

Metastatic Urothelial Carcinomas with high serum level of Ca19-9. apropos of three cases

Dionisios Aninos¹, Christina Kaprilian¹, Christos Kroupis², Niki Asimaki¹, Andreas C Lazaris³, Georgia-Eleni Thomopoulou¹

Abstract

CA 19-9 immunoexpression in cytological material, as shown by few case reports in the literature, may have a diagnostic significance in urothelial carcinoma, especially in cases of metastatic disease with unknown primary.

In the present paper, we present three cases of metastatic urothelial carcinoma with elevated CA 19-9 serum levels. The lesions were located in the lung, ascitic fluid and the skin of the right inguinal region. Paracentesis was done and ThinPrep smears were prepared for cytological diagnostic purposes. For immunocytochemical investigation, antibodies against cytokeratins 7 and 20 and GATA binding protein 3 (GATA-3) were included as well as the monoclonal antibody against CA19-9.

Morphological and immunocytochemical examination (positivity for CK7, GATA-3 and/or CK20), revealed the urothelial origin in these three cases. ABC-peroxidase staining also showed the presence of CA 19-9 in some tumor cells.

Metastatic urothelial carcinoma may express CA19-9, as detected immunocytochemically in our cases, and may also cause elevation of CA19-9 serum levels.

¹ Department of Cytopathology, University General Hospital "Attikon", Medical School, National and Kapodistrian University of Athens, Greece

² Department of Clinical Biochemistry and Molecular Diagnostics, Attikon University General Hospital, University of Athens Medical School

³ 1st Department of Pathology, School of Medicine, National and Kapodistrian University of Athens, Greece

Corresponding Author:

Georgia-Eleni Thomopoulou
Department of Cytopathology
University General Hospital "Attikon"
Medical School, National and Karodistrian University of Athens
Rimini 1, Chaidari
12243, Athens
Greece
E-mail : Cytodept@attikonhospital.gr

Introduction

Urothelial carcinoma (UC) usually does not present with metastatic disease, but when distant metastases occur, most commonly, they involve bone, lung and liver [1]. Metastatic UC can be early diagnosed with relative ease accuracy by combining the clinical presentation with the cytomorphologic appearance [2, 3]. In some instances, however, because of lack of tumor differentiation as well as of morphologic similarity among tumors of different origin, it may be difficult to recognize UC cells by routine stains. Today, for the most part, immunocytochemistry provides us with a useful tool in the diagnosis of neoplastic origin. The selection of appropriate immunocytological markers to aid in the diagnosis of an undifferentiated neoplasm depends on a number of factors including clinical findings, cytological appearance and differential diagnosis [4, 5]. ThinPrep processed smears are suitable for immunocytochemical studies. In general, some biomarkers have better sensitivity than does cytology alone. Although each single marker demonstrates low specificity, immunocytochemical markers have a potentially important role when used in combination [6, 7].

CA19-9 is a tumor marker which has been widely used for adenocarcinoma of the upper gastrointestinal tract, particularly primary adenocarcinoma of the pancreas; it also has been reported to be positive in gastrointestinal carcinomas, thyroid papillary carcinomas and endometrial adenocarcinomas [8]. In bladder cancer, CA19-9 has seldom been clinically used as a tumor marker. However, it has recently been reported that increased serum CA19-9 levels are associated with poor outcome in patients with urothelial carcinoma of the bladder [9, 10]. In the present report, we present three cases of metastatic UC with markedly elevated serum CA 19-9, including their immunocytochemical profile.

Presentation

Case (1)

A 74-year-old man admitted to the hospital with fever, cough and dyspnea of four days duration. Chest radiography and computerized tomography (CT) revealed lung lesions which were sampled by percutaneous fine needle aspiration (FNA) for cytological purposes. A Tru-cut needle biopsy was also taken for histological examination. The patient had previously been diagnosed with invasive UC, which had been treated with total cystectomy, ileal conduit diversion and urostomy, at the age of 70.

Case (2)

A 72-year-old man presented with abdominal swelling, lower abdominal pain, constipation and weight loss. Paracentesis of ascitic fluid was done and sent to the laboratory for cytological examination with the clinical indication that an intestinal primary was of a suspected intestinal primary carcinoma. Four years ago earlier, the patient was diagnosed with muscle-invasive bladder cancer (grade III and stage pT2N0M0), not eligible for cystectomy. Transurethral resection (TUR) was done and radiation therapy followed. The patient also had a history of resected colorectal adenomatous polyps.

Case (3)

An 80-year-old man presented with cutaneous swelling at the right inguinal region. The patient had a past history of radical cyctectomy. Fine needle aspiration (FNA) was done.

All these cases were characterised by elevated CA 19-9 serum levels (> 100U/ml).

For morphological diagnosis, the aspirated cytological material was washed in CytoLyt solution (a methanol-based, buffered solution to lyse red blood cells) and placed into a processor (ThinPrep processor2000, Cytoc Corporation) for only one thin layer slide preparation from each

case; the ThinPrep slides were immersed into sodium phosphate buffer solution (pH 7.2) for 5 minutes, followed by the laboratory's standard Pap staining protocol. Immunocytochemical staining was done on smears prepared from the residual material of the ThinPrep fixed cellular sample. The panel of monoclonal antibodies used, included CK20, CK7, GATA-3 and CA19-9. Immunoperoxidase staining procedure was performed using the automated Dako Autostainer (Dako, Glostrup, Denmark) and diaminobenzidine was used as chromogen (ChenMate Envision Detection Kit Peroxidase/DAB, Rabbit/Mouse).

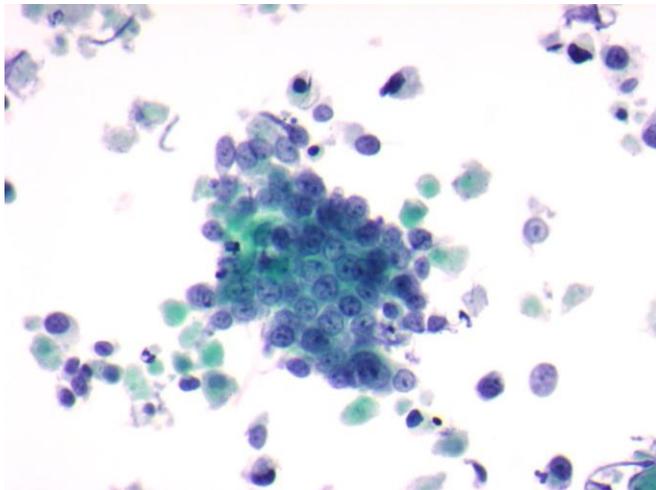


Figure 1. Fine needle aspiration of a lung mass shows malignant cells in a characteristic two dimensional pattern (Papanicolaou x 200)

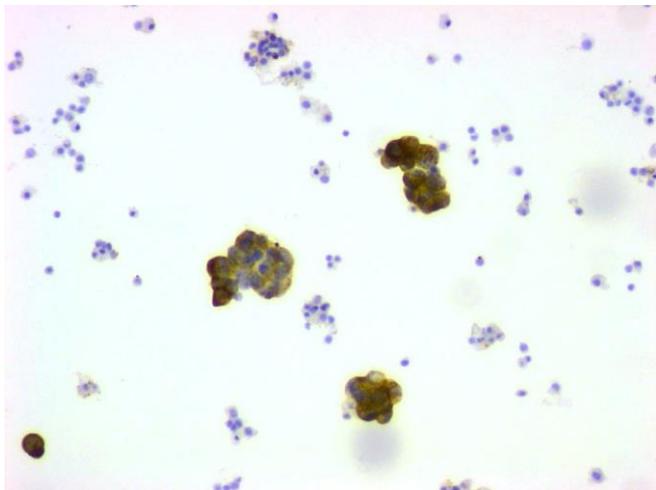


Figure 2. Ascitic fluid involved by metastatic UCC shows diffuse membrane CK 7 reactivity (anti-CK 7 with 3,3 diaminobenzidine (DAB) chromogen)

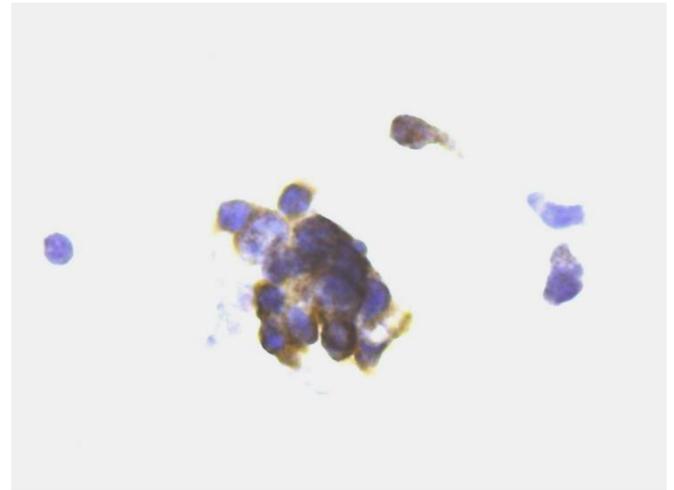


Figure 3. Positive cytokeratin 20 immunoreactivity (immunoperoxidase method x 200)

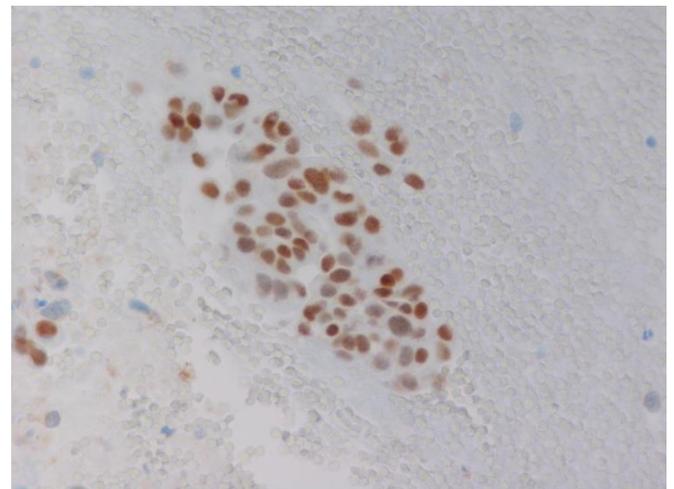


Figure 4. Strong nuclear expression of GATA-3 (IMMUNOPEROXIDASE METHOD X 200)

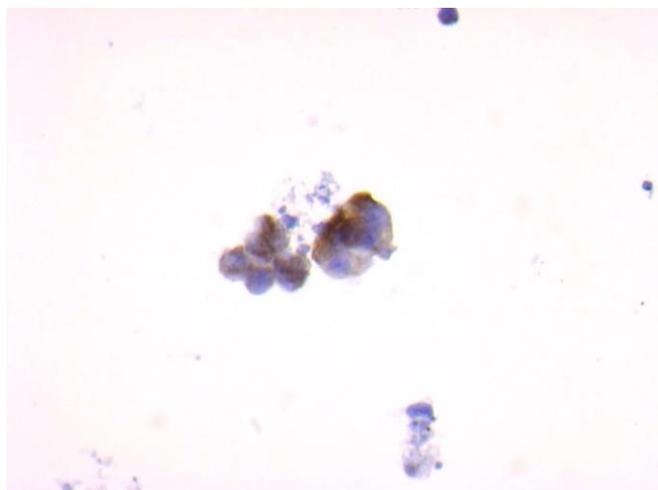


Figure 5. Malignant cells with focal granular cytoplasm staining with CA19-9 antibody (immunoperoxidase x 200)

Results

Cytological Finding

Morphologically, the first case was reported as a metastatic undifferentiated carcinoma. The second case was diagnosed as a metastatic carcinoma, probably of urothelial origin, while cytological diagnosis of the third case was metastatic urothelial carcinoma.

Immunocytochemical Results

All cases showed strong homogenous positive reaction to CK 7 and GATA-3, while the staining intensity against CK 20 was faint. Also, in all reported cases CA 19-9 immunopositivity was detected in a portion of the neoplastic population. Detailed immunocytochemical results are shown in Table 1.

Table 1. Antibodies contributed in the diagnosis of metastatic UCC

CASES/ MARKER	CK 7	CK 20	GATA-3 (nuclear positivity)	CA 19-9
Case 1	+++ >90%of neoplastic cells	+ >60%of neoplastic cells	+++ >80% of neoplastic cells	++ in 20%of neoplastic cells
Case 2	++ >80%of neoplastic cells	-	+++ >80% of neoplastic cells	+ in 15% of neoplastic cells
Case 3	++ >90% of neoplastic cells	+ >50% of neoplastic cells	+++ >60% of neoplastic cells	++ in 25% of neoplastic cells

+ : weak intensity, ++ : moderate intensity, +++: strong intensity

Discussion

A significant number of UCs of the bladder are primarily invasive or can subsequently progress to invasive and/or metastatic disease [11,12]. Flat tumors (carcinomas in situ, CIS) frequently progress to invasive disease [13]. Distant metastases occur largely in bone, lung and liver. Metastatic urothelial tumors may be difficult to distinguish from other poorly differentiated carcinomas because they are commonly positive for CEA [14]. Although a number of monoclonal antibodies directed against UCs have been produced in recent years, relatively few reports have been published on the definite use of such antibodies to diagnose UC. The study by G.P.Paner et al. [15] showed that GATA-3, S-100p, CK7, CK20, High Molecular Weight Cytokeratins and p63, in the appropriate differential diagnostic setting, may have important implications in the diagnosis of unusual bladder cancer histologies.

It is known that there are two different molecular pathways in UC pathogenesis:

- a) papillary pathway that leads to hyperplasia and superficial papillary tumors and
- b) non papillary pathway that leads to in situ lesions, invasive non papillary tumors and metastasis.

The utility of combined cytokeratin 7 and 20 immunoreactivity provides significant help in the identification of the primary site and the urothelial origin of malignancy. Tumors expressing, both CK7 and CK20, frequently but not always, correspond to UCs of the bladder; however, in one of our cases, CK20 was negative (Table 1). The percentage of positive CK20 in UC ranges from 15% to 97% in different studies [16]. Cytokeratin 7 can be used as an indicator for urothelial differentiation, especially in males, since it distinguishes urothelial malignancies from prostate cancer.

GATA binding protein 3 (GATA-3) is a transcription factor which belongs to a distinct family of tumor suppressor genes. It is involved in human cancer cell growth and differentiation, and plays an important role in cell proliferation and apoptosis. GATA-3 immunocytochemical negativity cannot also exclude UC especially when UC is derived from flat lesion (carcinoma in situ).

Moreover, although the monoclonal antibody to GATA-3 has been shown to be significantly expressed in most urothelial carcinoma variants, other tumors are known to express GATA-3 significantly i.e. breast carcinoma (lobular and ductal), basal cell carcinoma of the skin, malignant mesothelioma, paraganglioma, and chromophobe renal cell carcinoma, according to the study of Miettinen et al. [17].

In terms of metastatic UC to the lung, GATA-3 is a useful marker as the most common problem is discriminating UC from a primary pulmonary squamous cell carcinoma (SCC) [18]. Moreover, distinguishing invasive high-grade UC from other carcinomas occurring in the genitourinary tract, such as high-grade prostatic adenocarcinoma, spread from an anal SCC or spread from a uterine cervical SCC, can be challenging.

In addition GATA-3 level of expression was found to be an independent factor predicting cancer recurrence. Furthermore, strong GATA-3 expression was noted to have an effect on tumor size in patients with UC [19, 20].

Carcinoma Antigen 19-9 (CA 19-9) is a blood group-related carbohydrate antigen that reacts specifically with sialyl Lewis-containing glycolip. Blood group antigens are a group of carbohydrate structures bound to membrane lipids or proteins of red cells and certain epithelial tissues, including urothelium [8]. In normal urothelium, CA 19-9 is generally present in the cytoplasm, and it is reported to be expressed on cell membranes in non-invasive carcinomas, and in the cytoplasm of invasive cancer cells [14, 21]. CA 19-9 has been

clinically used as a tumor marker for pancreatic cancer, colorectal cancer; there is limited data on the use of CA 19-9 as a tumor marker in bladder cancer [22]. Recently, it has been reported that increased levels of urinary or serum CA 19-9 indicate poor prognosis in patients with bladder cancer [9, 10, 23, 24].

In the present report, all three cases exhibited markedly elevated serum CA 19-9 levels, so that, clinically, a pancreatic primary was suspected. However, the cytomorphologic findings combined with the immunohistochemical profile of the tumor cells and the clinical history of the patients permitted the diagnosis of metastatic UC of the bladder. Positive CA 19-9 cytoplasmic immunoreactivity was actually found in a number of carcinoma cells in all cases. There are rare reports in the English literature about combined serum and immunohistochemical detection of CA 19-9 in urothelial cancer; Camain and colleagues [17] reported the case of a 75-year-old woman with liver metastasis from urothelial carcinoma and marked increase in serum CA 19-9 levels [25]. Kuroda et al. reported a case of combined

small cell and conventional urothelial carcinoma of the bladder producing high levels of serum CA 19-9; in the latter, CA19-9 expression was confirmed in cancer cells of both tumor components [26]. The mechanism of enhanced CA 19-9 expression in bladder cancer is unclear. Increased production by tumor cells and decreased clearance due to urinary tract occlusion might be the cause. In a study by Nagao et al. overproduction of CA 19-9 by the bladder cancer cells was postulated to be the reason for the increased CA 19-9 urinary levels found in bladder cancer patients [27].

In conclusion, our cases reinforce the view that metastatic urothelial carcinoma may overexpress CA 19-9 leading to an elevation of the serum CA 19-9 levels, thus highlighting the ominous significance of serum CA 19-9 elevation in patients with bladder cancer. Immunohistochemical detection of CA 19-9 in cytological material from metastatic tumors of unknown origin may have some diagnostic significance indicating, in the proper setting, an urothelial primary, among other sites

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