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HER2 status in sinonasal intestinal-type adenocarcinoma

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ABSTRACT

Given that the prognosis of patients with sinonasal intestinal-type adenocarcinoma (ITAC) has not significantly changed recently, there is a desire for new therapeutic approaches to improve clinical management. HER2-targeted therapy has remarkably improved the overall survival of patients with HER2 amplified tumors. To date, HER2 assessment has produced contradictory results in ITAC. The aim of this study was to assess HER2 status at both protein and DNA levels in a large series of ITAC. HER2 status was assessed by immunohistochemistry (IHC) and chromogenic in situ hybridization (CISH) in forty-three patients that underwent surgical resection for ITAC at the Otorhinolaryngology Section, Padua University Hospital, between 2007 and 2016. IHC was evaluated using the four-tier score developed for gastroesophageal cancer. As for IHC, 83.7% (36/43) of ITAC were scored 0, 14% (6/43) 1+, and 2.3% (1/43) 2+. No HER2 amplification was detected by CISH. The present is the largest study of sinonasal ITAC tested with both IHC and CISH confirmation for HER2 status. No HER2 overexpression/amplification was detected. Contrary to previous studies, our findings seem to rule out any oncogenetic role of HER2 in ITAC pathogenesis.

1. Introduction

Intestinal-type sinonasal adenocarcinoma (ITAC) is an aggressive neoplasm, morphologically and immunophenotypically similar to primary colonic adenocarcinoma [1]. It accounts for about 18% of sinonasal cavities adenocarcinomas, with an incidence of less than 1 case per 100,000 individuals per year, and occurs primarily in males [2,3]. The majority of ITACs (88%) has been associated with occupational exposure to wood and leather dust, with a hundredfold higher risk in exposed versus non-exposed population [2–4]. Local spread of the disease and recurrences are responsible for clinical outcome, which has not significantly changed over the last decades [5].

Human epidermal growth factor receptor 2 (*HER2/neu,* also known as *CerbB-2*, or *ERBB-2*) is a proto-oncogene located on chromosome 17q21, which encodes a transmembrane protein with tyrosine kinase activity, and belongs to the HER receptor family (EGFR, named also HER1, HER3, and HER4) [6]. HER2 is involved in signal transduction pathways, leading to cell growth and differentiation, as well as anti-apoptotic activity. HER2 amplification/overexpression is a reliable tumor predictive marker in breast and gastroesophageal cancer (in the latter being much more common for the intestinal subtype) and HER2-targeted therapy has remarkably improved the overall survival of patients with HER2-positive tumors [7,8]. HER2 status has been found amplified with different frequency in adenocarcinomas of various sites [9]. In colorectal cancer (CRC) HER2 overexpression has been reported with rates of positivity from 2% to 11% [10]. In ITACs, the results are contradictory [11–14], thus deserve further investigation. Indeed, the detection of HER2 amplification/overexpression in ITACs could disclose the possible use of anti-HER2 therapy.

In relation to the morphological, immunophenotypical, and molecular similarities between CRC and sinonasal ITACs, the aim of this study is to assess HER2 status at both protein and DNA levels in a large series of ITACs. Our hypothesis was that the frequency of HER2 overexpression/amplification in ITAC could be similar to that of CRC.

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2. Materials and methods

2.1. Patients and specimen characteristics

The study was carried out on the formalin-fixed and paraffin-embedded (FFPE) specimens of ITAC patients undergoing surgical resection from 2007 to 2016 at the Otorhinolaryngology Section, Padua University Hospital. Only surgically resected, and not bioptic material was used. According to internal protocols, after the excision, the surgical material was immediately placed in formalin and sent to the Pathology Department. The inclusion criteria were: immunohistochemically confirmed sinonasal intestinal-type adenocarcinoma (CDX2 and CK20 positive) and availability of enough good quality non-decalcified FFPE material. Information about exposure settings, post-operative therapy, and follow-up have been recorded. All the patients were preoperatively staged by total-body PET-CT, according to the appropriate NCCN guidelines at the time of the first diagnosis [15]. Overall, 43 FFPE samples (one from each patient) were retrieved from the archives of the Surgical Pathology and Cytology Unit. Two pathologists (RC and VM), blinded to clinical information, reviewed all the cases and confirmed the diagnoses, according to the 4th World Health Organization classification of Head and Neck Tumors [16], and also applying Barnes [17] and Kleinsasser & Schroeder [18] classifications. These systems classify ITAC into five (papillary, colonic, solid, mucinous, and mixed) or four categories (papillary-tubular cylinder cell, alveolar-goblet cell, signet-ring cell, and transitional), respectively [17,18]. Tumors were staged according to the 8th UICC TNM system [19]. The mean follow-up period was of 31 ± 17.3 months (range: 12–94 months). The follow-up schedule was: i) clinical examination and sinonasal endoscopic evaluation 2 and 4 weeks after surgery and then every month in the first year, every 2 months in the second year, and every 3 months in the third, fourth, and fifth years; ii) MRI with contrast medium after 6 months and 1 year (additional MRI was performed in the clinical suspect of local recurrence). In patients without metastasis or tumor relapse, a clinical evaluation once a year was performed after the fifth year of follow-up. This study followed the REporting recommendations for tumor MARKer prognostic studies (REMARK) guidelines [20,21]. Descriptive statistics were used to summarize the demographic and clinical characteristics of the study cohort.

Table 1
Clinical and pathologic data of 43 sinonasal ITACs.

	N = 43 (%)
Male	37 (84)
Age, mean (years)	68.9
Tumor location	
Nasal cavity and/or ethmoid sinus	38 (88)
Maxillary sinus	5 (12)
Side	
Right	22 (51)
Left	16 (37)
Bilateral	4 (12)
Disease stage	
I	4 (9)
II	19 (45)
III	12 (28)
IVa	7 (16)
IVb	0 (0)
IVc	1 (2)
ITAC subtype	
Papillary	2 (4)
Colonic	19 (44)
Solid	3 (7)
Mucinous	11 (26)
Mixed	8 (19)
Risk (exposure)	
Absent exposure	9 (20.9)
Wood, leather, or both	34 (79.1)
Follow-up (months)	
Mean, range	31 (12 – 94)
Recurrence	
Absent	35 (81)
Present	8 (19)
Status	
Non evidence of disease	33 (77)
Alive with disease	2 (4.6)
Dead of disease	4 (9)
Dead for other causes	2 (4.6)
Missing	2 (4.6)
Adjuvant therapy (n = 33)	
Radiotherapy only	27 (81.8)
Radio- plus Chemotherapy	5 (15.2)
Surgery only	1 (3)

2.2. Immunohistochemistry

Immunohistochemistry was performed on 4 μm-thick FFPE freshly cut unstained sections from each sample. Staining was done automatically (Bond III, Leica Biosystems, Newcastle upon Tyne, UK), as described elsewhere [22–24], using the Bond Polymer Refine Detection kit (Leica Biosystems) with the Bond Oracle HER2 IHC System (clone CB11; Leica Biosystems; prediluted, EDTA buffer). Sections were then counterstained with hematoxylin. Appropriate positive and negative controls were run concurrently. HER2 IHC was evaluated using the four-tier score developed for gastroesophageal cancer surgical samples [25]: 0 = no reactivity or membranous reactivity in < 10% of tumor cells; 1+ = faint/barely perceptible reactivity in ≥10% of tumor cells in part of their membrane; 2+ = weak-moderate lateral/basolateral/complete membrane reactivity in ≥10% of tumor cells; 3+ = strong lateral/basolateral/complete membrane reactivity in ≥10% of tumor cells.

2.3. CISH assessment of HER2 status

Chromogenic in situ hybridization (CISH) was performed using the Dako Her2 CISH pharmDx kit (Dako, Glostrup, Denmark), according to the manufacturer's protocol, on FFPE cancer tissue specimens, as described elsewhere [24,26]. Hot spot areas containing the highest HER2 counts were identified assessing at least 40 nuclei by counting HER2 and centromeric probe 17 (CEP17). Only nonoverlapping nuclei with distinct nuclear borders were considered. The ratio between HER2 and CEP17 was calculated, and the HER2 gene was considered amplified when the ratio of gene-specific HER2 to CEP17 signals was ≥2.0. At least 1 CISH-positive spot was needed to assign a case to the HER2-amplified category [24,26].

3. Results

3.1. Study population: clinical and pathologic features

Clinical and pathological data of the patients are summarized in Table 1. The mean age was 68.9 ± 12.7 years (range: 42–88 years) and the male to female ratio was 6.2:1. Sixteen tumors were located in the nasal cavity, 6 in the ethmoid sinus, 16 in both sites, and 5 in the maxillary sinus. The right side (51.2%) was more common than the left side (37.2%) and a minority of tumors was bilateral (11.6%). Tumor histologic subtypes were as follows: 2 papillary type or papillary tubular cylinder cell I (PTCC-I), 19 colonic type (PTCC-II), 3 solid type (PTCC-III), 11 mucinous type, and 8 mixed (transitional) type (Fig. 1). T-staging was: T1 in 4 cases (9.3%), T2 in 19 cases (44.2%), T3 in 13 cases (30.2%), and T4 in 7 cases (16.3%). Neck dissection was performed in one patient, with latero-cervical nodal metastasis. Only one patient presented with multiple lung metastases. Positive surgical

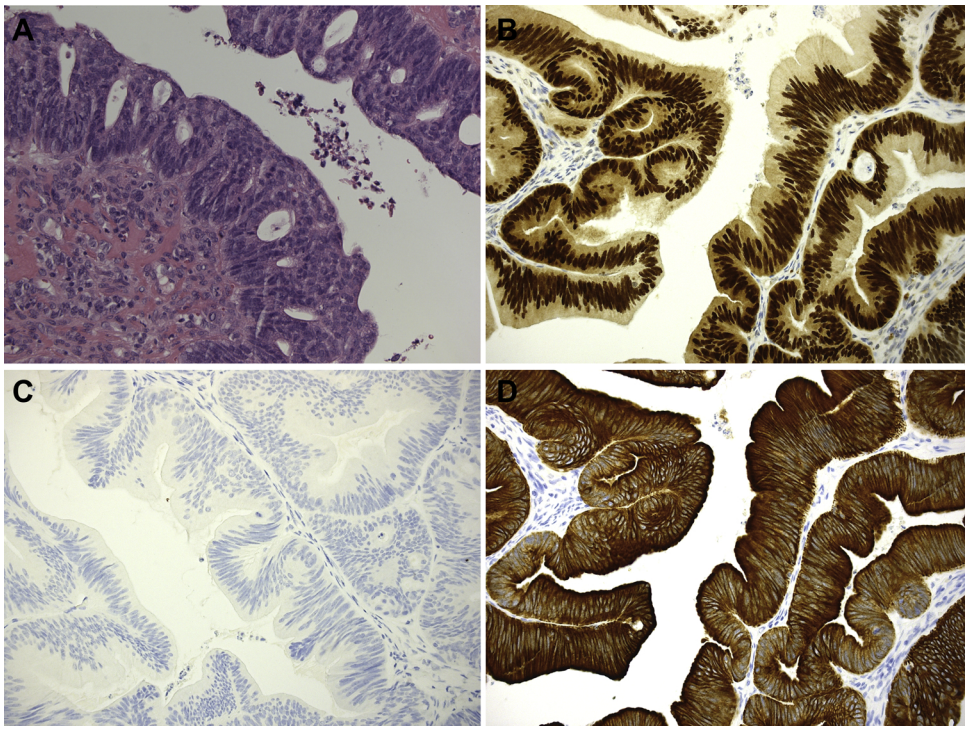

Fig. 1. Photomicrographs of a representative case of sinonasal intestinal-type adenocarcinoma from the investigated series. **A)** Hematoxylin and eosin (original magnification 20×). **B)** CDX2 nuclear positivity (original magnification 20×). **C)** CK7 negative staining (original magnification 20×). **D)** CK20 membrane staining (original magnification 20×).

margins were reported in 11 patients (25.6%). Data concerning post-operative therapy were available for 33 patients. Thirty-two patients (97%) received adjuvant radiotherapy, 5 of which also adjuvant chemotherapy. One patient was treated only with surgery. During the follow-up period we observed 8 local recurrences (18.6%) and 4 deaths of disease (9.3%). Most of the patients (79.1%) had a positive history of occupational exposures, especially to wood and leather dust.

3.2. HER2 status assessed by IHC and CISH

As for HER2 IHC (Fig. 2A and B), 36 (83.7%) tumors were scored 0, 6 (14.0%) 1+, and only one solid type tumor (2.3%) 2 + . No HER2 amplifications were detected by CISH (Fig. 2C and D).

4. Discussion

The present is one of the largest studies of sinonasal ITACs tested for HER2 status. The main result of the study is the absence of HER2

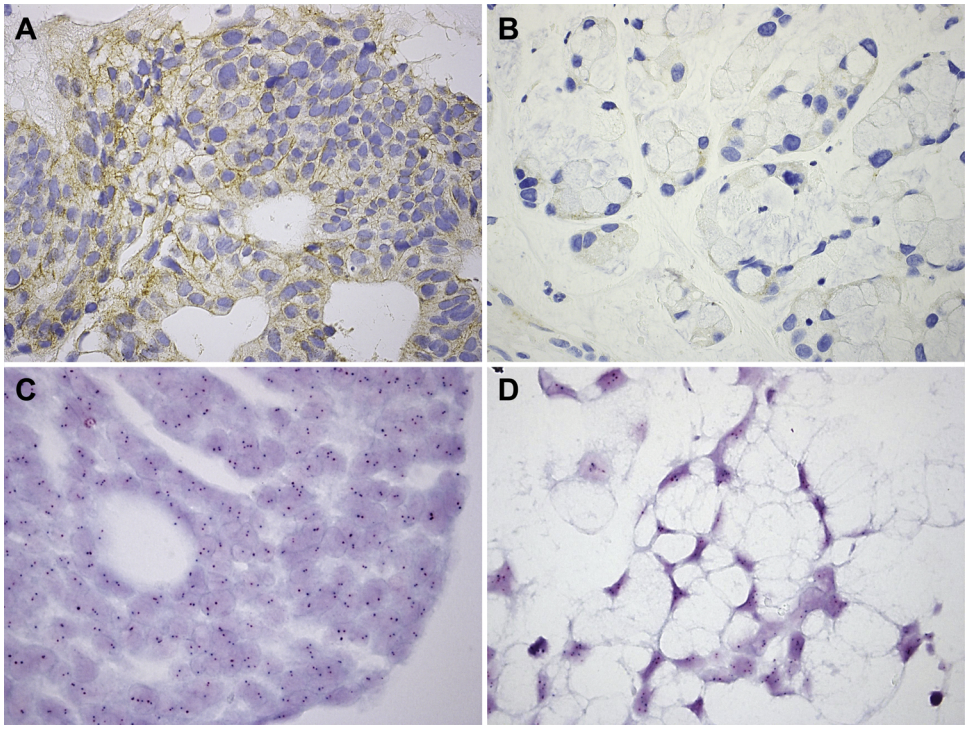

Fig. 2. Photomicrographs of two representative cases of sinonasal intestinal-type adenocarcinoma from the investigated series. **A)** HER2 immunostaining score 2+, defined as weak-moderate lateral/basolateral/complete membrane reactivity in ≥10% of tumor cells (case#4) (original magnification 40x). **B)** HER2 negative immunostaining, defined as no reactivity or membranous reactivity in < 10% of tumor cells (case#13) (original magnification 40x). **C & D)** Chromogenic in situ hybridization demonstrated the absence of HER2 amplification, respectively in case#4 and case#13 (original magnification 40x).

overexpression/amplification in all cases. Unlike other cancers, ITACs have few treatment options and evidence of specific targeted therapy in their management is scarce [27]. These tumors are rare and only a few studies have investigated a relevant number of cases.

Morphological and some genetic similarities between sinonasal ITAC and CRC suggested the hypothesis that analogous oncogenes, such as *HER2*, could be involved in the pathogenesis of both malignancies [11,28]. Contrasting results are available from the literature, with occasional ITACs reported to harbor HER2 overexpression/amplification [11–13]. Several factors may account for these differences, including the small sample size of the available series, the potential effect of preanalytical variables (such as cold ischemia, fixation duration, or decalcification), differences of methods (immunohistochemistry or in situ hybridization), different clones of antibodies used for IHC, lack of tumor-specific criteria for IHC interpretation and diverse scoring system, and analysis of subgroup of patients with heterogeneous clinico-pathologic characteristics.

Gallo and Vivanco-Allende immunohistochemically documented HER2 overexpression (along with some prognostic implications) respectively in 9 out of 28 [11] and in 5 out of 66 ITAC [12] cases. However, the definition of the HER2 assessment method used was somewhat ambiguous and the results obtained at the protein level were not supported by an analysis at the genetic level, which is the gold standard in other better-defined settings, namely breast and gastroesophageal cancer. Thus, the HER2 positive cases reported by these authors could not be determined by a gene amplification. Moreover, these studies did not discuss the eventual decalcification of the specimens and their cold ischemic time, which could be source of variability.

Bashir and colleagues, instead, reported a strong diffuse membrane HER2 immunoreaction (without stating if observed in more than 10% of cells) in 4 out of 11 cases of sinonasal primary non-salivary gland adenocarcinomas (2 intestinal type and 2 solid type cases). This finding was sustained by the detection of HER2 amplification using CISH only in one solid type tumor [13]. Another solid type adenocarcinoma resulted HER2 amplified, but with a 0 immunohistochemical score [13]. Of note, none of the amplified samples reported expressed CK20, more specific than CDX2 in the identification of ITAC [29], and CDX2 was not performed. These cases would be more appropriately classified as non-intestinal-type adenocarcinoma than ITAC [13,16]. Of the 2 intestinal type tumors with HER2 overexpression only one was CK20 positive. In the present series, 4 samples of solid type ITAC were tested and all were scored 0 with IHC and lacked HER2 amplification with CISH.

Nazar and colleagues reported a normal HER2 gene number in 13 primary and 2 recurrent ethmoid sinus adenocarcinomas, without any histological and immunohistochemical characterization, so a comparison with the present findings is impossible [14].

More recently, 552 head and neck carcinomas have been tested for HER2 status with both IHC and ISH techniques [9]. However, the histological types of the tumors were not reported, preventing a comparison with the present findings.

In the absence of a standardized method for HER2 evaluation in sinonasal ITAC, we chose to assess HER2 using the four-tier immunohistochemical score developed for gastroesophageal cancer given the similar morphology between intestinal type gastroesophageal carcinoma and ITAC [25]. This score is standardized, widely used, and reproducible. Among our cases, only one ITAC showed a 2 + HER2 score, and 6 of 43 cases were scored 1 + . Contrary with previous studies [11,12], we found lower HER2 expression levels with IHC. This variability could be explained by the difference in antibodies and diverse scoring systems. These previous studies used different clones than in our study, i.e. mouse monoclonal antibody mAbl (by Triton Diagnostic) [11], and HercepTest with a rabbit anti-human antibody [12]. Additionally, with regards to the scoring systems, the HER2 IHC criteria of the stomach are less restrictive than the breast. The percentage of positive cells (> 10%) used as a threshold is the same in these scoring systems and is coupled with a basolateral expression in the stomach, whereas requires a circumferential expression in the breast. So, we would not expect 3 + cases in our series using the breast scoring system. At the genetic level, we found no evidence of gene amplification in the complete series, but this result cannot be compared with the previous studies explained above. Therefore, the present is the first series of homogeneous sinonasal ITACs tested for HER2 status by cytogenetic technique. Moreover, overall concordance between IHC and CISH was excellent. This result supports HER2 IHC as a reliable tool in ITAC, as in breast and gastroesophageal cancer.

Besides morphological and immunophenotypical similarities between CRC and sinonasal adenocarcinomas, it is important to also consider the genetic differences [30–33]. The present study is in line with the idea that they are different entities, however, the number of cases does not supply enough evidence on this topic.

Patients diagnosed with ITAC, as other histological types in this anatomical site, have a poor prognosis, especially after relapse. The analysis of our study confirms that sinonasal ITACs are quite locally aggressive tumors, which rarely metastasize, often occurring in male patients with occupational exposure, mostly to wood and leather dust. The recurrence rate in our series (18.6%) is in line with existing literature, in the lower part of the reported range (17.6–49.6%) [34]. Four patients (9.3%) died of disease, 3 of which had been exposed to wood dust and relapsed before exitus, and all patients had been treated with surgical excision and adjuvant radiotherapy. Two cases were solid type, and the other 2 were colonic type ITACs. However the number of cases is too small to suggest any prognostic implication.

A strength of this study lies in the homogeneous features of the investigated population: i) all patients had similar occupational exposures; ii) initial treatment was the same for the entire population; iii) all patients were operated by the same surgical team; iv) scheduled follow-up visits were strictly observed. HER2 expression/amplification was analyzed only in surgical specimens (analogy of material) adhering to their current definitions and evaluating methods for gastroesophageal cancer (comparability of results). The main weaknesses concern the retrospective setting of the study and the relatively small sample size.

In conclusion, this is the largest study focused on HER2 status assessment with both IHC and CISH confirmation in patients with sinonasal ITAC. Both IHC and CISH techniques revealed no HER2 overexpression/amplification. Contrary to previous studies, the present findings seem to rule out any oncogenetic role of HER2 in sinonasal ITAC pathogenesis.

Authors' contribution

VM and RC: conception and design of the study, acquisition of data, analysis and interpretation of data, writing and revision of the manuscript. AZ: acquisition, analysis and interpretation of data. DC, EE and AM: acquisition of data. AF: study supervision, writing and revision of the manuscript. All the authors approved the final version of the manuscript.

Conflict of interest

The authors have no competing financial interests.

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