Signet-ring cells in pleural and peritoneal effusions identified on Wright stains – A diagnostic pitfall

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ABSTRACT

Objectives: Signet-ring cells (SRCs) in effusion specimens represent a diagnostic challenge. In this study, a consecutive series of pleural and peritoneal effusions with benign SRCs are examined and compared with malignant SRCs.

Material and Methods: We reviewed consecutive Wright-stained serous effusion slides and searched for cases with SRCs. Corresponding ThinPrep slides and clinical histories were reviewed. Cytology cases with known signet-ring adenocarcinoma were retrieved and reviewed.

Results: Four hundred Wright-stained serous effusions were reviewed. Eighteen cases were identified with SRC-like cells. Thirteen patients had liver cirrhosis, three patients had end-stage renal disease, one patient had a history of pancreatic adenocarcinoma, and one patient had endometrioid carcinoma. For the latter two patients, the primary tumor showed no histologic findings of signet-ring features. In all cases, no SRCs were found on the corresponding ThinPrep slides. Five cytology cases with malignant SRCs were reviewed. Benign SRCs have a uniformly pale and markedly distended cytoplasm, and the nuclei are thin and curved. The malignant SRCs showed larger non-curved nuclei and bubbly mucin-containing cytoplasm.

Conclusion: Mesothelial cells and histiocytes can mimic signet-ring adenocarcinoma cells on Wright-stained slides. Correlation with ThinPrep specimens is necessary before reporting, as the SRCs typically are not present in ThinPrep preparations.

Keywords: Atypical cells, Cytology, Effusions, Histiocytes, Mesothelial cells

INTRODUCTION

Signet-ring cell (SRC) carcinoma is one of the most subtle malignancies causing serous effusions. These cells typically present as single cells, with cytoplasmic vacuoles pushing the nucleus to the periphery of the cell. The nuclear-to-cytoplasmic ratio is low, and the nuclei can be bland with subtle atypia. These malignancies can be difficult to diagnose even when correlating with surgical specimens on histologic sections. SRCs are associated with many malignancies, most commonly gastric, breast, and ovarian carcinomas. SRCs have also been reported in many other carcinomas, including colonic adenocarcinoma, thyroid, squamous cell, prostatic adenocarcinomas, pancreatic colloid carcinoma, mucin-producing salivary gland adenocarcinomas, extramammary Paget’s, and lung adenocarcinoma, as well as tumors of alternate histogenesis including lymphoma, gastrointestinal stromal tumor, and melanoma. Diagnosis of malignant SRC in serous effusions can be very challenging, and ancillary studies are frequently utilized to confirm the diagnosis.

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SRC change is not only limited to mucin-producing tumors but has also been described in benign reactive processes.\textsuperscript{[14-16]} Cytoplasmic vacuolization can occur as a degenerative change in histiocytes and mesothelial cells, two ubiquitous components of serous effusions. These degenerative vacuoles can compress the nucleus and push it to the periphery of the cell, resulting in a perfect mimicker of malignant SRC. SRC change has also been observed as an artifact of cell shrinkage or procedural mechanical trauma to tissues, especially in Wright-stained slides.\textsuperscript{[17,18]} While mucin stains can be utilized to distinguish the secretory mucin of malignant SRC from the degenerative vacuoles, they are not always positive in SRC adenocarcinoma and can be difficult to interpret. Importantly, mucin stains are not available for the Wright-stained cytopsins, which are already known to be problematic for specific diagnosis.

Malignancy involving serous cavity is an unfavorable sign. Prognosis is poor, and most patients survive less than several months after presentation. Treatment choices are limited to palliative care for the majority of patients once diagnosis is confirmed. Thus, cytological diagnosis of SRC carcinoma is crucial for management decisions. Misinterpretation as SRC carcinoma without correlation may have serious consequences including premature overtreatment or inappropriate hospice referral.

The purpose of this study is to examine morphologic features of benign SRC mimickers in serous effusion, summarize features that distinguish benign from malignant SRCs, and understand the clinical scenarios in which benign SRCs are seen. We further investigate the appearance of benign SRCs in preparations commonly utilized by cytologic methods (Papanicolaou stain) versus methods typical of a hematology laboratory (Wright stain). Morphologic characteristics between the two methods are also compared.

**MATERIAL AND METHODS**

The Cleveland Clinic Institutional Review Board granted permission for this study. Consecutive cases ($n = 400$) of Wright-stained body fluid samples received in the hematology laboratory from October 2013 to January 2014 were reviewed. Serous effusions with SRC-like cells were retrieved. Corresponding cytology specimens that were collected at the same time or within 1 week of the hematology specimen were retrieved and reviewed. For patients with multiple specimens available, only the specimens submitted simultaneously to both hematology and cytology laboratories were selected for examination.

Body fluid received in the hematology laboratory was prepared by Cytospin (Thermo Scientific, Waltham, Massachusetts) method, and slides were Wright stained. Specimens received in the cytology laboratory were prepared using ThinPrep (Hologic, Marlborough, Massachusetts) method, and slides were Papanicolaou stained.

To compare the morphology of benign SRC-like cells and malignant SRC, serous effusion specimens with a cytology diagnosis of “positive for malignancy, adenocarcinoma with signet-ring cell features” were retrieved from the Anatomic Pathology CoPathPlus (Cerner Corporation, Kansas City, Missouri) database at Cleveland Clinic from April 2012 to April 2013. A total of six cases were identified. Both Wright-stained slides from hematology laboratory and the corresponding Papanicolaou-stained slides from cytology laboratory were reviewed. The clinical data were obtained from the institution's electronic medical record system (Epic Systems Corporation, Verona, Wisconsin).

**RESULTS**

**Clinical presentation**

Of 400 consecutive body fluid cases prepared in the hematology laboratory, we identified 18 cases with prominent SRC-like cells. Of these cases, 13 patients had a history of liver cirrhosis (alcoholic, hepatitis C virus [HCV] related, or combined alcoholic and HCV); three patients had end-stage kidney disease; one patient had endometrioid adenocarcinoma, status post total abdominal hysterectomy, bilateral salpingo-oophorectomy, and radiation therapy; and one patient had advanced unresectable pancreatic adenocarcinoma treated with chemotherapy.

The patient with endometrioid adenocarcinoma initially presented with Stage IV disease with peritoneal carcinomatosis. Fine-needle aspiration of the pancreatic tumor before chemotherapy showed adenocarcinoma with no SRC features. After chemotherapy, body fluid analysis and cytologic evaluation of peritoneal fluid were performed and showed SRC-like cells.

For all 18 cases with SRC-like cells and benign cytology, prominent lymphocytes and/or neutrophils were present in the cavity fluid. Except for the patient with sepsis, the patients generally lacked symptoms of peritonitis or pleuritis.

No SRC-like cells or malignant SRC cells were seen on the corresponding ThinPrep and Papanicolaou-stained preparations from the same body fluid samples across all 18 patients. Based on the clinical presentation as well as the
findings on ThinPrep slides, we think that these SRC-like cells seen on Wright stain are benign mimickers of SRCs.

**Morphology of SRC-like cells and malignant SRC**

On a Wright stain, SRC-like cells typically present as single cells within a chronic inflammatory cell background. The SRC-like cells have space-occupying cytoplasmic vacuoles that push the nucleus to a peripheral location. The nucleus is small, curved, and indented by the vacuole(s) [Figures 1 and 2]. Nucleoli are typically not seen. The majority of SRC-like cells have one large cytoplasmic vacuole; however, small numerous vacuoles can also be seen. In some cells, the vacuole overlies the partially folded nucleus.

Compared with SRC-like cells, malignant SRCs have larger, hyperchromatic nuclei with irregularly condensed chromatin. The nuclei are pushed to the periphery of the cells like their benign counterparts but one side of the nuclei is typically flat and not indented by the cytoplasmic vacuoles. The cytoplasmic vacuoles are usually small and numerous [Figures 3 and 4]. On Papanicolaou-stained slides, these cells show similar morphology, with large irregular nuclei and nuclear eccentricity, low nuclear-to-cytoplasmic ratio, abundant vacuoles in the cytoplasm, and numerous nucleoli. Some cases can be very challenging with subtle atypia and can be mistaken for mesothelial cells or histiocytes with degenerative changes. Knowledge of a history of malignancy and comparison to an existing surgical pathology specimen, if present, can greatly help to make the correct diagnosis.

**DISCUSSION**

The aim of this study was to examine pleural and peritoneal effusions with benign signet-ring cells and compare morphology to the malignant counterpart. The morphology of serous fluids across several different preparations has been well-described in the literature as well as recent reference books.[19] Of the 400 Wright-stained body fluids, 18 cases (4.5%) harbored prominent signet-ring cells. Of these cases, 13 cases (72.2%) had liver cirrhosis, 3 cases (16.7%) had end-stage renal disease (ESRD), one case had pancreatic adenocarcinoma (5.5%), and one case had uterine adenocarcinoma. Signet-ring cells were commonly found in patients with hepatic cirrhosis on examination of Wright-stained fluids [Figure 1]. An inflammatory cell background (lymphocytes and neutrophils) was noted in all of the benign cases. Hence, the finding of signet-ring cells can be the result of cellular degeneration secondary to inflammation in patients with chronic illnesses and repeated effusions, as demonstrated in this study in clinical settings of liver cirrhosis or ESRD. These cells were only identified on Wright-stained slides and Papanicolaou-stained smears and they were not identified on ThinPrep slides, indicating that those cells were likely disrupted by the ThinPrep method.

The typical morphology of SRC-like cells includes prominent cytoplasmic vacuolization and small, curved nuclei indented by
the vacuole(s) [Table 1]. Nucleoli are typically not seen. Most cells have one large vacuole, but small numerous vacuoles may also be seen. In contrast, the nuclei of malignant SRCs are larger with higher nuclear-to-cytoplasmic ratio, the cytoplasm more often contains multiple vacuoles, and the cells are not distended. Overall, the malignant cells have a more rigid appearance.

At Cleveland Clinic, body fluid prepared in the hematology laboratory is signed out by medical technologists and hematopathologists. Unless there are questions about the diagnosis, these slides are typically not reviewed by cytopathologists. As SRC-like cells are not uncommon findings in the hematology laboratory, both hematopathologists and laboratory technicians become immune to their sighting in daily practice. When there is morphologic suspicion for malignancy, correlation with the cytology preparation and cell block findings is warranted. This is especially important in cases with SRC-like morphology, which can have a bland cytologic appearance in cytology preparations and, paradoxically, an alarming cytologic appearance in the Wright-stained slides. Misclassification of these cells may lead to inappropriate treatment such as extensive workup to detect a metastatic primary, excessive treatment, or premature end-of-life decisions.

As many body fluid specimens are prepared by ThinPrep in the cytology laboratory, benign SRCs are not seen with Papanicolaou staining. Malignant SRCs present as a second population in serous effusions [Figure 3]. Still, some cases can be very challenging as they have only slightly larger nuclei with no or subtle atypia compared with mesothelial cells. Usually, these cells are noticed and dotted, yet a benign diagnosis is rendered by cytotecnologists. In addition, malignant signet-ring cells are not commonly present in serous effusions. Cleveland Clinic receives about 1000 serous effusion cytology samples every year, with a malignancy rate of 12.5%. In all of 2013, there were only six cases signed out as “adenocarcinoma with signet-ring cell features.” Of the six cases, five were high-grade adenocarcinomas with obviously malignant cells and diagnosis was not difficult. However, the remaining case was very challenging. The patient had a history of pancreatic adenocarcinoma with peritoneal carcinomatosis. The concurrent mesenteric biopsy showed infiltrating tumor cells forming glands. The tumor cells showed similar low nuclear-to-cytoplasmic ratio with abundant cytoplasmic mucin (peritoneal fluid) [Figure 3 and 4]. Without knowledge of the concurrent peritoneal biopsy, this case may have been signed out as atypical cells. These cells may have been considered to be reactive mesothelial cells/hiostocytes.

Signet-ring cell adenocarcinoma, lobular breast adenocarcinoma, small cell carcinoma, and mesothelioma represent the most subtle malignancies in serous effusion. Malignant serous effusions predict poor outcomes and poor survival rates in patients. Hence, the distinction of benign and malignant signet-ring cells is crucial in the diagnosis of such cases. As mentioned earlier, signet-ring cells have been identified in a number of malignancies, including gastric, breast, ovarian, lymphoma, gastrointestinal stromal tumor, prostatic adenocarcinomas, pancreatic, salivary gland adenocarcinomas, lung adenocarcinoma, and other malignancies. However, signet-ring cell change can occur as a degenerative change in histiocytes and mesothelial cells, common components of serous effusions. These represent benign mimickers of signet-ring cell

| Table 1: Morphologic features of benign versus malignant signet ring cells |
|---------------------------------|-----------------|-----------------|
| SRC-like mesothelial cells and histiocytes | SRC adenocarcinoma cells |
| Number of vacuoles | Single or multiple | Numerous vacuoles |
| Size of vacuoles | Variable size, usually large | Small |
| Border of vacuole | Well defined | Ill defined |
| Nuclear size | Small | Enlarged |
| Nuclear shape | Curved | One side is usually flat, rigid looking |
| Cell clusters | None, single cells | Occasional clusters |

Figure 4: Malignant SRCs on Papanicolaou-stained slides (right). These single cells may be subtle and can be missed on routine cytologic staining (×20). Wright stain (left) makes nuclear atypia more apparent (×20).
Signet-ring-like cells are a frequent finding in cytology specimens, particularly serous effusions. Wu et al. studied the cytopathologic significance of signet-ring cells in a wide range of cytology specimens including fine-needle aspiration specimens, washings, and fluids. There were 83 cases of signet-ring cell diagnosis over a 16-year period, and 13 of them (16%) were benign. Reactive mesothelial cells were the most common benign finding (10 of 13 cases; 77%). The other benign cases were due to reactive epithelium (two cases) and chronic bronchitis with goblet cells (one case). The specimens in which benign signet-ring cells were identified included paracentesis, brushing, effusions, and washes. However, the clinical presentation of the benign cases is not known, and the cytologic preparation used to diagnose those benign cases was not given.

The interpretation of benign signet-ring cells and their distinction from malignant signet-ring cells is crucial to avoid false-positive diagnosis of signet-ring carcinoma. Morphology can be useful in distinguishing benign from malignant SRCs. However, correlation with the ThinPrep specimen and knowledge of the clinical setting should be performed before reporting of SRCs on Wright-stained slides. According to this study, those cells are lost on ThinPrep prepared and Papanicolaou-stained slides. Careful integration of all available clinical data and pathologic material related to a patient procedure is necessary in this critical diagnostic situation.

Acknowledgments

The authors sincerely thank Joseph Peeples of Cleveland Clinic’s Robert J. Tomsich Pathology and Laboratory Medicine Institute for editorial assistance.

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The authors declared that they have no competing interest.

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LIST OF ABBREVIATIONS (In alphabetic order)

ESRD – End stage renal disease
SRC – Signet-ring cell carcinoma
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REFERENCES

1. Lui IO, Kung IT, Lee JM, Boey JH. Primary colorectal signet-ring cell carcinoma in young patients: Report of 3 cases. Pathology 1985;17:31-5.
2. Schroder S, Bocker W. Signet-ring-cell thyroid tumors. Follicle cell tumors with arrest of folliculogenesis. Am J Surg Pathol 1985;9:619-29.
3. Cramer SF, Heggeness LM. Signet-ring squamous cell carcinoma. Am J Clin Pathol 1989;91:488-91.
4. Che M, Tornos C, Deavers MT, Malpica A, Gershenson DM, Silva EG. Ovarian mixed-epithelial carcinomas with a microcystic pattern and signet-ring cells. Int J Gynecol Pathol 2001;20:323-8.
5. Saenz De Chirife AM, Rojas Bilbao E, Gimenez L, Marino L, Celeste F. Signet ring cell lymphoma mimicking mucin-producing carcinoma. Medicina (B Aires) 2004;64:521-4.
6. Suster S, Fletcher CD. Gastrointestinal stromal tumors with prominent signet-ring cell features. Mod Pathol 1996;9:609-13.
7. Kuroda N, Yamasaki I, Nakayama H, Tamura K, Yamamoto Y, Miyazaki E, et al. Prostatic signet-ring cell carcinoma: Case report and literature review. Pathol Int 1999;49:457-61.
8. Hiraki A, Ueoka H, Yoshino T, Tabata M, Kiura K, Tanimoto Y, et al. Primary signet-ring cell carcinoma of the lung with histochemical characterization. Anticancer Res 2002;22:1079-81.
9. Castro CY, Moran CA, Flieder DG, Suster S. Primary signet ring cell adenocarcinomas of the lung: A clinicopathological study of 15 cases. Histopathology 2001;39:397-401.
10. Ghannoum JE, Freedman PD. Signet-ring cell (mucin-producing) adenocarcinomas of minor salivary glands. Am J Surg Pathol 2004;28:89-93.
11. Gu M, Ghabari S, Lin F. Pap smears of patients with extramammary Paget's disease of the vulva. Diagn Cytopathol 2005;32:353-7.
12. Acsay NV, Pierson C, Sarkar F, Abrams J, Weaver D, Conlon KC, et al. Colloid (mucinous noncystic) carcinoma of the pancreas. Am J Surg Pathol 2001;25:26-42.
13. Sheibani K, Battifora H. Signet-ring cell melanoma: A rare morphologic variant of malignant melanoma. Am J Surg Pathol 1988;12:28-34.
14. Guerrero-Medrano J, Delgado R, Albores-Saavedra J. Signet-ring sinus histiocytosis: A reactive disorder that mimics metastatic adenocarcinoma. Cancer 1997;80:277-85.
15. Sidhu JS, Liu D. Signet-ring cells associated with pseudomembranous colitis. Am J Surg Pathol 2001;25:542-3.
16. Groisman GM, Amir M, Weiner P, Zamir D. Mucicarminophilic histiocytosis (benign signet-ring cells) and hyperplastic mesothelial cells: Two mimics of metastatic carcinoma within a single lymph node. Arch Pathol Lab Med 1998;122:282-4.
17. Arista-Nasr J, Romero-Lagarza P, Pichardo-Bahena R. Artifactual signet-ring-like cells in endoscopic biopsy of gastric lymphoma. Arch Pathol Lab Med 1997;121:623-5.
18. Wu ML, Natarajan S, Lewin KJ. Peculiar artifacts mimicking carcinoma. Arch Pathol Lab Med 2001;125:1473-6.
19. Shiham VB, Atkinson BE. Cytopathologic Diagnosis of Serous Fluids. 1st ed. Netherlands: Elsevier, WB. Saunders Company; 2009.
20. Zamboni G, Franzin G, Scarpa A, Bonetti F, Pea M, Mariuzzi GM, et al. Carcinoma-like signet-ring cells in gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Am J Surg Pathol 1996;20:588-98.
21. Chen KT. Benign signet ring cell aggregates in Peutz-Jeghers polyp: A diagnostic pitfall. Surg Pathol 1989;2:335-8.
22. Michal M, Chlumska A, Mukensnabl P. Signet-ring cell aggregates simulating carcinoma in colon and gallbladder mucosa. Pathol Res Pract 1998;194:197-200.

How to cite this article: Zhu H, Khattab R, Ondrejka SL, Reynolds JP. Signet-ring cells in pleural and peritoneal effusions identified on Wright stains – A diagnostic pitfall. CytoJournal 2022;19:12.

HTML of this article is available FREE at: https://dx.doi.org/10.25259/Cytojournal_97_2019