

Simultaneous detection of 3 cancer foci with histological heterogeneity at 3 different anatomical sites: is it a consequence of the phenotypic plasticity of tumor cells?

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ABSTRACT

The simultaneous (synchronous) detection of 3 primary tumors is rare in clinical practice. Recognizing and differentiating metastatic malignancies have always been challenging for clinicians and pathologists; however, treatment outcomes and prognoses highly depend on this recognition. Here, we used immunostaining and a new approach that, to our knowledge, few people know about based on an understanding of exosomal microRNAs (miRNAs), phenotypic plasticity, and the tumor microenvironment to reach a final diagnosis of multiple primary neoplasms.

Key words: Multiple primary cancer, Histological heterogeneity, Primary and metastatic tumors, Primary sites of squamous cell carcinoma, Phenotypic plasticity

INTRODUCTION

When faced with a case of detection of multiple malignancies at different anatomical sites at the time of diagnosis (synchronous), a frequent question for the clinician and pathologist is whether they are multiple primary cancers (MPCs) or metastatic masses. The challenge is even greater in the presence of histological heterogeneity among these tumors. However, this distinction profoundly affects the choice of treatment method and the patient's prognosis.

MPCs are rare in clinical practice, with studies reporting a prevalence between 0.7% and 11.7%^{1,2}. They are generally divided into two types: (1) synchronous, in which the cancers occur at the same time (within 2 months of each other), and (2) metachronous, in which the cancers occur in sequence (more than 2 months apart)³. In patients with cancer, the risk of developing a new malignancy is higher than in those without cancer⁴.

Histological heterogeneity is common in MPCs. In contrast, histological heterogeneity is rare in primary cancers and metastatic masses⁵⁻⁷. Therefore, it is easy to confuse the latter with multiple primary tumors. Several hypotheses have been proposed to explain the mechanism underlying histological heterogeneity between primary tumors and metastatic masses, including phenotypic plasticity, clonality, cancer stem cells, and the tumor microenvironment. These hypotheses are not mutually exclusive but complementary.

Among them, the theory of phenotypic plasticity is of great interest⁸. Under the influence of tumor microenvironment factors, the phenotype and genotype of tumor cells can be altered⁹, similar to the Darwinian theory of evolution.

Phenotypic plasticity refers to the change in phenotype caused by environmental factors. In metastatic tumors, malignant cells exhibit phenotypic plasticity in that they can change their phenotype under the influence of new habitats to survive and grow¹⁰. This report aims to raise awareness of the distinction between MPCs and metastatic masses, especially in the presence of histological heterogeneity between primary cancers and their metastases. Updating the information on the phenotypic plasticity of cancer cells is necessary to avoid misinterpreting metastatic masses as MPCs.

CASE REPORT

A 65-year-old male patient was admitted to the hospital on August 16, 2022, due to acute pain in the right iliac fossa and unintentional weight loss (lost 16 kg in about 3 months). The patient had a history of gastric ulcers and heavy drinking many years ago. About 6 months before admission, the patient sometimes experienced the following symptoms, the severity of which gradually increased: tiredness, transient chest pain, poor appetite, vague discomfort in the abdomen, and fullness after eating only a small amount,

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without dysphagia. Then, about 1 week before admission, the patient experienced increased abdominal pain. These symptoms are often attributed to inflammatory diseases of the gastrointestinal tract and are not specific to cancers of the esophagus, stomach, and cecum.

Liver and kidney function tests, coagulation tests, and electrolyte levels were within normal limits. Complete blood cell analysis revealed marked changes in some indicators: RBC (2.63 T/L), HBG (70 g/l), HCT (0.254 L/L), MCV (96.4 fL), MCH (26.6 pg), MCHC (275 g/L), CHCM (270 g/L), PLT (920 G/L), RDW-CV (17.8% CV), and PDW (36 g/dL). An abdominal ultrasound showed that the hepatobiliary system, pancreas, spleen, kidney, bladder, and prostate were within normal limits. The rectovesical pouch was 80 mm thick. The cecum contained a mixed echogenic mass measuring 6 cm × 5 cm containing little fluid; the mass border was infiltrated. A CT scan of the abdomen showed that the liver, gallbladder, spleen, kidney, pancreas, and bladder were within normal limits, and the scan was negative for abdominal lymph nodes. The ileocecal angle was dilated, measuring 21 cm in length, with indistinct boundaries. A contrast-enhanced CT scan showed heterogeneous density after contrast administration, and the normal structure of the gastrointestinal tract wall was lost.

Endoscopy of the esophagus and stomach revealed the following: the esophageal mucosa had 2 protrusions, 1 cm and 2.2 cm in size, located 2.5 cm and 3.6 cm from the gastroesophageal junction, respectively (**Figure 1**). The body and fundus of the stomach had a flat lesion extending from the large curvature to the vertical part of the small curvature, with a rough surface and scattered mucosal corrosion with raised margins (**Figure 2**). The duodenum was within normal limits. A colonoscopy only detected cecal lesions comprising large, ulcerated areas covered with pseudomembranous membranes, with a rough surface and firm ulcer base (**Figure 3**).

Biopsies of the esophagus, stomach, and cecum were performed. Histopathologically (**Figure 4**), squamous cell carcinoma was moderately differentiated and continuous with the esophageal mucosa. Several foci of moderate-grade dysplasia were seen in the esophageal squamous epithelium. Mixed-type gastric cancer consists of ductal glandular components and poorly adherent cell components. A mild dysplasia of the glandular epithelium was also present in the biopsies. The cecal tumor consisted of round, polymorphic cells that looked like poorly differentiated tumors. Immunostaining (**Figure 5**) was strong, diffuse-positive for LCA, CD20, PAX5, CD10, and

Bcl6, and negative for CK, CD3, CD5, S100, vimentin, EBV, CD43, and MUM1. *Final diagnosis*: Multiple primary neoplasms including squamous cell carcinoma with moderate differentiation and moderate-grade dysplastic foci of squamous epithelium (in the esophagus), gastric adenocarcinoma, mixed type (in the stomach), and diffuse large B-cell lymphoma, germinal-center type (in the cecum).

The patient dropped out of the next course of treatment because his condition was very poor.

DISCUSSION

Squamous cell carcinoma is similar in histological structure in many different anatomical locations, so it is difficult to determine its primary location¹¹. Thus, is esophageal squamous cell carcinoma the metastatic mass or the primary tumor in this case? Has the phenotypic plasticity of gastric adenocarcinoma cells been activated in the new metastatic microenvironment? Is this a morphological change to adapt to the new habitat? In our experience, two important criteria are used to distinguish primary and metastatic carcinomas: (1) reactive stroma is always present around the cluster of metastatic cells as opposed to the primary mass, and (2) some precancerous foci (epithelial dysplasia of different histological grades) are present in the primary tumor. In the present case, the reactive stroma around the squamous cell carcinoma was negative, while the precancerous lesion (squamous dysplasia) was positive. In gastric biopsies, similar lesions also presented as negative for reactive stroma and positive for mild dysplasia of the glandular epithelium.

In our experience, precancerous/dysplastic foci are often present in tissues or organs with primary cancer. Meanwhile, this phenomenon is rare in organs or tissues with secondary cancer. The known exosomal microRNAs (miRNAs) of cancer cells are short, non-coding RNA fragments functioning independently of the cancer cells. Some miRNAs, called oncomiRs, play an oncogenic role by impacting either oncogenes or tumor suppressor genes, while others, called metastamiRs, play a regulatory role in metastasis^{12,13}. Exosomes of cancer cells can communicate with adjacent healthy cells and pass oncomiRs and metastamiRs to them via pinocytosis^{13,14}. The long-term reception of cancer cell miRNAs leads to the progressive neoplastic transformation of healthy cells into precancerous lesions (*i.e.*, dysplasia). Thus, the esophagus and stomach were the primary sites of squamous cell carcinoma and mixed-type adenocarcinoma, respectively, in this case.

The histological type of the primary gastric tumor was a mixture of ductal and poorly adherent structures,

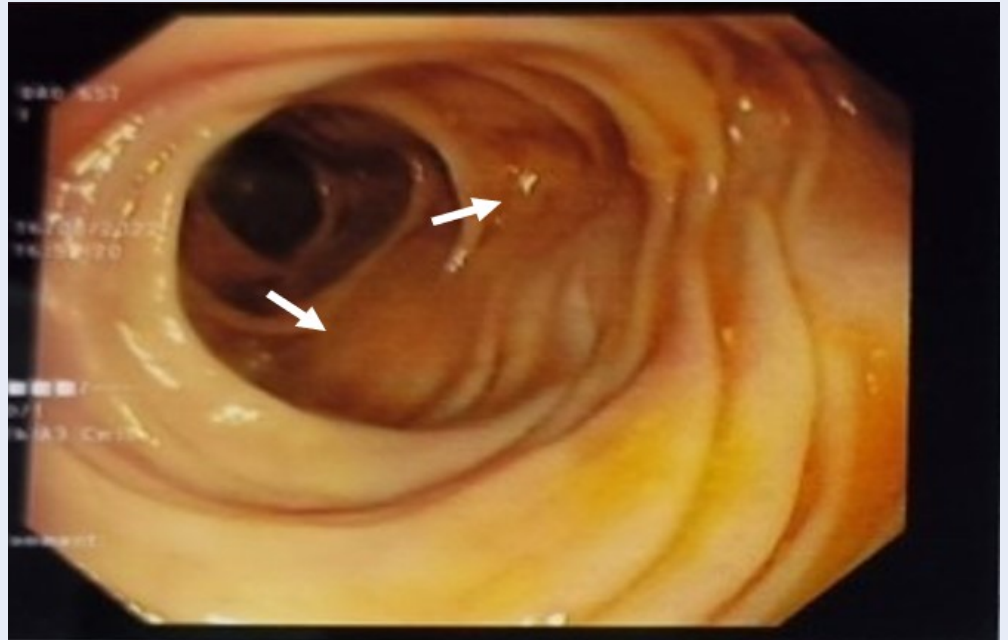


Figure 1: Esophagus: the esophageal mucosa has 2 protrusions of 1cm and 2.2cm in size.

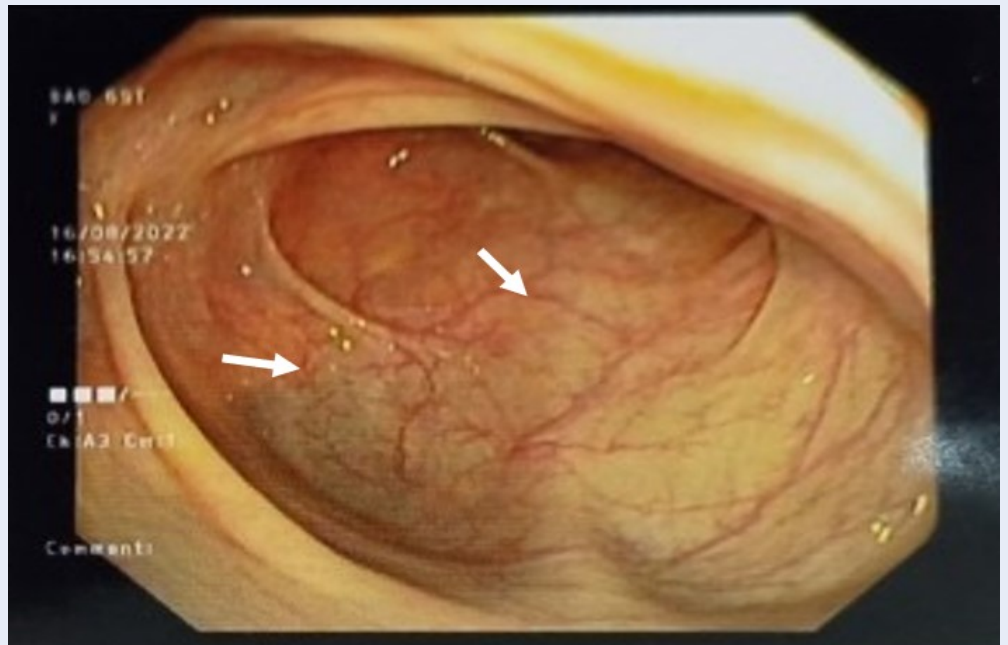


Figure 2: Gastroendoscopic image: the body and fundus have a flat lesion, rough surface, extending from the large curvature to the vertical part of the small curvature.



Figure 3: Colonoscopy: A large ulcerated area with pseudomembranous, rough surface, firm base in the cecum.

the latter consisting of rhabdoid and signet-ring cells. The histological type of the cecal tumor was round, polymorphic cells. The histological types of the 3 tumors in the present patient were completely different. Is the cecal tumor an independent primary mass or a metastatic mass of gastric or esophageal cancer?

The results of numerous studies comparing histological type between primary and secondary tumors indicate the common presence of histological homogeneity between the two sites. However, when comparing the histological type between the primary tumor and its corresponding metastatic tumor, rates of histological heterogeneity are not uncommon: 5% for renal cancer⁵, 9% for lung cancer⁶, and 22.7% and 14.3% for gastric cancer with liver metastasis and lymph node metastasis, respectively⁷.

To our knowledge, the histological type of the metastatic mass of differentiated squamous carcinoma is almost always the same as that of the primary tumor. Thus, the cecal tumor is not a metastasis of the squamous cell carcinoma of the esophagus. The possibility that the group of poorly adherent tumor cells of the gastric cancer metastasized to the cecum has not been ruled out yet. However, the morphological features of the tumor cells in the cecal tumors differed from those of the poorly adherent cells of the gastric cancer. In the present case, it is difficult to

distinguish primary and secondary tumors by H&E staining alone because the two morphological criteria mentioned above cannot be assessed.

Many hypotheses can explain the histological heterogeneity between the two tumors of unknown origin, among which the theory of the phenotypic plasticity of tumor cells is of great interest. Under the influence of different environments, certain genotypes are formed in organisms or cells that can produce different phenotypes; this phenomenon is called phenotypic plasticity. Plasticity is the factor that causes the expression of a new phenotype induced by the environment and the process of selecting the expression of that phenotype in a new environment⁹.

Thus, under the influence of the tumor microenvironment, the phenotype of cancer cells is changed to different degrees; in some cases, the cell phenotype of the secondary mass is completely different from that of the primary mass¹⁵. In general, in malignancies, there is always an interaction between cancer cells and their microenvironment that affects the shaping of malignant behavior and promotes the progression of malignancies¹⁵.

In the present case, as the cecal tumor consisted of round, polymorphic cells, the following immunological markers were usually our first choice: CK, LCA, S100, and vimentin. The results showed positivity for

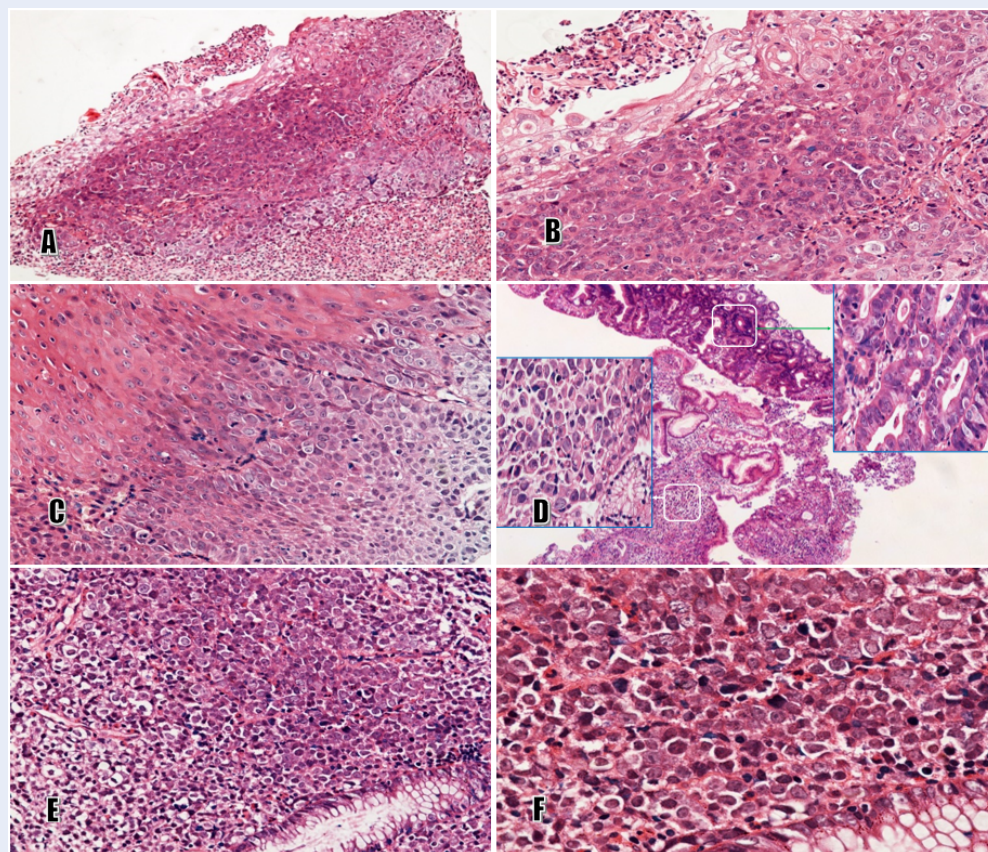


Figure 4: Multiple primary tumors. A, B, C- Squamous dysplasia with a moderate grade. **D-** Gastric adenocarcinoma, mixed type (ductal glandular and poorly adherent component). **E, F-** Diffuse large B cell lymphoma, with magnifications. H&E staining. Original magnification: 10x (A), 20x (others large box) and 40x (inserts).

LCA and negativity for CK, S100, and vimentin, so the malignancy of the cecum was determined to be non-Hodgkin lymphoma. Further immunomarker analysis was performed to reveal B- and T-cell lineage, germinal-center or non-germinal-center type, and the presence of EBV antigens, which revealed positivity for CD20, PAX5, CD10, and Bcl6 and negativity for CD3, CD5, CD43, MUM1, and EBV.

CONCLUSIONS

The patient, in this case, was ultimately diagnosed as having multiple primary tumors (synchronous) residing in 3 different anatomical sites: moderately differentiated squamous cell carcinoma (in the esophagus); gastric adenocarcinoma, mixed type (in the stomach); and diffuse large B-cell lymphoma, germinal-center type (in the cecum). A new approach with two important histological criteria, as described above, allowed us to identify the primary sites of the squamous cell carcinoma of the esophagus and the gastric adenocarcinoma, mixed type. Identifying the primary site

of squamous cell carcinoma, a difficult problem for pathologists, was achieved. Based on our experience, this new approach may be the perfect complement to immunostaining for carcinomas derived from covering epithelia when the amount of tissue samples and the number of H&E-stained sections are satisfactory.

ABBREVIATIONS

Bcl6: B-cell lymphoma 6, **CD10:** Cluster of differentiation 10, **CD20:** Cluster of differentiation 20, **CD3:** Cluster of differentiation 3, **CD43:** Cluster of differentiation 43, **CD5:** Cluster of differentiation 5, **CD79a:** Cluster of differentiation 79a, **CHCM:** Cell hemoglobin concentration, **CK:** Cytokeratin, **CT scan:** computed tomography scan, **EBV:** Epstein-Barr virus, **H&E:** Hematoxylin and Eosin, **HBG:** Hemoglobin, **HCT:** Hematocrit, **LCA:** Leukocyte common antigen, **MCH:** Mean corpuscular hemoglobin, **MCHC:** Mean corpuscular hemoglobin concentration, **MCV:** Mean corpuscular volume,

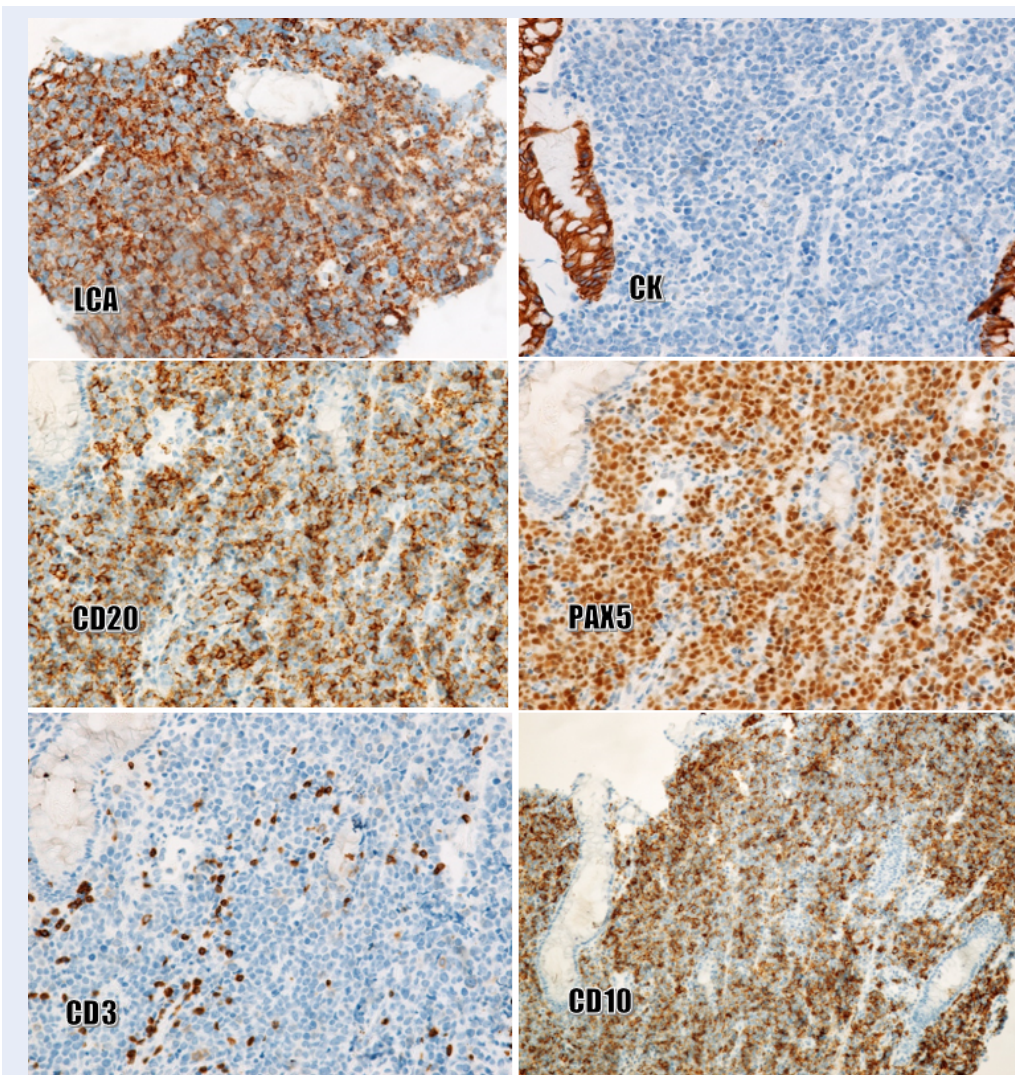


Figure 5: Multiple primary tumors: immunostaining have been labeled on the respective images. Strong and diffuse positive for LCA, CD20, PAX5, CD10, and Bcl6; Negative for CK, CD3, CD5, S100, vimentin, EBV, CD43, and MUM1. Original magnification: 20x; Images of S100, vimentin, CD5, MUM1, and EBV were not shown here.

MUM1: Multiple myeloma, **PAX5:** Paired box 5, **PDW:** Platelet distribution width, **PLT:** Platelet count, **RBC:** Red blood cells, **RDW-CV:** Red blood cell distribution width — coefficient of variation, **S100:** Protein S100

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AUTHOR'S CONTRIBUTIONS

NVH and NTT: Conceptualization, Methodology, Writing-Original draft preparation; NTT, PDD, TNM: Visualization, Methodology, Software; TNM:

Data curation, Writing-Original draft preparation; DTL: Validation, investigation, Supervision. NVH and NTT: These authors equally major contributed to this work. All authors read and approved the final manuscript.

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AVAILABILITY OF DATA AND MATERIALS

None.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was conducted in accordance with the amended Declaration of Helsinki. The institutional review board approved the study, and all participants provided written informed consent.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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