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Predictive value of CDKN2A/p16^{INK4a} expression in the malignant transformation of oral potentially malignant disorders: Systematic review and meta-analysis

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ABSTRACT

Background: Management of oral potentially malignant disorders (OPMDs) is still challenging. Despite the diagnostic ascertainment by bioptic examination, this method is poorly informative of the prognosis and subsequent malignant transformation. Prognosis is based on histological findings by grading of dysplasia. Immunohistochemical expression of p16^{INK4a} has been investigated in different studies, with controversial results. In this scenario, we systematically revised the current evidence about p16^{INK4a} immunohistochemical expression and the risk of malignization of OPMDs.

Material and methods: After a proper set of keywords combination, 5 databases were accessed and screened to select eligible studies. The protocol was previously registered on PROSPERO (Protocol ID: CRD42022355931). Data were obtained directly from the primary studies as a measure to determine the relationship between CDKN2A/P16^{INK4a} expression and the malignant transformation of OPMDs. Heterogeneity and publication bias were investigated by different tools, such as Cochran's Q test, Galbraith plot and Egger and Begg Mazumdar's rank tests.

Results: Meta-analysis revealed a twofold increased risk to malignant development (RR = 2.01, 95% CI = 1.36–2.96 - $I^2 = 0\%$). Subgroup analysis did not highlight any relevant heterogeneity. Galbraith plot showed that no individual study could be considered as an important outlier.

Conclusion: Pooled analysis showed that p16^{INK4a} assessment may arise adjunct tool to dysplasia grading, leading to an optimized determination of the potential progression to cancer of OPMDs. The p16^{INK4a} overexpression analysis by immunohistochemistry techniques has a multitude of virtues that may facilitate its incorporation in the day-to-day prognostic study of OPMDs.

1. Introduction

Oral squamous cell carcinoma (OSCC) remains as the most common and deadly neoplasia within head and neck region [1]. It affects more than 750,000 people worldwide per annum and, despite the efforts in terms of research as advances in therapies, during the last three decades the specific survival has marginally improved [2]. OSCC onset is often preceded by a group of pathologies, namely oral potentially malignant disorders (OPMDs) [3]. This group is made up of a plethora of pathologies, oral leukoplakia and lichen planus are the most ubiquitous conditions globally [4]. Worldwide prevalence of this group of disorders has been reported ranging from 2.6% to 4.1% with remarkable geographic variations [5]. Several reports have estimated a median malignant transformation rate from 7.9% to 12.1% with a great variability among

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OPMDs subtypes [6,7]. Clinically, management of patients diagnosed of OPMD is difficult since clinicians are still lacking means to accurately predict this risk of transformation.

Nowadays, OPMDs oncogenesis risk is mainly assessed by the keen evaluation of the presence and grading of oral epithelial dysplasia in a bioptic sample [8]. However, this intervention may present some drawbacks. Some studies highlighted inter- and intra-examiner variability [9,10]; moreover, it is well known that not all dysplasias will become malignancies, and on the other hand non-dysplastic lesions may eventually progress to carcinomas [11]. Due to advances in molecular biology, there is burgeoning interest in defining and characterizing the molecular factors that drive the malignant transition of these disorders [12]. This scenario calls for new biomarkers, aiming to guide therapies from a biological and molecular standpoint. In addition, these approaches may be able to predict from which tissue may arise a tumor [13].

Consistent with the multistep theory of field cancerization, the natural history of OSCC appears to evolve gradually through transitional precursors leading a progressive switch to carcinogenic phenotypes [14,15]. This process is due to the accumulation of genetic and epigenetic alterations [15–17]; indeed, DNA ploidy, and dysregulation in proteins, such as p53, podoplanin were found in patients with OPMDs, but with inconsistent patterns and heterogeneity, leading to limited clinical applications [12].

Cell cycle is the biological phenomena in which a cell replicates its contents and results in two twin cells from a genetic perspective. This molecular machinery in eukaryote cells is basically controlled by three groups of interferers: cyclins, cyclin-dependent kinases (CDKs) and CDK inhibitors [18]. In molecular oncology, these entities are classified as tumor suppression genes owed to their ability to knockdown cell proliferation. There are mainly two CDK inhibitors families, namely the CIP/KIP family and the INK4 family [19]. $P16^{INK4a}$ is a part of the INK4 family and exerts a pivotal role as a negative regulator of cell cycle progression by regulating the functions of the protein retinoblastoma. $P16^{INK4a}$ also down regulates the formation of cyclin D/CDK4,6 complexes by CDK4 and CDK6 interactions, which in turn controls pRb phosphorylation. Inactivation of $P16^{INK4a}$ has been classically described as an early event in OSCC [8,20]. Some authors have hypothesized that $p16^{INK4a}$ assessment may turn to be helpful as a predictive marker to evaluate the malignant potential of OPMDs [12]. However, many studies have suffered from significant drawbacks such as cross-sectional design or small sample sizes. In this sense, the ability of CDKN2A/-$p16^{INK4a}$ alterations in predicting malignant progression of OPMDs need to be clearly validated. Therefore, the objective of this systematic review was to evaluate published evidence on the $p16^{INK4a}$ expression as a predictive biomarker for the malignant transformation of OPMDs.

2. Material and methods

2.1. Data sources and search strategy

This systematic review and meta-analysis were performed following the PRISMA-P guidelines [21], as was registered in the PROSPERO database (ID: CRD42022355931). Two of the authors (AILP and MPS) independently performed searches on PubMed via Medline, Scopus, EMBASE, Web of Science and LILACS since their inception until December 2022 without language restriction.

A combination of thesaurus terms specific to each database and free text words were pooled through Boolean operators to build our search strategy. This next syntax was applied in PubMed: ("Tumor Suppressor Protein p16"[MeSH Terms] OR " Genes, p16"[MeSH Terms] OR "p16"[All Fields] OR "CDKN2A"[All Fields]) AND ("mouth"[MeSH Terms] OR "mouth"[All Fields] OR "oral"[All Fields] OR oropharyn*[All Fields]) AND ("carcinoma, squamous cell"[MeSH Terms] OR ("carcinoma"[All Fields] AND "squamous"[All Fields] AND "cell"[All Fields]) OR "squamous cell carcinoma"[All Fields] OR "dysplasia"[All Fields] OR "potentially malignant disorders"[All Fields] OR premalign* [All Fields] OR precancer* [All Fields] OR "leukoplakia"[All Fields] OR "erythroplakia"[All Fields] OR "lichen planus"[All Fields] OR "submucous fibrosis"[All Fields]) AND ("transformation"[All Fields] OR "progression"[All Fields] OR "risk"[All Fields]). Syntax was conveniently modified and optimized for each database (Appendix S1). To maximize sensitivity, references from related reviews and those of included studies were comprehensively checked to supplement manually database search.

The questions addressed was: What is the significance of CDKN2A/$p16^{INK4a}$ expression in the prediction of the malignant transformation risk of patients with oral potentially malignant disorders?

The question was addressed using the PICO methodology:

i. P (population): Patients diagnosed with oral potential malignant disorders (with or without epithelial dysplasia).
ii. I (intervention): CDKN2A/$p16^{INK4a}$ assessment using objective and quantitative methods, in biopsy specimens from the study population.
iii. C (comparison): Control group will be represented by the group of patients from the study population with low CDKN2A/$p16^{INK4a}$ expression.
iv. Differences in the expression of CDKN2A/$p16^{INK4a}$ will be categorized as overexpression for the exposition group, and low expression for the control group, based on the cut-off value chosen by the authors.
v. O (outcome): progression to OSCC.

2.2. Eligibility criteria

To be eligible for inclusions, studies had to: (i) evaluate CDKN2A/$P16^{INK4a}$ alterations in protein expression, assessed by immunohistochemistry in biopsies of OPMDs, (ii) and to correlate with their malignant transformation, including data on malignant development in studies with a longitudinal design, and (iii) providing Hazard ratio (ORs) or Risks Ratio (RRs) as their corresponding 95% confidence intervals (CIs) as a measurement of association or at least sufficient data for their calculation. In the case of results derived from an overlapping study population, the report providing more data was prioritized.

Exclusion criteria were: (i) studies including heterogeneous sampling with potentially malignant disorders not belonging to the mouth, (ii) withdrawn studies, review articles, case reports, commentaries, abstracts from scientific meetings, letters, or book chapters, (iii) studies based on animals or in vitro models, (iv) evaluation of CDKN2A gene alterations, and (v) cross-sectional studies.

In a two steps process, observers (AILP & APJ) independently assessed retrieved reports. First, titles/abstracts selected in our initial search were checked. Then, those apparently meeting set criteria were comprehensively read to reach a decision on their final inclusion or exclusion. Interrater reliability was evaluated by means of the κ statistic. Finally, $\kappa = 0.92$ was obtained. Minor disagreements among reviewers were carefully solved by discussion or with the inclusion of a third participant (MPS). All data management was performed with Mendeley Desktop v1.19.8 (Elsevier, London, UK).

2.3. Data extraction

Three authors (VCAC, AILP & OACI) separately extracted data from every record admitted in the final selection using a pilot-tested sheet, with the software Excel v.2021 (Microsoft. Redmond, WA, USA). From each included report, the following data was extracted: first author, year of publication, study country and continent, sample size, system and grading of dysplasia, type of OPMD, recruitment period, follow-up time, alteration evaluated CDKN2A/$P16^{INK4a}$, HPV presence/absence and method of assessment, methodology applied, alteration frequency, measure of effect and its adjustment. For immunohistochemistry

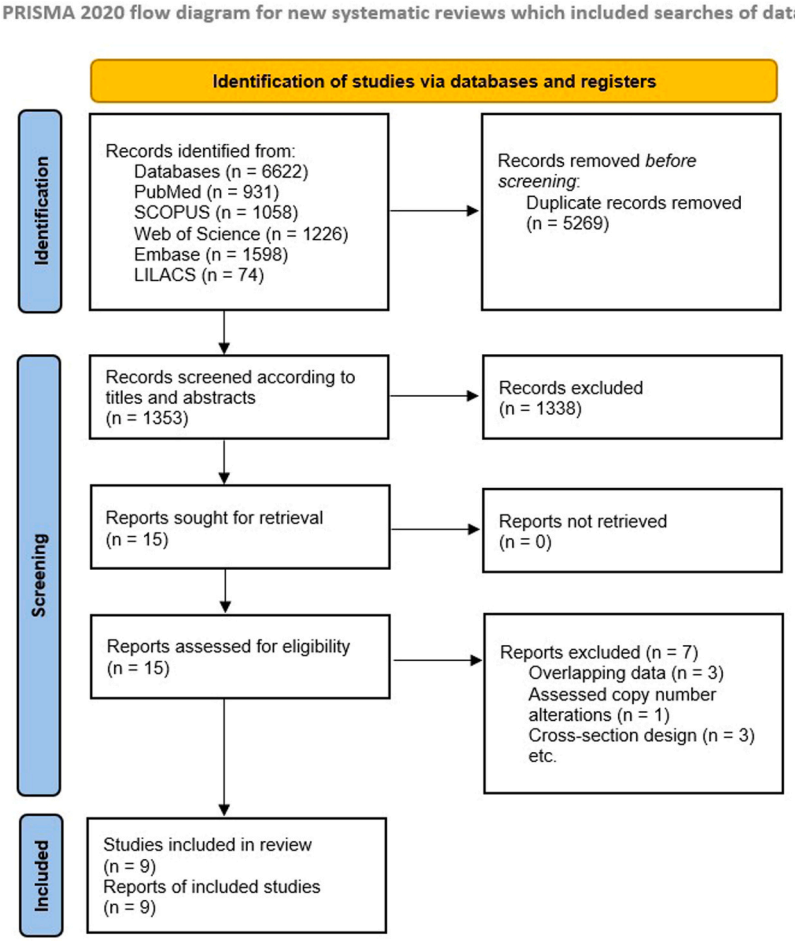

Fig. 1. : Prisma flow diagram of the searching processes.

reports, cut-off point for positivity, anti-P16^{INK4a} antibody, and cellular immunohistochemistry pattern used were also collected. Differences in the expression levels of CDKN2A/p16^{INK4a} were categorized as positive or negative based on the predefined cut-off value of each primary document. A RR value of one study was inquired obtaining a response that allowed inclusion [22].

2.4. Quality appraisal

The "QUality In Prognosis Studies" (QUIPS) tool was implemented to assess the risk of bias (RoB) of the included studies [23]. This tool consists of six domains:

i. Samples: a) Cohort (retrospective or prospective) study with a well-defined study population; b) Medical treatment applied to the patients was explained. Authors have explained if all patients have received the same treatment or not.
ii. Clinical data of the cohort: The basic clinical data such as age, gender, clinical stage, and histopathological grade was provided.
iii. Immunohistochemistry: Well-described staining protocol or referred to original paper.
iv. vi) Prognosis: The analyzed survival endpoints were well defined (e.g., OS and DFS).
v. Statistics: a) Cut-off point, which is used to divide the cases into risk groups was well described; b) Estimated effect describing the relationship between the evaluated biomarker and the outcome was provided; c) Adequate statistical analysis (e.g., Cox regression modelling) was performed to adjust the estimation of the effect of the biomarker for known prognostic factors.
vi. Classical prognostic factor: The prognostic value of other classical prognostic factors and its relationship with the studied factor was reported.

The RoB for each item was allocated as low or high. RoB assessment was performed independently by six authors, with each domain graded by a minimum of two authors. Two participants (AILP & VCAC) carried out RoB, and disagreements were resolved by consensus. An overall rating will be also assigned to individual studies for statistical purposes (i.e., to explore the potential influence of quality/risk of bias on pooled estimates).

2.5. Statistical analysis

Risk ratios and their related 95% confidential intervals were obtained directly from the primary studies as a measure to evaluate the relationship between CDKN2A/P16^{INK4a} overexpression and the malignant transformation of OPMDs. Both multivariate and univariate RRs were retrieved but, when available, the multivariate ones were chosen for further analysis. When RRs data were not reported, HRs were taken as an approximation of this measure [24]. In our output, study-specific RRs were weighted by the inverse of their variance to compute pooled RRs with their 95% CIs. Pooled analyses were obtained using Mantel–Haenszel method and DerSimonian and Laird method. In our computation, high heterogeneity warranted the use of inverse-variance models. On the other hand, fixed-effect models were applied in scenarios of non-significant heterogeneity. Forest plots were produced to graphically display our outputs.

Heterogeneity was evaluated with Cochran's Q test, using $p < 0.05$ as

Table 1
Extracted data from included reports. Abbreviations: F: female; M: male; MT: malignant transformation; N/R: non reported; NoMT: no malignant transformation; Buccal: buccal mucosa, FOM: Floor of the mouth.* Uses binary grading system for epithelial dysplasia instead of WHO grading system.

Study, year	Country	Sample size	Recruitment period	Follow-up (months)	Sex	Age	Tobacco	Alcohol	Type of OPMDs (n)	Anatomical subsite (n)	Grading of epithelial dysplasia	Progressed (MT)	Not progressed (NoMT)	$p16^{INK4a}$ antibody for IHC	IHC pattern	Cut-off (%)
Shah et al. 2007	India	60	2000–2003	N/R	6 F, 54 M	18–75	53	N/R	Leukoplakias (45)	Buccal mucosa (60)	16 Mild, 5 Moderate and 4 Severe grade transformed	25	35	Clone SC-166 (Monoclonal)	Nuclear	11
Kaur et al. 2013	Canada	110	2000–2010	43	MT: 21 F, 18 M NoMT: 30 F, 41 M	59 (30–88)	MT: 15, NoMT: 31 Yes, 28 No	N/R	Unspecified	Tongue:(26 MT, 53 NoMT) Others (13 MT, 18 NoMT)	Mild (12 MT, 46 NoMT) Moderate (18 MT,21 NoMT), Severe (9 MT, 4 NoMT)	39	71	Clone SC-166 (Monoclonal)	Nuclear	N/R
Nankivell et al. 2014	United Kingdom	148	1996–2008	42	72 F, 76 M	62 ± 14	78	81	Unspecified	Tongue (69), Buccal (38), FOM (20), Other (21)	N/R	39	148	E6H4T (monoclonal)	Nuclear/ cytoplasmic	75
Bazarsad et al. 2017	Sri Lanka	36	N/R	78	MT: 3 F, 2 M NoMT: 9 F, 22 M	N/R	N/R	N/R	Unspecified	Tongue (1 NoMT), Buccal (4 MT, 28 NoMT), Other (2 MT, 1 NoMT)	9/12 dysplastic 22/24 non-dysplastic did not transform	5	31	N/R	Nuclear/ cytoplasmic	5
Zhang et al. 2017	South Korea	160	1994–2009	135	MT: 8 F, 2 M NoMT: 6 F, 4 M	52 (13–89)	N/R	N/R	Leukoplakias: 22 MT 138 No MT	Tongue (10 MT y 34 NoMT) Buccal (6 MT, 38 NoMT) Gingiva (6 MT, 66 NoMT)	13/24 with high grade 45/54 with low grade 80/82 without dysplasia did not transform*	22	138	N/R	Nuclear/ cytoplasmic	N/R
Upadhyaya et al. 2018	USA	20	1994–2016	92	MT: 8 F, 2 M NoMT: 6 F, 4 M	62 (34–87)	MT: 4 Yes, 4 No NoMT: 7 Yes,1 No	N/R	Proliferative verrucous leukoplakia	Multiple affected regions	N/R	10	10	N/R	Nuclear/ cytoplasmic	75
Wu et al. 2019	China	76	2000–2015	72	MT: 27 F, 14 M NoMT: 24 F, 11 M	60	18	10	Leukoplakias: 41 MT 35 no MT	Tongue (30 MT, 29 NoMT), Buccal (9 MT, 5 NoMT), Gingiva (2 MT, 1 NoMT)	Mild (15 MT) Moderate (17 MT) Severe (9 MT, 35 NoMT)	41	35	N/R	Nuclear/ cytoplasmic	> 70
Monteiro et al. 2022	Portugal	52	1995–2006	32	46 M 18 F	58.1 ± 16.8	17	15	Leukoplakias: 6 MT 46 no MT	Tongue (27), Others (11), FOM (2), Buccal (13), Gingiva (11)	41 low grade and 11 high grade *	6	46	OA315 (Monoclonal)	Nuclear/ cytoplasmic	N/R

Table 2
Quality appraisal using the Quality in Prognosis Studies tool.

Author	Year	1. Study Participation	2. Study Attrition	3. Prognostic Factor Measurement	4. Outcome Measurement	5. Study Confounding	6. Statistical Analysis and Reporting	Overall
Shah et al.	2007	Low	High	High	Moderate	High	Low	High
Kaur et al.	2013	Low	Low	Moderate	Low	Low	Low	Low
Nankivell et al.	2014	Low	Low	Low	Low	Moderate	Low	Low
Bazarsad et al.	2017	Moderate	Low	Low	Low	Moderate	High	High
Zhang et al.	2017	Low	Low	Moderate	Low	Low	Low	Low
Upadhyaya et al.	2018	Low	Low	Low	Low	Low	High	High
Wu et al.	2019	Low	Low	Low	Low	Moderate	Low	Low
Monteiro et al.	2022	Low	Low	Moderate	Low	Low	Low	Low

significant threshold. We also used Higgins I^2 index. We considered the values of 25%, 50%, and 75% to inform about low, moderate, and high heterogeneity [25]. Later, the origin of heterogeneity was studied by subgroup analysis bearing in mind relevant predefined study characteristics (i.e., OPMD type, continent, anti-$P16^{INK4a}$ antibody, cut-off point, adjustment, inmunohistochemical pattern, quality appraisal). Ad hoc Galbraith plot was built to assess the relative contribution of each individual study to global heterogeneity more formally. We also used this method to check for the potential presence of statistical outliers [26].

Publication bias was also assessed using funnel plots. Statistically, we used the regression test proposed by Egger as Begg & Mazumdar's rank correlation test. The trim-and-fill method proposed by Duval et al. [27] was also used. Reviewer Manager 5.4 (Cochrane Collaboration, Copenhagen, Denmark) and Stata 16.1 (Stata Corp, College Station, TX, USA) were employed for data management. A value of $p < 5\%$ was considered significant.

3. Results

3.1. Study selection

The step wise search protocol is depicted in Fig. 1. Globally, 6622 records were initially retrieved. Each database retrieved the following number of documents: 931 from Medline via PubMed, 1058 from Scopus, 1598 from Embase, 1226 from WOS, , 74 from LILACS and 1735 from hand-searching and grey literature. No additional studies were retrieved based on the complementary manual handsearching. Elimination of duplicates resulted in 1353 studies. After, 1338 records were screened according to title/abstract. Among 15 potentially eligible papers selected for full-text assessment, 7 did not meet predefined criteria. Finally, 8 manuscripts fulfilled the inclusion criteria and were yielded for both qualitative and quantitative synthesis [22,28–34].

3.2. Study features and quality appraisal within studies

The 8 included publications encompassed only retrospective cohort studies. Studies were performed across three continents, particularly 4 in Asia [28,31,32,34]; 2 in America [29,33] and 2 in Europe [22,30]. All documents were published between 2007 and 2022 [22,28]. Studies comprised 496 patients with OPMDs; ranging from 20 to 160 patients [32,33]. Out of 8 studies, 3 included mixed OPMDs [28–30], 3 oral leukoplakias [22,32,34], 1 oral submucous fibrosis [31] and 1 included proliferative verrucous leukoplakias [33]. All studies ascertained 16^{INK4a} expression by immunohistochemistry techniques on formalin-fixed paraffin-embedded tissues with different cut-off points to establish positivity as a variety of anti-$p16^{INK4a}$ antibodies (Table 1).

The RoB evaluation ended in 3 studies (37.5%) with a high score, whilst it was low in 5 (62.5%). Most potential biases emerged due to failure in not considering relevant cofounders and due to the use of non-meticulous statistic approaches. Results of the RoB assessment are reported in Table 2.

3.3. Quantitative analysis

Pooled analysis of eight studies evaluating the overexpression of $P16^{INK4a}$ in OPMD revealed a twofold risk to progress into OSCC (RR = 2.01, 95% CI = 1.36–2.96). The rate of between-study heterogeneity was negligible ($I^2 = 0\%$), and for such reason, a fixed-effects model was computed. Individual and pooled RRs are depicted in Fig. 2A. Subgroup analysis pointed out that association was preserved in most of the strata: Asian studies (RR = 1.81, 95%CI 1.06–3.10), studies that used other antibody instead of SC-166 (RR = 1.57, 95%CI 0.76–3.22), studies involving a heterogeneous group of OPMDs (RR = 1.96, 95% CI 1.09–3.50) and those evaluating joint nuclear and cytoplasmatic staining patterns (RR = 2.22, 95%CI 1.40–3.52). No relevant heterogeneity subsided according to our subgroup analysis Complementary, Galbraith plot showed that no individual study could be considered as an important outlier. In this sense, overall heterogeneity was not disproportionately affected by any primary-level study in our pooled analysis (Fig. 2B).

The funnel plot is moderately skewed to the right (Fig. 3). However, the existence of publication bias was not confirmed by Eggers's regression (p = 0.977), nor by the Begg & Mazumdar's correlation test (p = 0.229). However, implementation of the trim-and-fill methodology ended up in the addition of a new "missing" RR. The inclusion not substantively altered our pooled analysis, yielding a close estimate (RR = 1.83, 95% CI = 1.42–2.24). Another simulation was performed to evaluate the robustness of our quantitative synthesis. We calculated how many unpublished negative reports (i.e., RR = 1) with as many cases and controls as the average number of the ones included in our primary studies were necessary to reverse our estimate. Our simulation showed the need of 329 negative studies to obtain a pooled RR equal to the unit and non-significant.

4. Discussion

The progression from normal oral epithelium to neoplasia usually happens in a stepwise process [4]. Accumulation of cellular and histological alterations preceding its development eventually manifest as clinically visible lesions on the topography of the mouth. These OPMDs have potential for reversion as well as for malignant development depending on factors such as cellular stress and tumor microenvironment [11]. Oral carcinogenesis is a complex multistep process requiring both genetic and epigenetic variations. Several related hallmarks represent a cornerstone to properly understand its biology, such as cell cycle. Inside the molecular machinery of the cell cycle, tumor suppression genes such as $P16^{INK4a}$ play pivotal roles in oncogenesis as in the transition of precursor lesions [20].

Nonetheless, no attempt has been made to systematically address the current evidence on $p16^{INK4a}$ as a predictive marker of OPMDs. The current study houses the largest pooled sample analyzed to date (8

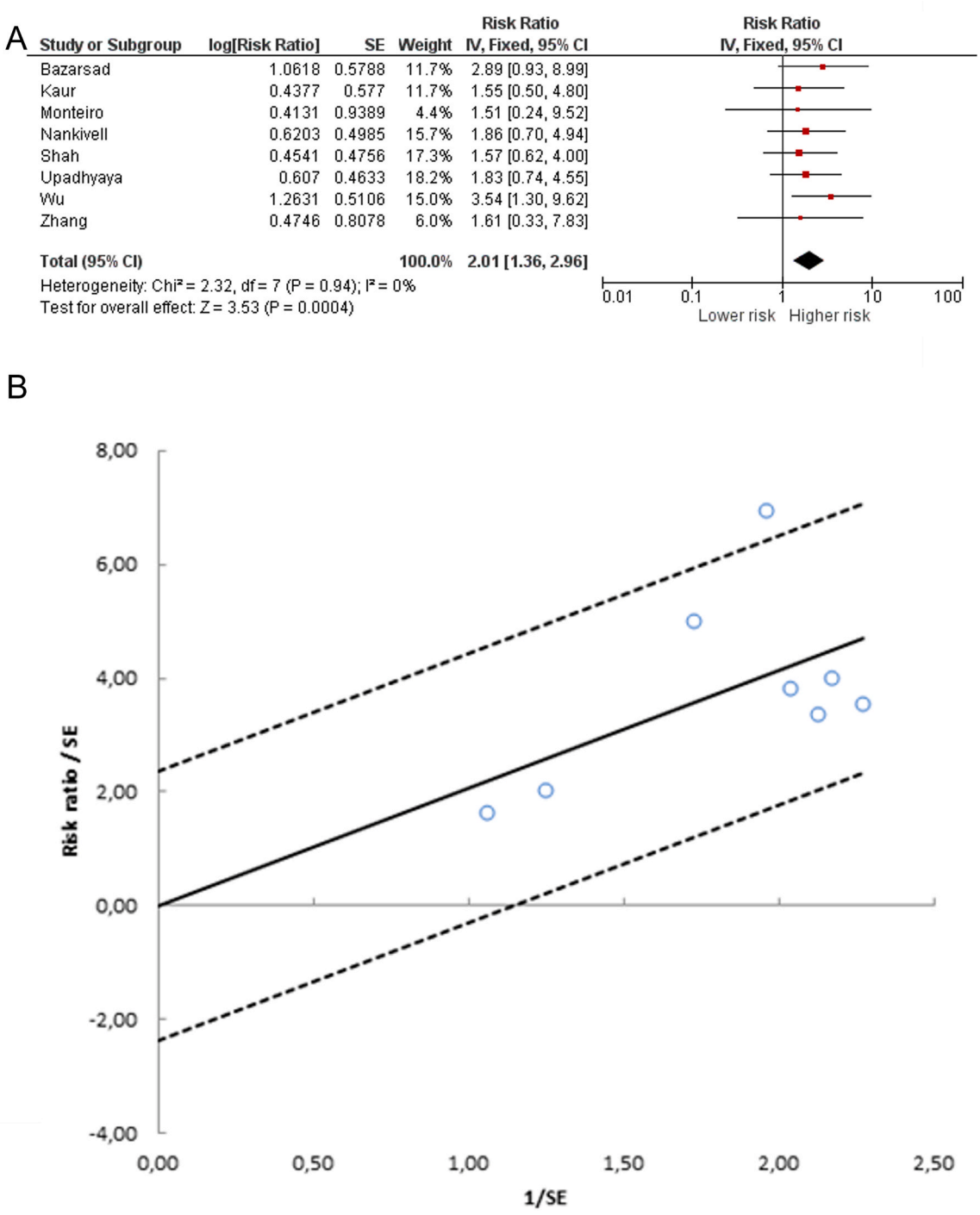

Fig. 2. : A) Forest plot displaying risk of malignant progression in oral potentially malignant disorders positive for P16INK4a overexpression. B) Galbraith plot depicting the individual contribution of studies to the overall heterogeneity. X axis represents individual risk ratios standardized according to standard errors following the next formula, y = RR/SE(RR), whilst the y axis displays precision (x = 1/SE[RR]). The regression diagonal line is projected from the origin of the coordinate system (i.e., x = 0, y = 0). Approximate 95% confidence intervals run between the two parallel lines at ± 2 units around diagonal line.

studies/496 patients), representing the first pooled analysis dealing with prognostic ability of $p16^{INK4a}$ alterations to predict the malignant transformation. Overall, this meta-analysis suggests that $p16^{INK4a}$ overexpression in OPMDs is significantly associated with a greater risk of malignant transformation to OSCC (RR = 2.01, 95% CI = 1.36–2.96) with a negligible heterogeneity among the studies as evidenced by the value of the I^2 index, 0% (Fig. 2, Table 3). In addition to the predictive value attributable to $p16^{INK4a}$ overexpression, a significant fraction of its value as a predictor for malignant transformation of OPMDs, may be caused by distortion derived from residual confounding. It was evidenced that some of the primary studies included in our data synthesis did not provide adjusted RRs estimates for relevant cofounding factors such as for oral epithelial dysplasia or human papilloma virus infection. Nonetheless, overexpression of $p16^{INK4a}$ is known to be indicative of HPV infection [35]. To this regard, only 3 included studies investigated HPV infection in OPMDs [29,33,34], with inconclusive results. Indeed, there is discrepancy in the prevalence of HPV in the mouth, and its role in the oral carcinogenetic process is still under-explored [36,37].

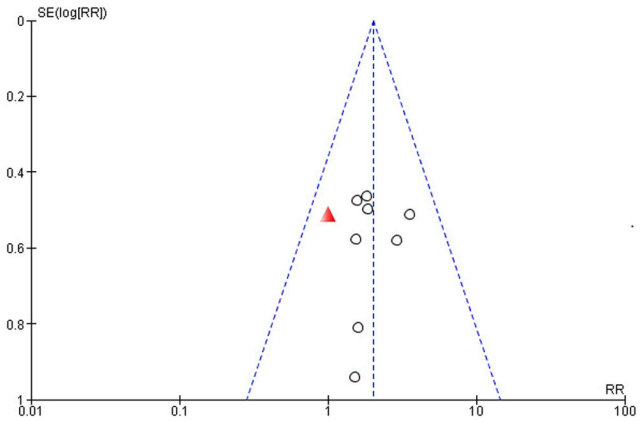

Fig. 3. : Funnel plot. The log RRs of malignant transformation is represented on the x axis, whereas its standard error (SE) values are represented on the y axis. The blue bubbles represent the 8 studies from primary literature and the triangle represents the "new study" added by the trim-and-fill methodology.

Moreover, a growing body of literature points out that the p16 overexpression does not unequivocally represent the real presence or more importantly the active transcription of HPV in these disorders since other mechanisms related to cell cycle and other molecular pathways may fuel the positive staining for $p16^{INK4a}$ [38,39].

We acknowledge some limitations of this systematic review beginning by the plausible presence of publication bias. Small study effects is an unlikely explanation for our outputs due to the asymmetry tests used as the clear interpretation of our funnel plot. We also emphasized that the trim-and-fill method detected one apparently "missing" study (Fig. 3). Its inclusion in our pooled estimate did not appreciably alter our final output (RR = 1.83, 95% CI = 1.42–2.24). Moreover, our quantitative synthesis achieved robust results with almost null heterogeneity, as evidenced by our subgroup analysis and the Galbraith plot interpretation. (Fig. 2, Table 3). The apparent absence of these statistical artefacts is of paramount importance due to in oncological literature on biological markers with prognostic or predictive value there is consistently a relevant tendency to find exclusively positive results [40].

In our subgroup analyses, we were unable to identify a specific rationale that accounted for study heterogeneity due its negligible degree across our global computation. Specifically, we could not trace relevant factors related to immunohistochemistry techniques such as cut-off point, $p16^{INK4a}$ antibodies or histological patters that could enhance the predictive value of this biomarker. Moreover, a specifical OPMD subtype in which $p16^{INK4a}$ assessment could have a stronger predictive value for carcinogenesis was not identified, this assumption may be derived from residual confounding given that several reports included pooled data about unspecified groups of OPMDs (Table 3).

While this study provides valuable insights into the role of $p16^{INK4a}$ immunohistochemical expression in predicting malignant transformation of OPMDs, several drawbacks should be considered. Firstly, the analysis was based on the available published literature, which might be subject to publication bias. Despite efforts to investigate publication bias and assess statistical power, the potential presence of unpublished or inaccessible studies cannot be completely ruled out. Secondly, the number of primary studies included in the meta-analysis was relatively limited, which might affect the statistical power and generalizability of the findings. Additionally, the heterogeneity among the included studies was not significant, but variations in study design, patient characteristics, and methodologies might have influenced the overall results. Additionally, a generalized lack of patients' treatment information as follow-up evaluation and outcome evaluation hindered the possibility to run a more detailed analysis. Moreover, although the use of immunohistochemical expression of $p16^{INK4a}$ shows promise as a prognostic tool, its practical implementation in routine clinical practice may require further validation and standardization across different laboratories and institutions. Lastly, it is important to note that this study focused specifically on $p16^{INK4a}$ and its association with malignant transformation, and other biomarkers or factors that might contribute to the prognosis of OPMDs were not extensively explored.

By and large, the findings of this study have practical implications of paramount importance for the management of OPMDs. The use of $p16^{INK4a}$ immunohistochemical expression as an adjunct tool to dysplasia grading can significantly improve the assessment of the potential progression of OPMDs to cancer. The twofold increased risk of malignant development identified in the meta-analysis underscores the significance of considering $p16^{INK4a}$ expression in the clinical evaluation of patients with OPMDs. Incorporating $p16^{INK4a}$ immunohistochemistry into the day-to-day prognostic study of OPMDs can provide clinicians with valuable information for risk stratification and treatment planning. By enhancing the accuracy of prognostic assessments, this approach may help identify individuals at higher risk of malignant transformation, allowing for early intervention and appropriate management strategies. Furthermore, the multitude of advantages associated with $p16^{INK4a}$ overexpression analysis, such as its availability, simplicity, and cost-effectiveness, make it a feasible and valuable addition to routine clinical practice in the evaluation of OPMDs.

In conclusion, our pooled analysis showed that $p16^{INK4a}$ may be a remarkable adjunct tool to dysplasia assessment leading to a better biological characterization of the potential progression to cancer of OPMDs complementing it from a molecular perspective. Future research

Table 3
Subgroup analysis.

				Pooled data		Heterogeneity		
Subgroups		**No. of studies**	**Stat. model**	**RR (95% CI)**	**P**	**Q**	**P_{het}**	**I^2 (%)**
All		8	F	2.01 (1.36–2.96)	< 0.001	0.941	0.93	0
Continent	Asia	4	F	1.81 (1.06–3.10)	0.03	0.837	0.84	0
	America	2	F	1.72 (0.84–3.48)	0.135	0.050	0.82	0
	Europe	2	F	1.78 (0.75–4.21)	0.192	0.038	0.84	0
Cutoff point	75%	2	F	1.84 (0.94–3.58	0.071	0.001	0.98	0
	Other	6	F	1.74 (1.09–2.78)	0.020	0.923	0.96	0
Antibody	SC-166	2	F	1.57 (0.76–3.22)	0.22	0.001	0.98	0
	Other	6		2.22 (1.40–3.52)	0.001	1.669	0.89	0
Type of OPMDs	Oral leukoplakia	3	F	1.57 (3.29–3.29)	0.23	0.003	0.99	0
	Mixed OPMDs	3	F	1.96 (1.09–3.50)	0.023	0.666	0.72	0
	OSMF	1	F	2.89 (0.93–8.99)	0.001			
	PVL	1	F	1.83 (0.74–4.55)	0.658			
Immunohistochemical pattern	Nuclear/ cytoplasmic	7	F	2.22 (1.40–3.52)	< 0.001	1.669	0.89	0
	Nuclear	2	F	1.57 (0.76–3.22)	0.22	0.001	0.98	0

Abbreviations: CI, confidence intervals; F, Fixed-effect model; OPMDs, oral potentially malignant disorders; RR, risk ratio; OSMF, oral submucous fibrosis; PVL, proliferative verrucous leukoplakia.

should aim to address identified gaps of knowledge and provide a more comprehensive understanding of the prognostic value of $p16^{INK4a}$ and other related markers in the context of OPMDs.

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CRediT authorship contribution statement

Alejandro I. Lorenzo-Pouso: Data curation; Formal analysis; Investigation; Visualization; Writing-original draft. **Vito Carlo Alberto Caponio:** Investigation; Writing-review & editing. **Fábio França-Vieira-E-Silva:** Formal analysis; Software. **Alba Pérez-Jardón:** Methodology; Visualization. **Óscar Álvarez-Calderón-Iglesias:** Methodology; Writing-review & editing. **Pilar Gándara-Vila:** Supervision. **Giuseppe Pannone:** Conceptualization. **Mario Pérez-Sayáns:** Project administration; Supervision; Writing-review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prp.2023.154656.

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