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The Clinical Value of Image Cytometry DNA Analysis in Distinguishing Branchial Cleft Cysts From Cystic Metastases of Head and Neck Cancer

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Objectives/Hypothesis: **A branchial cleft cyst presents as a lump in the neck that, generally, is easily cured by surgical excision. The preoperative diagnosis is based on clinical examination and, especially in the Scandinavian countries, fine-needle aspiration cytology. However, at times, the histopathological analysis of the excised cyst reveals a cystic metastasis of squamous cell carcinoma of the head and neck. If adequate diagnosis could be obtained preoperatively, patients would most likely fare better. The study was performed to investigate whether the diagnostic accuracy for these lesions could be improved preoperatively by image cytometry DNA analysis of the fine-needle aspiration cytology specimen.** ***Study Design:*** **Image cytometry DNA analysis was performed on the preoperative fine-needle aspiration cytology specimen and the surgical specimens from 51 patients with solitary cysts in the lateral region of the neck. Thirty-six patients were selected because there was a discrepancy between findings on fine-needle aspiration cytology and the final histopathological diagnosis or an uncertain cytological diagnosis. There were 25 metastatic squamous cell carcinomas and 3 thyroid cancers, there was 1 lymphoma and 1 sialoadenitis, and there were 21 branchial cleft cysts.** ***Methods:*** **The cytodiagnostic Giemsa-stained slides were destained in Methanol and then stained with Schiff's reagent. The paraffin-embedded material from excised cysts were cut and deparaffinized and then stained with Schiff's reagent. Ahrens image analysis was used for DNA analysis and lymphocytes were used as control cells. DNA valves exceeding 5c was regarded as aneuploid.** ***Results:*** **Image cytometry DNA analysis of the preoperative cytological specimen was possible in 41 of 51 patients. We found that in 53% of the cases with cystic metastasis, image cytometry DNA analysis, when possible, revealed aneuploidy, thus indicating malignancy. DNA analysis showed diploidy in all benign cases.** ***Conclusions:*** **Aneuploidy is highly specific for malignancy. Image cytometry DNA analysis increases the diagnostic sensitivity for malignant cystic metastasis and therefore is a valuable supplement to conventional cytological study for these lesions.** ***Key Words:*** **Image cytometry DNA analysis, branchial cleft cysts, head and neck neoplasm, cystic metastasis.**

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INTRODUCTION

The most common cystic lesion occurring in the neck is the branchial cleft cyst (BCC). Branchial cleft cysts occur most frequently in the third decade of life and are more frequent in male patients, and more than 75% of cases present in the anterior triangle of the neck as a persistent cystic swelling. Squamous cell carcinoma of the head and neck (HNSCC) produces regional metastasis in the deep cervical nodes. In the majority of cases, these cases present as firm, solid masses in one of the designated lymph node levels.[1,2] However, the metastatic carcinoma may undergo cystic degeneration and present as a cervical cyst that mimics a BCC, especially when they occur at levels II to IV and the primary cancer lesion is not evident.[3–5]

The differential diagnosis depends on the age of the patient, and especially in patients over the age of 40 years, the possibility of a metastatic cervical lymph node should be considered.[6,7] There is also the possibility of a primary cervical neoplastic cyst or "branchiogenic carcinoma," although this is rare.[8,9] Detecting malignancy within a cyst can be difficult. Radiological examination is generally not helpful, and fine-needle aspiration cytology (FNAC) is not always reliable.[10] The false-negative rate of FNAC in cystic lesions (i.e., when malignancy remains undetected) has

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been reported to range from 50% to 67%.[6,11,12] The mistaken diagnosis of a cystic metastasis has serious consequences: The patient may not be urgently treated, the search for a possible primary tumor is delayed, and the surgical technique for a BCC differs considerably from what is adequate surgical treatment when dealing with neck metastasis.

Aberrant DNA values are seen in the majority of cases of HNSCC when image cytometry (ICM) technique is used in instances when single cell nuclei are investigated.[13] The present investigation was undertaken to clarify whether ICM DNA analysis contributes any diagnostic information to conventional cytomorphological study of FNAC specimens when a distinction between BCC and cystic metastasis of HNSCC is required.

MATERIAL AND METHODS

Fifty-one patients (24 female and 27 male patients with a mean age of 50.2 y) with a solitary cervical cystic lesion in the lateral region of the neck who were referred to the departments of otorhinolaryngology of the Karolinska Hospital (Stockholm, Sweden) and Umeå Hospital (Umeå , Sweden) from 1988 to 2000 were included in the study. None of these patients presented any clinically apparent head and neck cancer at the time of admission. Thirty-six of these patients were selected because of a discrepancy between results of FNAC and the histopathological findings of the excised cysts or uncertain cytological analysis, and 15 patients in whom both FNAC and histopathological examination showed BCC were also investigated. Image cytometry DNA analysis was performed on the FNAC specimen obtained before surgery, as well as on the surgically excised specimen from all patients.

DNA Analysis

The cytodiagnostic slides (FNAC) stained with Giemsa were destained in concentrated methanol. The slides were stored in buffered 4% formalin overnight. The slides were rinsed in distilled water and subjected to acid hydrolysis (5N HCl, 22°C for 1 h). After being rinsed in distilled water, they were stained with Schiff's reagent at room temperature for 2 hours. They were washed in distilled water and thereafter washed three times for 10 minutes in sodium bisulfite (10 mL 10% $Na_2S_2O_5$, 10 mL 1 mol/L HCl, 180 mL distilled water) and washed in tap water and then in distilled water, and dehydrated in ethanol series and xylene.[14] The archival formalin-fixed, paraffin-embedded material from the excised cysts was cut (4 μm), deparaffinized in xylene, immersed in a descending ethanol series, and then stored in buffered 4% formalin overnight. Thereafter, the slides were treated as previously mentioned.

Evaluation of Nuclear DNA Content

For the quantitative DNA analysis, a densitometric device, the Ahrens image analysis system (Bargtheide, Hamburg, Germany), was used. This is a TV-based system equipped with a charge-coupled device (CCD) video camera connected to a Nikon microscope. In the excised specimen the DNA content of 100 cell nuclei per specimen was registered. Lymphocytes were used as control cells to establish the normal diploid 2c value.[15] Normal diploid epithelial cells contain 2c DNA. Before mitosis the cell increases its DNA content to 4c, but a nuclear DNA content exceeding 5c is not normally seen in non-tumorous cell and is therefore regarded as aneuploid. A surgically resected cyst in which DNA analysis revealed greater than 5% aneuploid cell nuclei was regarded as aneuploid, whereas in the FNAC specimen, the lesion was considered aneuploid if any aneuploid cell nucleus was found.

RESULTS

Image cytometry DNA assessments of the FNAC specimen was possible in 41 of 51 cases. In the remaining specimens, hypocellularity, cellular debris, inflammatory cells, or initial handling and fixation made Feulgen staining and DNA analysis impossible.

Thirty-five patients had an initial diagnosis of BCC after FNAC, but histopathological examination of the excised cyst revealed squamous cell carcinoma (SCC) in 18 of them. Twelve of these 18 FNAC specimens could be DNA analyzed, and 6 of them showed aneuploidy.

Eight patients had an inconclusive diagnosis after FNAC (i.e., BCC or SCC). The histopathological examination of the excised cysts revealed SCC in six cases; one was a BCC and one was a lymphoma. Image cytometry DNA analysis of the FNAC specimens could be performed in five cases. In three of the five cases of SCC, ICM DNA analysis showed aneuploidy (60%), and the BCC specimen on FNAC was diploid.

Six patients were diagnosed with SCC after FNAC, but in five of them, histopathological examination of the excised cysts revealed BCC. Image cytometry DNA analysis was diploid in all of these cases.

Image cytometry DNA analysis was possible in all the excised cysts. Eighteen of 25 (72%) of the cystic metastases showed aneuploidy. All 15 patients (mean age, 39 y [age range, 18–58 y]) in whom finding on both FNAC and histopathological examination were benign were diploid at DNA analysis (100%). The mean age of patients with malignant cysts was higher (54.9 y) than for patients with benign cysts (39.0 y) (Table I).

CASE REPORTS

Case 1

A 26-year-old woman (patient 5) presented with a few-weeks' history of a cervical swelling on the left anterolateral region of the neck in December 1988. A complete otorhinolaryngological examination did not reveal any malignancy. The 7 × 5-cm mass was not tender, was movable, and was lying in the anterosuperior triangle. Results of FNAC showed normal squamous cells, macrophages, and inflammatory cells. The cytological diagnosis was BCC. The cyst was excised in February 1989. The histopathological diagnosis was metastatic SCC. Subsequent panendoscopy and random biopsies could not localize any primary tumor site. The patient was given radiation therapy. In December 1995, an SCC lesion at the left side of the tongue base was noticed. The patient underwent a tongue base–pharyngeal resection and had reconstruction with a free radial flap. DNA analysis of the preoperative FNAC specimen in December 1988 and the excised cyst in February 1989 showed aneuploidy.

Case 2

A 79-year-old woman (patient 30) noticed a cervical swelling on the right side in April 1999. She was referred to an otorhinolaryngologist in May 1999 after findings on FNAC showed SCC. Clinical examination did not reveal any primary tumor within the head and neck region. Computed tomography (CT) scan of the neck did not show any disease other than the solitary cystic tumor in the right anterolateral region of the neck. A new finding on FNAC in

TABLE I.
Clinical, Cytologic, Histologic, and DNA-analysis Data of All Patients.

Patient No.	Sex	Age (y)	FNAC	Histology of Excised Cyst	DNA-content of FNAC	DNA-content of Excised Cyst	Primary Tumor
1	M	66	BCC	SCC	Aneuploid	Aneuploid	Tonsil
2	M	72	BCC	SCC	Aneuploid	Diploid	Unknown
3	F	43	BCC	SCC	Aneuploid	Aneuploid	Tonsil
4	M	58	BCC	SCC	Aneuploid	Aneuploid	Tonsil
5	F	26	BCC	SCC	Aneuploid	Aneuploid	Tongue base
6	M	65	BCC	SCC	Aneuploid	Aneuploid	Tongue base
7	M	49	BCC	SCC	Diploid	Diploid	Unknown
8	F	52	BCC	SCC	Diploid	Aneuploid	Tonsil
9	M	37	BCC	SCC	Diploid	Aneuploid	Tonsil
10	M	52	BCC	SCC	Diploid	Diploid	Unknown
11	F	57	BCC	SCC	Diploid	Aneuploid	Unknown
12	M	52	BCC	SCC	Diploid	Diploid	Unknown
13	F	35	BCC	Thyroid cancer	Diploid	Diploid	
14	F	24	BCC	Thyroid cancer	Diploid	Diploid	
15	M	52	BCC	SCC	Not possible	Aneuploid	Tonsil
16	M	53	BCC	SCC	Not possible	Aneuploid	Unknown
17	M	47	BCC	SCC	Not possible	Aneuploid	Nasopharynx
18	M	70	BCC	SCC	Not possible	Aneuploid	Tonsil
19	F	57	BCC	SCC	Not possible	Aneuploid	Palate
20	M	58	BCC	SCC	Not possible	Aneuploid	Tonsil
21	F	66	BCC/SCC	SCC	Aneuploid	Aneuploid	Tongue base
22	F	65	BCC/SCC	SCC	Aneuploid	Aneuploid	Unknown
23	M	44	BCC/SCC	SCC	Aneuploid	Aneuploid	Tonsil
24	M	64	BCC/SCC	SCC	Diploid	Diploid	Tonsil
25	F	40	BCC/SCC	BCC	Diploid	Diploid	
26	M	63	BCC/SCC	SCC	Diploid	Diploid	Unknown
27	M	72	BCC/SCC	SCC	Not possible	Diploid	Nasopharynx
28	M	47	BCC/SCC	Lymphoma	Not possible	Diploid	
29	F	61	SCC	BCC	Diploid	Diploid	
30	F	79	SCC	BCC	Diploid	Diploid	
31	M	59	SCC	BCC	Diploid	Diploid	
32	F	64	SCC	BCC	Not possible	Diploid	
33	F	61	SCC	BCC	Diploid	Diploid	
34	F	68	SCC	Sialoadenitis	Diploid	Diploid	
35	M	70	Sialoadenitis	SCC	Not possible	Diploid	Tongue base
36	F	39	Thyroid cyst	Thyroid cancer	Diploid	Diploid	

BCC = branchial cleft cyst; SCC = squamous cell carcinoma.

July 1999 persisted in metastatic SCC. Results on panendoscopy and nasopharyngeal as well as tongue base and ipsilateral tonsillectomy in August 1999 were negative. The patient underwent a neck dissection of the right side in September 1999. The histopathological diagnosis was BCC. DNA analysis of the preoperative FNAC specimen as well as the BCC showed diploidy.

DISCUSSION

Metastases from certain primary tumors of the oropharynx, particularly SCC of the tonsil, are often cystic.[6,16] The tendency for this tumor site to produce solitary cystic metastasis while the primary tumor is still occult is well documented and has led to misdiagnosis clinically and cytologically.[8,17] Palpation alone cannot distinguish between benign and malignant cysts, and the absence of a clinically detectable primary tumor makes the diagnosis difficult.

Fine-needle aspiration cytology is a useful technique when applied to solid cervical nodes because the false-positive and false-negative rate is low (1%–3%).[1,18] However, in cystic lesions it may be impossible to distinguish the cytological features of a BCC from a cystic metastasis of SCC.[3] The aspirate from a cystic lesion can be difficult to interpret as a result of hypocellularity, large quantities of inflammatory cells, and cellular debris.[19] Granström and Edström[6] in 1989 showed that cytological assessment of the aspirate from cystic metastasis had a high false-

negative rate. They recommended ipsilateral tonsillectomy in patients older than 40 years of age with a cervical cyst because in their study, 80% of cystic metastases originated from an occult tonsillar carcinoma.[6,20] A false-negative finding on FNAC leads to delay in searching for the primary tumor site, as well as delay in the onset of adequate oncological treatment. The surgeon violates the neck while performing cyst excision and misses the chance to take biopsy specimens from the most likely primary tumor sites (i.e., tonsil, base of the tongue, and nasopharynx) while the patient is under general anesthesia. Some studies have shown that violating a neck by performing an open biopsy of the cyst could not only delay the definitive treatment but also jeopardize cure.[21] However, this fact has also been questioned.[22] On the other hand, when the finding on FNAC is false-positive for malignancy, the overtreatment that will be performed may cause unnecessary morbidity.

Our study demonstrates the difficulties in assessing a correct diagnosis of cystic neck lesions with only cytological examination, and that DNA analysis allows improved diagnostic accuracy for these lesions. We had four different groups of patients. In the first group of 20 patients, the finding on FNAC was benign (i.e., BCC) but histopathological examination of the excised cysts revealed malignancy. Eighteen cases were SCC; DNA analysis was possible in 12 cases and 6 of the 12 (50%) showed aneuploidy. In the second group of eight patients, the finding on FNAC was inconclusive BCC or SCC. Later histopathological examination revealed SCC in six cases, and DNA analysis showed aneuploidy in three of the five lesions (60%) in which DNA analysis was possible. In the third group of six patients, FNAC did indicate malignancy but the results of histopathological examination were benign. DNA analysis showed diploidy in all five lesions in which analysis was possible (100%). Thus, if thyroid cancer cases are excluded, two-thirds (65%) of the patients in whom it was possible to analyze the FNAC specimen would have had benefit from an ICM DNA analysis preoperatively. Furthermore, all patients with BCC both on FNAC and on histopathological examination displayed only diploid cells.

Image cytometry DNA analysis was performed on all the FNAC specimens, but interpretation was possible in 41 of 51 patients, because of the sparse amount of cells obtained by aspiration, necrosis, and technical difficulties resulting from inadequate fixations, need for destaining, and so forth. Our study is a retrospective study that was performed on archived cytological material, and we suggest that the cytologist should aspirate enough material for at least three or four slides so that both conventional cytological analysis and Feulgen staining for DNA analysis can be performed primarily. The initial handling with immediate rinsing from the specimens of blood, mucus, and external contaminants and adequate fixation is important. DNA analysis is easier to perform on freshly obtained FNAC specimens than on archival material because the destaining procedure itself is difficult and time-consuming and may hamper the Feulgen staining.

When DNA analysis is performed on a resected cyst, it is in fact the cyst wall that is examined as a histopathological specimen. Because of the risk for overlapping of cell nuclei, a 5c exceeding rate greater than 5% is requested for aneuploidy. The cytological material from the fine-needle aspirations in general contains a sparse amount of cell nuclei for analysis, and the risk for undetected overlap of nuclei is low, which is why the lesion was classified as aneuploid if any aneuploid cell nuclei could be detected in the FNAC specimen.

In the present study, 10 of 25 patients (40%) with malignant histological findings (i.e., SCC) had the tonsil as the primary site of the tumor. Although this is a lower proportion than that found by Granstöm and Edström,[6] this confirms the theory that malignancies in the tonsils give cystic metastases to the neck in a higher rate than other malignancies in the head and neck region.

We do not know the incidence of SCC metastasis to the neck masquerading as a BCC. In the Stockholm/Gotland area of Sweden, a population of two million inhabitants, approximately 40 patients with BCC are surgically treated annually. Cystic metastasis from head and neck cancer was found in 1 patient in the year 2000 and in 4 patients in the year 2001. There is reason to believe that the risk increases with age and known risk factors for head and neck cancer (i.e., smoking and alcohol use). However, in our material we found both benign and malignant cystic lesions broadly overlapping age ranges; two patients younger than 40 years of age had cystic metastases, and five patients older than 59 years of age had benign branchial cleft cysts. As a result of our findings, the Stockholm Head and Neck Cancer Center has decided to recommend ICM DNA analysis on the FNAC specimen from all patients older than 30 years of age with a cystic lesion in the lateral region of the neck. Furthermore, before surgical resection of the lesion, a panendoscopy is performed to minimize the risk of missing a primary cancer lesion within the head and neck region.

CONCLUSION

Image cytometry DNA analysis appears to add valuable information to conventional cytological examination in the diagnostic assessment of cystic lesions in the neck. In our study, we found that 53% of SCC metastases showed aneuploidy on FNAC indicating malignancy. The risk for malignancy increases with age and risk factors, and we recommend that ICM-DNA analysis should be considered on all FNAC specimens from patients older than 30 years of age as a supplementary tool in the clinicopathological assessment of these lesions so that appropriate therapy can be instituted primarily. Further, panendoscopy preoperatively and, in suspect cases, frozen-section analysis preoperatively should be used. Because approximately 30% of SCC of the head and neck and many thyroid cancer lesions are diploid, the ICM DNA analysis will not be able to detect all malignant cystic lesions, and a diploid result does not rule out malignancy. However, our clinical experience clearly shows that the method is of considerable use because when aneuploidy is found, malignancy should be expected and the patient treated accordingly.

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