 years female, non-hypertensive/ non-diabetic/ non-alcoholic housewife was apparently unwell for about last 3 months, presenting with complaints of:

- Tingling and mild burning sensation of bilateral distal upper and lower limbs for about 3 months
- Progressive difficulty in walking for about last 2 months
- Progressive diminished sensation of bilateral lower limbs below waist for about last 6 weeks
- Cannot perceive touch or differentiate hot water from cold for about last 6 weeks
- Developed bladder as well as bowel incontinence after 10 days of admission at the hospital.

The patient had no similar history in the past, is non-vegetarian, had not undergone any gastrointestinal surgery, no history of headache, cognitive impairment, radicular pain or chronic diarrhea, but had a long history of dyspepsia. She had no major illness in the past and no chronic medication.

Clinically the patient was afebrile with pallor (anemia), and non-icteric. No dehydration or lymphadenopathy was found. Pulse: 90 per min; BP: 110/66. Pigmentation over knuckles of both hands (recent onset; figure 1) along with beefy tongue was noted. Chest/ abdomen/ cardiovascular system were normal.



Figure 1: Knuckle Pigmentation

### Investigations

Pathological Findings

- Hemoglobin – 8.6 gm/dl ↓
- Leucocytes (tc) – 4260 per cu mm ↓
- MCV – 109.5 fl ↑
- RBC – macrocytic
- Urea & Creatinine – normal
- LFT – normal
- HIV – negative
- Plasma Glucose – normal
- Sodium & Potassium – normal
- Serum Folate – >24 ng/ml
- Vit B12 – 146 pg/ml ↓
- Anti Parietal Cell Antibody (APCA) – strong positive

### Neurologic Examination

**Motor System:** Power of lower limb all muscles 4/5, bilateral knee jerks & ankle jerk absent, planter bilateral extensor

**Sensory System:** Bilateral pain, touch and temperature perception impaired from about T12 level downwards, bilateral upper and lower limb joint, position and vibration sense impaired, Romberg test: positive

**Cerebellar Function:** Normal

**Nerve Conduction Velocity:** shows features of peripheral neuropathy involving sural, tibial and peroneal nerves

### Upper GI Endoscopy shows Antral Gastritis

MRI — of the spine showed intramedullary hyper intensity on T2-weighted images in the posterior column of the cervico—dorsal spinal cord (figure 2)

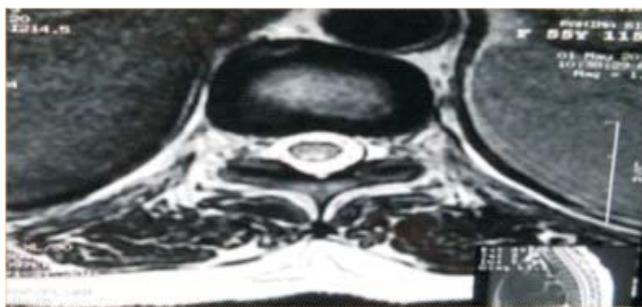


Figure 2: MRI of spine showed intramedullary hyper intensity seen on T2-weighted images in posterior column of cervico-dorsal spinal cord. Diagnosis of SCD of spinal cord was considered.

### Differential Diagnosis

Based on neuroimaging: HIV vacuolar myelopathy/ Herpes viruses myelitis/ Vitamin E deficiency/ Copper deficiency/ Tabes Dorsalis

### Treatment

Patient was given injection Hydroxy Cobalamin 1000µg IM weekly for 1 month, then continued monthly.

### Outcome and Follow-up

After 3 months patient was able to walk without any help. Bladder symptoms improved.

### Discussion

Vitamin B12 deficiency is a systemic disease that often affects the nervous and hematological systems. Megaloblastic anemia is a common early manifestation pointing to an underlying B12 deficiency, although neurological symptoms may occur in the absence of hematological abnormalities. The most frequent neurologic manifestations are the SCD of the spinal cord and polyneuropathy.

ANATOMY: Plantar extensor: CORTICO SPINAL TRACT

involvement. Loss of proprioception and band like sensation: DORSAL/ POSTERIOR COLUMN involvement (1). Loss of jerks without root pain: PERIPHERAL NERVE involvement. Upper motor neuron lesion found so jerk should increase and plantar extensor should be there, but due to vitamin B12 deficiency which causes peripheral nerve damage, causing jerks to be absent. Loss of pain, touch, and temperature: SPINO THALAMIC TRACT or PERIPHERAL NERVE involvement so impression is SUBACUTE ONSET OF PROGRESSIVE MYELO NEUROPATHY means Spinal Cord and peripheral nerve involvement (2). Cerebellar Functions like finger nose or heel shin test normal, dyssynergia or nystagmus not noted.

**PATHOLOGY:** Toxic/ Metabolic/ Nutritional – might be possible. APCA strong positive indicates vitamin B12 deficiency (3) because intrinsic factor needed for B12 absorption is produced from parietal cells so antibodies against parietal cells will make intrinsic factor production low thus resulting in low B12 absorption. Presence of antral gastritis supports impaired absorption of vitamin B12, as it is an autoimmune problem causing inflammation to only one region of the stomach, can be caused after a treatment of radiation for cancer, autoimmune diseases, pernicious anemia, and chronic vomiting. In cases of high Folate and Vitamin B12 deficiency, high Folate interferes with Vitamin B12 containing enzymes, thus resulting in worsening of Vitamin B12 deficiency (4). Degenerative diseases like Parkinson s, Alzheimer s, Motor Neuron diseases etc – unlikely. Primary demyelination – unlikely.

### Conclusion

Therefore in presence of pallor, beefy tongue, knuckle pigmentation & spinal cord lesion along with the aforementioned signs and symptoms with Vitamin B12 deficiency, a diagnosis of SUB ACUTE COMBINED DEGENERATION OF SPINAL CORD should be considered.

### Learning Points

- The case was diagnosed early and clinical and radiological improvements were observed during follow up.
- Diagnostic delay and/ or late initiation of therapy may result in permanent irreversible injury to the spinal cord with little or no improvement on treatment.
- A prompt diagnosis of SCD and early vitamin B12 treatment could avoid irreversible neurologic damages and prevent disability.

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## Erythrophagocytosis in Bone Marrow as a Clue to the Diagnosis of Typhoid

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### Summary

A 17-year-old boy presented with fever. The presence of bone marrow granulomas with erythrophagocytosis and Gram negative bacilli detected on modified Brown and Hopp stain helped in clinching the diagnosis of Typhoid infection. It was confirmed when the bone marrow aspirate sample collected in EDTA as well as the blood culture grew *Salmonella typhi*. The patient recovered after starting treatment for Typhoid and was discharged in afebrile condition.

### Background

This article has been written to highlight the significance of erythrophagocytosis in bone marrow in diagnosing Typhoid infection in the setting of fever and presence of granulomas in the bone marrow.

We also want to highlight the use of modified Brown and Hopp's stain on bone marrow biopsy which helped in confirming the presence of Gram negative bacilli within the granulomas. It is a simple stain which can be used on the biopsy samples for identifying Gram positive and Gram negative bacteria.

### Case Presentation

17 yr old boy was admitted with history of:

- Fever with chills and rigors off and on since 2 months
- Throat pain

During this period he had been treated for Typhoid fever with various antibiotics in the OPD.

*Past Medical History:* Diagnosed as Seizure Disorder at 3 months of age, was on tablet, Gardenal.

Relevant Examination findings:

### On admission:

General condition was poor with cold peripheries and peripheral cyanosis

Temperature: 103.4°C

Pulse: 139/min, feeble pulse

P/A- Soft,

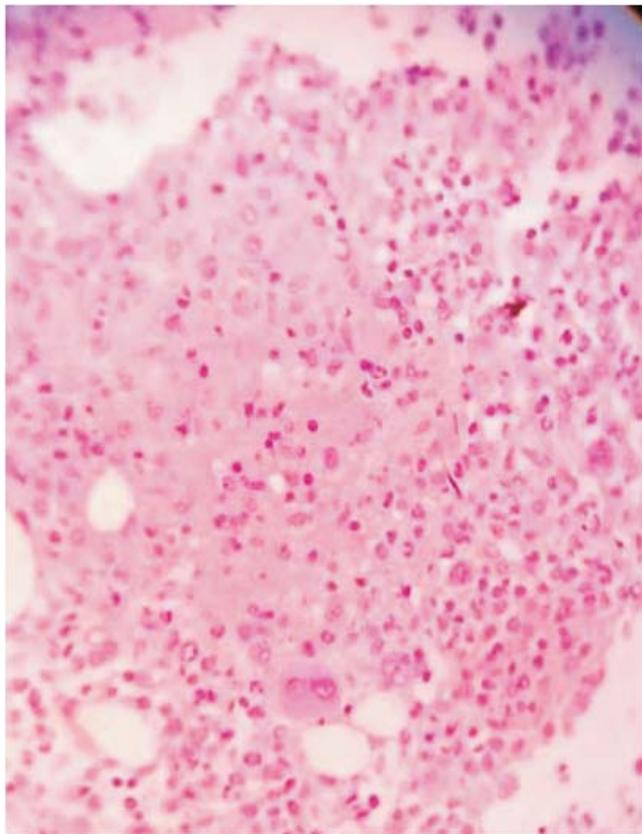
Liver-6-7 cm below subcostal margin, non-tender

Spleen- 3-4 cm below subcostal margin, non-tender

Chest-Bilateral spasm, wheeze present

### Investigations

The investigations revealed Hb 8.3g/dL, TLC 4820/ $\mu$ L, DLC:N66 L27 E00 M07, PLT: 468000/ $\mu$ L, ESR:22 mm/1st hr, S.ALT:84 IU/L, AST: 59 IU/L, S. Creatinine: 0.61 mg/dL, Malarial Antigen: Negative, HsCRP:124 mg/L. Ultrasound abdomen revealed lymphadenopathy in the periportal, portocaval and peri ascending colic region and right paracolic gutter. Mural thickening of the terminal ileum, ileocaecal valve and cecum was noted. Gall bladder wall was edematous with mild peri-portal oedema. Mild ascitis was noted. To rule out tuberculosis, Quantiferon Gold test was carried out, which was negative. Bone marrow aspiration smears revealed normoblastic and megaloblastic erythropoiesis. Eosinophils, plasma cells and megakaryocytes were marginally increased in number. Reduced bone marrow storage iron Grade 1+ (on 0-6+ scale). No granulomas were seen. The bone marrow biopsy revealed many granulomas located mainly interstitially, few were seen in the paratrabeular regions too. The granulomas were composed of epithelioid cells, histiocytes, few lymphocytes and occasional plasma cells. Erythrophagocytosis was noted in some granulomas. Necrosis was seen in some granulomas.



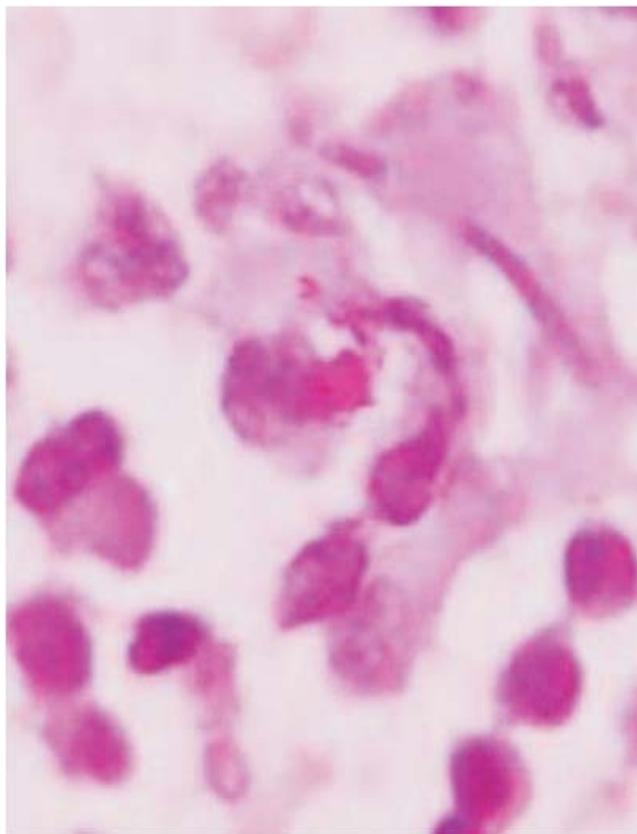
*Bone marrow biopsy showing epithelioid granuloma with erythrophagocytosis*

ZN stain did not reveal any acid fast bacilli. PAS stain was negative for fungal organism. Since erythrophagocytosis and granulomas have been described in Typhoid fever, an attempt was made to search for Gram negative bacilli within the granulomas in the bone marrow biopsy. Brown and Hopp's method with modification was used. We used the modified technique as developed by Engbaek K et al (1), with one further modification: picric acid acetone was replaced by hematoxylin as we did not have picric acid. It revealed occasional Gram negative bacilli within the granulomas. We decided to culture the 2-day-old bone marrow sample received in EDTA. *Salmonella typhi* was grown in the culture. *S.typhi* was also isolated from blood culture. The bone marrow was reported as Granulomatous inflammation due to Typhoid fever involving the bone marrow.

#### Differential Diagnosis

- In a case of prolonged fever with granulomas in the bone marrow, tuberculosis is the first cause that should be ruled out in our country. Our patient's bone marrow showed epithelioid granulomas with necrosis in some granulomas. However, there were no Langhans giant cells; no caseation necrosis and no AFB were seen on ZN stain. It has been shown that in cases of miliary tuberculosis, 33-100% bone marrow biopsies show granulomas, yet caseating necrosis is uncommon and finding of AFB on ZN stain is rare (2) so the possibility of tuberculosis cannot completely be ruled out.

- Disseminated fungal infections are also high in the differential diagnosis of bone marrow granulomas. However, stains for fungus were negative.



*Gram negative bacilli stained with modification of Brown and Hopp's stain*

#### Treatment

The patient was treated with injection Monocef 1.5 gram twice a day for 5 days and Tab Azithral 500mg twice a day for 10 days. He was discharged in afebrile condition.

#### Discussion

Shin et al (3) have described the sequential changes in bone marrow pathology according to the stage of the disease based on the onset of symptoms. The early stage, which is until 10 days from the onset of symptoms showed granulocytic hyperplasia as the predominant bone marrow finding along with mild mono-histiocytic proliferation. It is just the beginning of hemophagocytosis. The next stage is the proliferative stage which lasts until 25 days. During this stage granulomatous inflammation and hemophagocytosis are important findings. The last (lysis) stage begins after 25 days. In this stage chronic granulomatous inflammation is the typical finding in most cases. The granulomatous inflammation can be well-formed and ill defined. Our case showed well formed granulomas composed of epithelioid cells, histiocytes, few lymphocytes and occasional plasma cells. Necrosis was also noted in some granulomas with cellular debris. Erythrophagocytosis was also seen in some granulomas even though our case was in the lysis stage as his onset of fever was about 2 months ago. However since he had

received partial treatment for Typhoid, the morphology may have been modified or this could have been a recurrence.

Muniraj K et al (4) have highlighted the importance of erythrophagocytosis in bone marrow in detecting typhoid infection. According to Garg N et al (5) the simple finding of erythrophagocytosis in the bone marrow even in the midst of atypical findings, can point towards the right diagnosis.

#### Learning Points

- Bone marrow granulomas with erythrophagocytosis should be taken as pointers to the diagnosis of Typhoid.
- Salmonella typhi can be cultured from EDTA bone marrow.
- Modified Brown and Hopp's method of histological Gram stain can be used to pick up Gram negative Salmonella typhi on bone marrow biopsy samples.

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## Primary Retroperitoneal Cavernous Hemangioma – A Rare Case

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### Summary

An elderly female with known case of chronic ITP presented with complaints of pain in abdomen, loss of appetite and weight loss since 4 weeks. Imaging studies revealed a large lobulated well encapsulated solid cystic mass with multiple fluid levels within the retroperitoneum on the left side measuring 13x8.7x12.9 cms showing large areas of haemorrhage and on histopathology reported as a Hemangioma. The retroperitoneum is a very rare site for a Hemangioma with only one case reported previously in the literature.

### Background

Vascular tumours are non epithelial tumours which are common in childhood. Hemangiomas have a rare presentation in the retroperitoneum. Among retroperitoneal tumours, cavernous hemangioma is even rarer (1, 2). Majority of reported retroperitoneal hemangiomas in adults originated from kidneys, adrenal glands and pancreas (3, 4, 5). It is difficult to make a clinical diagnosis and difficult to diagnose even by various imaging modalities. Diagnosis is confirmed only by histopathological examination as was done in our case.

### Case Presentation

66-year-old female, known diabetic, hypertensive and an old diagnosed case of chronic idiopathic thrombocytopenic purpura (ITP) presented with complaints of pain, abdominal discomfort, loss of appetite and weight loss of 17 kgs since 4 weeks. On physical examination she was found to be conscious and alert. There was tenderness in left abdomen with normal bowel sounds. She had undergone ovarian cystectomy, appendectomy and cholecystectomy in the past. She was not receiving any active treatment for ITP.

### Investigations

At time of admission, complete blood counts were: hemoglobin- 9.8 mg/dl, total leucocyte count- 7900 /  $\mu$ l with predominance of neutrophils (90%), platelet count- 35000/  $\mu$ l. She was given PRBC S and RDP units. Simultaneously her pain in abdomen increased and a contrast enhanced MRI (CEMRI) of whole abdomen was done which revealed a large lobulated well encapsulated solid cystic mass with multiple fluid levels within the

retroperitoneum on the left side measuring 13x8.7x12.9 cms. Large areas of haemorrhage were seen. No calcification or fat stranding noted within the mass. Few well defined cysts also seen at the periphery of the mass. The mass was extending from level of splenic hilum superiorly (L1 vertebral body) upto the level of lower pole of left kidney inferiorly. The mass was displacing the distal body and tail of pancreas superiorly and anteriorly and abutting lower pole of spleen superolaterally. The splenic vascular flow is well maintained. Posteriorly, mass is abutting anterior cortex of left kidney and left renal vessels. Anteriorly mass is indenting posterior wall of stomach. Laterally focal abudment of descending colon is noted. Intervening fat planes are well preserved. Medially, mass is in close proximity with proximal jejunal loops and DJ flexure with indistinct fat planes. Few subcentimetric retroperitoneal lymph nodes are noted in para aortic, aortocaval and renal hilar regions, largest measuring 15.5x9.5 mm. An exploratory laparotomy was done comprising of wide tumour excision with splenectomy and a left adrenalectomy. Intra operatively a retroperitoneal tumour was seen abutting the pancreas, spleen, left kidney, transverse colon, descending colon and deudojejunal flexure with maintained fat planes. A mid line incision was given and tumour mobilised from the retroperitoneum. Tumour was closely adherent to spleen and left adrenal and as a result they had to be resected. Specimen was sent for histopathological examination. Gross examination revealed a gray brown mass measuring 13x12x7.0 cms. External surface was well encapsulated and bosselated. Cut section showed numerous cystic spaces filled with haemorrhagic fluid. Few solid irregular areas were seen. Spleen and adrenal gland appear unremarkable. Light microscopy showed large dilated vascular channels lined by flattened endothelial cells lying in a loose myxoid connective tissue stroma showing foci of chronic inflammation. No atypical cells were seen. Periphery of the lesion showed a tiny portion of normal pancreatic tissue. Immunohistochemical studies were done: CD34 was found to be positive in endothelial cells lining the vascular channels. Vimentin was positive in stromal cells. Ki67 was ~ 2% indicating a low proliferative activity. Sections from spleen and adrenal were unremarkable.



Figure 1: Gross specimen: Cut section shows numerous cystic spaces filled with hemorrhagic fluid

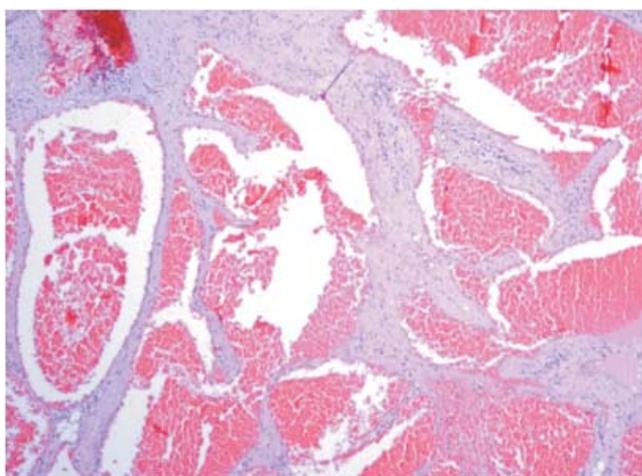


Figure 2: Multiple dilated vascular channels surrounded by fibroconnective stroma (H&E x10)



Figure 3: Large lobulated hypochoic mass seen in retroperitoneum on contrast enhanced MRI (CEMRI)

### Differential Diagnosis

A large cystic tumor originating from the retroperitoneum, the differential diagnosis includes malignancies such as liposarcoma, malignant fibrous histiocytoma,

leiomyosarcoma, and neuroblastoma, or benign lesions such as paraganglioma, neurofibroma, lipoma, teratoma, and neurilemoma. We arrived at a diagnosis of primary retroperitoneal cavernous hemangioma based on the histomorphology.

### Treatment

Surgical resection is a curative treatment for primary retroperitoneal cavernous hemangioma which reduces the risk of hemorrhage and relieves the pressure on neighbouring organs.

### Outcome and Follow-Up

Patient's outcome was good following surgery. His symptoms have been relieved and on follow up he is doing well.

### Discussion

Cavernous hemangioma is a benign vascular tumor, commonly involving the skin or mucosa. Other visceral cavernous hemangiomas originate from the liver, spleen, kidney, adrenal gland, and pancreas. Adult hemangiomas are uncommon in retroperitoneal organs, and adult primary retroperitoneal cavernous hemangioma is even rarer with only one case reported previously in the literature.

Our patient was a middle-aged female. No gender predilection has been found for retroperitoneal cavernous hemangiomas except for tumors in the adrenal gland. Imaging studies (CEMRI) in our case revealed a large lobulated well encapsulated solid cystic mass with multiple fluid levels within the retroperitoneum on the left side measuring 13x8.7x12.9 cms showing large areas of haemorrhage [figure 3]. Our first impression in this case was of a cystic tumor originating from the retroperitoneum. For such cases, the differential diagnosis includes malignancies such as liposarcoma, malignant fibrous histiocytoma, leiomyosarcoma, and neuroblastoma, or benign lesions such as paraganglioma, neurofibroma, lipoma, teratoma, and neurilemoma [1]. Surgery was performed and the retroperitoneal mass was sent along with the adrenal gland and spleen. Pathologically, the gross findings of the mass included cystically dilated spaces filled with blood [figure 1]. Microscopically, the mass consisted of variously sized vascular spaces lined by a single layer of flattened cells, which stained positive with CD34 and vimentin. Mitotic activity was low, KI67 ~ 2%. A final diagnosis of primary cavernous hemangioma was given. Postoperative period was tolerated well by the patient and she has no complaints on follow up.

**Take Home Messages**

1. Any large cystic mass detected on imaging studies in the retroperitoneum, one must also keep a differential diagnosis of benign conditions such as a hemangioma.
2. Prognosis of hemangioma is very good.

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## Intraoral Malignant Melanoma in a Young Male: A Very Rare Entity

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**Introduction**

Primary malignant melanoma of the oral mucosa is an extremely rare condition. The exact incidence rates of oral melanoma are not available. However, it is estimated to represent 1-2% of all oral malignancies and accounting for about 0.2-8% of all melanomas. It is frequent in countries like Japan, Uganda, and India (1-3). Among the Japanese, oral melanoma accounts for 11-14% of all cases of melanomas (3-5). In Australia, primary malignant melanoma of the oral mucosa is rare (6). In the East, mucosal malignant melanoma seems to be more common than the West (7, 8). Primary oral melanomas are extremely rare in the United States and account for less than 2% of all melanomas. At 1.2 cases per 10 million people per year, the annual incidence of oral melanoma is very low (9).

We here report a case of primary oral malignant melanoma recently seen by us.

**Case Presentation**

A 38-year-old male presented with brown to black swelling over palate with history of occasional bleeding for 3 months (clinical photo). There were no other symptoms e.g., pain, burning, fever. Patient denied any history of preexisting mole or black patch over the area of swelling. He was otherwise healthy with no history of addiction to tobacco, paan etc. On examination, a black colored boggy irregular swelling of approx 5cm x 3 cm was seen over left frontal aspect of hard palate and adjoining gum.

The swelling had a bosselated surface. Some satellite hyperpigmented areas were seen at the periphery of the mass, particularly in the upper molar gingiva. No bleeding points or frank hemorrhage was, however, visible at the time of examination. Submental, submandibular and cervical lymph nodes were not enlarged.



*Clinical Presentation*

CT and MRI of head and neck did not show any metastatic lymph nodes.

Various differential diagnosis given were 1) Kaposi's sarcoma, 2) Malakoplakia 3) squamous papilloma

Gross: Incision biopsy of the lesion was sent to laboratory for histopathological examination. Grossly tiny bit of brownish mucosal tissue measuring 0.5x0.3x0.2cm was received.

Microscopy: Sections studied revealed a polypoid tumour lined by stratified squamous epithelium. The underlying stroma was infiltrated by sheets of pleomorphic spindle cells which had hyperchromatic nuclei, some with prominent nucleoli. The cells were heavily pigmented. Nests of pigmented cells were also present in the epithelium (Figure 1 & 2).

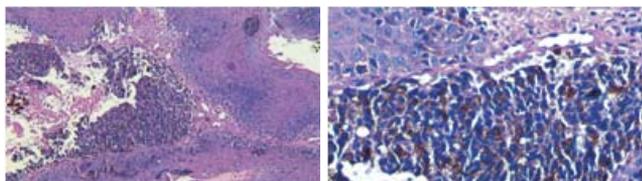


Figure 1

Figure 2

Immunohistochemistry for S-100 was strongly positive in the tumour cells (Figure 3).

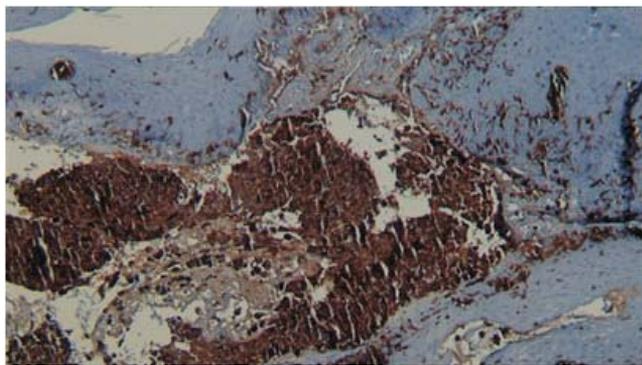


Figure 3

### Discussion

Various differential diagnosis for oral malignant melanoma are oral melanotic macule, smoking-associated melanosis, medication-induced melanosis (antimalarial drugs and minocycline), melanoplakia, postinflammatory pigmentation, melanoacanthoma, melanocytic nevi of the oral mucosa, and blue nevi (8-11). Recent and major investigations in Africa showed oropharyngeal melanoma as 1.7% of all melanomas in Sudan and oral melanomas as 0.9% of all melanomas of Nigeria. It was suggested that mouth is a common site for melanoma in Uganda, with a frequency of 8% in 125 melanomas (12-15).

In a study by Jackson and Simpson, primary malignant melanoma of the mouth represented less than 2% of all malignant melanomas (16).

Robertson et al. and Reddy et al. showed that primary malignant melanoma of the oral cavity comprises 0.4-1.3% of all malignant melanomas (7, 8). Van der Wall showed that only 2.5% of all melanomas were oral

primaries (6). Subsequent studies confirm the predominance of oral melanoma in American, Caribbean, African, and Indian population. Indian studies show between 20.41% and 34.4% of all melanomas at mucosal surface and up to 16% of these tumors are intraoral (8).

Oral melanoma is excessively uncommon at any site in prepubertal children of all races (12, 14). This malignancy is a lesion of the adulthood, rarely occurring under the age of 20 years. In different studies, the highest incidence of malignant melanoma is in the fifth decade of life i.e. 40-70 years (1-4, 8-12). Our patient was in late 4th decade of life (38 years) and a male. Males appear to be more often affected than females (2-5, 7-13).

Morris and Horn found that malignant melanomas are 4.4 times more common in Whites than in Negroes. Reports of cases of oral melanomas in Blacks are infrequent (12).

Primary melanoma occurs most frequently in the hard palate and maxillary gingiva; other oral sites are mandible, tongue, buccal mucosa, and upper and lower lip (1-7, 10-15). The microscopy of oral mucosal melanomas is different from cutaneous melanoma because biopsy is often difficult in terms of orientation and Clarke's or Breslow's depth is of little use in them. The AJCC system is commonly used to stage mucosal melanomas. The diagnosis can be easily made by H&E stain when the cells are pigmented. Amelanotic melanomas are relatively difficult to diagnose by H&E alone and IHC for S100 and HMB45 then become very useful for confirming diagnosis.

### Conclusion

Primary melanoma arising in the mucous membranes is an aggressive disease. The best likelihood for favorable outcome is early detection and excision, but many patients present with advanced disease at diagnosis because the lesion is often hidden from sight. We present this case because of its rarity and to create awareness about its possibility amongst dental surgeons, dermatologists and histopathologists.

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## Penicillium Marneffeii Infection in an HIV Positive Patient

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### Summary

A 42-year-old male from Tripura, reported to Tripura Medical College with nodular skin lesions on multiple sites. Histology and culture of punch biopsy from a facial skin lesion established the diagnosis of *Penicillium marneffeii* (*P. marneffeii*). This dimorphic fungus is endemic in Southeast Asia. It is an opportunistic pathogen which has emerged to become an acquired immune deficiency syndrome (AIDS)-defining illness in the endemic areas. Early diagnosis with prompt initiation of treatment is crucial for its management. Prompt diagnosis can often be established through careful cytological and histological examination of clinical specimens although microbiological culture remains the gold standard for its diagnosis. Standard antifungal treatment for AIDS patients with penicilliosis is well established. Highly active anti retroviral therapy should be started early together with the antifungal treatment. Special attention should be paid to potential drug interaction between anti-retroviral and antifungal treatments. Secondary prophylaxis may be discontinued with a low risk of relapse of the infection once the immune dysfunction has improved.

### Background

*P. marneffeii*, the only dimorphic species of the genus *Penicillium* is the etiological agent of a potentially life-threatening opportunistic fungal infection in human immunodeficiency virus (HIV)-infected and other immunocompromised patients (1, 2). The disease is endemic in several regions of Southeast Asia including Thailand, Malaysia, South China, Indonesia and Vietnam (2). *P. marneffeii* is also endemic in Manipur state in Northeast region of India as evidenced by reports of numerous autochthonous cases from this state. A few imported cases of *P. marneffeii* infection have been reported from non-endemic areas of India (3). Four species of bamboo rats have been found to serve as carriers of *P. marneffeii*: *Rhizomys sinensis*, *R. pruinosus*, *R. sumatrensis* and *Cannomys badius* in Southeast Asia (1). However, the incidence of penicilliosis has increased in recent times with the development of HIV pandemic and the infection has become one of the commonest AIDS-defining illnesses among HIV-positive patients in endemic areas.

### Case Presentation

1. A 42-year-old male reported to Tripura Medical College with nodular skin lesions over face and whole back for last three weeks.
2. He was reactive for HIV and was undergoing anti-retroviral therapy for last 1 month.

### Investigation

1. Hematoxylin and Eosin (H & E) stained tissue sections of punch biopsy of the skin lesion showed unremarkable epidermis. The dermis showed ill defined epitheloid cell granulomas and mixed inflammatory cells. The epitheloid cells and histiocytes showed intracellular small spore like structures. Spores stained positively by PAS stain.
2. Culture report of the skin biopsy showed pure growth of *P. marneffeii*, its identification being based on gross morphological and microscopic features, distinctive red pigment and conversion of the isolate to fission yeast form when grown on Sabouraud dextrose agar at 25°C.



Figure 1: Granular colony of *P. marneffeii* with a characteristic red diffusible pigment on Sabouraud's dextrose agar after 7 days incubation at 25°C



Figure 2: Yeast-like colony of *P. marneffeii* without red diffusible pigment on Sabouraud's dextrose agar after 7 days of incubation at 35°C

3. Under the microscope, the mold phase looks like a typical *Penicillium*, with hyaline, septate and branched hyphae; the conidiophores located both laterally and terminally. Each conidiophore gives rise

to three to five phialides, where chains of lemon-shaped conidia are formed.

4. On the 37°C plate, microscopically, sausage-shaped cells are mixed with hyphae-like structures. As the culture ages, segments begin to form. The cells divide by binary fission, rather than budding. The cells are not yeast cells, but rather arthroconidia.

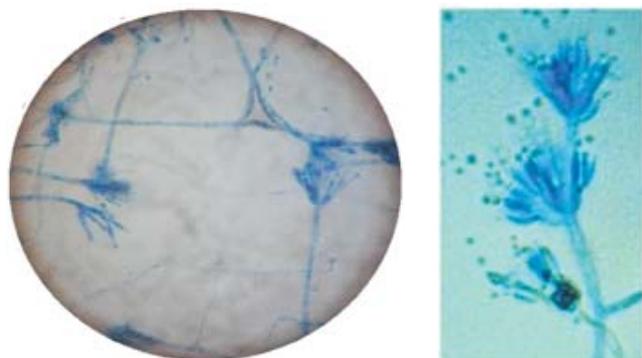


Figure 3: Microscopy of the mold form of *P. marneffei* showing septated hyaline hyphae and fruiting structures composing of branching metulae and phialides with spherical conidia in chains (lactophenol cotton blue).

Thus a diagnosis of *P. marneffei* was confirmed.

#### Treatment

The patient was treated with 0.7 mg/kg amphotericin B for 14 days and later changed to itraconazole 400 mg in two divided doses for two weeks. Thereafter the patient was put on HAART.

#### Outcome and Followup

He responded well with the regression of skin lesions, and was discharged from the hospital on request one month later with advice to continue with itraconazole and HAART.

#### Discussion

*P. marneffei* is a third most common opportunistic infection after tuberculosis and cryptococcosis in some parts of Southeast Asia. Its occurrence in AIDS patients has increased in recent years with 10% having penicilliosis as the primary AIDS defining illness (1, 2). This infection has been reported from non-endemic regions of India such as states of Tamil Nadu, Maharashtra, Assam, Meghalaya and Delhi (3). The case from Delhi was a patient of immune restoration syndrome and was of Manipur origin. The lesser bamboo rat (*Cannomys badius*), which is a carrier of *P.*

*marneffei* in Manipur occurs in other Northeastern states in India, viz. Arunachal Pradesh, Assam, Meghalaya, Mizoram and Nagaland, and as well as in the neighboring countries viz. Myanmar, Nepal, Bhutan and Bangladesh. Thus it is possible that these regions have endemic foci but the cases have not been reported from there. The present communication constitutes the first report of a case of *P. marneffei* from Tripura.

#### Learning Points

1. A high index of suspicion of *P. marneffei* is imperative in patients with subacute febrile illness with pulmonary infiltration and characteristic skin lesions especially if they originate from areas endemic for the disease.
2. Further it is possible that many undiagnosed cases of *P. marneffei* exist in Northeastern migrants in metropolitan cities such as Delhi, Mumbai, Calcutta and Chennai.
3. When an HIV positive patient presents with systemic infection, *P. marneffei* infection must be considered, particularly due to the increase in incidence of *P. marneffei* in immunocompromised hosts.
4. Increased attention by the clinician and patient is essential to manage the infection, and antifungal treatment is of great importance.
5. To prevent recurrence, long-term effective antifungal therapy is notably important; therapy withdrawal requires strict and systemic evaluation. Treatment follow-up after the review is essential.

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Answer for 'Make a Diagnosis' Photo Quiz (June 2018)

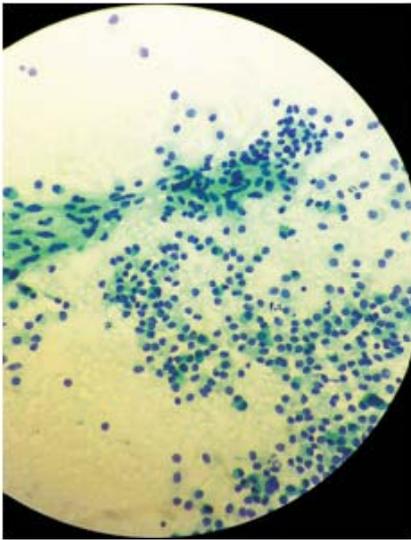
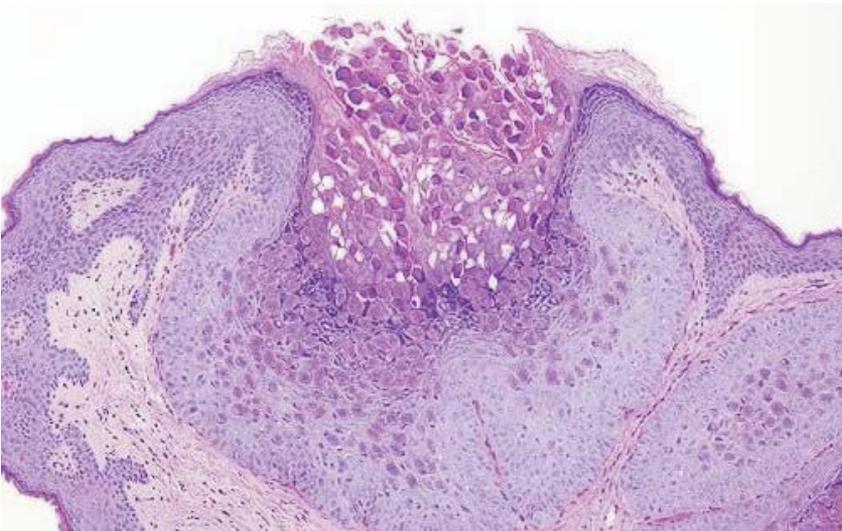


Image - EBUS TBNA Endobronchial Lung Mass; 61 yrs/M

Answer: **Low grade neuroendocrine tumor – Carcinoid**

Diagnose this

Tissue from a patient with skin infection. The lesions consist of multiple pearly round papules measuring 3 to 5 mm in size with central umbilication.



## SRL Activities

### Recent Trends in Diagnostics

Test	Specialty	Significance
Liquid Biopsy	Oncology	A test done on a sample of blood to look for cancer cells from a tumor that are circulating in the blood or for pieces of DNA from tumor cells that are in the blood. A liquid biopsy may be used to help find cancer at an early stage. It may also be used to help plan treatment or to find out how well treatment is working or if cancer has come back. Being able to take multiple samples of blood over time may also help doctors understand what kind of molecular changes are taking place in a tumor.
Preeclampsia (PE) Testing	Maternal Testing	Blood markers, placental growth factor (PlGF) and soluble fms-like tyrosine kinase 1 (sFlt1) and their ratio aid in prediction and diagnosis of PE and show improved accuracy when used in combination with first trimester prenatal screening.
FMF Certified Double Marker	Prenatal Testing	NT measured using FMF guidelines and the FMF accredited software increases the detection rate up to 91%. It provides comprehensive informatics package for maternal health monitoring, risk assessment and data management.
Lung Cancer NGS	Oncology	Cancer panels are multi-biomarker targeted next-generation sequencing (NGS) based test that detects genomic alterations in cancer-related genes. They are part of an end-to-end workflow that includes simple, scalable sequencing and optimized bioinformatics and reporting with the OncoPrint Knowledgebase Reporter. It not only enables the analysis of multiple biomarker types fusions, insertion/deletions (indels), single nucleotide variants, and copy number variations, but the test results are also intended to aid in therapy management in patient.
Solid Tissue Cancer DNA & RNA on NGS		

## Recent Publications

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12. Preethi Nair, Aparna Bhanushali and B R Das. Thalassemia- Importance of Mutation Testing – Pre-marital and Prenatal. *Medchrome*, July 2018
13. Shaloo Kapoor. Technology and Trends of Coagulation Analyzers. *Medical Buyer*, November 2018
14. Shaloo Kapoor. POCT –An Overview of Current and Future Scenario. *Medical Buyer*. August 2018
15. N K Joshi, Arnab Roy. Parkinson's Disease: The Story of a Survivor. *Medchrome.com*, Dec 16, 2018.

