

ORIGINAL ARTICLE

## Intestinal-type Sinonasal Adenocarcinomas. Immunohistochemical Profile of 66 Cases<sup>☆</sup>

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### KEYWORDS

Sinonasal adenocarcinoma;  
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p53;  
p16;  
β-Catenin;  
E-Cadherin;  
Epidermal growth factor receptor;  
Human epidermal growth factor receptor 2;  
Cyclooxygenase-2

### Abstract

**Introduction and objectives:** Intestinal-type sinonasal adenocarcinomas are malignant epithelial tumours. Around 8%–25% of all sinonasal malignant tumours are intestinal-type adenocarcinomas, which are related to wood dust exposure. Four histological subtypes have been described: papillary, colonic, solid and mucinous. We performed a pathological and immunohistochemical study in order to describe characteristics with prognostic, diagnostic and therapeutic value, and also to compare our results with previous studies.

**Methods:** Sixty-six tumour samples were analysed and protein expression of p53, p16, E-cadherin, β-catenin, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2/neu) and cyclooxygenase-2 (COX-2) was performed by tissue microarray blocks.

**Results:** The 63% of cases were p53 positive; 37% showed nuclear staining with β-catenin and 100% with E-cadherin, while 98% showed membrane staining with β-catenin, 7% with EGFR, 8% with HER2/neu and 52% with COX-2; and 59% of the cases lost p16 expression.

**Conclusions:** Intracranial invasion was the worst prognostic associated event. Solid and mucinous tumours were the most aggressive histological subtypes. Intracranial invasion was more frequent in mucinous subtype tumours. Immunohistochemical results were similar in all tumour subtypes, except for mucinous tumours, which showed weak expression of E-cadherin and β-catenin. Comparing with previous studies, we found a lower expression of EGFR, HER2/neu and COX-2. The p16 expression was associated with worse survival and metastatic disease.

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**PALABRAS CLAVE**

Adenocarcinoma nasosinusal;  
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 p53;  
 p16;  
 $\beta$ -catenina;  
 E-cadherina;  
 Receptor del factor de crecimiento epidérmico;  
 Receptor 2 de factor de crecimiento epidérmico humano;  
 Cyclooxygenasa-2

**Adenocarcinomas nasosinuales tipo intestinal. Perfil inmunohistoquímico de 66 casos****Resumen**

*Introducción y objetivos:* Los adenocarcinomas nasosinuales tipo intestinal son tumores epiteliales malignos, que suponen el 8–25% de los tumores malignos nasosinuales. Se relacionan con la exposición al polvo de la madera. Se subdividen histológicamente en papilares, colónicos, sólidos y mucinosos. Realizamos un estudio patológico e inmunohistoquímico con el fin de establecer características con significado pronóstico, diagnóstico e incluso terapéutico, así como comparar con estudios previos.

*Métodos:* Estudiamos 66 muestras tumorales mediante matrices tisulares. Realizamos tinciones inmunohistoquímicas para p53, p16,  $\beta$ -catenina, E-cadherina, receptor del factor de crecimiento epidérmico (EGFR), receptor 2 de factor de crecimiento epidérmico humano (HER2/neu) y ciclooxigenasa 2 (COX-2).

*Resultados:* Un 63% de los casos son positivos para p53, el 37% para  $\beta$ -catenina nuclear, el 100% para E-cadherina, el 98% para  $\beta$ -catenina membranosa, el 7% para EGFR, el 8% para HER2/neu, el 52% para COX-2 y el 59% pierden la expresión de p16.

*Conclusiones:* La invasión intracraneal es el factor clínico pronóstico más importante. Los tumores de tipo sólido y mucinoso son los que muestran un comportamiento más agresivo, siendo los mucinosos los que mayor invasión intracraneal muestran. No existen diferencias inmunohistoquímicas entre los distintos subtipos histológicos, únicamente la tinción débil para E-cadherina y  $\beta$ -catenina, más frecuente en los de tipo mucinoso. El EGFR, HER2/neu y COX-2 muestran una positividad menos frecuente que en series previas. La positividad para p16 se asocia a una menor supervivencia y mayor frecuencia de enfermedad metastásica.

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**Introduction**

Sinonasal intestinal-type adenocarcinomas (ITAC) are rare, primary, malignant, epithelial tumours of the nasal cavity. They represent between 8% and 25% of sinonasal malignancies.<sup>1</sup> Their incidence in our environment is 0.19 cases per 100 000 inhabitants per year.<sup>2</sup> Their most common location (85% of cases) is the ethmoid region and upper part of the nasal cavity, followed by the maxillary sinus (10%), and exceptionally in the rest of sinus cavities.<sup>3</sup> The age of onset is between 50 and 60 years. It has been linked with a prolonged exposure to wood dust or leather; it is estimated that workers with such exposures have a 500 times higher risk of developing these tumours than the unexposed male population, and almost 900 times higher than the general population.

Sinonasal adenocarcinomas are divided into 2 main groups: ITAC and non-intestinal type sinonasal adenocarcinomas. In turn, ITAC are divided into 5 categories according to the classifications by Barnes and Kleinsasser: papillary (papillary-tubular cylinder cell [PTCC type I]), colonic (PTCC II), solid (PTCC III), mucinous (alveolar type with goblet cells and signet ring cells) and mixed (transitional).<sup>1,4</sup> Their survival at 5 years is between 20% and 50%. The main cause of mortality is due to local recurrence.<sup>1,5</sup>

Very little is known about the molecular alterations of ITAC. Comparative genomic hybridisation (CGH) shows frequent gains in chromosomal regions 7q, 8q, 11q, 12p, 20q, 1q, and 22, as well as losses in 4q31-qter, 8p23, 18q12–22, 5q11-qter, 8p, 9p and 17p.<sup>6–8</sup> Multiplex Ligation-dependent Probe Amplification (MLPA) has revealed losses in 18q, 5q, 13q, less frequent in 17p and gains in 8q, Xq, 12p, 1p, 11p and 19q, the last 3 not observed in other studies.<sup>9,10</sup>

ITAC present their own pattern of chromosomal gains and losses, which does not resemble that of squamous cell carcinomas (SCC) or colorectal adenocarcinomas (CAC), despite their histological similarity.

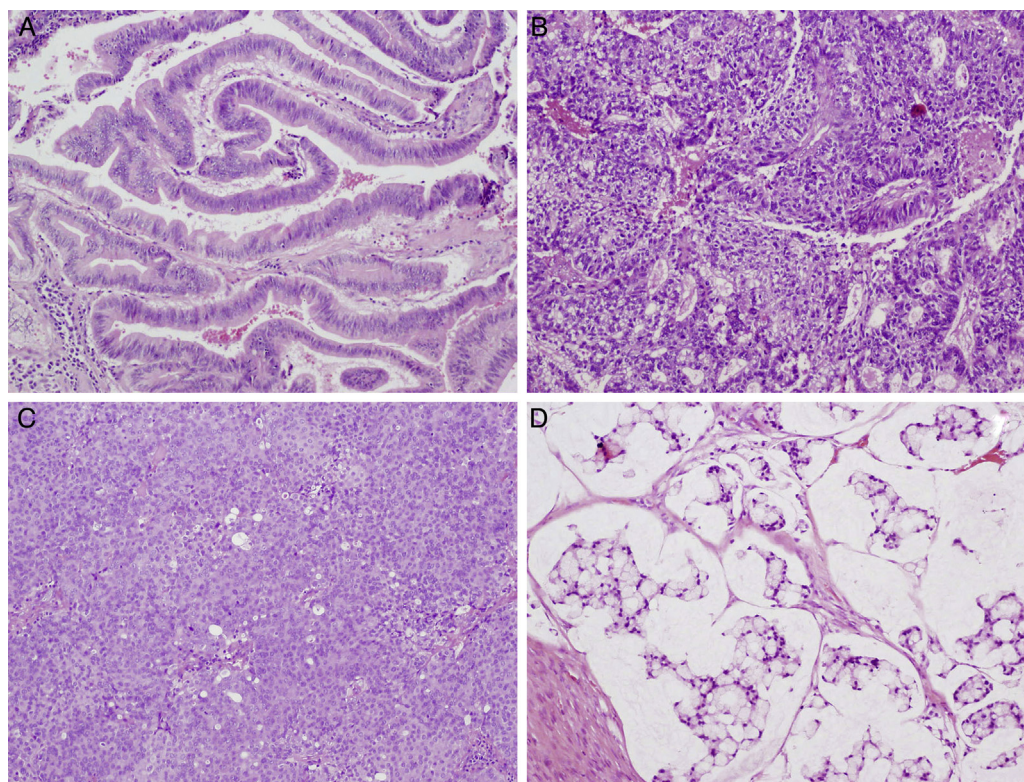
The immunohistochemical (IHC) profile of ITAC is characterised by positivity for pancytokeratin, epithelial membrane antigen, B72.3, Ber-EP4, BRST-1, Leu-M1 and "human milk fat globule" (HMFG-2),<sup>11</sup> for cytokeratin (CK) 20 in 73% and variable reactivity for CK7, between 43% and 93%. CDX-2 is frequently expressed in ITAC, reaching 90% in some series.<sup>12,13</sup>

In this study, we sought to establish the clinical, pathological and IHC profile of 66 intestinal-type sinonasal adenocarcinomas in patients exposed and not exposed to wood dust through tissue matrices. We selected markers implicated in tumourigenesis of CAC, like the products of oncogenes p53, p16 and  $\beta$ -catenin (WNT pathway). The enzyme COX-2 regulates the synthesis of prostaglandins implicated in tumourigenesis of CAC. Moreover, we also verified markers associated with aggressive tumour behaviour, such as E-cadherin, receptor 2 of the human epidermal growth factor (HER2/neu), and epidermal growth factor receptor (EGFR).

**Materials and Methods****Patients**

We conducted a retrospective study of 66 patients operated at our hospital with a diagnosis of ITAC between 1981 and 2006.

The sample consisted of 64 males (97%) and 2 females (3%) with a mean age of 63 years (range: 45–92 years). Of



**Figure 1** ITAC, histological subtypes (haematoxylin–eosin 200 $\times$ ): (A) papillary subtype with papillary architecture, minimal cytological atypia and scarce mitosis; (B) colonic subtype with tubuloglandular architecture plus pleomorphism and mitotic figures; (C) poorly differentiated solid subtype with solid or trabecular growth, marked pleomorphism and frequent mitotic figures; (D) mucinous subtype with mucus pools separated by thin fibrous septa and signet ring cells.

these, 54 cases (82%) presented a history of exposure to wood dust, with a mean exposure time of 25 years (range: 1–55 years). A total of 5 patients received preoperative treatment (7%): 3 of them with radiotherapy and 2 with incomplete surgery. After surgery, 49 patients (74%) received complementary radiotherapy. The monitoring of patients lasted for a mean 41 months (range: 1–217 months). During this period, 29 patients (44%) died due to the disease and 8 (12%) through other reasons. Locoregional recurrence took place in 34 cases (51%), distant metastases in 9 cases (14%) and intracranial extension in 14 cases (21%). The disease-free time presented a mean value of 29 months (range: 0–217 months).

Patients who received preoperative radiotherapy were excluded from the analysis of immunohistochemical results.

## Method

We used tumour tissue samples fixed in formol and embedded in paraffin. We reclassified cases according to the Barnes classification<sup>1</sup> (Fig. 1). We took 2 or 3 cylinders of 1 mm diameter from the most representative tumour area in each case and prepared 2 multi-tissue blocks measuring 2 cm $\times$ 2.5 cm. In total, we obtained 83 cylinders in each block and 66 cases.

We performed IHC staining for p53, p16, E-cadherin,  $\beta$ -catenin, EGFR, Her2/neu and COX-2.<sup>14,15</sup> Table 1 shows the antibodies and immunohistochemical techniques

employed. Fig. 2 shows the images of each of the dyes.

## Immunohistochemical Assessment

- P16: the percentage of positive tumoural cells was assessed by establishing 3 groups: 0:  $\leq$ 10%; 1: >10%–50%; and 2: >50%.
- P53 and EGFR: we used an IRS score (classification by immunoreaction) based on the staining intensity and the percentage of stained cells.

For p53 we used:

- Intensity: 0: negative; 1: weak positive; 2: moderate positive; and 3: intense positive.
- Percentage: 0:  $\leq$ 10%; 1: >10%–30%; 2: >30%–50%; 3: >50%–70%; and 4: >70%.

According to the IRS score=intensity $\times$ percentage of stained cells we classified into values: 1: 0–3; 2: 4–7; and 3: 8–12.

For EGFR we used:

- Intensity: 0: negative; 1: weak positive; 2: moderate positive; and 3: intense positive.
- Percentage: 0:  $\leq$ 10%; 1: >10%–30%; 2: >30%–50%; and 3: >50%.

**Table 1** Immunohistochemical Techniques Employed.

Staining	ARS	Ab	Dilution	IT, min
p53	pH9	p53 Clone Do7	1/100	15'
p16	pH9	p16 Clone E6H4	Cintec Histology kit®	
β-Catenin	pH9	β-Catenin Clone β-catenin 1	1/200	25'
EGFR	Proteinase K	Monoclonal EGFR Clone 2-18C9	Kit EGFR pharm Dx®	30.5'
E-cadherin	High pH	E-cadherin Clone NCH-38	1/50	20'
COX-2	pH9	COX-2 Clone CX-294	1/100	20'
Hercep test	ARS 1/10 95–99° 40 min	<i>Rabbit anti-human HER 2 protein</i>		

Ab: antibody; ARS: antigen recovery solution; IT: incubation time.

According to the IRS score=intensity×percentage of stained cells we classified into: 1: 0–3; 2: 4–6; and 3: 7–9.

We considered scores of 2 and 3 as positives and divided the sample into positive and negative cases.

- E-cadherin showed a diffuse membrane staining which we assessed as: negative (–): absence of staining; weak positive (+); positive (++)
- We performed 2 assessments for β-catenin:
  - Membrane staining with 3 groups: negative (–); weak positive (+); and positive (++)
  - Nuclear staining with 2 groups: negative (–) and positive (+).<sup>16</sup>
- COX-2: we observed a cytoplasmic staining with paranuclear reinforcement and constant intensity. We divided cases into negative (–) and positive (+) establishing the limit for positivity at the presence of at least 10% positive cells.
- HER2/neu: we assessed the presence or absence of membrane or cytoplasmic positivity.<sup>16</sup>

### Statistical Analysis

The clinical, pathological and IHC data were statistically analysed using the software package SPSS® version 19.0 for Windows (SPSS® Inc, Illinois, USA). Comparison of categorical variables was performed using the chi-square ( $\chi^2$ ) test considering as significant a value of  $P < .05$ . We estimated survival curves by the Kaplan–Meier method and analysed differences using the log-rank statistic. We considered as significant a value of  $P < .05$ .

## Results

### Tumour Stage and Histological Subtype

All but 1 case (M1) presented localised disease at the time of diagnosis. Regarding the local stage, 15 cases were diagnosed as T1 (22.7%), 6 as T2 (9.1%), 26 as T3 (39.4%), 11 as T4a (16.7%) and 8 as T4b (12.1%).

A total of 10 tumours presented papillary histology (15.2%), 30 presented colonic histology (45.5%), 10 were of solid type (15.2%) and 16 of mucinous type (24.2%). Cases with mixed histology were reclassified according to the histological type with a worse prognosis.

When we grouped the histological subtypes papillary with colonic and solid with mucinous, we observed that in stages T1, T2 and T3, the majority of tumours belonged to the first subgroup (66% in T1, 83% in T2 and 69% in T3). The opposite was true in stages T4a and T4b, that is, most tumours were of solid and mucinous type (54.5% in T4a and 75% in T4b) ( $P = .039$ ).

### Immunohistochemical Profile

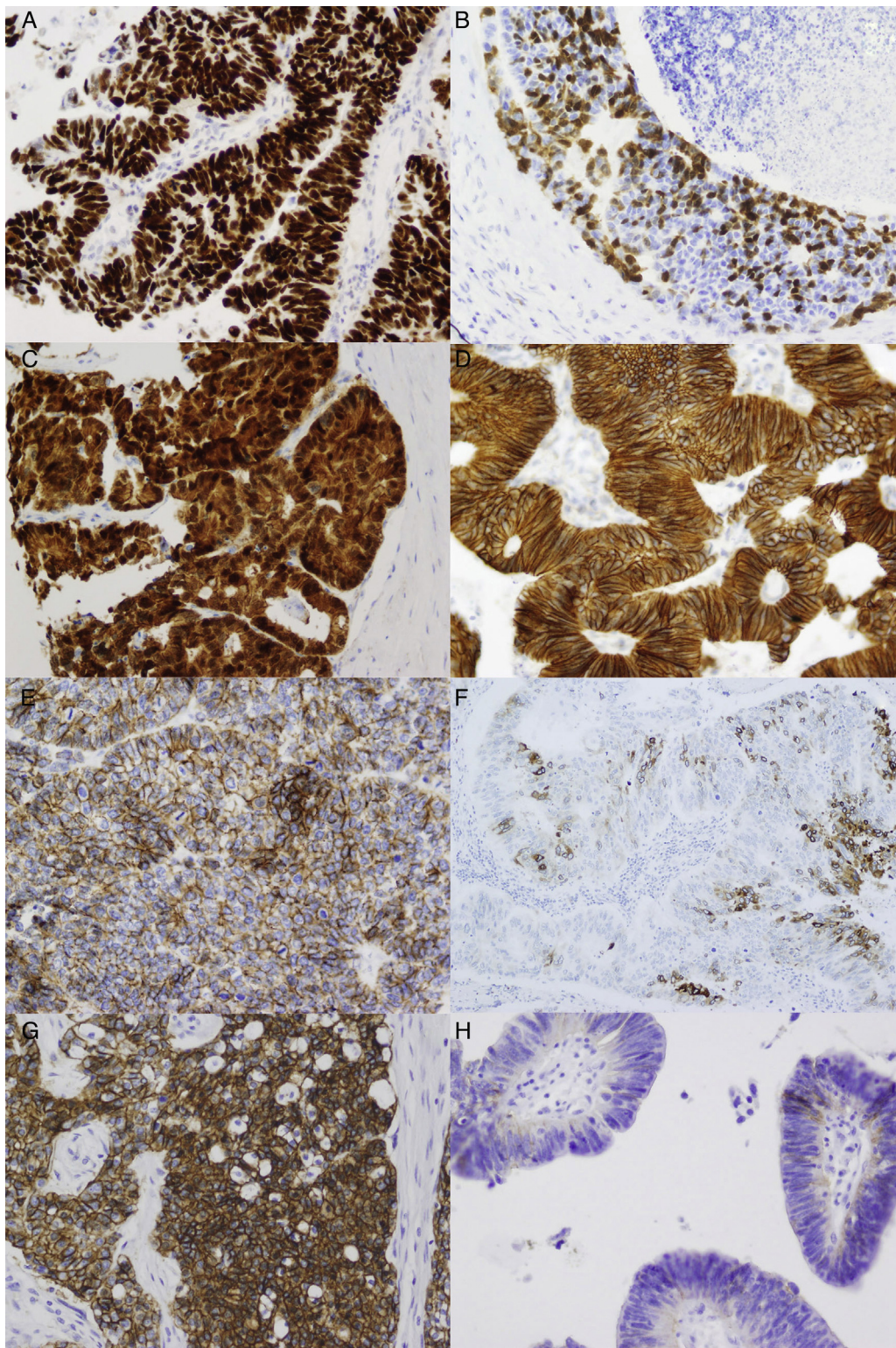
The immunohistochemical study excluded cases which received preoperative radiotherapy. A total of 63 cases were considered valid for immunohistochemical study.

For p53 we observed positivity in 63% of cases (46% with a score of 3). For p16 we noted a loss of positivity in 59%. Membrane staining with β-catenin took place in 98%. Nuclear staining with β-catenin was observed in 37%. Up to 7% of cases were positive for EGFR and all tumours expressed E-cadherin with variations in intensity. Staining for COX-2 presented a similar number of positive and negative cases, with the percentage of positives being 52%. As for c-erbB-2, the results reflected a small percentage of positive cases (8%) whose staining pattern was very dim. There were no significant differences in the expression of these immunohistochemical markers between the different histological subtypes. Neither were there differences between those cases with a history of exposure to wood dust and those without it.

The results of the IHC study of the various proteins are shown in Table 2.

### Association Between the Immunohistochemical Profile and the Clinical–Pathological Parameters

There was an increased frequency of distant metastases among the solid and mucinous histological subtypes. Up to



**Figure 2** Immunohistochemical markers (haematoxylin 200 $\times$ ): (A) p53: intense and diffuse nuclear staining; (B) p16: intense focal cytoplasmic and nuclear staining; (C) nuclear  $\beta$ -catenin: intense and diffuse nuclear and cytoplasmic staining; (D) membrane  $\beta$ -catenin: continuous membrane staining; (E) E-cadherin: diffuse membrane staining; (F) COX-2: cytoplasmic staining with focal paranuclear reinforcement; (G) EGFR: intense, complete membrane staining; (H) Hercep-test: minimum focal cytoplasmic staining.

**Table 2** Result of the Immunohistochemical Techniques Throughout the Entire Sample and Among the Different Histological Subtypes. Frequency (Percentage).

IHC		Sample No., %	Papillary No., %	Colonic No., %	Solid No., %	Mucinous No., %	Exposure to wood dust No., %	No exposure to wood dust No., %
p53	1	22 (37)	2 (22)	8 (30)	6 (67)	6 (43)	17 (34)	5 (56)
	2	10 (17)	2 (22)	6 (22)	0	2 (14)	8 (16)	2 (22)
	3	27 (46)	5 (56)	13 (48)	3 (33)	6 (43)	25 (50)	2 (22)
p16	<10%	34 (59)	6 (67)	18 (69)	2 (22)	8 (58)	29 (59)	5 (56)
	10%–50%	18 (31)	3 (33)	6 (23)	6 (67)	3 (21)	15 (31)	3 (33)
	>50%	6 (10)	0	2 (8)	1 (11)	3 (21)	5 (10)	1 (11)
Nuclear $\beta$ -catenin	–	36 (63)	5 (56)	15 (58)	6 (67)	10 (77)	30 (61)	6 (75)
	+	21 (37)	4 (44)	11 (42)	3 (33)	3 (23)	19 (39)	2 (25)
E-cadherin	+	2 (3)	0	0	0	2 (13)	2 (4)	0
	++	57 (97)	9 (100)	26 (100)	9 (100)	13 (87)	49 (96)	8 (100)
Membrane $\beta$ -catenin	–	1 (2)	0	0	0	1 (8)	1 (2)	0
	+	10 (17)	1 (11)	4 (15)	1 (11)	4 (31)	9 (18)	1 (13)
EGFR	++	46 (81)	8 (89)	22 (85)	8 (89)	8 (61)	39 (80)	7 (87)
	–	55 (93)	8 (89)	26 (96)	7 (78)	14 (100)	47 (94)	8 (89)
HER2	+	4 (7)	1 (11)	1 (4)	2 (22)	0	3 (6)	1 (11)
	–	58 (92)	8 (89)	25 (86)	10 (100)	15 (100)	48 (91)	10 (100)
COX-2	+	5 (8)	1 (11)	4 (14)	0	0	5 (9)	0
	–	30 (48)	2 (22)	15 (54)	5 (50)	8 (53)	25 (48)	5 (50)
	+	32 (52)	7 (78)	13 (46)	5 (50)	4 (47)	27 (52)	5 (50)

IHC: immunohistochemical analysis.

28% of these subtypes presented metastases, compared with 5% of the papillary and colonic subtypes ( $P=.012$ ). There was a significant association between the histological type and the presence of intracranial disease. Up to 66.6% of cases with intracranial invasion were of mucinous or solid type ( $P=.034$ ). We also noted the absence of metastases (except in 1 case) among p16-negative tumours, compared to 33% of metastases among tumours which retained expression ( $P=.007$ ).

#### Association Between Immunohistochemical Markers

No statistically significant associations were observed.

## Survival Studies

### Overall Survival

The colonic and papillary types presented a significantly better survival than the solid and mucinous types ( $P<.0001$ ) (Fig. 3). Intracranial extension was the clinical factor associated with the greatest decrease in overall survival ( $P<.0001$ ) (Fig. 4).

Loss of expression (–) and weak positivity (+) of membrane  $\beta$ -catenin ( $P=.042$ ), as well as weak positivity (+) for E-cadherin ( $P=.009$ ) and preserved positivity for p16, especially in over 50% of tumour cells ( $P=.013$ ), were the only IHC markers associated with shorter overall survival (Fig. 5).

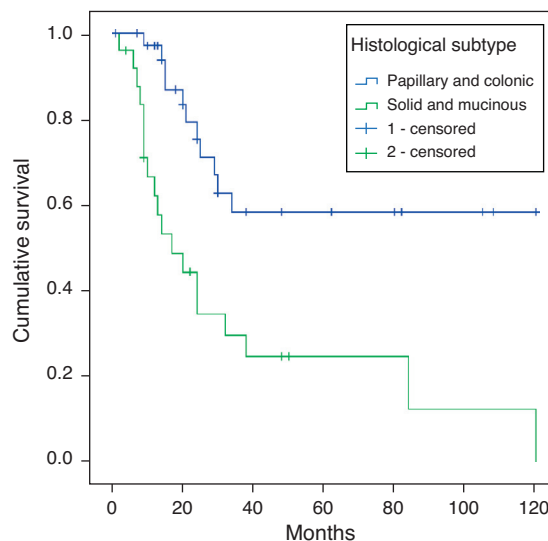
### Disease-free Period

The colonic and papillary subtypes presented a longer disease-free period than the solid and mucinous subtypes

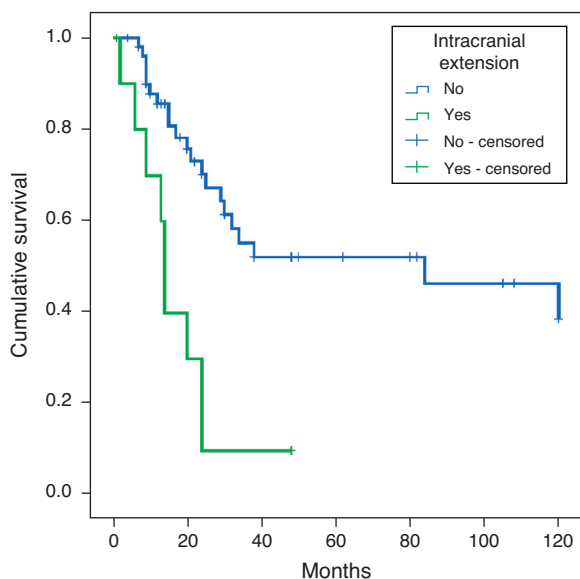
( $P<.0001$ ). P16-positive tumours ( $P=.021$ ) were associated with a shorter disease-free period. In clinical terms, those patients who received postoperative treatment had a longer disease-free period ( $P=.015$ ).

## Discussion

ITAC are tumours which mainly appear among elderly patients and those with a history of exposure to wood dust or leather. Histologically, they present a strong resemblance to intestinal adenocarcinomas. Regarding the histological subtypes, the most common is the colonic subtype, followed by



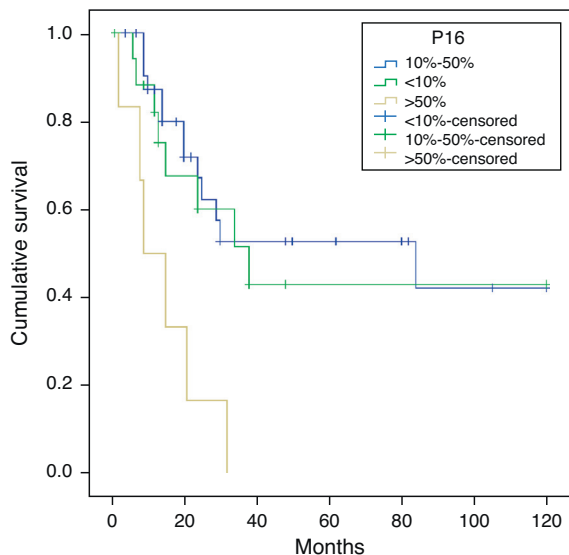
**Figure 3** Histological subtype and overall survival ( $P<.0001$ ).



**Figure 4** Intracranial extension and overall survival ( $P < .0001$ ).

the papillary, mucinous and solid. This histological classification has clear prognostic implications; survival is lower with solid subtypes, followed by mucinous, colonic and papillary.

Mutations in TP53 have been described in 44%–60% of ITAC cases.<sup>17,18</sup> TP53 mutations are associated with a more stable p53 protein and, therefore, with an increased immunohistochemical expression. IHC expression of p53 in ITAC is between 52% and 80%.<sup>19–22</sup> In our series, 63% of cases presented expression of p53. A series of alterations in TP53 have been described in response to oxidative stress produced by exposure to wood dust,<sup>23</sup> but we did not observe differences in expression among patients exposed to wood dust or not. Furthermore, different positivity rates have been described among histological subtypes, with mucinous types presenting the least positivity.<sup>19</sup> In our series, the tumoural subtype with greater positivity for p53 was the papillary (78% of cases), followed by the colonic (70%),



**Figure 5** Expression of p16 and overall survival ( $P = .013$ ).

mucinous (57%) and solid (33%) subtypes, without statistically significant differences.

The only work evaluating IHC expression of p16 in ITAC reported a loss of staining in 65% of cases,<sup>19</sup> a similar figure to that of promoter hypermethylation. Promoter hypermethylation is the most common scenario in ITAC, and this implies a loss of expression of p16.<sup>17</sup> In our series, 59% of cases were negative. We observed no differences between histological subtypes or between patients exposed and not exposed to wood dust, despite reports of alterations in p16 secondary to genotoxicity induced by wood dust.<sup>23</sup> Interestingly, conserved positivity for this marker was associated with decreased survival ( $P = .013$ ), as well as a higher frequency of metastatic disease ( $P = .007$ ).

The E-cadherin/ $\beta$ -catenin complex is essential in establishing intercellular junctions and for the integration of inter- and intracellular signals. Cell dissociation is a critical step in the metastatic cascade. In ITAC, Perez-Ordoñez et al. found positivity for E-cadherin in all cases, with variations in intensity and for membrane  $\beta$ -catenin.<sup>24</sup> There is only 1 series which observed loss of E-cadherin expression in more than 20% of tumour cells, in 45% of cases.<sup>19</sup> In our case, E-cadherin was always positive, diffusely and with intensity variations. Papillary and colonic tumours always present intense staining. The mucinous and solid types present weak staining more frequently. Regarding  $\beta$ -catenin, the only negative case was of mucinous type, and this subtype also presented weak staining intensity most frequently. Histologically, mucinous tumours are composed of scarcely cohesive cells, sometimes arranged as a signet ring or as scattered mucus pools. Negativity (–) or weak positivity (+) for  $\beta$ -catenin and E-cadherin are associated with decreased survival ( $P = .042$  and  $P = .009$ , respectively).

In addition to being a subunit of the cadherin-catenin protein complex,  $\beta$ -catenin can also function as a transcription gene if translocated to the nucleus. The nuclear expression of  $\beta$ -catenin has been studied in ITAC with different results, from an absence of nuclear staining up to 40% of cases with staining.<sup>24</sup> It is considered a marker of the activation of the WNT pathway and is associated with a worse prognosis.<sup>25</sup> In our series, nuclear positivity for  $\beta$ -catenin appeared in 37% of cases, being slightly more frequent among patients exposed to wood dust. It is more common in papillary and colonic tumours, where staining is identified in up to 44% and 42% of cases, respectively. It is less frequent among mucinous and solid types. It is believed that mucinous-type ITAC have different molecular alterations from the rest of ITAC,<sup>19</sup> but in our case  $\beta$ -catenin was negative in 77% of mucinous tumours. This figure was slightly higher than that observed in colonic and papillary tumours, with no statistically significant differences. Under normal conditions EGFR requires a ligand to become activated, but in tumour cells there are other activation mechanisms, such an increase in the number of copies or activating mutations. In ITAC, the only determination of EGFR showed expression in 80% of tumours.<sup>26</sup> Only 7% of our patients presented membrane positivity for EGFR, the majority with focal staining with intensity variations. Our study had the limitation of only evaluating the tumoural representation selected for the tissue matrix, considering that EGFR staining presents considerable intratumoral variation. Staining was not associated with any clinical–pathological feature or prognosis.

Her-2/neu (c-Erb-B2 or HER2) is a member of the growth factor receptor family and also a proto-oncogene. The over-expression/amplification of HER2 has been associated with prognostic and predictive factors of response to treatment, such as resistance to conventional chemotherapy or tamoxifen, and a good response to selective treatments, such as trastuzumab.<sup>27</sup> In the case of ITAC, a 32% positivity associated with an increased risk of recurrence has been observed.<sup>28</sup> In addition to an expression study, Bashir et al. determined the number of copies by chromogenic *in situ* hybridisation (CISH) and found only 2 out of 11 cases with an increased number of copies, with both tumours being of the solid type.<sup>21</sup> In our series, only 5 cases (8%) presented minimal membrane staining and sometimes cytoplasmic for HER2. In the HERCEPT assessment criteria for breast cancer these would fall within 1+, that is, they would be considered negative. All positive cases worked with wood and presented colonic or papillary histological subtypes. As in CAC, it appears that overexpression of HER2 is not a key event in tumoural onset or progression.

Finally, the COX2 gene is an immediate response gene induced by a variety of stress signals such as growth factors,<sup>29</sup> cytokines and other mediators of inflammation, tumour promoters, oxidising agents and DNA damaging agents.<sup>30</sup> Positivity in ITAC has been described in up to 92% of cases,<sup>22</sup> contrasting with 52% of positive cases observed in our sample, with no link to histological subtype or prognosis. The expression of COX-2 in ITAC presents a significantly higher percentage of positivity than among squamous carcinomas in the same location.<sup>22</sup> The COX-2 gene is activated by DNA damage, either by alterations in genes such as p53 or by genome damage by chronic inflammation. There is no relationship between p53 and COX-2, nor between COX-2 and EGFR, which, as previously mentioned, is capable of activating COX-2.

## Conclusions

In summary, intracranial invasion was the most important prognostic clinical factor. The histological subtypes with decreased survival were the solid and mucinous, with the latter presenting the greatest rate of intracranial invasion. Postoperative radiotherapy increased the disease-free period. The immunohistochemical profile did not differ significantly between different histological subtypes or between those patients exposed and not exposed to wood dust. Weaker  $\beta$ -catenin and E-cadherin staining was only observed in mucinous tumours. Compared with previous studies, there were clear differences in the expression of EGFR, HER2/neu and COX-2, less common in our series. P16 positivity was clearly associated with decreased survival and metastatic disease, in contrast to the reports by previous studies, which pointed to loss of p16 expression as secondary to hypermethylation or deletion, and associated with a worse prognosis.

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## Conflict of Interest

The authors have no conflict of interest to declare.

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