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Occupational exposure and risk clusters in malignant transformation of inverted sinonasal papilloma

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Inverted sinonasal papilloma (ISP) is a benign epithelial neoplasm with potential for malignant transformation into squamous cell carcinoma (ISP-SCC). While high-risk HPV infection has been studied as a possible driver, other factors, including occupational exposures, may play a role. Thirty-two cases ($n = 32$) of ISP and ISP SCC were retrospectively analysed. These cases had been diagnosed between 2010 and 2022. We assessed their occupational history through the Italian Sino-Nasal Cancer Registry questionnaire and performed p16/p53 immunohistochemistry, HPV DNA and RNA testing. Overall, 41% of ISP and 40% of ISP-SCC cases reported exposure to IARC Group 1–2 A carcinogens, and 69% had a history of smoking. Transcriptionally active HPV was detected in only one case (3.1%). Conventional bivariate analyses did not reveal significant associations between occupational exposure and malignant transformation. However, hierarchical cluster analysis identified three distinct phenotypes. Cluster 1 included predominantly male smokers with high cumulative occupational and non-occupational exposure, with ISP-SCC in 17%. Cluster 2, characterized by the highest occupational exposure and lowest smoking prevalence, consisted exclusively of ISP-SCC cases (100%), suggesting occupational carcinogens as a possible independent driver. Cluster 3 had minimal exposure, inflammatory features, and no malignant transformation. These findings highlight the potential of multidimensional analytical approaches to identify high-risk subgroups and inform targeted preventive and surveillance strategies in exposed populations.

Keywords Inverted sinonasal papilloma, Malignant transformation, Occupational exposures

Abbreviations

SP	Sinonasal papilloma
ISP	Inverted sinonasal papilloma
ISP-SCC	Inverted sinonasal papilloma with squamous cell carcinoma
ESP	Exophytic papilloma

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OSP	Oncocytic sinonasal papilloma
SCC	Squamous cell carcinoma
hrHPV	High-risk HPV
lrHPV	Low-risk HPV

Sinonasal papillomas (SP) are benign tumours originating from ectoderm-derived pseudostratified ciliated columnar epithelium[1] and represent approximately 0.4 to 4.7% of all tumours of the nasal cavity and paranasal sinuses[2]. According to the Head and Neck WHO classification, 5th edition, SPs are divided into three subtypes: inverted sinonasal papilloma (ISP), exophytic sinonasal papilloma (ESP), and oncocytic sinonasal papilloma (OSP). Among these, ISP is the most frequent subtype, accounting for 47 to 78% of all SP cases[3]. ISPs typically develop on the lateral nasal wall and maxillary sinus of patients aged 40 to 70 years, with prevalence of male individuals. On histology, ISPs are characterised by the inward proliferation of immature squamous and respiratory epithelial cells into the underlying stroma. Several etiopathogenic factors have been investigated, including exposure to occupational hazards, yet the association with tobacco use remains controversial[4,5]. Earlier studies identified both low- and high-risk HPV DNA in subsets of ISP[6–8], while more recent RNA in situ hybridization analyses demonstrated transcriptional activity of low-risk HPV E6/E7 transcripts only, with consistent absence of high-risk HPV[9,10]. In this context, a distinctive subset of ISPs with condylomatous morphology has recently been described[11]. These lesions, associated with transcriptionally active low-risk HPV and characterised histologically by hyperkeratosis, cytoplasmic clearing, and koilocytic changes, share features with low-risk HPV–related anogenital condylomas and may represent a specific morphologic variant within the ISP spectrum. Recent molecular investigations have established EGFR mutations as key oncogenic drivers in the development of ISP. These alterations, reported in up to 88% of benign ISPs according to the WHO Classification of Head and Neck Tumours (5th edition), most frequently consist of in-frame insertions or deletions within exon 20[12,13]. EGFR-mutated ISPs are generally mutually exclusive with low-risk HPV infection, suggesting that these two mechanisms represent distinct and alternative oncogenic pathways in ISP pathogenesis[10,14].

ISPs can undergo malignant transformation in 2% to 27% of cases. ISP malignancy can be synchronous, thus being present at presentation, or metachronous, appearing in recurrent ISP. The most frequent histotype is squamous cell carcinoma SCCs (ISP-SCC)[15–17], even if other histotypes have been described. Various risk factors are believed to contribute to the ISP malignant transformation; these include high-risk HPV (hrHPV) infection, smoking habit[18], EGFR/KRAS gene mutations[19] and changes in DNA methylation[20], but the exact mechanisms are not yet fully understood. High-risk human papillomavirus (hrHPV) is widely recognized as a key etiological and prognostic factor in carcinomas of several organs. The potential involvement of hrHPV in the development of ISP and its role in malignant transformation has been extensively studied since the early 1980s. Research findings have reported a broad range of HPV detection rates, varying from 0% to 100%[9–22]. In addition, three meta-analyses reported data in favor of the role of HPV in ISP malignant transformation[23–25]. Infection with HPV-16, HPV-18, and combined HPV-16/18 has been shown to significantly elevate the risk of malignant transformation in ISP. However, the other side, the meta-analysis by Ferreli et al.[26] investigated the link between hrHPV did not confirm a definitive causal relationship. In most instances, hrHPV was identified after ISP had already undergone malignant transformation. As a result, a clear causal role of hrHPV infection in the onset of ISP malignancy has yet to be conclusively demonstrated[26,27].

While HPV infection has long been investigated as a potential driver of malignant transformation in ISP, evidence for its role in direct causation remains unclear, and other contributing factors must be considered. Among these, occupational exposures have emerged as potential environmental cofactors, although data are still limited. A British study reported that 54% of ISP cases involved workers in the steel industry[28], and similar case-control studies observed increased exposure to inhaled toxic agents and a higher prevalence of employment in manufacturing[4,5]. Specific substances such as organic solvents, welding fumes, and nickel compounds have also been associated with elevated ISP risk[29].

Recent studies suggest that genetic and epigenetic alterations—rather than exposure alone—may be critical for progression. For instance, polycyclic aromatic hydrocarbons and benzene have been shown to induce DNA methylation changes[30], and LINE-1 hypomethylation has been proposed as a marker of more aggressive SCCs related to occupational exposures[22].

Beyond occupational exposures, tobacco smoking represents another environmental factor of interest in the context of ISP malignant transformation, due to its known association with squamous carcinomas in the upper aerodigestive tract. The role of smoking in the malignant transformation of inverted papillomas (IPs) remains controversial. While some studies have reported a correlation between smoking history and increased risk of malignancy[31], others have not confirmed this association[32,33]. Additionally, smoking has been linked to a higher risk of ISP recurrence following surgical excision[34].

This study aimed to investigate the possible role of occupational hazards exposure, smoking habits, patients' respiratory diseases and the presence of transcriptionally active low-risk (lrHPV) and high-risk (hrHPV) HPV in ISP and ISP with malignant transformation.

Materials and methods

Case selection

Study cohort and clinicopathologic data

The study was approved by the local research ethics committee Comitato Etico-Area Vasta Emilia Centro (97-2022-OSS-AUSLBO).

Cases were retrieved from the files of the Units of Anatomic Pathology and of Otolaryngology, at Bellaria Hospital (Bologna), in the period between January 2013 and December 2020. Cases were included if the following criteria were met: availability of blocks and slides for review, availability of data regarding surgical intervention

and follow-up, and patient consent to participate in the interview. In addition, the following data were collected: allergic rhinitis, nasal septal deviation, turbinate hypertrophy, and use of nasal spray medicaments.

All cases were reviewed and classified according to the Head and Neck WHO classification (5th edition) by pathologists with expertise in of head and neck tumours (GQ and MPF). Lesions exhibiting an inverted growth pattern were classified as ISP, irrespective of the extent of inversion or any accompanying surface growth pattern. Specimens containing a distinct component of ISP alongside areas of overt carcinoma, either on presentation or in recurrences, were categorised as ISP with malignant transformation. Cases in which all tumour blocks underwent acid decalcification were excluded from the study. Any discrepancies in classification were resolved through consensus review. As part of the histological reassessment of ISP, we evaluated the frequency of ISP and IPS-SCC cases displaying a thickened tumour epithelium with dispersed koilocyte-like cells in the mid to upper layers, clear cytoplasm, and occasional binucleated cells—features reminiscent of anal condylomas, as previously described by Mehrad et al.[10]. These lesions were classified as Condylomatous Sinonasal Papilloma according to the inclusion and exclusion criteria described in the study by Mehreen et al.[35]. Patients were divided in two cohorts according to the absence (ISP cohort) or presence of malignant transformation (ISP-SCC cohort). Immunohistochemical analysis of p53 and p16 was performed on 4-micron formalin-fixed, paraffin-embedded (FFPE) sections using the Benchmark Ultra platform Ventana, and antibodies were employed in combination with the OptiView DAB detection system (Ventana Diagnostic Systems).

Immunohistochemical markers

Scoring p16

The expression of p16 was evaluated by considering both nuclear and cytoplasmic staining. Cases showing a strong and diffuse nuclear and cytoplasmic staining in more than 70% of tumour cells were classified as positive. Tumours displaying variable or moderate staining intensity, including those with 50 to 70% of tumour cells showing moderate to strong nuclear and cytoplasmic staining, or a diffused but weak staining pattern, were classified as equivocal. The complete absence of staining in both the nucleus and cytoplasm was interpreted as negative[36–38].

Scoring p53

p53 expression was evaluated and categorised based on the pattern and extent of nuclear staining in tumour cells. A diffused and strong nuclear staining observed in more than 80% of tumour cell nuclei was interpreted as diffuse strong positivity. Cases showing a complete absence of nuclear staining were classified as having total loss of expression. However, those displaying variable nuclear staining in 1% to 80% of tumour cells were considered patchy positive[36]. In the ISP cohort, the evaluation of p53 expression was performed according to the seven patterns described by Novack et al. These included four wild-type patterns: scattered basal, patchy basal/parabasal, null-like/basal sparing, and mid-epithelial/basal sparing, and three abnormal or mutant patterns: overexpression basal/parabasal only, overexpression basal/parabasal to diffuse, and null[39].

HPV nucleic acids: extraction and detection

Molecular analysis of HPV DNA and mRNA was performed on 10-micron formalin-fixed biopsies, paraffin-embedded (FFPE) after removing the paraffin with deparaffinisation solution (Qiagen).

Automated DNA and RNA extraction using the ELITeInGenius SP 200 Kit (ELITechGroup, Italy) on the ELITeInGenius® instrument was performed following the manufacturer's instructions.

HPV genotyping was performed using Allplex HPVHR assay (Seegene, Korea). The amplification assays were carried according to the manufacturer's instructions. Allplex HPVHR targets the E6/E7 region for DNA detection of HPV 16 and HPV 18, and the L1 gene for DNA detection of 12 other hrHPV types (i.e., HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). The beta-globin gene is then added as an internal control. Finally, targets are detected separately in five channels with amplification of fluorescent signals (HEX, FAM, Cal Red 610, Quasar 670, and Quasar 705), providing Ct values as output. Analysis is performed with Seegene Viewer software.

All DNA samples yielding internal control Ct values greater than 32 were analysed for the presence of individual HPV genotypes by the HPV SPF10 PCR and LiPA (Line Probe Assay) (INNO-LiPA® HPV Genotyping Extra II, Fujirebio, Europe, Ghent, Belgium.)

SPF10 Plus permits simultaneous detection of multiple genotypes in a single sample and provides high test sensitivity due to the precision of the short 65-base pair PCR product, even with degraded DNA (FFPE samples) and low viral load. LiPAassay is based on the reverse hybridization principle, which is designed for the identification of 32 different genotypes of HPV.

Detection of HPV E6 mRNA was performed only on nucleic acids extracted from biopsies which tested positive for HPV DNA and were treated with DNase. The HPV mRNA E6 expression of HPV 16, 18, 31, 33, 45, 52 and 58 was processed independently. The details of the experimental procedures have been described previously[40].

Evaluation of exposure to occupation hazards

Exposure history was evaluated by Occupational Safety and Prevention Unit personnel (AC, MM, PG) through the administration of a standardised questionnaire developed by the Italian National Institute for Accident Insurance at Work (INAIL) as part of the activities carried out by Italian Sino-Nasal Cancer Registry. The questionnaire was conducted by phone contact with the patient or, if deceased or unable to answer, with a next of kin. The questionnaire can be found in the PDF of the operational manual from pp. 29 to 77. The interview investigates professional and extra-professional risk factors (such as smoking habits, sinonasal pathologies, environmental exposure, hobbies, and family-related exposure). Working history was explored by gathering

information on each job task patients had performed throughout their life. Furthermore, the interviewer used additional standardised forms for specific sectors/jobs to identify particular aspects of each work activity.

Exposure to known/suspected carcinogens was then assessed by a panel of Occupational Safety and Prevention Unit personnel (AC, MM, PG). The carcinogens considered in defining exposure are IARC agents classified as Group 1 and 2 A[41] for nose and nasal cavities (Supplementary Table 1). According to the IARC classification, group 1 includes all substances, chemical mixtures, and exposure circumstances classified as carcinogenic based on sufficient evidence of carcinogenicity in humans. Whereas group 2 A includes all substances and exposure circumstances classified as probable carcinogens, based on limited evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in experimental animals. Cumulative exposure has also been collected for each agent.

Furthermore, data on exposure to the following suspected carcinogenic agents for the sinonasal site have been collected, which included welding fumes (not stainless steel)[42,43] and solvents[29]. Other suspected agents considered by the operative manual of Italian Sino-Nasal Cancer Registry have been included, such as polycyclic aromatic hydrocarbons (PAHs), adhesives, arsenic, silica and asbestos[44].

Evaluators assigned a judgement for each working period, ultimately providing a coherent overall assessment, according to the method for exposure assessment used in the Nasal and Paranasal Sinus Cancer Registry[44]. This method outlines that exposure to carcinogens may be classified as *occupational* and *non-occupational*. Occupational exposure can be further classified as *certain* (subjects who have been engaged in work activities involving exposure to the considered causal agent, and such exposure is documented), *probable* (subjects who have worked in an industry or workplace where the considered agent was definitely present, but where it's impossible to securely document/assess it, due to lack or inconsistency of information from the questionnaire) and *possible* (subjects who have worked in an industry or workplace belonging to an economic sector where exposure to the considered agent may have occurred, but there is insufficient information to document such exposures). Non-occupational exposure pertains to subjects exposed in *do-it-yourself* (DIY) activities in familiar or environmental settings. More detailed information about the questionnaire and assessment methods mentioned above can be found in the following volume: *Renatuns Sorveglianza epidemiologica dei tumori naso-sinusali – Manuale operativo – INAIL, Ed. ottobre 2020*[44].

Statistical analysis

A comprehensive description of the data was provided using percentage distributions or means and standard deviations according to the nature of each variable. Given the limited number of observations, relationships between variables were analysed using a bivariate, non-parametric approach: two-tailed exact Fisher's tests were used for intergroup comparisons of categorical variables. To address the complexity of the phenomena, a descriptive multidimensional approach was employed, leveraging machine learning methods for hierarchical clustering (HC), using complete linkage and Jaccard's distance metric. Some variables such as smoking habits, gender, allergic rhinitis, nasal septal deviation, turbinate hypertrophy, use of nasal spray medicaments, occupational exposure and other types, were incorporated into an unsupervised learning algorithm, which grouped patients into distinct clusters characterised by high intra-cluster similarity and low inter-cluster similarity. For the purpose of both bivariate and clustering analyses, "probable" and "possible" exposures were grouped within the "exposed" category. However, "certain" exposures were included in their own category to allow for consistent comparison with non-exposed patients. All these potential factors, including both anamnesis-related factors and risk factors, were included in the clustering algorithm, except for the variable representing neoplastic transformation, which was excluded. Specifically, variables such as HPV information and chronic sinusitis were excluded from the clustering analysis due to their low incidence within the sample. Including variables with very limited variability may reduce the discriminatory power of the clustering algorithm and compromise the reliability of cluster assignment. Therefore, only variables with sufficient distribution across the cohort were retained to ensure meaningful and interpretable cluster formation.

To assess the internal consistency and robustness of the clustering model, the average silhouette score was calculated. This metric evaluates how well each observation fits within its assigned cluster compared to others, with values above 0.5 indicating good separation. Additionally, a bootstrap analysis was performed by repeating the clustering process on 1,000 random resamples of the dataset. Agreement across solutions was measured using the Adjusted Rand Index (ARI) to evaluate the reproducibility of the cluster structure. These steps allowed us to estimate the reliability of the unsupervised classification.

The percentage distribution of patients with malignant transformation was evaluated post-clustering. This methodology allowed us to identify clusters of patients sharing similar anamnestic characteristics and occupational and extra-occupational exposures and subsequently assess the percentage of patients with malignant transformation within each cluster. By excluding the variable coding for the malignant outcome during clustering and examining its percentage distribution across the clusters afterward, we were able to determine whether classifications based on anamnestic characteristics also reflected differences in clinical outcomes. Conversely, variables such as HPV status were excluded from the clustering process due to their limited discriminatory power. In preliminary analyses, HPV status was included as a potential variable; however, it demonstrated a very limited ability to differentiate between patients, primarily due to the small overall sample size and the very low prevalence of HPV-positive cases. Both factors substantially reduced the variability of the HPV variable, limiting its contribution to patient differentiation within clusters. Consequently, HPV status was excluded from the final specification of the clustering analysis, which prioritized variables based on their discriminatory capacity. Importantly, the exclusion of HPV status from the final analysis does not allow any conclusion regarding the true role of HPV in the malignant transformation of ISP, as it is reasonable to assume that the limited discriminatory power observed was due to the combined effects of low sample size and low HPV incidence.

To identify features with high discriminatory power, we examined the variables included in the clustering process. Depending on the variable type, either Fisher's exact test or the Mann-Whitney U test was employed.

Results

A total of 32 patients provided consent for this study (22 with ISP and 10 with ISP-SCC).

p16 and p53 immunohistochemical analyses, as well as HPV DNA testing, were performed in 31 out of 32 patients (22 in the ISP cohort and 9 in the ISP-SCC cohort). One case was excluded due to the unavailability of histological material, as the patient transferred care to another institution and withdrew the sample. HPV RNA testing was conducted only in the two cases that tested positive for HPV DNA (one from each cohort). Exposure results are summarised in Tables 1 and 2.

ISP cohort consisted of 22 patients,15 of whom were males (68.2%), with a mean age of 61.1 years (range 45–80) (Table 2). The predominant ISP sites were the nasal cavity and the maxillary sinus (27.3% each), followed by the frontal (22.7%), ethmoidal (18.2%) and sphenoidal (4.6%) sinuses (Table 2). In the ISP cohort, histological evaluation revealed that 5 out of 22 cases exhibited condylomatous features of the surface epithelium and were therefore classified as condylomatous ISP.

P16 immunostaining: p16 was negative in all examined cases: in 17 cases, nuclear and cytoplasmic positivity was observed in the lower one-third of the epithelial thickness, while in 5 cases, only occasional positive cells were present.

P53 immunostaining all 22 ISP cases displayed 'wild type' p53 expression patterns. Among them 14 cases showed a 'patchy basal/parabasal' expression, while 8 exhibited a 'scattered basal' pattern.

HPV genotyping status one case tested positive for hr-HPV (HPV58) and belonged to the subset of five ISP cases that exhibited a condylomatous surface architecture (condylomatous ISP) (Supplementary Fig. 1). This case was not transcriptionally active and tested negative for p16.

Exposure results 9 out of 22 patients (41%) were exposed to IARC Group 1 and 2 A agents, with 8 of these occurring in occupational settings. Specifically, 4 patients were exposed to wood dust (2 confirmed exposures in furniture and wood product manufacturing, and 2 possible exposures in construction and woodworking environments, respectively). Additionally, 2 patients working in the leather footwear manufacturing sector were exposed to leather dust (one confirmed and one probable exposure). One patient had possible exposure to nickel and chromium VI compounds in a steel-working environment, while another worked in textile manufacturing, with exposure to cotton.

In the remaining 13 cases, no exposures to IARC Group 1–2 A carcinogens were identified. However, it is worth mentioning occupational exposures to other agents were reported in 3 of these cases (see Table 1 for details).

In addition, 17 out of 22 (77%) patients in this cohort had a history of smoking (either current or former). The only non-occupational exposure identified involved low-intensity and occasional exposure to wood dust through DIY activities.

ISP-SCC cohort consisted of 10 cases, 5 of whom were males (50%), with a mean age of 64.4 years (range 46–77) (Table 3). Similar to the ISP cohort, the predominant sites were the nasal cavity (40%) and the maxillary sinus (30%). Histologically, all carcinomas were keratinising SCC and no other histotypes were observed. Among the 10 cases, an in-situ squamous cell carcinoma (SCC) component was identified in 5 cases: in 1 case as an isolated in-situ lesion, and in 4 cases in association with an invasive component (Fig. 1). In the remaining 5 cases, no in-situ component was observed. The invasive component was confined within the ISP in 3 cases, while it extended to the surrounding tissues in 6 cases. Vascular infiltration was present in 1 case, while perineural infiltration was observed in 2 cases.

p16 immunostaining One case showed diffused, block-type positivity for p16. The remaining eight cases were negative, with only occasional scattered positive cells.

p53 immunostaining revealed a mutant-type pattern in 5 out of 9 ISP-SCC cases. Specifically, four cases showed diffuse strong nuclear staining in more than 80% of tumour cell nuclei, consistent with an overexpression pattern, while one case exhibited a 'null' pattern, characterised by complete absence of nuclear staining (Fig. 2).

HPV genotyping status lrHPV DNA was detected in two cases (HPV6 and HPV11); hrHPV DNA (HPV45) was identified in one case and was associated with transcriptionally active RNA.

Exposure results 4 out of 10 patients (40%) were exposed to IARC Group 1–2 A agents; one case involved probable occupational exposure to formaldehyde in a healthcare setting, and another involved possible occupational exposure to wood dust in an agricultural environment. Two additional cases involved low-frequency, low-intensity exposure to wood dust from DIY activities, performed without respiratory protection.

Among the 3 cases in the ISP-SCC cohort with no exposures to IARC Group 1–2 A carcinogens, occupational exposures to other agents were documented (refer to Table 2). Additionally, 5 out of 10 patients (50%) in this cohort had a history of smoking.

The bivariate analysis revealed no significant differences between the ISP and ISP-SCC cohorts. Specifically, no significant correlation was found between malignant transformation and occupational exposure to carcinogenic agents ($p = 0.636$) or other agents ($p = 0.488$). Similarly, smoking habits and the number of pack-years did not appear to be associated with the SCC development in ISP ($p = 0.130$ and $p = 0.552$, respectively). Furthermore, malignant transformation was not significantly associated with HPV DNA ($p = 0.069$), HPV RNA ($p = 0.500$), gender ($p = 0.275$), allergic rhinitis ($p = 0.310$), turbinate hypertrophy ($p = 0.203$), or nasal septal deviation ($p = 0.450$) (refer to Table 3).

The hierarchical clustering (HC) analysis identified three distinct clusters (refer to Table 4): the first comprising 18 patients, and the second and third each comprising 7 patients. The variables included in the analysis demonstrated strong discriminatory power for defining the clusters. The quality of the clustering solution was evaluated through internal validation metrics. The average silhouette score across all patients was 0.61,

ISP cohort	Sex	Diagnosis age	Site	Overall exposure to carcinogenic agents IARC 1–2 A	Carcinogenic agents IARC 1–2 A	Job task	Years of exposure to carcinogenic agents IARC 1–2 A	Smoke (pack/year)	OCCUPATIONAL EXPOSURE TO OTHER AGENTS	HPV DNA	HPV RNA
1	***M***	59	*Nasal Cavity*	***CERTAIN OCCUPATIONAL***	*TEXTILE INDUSTRY*	*Textile production worker (cotton)*	*16*	*15*	-	0	
2	***M***	80	*Nasal Cavity*	***NO****exposure*	-	-	-	*17*	-	0	
3	***M***	69	*Nasal Cavity*	***NO****exposure*	-	-	-	*5*	***ASBESTOS***, ***PAHs***, ***SOLVENTS***	0	
4	***F***	65	*Maxillary sinus*	***CERTAIN OCCUPATIONAL***	*WOOD DUST*	*Wooden chair sanding worker*	*4*	*1*	-	0	
5	***M***	65	*Maxillary sinus*	***NO****exposure*	-	-	-	*48*	***PAHs***	HPV 58	Negative
6	***M***	45	*Nasal Cavity*	***NO****exposure*	-	-	-	-	-	0	
7	***M***	67	*Ethmoidal sinus*	***NO****exposure*	-	-	-	*22,5*	-	0	
8	***M***	60	*Frontal sinus*	***NO****exposure*	-	-	-	*50*	-	0	
9	***M***	68	*Ethmoidal sinus*	***NO****exposure*	-	-	-	*35*	-	0	
10	***F***	56	*Sphenoidal sinus*	***NO****exposure*	-	-	-	-	-	0	
11	***M***	59	*Ethmoidal sinus*	***DIY ACTIVITIES***	*WOOD DUST*	-	-	*7,5*	***PAHs***	0	
12	***M***	63	*Frontal sinus*	***NO****exposure*	-	-	-	*35*	-	0	
13	***M***	63	*Nasal Cavity*	***POSSIBLE OCCUPATIONAL***	*NICKEL and CROMIUM COMPOUNDS*	*Steel mill clerk*	*35*	*50*	-	0	
14	***F***	45	*Ethmoidal sinus*	***NO****exposure*	-	-	-	-	-	0	
15	***M***	56	*Maxillary sinus*	***POSSIBLE OCCUPATIONAL***	*WOOD DUST*	*Construction carpenter*	*43*	*40*	-	0	
16	***F***	64	*Maxillary sinus*	***NO****exposure*	-	-	-	-	-	0	
17	***F***	46	*Frontal sinus*	***PROBABLE OCCUPATIONAL***	*LEATHER DUST*	*Footwear assembly and packaging worker (leather and synthetic shoes)*	*10*	-	***ADHESIVES***, ***SOLVENTS***	0	
18	***M***	48	*Frontal sinus*	***CERTAIN OCCUPATIONAL****exposure*	*WOOD DUST*	*Wood cutting operator*	*3*	*80*	-	0	
19	***F***	79	*Frontal sinus*	***CERTAIN OCCUPATIONAL***	*LEATHER DUST*	*Footwear production worker*	*10*	*10*	-	0	
20	***M***	56	*Maxillary sinus*	***POSSIBLE OCCUPATIONAL***	*WOOD DUST*	*Furniture designer with access to woodworking laborator*	*30*	*22,5*	-	0	
21	***F***	62	*Nasal Cavity*	***NO****exposure*	-	-	-	*1*	-	0	
22	***M***	71	*Maxillary sinus*	***NO****exposure*	-	-	-	*48*	-	0	

Table 1. ISP cohort: contains Raw data for patients with benign inverted sinonasal papillomas (ISP), including age, gender, tumour site, smoking habits, occupational exposure to carcinogenic agents, and cumulative exposure years. *PAHs* polycyclic aromatic hydrocarbons.

indicating good internal consistency and adequate separation between clusters. Cluster-specific silhouette values ranged from 0.55 to 0.68. Additionally, cluster stability was assessed using a bootstrap resampling approach with 1,000 iterations. The mean (ARI) between resampled solutions and the original clustering was 0.71, suggesting robust reproducibility of the identified cluster structure. Statistical tests confirmed significant differences in each variable across the cluster.

ISP-SCC cohort	Sex	Diagnosis AGE	Site	Overall exposure to carcinogenic agents IARC 1–2 A	Carcinogenic agents IARC 1–2 A	Job task	Years of exposure to carcinogenic agents IARC 1–2 A	Smoke (Pack/year)	Occupational exposure to other agents	HPV DNA	HPV RNA
1	***M***	46	*Nasal Cavity*	***NO****exposure*	-	-	-	*63*	***SILICA***	*0*	
2	***M***	59	*Nasal Cavity*	***NO****exposure*	-	-	-	-	***WELDING FUMES***	*HPV 6*	
3	***M***	66	*Ethmoidal-Frontal sinus*	***NO****exposure*	-	-	-	*5*	-	*0*	
4	***F***	68	*Ethmoidal sinus*	***NO****exposure*	-	-	-	*17*	***ADHESIVES***	*Not performed*	
5	***M***	71	*Nasal Cavity -Maxillary sinus*	***NO****exposure*	-	-	-	-	-	*0*	
6	***F***	77	*Maxillary sinus*	***POSSIBLE OCCUPATIONAL****exposure*	*WOOD DUST*	*Cattle farmer and breeder (wood sawing)*	16	-	-	*0*	
7	***F***	68	*Nasal Cavity*	***DIY ACTIVITIES****exposure*	*WOOD DUST*	-	-	*11*	-	*HPV 11*	
8	***F***	61	*Maxillary sinus*	***PROBABLE OCCUPATIONAL****exposure*	*FORMALDEHYDE*	*Health care assistant*	*6*	*13*	-	*0*	
9	***M***	64	*Maxillary sinus*	***DIY ACTIVITIES****exposure*	*WOOD DUST*	-	-	-	-	*0*	
10	***F***	64	*Nasal Cavity*	***NO****exposure*	-	-	-	-	-	*HPV 45*	*Positive*

Table 2. ISP-SCC cohort: contains Raw data for patients with of inverted sinonasal papilloma with malignant transformation (ISP-SCC), detailing age, gender, tumour site, smoking habits, occupational exposure to carcinogenic agents, and cumulative exposure years.

Cluster 1 was characterized by a high prevalence of smokers (94%), a low proportion of females (17%), and elevated mean levels of occupational and extra-occupational exposure (44% and 33%, respectively). Within this cluster, 17% of the patients were diagnosed with ISP-SCC.

Cluster 2 exhibits the lowest proportion of smokers (28%), with more than half (57%) being female. All patients in this cluster have sinusitis. This cluster shows the highest level of occupational exposure (57%) but shares a similar level of extra-occupational exposure with Cluster 3 (14%). Notably, all patients (100%) in Cluster 2 were diagnosed with ISP-SCC.

Cluster 3 includes patients of whom nearly half are smokers (43%), predominantly female (71%). The majority presents with nasal septal deviation (57%), turbinate hypertrophy (43%), sinusitis (71%) and frequent use of medical nasal sprays (71%). Both occupational and extra-occupational exposure levels are low (14% for each variable). None of the patients in this cluster (0%) were diagnosed with ISP-SCC.

Discussion

ISP is a distinct benign neoplasm that can undergo malignant transformation, more frequently consisting in SCC. ISP pathogenesis and the genetic mechanisms driving its development and malignant transformation remain unclear. The present study evaluated the possible influence of several factors in ISP development and malignant transformation. The results obtained here did not highlight the impact any single agent but rather the importance of multiple factors for ISP development and malignant transformation. While classical univariate or bivariate analyses often fail to capture the complexity of interacting risk factors, our unsupervised multidimensional clustering approach allowed us to identify three well-defined patient clusters, each characterised by distinct clinical, anamnestic, and exposure-related profiles, thereby offering a more nuanced stratification of transformation risk. This approach, which deliberately excluded clinical outcome (malignant transformation) from the clustering process, revealed that specific combinations of factors may be associated with different risks of neoplastic transformation. Moreover, it represents an important methodological innovation in the study of ISP, grounded in a necessarily multidimensional perspective. The need for such an approach is underscored by the algorithm's ability to identify meaningful patient patterns. Cluster 1 reflects a cumulative risk profile (tobacco + exposure) composed predominantly of male patients, smokers (94%), and individuals with high levels of both occupational (44%) and non-occupational (33%) exposure to carcinogens. This configuration corresponds to a "classical" high-risk profile, already suggested in the literature but rarely analysed through a combined and systematic lens. The co-occurrence of tobacco usage, and exposure to IARC-classified carcinogens suggests a possible synergistic effect of external agents in promoting malignant transformation. Cluster 2 (exposure) potentially represents a new high-risk category driven by occupational exposure and it consists exclusively of

Variable	Whole cohort (ISP + ISP-SCC)	ISP cohort	ISP-SCCcohort	*p*-value
Gender (n, %)				
Female	12 (37.5)	7 (31.8)	5 (50.0)	0.28
Allergic rhinitis (n, %)				
Yes	3 (9.4)	3 (13.6)	0 (0.0)	0.31
Nasal septal deviation (n, %)				
Yes	9 (28.1)	7 (31.8)	2 (20.0)	0.45
Turbinate hypertrophy (n, %)				
Yes	4 (12.5)	4 (18.2)	0 (0.0)	0.20
Use of nasal spray medicaments (n, %)				
Yes	6 (18.8)	5 (22.7)	1 (10.0)	0.37
Malignant transformation (n, %)				
Yes	10 (31.3)	NA	NA	NA
Site of cancer (n, %)				
Ethmoidal	2 (20.0)			
Nasal cavity	5 (50.0)			
Maxillary sinus	3 (30.0)	NA	NA	NA
Smoking habits (n, %)				
Yes	22 (68.8)	17 (77.3)	5 (50.0)	0.13
Working exposure (n, %)				
Yes	13 (40.6)	9 (40.9)	4 (40.0)	0.64
Exposure to other agents (n, %)				
Yes	8 (25.0)	5 (22.7)	3 (30.0)	0.49
HPV DNA				
Yes	4/31 (12.9)	1/22 (4.5)	3/9 (33.3)	0.06
HPV RNA				
Yes	1/2 (50.0)	0/1 (0.0)	1/1 (4.5)	0.50

Table 3. Descriptive statistics and tests for differences between ISP and ISP-SCC cohorts: bivariate analysis results: compares characteristics between the ISP and ISP-SCC cohort, including gender, smoking habits, occupational exposure and other anamnestic factors. Note P-values were obtained from two-tailed Fisher's exact tests conducted for intergroup comparisons of categorical variables. * HPV DNA testing was performed in 31 out of 32 patients (22 in the ISP cohort, 9 in the ISP-SCC cohort); one case was excluded due to unavailability of histological material. HPV RNA testing was performed only in the two cases positive for HPV DNA (one per cohort).

patients with malignant transformation (100% ISP-SCC). It is characterized by the highest level of occupational exposure (57%) and the lowest prevalence of smoking (28%). Although outcome data were excluded from the clustering process, the strong association with malignancy, suggests that occupational exposure may identify a favourable substrate for neoplastic progression even in the absence of traditional risk factors such as tobacco use. Cluster 3 emerges as a low-risk group dominated by inflammatory features but lacking relevant exposures, and no cases of ISP-SCC. These findings underscore the importance of adopting an integrated, multifactorial approach when assessing the risk of malignant transformation in ISP. They also lay the groundwork for future studies that combine anamnestic, environmental, and clinical data to validate these phenotypes and develop more targeted surveillance and prevention strategies. The present study supports the concept that ISP development and malignant progression cannot be attributed to a single dominant etiological agent. Rather, our findings point to a multifactorial model, in which environmental exposures, behavioural habits, inflammatory conditions, and molecular alterations interact in complex and often synergistic ways to shape individual risk profiles. This perspective is reinforced by our stratification model, which shows that malignant transformation arises not from the isolated presence of a specific factor, but from constellations of co-occurring risks. Among these, tobacco use has traditionally been regarded as a major contributor to ISP pathogenesis. According to the 2023–2024 data from the PASSI surveillance system of the Italian Health Ministry, 23.8% of Italians are smokers, with an average daily consumption of about 12 cigarettes, while the percentage of former smokers stands at 16.8%[45]. Data from a recent meta-analysis conducted by our group suggest that smoking has a significant impact on this transformation[25], as also supported by the study by Hong S et al.[46]. However, other studies have not found a significant association[4,5,47]. Additionally, Moon et al. found that smoking was associated with ISP recurrence after surgical excision[31]. In the present series, smoking was prevalent in 69% of the overall sample, with higher rates in ISP cases (77%) compared to ISP-SCC cases (50%), suggesting that tobacco may play a greater role in tumour initiation, while its contribution to malignant transformation may depend on the presence of additional co-occurring risk factors. Among these, occupational exposure to IARC-classified carcinogens (e.g., wood and leather dust, nickel compounds, formaldehyde) warrants particular attention. This exposure has been

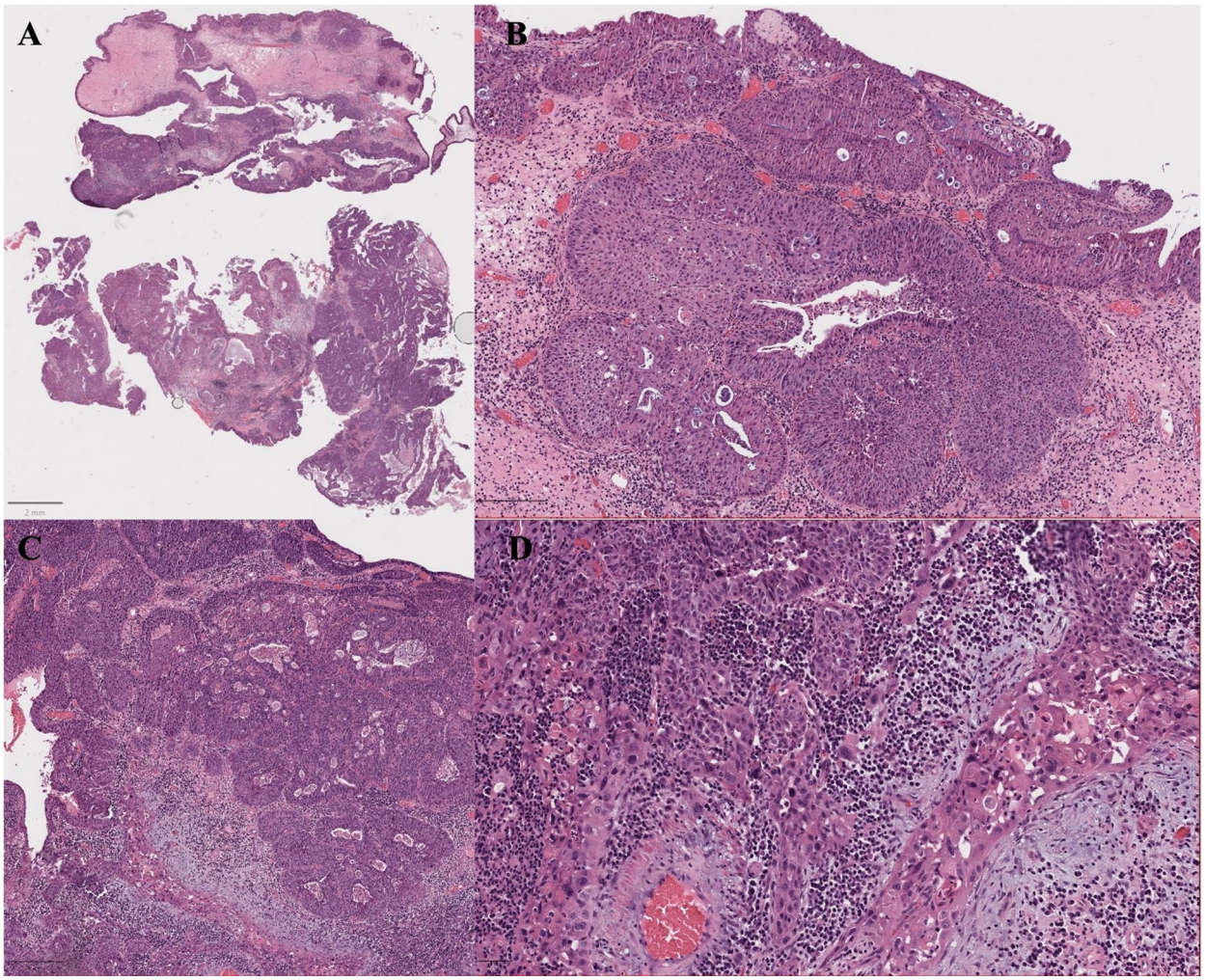

Fig. 1. (**A**–**D**) Histological images of an IPS-SCC. (**A**) The tumour is composed of nests and ribbons of hyperplastic, immature squamous epithelium (previously known as transitional epithelium) within a hypocellular, edematous stroma. (**B**) In the in-situ component, these nests are rounded and enclosed by an intact basement membrane. A hallmark feature is the marked presence of transmigrating neutrophils within the epithelium. The epithelium shows dysplasia characterized by abnormal squamous maturation, cellular pleomorphism, and increased mitotic figures; scattered superficial ciliated and mucous columnar cell are seen. (**C**) In the left lower corner, there are infiltrating irregular nests of atypical squamous epithelial cells involving the subepithelial stroma. (**D**) At high power view, this area contains keratinizing squamous cell carcinoma with irregular, infiltrating clusters of atypical squamous epithelial cells extending into the subepithelial stroma. This carcinoma arises in connection with an inverted sinonasal papilloma.

consistently associated with sinonasal carcinogenesis in both SCC and adenocarcinoma, although its link with ISP transformation has been less well defined[48]. In our cohort, 41% of patients with benign ISPs were exposed to IARC Group 1–2 A carcinogenic agents, while 77% (17 cases) reported a current or previous history of tobacco smoking. Only 4 cases had no exposure to either carcinogenic agents or tobacco smoke. In patients with malignant transformation, 50% were smokers, and 40% had exposure to carcinogenic agents, predominantly those classified in Group 1. While bivariate analyses did not reveal a statistically significant correlation between exposure and malignancy, our clustering model was able to isolate a subgroup, Cluster 2, composed entirely of ISP-SCC cases, and distinguished by high occupational exposure and low smoking prevalence. This suggests that chronic workplace exposure may act as an independent risk factor for transformation, possibly by creating a permissive biological environment even in the absence of traditional cofactors. The broader literature supports this interpretation. Case-control studies have reported an increased risk of ISP among individuals working in manufacturing settings or exposed to industrial chemicals, including welding fumes, organic solvents, and nickel compounds[4,5,24,34,42]. These findings highlight the need for epidemiological surveillance in occupationally exposed populations, a priority already recognised in Italy through the ReNaTuNS registry, which monitors sinonasal cancers and occupational etiologies across the national territory[44].

Another agent of interest is human papillomavirus (HPV). Its oncogenic potential in head and neck squamous cell carcinomas is well established, and its presence in ISP tissue has been documented with variable

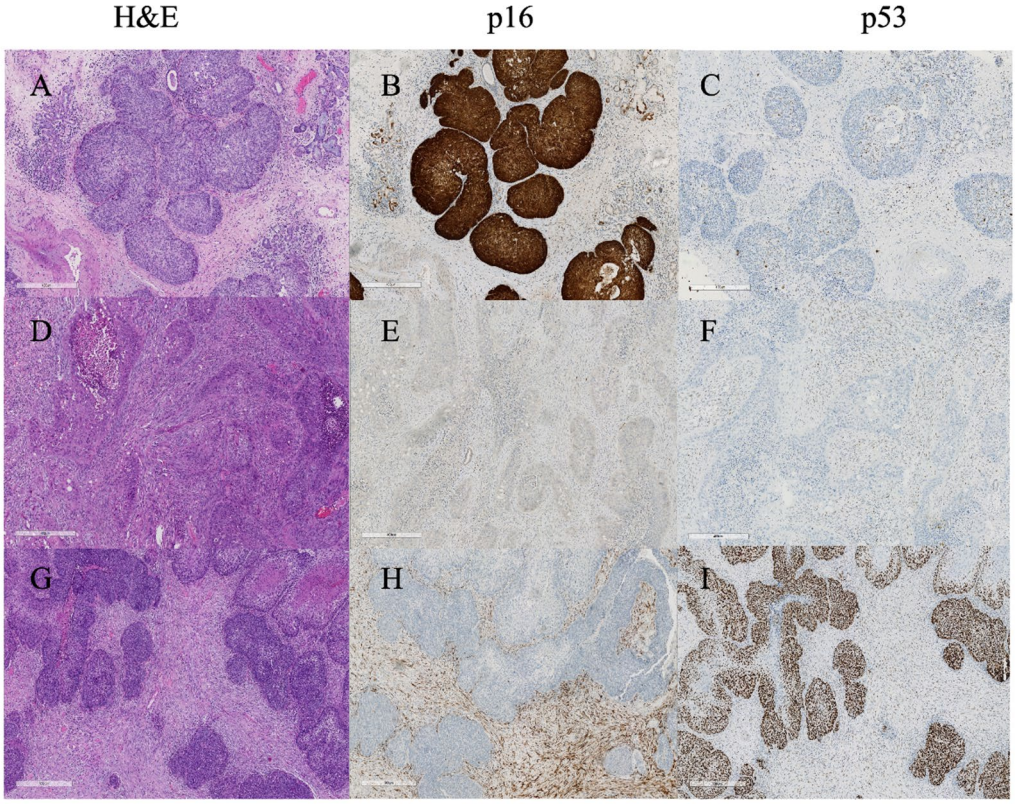

Fig. 2. (**A–I**) Histological images from three different cases in the ISP-SCC cohort, showing the distinct expression patterns of p16 and p53. (**A**) Hematoxylin and eosin (H&E) staining shows nests of neoplastic cells within a desmoplastic stroma. (**B**) p16 immunohistochemistry reveals strong, block-type positivity ("en bloc" pattern). (**C**) p53 shows a wild-type expression pattern with only occasional positive tumour cells. (**D**) H&E staining of a different area highlights solid epithelial proliferation. (**E**) p16 is negative. (**F**) p53 shows a "null-type" pattern: complete absence of staining in neoplastic cells, with positive internal control in stromal cells. (**G**) H&E staining shows neoplastic nests of carcinoma with biphasic features—keratinizing and basaloid—associated with a desmoplastic stroma. (**H**) p16 remains negative, while p53 demonstrates an overexpression pattern in tumour cells (**I**).

frequency. Some meta-analyses report HPV DNA detection in up to 38% of ISP and 32% of ISP-SCC cases[8,23–25], but these data often derive from PCR-based studies that do not distinguish between latent and transcriptionally active infection. In our series, transcriptionally active HPV was confirmed in only one case (3.1%), based on E6/E7 mRNA detection and p16 immunopositivity. This case belonged to the ISP-SCC group. These findings are in line with other studies reporting positivity rates between 0% and 13%[49–51] in selected IPS-SCC cohorts. At the molecular level, TP53 and CDKN2A alterations have emerged as possible mediators of the shift from benign to malignant phenotype. In our cohort, aberrant p53 expression patterns were observed only in ISP-SCC cases, while all benign ISP samples retained a wild-type profile. These results align with findings from Brown[19], and Kwon[36]. In contrast, Yasukawa et al. observed that most p53 mutations detected in dysplasia and SCC were already present in the associated ISP, with only minimal differences in the mutational profiles between ISP and SCC[52]. Taken together, these findings suggest that p53 and CDKN2A alterations may occur early in malignant transformation and could serve as potential biomarkers for the early detection of malignant progression in ISP.

Limitations and implications

The present study has several limitations. The sample size is relatively small, mainly due to the exclusion of patients whose ISP tissue blocks underwent complete acid decalcification, as well as the limited number of individuals who agreed to participate in the occupational exposure questionnaire.

The small sample size restricts the ability to draw causality conclusions. Given the limited sample size, we deliberately avoided inferential or causal statistical models, which would have required specific power assumptions and could have led to overinterpretation of the results. Instead, we employed a multivariate machine learning approach based on unsupervised clustering, which does not depend on statistical power prerequisites

	Cluster 1	Cluster 2	Cluster 3	C1 vs. C2	C1 vs. C3	C2 vs. C3
N	18	7	7			
Variables included in HC process						
Smoke habits						
Yes	94%	28%	43%	***	***	**
Gender	17%	57%	71%	***	***	*
Female						
Allergic rhinitis	5%	0%	28%	***	***	***
Yes						
Nasal septal deviation	17%	14%	57%		***	***
Yes						
Turbinate hypertrophy	5%	0%	43%	***	***	***
Yes						
Use of nasal spray medicaments	5%	0%	71%	***	***	***
Yes						
Working exposure	44%	57%	14%		***	***
(Yes)						
Other exposure (Yes)	33%	14%	14%	***		***
Variable NOT included in HC process						
Malignant transformation						
Yes	17%	100%	0%	***	***	***
Silhouette mean (SD)	0.62 (0.09)	0.57 (0.10)	0.64 (0.07)			

Table 4. Differences between clusters identified by hierarchical clustering (HC): multivariable analysis results: highlights the distinguishing characteristics of clusters derived from multidimensional analysis, including smoking habits, occupational exposure, and other anamnestic factors. Results from HC procedures. Statistical significance of differences between clusters was assessed by Fisher's exact test. P-value was reported according to this legend:0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' '.

and allows the extraction of meaningful, data-driven patterns even in small cohorts. This methodological choice was aimed at maximizing the informational yield while minimizing the risk of spurious inference. Regarding exposure assessment, the limitations consist in the lack of personal or environmental sampling, which are seldom available for exposures that occurred many years earlier. Therefore, the exposure level usually lays on two criteria: the interviewer's expertise on occupational risks in workplaces and a self-reported, retrospective occupational history, that can also cause an underestimation of short-term or low-intensity exposures.

Another limitation of the study, stemming from the small sample size, is the exclusion of low-incidence variables—such as HPV and sinusitis—from the multivariate analysis. As a result, it was not possible to investigate their potential role in clusters identification process. Furthermore, exposure was defined as cumulative exposure lasting six months or more, potentially overlooking shorter but potentially clinically significant exposures.

Conclusion

Although this study, due to its limited sample size, does not allow for causal inferences or the identification of specific risk factors associated with malignant transformation, the results offer novel insights for future research. It highlights the likely involvement of external carcinogenic exposure, particularly of occupational origin, in the malignant evolution of ISP. It clearly emerges that the malignant transformation of ISP is a multifactorial process, involving both external agents and factors related to the patient's anamnestic and inflammatory background. The data here shown provide further evidence that occupational exposures contribute to the development of benign ISPs but are not sufficient to trigger malignant transformation. From a clinical standpoint, incorporating occupational and environmental exposure assessment into the diagnostic and follow-up pathways may improve risk stratification and allow early identification of patients at higher risk for recurrence or malignant transformation. Individuals with documented exposure to IARC Group 1–2 A carcinogens may benefit from intensified endoscopic and radiologic surveillance, even in the absence of traditional risk factors such as tobacco use. During follow-up, smoking cessation should be strongly recommended for all patients, with activation of specialized anti-smoking centers to provide structured counseling and pharmacologic support when appropriate. Given the complex and multifactorial nature of ISP and ISP-SCC pathogenesis, molecular studies across different populations could help identify progressive molecular alterations leading to malignant transformation, potentially defining a pathogenic model of disease development and possibly unveiling specific molecular targets for future personalized or targeted therapies. These considerations highlight the importance of a multidisciplinary approach involving pathologists, otolaryngologists, molecular biologists, and occupational medicine specialists to optimize patient management and advance understanding of disease mechanisms.

Our results emphasize the importance of investigating this phenomenon from a multidimensional perspective, considering occupational exposure alongside other anamnestic factors. Therefore, these observations underscore

the need for further studies on larger cohorts that integrate environmental, clinical, and occupational data to achieve a better understanding of the pathogenic mechanisms, a more accurate risk stratification and inform preventive strategies.

Data availability

The data of this study are available from the corresponding author upon reasonable request to the corresponding author at: [https://zenodo.org/records/16081410] (https:/zenodo.org/records/16081410) .

Code availability

Cluster analysis was performed using R (version 4.3.1). No extensive custom code was developed; standard R functions and packages were used.

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Author contributions

G.Q, A.C, A.C.L and M.P.F performed the study concept and design and wrote the manuscript; G.Q, M.P.F performed the histologic revision; A.B. performed immunohistochemical tests; L.G, S.V performed molecular analysis, E.P, P.F, L.A, E.D.A, E.D, A.T, L.R. provided clinical data; A.C, M.M, P.G, provided occupational exposure data; A.C.L performed data analysis; G.Q, M.P.F, A.C.L, A.C, A.A.S wrote the manuscript and performed the revisions. All the Authors read and approved the final version of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

The study was performed according to the Declaration of Helsinki and following Italian law for studies based only on retrospective analyses of routine archival FFPE tissue; written informed consent from the living patient, following the indication of Italian D. Lgs. No. 196/03 (Codex on Privacy), as modified by UE 20167679 law of the European Parliament and Commission, was obtained at the time of surgery.

Consent for publication

Consent for publication was obtained for the patients included in the study.

Consent to participate

Informed consent was obtained from the patients included in the study.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-33299-7.

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