

# Immunohistochemical diagnosis of Krukenberg tumors

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## Abstract

The diagnosis of Krukenberg tumors, as in other types of metastatic tumors of unknown primary origin, can often be a challenge for clinicians. In many cases, traditional diagnostic methods are insufficient, requiring immunohistochemistry analysis for identifying the origin of metastatic tumors. In our study, we examined a total of 34 female patients with Krukenberg tumors with different sites of the primary tumor: gastric ( $n=18$ ), colorectal ( $n=6$ ) or breast ( $n=7$ ) and tumors with unknown origin ( $n=3$ ). Cytokeratin (CK) 7 and CK20, carcinoembryonic antigen (CEA) and cancer antigen (CA) 125 were applied. The analysis of immunohistochemical profiles for CEA and CA125 showed that, regardless of the histological origin, the predominant immunohistochemical profile was CEA(+)/CA125(-). CK7/CK20 profile was different depending on the histological origin of the Krukenberg tumors. Thus, for the cases of gastric origin, CK7(-)/CK20(-) was present in 66.7% (12/18) of the cases. For the cases with colorectal origin, the predominant immunohistochemical profile was CK7(-)/CK20(+), in a percentage of 66.7% (4/6). The combination CK7(+)/CK20(-) was found in 85.7% (6/7) among cases of breast origin. Consequently, the immunohistochemical profile CK7/CK20 can have a key role in identifying the primary tumor in patients with Krukenberg tumors of unknown origin.

**Keywords:** Krukenberg tumors, cytokeratin 7, cytokeratin 20.

## Introduction

The Krukenberg eponym dates from 1896, when he describes five cases of ovarian tumors that it considers primitive neoplasms and which it calls “fibrosarcoma of the ovary mucocellular carcinomatodes” [1]. By studying other ovarian tumors that correspond to this description, Schlagenhauser shown that they are, in fact, metastatic tumors of epithelial cancers. Despite this specification, the term “Krukenberg tumors” was used later in various ways, from any ovarian tumor coexisting with another cancer to the strict sense of ovarian metastasis of gastric cancer [2]. Thus, since the definition was necessary in 1973, Krukenberg tumors are defined by the *World Health Organization* (WHO) as ovarian carcinomas characterized by the presence of stromal involvement, mucin-producing neoplastic signet ring cells, and ovarian stromal sarcomatoid proliferation [3].

The realization of a complete epidemiological picture of the mortality and incidence of Krukenberg tumors, however, faces a number of difficulties. Krukenberg’s tumor definition criteria vary from study to study, or sometimes are not specified. The frequency of these tumors is reported in a variety of ways, both in terms of total digestive cancers or as a proportion of all ovarian neoplasia or ovarian metastases alone. Deciphering the epidemiology of Krukenberg tumors could be facilitated by the theoretical premise that their epidemiological parameters vary with the epidemiological parameters of the origin cancers, their mortality and incidence being directly proportional to the cancers that constitute their starting points. In this context, the literature states that it occurs in 3–14% of patients with digestive cancers, or between 1–18% of all ovarian tumors [4–6].

The majority of the patients are between the ages of

20 years and 60 years [7] and it is more common in premenopausal women than in postmenopausal [8]. Diagnosis of the Krukenberg tumor does not usually raise problems in the situation where the metastatic tumor appears as a poor evolution of a previously known digestive tumor. Also, diagnosis of Krukenberg tumors is easy when they occur with a digestive tumor. The situation changes when initially identifying a malignant ovarian tumor mass that requires a prompt answer to the question: is the tumor primary or metastatic?

Therefore, diagnosis of Krukenberg tumors can sometimes be a challenge for clinicians. The most commonly used tumor markers in immunohistochemical diagnosis of metastatic ovarian tumors are cytokeratins: CK20 and CK7. With the desire to respond as accurately as possible to the question of “where does this ovarian mass come from?”, we tried to identify the immunohistochemical profile with the best predictive value for the most common types of cancers (gastric, colorectal and breast) which develop in their evolution Krukenberg tumors.

## Patients, Materials and Methods

In our study was analyzed retrospectively an important oncological casuistry, represented by the female population with digestive and genital oncological pathology, which addressed to the Surgery Clinic IV, “Iuliu Hațieganu” University of Medicine and Pharmacy, Cluj-Napoca, Romania, in 2006–2016. Were analyzed 34 cases with Krukenberg tumors, which performed total bilateral hysterectomy with bilateral adnexectomy, in addition to the multimodal treatment of the origin tumor. The patients studied were aged between 23 and 74 years, and only those cases that met the criteria of the WHO for the definition of Krukenberg tumors were included in the study.

Patients had the origin tumor localized at the gastric ( $n=18$ ), colorectal ( $n=6$ ), or breast ( $n=7$ ). There were also cases whose primary tumor could not be specified ( $n=3$ ).

Paraffin blocks were identified for the 34 patients and stainings for CK7, CK20, carcinoembryonic antigen (CEA) and cancer antigen (CA) 125 were performed. A block of paraffin containing a representative fragment of the ovarian tumor was selected for each case. Sections of 3  $\mu$ m of paraffin blocks and stained by the usual Hematoxylin–Eosin method were performed. Immunohistochemistry was performed on 3  $\mu$ m sections of tissues fixed in 10% buffered formalin and included in paraffin blocks by indirect technique performed with a polymer detection system. The sections of the tumor tissue were spread on poly-L-lysine treated slides, deparaffinized then in xylene and rehydrated with alcohol. Antibody unmasking was performed by boiling in the microwave oven. Endogenous peroxidase was blocked on each section by incubation for 20 minutes with hydrogen peroxide. The sections were then incubated with the primary antibody: cytokeratin 7 (Dako, 1:50 dilution, clone OV TL 12/30), cytokeratin 20 (Leica, 1:100 dilution, clone PW31), CA125 (Leica, 1:100 dilution, clone 1) and CEA (Leica, 1:200 dilution, clone 12-140-10), at room temperature, for one hour. The polymer (Max Polymer Detection System – Leica Ref. RE 7280-k) was then applied for 30 minutes. Finally, the sections were incubated for 5 minutes with 3,3'-diaminobenzidine (DAB), stained with Mayer's Hematoxylin, clarified and mounted. The slides thus prepared were examined and photographed on a Zeiss Axioscope 2+ microscope. Negative control was obtained by replacing the primary antibody with a non-immune serum. For positive control, sections of colon carcinomas (for CK20 and CEA), lung carcinomas (for CK7) and pancreatic carcinomas (for CA125) were used. All diffuse and focal cytoplasmic and membrane diffusion stainings were considered positive regardless of intensity (+, ++ or +++).

The statistical analysis of the results (sensitivity, specificity and positive predictive value) was made by  $\chi^2$  (chi)-square test.

## Results

Immunohistochemical stainings were carried out with antibodies for CK7, CK20, CEA and CA125, in all the 34 cases, and the results obtained for each case, depending on the origin of the primary tumor, are shown in Table 1.

Immunohistochemistry analysis of CEA and CA125 markers indicates that of the 34 cases investigated, 97% (33/34) showed positive values for the CEA marker, including those of breast origin. One case, whose origin was not specified, had the negative CEA marker. In contrast, CA125 marker values were mostly negative, respectively 88.9% (16/18) of Krukenberg tumors of gastric origin, 66.7% (4/6) of the cases of colorectal origin and 57.2% (4/7) of the cases with breast origin. The analysis of the immunohistochemical profiles for these two markers depending on the histological origin of the Krukenberg tumor shows that for the cases of gastric origin, the most often noticed profile was CEA(+)/CA125(-) at the rate of 88.9%. For the Krukenberg

tumors of colorectal origin, the most common profile has also been CEA(+)/CA125(-) at a rate of 66.7% of the cases. At the cases of breast origin, the CEA(+)/CA125(-) profile was noticed in a percentage of 57.2% (Figure 1, A and B).

The results are summarized in Table 2. The sensitivity, specificity and the positive predictive values were computed with *chi*-square test.

**Table 1 – Immunohistochemical stainings and the origin of the primary tumor**

Origin	CK7	CK20	CEA	CA125
<b>Gastric</b>				
Positive	0+	6+	18+	2+
Negative	18-	12-	0-	16-
<b>Colon</b>				
Positive	0+	4+	6+	2+
Negative	6-	2-	0-	4-
<b>Breast</b>				
Positive	6+	0+	7+	3+
Negative	1-	7-	0-	4-
<b>Unknown</b>				
Positive	0+	1+	2+	1+
Negative	3-	2-	1-	2-

CK: Cytokeratin; CEA: Carcinoembryonic antigen; CA: Cancer antigen.

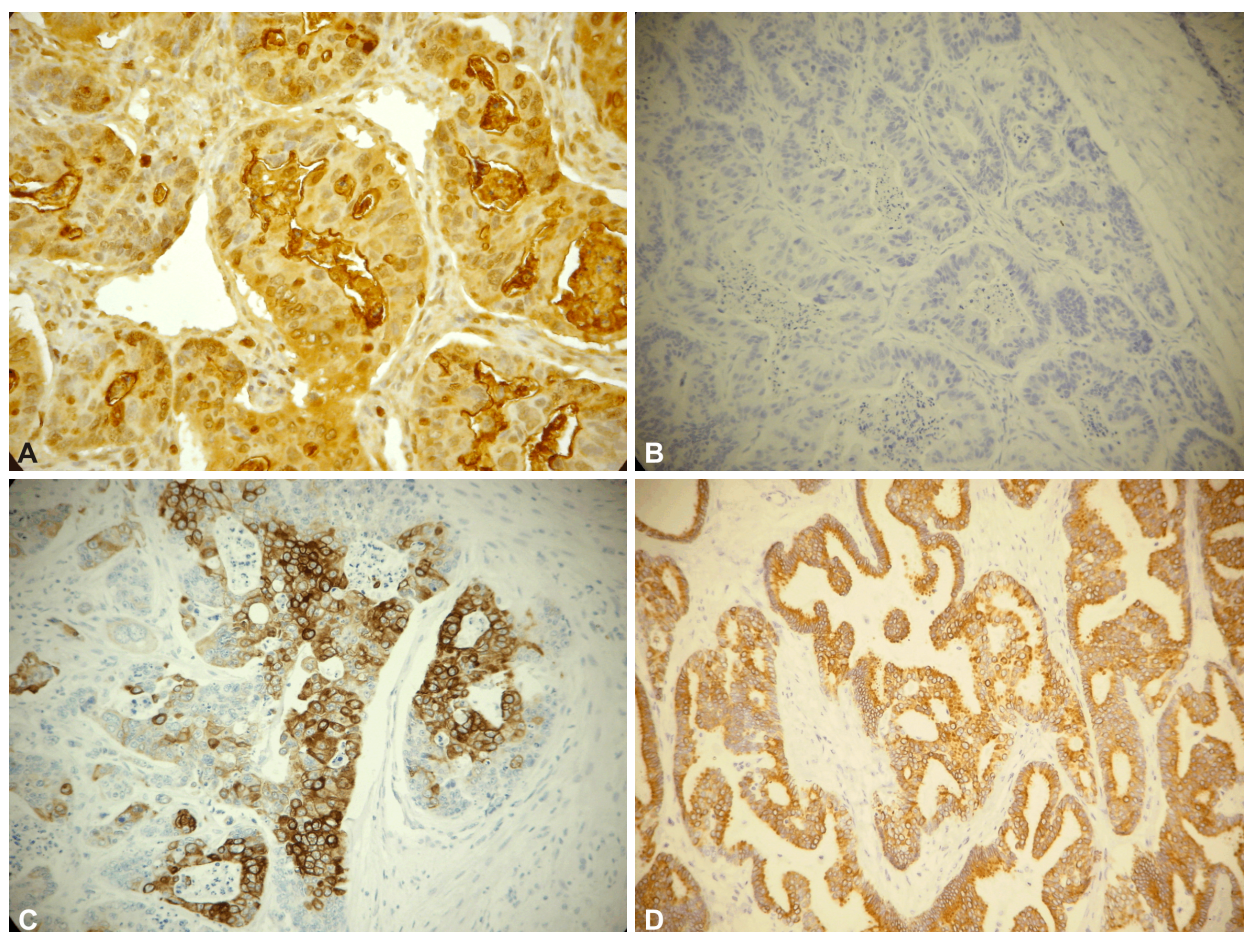
**Table 2 – Immunohistochemical profile CEA/CA125 and the origin of the primary tumor**

Origin of cases	Immunohistochemical profile CEA/CA125	Sensitivity [%]	Specificity [%]	Positive predictive value [%]
Gastric	CEA(+)/CA125(-)	88.9	38.5	66.7
Colorectal	CEA(+)/CA125(-)	66.7	20	16.7
Breast	CEA(+)/CA125(-)	57.2	16.7	16.7

CEA: Carcinoembryonic antigen; CA: Cancer antigen.

CK7 and CK20 were analyzed separately, based on the histological origin (Figure 1, C and D).

It was found that CK7 was negative in 24/34 cases, respectively 100% (6/6) of the cases of colorectal origin, 77.7% (14/18) of the cases of gastric origin and in all cases of unknown origin (100%, 3/3). In contrast, CK7 was positive in 85.7% (6/7) of cases of breast origin. CK20 was negative in 66.7% (12/18) of the cases of gastric origin, in 100% (7/7) of cases of breast origin and in 33.3% (2/6) of the cases of colorectal origin. CK20 was also positive in 33.3% (6/18) of the cases of gastric origin, 66.7% (4/6) of those of colorectal origin, and 33.3% (1/3) of the cases of breast origin. CK7/CK20 profile was different depending on the histological origin of the Krukenberg tumor. Therefore, for the cases of gastric origin, the most frequent immunohistochemical profile was CK7(-)/CK20(-), present in 66.7% (12/18) of the cases. For the cases of colorectal origin, the predominant immunohistochemical profile was CK7(-)/CK20(+), observed in 66.7% (4/6) of the cases. Among cases of breast origin, almost all (85.7%, 6/7) of the cases showed the CK7(+)/CK20(-) combination. Thus, in this study we identified the predominant immunohistochemical profiles for each origin, as Table 3 shows.



**Figure 1 – Krukenberg tumor, immunohistochemical analysis on surgical specimen: (A) CEA positive ( $\times 400$ ); (B) CA125 negative ( $\times 200$ ); (C) CK20 positive ( $\times 400$ ); (D) CK7 positive ( $\times 200$ ).**

**Table 3 – Immunohistochemical profile CK7/CK20 and the origin of the primary tumor**

Origin of cases	Immunohistochemical profile CK7/CK20	Sensitivity [%]	Specificity [%]	Positive predictive value [%]
Gastric	CK7(-)/CK20(-)	66.7	76.9	78.5
Colorectal	CK7(-)/CK20(+)	66.7	88	57.1
Breast	CK7(+)/CK20(-)	85.7	95.8	85.7

CK: Cytokeratin.

According to data obtained in this study conducted on 34 excised tissues from 34 female patients with Krukenberg tumors, it can be observed that CEA and CA125 tumor markers bring no benefit in differentiating metastatic tumors of gastrointestinal (gastric or colorectal) or breast origin, as the predominant immunohistochemical profile is identical in all three sites of the primary tumors. However, their analysis brings a significant benefit in differentiating metastatic tumors from the primary ovarian tumors. Immunohistochemical profiles of CK7 and CK20 are important milestones in the diagnosis of tumors of origin of Krukenberg metastases.

## Discussion

A metastatic tumor can often be the first manifestation of a neoplastic process, and in many cases, the primary

tumor remains unidentified despite the numerous investigations [9]. Not much is known about the factors that determine the variation in expression of CK7 and CK20 patterns present in gastric and colorectal carcinomas, but it is known that these cytokeratins are frequent negative in tumors of non-epithelial origin (lymphomas, sarcomas) [10].

In literature, few studies have evaluated these cytokeratins in Krukenberg tumors, the latest studies are rather case presentations. However, an important literature study [11] has analyzed the expressions of CK7 and CK20 for a large number of gastric and colorectal cancer cases. The proportion of CK7(+)/CK20(-) was the most common in gastric carcinomas and 46%, respectively, and was independent of Lauren's histological classification (46% in the intestinal type, 45% in the diffuse type). The CK7(-)/CK20(+) ratio was the most common in colorectal cancers (68%) and was dependent on histological grade (75% low vs. 52% high) tumor (46% on the right vs. 76% on the left). Furthermore, 42% of ovarian metastases of gastric origin were CK7(+)/CK20(-). All ovarian metastases of colorectal origin were CK7(-)/CK20(+), except for a case that was CK7(-)/CK20(-). In conclusion, in a large study, the expression of CK7 and CK20 patterns in gastric carcinomas varies greatly, whereas colorectal tumors are important for histological and tumor localization. The CK7(-)/CK20(+) pattern is specific for ovarian metastases of colorectal origin but has a low predictability for colorectal origin in metastatic ovarian carcinomas [11].



Also, another major study [12] analyzed 179 ovarian mucosal tumors, of which 53 primary tumors and 126 metastatic tumors. It was found that CK7(+)/CK20(+) was the most commonly encountered profile for primary ovarian tumors (74%), high (78%) and endocervical (88%) gastrointestinal tract. The immunohistochemical profile characteristic for distal intestinal tract tumors was CK7(-)/CK20(+), met in the proportion of (79%). CK7(+)/CK20(-) was found in 23% in primary ovarian tumors, 13% in high gastrointestinal tract and 13% in endocervical tumors but never in colorectal tumors [12].

Unlike previous studies conducted on a larger number of cases [11, 12], in our study the predominant immunohistochemical profile of ovarian tumors with gastric origin was CK7(-)/CK20(-), present in 12/18 (66.7%) cases. However, previous studies have failed to reach a consensus regarding the immunohistochemical profile of Krukenberg tumors of gastric origin. Furthermore, it is difficult to identify a single characteristic immunohistochemical phenotype for ovarian metastases of gastric cancer since the primary gastric site has different immunohistochemical phenotypes. Another major study in the literature [13], identified for gastric cancer developed on intestinal metaplasia the following immunohistochemical profiles: 66% CK7(+)/CK20(+), 24% CK7(-)/CK20(-), 2% CK7(+)/CK20(-) [13]. The CK7(+)/CK20(-) profile has been shown to be characteristic of the adenocarcinomas developed on the Barrett's esophagus [14].

There have been studies in which the expression of gynecological cytokeratins according to the Goseki classification was examined, with a high variability of CK20 positivity in 18% of grade I (intestinal), 24% of grade II (mucinous) and 31% in grade IV (with ringed cell), and did not show CK20 immunoreactivity for grade III cancers (diffuse infiltrative type, mucin poor). In fact, the phenotype with CK7(-)/CK20(+) was observed in 20% of grade IV and 66% of grade II and was absent for grade I or grade III [15].

In conclusion, the high variability of CK7/CK20 profiles in gastric cancer was only partially explained in the literature by the histological subtypes. From a practical point of view, it is impossible to define a phenotype whose predictive value is sufficient to indicate the gastric origin of ovarian metastases. In fact, compared to other digestive or mammary origins, the different immunohistochemical algorithms for gastric origin have consistently recorded the lowest sensitivity [16].

In our study, this most unusual CK7(-)/CK20(-) profile had some of the best values of sensitivity, specificity and predictive values in the literature for gastric site. For cases of colorectal cancer, the cytokeratin profile was consistent with that in the literature, including the rate of sensitivity, specificity, and predictive value. There are no studies in the literature on the immunohistochemical profile for cases of Krukenberg tumors from tumors of mammary origin. For our seven cases of mammary origin, the immunohistochemical profile was CK7(+)/CK20(-). In the current study batch, three cases of Krukenberg tumors whose origin was not previously determined were included. Even in these cases, the definition of Krukenberg tumors according to the WHO has been respected. In addition, in all three cases, CK7 was negative, thus

excluding their ovarian origin, knowing that primary ovarian tumors (serous, endometrioid, mucinous, transitional cell carcinoma, or clear cells) show positive CK7. By interpreting the three cases of undetermined origin according to the profiles determined in our study, it can be assumed that two of them had gastric and colorectal origin.

Regarding CEA and CA125 tumor markers (as it can be seen in Table 2), both cases of digestive origin and those of non-digestive, respectively breast origin, have the same immunohistochemical profile, without being able to distinguish, with their help, between the gastric, colorectal or breast origin, as the specificity of this profile is reduced. According to the literature data, this profile is reversed for the primary ovarian tumors, namely CEA(-)/CA125(+) [17–20].

Therefore, the analysis of CEA and CA125 markers can be essential in determining the metastatic or primary origin of ovarian tumors and must be prior to or at least concomitant to other immunohistochemical assays targeting the differential diagnosis.

## Conclusions

Although many studies tried to identify an immunohistochemical phenotype, the expression of CK7 and CK20 patterns in gastric cancers varies widely. In practical terms, this translates into the impossibility to define a phenotype whose predictive value be sufficient to indicate the origin of gastric ovarian metastases. However, in our current study, this CK7(-)/CK20(-) profile presented among the best values of sensitivity, specificity and predictive values among in the literature for gastric site (66.7%; 76.9%; 78.5%). For colorectal origins, the cytokeratin profile was consistent with that of the literature, namely CK7(-)/CK20(+), and the immunohistochemical profile identified by us for tumors of mammary origin was CK7(+)/CK20(-). The immunohistochemistry analysis of CEA and CA125 markers may be instrumental in determining the metastatic or non-metastatic origin of ovarian tumors.

## Conflict of interests

The authors declare that they have no conflict of interests.

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