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ESCUELA DE DOCTORADO INTERNACIONAL DE LA USC

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Tesis doctoral

Estudio del mecanismo de cancerificación y opciones terapéuticas en la Queilitis Actínica

Santiago de Compostela, 2022

PD en Investigación Clínica en Medicina

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TESIS DE DOCTORADO

ESTUDIO DEL MECANISMO DE CANCERIFICACIÓN Y OPCIONES TERAPÉUTICAS EN LA QUEILITIS ACTÍNICA

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ESCUELA DE DOCTORADO INTERNACIONAL DE LA UNIVERSIDAD DE SANTIAGO DE COMPOSTELA

PROGRAMA DE DOCTORADO EN INVESTIGACIÓN CLÍNICA EN MEDICINA

SANTIAGO DE COMPOSTELA
2022

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Estudio del mecanismo de cancerificación y opciones terapéuticas en la Queilitis Actínica

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AGRADECIMIENTOS

Al doctor Manuel Pereiro Ferreiros mi Tutor y Director de Tesis por su inestimable ayuda, para conseguir los objetivos fijados en este trabajo, por su disponibilidad y dedicación durante todo el proceso.

Al doctor Manuel Almagro por su ejemplo y por ser un referente profesional y académico. También, por su colaboración y ayuda para llevar la Tesis a buen puerto.

Mi agradecimiento a todo el equipo de investigación por sus enseñanzas, rigurosidad y colaboración, que ha sido imprescindible durante todo el camino recorrido.

A mi familia, por inculcarme su pasión por esta profesión, las ganas y el espíritu de superación, así como por sus constantes consejos y ayuda.

A mis jefes y a los compañeros del Servicio de Reumatología del Hospital Universitario de Ciudad Real, por su docencia y ejemplo profesional y académico.

Finalmente, mi agradecimiento a mi novia Sara, por su constante estímulo, por proporcionarme toda la motivación y por ser inspiradora de la creatividad y el esfuerzo.

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1 RESUMO

1.1 INTRODUCIÓN. CONSIDERACIÓNS XERAIS

Empregouse unha terminoloxía moi ampla para definir esta patoloxía, aínda que a Queilitis Actínica (QA) é a máis frecuentemente utilizada, tamén se utilizou como sinónimo o termo de Queilitis Solar. Ademais, acuñáronse os termos de Queilosis Actínica e Queilosis Solar, para salientar a natureza non necesariamente inflamatoria desta patoloxía.

A evidencia dispoñible sobre o potencial de malignización desta lesión baséase na concorrencia co carcinoma labial de células escamosas. Con todo, apenas existen estudos con deseños lonxitudinais que permitan determinar a taxa de malignización anual en pacientes non tratados. O único metaanálisis dispoñible publicado en 2018, en base a un estudo, moi débil metodologicamente, asigna unha taxa de malignización do 3,07%. Con todo, ata 89 xenes diferentes implicáronse na progresión de QA a carcinoma labial. En calquera caso, no momento actual, esta patoloxía foi considerada no contexto das desordes orais potencialmente malignos. Tamén foron utilizadas outras terminoloxías taxonómicas, como desorde ou lesión labial potencialmente maligna, lesión precursoras ou preinvasivas e precáncer labial. Considerados globalmente, as desordes orais potencialmente malignos presentan unha prevalencia do 4,47% . Con todo, a prevalencia e as taxas de incidencia de Queilitis Actínica mostran unha ampla variabilidade en dependencia dos criterios diagnósticos utilizados. Especificamente en

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España, utilizando criterios clínicos, publicouse recentemente unha prevalencia do 31,3%

A exposición continuada á radiación solar considérase o principal factor etiolopatogénico desta lesión. A radiación ultravioleta RUV-A (315-400 nm) e a radiación ultravioleta RUV- B (280-315 nm), demostrou en humanos e en experimentación animal a súa capacidade carcinogenética. a RUV dana directamente o DNA, e produce #estrés oxidativo e inflamación. Ademais, identificáronse como factores de risco, unha idade maior a 60 anos, ter unha pel con fototipo II, traballos ao aire libre durante polo menos 25 anos, e unha historia previa de cancro cutáneo non-melanoma. Globalmente esta patoloxía observouse con máis frecuencia en homes e poboación de raza branca. O tempo de traballo "ao aire libre" (agricultores, pescadores, xardineiros, etc…), sen protección solar tamén se considera un forte factor de risco para o desenvolvemento desta patoloxía. Especificamente este factor de risco mostrou unha asociación positiva con significación estatística para a atrofia labial, a falta de definición de limítelos do bermellón, a aparición de fisuras verticais, as manchas brancas e vermellas e a perda de elasticidade e induración labial.

1.2 CLÍNICA E DIAGNÓSTICO

A QA, mostra unha clínica proteiforme con cambios leves, como secura do bermellón, descamación e aparición de fisuras e engurras perpendiculares ao eixo do beizo. Tamén pode aparecer palidez da semimucosa, perdida de elasticidade e dos contornos que definen o bermellón; así como, lesións costrosas, erosivas ou ulceradas, únicas ou múltiples, homoxéneas ou non. Ocasionalmente aparecen placas

brancas, vermellas ou unha mestura de ambas, que se desenvolven sobre este fondo patolóxico.

As QA frecuentemente aparecen cunha ou dúas manifestacións clínicas e só excepcionalmente con mais de 3. Os signos/síntomas máis frecuentes son a aparencia moteada, a descamación e o eritema. Mentres a aparición lesiones erosivas/ ulceradas e de placa branca adoitan ter unha menor prevalencia.

Coa finalidade de facilitar o recoñecemento e a reproductibilidad no diagnóstico clínico propúxose unha gradación dos criterios clínicos.

Grao I: Secura do bermellón labial; Grao II: Atrofia dos bordos labiales, palidez do bermellón, desdibujamiento de limítelos do bermellón labial, e tenues lineas pigmentadas (non efelides); Grao III: beizo heteroxéneo con áreas rugosas, areas hiperqueratósicas e secas; Grao IV: Ulceraciones nunha ou máis localizacións do beizo, placas brancas, areas traumatizadas e induradas. Estas poderían considerarse como signos/síntomas de alarma no proceso de malignización Tamén, queilitis actínica que se presenta como unha lesión pobremente delimitada pode sufrir cambios histolóxicos distribuídos de forma irregular por todo o bermellón labial.

1.3 DIAGNÓSTICO E AXUDAS AO DIAGNÓSTICO CLÍNICO

Son moi controvertidos os aspectos diagnósticos desta lesión. Un amplo grupo de autores asume o concepto clásico de que QA é un termo clínico, polo que se asume que os cambios macroscópicos asociados a esta entidade son suficientes para establecer un diagnóstico definitivo. Esta abordaxe é o maioritariamente empregado nos estudos

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de prevalencia. Con todo, a falta de correlación clínico-patolóxica debilita sensiblemente este abordaxe diagnóstico.

As QA teñen unha presentación uni ou multifocal e o substrato histopatológico é completamente diferente con independencia das características clínicas da mesma. Isto é "non hai maneira fiable de determinar o grao de cambio histolóxico epitelial cando nos atopamos #ante unha lesión calquera que fose a súa presentación clínica"

Estas circunstancias propiciaron que outra corrente de opinión considere indispensable a información histolóxica para establecer un diagnóstico definitivo de QA. Neste caso, a histología abarca desde un moderada acantosis ou hiperqueratosis, ausencia ou presenza de diferentes graos de displasía epitelial, incluso a coexistencia dun carcinoma labial de células escamosas microinvasivo ou invasivo. Particularmente a presenza de displasia epitelial é considerada por outros autores, un trazo histolóxico indispensable para establecer o diagnóstico. En calquera caso, esta controversia permanece activa no momento presente.

Tamén hai que engadir a falta de acordo na clasificación da displasia epitelial. Particularmente a displasia moderada constitúe un reto para a categorización destas lesións. A proposta de utilizar unha gradación binaria (baixo risco e alto risco) simplificaría a categorización diagnóstica, pero necesita ser amplamente validada.

En base á heteroxeneidade lesional das QAs diferentes procedementos ópticos parecen potencialmente útiles para mellorar a avaliación visual desta patoloxía e axudar na toma de decisións respecto a a biopsia.

Actualmente non existen suficientes estudos que explorase o potencial en termos de sensibilidade e especificidade diagnóstica, da

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microscopía confocal in vivo para as QA e tampouco se coñece con precisión a utilidade diagnóstica da Dermoscopia.

Pola contra, os procedementos de análises da autofluorescencia de tecidos, demostraron utilidade clínica para identificar diferentes patróns patolóxicos de imaxe, en ata no 95% das QAs, e capacidade de dirixir as biopsias incisionales cara ás áreas con maior displasía.

A biopsia labial tamén representa outro motivo de discrepancia, a falta de sensibilidade clínica propicia que leve a cabo unha ou máis biopsias para o diagnóstico histolóxico e pre-tratamento. Con todo, cando se programa unha bermellectomía "queda en dúbida" a toma previa de biopsia. Neste sentido, moitos autores consideran á vermellectomía como unha biopsia excisional que permite unha análise seriada da lesión ao completo.

Frecuentemente, ata no 40% dos casos, as biopsias incisionales infradiagnostican o grao de displasia epitelial. Desgraciadamente, tampouco podemos contestar á pregunta de se é posible que os biomarcadores poidan distinguir entre os diferentes graos de displasía epitelial nas QA, dado que un metaanalisis recente mostrou unha ampla heteroxeneidade entre estudos e uns resultados inconsistentes.

1.4 TRATAMENTO

A prevención primaria e secundaria mediante o uso de protectores labiales é indispensable para a abordaxe das QA. Diferentes modalidades terapéuticas poden empregarse para tratar as QA, tanto de forma cirúrxica como non cirúrxica. Así, a bermellectomía con bisturí frio ou con láser CO2 e as vaporizaciones con láser a diferentes lonxitudes de onda foron os procedementos cirúrxicos máis frecuentemente

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empregados. Estes procedementos achegan maior radicalidad e morbilidade cirúrxica. Doutra parte, a terapia fotodinámica e a aplicación tópica de diferentes fármacos (acido retinoico, 5- Fluoracilo, acido tricloroacético, etc…) poderían presentar unha menor morbilidade, maior taxa de recorrencia, mellores resultados estéticos e unha efectividade terapéutica dubidosa en QAs con displasía epitelial. Tamén a fototerapia combinada con imiquimod ao 5%, representa unha combinación con alta efectividade terapéutica. Con todo, os protocolos terapéuticos son moi diferentes en dependencia da contorna clínica en que se diagnostica e trata a enfermidade.

1.5 IDENTIFICACIÓN DE BRECHAS NA INVESTIGACIÓN EN QAS.

Un gran número de estudos cubriron distinto aspectos da QAs, achegando abundante información de carácter epidemiolóxico, etiopatogénico e clínico. Con todo, a información terapéutica sobre QA é tremendamente discordante e en base a pequenas series de casos, xeralmente con pacientes tratados cunha única modalidade terapéutica e con escaso seguimento, o que impide confeccionar guías clínicas con recomendacións baseadas na evidencia científica.

Tamén se identificou a ausencia de consenso na terminoloxía e taxonomía da lesión (QA), mesmo nos criterios diagnósticos da mesma. Así mesmo, é deficitaria a investigación relativa á identificación de factores de risco de malignización das QA e á predición mediante o uso de biomarcadores con potencial utilidade para identificar as QA que evolucionarán cara á malignización do epitelio labial.

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1.6 HIPÓTESE E OBXECTIVOS

En base ao previamente exposto parece necesario alcanzar consensos entre expertos encamiñados a utilizar unha única terminoloxía, criterios diagnósticos claros que permitan a comparación entre estudos e recomendacións terapéuticas, en base á experiencia clínica e á evidencia científica dispoñible.

Ao tratarse dunha lesión con potencial de malignización é particularmente necesario identificar marcadores con capacidade pronóstica/preditiva. Para iso, poderían resultar idóneos os deseños prospectivos de seguimento de QA, pero este tipo de estudos ten serias limitacións éticas, como sería o deixar evolucionar QAs sen tratar, ata a aparición do evento (malignización). Ademais, este evento ten unha baixa prevalencia e pode ocorrer ata varias décadas despois do diagnóstico da QA.

Para liquidar estes inconvenientes utilizouse o modelo de " Epitelio non tumoral adxacente ao cancro" (#ANTE), isto é, identificar cambios moleculares no epitelio peritumoral e asumir que se trata de cambios precoces na carcinogénesis e potencialmente útiles para identificar epitelio en risco de malignización. Este modelo (#ANTE) foi amplamente usado con esta finalidade no carcinoma oral de células escamosas con resultados esperanzadores, con todo descoñécese a súa potencial utilidade para o diagnóstico de eventos precoces na carcinogenesis labial.

Por tanto, os obxectivos do presente traballo de Tese Doutoral foron os seguintes:

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1.6.1 Obxectivos

1. Facilitar e alcanzar consenso sobre a terminoloxía, os criterios diagnósticos e actitudes terapéuticas aplicables á Queilitis Actínica.
2. Avaliar a efectividade terapéutica das distintas abordaxes cirúrxicas e non cirúrxicos na Queilitis Actínica.
3. Identificar a inmunoreactividad do epitelio non tumoral adxacente ao cancro de beizo.

1.7 CAPÍTULO I. CONSTRUINDO CONSENSO SOBRE QUEILITIS ACTÍNICA. ESTUDO DELPHI

Coa finalidade de cumprimentar e primeiro obxectivo deseñouse un estudo Delphi, de dúas roldas, onde se recrutou un grupo de 21 expertos (9 dermatólogos, 9 especialistas en medicina oral e 3 patólogos orais), 11 deles procedentes do ámbito académico e 10 do ámbito clínico, con diferente procedencia xeográfica. O panel de expertos foi seleccionado aplicando criterios bibliográficos e de experiencia clínica (> de 15 anos de experiencia no tratamento da QA e/ou polo menos unha publicación sobre QA).

Elaborouse un cuestionario con 34 propostas pechadas (9 -sobre nomenclatura e taxonomia-, 5 -sobre potencial de malignización de QA-, 12 -sobre aspectos diagnósticos en AQ-e 8 -sobre tratamento de QA-), e 8 preguntas abertas, que foi remitido por correo electrónico, ao panel de expertos.

Para a primeira rolda (R1: marzo 2020), nas propostas de resposta pechada valorouse o nivel de acordo, nunha escala likert de 1-7,

(1:forte acordo; 7: forte desacordo).Tamén se preguntou aos expertos polo grao de confianza nas súas respostas utilizando unha escala likert diferente (1: máxima incerteza; 7: máxima certeza). Unha vez analizada e sintetizada a información, e en base á retroalimentación do panel de expertos, transformáronse as preguntas abertas, en preguntas pechadas e iniciouse a segunda rolda (R2: Xuño-2020). O panel de expertos volveu recibir o cuestionario, con información sobre a resposta global do grupo de expertos (R1), e en caso de ausencia de consenso co grupo e para facilitar o mesmo, solicitouse ao experto que reconsiderase a puntuación da súa primeira resposta. Este procedemento permitiu ao experto reposicionarse ou manter a súa puntuación previa.

Consenso foi definido a priori, para as cuestións pechadas, como un acordo 75% e cun coeficiente de variación <50, para as propostas do comité organizador.

1.7.1 Resultados

A técnica Delphi permitiu alcanzar consenso en 25/34 (73.5%) das propostas pechadas e en 5/8 (62.5%) das preguntas pechadas. Non houbo perdas nas respostas entre a primeira e a segunda rolda. Queilitis actínica é o termo que mostrou un maior consenso entre os expertos, e unha menor dispersión nas súas puntuacións (CV=21.33), alcanzando unha mediana de 7-forte acordo- (RIQ :6-7). Respecto a a taxonomía, “desorde oral potencialmente maligno” foi o grupo máis valorado para categorizar esta entidade, cunha mediana de 7, co que o 85.6 % dos expertos mostraron algún grao de acordo (CV<50).

O 66.7% dos expertos consideran que a QA é un termo clínico e non patolóxico (mediana:7; RIQ:4-7) e que pode establecerse un diagnóstico definitivo só con criterios clínicos (mediana: 6; RIQ: 5-7). Particularmente, a inspección e palpación permiten establecer un di-

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agnóstico clínico definitivo (mediana:5 ; RIQ: 4-6). Con todo, tamén consideran, aínda que cunha menor puntuación, que é obrigatoria a confirmación histologica no manexo de QA (mediana:5; RIQ: 2.5-7), podendo estar mesmo indicada en lesións homoxéneas (mediana:5; RIQ: 3.5-6).

Ademais, conseguiuse acordo para todas as propostas relativas ao tratamento de QA (CV<50).

1.7.2 Implicacións para a clínica e a investigación

A discordancia entre a clínica e a histología parece obrigar ao clínico a un estrito seguimento e á realización dunha ou varias biopsias incisionales #ante calquera signo/síntoma que alerte dunha potencial malignización da QA. A selección da abordaxe terapéutica debería adaptarse ao aspecto clínico e ao substrato histologico da desorde, priorizando os tratamentos cirúrxicos para lesións con displasia moderada/severa. Nestes casos, e #ante lesións difusas ,a vermellectomía con análise seriada do bermellón podería ser a mellor opción. As AC con displasia leve ou sen displásia poden beneficiarse de non-surgical treatment

1.8 CAPÍTULO II. APROXIMACIÓNS TERAPÉUTICAS Á QUEILITIS ACTÍNICA: REVISIÓN SISTEMÁTICA DA LITERATURA

Coa finalidade de cubrir o segundo obxectivo da Tese Doutoral, deseñouse unha revisión sistemática que se realizou seguindo as directrices PRISMA (Preferred Reporting Items para Revisións Sistemáticas e Meta-análises). A revisión seguiu os criterios PICO e considerou exposición a calquera tratamento médico ou cirúrxico con intención cura-

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tiva. As comparacións establecéronse entre pacientes tratados con procedementos cirúrxicos e non cirúrxicos. Os resultados analizados foi a melloría clínica e histolóxicas, así como a maligmnización despois do tratamento das queilitis actínicas.

Utilizáronse #basear de datos de MEDLINE (desde 1966), EMBASE (desde 1980) e Proceedings Web of Science (censos de conferencias CCI) A estratexia de procura foi: ((" actínic" ou "solar") e (" cheilitis")) utilizando tanto descriptores médicos (MeSH) como texto libre. Última procura actualizouse en abril de 2019. Consideráronse elegibles calquera tipo de estudos, sen limitación de idioma, que incluísen datos orixinais, series de casos (≥10 pacientes) con diagnóstico patolóxico de QA e tratados mediante procedementos cirúrxicos ou non cirúrxicos, que considerasen resposta clínica e/ou patolóxica como variable resultado do estudo. Excluíronse estudos transversais sen seguimento do paciente, e a calidade metodolóxica dos estudos incluídos avaliouse mediante a lista de Downs e Black.

1.8.1 Resultados

Identificáronse un total de 392 artigos potencialmente elegibles e finalmente só 20 deles foron incluídos na síntese cualitativa. 10 publicacións de calidade moderada, que agruparon 227 pacientes, centráronse no tratamento cirúrxico. A bermellectomia con bisturí frio e con láser carbónico, así como a vaporización, conduciron á resolución completa da lesión, pero xeraron disestesias labiales como efectos adversos do procedemento. Con todo, non se produciron recorrencias ou estas foron moi baixas. Doutra parte, 11 estudos que incluíron 187 pacientes foron tratados mediante terapia fotodinámica (9 estudos), outro estudos tentou un tratamento tópico con imiquimod e uns último estudos prospectivo comparou a abordaxe cirúrxica con terapia non

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cirúrxica (fluoracil e acedo tricloroacético). Ademais, a taxa de transformación maligna despois de tratamentos cirúrxicos avaliáronse en 6 estudos lonxitudinais, catro dos cales non informaron ningún caso de malignización, mentres que os estudos utilizando diferentes protocolos para a vaporización con láser de CO2 mostraron baixas taxas de malignización nas zonas tratadas.

1.8.2 Limitacións desta revisión sistemática

As limitacións inherentes á calidade moderada dos estudos individuais considerados nesta revisión, xunto cos potenciais rumbos de selección (diferentes criterios utilizados para definir un caso), deberían ser considerados ao examinar os nosos resultados. Os estudos incluídos nesta revisión son principalmente series de casos observacionales retrospectivas/ prospectivas, ou estudos case-experimentais, o noso estudo é a primeira revisión sistemática que compara diferentes enfoques terapéuticos na QA, que inclúe estudos con seguimento de pacientes e resultados de malignización despois do tratamento e descrición dos efectos adversos asociados á terapéutica.

1.8.3 Implicacións clínicas e recomendacións para futuras investigacións

A limitada fiabilidade das biopsias incisionales para o diagnóstico das QA e a imposibilidade dunha curación completa das lesións displásicas pola terapia non cirúrxica debería implicar modificacións nas estratexias de manexo das QA, evitando recomendar o 5 - fluorouracilo e o imiquimod para tratar áreas clinicamente sospeitosas (> 0,5 cm) con displasia, ou cando se trata de lesións difusas, leucoplasia ou atrofia con displasia.

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O uso de procedementos ópticos auxiliares á biopsia e/ou a toma de biopsias múltiples podería incrementar substancialmente a certeza diagnóstica e axudar a personalizar os tratamentos; así como para evitar rumbos de selección en futuras investigacións. Tamén, son necesarios novos ensaios clínicos mellor deseñados, que consideren a taxa de malignización entre os seus resultados e así poder proporcionar probas máis sólidas sobre as diferentes opcións de tratamento, en particular para os casos de procedementos non invasivos.

1.9 CAPÍTULO III. ESTUDO INMUNOHISTOQUÍMICO DO EPITELIO NON TUMORAL ADXACENTE AO CANCER DE BEIZO. METAANALISIS

Para satisfacer o terceiro obxectivo proposto nesta Tese, levouse a cabo unha revisión sistemática con metaanálisis.

1.9.1 Metodoloxía

O protocolo seguiu a metodoloxía PRISMA para revisións sistemáticas e solicitouse o rexistro en PROSPERO (International Prospective Register of Systematic Reviews). O estudo deseñouse co obxectivo identificar o patrón inmunohistoquímico do epitelio peritumoral ao cancro de beizo e como obxectivo secundario avaliar a inmunoreactividad do carcinoma labial de células escamosas. Utilizáronse as bases MEDLINE (desde 1966), EMBASE (desde 1980) e Proceedings Web of Science (Conference Proceedings Citation Index- Science (CPCI- S), desde1990). Para a estratexia de procura utilizáronse tanto termos MESH como termos libres. Ademais utilizouse

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a base de datos Open Gray e WorldCat. A estratexia de procura utilizada foi a seguinte:

((lip cancer OR lip neoplasm OR oral cancer OR oral neoplasm) and (non-tumor epithelium OR adjacent epithelium OR surrounding epithelium OR surrounding dysplasia) and (immunohistochemistry))

1.9.2 Criterios de elixibilidade

Como criterios de inclusión consideráronse todos os estudos inmunohistoquímicos que incluísen mostras de carcinoma labial e do epitelio peritumoral e proporcionasen resultados cuantitativos ou semicuantitativos da tinción (nuclear, citoplásmatica ou de membrana) nos tecidos analizados. Como criterios de exclusión: i) Estudos que considerasen análises histolóxicas, pero non inmunohistoquímicos, Ii) Estudos en que a mostra do carcinoma labial e do epitelio adhyacente non tumoral procedan de pacientes diferentes.,iii). Non se consideraron estudos con datos secundarios, casos clínicos e revisións narrativas ou sistemáticas.

Procedeuse a unha avaliación da calidade dos estudos incluídos na (RS) e o metaanalisis levou a cabo usando MetaXL software, a versión 5.3 dispoñible en https://www.epigear.com/ index_ files/ metaxl.html.

1.9.3 Resultados

Identificáronse 339 artigos, dos que se consideraron finalmente 7 para a síntese cualitativa e tan só 4 con inmunoreactividad para p53, incluíronse no metaanálisis. A prevalencia agrupada de p53, no

#ANTE foi de 0,74% (IC95%: 0,66-0,82), mentres no epitelio tumoral (cancro de beizo) foi de 0,83% (IC95%: 0,75-0,89)

1.9.4 Discusión

A pesar de que diferentes marcadores demostraron capacidade para identificar alteracións moleculares previas á aparición do cancro de beizo. O potencial desenvolvemento deste cancro sobre epitelios non displásicos ou con displasia leve, parece obrigar ao seguimento de pacientes con factores de risco claros ou con QA, onde o uso de biomarcadores probablemente tería utilidade pronóstica. Tamén poderían resultar de utilidade na planificación do tratamento cirúrxico e non cirúrxico. Todos os estudos desta SR foron estudos transversais, con mostras pequenas, que non reportaron datos clínicos e que evidencian defectos metodolóxicos, que deberían ser evitados en futuras investigacións. Neste sentido propúxose levar a cabo estudos prospectivos, multicéntricos e con estratificación dos pacientes segundo a terapeutica empregada, para proporcionar datos de calidade. A definición de caso debería ser establecida con claridade, e diferenciar e estandarizar os criterios clínicos e histologicos. Teniendo en cuenta os distintos factores etiológicos e da carcinogenesis labial, os futuros estudos deberían incluír tamén información sobre factores de risco e susceptibilidade do paciente. Ademais, coa finalidade de incrementar a replicabilidad nestes estudos con biomarcadores, debería describirse a inmunoreactivity nos distintos estratos epiteliales, estandarizarse e especificarse o procedemento de contaxe, e desenvolver estratexias para minimizar a variabilidade interobservador. O incorporar grupo control (epitelio labial normal) aos estudos sobre biomarcadores neste ámbito, probablemente proporcionaría información suplementaria sobre o proceso da carcinogenesis labial

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Conclúese que os biomarcadores inmunohistoquímicos mostraron unha escasa capacidade de diferenciar entre os distintos graos de displasia no epitelio peritumoral ao cancro de beizo. Ademais parece existir similitude na inmunoexpresión de p53, no epitelio adxacente e no epitelio tumoral, mesmo en epitelios de aparencia normal ou con leves alteracións histolóxicas. Con todo, o emprego destes biomarcadores no seguimento de queilitis actínica, como alerta dunha potencial malignización ou para a toma de decisións terapeuticas, require de máis estudos, metodologicamente adecuados e desenvolvidos nunha contorna clínica.

1.10 CONCLUSIÓNS DA TESE DOUTORAL

1.10.1 Primeira:

En base a estudos dunha calidade limitada, o tratamento cirúrxico parece ser a primeira liña terapéutica nas Queilitis Actínicas, dado que demostrou ser útil para o control clínico e patolóxico desta entidade. Con todo, non hai evidencia sobre ningún tratamento efectivo para previr as transformacións malignas asociadas á Queilitis actínica. Os procedementos non cirúrxicos mostraron resultados menos consistentes no control histolóxico da lesión, aínda que a terapia farmacolóxica podería optimizar os resultados obtidos por outros enfoques terapéuticos.

1.10.2 Segunda:

A Queilitis Actínica é unha desorde potencialmente maligno, que mostrou unha importante falta de acordo na comunidade científi-

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ca, respecto a os criterios e procedementos diagnósticos, as indicacións de biopsia e a importancia dos procedementos auxiliares como axuda á biopsia. Con todo, a pesar da insuficiente evidencia científica, alcanzouse un maior consenso respecto ao manexo terapéutico desta entidade. Este consenso podería ser aproveitado polos clínicos como axuda na toma de decisións.

1.10.3 Terceira:

Os biomarcadores inmunohistoquímicos mostraron unha escasa capacidade de diferenciar entre os distintos graos de displasia no epitelio peritumoral ao cancro de beizo. Ademais, parece existir similitude na inmunoexpresión de p53, no epitelio adxacente e no epitelio tumoral, mesmo en epitelios de aparencia normal ou con leves alteracións histolóxicas. Con todo, o emprego destes biomarcadores no seguimento das queilitis actínicas, como alerta dunha potencial malignización ou para a toma de decisións terapeuticas, require de máis estudos metodologicamente adecuados e desenvolvidos nunha contorna clínica.

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2 RESUMEN

2.1 INTRODUCCIÓN. CONSIDERACIONES GENERALES

Se ha empleado una terminología muy amplia para definir esta patología, si bien la Queilitis Actínica (QA) es la más frecuentemente utilizada, también se ha utilizado como sinónimo el término de Queilitis Solar. Además, se han acuñado los términos de Queilosis Actínica y Queilosis Solar, para enfatizar la naturaleza no necesariamente inflamatoria de esta patología

La evidencia disponible sobre el potencial de malignización de esta lesión se basa en la concurrencia con el carcinoma labial de células escamosas. Sin embargo, apenas existen estudios con diseños longitudinales que permitan determinar la tasa de malignización anual en pacientes no tratados. El único metaanálisis disponible publicado en 2018, en base a un estudio, muy débil metodologicamente, asigna una tasa de malignización del 3,07%. Sin embargo, hasta 89 genes diferentes se han implicado en la progresión de QA a carcinoma labial. En cualquier caso, en el momento actual, esta patología ha sido considerada en el contexto de los desórdenes orales potencialmente malignos. También han sido utilizadas otras terminologías taxonómicas, como desorden o lesión labial potencialmente maligna, lesión precursoras o preinvasiva y precáncer labial. Considerados globalmente, los desórdenes orales potencialmente malignos presentan una prevalencia del 4,47% . Sin embargo, la prevalencia y las tasas de incidencia de Queilitis Actínica muestran una amplia variabilidad en dependencia de los criterios diag-

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nósticos utilizados. Específicamente en España, utilizando criterios clínicos, se ha publicado recientemente una prevalencia del 31,3%

La exposición continuada a la radiación solar se considera el principal factor etiolopatogénico de esta lesión. La radiación ultravioleta RUV-A (315-400 nm) y la radiación ultravioleta RUV-B (280-315 nm), ha demostrado en humanos y en experimentación animal su capacidad carcinogenética. la RUV daña directamente el DNA, y produce estrés oxidativo e inflamación. . Además, se han identificado como factores de riesgo, una edad mayor a 60 años, tener una piel con fototipo II, trabajos al aire libre durante al menos 25 años, y una historia previa de cáncer cutáneo no-melanoma. Globalmente esta patología se ha observado con más frecuencia en varones y población de raza blanca. El tiempo de trabajo "al aire libre" (agricultores, pescadores, jardineros, etc…), sin protección solar también se considera un fuerte factor de riesgo para el desarrollo de esta patología. Específicamente este factor de riesgo ha mostrado una asociación positiva con significación estadística para la atrofia labial, la falta de definición de los limites del bermellón, la aparición de fisuras verticales, las manchas blancas y rojas y la pérdida de elasticidad e induración labial.

2.2 CLÍNICA Y DIAGNÓSTICO

La QA, muestra una clínica proteiforme con cambios leves, como sequedad del bermellón, descamación y aparición de fisuras y arrugas perpendiculares al eje del labio. También puede aparecer palidez de la semimucosa, perdida de elasticidad y de los contornos que definen el bermellón; así como, lesiones costrosas, erosivas o ulceradas, únicas o múltiples, homogéneas o no. Ocasionalmente aparecen placas blancas, rojas o una mezcla de ambas, que se desarrollan sobre este fondo patológico.

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Las QA frecuentemente aparecen con una o dos manifestaciones clínicas y solo excepcionalmente con mas de 3. Los signos/síntomas más frecuentes son la apariencia moteada, la descamación y el eritema. Mientras la aparición lesiones erosivas/ulceradas y de placa blanca suelen tener una menor prevalencia.

Con la finalidad de facilitar el reconocimiento y la reproductibilidad en el diagnóstico clínico se ha propuesto una gradación de los criterios clínicos.

Grado I: Sequedad del bermellón labial; Grado II: Atrofia de los bordes labiales, palidez del bermellón, desdibujamiento de los limites del bermellón labial, y tenues lineas pigmentadas (no efelides); Grado III: labio heterogéneo con áreas rugosas, areas hiperqueratósicas y secas; Grado IV: Ulceraciones en una o más localizaciones del labio, placas blancas, areas traumatizadas e induradas. Estas podrían considerarse como signos/síntomas de alarma en el proceso de malignización También, queilitis actínica que se presenta como una lesión pobremente delimitada puede sufrir cambios histológicos distribuidos de forma irregular por todo el bermellón labial.

2.3 DIAGNÓSTICO Y AYUDAS AL DIAGNÓSTICO CLÍNICO

Son muy controvertidos los aspectos diagnósticos de esta lesión. Un amplio grupo de autores asume el concepto clásico de que QA es un término clínico, por lo que se asume que los cambios macroscópicos asociados a esta entidad son suficientes para establecer un diagnóstico definitivo. Este abordaje es el mayoritariamente empleado en los estudios de prevalencia. Sin embargo, la falta de correlación clínico-patológica debilita sensiblemente este abordaje diagnóstico.

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Las QA tienen una presentación uni o multifocal y el sustrato histopatológico es completamente diferente con independencia de las características clínicas de la misma. Esto es "no hay manera fiable de determinar el grado de cambio histológico epitelial cuando nos encontramos ante una lesión cualquiera que fuese su presentación clínica"

Estas circunstancias han propiciado que otra corriente de opinión considere indispensable la información histológica para establecer un diagnóstico definitivo de QA. En este caso, la histología abarca desde un moderada acantosis o hiperqueratosis, ausencia o presencia de diferentes grados de displasía epitelial, incluso la coexistencia de un carcinoma labial de células escamosas microinvasivo o invasivo. Particularmente la presencia de displasia epitelial es considerada por otros autores, un rasgo histológico indispensable para establecer el diagnóstico. En cualquier caso, esta controversia permanece activa en el momento presente.

También hay que añadir la falta de acuerdo en la clasificación de la displasia epitelial. Particularmente la displasia moderada constituye un reto para la categorización de estas lesiones. La propuesta de utilizar una gradación binaria (bajo riesgo y alto riesgo) simplificaría la categorización diagnóstica, pero necesita ser ampliamente validada.

En base a la heterogeneidad lesional de las QAs diferentes procedimientos ópticos parecen potencialmente útiles para mejorar la evaluación visual de esta patología y ayudar en la toma de decisiones respecto a la biopsia.

Actualmente no existen suficientes estudios que haya explorado el potencial en términos de sensibilidad y especificidad diagnóstica, de la microscopía confocal *in vivo* para las QA y tampoco se conoce con precisión la utilidad diagnóstica de la Dermoscopia.

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Por el contrario, los procedimientos de análisis de la autofluorescencia de tejidos, han demostrado utilidad clínica para identificar diferentes patrones patológicos de imagen, en hasta en el 95% de las QAs, y capacidad de dirigir las biopsias incisionales hacia las áreas con mayor displasía.

La biopsia labial también representa otro motivo de discrepancia, la falta de sensibilidad clínica propicia que se lleve a cabo una o más biopsias para el diagnóstico histológico y pre-tratamiento. Sin embargo, cuando se programa una bermellectomía "queda en entredicho" la toma previa de biopsia. En este sentido, muchos autores consideran a la vermellectomía como una biopsia excisional que permite un análisis seriado de la lesión al completo.

Frecuentemente, hasta en el 40% de los casos, las biopsias incisionales infradiagnostican el grado de displasia epitelial. Desgraciadamente, tampoco podemos contestar a la pregunta de si es posible que los biomarcadores puedan distinguir entre los diferentes grados de displasía epitelial en las QA, dado que un metaanalisis reciente ha mostrado una amplia heterogeneidad entre estudios y unos resultados inconsistentes.

2.4 TRATAMIENTO

La prevención primaria y secundaria mediante el uso de protectores labiales es indispensable para el abordaje de las QA. Diferentes modalidades terapéuticas pueden emplearse para tratar las QA, tanto de forma quirúrgica como no quirúrgica. Así, la bermellectomía con bisturí frio o con láser CO2 y las vaporizaciones con láser a diferentes longitudes de onda han sido los procedimientos quirúrgicos más frecuentemente empleados. Estos procedimientos aportan mayor radi-

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calidad y morbilidad quirúrgica. De otra parte, la terapia fotodinámica y la aplicación tópica de diferentes fármacos (acido retinoico, 5-Fluoracilo, acido tricloroacético, etc…) podrían presentar una menor morbilidad, mayor tasa de recurrencia, mejores resultados estéticos y una efectividad terapéutica dudosa en QAs con displasía epitelial. También la fototerapia combinada con imiquimod al 5%, representa una combinación con alta efectividad terapéutica. Sin embargo, los protocolos terapéuticos son muy diferentes en dependencia del entorno clínico en que se diagnostica y trata la enfermedad.

2.5 IDENTIFICACIÓN DE BRECHAS EN LA INVESTIGACIÓN EN QAS

Un gran numero de estudios han cubierto distinto aspectos de la QAs, aportando abundante información de carácter epidemiológico, etiopatogénico y clínico. Sin embargo, la información terapéutica sobre QA es tremendamente discordante y en base a pequeñas series de casos, generalmente con pacientes tratados con una única modalidad terapéutica y con escaso seguimiento, lo que impide confeccionar guías clínicas con recomendaciones basadas en la evidencia científica.

También se ha identificado la ausencia de consenso en la terminología y taxonomía de la lesión (QA), incluso en los criterios diagnósticos de la misma. Así mismo, es deficitaria la investigación relativa a la identificación de factores de riesgo de malignización de las QA y a la predicción mediante el uso de biomarcadores con potencial utilidad para identificar las QA que evolucionarán hacia la malignización del epitelio labial.

2.6 JUSTIFICACIÓN Y OBJETIVOS

En base a lo previamente expuesto parece necesario alcanzar consensos entre expertos encaminados a utilizar una única terminología, criterios diagnósticos claros que permitan la comparación entre estudios y recomendaciones terapéuticas, en base a la experiencia clínica y a la evidencia científica disponible.

Al tratarse de una lesión con potencial de malignización es particularmente necesario identificar marcadores con capacidad pronóstica/predictiva. Para ello, podrían resultar idóneos los diseños prospectivos de seguimiento de QA, pero este tipo de estudios tiene serias limitaciones éticas, como sería el dejar evolucionar QAs sin tratar, hasta la aparición del evento (malignización). Además, este evento tiene una baja prevalencia y puede ocurrir hasta varias décadas después del diagnóstico de la QA.

Para solventar estos inconvenientes se ha utilizado el modelo de "Epitelio no tumoral adyacente al cáncer" (ANTE), esto es, identificar cambios moleculares en el epitelio peritumoral y asumir que se trata de cambios precoces en la carcinogénesis y potencialmente útiles para identificar epitelio en riesgo de malignización. Este modelo (ANTE) ha sido ampliamente usado con esta finalidad en el carcinoma oral de células escamosas con resultados esperanzadores, sin embargo se desconoce su potencial utilidad para el diagnóstico de eventos precoces en la carcinogenesis labial.

Por tanto, los objetivos del presente trabajo de Tesis Doctoral han sido los siguientes:

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2.6.1 Objetivos

1. Facilitar y alcanzar consenso sobre la terminología, los criterios diagnósticos y actitudes terapéuticas aplicables a la Queilitis Actínica.
2. Evaluar la efectividad terapéutica de los distintos abordajes quirúrgicos y no quirúrgicos en la Queilitis Actínica.
3. Identificar la inmunoreactividad del epitelio no tumoral adyacente al cáncer de labio.

2.7 CAPÍTULO I. CONSTRUYENDO CONSENSO SOBRE QUEILITIS ACTÍNICA. ESTUDIO DELPHI.

Con la finalidad de cumplimentar e primer objetivo se ha diseñado un estudio Delphi, de dos rondas, donde se ha reclutado un grupo de 21 expertos (9dermatólogos, 9 especialistas en medicina oral y 3 patólogos orales), 11 de ellos procedentes del ámbito académico y 10 del ámbito clínico, con diferente procedencia geográfica. El panel de expertos fue seleccionado aplicando criterios bibliográficos y de experiencia clínica (> de 15 años de experiencia en el tratamiento de la QA y/o al menos una publicación sobre QA).

Se ha elaborado un cuestionario con 34 propuestas cerradas (9 -sobre nomenclatura y taxonomia-, 5 -sobre potencial de malignización de QA-, 12 -sobre aspectos diagnósticos en AQ-y 8 -sobre tratamiento de QA-), y 8 preguntas abiertas, que fue remitido por correo electrónico, al panel de expertos.

Para la primera ronda (R1: marzo 2020), en las propuestas de respuesta cerrada se ha valorado el nivel de acuerdo, en una escala

likert de 1-7, (1:fuerte acuerdo; 7: fuerte desacuerdo).También se ha preguntado a los expertos por el grado de confianza en sus respuestas utilizando una escala likert diferente (1: máxima incertidumbre; 7: máxima certeza). Una vez analizada y sintetizada la información, y en base a la retroalimentación del panel de expertos, se transformaron las preguntas abiertas, en preguntas cerradas y se inició la segunda ronda (R2: Junio-2020). El panel de expertos volvió a recibir el cuestionario, con información sobre la respuesta global del grupo de expertos (R1), y en caso de ausencia de consenso con el grupo y para facilitar el mismo, se solicitó al experto que reconsiderase la puntuación de su primera respuesta. Este procedimiento permitió al experto reposicionarse o mantener su puntuación previa.

Consenso fue definido a priori, para las cuestiones cerradas, como un acuerdo 75% y con un coeficiente de variación <50, para las propuestas del comité organizador.

2.7.1 Resultados

La técnica Delphi permitió alcanzar consenso en 25/34 (73.5%) de las propuestas cerradas y en 5/8 (62.5%) de las preguntas cerradas. No hubo pérdidas en las respuestas entre la primera y la segunda ronda. Queilitis actínica es el término que ha mostrado un mayor consenso entre los expertos, y una menor dispersión en sus puntuaciones (CV=21.33), alcanzando una mediana de 7-fuerte acuerdo- (RIQ :6-7). Respecto a la taxonomía, “desorden oral potencialmente maligno” ha sido el grupo más valorado para categorizar esta entidad, con una mediana de 7, con el que el 85.6 % de los expertos han mostrado algún grado de acuerdo (CV<50).

El 66.7% de los expertos consideran que la QA es un término clínico y no patológico (mediana:7; RIQ:4-7) y que puede establecerse

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un diagnóstico definitivo solo con criterios clínicos (mediana: 6; RIQ: 5-7). Particularmente, la inspección y palpación permiten establecer un diagnóstico clínico definitivo (median:a5 ; RIQ: 4-6). Sin embargo, también consideran, aunque con una menor puntuación, que es obligatoria la confirmación histologica en el manejo de QA (mediana:5; RIQ: 2.5-7), pudiendo estar incluso indicada en lesiones homogéneas (mediana:5; RIQ: 3.5-6).

Además, se ha conseguido acuerdo para todas las propuestas relativas al tratamiento de QA (CV<50).

2.7.2 Implications para la clinica y la investigación

La discordancia entre la clínica y la histología parece obligar al clínico a un estricto seguimiento y a la realización de una o varias biopsias incisionales ante cualquier signo/síntoma que alerte de una potencial malignización de la QA. La selección del abordaje terapéutico debería adaptarse al aspecto clínico y al sustrato histologico del desorden, priorizando los tratamientos quirúrgicos para lesiones con displasia moderada/severa. En estos casos, y ante lesiones difusas ,la vermellectomía con análisis seriado del bermellón podría ser la mejor opción. Las AC con displasia leve o sin displásia pueden beneficiarse de non-surgical treatment

2.7.3 Conclusiones

Queilitis actínica es un desorden potencialmente maligno que ha mostrado una importante falta de acuerdo en la comunidad científica, respecto a los criterios y procedimientos diagnósticos, las indicaciones de biopsia y la importancia de los procedimientos auxiliares como asistentes a la biopsia. Sin embargo, a pesar de la insuficiente

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evidencia científica, se ha alcanzado un mayor consenso respecto al manejo terapéutico de esta entidad. Este consenso puede ser aprovechado por los clínicos como ayuda en la toma de decisiones.

2.8 Capítulo II. Aproximaciones terapéuticas a la Queilitis Actínica: Revisión sistemática de la literatura

Con la finalidad de cubrir el segundo objetivo de la Tesis Doctoral, se ha diseñado una revisión sistemática que se realizó siguiendo las directrices PRISMA (Preferred Reporting Items para Revisiones Sistemáticas y Meta-análisis). La revisión siguió los criterios PICO y consideró exposición a cualquier tratamiento médico o quirúrgico con intención curativa. Las comparaciones se establecieron entre pacientes tratados con procedimientos quirúrgicos y no quirúrgicos. Los resultados analizados fue la mejoría clínica e histológicas, así como la maligmnización después del tratamiento de las queilitis actínicas.

Se utilizaron las las bases de datos de MEDLINE (desde 1966), EMBASE (desde 1980) y Proceedings Web of Science (censos de conferencias CCI) La estrategia de búsqueda fue: (("actínic" o "solar") y ("cheilitis")) utilizando tanto descriptores médicos (MeSH) como texto libre. Última búsqueda se actualizó en abril de 2019. Se consideraron elegibles cualquier tipo de estudios, sin limitación de idioma, que incluyeran datos originales, series de casos (≥10 pacientes) con diagnóstico patológico de QA y tratados mediante procedimientos quirúrgicos o no quirúrgicos, que hayan considerado respuesta clínica y/o patológica como variable resultado del estudio. Se excluyeron estudios transversales sin seguimiento del paciente, y la calidad metodológica de los estudios incluidos se evaluó mediante la lista de Downs y Black.

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2.8.1 Resultados

Se identificaron un total de 392 artículos potencialmente elegibles y finalmente solo 20 de ellos fueron incluidos en la síntesis cualitativa. 10 publicaciones de calidad moderada, que agruparon 227 pacientes, se centraron en el tratamiento quirúrgico. La bermellectomia con bisturí frio y con láser carbónico, así como la vaporización, condujeron a la resolución completa de la lesión, pero generaron disestesias labiales como efectos adversos del procedimiento. Sin embargo, no se produjeron recurrencias o estas fueron muy bajas. De otra parte, 11 estudios que incluyeron 187 pacientes fueron tratados mediante terapia fotodinámica (9 estudios), otro estudios intentó un tratamiento tópico con imiquimod y un último estudios prospectivo comparó el abordaje quirúrgico con terapia no quirúrgica (fluoracil y ácido tricloroacético). Además, la tasa de transformación maligna después de tratamientos quirúrgicos se han evaluado en 6 estudios longitudinales, cuatro de los cuales no informaron ningún caso de malignización, mientras que los estudios utilizando diferentes protocolos para la vaporización con láser de CO2 mostraron bajas tasas de malignización en las zonas tratadas.

2.8.2 Limitaciones de esta revisión sistemática

Las limitaciones inherentes a la calidad moderada de los estudios individuales considerados en esta revisión, junto con los potenciales sesgos de selección (diferentes criterios utilizados para definir un caso), deberían ser considerados al examinar nuestros resultados. Los estudios incluidos en esta revisión son principalmente series de casos observacionales retrospectivas/prospectivas, o estudios cuasi-experimentales, nuestro estudio es la primera revisión sistemática que compara diferentes enfoques terapéuticos en la QA, que incluye estudios con seguimiento de pacientes y resultados de malignización después del tratamiento y descripción de los efectos adversos asociados a la terapéutica.

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2.8.3 Implicaciones clínicas y recomendaciones para futuras investigaciones

La limitada fiabilidad de las biopsias incisionales para el diagnóstico de las QA y la imposibilidad de una curación completa de las lesiones displásicas por la terapia no quirúrgica debería implicar modificaciones en las estrategias de manejo de las QA, evitando recomendar el 5 -fluorouracilo y el imiquimod para tratar áreas clínicamente sospechosas (> 0,5 cm) con displasia, o cuando se trata de lesiones difusas, leucoplasia o atrofia con displasia.

El uso de procedimientos ópticos auxiliares a la biopsia y/o la toma de biopsias múltiples podría incrementar sustancialmente la certeza diagnóstica y ayudar a personalizar los tratamientos; así como para evitar sesgos de selección en futuras investigaciones. También, son necesarios nuevos ensayos clínicos mejor diseñados, que consideren la tasa de malignización entre sus resultados y así poder proporcionar pruebas más sólidas sobre las diferentes opciones de tratamiento, en particular para los casos de procedimientos no invasivos.

2.8.4 Conclusiónes

En base a estudios de una calidad limitada, El tratamiento quirúrgico parece ser la primera línea de tratamiento para las QA dado que ha demostrado ser útil para el control clínico y patológico de esta entidad. Sin embargo, no hay evidencia de ningún tratamiento efectivo para prevenir las transformaciones malignas asociadas a Queilitis actínica. Los procedimientos no quirúrgicos han mostrado resultados menos consistentes en el control histologico de la lesión, aunque la terapia con fármacos podría optimizar los resultados obtenidos por otros enfoques terapéuticos.

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2.9 CAPÍTULO III. ESTUDIO INMUNOHISTOQUÍMICO DEL EPITELIO NO TUMORAL ADYACENTE AL CANCER DE LABIO. METAANALISIS

Para satisfacer el tercer objetivo propuesto en esta Tesis, se ha llevado a cabo una revisión sistemática con metaanálisis.

2.9.1 Metodología:

El protocolo siguió la metodología PRISMA para revisiones sistemáticas y se solicitó el registro en PROSPERO (International Prospective Register of Systematic Reviews). El estudio se diseñó con el objetivo identificar el patrón inmunohistoquímico del epitelio peritumoral al cáncer de labio y como objetivo secundario evaluar la inmunoreactividad del carcinoma labial de células escamosas. Se han utilizado las bases MEDLINE (desde 1966), EMBASE (desde 1980) y Proceedings Web of Science (Conference Proceedings Citation Index-Science (CPCI-S), desde1990). Para la estrategia de búsqueda se han utilizado tanto términos MESH como términos libres. Además se ha utilizado la base de datos Open Gray y WorldCat. La estrategia de búsqueda utilizada fue la siguiente:

((lip cancer OR lip neoplasm OR oral cancer OR oral neoplasm) and (non-tumor epithelium OR adjacent epithelium OR surrounding epithelium OR surrounding dysplasia) and (immunohistochemistry))

2.9.2 Criterios de elegibilidad

Como criterios de inclusión se consideraron todos los estudios inmunohistoquímicos que incluyesen muestras de carcinoma labial y

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del epitelio peritumoral y proporcionasen resultados cuantitativos o semicuantitativos de la tinción (nuclear, citoplásmatica o de membrana) en los tejidos analizados. Como criterios de exclusión: i)Estudios que hayan considerado análisis histológicos, pero no inmunohistoquímicos, Ii) Estudios en que la muestra del carcinoma labial y del epitelio adhyacente no tumoral procedan de pacientes diferentes.,iii). No se consideraron estudios con datos secundarios, casos clínicos y revisiones narrativas o sistemáticas.

Se procedió a una evaluación de la calidad de los estudios incluidos en la (RS) y el metaanalisis se llevó a cabo usando MetaXL software, la versión 5.3 disponible en https://www.epigear.com/ index_files/ metaxl.html.

2.9.3 Resultados

Se identificaron 339 artículos, de los que se consideraron finalmente 7 para la síntesis cualitativa y tan solo 4 con inmunoreactividad para p53, se incluyeron en el metaanálisis. La prevalencia agrupada de p53, en el ANTE fue de 0,74% (IC95%: 0,66-0,82), mientras en el epitelio tumoral (cáncer de labio) fue de 0,83% (IC95%: 0,75-0,89)

2.9.4 Discusión

A pesar de que diferentes marcadores han demostrado capacidad para identificar alteraciones moleculares previas a la aparición del cáncer de labio. El potencial desarrollo de este cáncer sobre epitelios no displásicos o con displasia leve, parece obligar al seguimiento de pacientes con factores de riesgo claros o con QA, donde el uso de biomarcadores probablemente tendría utilidad pronóstica. También

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podrían resultar de utilidad en la planificación del tratamiento quirúrgico y no quirúrgico. Todos los estudios de esta SR han sido estudios transversales, con muestras pequeñas, que no han reportado datos clínicos y que evidencian defectos metodológicos, que deberían ser evitados en futuras investigaciones. En este sentido se ha propuesto llevar a cabo estudios prospectivos, multicéntricos y con estratificación de los pacientes según la terapeutica empleada, para proporcionar datos de calidad. La definición de caso debería ser establecida con claridad, y diferenciar y estandarizar los criterios clínicos e histologicos. Teniendo en cuenta los distintos factores etiológicos y de la carcinogenesis labial, los futuros estudios deberían incluir también información sobre factores de riesgo y susceptibilidad del paciente . Además, con la finalidad de incrementar la replicabilidad en estos estudios con biomarcadores, debería describirse la inmunoreactivity en los distintos estratos epiteliales, estandarizarse y especificarse el procedimiento de contaje, y desarrollar estrategias para minimizar la variabilidad interobservador. El incorporar grupo control (epitelio labial normal) a los estudios sobre biomarcadores en este ámbito, probablemente proporcionaría información suplementaria sobre el proceso de la carcinogenesis labial

Se concluye que los biomarcadores inmunohistoquímicos han mostrado una escasa capacidad de diferenciar entre los distintos grados de displasia en el epitelio peritumoral al cáncer de labio. Además parece existir similitud en la inmunoexpresión de p53, en el epitelio adyacente y en el epitelio tumoral, incluso en epitelios de apariencia normal o con leves alteraciones histológicas. Sin embargo, el empleo de estos biomarcadores en el seguimiento de queilitis actínica, como alerta de una potencial malignización o para la toma de decisiones terapeuticas, requiere de más estudios, metodologicamente adecuados y desarrollados en un entorno clínico.

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2.10 Conclusiones de la Tesis Doctoral

2.10.1 Primera:

En base a estudios de una calidad limitada, el tratamiento quirúrgico parece ser la primera línea terapéutica en las Queilitis Actínicas, dado que ha demostrado ser útil para el control clínico y patológico de esta entidad. Sin embargo, no hay evidencia sobre ningún tratamiento efectivo para prevenir las transformaciones malignas asociadas a la Queilitis actínica. Los procedimientos no quirúrgicos han mostrado resultados menos consistentes en el control histológico de la lesión, aunque la terapia farmacológica podría optimizar los resultados obtenidos por otros enfoques terapéuticos.

2.10.2 Segunda:

La Queilitis Actínica es un desorden potencialmente maligno, que ha mostrado una importante falta de acuerdo en la comunidad científica, respecto a los criterios y procedimientos diagnósticos, las indicaciones de biopsia y la importancia de los procedimientos auxiliares como ayuda a la biopsia. Sin embargo, a pesar de la insuficiente evidencia científica, se ha alcanzado un mayor consenso respecto al manejo terapéutico de esta entidad. Este consenso podría ser aprovechado por los clínicos como ayuda en la toma de decisiones.

2.10.3 Tercera:

Los biomarcadores inmunohistoquímicos han mostrado una escasa capacidad de diferenciar entre los distintos grados de displasia en el epitelio peritumoral al cáncer de labio. Además, parece existir

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similitud en la inmunoexpresión de p53, en el epitelio adyacente y en el epitelio tumoral, incluso en epitelios de apariencia normal o con leves alteraciones histológicas. Sin embargo, el empleo de estos biomarcadores en el seguimiento de las queilitis actínicas, como alerta de una potencial malignización o para la toma de decisiones terapeuticas, requiere de más estudios metodologicamente adecuados y desarrollados en un entorno clínico.

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3 SUMMARY

3.1 INTRODUCTION. GENERAL CONSIDERATIONS

Among the multiple terms used to describe this condition, actinic cheilitis (AC) is the most frequently found in the literature, followed by solar cheilitis. Moreover, the terms actinic cheilosis and solar cheilosis have been coined to emphasise the not necessarily inflammatory nature of this pathology.

The available evidence on the potential for malignancy of this lesion is built on its co-occurrence with squamous cell carcinoma of the lip, although few longitudinal studies allow determining the annual malignancy rate in untreated patients. A single meta-analysis published in 2018 and based on a methodologically weak study, determined that the malignant transformation rate of AC is 3.07%. However, up to 89 genes have been linked with the progression of AC to lip carcinoma. In any case, to date, this pathology is considered an oral potentially malignant disorder (OPMD). Other terms to describe OPMDs are *lip potentially malignant disorder* or *lesion*, *precursor or pre-invasive lesion*, and *lip pre-cancer*. The overall prevalence of OPMDs is 4.47%. Nonetheless, AC prevalence and incidence rates vary enormously depending on the diagnostic criteria. Based on clinical criteria, a prevalence of 31.3 % has been recently reported in Spain

Continuous exposure to solar radiation is considered the main factor in the etiopathogenesis of AC. The potential carcinogenic risk of ultraviolet A radiation (UVA) (315-400 nm) and ultraviolet B ra-

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diation (UVB) (280-315 nm) has been shown in humans and animal models. Ultraviolet radiation causes direct DNA damage and produces oxidative stress and inflammation. Being older than 60 years, with Fitzpatrick II skin type, working outdoors for at least 25 years, and a history of non-melanoma skin cancer have also been identified as risk factors for AC. Globally, this condition has been observed more frequently in males and fair-skinned people. The time spent working outdoors (farmers, fishermen, gardeners, etc.) without sun protection is also considered a relevant risk factor for developing AC. The later risk factor has been shown to have a statistically significant positive relationship to atrophy of the lip, blurring of lip borders, formation of vertical fissures, white and red blemishes, loss of elasticity, and lip induration.

3.2 CLINICAL BEHAVIOUR AND DIAGNOSIS

AC has protean clinical manifestations with mild changes, e.g., dryness of the vermilion border, desquamation, fissures, and wrinkles perpendicular to the axis of the lips. Other expressions of this condition may be a pale semimucosa, loss of elasticity and blurring of the vermilion border, as well as crusted, eroded, or ulcerated lesions at one or more sites, homogeneous or not. White, red, or combined red-and-white plaques may sometimes develop.

AC usually presents with one or two clinical features and only exceptionally with more than three. The most common signs/symptoms are a spotted appearance, desquamation, and erythema of the lip, while the development of eroded/ulcerated lesions and the white plaque are less prevalent.

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In pursuit of a better identification and reproducibility of the clinical diagnosis a grading for the clinical criteria has been proposed.

Grade I: dryness of the vermilion border of the lips; Grade II: atrophy of the border of the lips, a pale vermilion border, blurring of lip borders, and faint pigmented lines (not ephelides); Grade III: lips have a heterogeneous appearance with rough, hyperkeratotic, and/or dry areas; Grade IV: one or more sites of the lips appear ulcerated, white plaques, traumatized and indurated areas. These may be considered as warning signs/symptoms for malignant transformation. A poorly defined AC may show histological changes unevenly distributed along the vermilion border of the lips.

3.3 DIAGNOSIS AND AIDS FOR CLINICAL DIAGNOSIS

The diagnostic aspects of AC are very controversial. A large group of authors have assumed the traditional concept of AC as a clinical term, thus accepting that the macroscopic changes associated to this entity are sufficient to establish a definitive diagnosis. This approach is mostly used in prevalence studies. However, the lack of clinical-pathological correlations noticeably weakens this diagnostic criterion.

AC may be solitary or multifocal, and the histopathological substrate is completely different regardless the clinical characteristics. I.e., there is no reliable way to determine the degree of histological change in the epithelium when evaluating a lesion whatever its clinical presentation.

This has encouraged researchers with another school of thought to regard histological information essential to establish a de-

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finitive diagnosis of AC. In this case, histology encompasses moderate acanthosis or hyperkeratosis, absence or presence of different degrees of epithelial dysplasia, and even the coexistence of a micro-invasive or invasive squamous cell carcinoma of the lip. The presence of epithelial dysplasia is especially considered by other authors, a key histological feature to establish a diagnosis. In any case, to date this controversy remains active.

Moreover, there is a lack of agreement on the classification of epithelial dysplasia. In particular, moderate dysplasia is a challenge when classifying these lesions. A binary classification (low risk and high risk) may help simplify the diagnostic categorization, but for this, previous thorough validation is necessary.

As AC are heterogeneous lesions, using different optical methods may be potentially useful for improving visual evaluation and help in the decision-making process regarding a biopsy.

To date, only few studies have investigated the potential of *in vivo* confocal microscopy for AC in terms of diagnostic sensitivity and specificity. Similarly, the diagnostic benefit of dermoscopy remains unknown.

Contrarily, tissue autofluorescence analytical procedures have proven their clinical usefulness in identifying different pathological patterns through imaging in up to 95% of AC cases, as well as a capacity to direct incisional biopsies towards areas with greater dysplasia.

Lip biopsy is another point of disagreement. The lack of clinical sensitivity promotes that one or more biopsies are taken to make a histological diagnosis and establish a pre-treatment plan. However, when scheduling a vermilionectomy, a biopsy before the procedure *is*

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open to doubt. Thus, many authors consider vermilionectomy an excisional biopsy that allows a serial analysis of the whole lesion.

The degree of epithelial dysplasia is frequently underdiagnosed with incisional biopsies (up to 40% of the cases). Unfortunately, based on the results of a recent meta-analysis that showed large heterogeneity between studies and inconsistent results, it is not possible to confirm if biomarkers can distinguish between the different types of epithelial dysplasia in AC.

3.4 TREATMENT

Primary and secondary prevention with lip protectors is essential for the therapeutic approach of AC. There are several surgical and non-surgical modalities of treatment for the management of AC. Vermilionectomies using either a cold scalpel or a carbon dioxide laser and laser vaporization at different wavelengths are the most frequently used procedures. These interventions are more radical and result in less surgical morbidity. On the other hand, photodynamic therapy and topical use of different drugs (retinoic acid, 5-fluorouracil, trichloroacetic acid, etc.) is associated with lower morbidity, higher rates of recurrence, better aesthetic results, and doubtful therapeutic effectiveness in AC with epithelial dysplasia. Phototherapy in combination with 5% imiquimod is highly effective. However, treatment protocols are very different depending on the clinical setting at which the condition is diagnosed and treated.

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3.5 IDENTIFICATION OF RESEARCH GAPS IN ACTINIC CHEILITIS

The literature provides a large number of studies covering different aspects of AC with abundant epidemiological, etiopathogenic, and clinical information. However, the available therapeutic data is very contrasting and based on small series of cases, usually including subjects treated with a single therapeutic modality and little follow-up, which makes designing clinical guidelines that include recommendations based on scientific evidence difficult.

We detected a lack of consensus on AC terminology and taxonomy, even in the diagnostic criteria. Research aiming to identify the risk factors for malignancy of AC or to predict the potential development of malignancy of the lip epithelium using biomarkers is also scarce.

3.6 RATIONALE AND OBJECTIVES

There is a need to achieve a consensus among experts aiming the use of a single terminology, clear diagnostic criteria that allow inter-study comparisons, and therapeutic recommendations based on the available scientific evidence.

AC is a lesion with a potential for malignancy, hence, it is necessary to identify markers with prognostic/predictive ability. Prospective studies and follow-up in AC patients are ideal for this purpose, although this type of research has important ethical limitations, such as allowing the AC to progress (in untreated subjects) until it becomes malignant, and the fact that this event is of low prevalence and may occur decades after AC diagnosis.

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To overcome these difficulties, the adjacent non-tumour epithelia (ANTE) model was used, i.e., identify molecular changes in peritumoral epithelium and assume they are early carcinogenic changes potentially useful to recognize an epithelium at risk of malignancy. The ANTE model has been widely used for this purpose in oral squamous cell carcinoma with encouraging results, but its potential usefulness in the diagnosis of early events in lip carcinogenesis remains unknown.

Thus, the aims of this PhD thesis are:

3.6.1 Objectives

1. To facilitate and reach a consensus for actinic cheilitis terminology, diagnostic criteria, and therapeutic strategies/approaches.
2. To assess the therapeutic effectiveness of the different surgical and non-surgical approaches for actinic cheilitis.
3. To identify immune response in non-tumour epithelium adjacent to a cancer on the lip.

3.7 Chapter I. Creating consensus on actinic cheilitis. The Delphi survey.

In order to meet the first objective of this study, a two-round Delphi survey was designed. A team of 21 experts was recruited (nine dermatologists, nine specialists in oral medicine, and three oral pathologists), 11 from the academic field and 10 from the clinical field, from different geographical regions. The panel of experts was

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selected based on bibliographic criteria and clinical experience (more than 15 years of experience treating AC and/or at least one publication on AC).

A questionnaire with 34 close-ended proposals (nine on terminology and taxonomy, five on potential for malignancy of AC, 12 on diagnostic AC aspects, and eight on AC treatment) and eight open-ended questions was created and sent by email to the panel of experts.

In the first round (R1: March 2020), the level of agreement for the close-ended answers proposals was assessed, based on a seven-point (1-7) Likert scale (1: agree strongly; 7: disagree strongly). The degree of confidence the experts had regarding their answers was also measured using a different Likert scale (1: maximum uncertainty; 7: maximum certainty). Once the information was analysed and synthetized, and based on the feedback of the panel of experts, the open-ended questions were transformed to close-ended questions and a second round was initiated (R2: June 2020). The members of the panel of experts received the questionnaire again with information on the global response given by the experts (R1); in case a consensus with the group had not been reached, the expert was asked to reconsider the scoring of his/her initial answer, aiming a consensus. This allowed the expert to change or maintain the scoring.

The consensus was defined *a priori* for close-ended questions by an agreement of 75% and coefficient of variation (CV) < 50 for the proposals of the organizing committee.

3.7.1 Results

The Delphi method allowed reaching a consensus in 25/34 (73.5%) of the close-ended proposals and in 5/8 (62.5%) of the

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closed-ended questions. There were no losses in the answers between the first and the second round. Actinic cheilitis was the term with the highest consensus between the experts and lowest score spread (CV = 21.33), with a mean value of 7 (strong agreement) (RIQ: 6-7). Regarding taxonomy, the best valued group to categorize this entity was *potentially malignant oral disorder* (mean value of 7), with at least some level of agreement in 85.6% of the experts (CV < 50).

Sixty-six point seven per cent of the experts consider AC a clinical term (not a pathological one) (mean: 7; RIQ: 4-7) and that a definitive diagnosis can be made with clinical criteria (mean: 6; RIQ: 5-7). More specifically, inspection and palpation allow establishing a definitive clinical diagnosis (mean: a5; RIQ: 4-6). However, experts also consider, although with a lower scoring, that histological confirmation is mandatory in the management of AC (mean: 5; RIQ: 2.5-7), and even indicated in homogeneous lesions (mean: 5; RIQ: 3.5-6).

Additionally, agreement was achieved for all AC treatment proposals (CV<50).

3.7.2 Consequences for clinical management and research

The discrepancies between clinical and histological features seem to pressure the physician to strictly monitor the patient and to perform one or several incisional biopsies in case of any signs/symptoms alerting on a progression to malignancy of the AC. The type of therapeutic approach should adapt to the clinical findings and histological substrate of the condition, prioritizing surgical treatments in lesions with moderate/severe dysplasia. In these cases, as well as with diffuse lesions, vermilionectomies with serial analyses of the vermillion is probably the best treatment option. Non-surgical treatment may be sufficient for AC with mild or no dysplasia.

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3.7.3 Conclusions

Actinic cheilitis is a potentially malignant disorder for which there is a significant lack of agreement on the diagnostic criteria and procedures, indications for biopsies, and relevance of auxiliary procedures (e.g., asistente a la biopsia) among the scientific community. Nonetheless, despite the scarce scientific evidence, greater consensus has been achieved on the therapeutic management of this entity. Clinicians may use this consensus to help them in their decision-making.

3.8 CHAPTER II. THERAPEUTIC APPROACH FOR ACTINIC CHEILITIS: SYSTEMATIC REVIEW OF THERAPEUTIC EFFECTIVENESS AND MALIGNANT TRANSFORMATION AFTER TREATMENT

To meet the second objective of this PhD thesis, a systematic review following the guidelines of the Preferred Reporting Items for Systematic Review and meta-Analysis (PRISMA) was designed. The PICO criteria were used for the review and exposure to any medical or surgical treatment with the intent of healing was considered. Comparisons were made between patients treated with surgical and non-surgical procedures. Clinical and histological improvements were analysed, as well as malignant transformation after the treatment of the AC.

The following databases were used: MEDLINE (from 1966), EMBASE (from 1980), and Web of Science proceedings (Conference Citation Index - CCI). The used search strategy was: (*actinic* or *solar*) and (cheilitis) using medical descriptors (MeSH) as well as free terms. The last search was updated on April 2019. Any type of study (no language limitation) with original data, case series (≥10 patients) with

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pathological diagnosis of AC treated with surgical and non-surgical procedures was eligible for inclusion. The studies had to consider clinical and/or pathological response as a result variable. Transversal studies without patient follow-up were excluded and the methodological quality of the included studies was evaluated with the Downs and Black checklist.

3.8.1 Results

Three hundred and ninety-two potentially eligible articles were identified, from which 20 were included in the qualitative synthesis. Ten moderate quality publications that grouped 227 patients focused on surgical treatment. Complete resolution of lesions was achieved with vermilionectomies using either a cold scalpel or a carbon dioxide laser and laser vaporization, although it caused lip dysesthesia (adverse effect of the procedure). Few or no recurrences were reported. On the other hand, 11 studies that included 187 patients used photodynamic therapy (nine studies studies), one tried treating patients with topical use of imiquimod, and another was a prospective study that compared surgical versus non-surgical approaches (5-fluorouracil and trichloroacetic acid). Furthermore, the rate of malignant transformation after surgical treatment was assessed in six longitudinal studies, four of which did not report any malignancy case, while the studies that used different carbon dioxide laser vaporization protocols reported low malignancy rates in the treated areas.

3.8.2 Limitations to this systematic review

Limitations include the moderate quality inherent to the individual studies considered for the review and possible selection

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biases (different criteria used for defining a case). These limitaions should be taken into account when examining the results. The works considered in this review are mainly retrospective/prospective observational case series or quasi-experimental studies. To the best of our knowledge, this is the first systematic review that compares different therapeutic approaches in AC and includes studies with patient follow-up, results on malignancy after the treatment, and description of adverse events associated to the therapy.

3.8.3 Clinical implications and recommendations for future research

Incisional biopsies have limited reliability for the diagnosis of AC and complete healing of dysplastic lesions is not achieved with non-surgical treatments. Considering this, changes should be made in the strategies for managing AC, e.g., avoid recommending 5-fluorouracil and imiquimod to treat clinically suspicious lesions (> 0.5 cm) with dysplasia, or cases with diffuse lesions, leukoplakia, or atrophy with dysplasia.

The use of optical approaches supplementary to biopsy and/or performing multiple biopsies may significantly improve the diagnostic certainty and help personalize treatment. Moreover, this may prevent selection biases in future research studies. Further clinical trials with better designs in which the malignancy rate is considered in their results are required to provide more robust evidence on the different treatment options, particularly in cases for which non-invasive procedures are used.

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3.8.4 Conclusions

Based on studies of poor quality, surgical treatment seems to be the first therapeutic choice for AC, as it has shown to be useful for the clinical and pathological control of this entity. However, there is no evidence on an effective treatment to prevent malignant transformation associated to actinic cheilitis. Non-surgical procedures show less consistent results in the histological control of the lesion, although pharmacological therapy may optimize the results obtained by other therapeutic approaches.

3.9 CHAPTER III. IMMUNOHISTOCHEMICAL STUDY OF THE NON-TUMOUR EPITHELIUM ADJACENT TO A LIP CANCER. META-ANALYSIS

For the third objective, we carried out a systematic review with meta-analysis.

3.9.1 Methodology:

The PRISMA protocol was followed for the systematic reviews and registration in the International Prospective Register of Systematic Reviews (PROSPERO) was requested. The study was designed to identify the immunohistochemical pattern of non-tumoral epithelium adjacent to lip cancer and assess the immune reactivity of squamous cell carcinoma of the lip. The following databases were used: MEDLINE (from 1966), EMBASE (from 1980), and Web of Science proceedings (Conference Proceedings Citation Index - Science (CPCI-S), from 1990). Search strategies were applied using MeSH and free terms. The Open Gray and WorldCat databases were

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also used. Search strategy was: ((lip cancer OR lip neoplasm OR oral cancer OR oral neoplasm) and (non-tumour epithelium OR adjacent epithelium OR surrounding epithelium OR surrounding dysplasia) and (immunohistochemistry)).

3.9.2 Eligibility criteria

The following criteria had to be met for inclusion: immunohistochemical studies that included lip cancer and peritumoral epithelium samples and provided quantitative or semi-quantitative staining results (nuclear, cytoplasmic, or membrane) of the analysed tissues. Exclusion criteria: i) Studies that included histological analyses but not immunohistochemical; ii) studies in which lip cancer and peritumoral epithelium samples were from different patients; iii) studies with secondary data, clinical cases, and narrative or systematic reviews were not considered.

The quality of the studies included in the systematic review was evaluated. The MetaXL software version 5.3 was used for the meta-analysis, available at https://www.epigear.com/index_files/ metaxl.html.

3.9.3 Results

Three hundred and thirty-nine articles were identified, from which seven were considered for the qualitative synthesis and only four with immune reactivity to p53 were included in the meta-analysis. Grouped p53 prevalence was 0.74% (95% CI: 0.66-0.82) in ANTE and 0.83% (95% CI: 0.75-0.89) in tumour epithelium (lip cancer)

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3.9.4 Discussion

Several markers have proven their utility in identifying molecular changes before the appearance of lip cancer. The potential development of this cancer on non-dysplastic epithelium or with mild dysplasia seems to pressure the physician to monitor patients with clear risk factors or with AC. In these cases, biomarkers may be of prognostic value. These markers may also be of help for planning surgical or non-surgical treatments. All the studies in the systematic review are transversal, with small samples, did not report clinical data, and had methodological weaknesses that should be corrected in future research works. Prospective, multicenter, stratified (based on the therapeutic approach) studies have been proposed aiming to ensure quality data. Case definition should be clearly established, and clinical and histological criteria differentiated and standardized. Taking into account the different etiological and lip carcinogenesis factors, future studies should also include information on patient´s risk factors and susceptibilities. Furthermore, to improve reproducibility of studies using biomarkers, immune reactivity in the different layers of the epithelium should be described, the count procedure standardized and specified, and strategies to reduce inter-observer variability developed. The inclusion of a control group (normal lip epithelium) in studies evaluating biomarkers in this field may provide supplementary information on the process of lip carcinogenesis.

In short, immunohistochemical markers have shown to have a limited ability to distinguish between different degrees of dysplasia in the epithelium adjacent to lip cancer. Moreover, there seems to be similar immune expression of p53 in peritumoral and tumoral epithelia, even in epithelium with normal appearance or with mild histological changes. To use these biomarkers in the follow-up of patients with

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actinic cheilitis as alert for potential malignancy or for therapeutic decision-making requires the development of additional studies with the appropriate methodology and in clinical settings.

3.10 CONCLUSIONS

3.10.1 First:

Actinic cheilitis is a potentially malignant disorder for which there is a significant lack of agreement on the diagnostic criteria and procedures, indications for biopsies, and relevance of auxiliary procedures (e.g., asistente a la biopsia) among the scientific community. Nonetheless, despite the scarce scientific evidence, greater consensus has been achieved on the therapeutic management of this entity. Clinicians may use this consensus to help them in their decision-making.

3.10.2 Second:

Based on studies of poor quality, surgical treatment seems to be the first therapeutic choice for AC, as it has shown to be useful for the clinical and pathological control of this entity. However, there is no evidence on an effective treatment to prevent malignant transformation associated to actinic cheilitis. Non-surgical procedures show less consistent results in the histological control of the lesion, although pharmacological therapy may optimize the results obtained by other therapeutic approaches.

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3.10.3 Third:

Immunohistochemical markers have shown to have a limited ability to distinguish between different degrees of dysplasia in the epithelium adjacent to lip cancer. In addition, there seems to be similar p53 immune expression in peritumoral and tumoral epithelia, even in epithelium with normal appearance or mild histological changes. To use these biomarkers in the follow-up of patients with actinic cheilitis as alert for potential malignancy or for therapeutic decision-making, requires that additional studies with the appropriate methodology and in clinical settings are conducted.

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4 INTRODUCTION. ACTINIC CHEILITIS

4.1 TAXONOMY

Many terms have been used to define this condition. Actinic cheilitis (AC) and solar cheilitis are indifferently used, although the first is more extensively used. Moreover, the terms actinic cheilosis and solar cheilosis have been coined to emphasise the not necessarily inflammatory nature of this pathology (Jadotte, 2012).

The evidence available on the potential for malignancy of this lesion is based on its co-occurrence with squamous cell lip carcinoma, but only few longitudinal studies allow to determine the annual malignancy rate in untreated patients. According to some authors, the level of certainty of the lesion becoming malignant is low (Ben Slama, 2001; van Der Waal, 2003). However, up to 89 genes have been linked with the progression of AC to lip carcinoma (Jadotte YT, 2012). In any case, to date, this pathology is considered an oral potentially malignant disorder (OPMD) (Warnakulasuriya, 2009; van Der Waal, 2015). Other terms are also used to describe OPMD, such as lip potentially malignant disorder or lesion, precursor or pre-invasive lesion, and lip pre-cancer.

The only meta-analysis on the topic, published in 2018, determined that the malignant transformation rate of AC is 3.07 % based on a methodologically weak study (Dancynger et al., 2018).

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4.2 ETIOPATHOGENESIS AND EPIDEMIOLOGY OF ACTINIC CHEILITIS

Continuous exposure to solar radiation is considered the main factor in the etiopathogenesis of this lesion. The potential carcinogenic risk of ultraviolet A radiation (UVA) (315-400 nm) and ultraviolet B radiation (UVB) (280-315 nm) has been shown in humans and animal models. Ultraviolet radiation causes direct DNA damage, and produces oxidative stress and inflammation (Dancynger et al., 2018).

The overall prevalence of OPMDs is 4.47% (Mello, 2018). However, AC prevalence and incidence rates vary enormously depending on the diagnostic criteria. Based on clinical criteria, a prevalence of 31.3 % has been recently published in Spain (Rodriguez-Blanco I). Being older than 60 years, with Fitzpatrick II skin type, working outdoors for at least 25 years, and a history of non-melanoma skin cancer have also been identified as risk factors for AC (Rodriguez-Blanco et al., 2018). Globally, this condition has been observed more frequently in males and fair-skinned people. The time spent working outdoors (farmers, fishermen, gardeners, etc…) without sun protection is also considered a relevant risk factor for developing AC (van Der Waal, 2003). The later risk factor has been shown to have a statistically significant positive relationship to atrophy of the lip, blurring of lip borders, formation of vertical fissures, white and red blemishes, loss of elasticity, and lip induration (Santos et al., 2018).

4.3 CLINICAL BEHAVIOUR

AC may present an acute clinical course in limited periods of sun exposure. In these cases, the lesion manifests with a variable spectrum throughout several weeks, usually disappearing at the end of the

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summer season. Contrarily, long hours in the sun leads to chronic permanent clinical changes of the vermilion border of the lips (Picascia et al., 1987).

AC has protean clinical manifestations with mild changes, e.g., dryness of the vermilion border, desquamation, fissures, and wrinkles perpendicular to the axis of the lips. Other expressions of this condition may be a pale semimucosa, loss of elasticity and blurring of the vermilion border, as well as crusted, eroded, or ulcerated lesions at one or more sites, homogeneous or not (Fig.1). White, red, or combined red-and-white plaques may sometimes develop.

Figure 1: Different clinical forms of Actinic Cheilitis. Image 1A shows an impetiginized Actinic cheilitis. Image 1B demostrate a homogeneous Actinic cheilitis with slight blurring of the vermilion border. Image 1C presents a slight loss of lip color and the presence of lip fissures. Image 1D evidence a marked loss in the delimitation of the vermilion border as well as labial pallor.

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AC usually presents with one or two clinical features and only exceptionally with more than three (Rodriguez Blanco et al., 2019). The most common signs/symptoms are a spotted appearance, desquamation, and erythema of the lip, while the development of eroded/ulcerated lesions and the white plaque are less prevalent (Rodriguez Blanco et al., 2019).

In pursuit of a better identification and reproducibility of the clinical diagnosis Poitevin and collaborators (Poitevin et al., 2017) proposed a grading for the clinical criteria.

Grade I: Dryness of the vermilion border of the lips. Grade II: Atrophy of the border of the lips, a pale vermilion border, blurring of lip borders, and faint pigmented lines (not ephelides). Grade III: lips have a heterogeneous appearance with rough, hyperkeratotic, and/or dry areas. Grade IV: One or more sites of the lips appear ulcerated, white plaques, traumatized and indurated areas. These may be considered as warning signs/symptoms for malignant transformation (Poitevin et al., 2017). A poorly defined AC may show histological changes unevenly distributed along the vermilion border of the lips (Simonsen et al., 2007).

4.4 DIAGNOSIS

The diagnostic aspects of AC are very controversial. A large group of authors have assumed the traditional concept of AC as a clinical term, thus accepting that the macroscopic changes associated to this entity are sufficient to establish a definitive diagnosis. This approach is mostly used in prevalence studies. However, the lack of clinical-pathological correlations noticeably weakens this diagnostic criterion.

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AC may be solitary or multifocal, and the histopathological substrate is completely different regardless the clinical characteristics. I.e., there is no reliable way to determine the degree of histological change in the epithelium when evaluating a lesion whatever its clinical presentation (van Der Waal, 2003).

These circumstances have encouraged researchers with another school of thought to consider histological information as essential to establish a definitive diagnosis of AC. Histology encompasses moderate acanthosis or hyperkeratosis, absence or presence of different degrees of epithelial dysplasia, and even the coexistence of a microinvasive or invasive squamous cell lip carcinoma Fig.2 (Melo et al., 2019). The presence of epithelial dysplasia is particularly considered by other authors, a key histological feature to establish a diagnosis (Kaugars, 1999). In any case, the controversy remains active to date.

Moreover, there is a lack of agreement on the classification of epithelial dysplasia, particularly regarding moderate dysplasia, a challenge when classifying these lesions. A binary classification (low risk and high risk) may help simplify the diagnostic categorization, but for this, previous thorough validation is necessary (Cámara, 2016).

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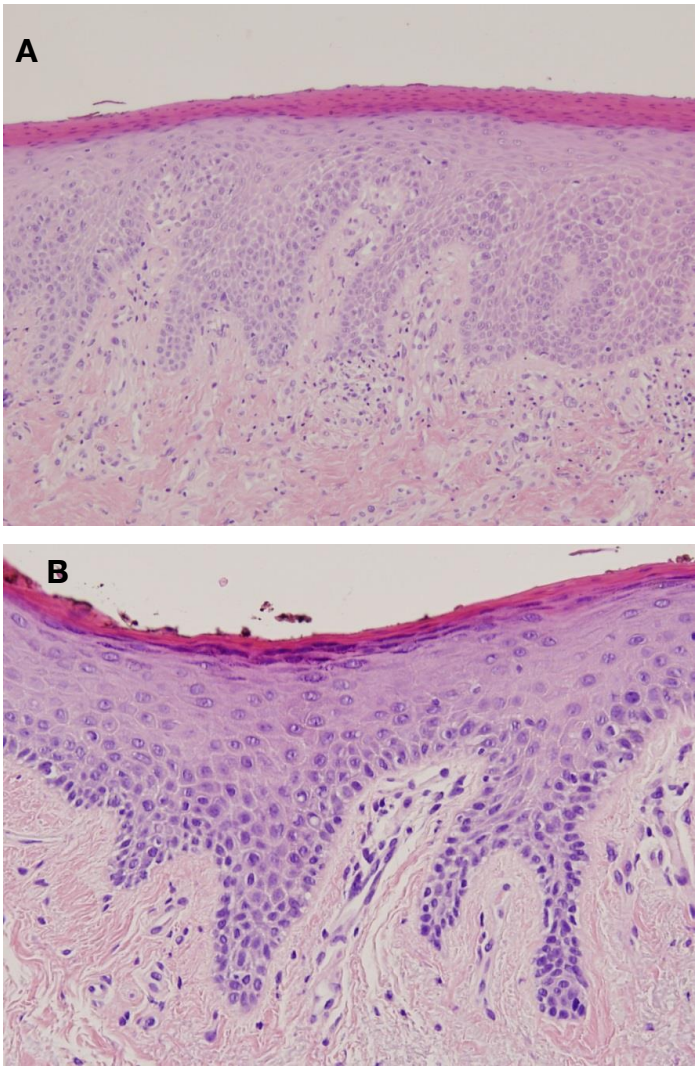

Figure 2: Image A shows an epidermal hyperplasia with an irregular elongation of the epithelial rete pegs, with thickening of the corneal layer showing the occassional persistence of nuclei. (HE, 10X). Image B shows irregular hyperplasia of the epithelial rete pegs with scarce inflammatory infiltrate and solar elastosis.

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4.5 DIFFERENTIAL DIAGNOSIS

Clinically, AC is easily recognized, although it requires a differential diagnosis from other cheilitis with different aetiologies or associated to a systemic pathology (Lugović-Mihić et al., 2018; Samimi et al., 2016). Other non-actinic cheilitis frequently reported are angular cheilitis, those associated with irritating products, retinoid-induced cheilitis, granulomatous cheilitis, glandular cheilitis, and plasma cell cheilitis. The proposed new classification of cheilitis may help in the differential diagnosis of this entity (Lugovic-Mihic et al., 2018). Table 1.

Table 1. Cheilitis classification proposed by Lugović-Mihić (2018)

Mostly reversible cheilitis	Mostly persistent cheilitis	Cheilitis in association with other dermatoses and systemic pathologies
Cheilitis simplex	Actinic cheilitis	Lupus erythematosus
Angular/ infective cheilitis	Granulomatous cheilitis	Lichen planus
Eczematous/ contact cheilitis	Glandular cheilitis	Angioedema
Exfoliative cheilitis	Plasma cell cheilitis	Pemphigoid/pemphigus
Drug-related cheilitis		Xerostomia
Actinic cheilitis		Erythema multiforme
		Crohn's disease

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4.6 AIDS FOR CLINICAL DIAGNOSIS

As AC are heterogeneous lesions, using different optical procedures seems to be potentially useful for improving a visual evaluation and to help in the decision-making process regarding a biopsy.

To date, only few studies have investigated the potential of *in vivo* confocal microscopy for AC in terms of diagnostic sensitivity and specificity (Nguyen et al., 2016). Similarly, the diagnostic benefit of dermoscopy remains unknown (Ito, 2019)

Contrarily, tissue autofluorescence analytical procedures have proven their clinical usefulness in identifying different pathological patterns through imaging in up to 95% of AC cases, as well as a capacity to direct incisional biopsies towards areas with greater dysplasia (Takahama et al., 2013).

Lip biopsy is another point of disagreement. The lack of clinical sensitivity promotes that one or more biopsies are taken to make a histological diagnosis and establish a pre-treatment plan. However, when scheduling a vermilionectomy, a biopsy before the procedure "is open to doubt" (van Der Waal, 2003). Thus, many authors consider vermilionectomy an excisional biopsy that allows a serial analysis of the lesion (Vieira et al., 2012; Vasconcelos et al., 2019).

The degree of epithelial dysplasia is frequently underdiagnosed with incisional biopsies (up to 40% of the cases) (Simonsen Nico MM et al., 2007). Moreover and unfortunately, based on the results of a recent meta-analysis that showed large heterogeneity between studies and inconsistent results, it is not possible to confirm if biomarkers can distinguish between the different types of epithelial dysplasia in AC (Santana et al., 2020).

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4.7 TREATMENT

Primary and secondary prevention with lip protectors is essential for the therapeutic approach of AC (Rodriguez-Blanco et al., 2019).

There are several surgical and non-surgical modalities of treatment for the management of AC. Vermilionectomies using either a cold scalpel or a carbon dioxide laser and laser vaporization at different wavelengths are the most frequently used procedures. These interventions are more radical and result in less surgical morbidity (Jadotte et al., 2012). On the other hand, photodynamic therapy and topical use of different drugs (retinoic acid, 5-fluorouracil, trichloroacetic acid, etc.) is associated with lower morbidity, higher rates of recurrence, better aesthetic results, and doubtful therapeutic effectiveness in AC with epithelial dysplasia (Yazdani et al., 2015). Phototherapy in combination with 5% imiquimod is highly effective (Lai et al., 2019; Salgueiro, 2019; Shah et al., 2010; Jadotte et al., 2012).

Treatment protocols are very different depending on the clinical setting at which the condition is diagnosed and treated. Overall, better control and remission of the lesion is achieved with surgical procedures (up to 92.8%) in comparison to non-surgical procedures (65.9%). Moreover, the rate of recurrence is higher in non-surgical procedures (19.2%) (Vasconcelos, 2019).

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4.8 IDENTIFICATION OF RESEARCH GAPS IN ACTINIC CHEILITIS

The literature provides a large number of studies covering different aspects of AC with abundant epidemiological, etiopathogenic, and clinical information. However, the available therapeutic data is very contrasting and based on small series of cases, usually including subjects treated with a single therapeutic modality and little follow-up, which makes difficult the design of clinical guidelines that include recommendations based on scientific evidence.

We detected a lack of consensus on AC terminology and taxonomy, including in the diagnostic criteria. Research aiming to identify the risk factors for malignancy of AC or to predict the potential development of malignancy of the lip epithelium using biomarkers is also scarce.

Thus, there is a need to achieve a consensus among experts that aims the use of just a single terminology, clear diagnostic criteria that allow inter-study comparisons, and therapeutic recommendations based on the available scientific evidence.

AC is a lesion with a potential for malignancy, hence, it is necessary to identify markers with prognostic/predictive ability. Prospective studies and follow-up in AC patients are ideal for this purpose, although this type of research has important ethical limitations, such as allowing the AC to progress (in untreated subjects) until it becomes malignant, and the fact that this event is of low prevalence and may occur years after AC diagnosis.

To overcome these difficulties, the adjacent non-tumour epithelia (ANTE) model was used, i.e., identify molecular changes in peritumoral epithelium and assume they are early carcinogenic chang-

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es potentially useful to recognize an epithelium at risk of malignancy. The ANTE model has been widely used for this purpose in oral squamous cell carcinoma (OSCC).

Non-tumoral epithelium adjacent to oral squamous cell carcinoma

Many studies focus on the comparison between the immunohistochemical expression of the epithelium adjacent to oral cancer and OSCC in the same patient (35-69). The different defining criteria should be noted, which noticeably limits the external validity of these studies, and the large number of employed immune markers, the most frequently used being p53.

The histological spectrum of the peritumoral epithelium is wide. I.e., an epithelium with a normal appearance or different degrees of hyperplasia and epithelial dysplasia may be found. Biomarker expression is also heterogeneous in non-tumour epithelium; significant differences or great similarities may be found when compared to a tumour epithelium. This information is summarized in Table 2. The heterogeneity observed in the results, in the defining criteria and in their assessment have not allowed extrapolating these findings nor use them in the standard of care.

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Table 2. Immunomarkers in the non-tumor epithelium adjacent to oral squamous cell carcinoma

Author/ country	Sample SCC Cases (n)	Age M/F	ANTE	Stage	Control (Scoring)	Antibody	Prognostic value	OSCC
Wang et al, 2019 China	OSCC=90 ANTE=30	NR.	Adjacent normal tissue	WHO-Histological Grading TNM-Stage	Semiquantitative scoring Granulos citoplasmaticos	Anti SNCG Mab1	+= : 7/30 (23.3%)	+=61/94 (64.89%)
Ramos-García, et al; 2019;Spain	OSCC=68	63 yr.	Close ANTE=58 Distant ANTE= 41	TNM-Stage Histological Grading; Site	ANTE: + epithelial strata % + for each layer Semiquantitative scoring	Anti-Cyclin D! (Clone EP-12); Anti-Ki67 (MIB-1)	Associacion de Ciclina D1 ANTE y en el tumor (nuclei + in basal, parabasal & middle third)	Cycli D1 28.7%
Nasry et al, 2019; Canada	OSCC=39 (Biopsy specimens)	40 to >80 28M/11F	Adjacent oral epithelium	Site	Semiquantitative % positive cells ANTE &Tumor	Anti-COX-2 Anti-CD147	Significant higher COX-2 expresión en tumor que en ANTE. No diferencias significativas en CD-147 expression entre ANTE y Tumor	COX-2 + 37/39
Nair et al, 2016; India	OSCC=50	49.92yr.	AANM (apparently normal mucosa) (1cm. away margin)	TNM-Stage Histological Grading;	TGF-b1 Semiquantitative: Intensity and number of cells stained.	CD 105 /AM441-5M TGF-b1 +/6667E-100	AANM (TGF-b1 + =30/50 (60%) Diferencia significativa con la expresión en OSCC	TGF-b1 + (41/50; 82%)
Wen et al, 2015; China	OSCC =78 (tongue) ANTE=27	³50 =49 63M/15F	Adjacent non cancerous epithelium	WHO-Histological Grading TNM-Stage	Semiquantitative score Staining intensity and extent.	FLOT-2 protein	+= 7/27 (25.9%) Weak staining	+=58/78 (74.4%)
Matsuhira et al, 2015; Japan	OSCC=12 (tongue) ANTE=	61.5 yr. 7M/5F	Epithelial dysplasia	WHO-Histological Grading TNM-Stage	Cell counter software % cells positive (CK13,CK-17); positive nuclei (Ki-67,p53)	Monoclonal AB CK13,CK17,Ki67,p53	Normal epithelium (n=12) LN+ =Ki67,p53 / Displasia moderada (n=12) LD + = CK17, Ki67,p53	(n=12) CK17,Ki67, p53 (+)
Purgina et al, 2015; Canada	OSCC =55 (DSIN:intra epithelial neoplasia)	NR.	Tipe 1: inmediately to invasive SCC Tipe 2: surrounding epithelium	Site	p53 & ki67: strong nuclear staning to be + P16: extensive nuclear & cytoplasmic staining	p53 (DO7 clone) p16 (JC8 clone) Ki67 (bcl-2/100 /D5)	Type 1 and Tipe 2: p53 and Ki67 + (parabasal layer). Typr 1 and Type 2: p53+=46/55	P53+= 40/55

Author/ country	Sample SCC Cases (n)	Age M/F	ANTE	Stage	Control (Scoring)	Antibody	Prognostic value		OSCC
Fu et al, 2015; Taiwan	OSCC=436	51.8 yr. 402M/34F	Tumor adjacent normal tissue CTAN=362	TNM-Stage; Histological Grading; Site	Semiquantitative Intensity & percentage	Anti-OCT4 Anti-SOX2 NANOG	CTAN (n=348): Expresión SOX2 and OCT4 *> que OSCC; SOX expresión en CTAN * > que normal epithelium		(n=348) NANOG exp. CTAN= OSCC
Cantarutti et al, 2014; Brazil	OC=13; OPC=13	Under-76 16M/10F	Dysplastic & normal epithelium adyacente SCC	TNM-Stage; Site	Semiquantitative (% of stained cells) distribution/location	$P16^{INQ4a}$ (E6H4 clone)	NE. P16+= 22/26 (84.6%)	DE= p16+=15/26 (57.7%)	P16+= 10/26 (38.56%)
Kauppila et al, 2013; Finland	OSCC=119 (Tongue)	66 yr.	Surrounding normal lingual epithelium	Histological Grading TNM-Stage	Intensity (0-3) Extent (% positive cells)	Antibody IMG-664[a] (Clone 19D759.2)	NE+ =84/101 (83.2%) Citoplasmatic Basal layer	NR.	+ :118/119 (99.2%)
Kale et al, 2012; India	OSCC=20	NR.	Apparently normal mucosa (1cm. away from tumor)	NR.	Scoring= Staining intensity + staing area Positive if ³ 3	CK 19 (AM246-5M) CK 8/18 (AM131-5M); MMP-9 (AN504-5M)	n= 0	n=4 n=10 n= 6 90% MMP-9 positivo	MMP9 + = (100%); CK (/18 + = (10%)
Gonzalez-Moles et al, 2012; Spain	OSCC=77	62 yr. 51M/26F	ANTE=61; Close vs. Distant=more or less than 1 cm.)	TNM-Stage Sites	Semiquantitative % stained cells (Cell membrane)	Anti E-Cadherin (NHC-38 clone)	Close ANTE= lost in (48% cases) basal, and (43%) parabasal layers; Distant ANTE= lost in (47%) basal and (38%) parabasal layers.		50% (lost Expression)
Fan et al, 2012; China	OSCC=48 (Tongue)	³55=58.3% 36M/12F	2.0-2.5 cm. away from primary tumor (Normal & Displasia)	TNM-Stage Histological Trading; Site	Semiquantitative Integral optical density	MMP2 (MAB-0244) MMP-9 (MAB-0245) CoLLV (MAB-0025)	MMP-2/MMP-9 Negative or weak positive	DE= MMP-9 /MMP-2 Positivo in basal layer	MMP-2 and MMP-9 peor pronostico
Matizonkas et al, 2011; Brazil	OSCC=46	40-80 yr. 39M/7F	Tumor free	Histologic grade; Site	Semiquantitatively (% tumor cells stained)	Antibody TGIF1 (H-172)	NT + = (100%) 50% (all epithelial layer	NR.	27/(58.7%) (>75% +cells)

Author/ country	Sample SCC Cases (n)	Age M/F	ANTE	Stage	Control (Scoring)	Antibody	Prognostic value			OSCC
Thode et al, 2010; Denmark	OSCC=18	NR.	Normal mucosa adyacente to tumor	NR.	NR.	Antihuman p53 (DO1)	NE= 7/18 p53+=6/7 Basal & parabasal	DE=7/18 p53+=4/7 basal & parabasal & more differenciated cells		P53+= 13/18
Huang et al, 2009; China	OSCC=68 (Tongue)	52.3 yr. 44M/24F	Epithelium adjacent to carcinoma	TNM-Stage Histological Grading; Site	Categotizados (% cells +)	Anti-EMMPRIN	ANTE: EMMPRIN + 16/68			EMMPRIN 46/68
Yang et al, 2009; China	OSCC=30	53.8 yr. 21M/9F	Adjacent normal epithelia ³ 2cm. from the Edge of tumor	TNM-Stage Histological Grading; Site	Semiquantitative (% of tumor cells)	Cathepsin B Anti-CTSB (Cytoplasm)	Non-malignant epithelia: CTSB negativo o débilmente positivo en 29/30 (97%)			CTBS + 30/30 (100%)
Bilde et al, 2009; Denmark	OSCC=16	38-66 yr. 12M/4F	Clear (epithelial surgical margin) (³ 5mm)	TNM-Stage, Histological Grading; Site	NR.	Anti p53, Anti p16, Anti-Chk2, Anti- laminin 5	p53 += 12/16 p16+= 11/16	DE=5; p53 + =4/5; p16+ =2/5		p53+= 12/16 p16 = 1/16
Lindberg et al, 2006; Denmark	OSCC (n=20)	NR.	Adjacent areaas of normal/hyperplastic or dysplastic epithelium	TNM-Staging; Histologic Grading; Site	Number or lesions + immunoreactivity/Number of lesions	Anti-PAI-1, Anti-uPAR, Anti-lam-g2	Apparenly non-malignant mucosa Normal: PAI-1,uPAR, lam-g2 = 0/17 Dysplasia/Hyperplasia (n=19): PAI-1 + few of the cases.			Invasive-SCC PAI-1,uPAR lam-g2 (n=12;100%)
Gonzalez-Moles et al; 2004, Spain	OSCC=32 (Tongue)	60.0 yr. 28/4F	ANTE=32 (Normal/Dysplasia)	pTNM-Stage	Semiquantitative (% + cells/Total 20 fields) (+ Epithelial strata)	Anti-CD-44 (DF1485 Clone)	NE= 22 NE=+19/59.4 %	DE=10; DE= +7 (21.9%) DE= -3 (9.4%)		³ 75% positivos 15 (46.8%)
Gonzalez-Moles et al, 2002;Spain	OSCC=78 (OSCC=40; Lip cáncer =37)	62.7 yr.	ANTE=53 (67.9%)	Histological Grading; Site	Semiquantitative % p53+ Tumor cells (+ Epitelial strata)	Anti-p53 protein (Bp53-12)	NE=6 (7.6%) HE=24 (30.7%)	23 47.9%	23 47.9%	2 4. 1% P53-33(44.6%)
Bánkfalvi et al, 2002; Germany	OSCC=75	NR.	Adjacent normal and/or dysplastic mucosa	TNM-Stage; Histological Grading,; Site	Semiquantitative (% of tumor cells) non-neoplastic: E. strata	Anti E-cad (HECD-1) Anti b-cat (clone 14) Anti-EGFR (E30)	NE=EGFR+ basal/ parabasal layers	EGFR + all displastic layer		Anti E-cad + Anti b-cat + Anti-EGFR +

Author/ country	Sample SCC Cases (n)	Age M/F	ANTE	Stage	Control (Scoring)	Antibody	Prognostic value	OSCC
Gonzalez-Moles et al, 2002; Spain	OSCC=81	58 yr. 63M/17F	ANTE =33 Normal/Dysplasia Histological analysis	p-TNM; Site	Semiquantitative (% of tumor cells) nuclear staning	p16 protein	NE=25 p16+ =4/25 (16%) DE=8 ; p16-= 8/8 (100%)	P16+ 34/50 (68%)
Bei et al, 2001; USA	OSCC=32 in situ=11	NR.	Normal mucosa adjacent=22	Histological Grading; Site	Semiquantitative (% of tumor cells)	Anti-EGFR (E/); Anti Erb 2,3,4	Histologically normal mucosa EGFR (10/28);Erb2 (4/28); Erb4 (3/28)	EGFR+ OSCC=29/32 In situ= 8/11
Jin et al, 2001; China	OSCC=29	NR.	Epithelium adjacent to carcinoma (EAC) =10	Histological Grading	Intensity and distribution (% + cells)	BMP-2/4; BMP-5; BMPR-IA; bBMP-McAb	EAC = BMP-5 =10/10;; BMPR-IA + =10/10; bBMP+ =10/10; BMP-2/4 + =8/10)	BMP-2/4 + = 26/29
Cruz et al, 2000; Netherland	OSCC=43	28 -99 yr. 22M/21F	Non-Malignant: Immediatly adyacente OSCC	Histological Grading; Site	Semiquantitative (% +) Non-malignant (strata staining)	P53 (DO7 clone)	No/mild DE= 23/43 DE mod/sev=17/43 P53+ =33/43 at basal or parabasal layers	p53 +=27/43
Gonzalez-Moles et al,1999;Spain	OSCC=74	62.4 yr 68M/6F.	ANTE: (NE,HE, cathegories of dysplasia)	NR.	KI67 (Absent,Basal,suprabasal)	Ki67 (MIB-1)	NE=19;HE=38. NE +(47.4%%) & HE +(47.4%) DES=20 DEM=12 DES=5 DE Ki67+ suprabasal 27(73%)	NR.
Kuo et al, 1999; Taiwan	OSCC=88	76M/12F	Normal appearing epithelium immediatly adjacent	TNM-Stage; Fistological Grading; Site	% positive cells (entire section)	Anti-Cyclin D1	Normal appearing epithelium adjacent Cyclin D1 + =54/73 (74%)	Cyclin D1 + 73/88 (83%)
Stoll et al. 1999; Germany	O&OPSCC= 99	56.7yr. 76M/23F	Non-neoplastic epithelium (outside tumor)	p-TNM-Stage; Fistological Grading; Site	Semiquantitative (% positivity cells >50%)	Anti-CD44 (v4,v5,v6,v7)	+CD44 (Basal, parabasal,& tratum spinosum)	CD44v6 + 96/97 (100%)
Van Heerden et al, 1999; USA	OSCC=32	NR.	Adjacent epithelium	Histological grading	Intensitiy & stent (% + cells)	Anti-GST-FHIT	NE mostró fuerte positividad Fhit proteína Dysplasia severa absence of Fhit expression	Fhit = Lost expression 60%

Author/ country	Sample SCC Cases (n)	Age M/F	ANTE	Stage	Control (Scoring)	Antibody	Prognostic value		OSCC
Van Oijen et al, 1998; Netherlands	HNSCC=42 O&OPC=30	53 yr/68 yr 16M/9F* 5M/12F**	Adyacente T-Histologically normal mucosa	Sites	Semiquantitative scoring Epithelium breadthwise (%)	Monoclonal antibody EGFR	NE >extended CG. Citoplasmatic staining	(no se incluyeron casos de displasia)	EGFR+ = 30/30 (100%)
Yook et al, 1998; Korea	OSCC=20	36-74yr. 14M/6F		TNM-Stage	Categorica (absent, focal, strong)	p53 (D0-7) p21WAF1/CIP1	Adjacent HE/DE = p53- Adjacent HE/DE = p21WAF1/CIP1 + (Strong expresión)		p53 (10/20) p21 (15/20)
Jordan et al,1996; UK.	OSCC=30	57 yr. 18m/12F	Perilesional tissues	Histological Grading; Site TNM-Stage	Semiquantitatively (% tumor cells stained)	Anti-bcl-2	NE= Bax + at all layers; Bcl-2 +at basal layer	DE ; bcl-2+=full thichness; bax reduced expresion	+bcl-2= 60% +bax=63%.
Kusukawa et al,1995; Japan	OSCC=65	60.8 yr.38M/27 F		TNM-Stage Histological Grading;Site	Intensity (+ *vs* -) Respecto a la expresión del epitelio normal	Anti-MMP-3	Normal adjacent epithelium MMP-3 negativo		MMP-3 + 30/65 (46.2%)
Slootweg et al, 1994; Netherlands	HNSCC=15 (OSCC=13)	59.3 yr.	Epithelial dysplasia	NR.	NR.	Antibody MIB-1 BP53-12-1	EN= p53 - EN= Ki67 + (basal layer)	DE=+p53 =9/15 (60%) DE= +Ki67 (por encima de basal layer).	P53+=9/15 (60%)

ANTE: Adjacent non-tumor epithelium; BAX= gene encodes bcl-2 ; BMP=bone morphogenetic proteins; CD= Adhesion molecule; CK= Cytokeratins ; CTAN= corresponding tumor adjacent normal tissues; CCOX= Cyclooxygenase ; DE: Dysplastic epithelium; EGFR= epidermal growth factor receptor; EMMPRIN= Extracellular matrix metalloproteinase inducer; F: Female; FLOT= Flotillin; GST-FHIT=fragile histidine triad; HE: Hyperplastic epithelium; LD: Lower Layer of epithelial dysplasia; LN:lower layer of normal epithelium; M: Males; ;MMP= Matrix metalloproteinases; M= male; NANOG= Transcription factor; NE:Normal epithelium; NR: not reported; OCT= Octamer-binding protein 4 ; OSCC: oral squamous cell carcinomas; SNCG= synuclein-ɣ; SOX= SRY-related HMG-box gene 2; TGF= transforming growth factor; TGIF= Homeobox gene; TLR5=Toll-like receptors (Flagellin); WHO: world health organization;

4.9 RATIONALE AND OBJECTIVES

AC is a highly prevalent condition that can affect up to 30% of the general population, and is particularly associated to long-term sun exposure. Moreover, when AC is present with squamous cell lip carcinoma, the lesion has a potential to malignancy, which has not been adequately quantified.

Despite the high prevalence of AC, there is still great disagreement on the terminology, the diagnostic criteria (case definition), and especially on the different therapeutic approaches. In addition, the rate of malignancy after the treatment remains to be determined. On the other hand, while the use of biomarkers in non-tumour epithelium adjacent to oral cancer and its usefulness in predicting malignancy in potentially malignant oral disorders is well known, few studies have been performed on the immune reactivity of ANTE in lip cancer and its potential usefulness for the diagnosis of early events in lip cancer in unknown. The aims of this PhD thesis are:

4.9.1 Objectives

1. To reach a consensus for AC terminology and diagnostic therapeutic criteria.
2. To assess the therapeutic effectiveness of the different surgical and non-surgical approaches for AC.
3. To identify immunoexpression in non-tumour epithelium adjacent to a cancer on the lip.

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4.10 PAPER´S CONTENTS (FIG. 1C & FIG 3A)

Seoane Romero J, García Caballero L, Pereiro-Ferreiros M. Queilitis Actínica. Modalidades Terapéuticas, en Cáncer oral para dentistas. Edita Fundación Dental Española. Consejo General de Dentistas de España. 2019. Primera edición. ISBN: 978-84-948717-9-5; Depósito Legal: C 296-2019.

5 CAPITULO I. ASSEMBLING A CONSENSUS ON ACTINIC CHEILITIS. A DELPHI STUDY

Highlights

- Actinic cheilitis is the term which reached the highest level of consensus among the experts, and *"potentially malignant disorder"* was the most favoured by the group for classifying this entity.
- Most experts consider AC a clinical term, not a histopathological description and believe a definitive diagnosis can be made solely by clinical criteria.
- An incisional biopsy of AC should be performed when a clinical diagnosis is established and when clinical changes or signs of suspicion for malignant transformation are detected during follow-up.
- Treatment should be started once the diagnosis is established (including preventive measures), and there after follow-up should include clinical visits every 3 or 6 months, depending on the clinical presentation and on the severity of the epithelial dysplasia

Abstract (250w)

The objectives of this investigation were: i) to discuss and agree upon the most appropriate terminology to define and classify this entity; and ii) to build a consensus opinion about the diagnostic and therapeutic approaches to Actinic Cheilitis (AC).

Materials and methods

A two-round Delphi study based on the opinion of experts to achieve a consensus on AC was designed. a questionnaire was built which included 34 closed sentences (9 on terminology and taxonomy, 5 on potential for malignant transformation, 12 dealing with diagnostic aspects, as well as 8 state-

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ments on AC treatment) together with 8 open questions. El comité de expertos (n=21) respondió expresando su nivel de acuerdo mediante una escala likert (1-7). Se utilizó la mediana como medida de tendencia central y el coeficiente de variación como medida de dispersión de las puntuaciones entre expertos.

Results

The Delphi technique permitted a consensus on 24 out 34 statements (73.5%) and on 5 out of 8 (62.5%) close-ended questions. The response rate was identical in both rounds, with an attrition of 0%. Actinic cheilitis is the term which reached the highest level of consensus among the experts, with the lowest dispersion of scores (VC=21.33) and a median of 7 (strongly agree) (IQR: 6-7). Regarding taxonomy, *"potentially malignant disorder"* was the most favoured by the group for classifying this entity, with a median of 7 and 85.6% of participants showing some level of agreement (CV<50). Most experts (66.75%) consider AC a clinical term, not a histopathological description (median: 7; IQR: 4-7) and believe a definitive diagnosis can be made solely by clinical criteria (median: 6; IQR: 5-7), particularly by inspection and palpation (median: 5; IQR: 4-6). However, they find histopathological confirmation mandatory for the management of AC (median: 5; IQR: 2.5-7), even for homogeneous lesions (median: 5; IQR: 3.5-6).

Consensus was reached on all eight statements related to the treatment of AC (VC<50).

Conclusions

AC is a potentially malignant disorder about which there is a significant lack of agreement on diagnostic criteria and procedures and about biopsy indications and the importance of ancillary techniques to assist in biopsy. However, and despite the insufficient scientific evidence, a consensus was reached on the nomenclature and about the therapeutical management of this disorder,

Keywords: Actinic cheilitis, Oral potentially malignant disorder, Delphy study, Consensus

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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5.1 INTRODUCTION

In the scientific literature the term Actinic Chelitis (AC) is used to describe changes induced by chronic exposure to sunlight (ultraviolet A wavelength 320-400 nm, and largely ultraviolet B wavelength 290-320 nm) mostly affecting the lower lip (de Visscher et al, 1998). However, AC is considered a misnomer by some authors who use alternative terms, for example solar cheilitis (Cataldo et al, 1981), solar cheilosis (Jadotte a, et al., 2012), solar keratosis (shah et al., 2010) or actinic keratosis of the lip (Picascia et al., 1987). This controversy over the terminology is based on the understanding of the patho-biology of the condition as primarily inflammatory (de Visscher et al, 1998) or non-inflammatory (Cataldo et al, 1981) and on the appellation (actinic: “a ray” vs. solar) of the aetiologic radiation (Cataldo et al, 1981; Jadotte a, et al.,2012; Shah et al., 2010; Picascia et al., 1987).

There is also a lack of consensus on the nature of AC as either a “precancerous or potentially malignant” (van Der Waal, 2014; van Der Waal, 2015) or a primarily neoplastic (intra-epithelial neoplasia) condition (Jadotte et al., 2012; Schwartz et al., 2008; Menta et al., 2007; Heaphy et al., 2000), which may explain the divergences in case-definition, diagnostic criteria and management. In addition, existing literature shows a disagreement on whether AC should be used as a clinical term where biopsy is not always recommended (Mello et al., 2018) or used a preliminary clinical diagnosis for which histopathological examination is mandatory to reach a definitive diagnosis (Jadotte et al., 2012). Even the requirement of epithelial dysplasia of the lip epithelium to arrive at a diagnosis of AC is a matter of debate (Kaugars et al., 1999; de Santana et al., 2014).

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These uncertainties may well explain the wide differences in reported AC prevalence when estimated in terms of histopathological (2.08%; 95%CI: 0.94-3.67) (Mello et al, 2018) or clinical (31.3%; 95%CI: 30.5-35.7) criteria (Rodriguez Blanco et al, 2008), and highlight the need for a consensus on an unequivocal definition of AC.

A range of medical and surgical procedures (5% topical 5-FU, 5% topical imiquimod, 3% diclofenac gel, chemical peels, dermabrasion, photodynamic therapy, cryotherapy, electrosurgery, Er:YAG laser, and vermilionectomy (cold blade and CO2 laser), together with Mohs micrographic surgery) have been used for treating AC (Jadotte et al, 2012; Varela-Centelles et al., 2020; de Vasconcelos et al., 2018; Salgueiro et al., 2019; Lai et al., 2019). However, and despite the better performance of surgery compared with medical therapies (92.8% vs. 65.9% showing remission with lower recurrence rates (8,4% vs. 19.2%) (de Vasconcelos et al., 2018), there is no consensus on the optimal strategy for treatment of AC (de Vasconcelos et al., 2018; Salgueiro et al., 2019), because both approaches have benefits and drawbacks (Brignardello, 2019).

Thus, the current state of comprehension regarding AC clearly requires an effort to minimize disparities and reach agreements on terminological and taxonomical aspects, as well as in the diagnosis and treatment of this disorder. In this vein, an expert consensus (Delphi method) approach can support clinical decision-making, particularly when evidence about a given topic is contradictory (Schwendicke et al., 2019). The Delphi method is aimed at structuring a consensus and it is based upon the anonymity of the experts among themselves and on the iteration with controlled feed-back (experts are consulted more than once) (Jünger et al., 2017; Loblaw et al., 2012; McPerson et al., 2018; Diamond et al., 2014; Cramer et al., 2008; Medianos et al., 2016). This technique is considered as a procedure for

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group communication meant to reach a convergence of opinion on a specific “real world” issue (Medianos et al., 2016).

The objectives of this investigation were: i) to discuss and agree upon the most appropriate terminology to define and classify this entity; and ii) to build a consensus opinion about the diagnostic and therapeutic approaches to AC.

5.2 MATERIAL AND METHODS

A two-round Delphi study based on the opinion of experts to achieve a consensus on AC was designed according to the guidance on conducting and reporting Delphi studies (Jünger et al, 2017). A steering committee was established and 21 experts (9 dermatologists, 9 specialists in Oral Medicine, and 3 oral pathologists) were recruited from a consortium of 11 academics and 10 clinical specialists across Europe and Latin America. Experts were selected through a bibliographical search (contributing at least one publication on this topic) and from their clinical experience (>15 years of treating AC).

Our previously published systematic review (Varela-CEntelles et al., 2020) permitted the identification of terminological and taxonomical inconsistencies, along with discordances in diagnostic and therapeutic criteria for AC. As a result, a questionnaire was built which included 34 closed sentences (9 on terminology and taxonomy, 5 on potential for malignant transformation, 12 dealing with diagnostic aspects, as well as 8 statements on AC treatment) together with 8 open questions. The instrument was electronically circulated to the members of the panel.

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The first round (R1: March 2020) used a Likert scale (1 to 7; 1: strongly agree – 7: strongly disagree) to assess the level of concordance with the closed sentences presented to the experts (Medianos et al., 2016). Participants were also asked to cast their degree of confidence on their responses using another Likert scale (1: very uncertain – 7: very certain). Once the obtained information was analyzed and synthesized, and considering the feedback from the panel, the open questions in the instrument were changed into close-ended ones and the second round (R2: June 2020) was initiated. In addition to the second questionnaire, the experts received information about the overall response of the group in R1. In case a participant disagreed in one or more of his/her responses with the panel (agree/strongly agree or disagree/strongly disagree), and to favour consensus, the person was asked to reconsider the score allocated to his/her R1 response(s). This permitted the expert to bring his/her responses closer to the group's opinion or to remain with his/her previous response and to justify his/her score.

For purposes of this study, a consensus for close-ended questions was defined as ≥ 75% agreement by the experts. With reference to the sentences, a <50 variation coefficient (minor variability among experts) was considered an indicator for agreement. The criterion for dropping items at R1 was pre-established considering a minimum acceptable level of confidence in expressing an opinion (≥ 5).

This study was approved by the University of Santiago de Compostela Research Ethics Committee (USC-17/2020). A declaration of interest was completed by all participants indicating any interests.

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5.2.1 *Statistical analysis*

A quantitative analysis of the results of both R1 and R2 was undertaken using the SPSS v.24 statistical package (SPSS Inc. Chicago, IL). The descriptive study of the statements used the median as a central trend indicator and the interquartile range as a measure of spread. The variability of the experts' scores (dispersion) for a given item was calculated using the coefficient of variation (standard deviation/mean). The level of agreement (percentage) was also analyzed for close-ended questions. The opinions of the experts were also considered for non-consensual questions.

5.3 RESULTS

25 experts were invited and 21 agreed to participate in the study, resulting a recruitment rate of 84%.

The Delphi technique permitted a consensus on 24 out 34 statements (73.5%) and on 5 out of 8 (62.5%) close-ended questions. The response rate was identical in both rounds, with an attrition of 0%, and just one statement was dropped because of a low level of confidence among the participants in their responses. Results are displayed in Tables 1 to 4.

5.3.1 *Nomenclature and taxonomy*

Actinic cheilitis (AC) is the term which reached the highest level of consensus among the experts, with the lowest dispersion of scores (VC=21.33) and a median of 7 (strongly agree) (IQR: 6-7). Conversely, solar cheilosis was the term that gathered the strongest

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disagreement, with 85.7% of experts showing some level of discrepancy and a median of 1 (strongly disagree) (IQR: 1-3). Actinic cheilosis and solar cheilitis have also been discarded as inappropriate terms, with CV >50.

Regarding taxonomy, *"potentially malignant disorder"* was the most favoured by the group for classifying this entity, with a median of 7 and 85.6% of participants showing some level of agreement (CV<50). Other options, such as "*precancerous lip lesion*", "*precursor lesion*", or "*preinvasive malignant lesion*" have demonstrated lack of agreement (CV>50) (table 1). However, 23.8% of experts considered AC a primarily malignant lesion. Furthermore, the panel exhibited their agreement with the statement that histopathological findings are not sufficient to predict a malignant transformation (median 6; IQR: 4-6) and also with the absence of methodologically sound prospective studies reporting on malignant transformations of AC (median: 7; IQR: 5-7) (Table 1).

From a clinical standpoint, the presence of erosions or ulcerations, white patches, and heterogeneous lesions were considered the best clinical predictors for malignant transformation in AC (76.9%). In addition, the presence of crusts, hyperkeratotic lesions, paleness, and blurring of vermillion border can also indicate clinical suspicion for malignant transformation (80.95%) (Table 1).

5.3.2 *Diagnosis of actinic cheilitis*

Most experts (66.75%) consider AC a clinical term, not a histopathological description (median: 7; IQR: 4-7) and believe a definitive diagnosis can be made solely by clinical criteria (median: 6; IQR: 5-7), particularly by inspection and palpation (median: 5; IQR: 4-6). However, they find histopathological confirmation mandatory for the

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management of AC (median: 5; IQR: 2.5-7), even for homogeneous lesions (median: 5; IQR: 3.5-6).

An incisional biopsy of AC should be performed when a clinical diagnosis is established and when clinical changes or signs of suspicion for malignant transformation are detected during follow-up (95.23%). In this vein, an adequate histopathological diagnosis may be critical for selecting therapeutic approaches (median: 6; IQR: 3.5-7). However, the maximum diagnostic certainty for AC is achieved by surgical excision (vermiliectomy) after incisional biopsy (i.e. by histopathological al assessment of the whole specimen) (median: 7; IQR: 6.5-7).

Moreover, the experts consider solar elastosis as a pathognomonic microscopic finding in AC (median: 7; IQR: 5-7), but no consensus was reached about the requirement of epithelial dysplasia for a histopathological diagnosis of AC (median: 3; IQR: 1-4; VC: 72.75; table 2), with dermatologists consistently assigning significantly higher scores in favour of this diagnostic criterion as a requirement (p=0.019).

5.3.3 *Therapeutic approaches*

Consensus was reached on all eight statements related to the treatment of AC (VC<50). According to the panel, treatment should be started once the diagnosis is established (including preventive measures) (80.95%), and there after follow-up should include clinical visits every 3 or 6 months, depending on the clinical presentation and on the severity of the epithelial dysplasia (85.71%), Tables 3,4.

In mild AC cases, once clinical diagnosis has been established, treatment could be started without awaiting histopathological al confirmation (median: 5; IQR: 2.5-7). Dermatologists elicited a higher level of agreement with this approach than did experts in Oral Medi-

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cine and Pathology (p=0.014). Particularly, non-dysplastic AC lesions could be treated by non-surgical approaches (drugs and/or photodynamic therapy) (median: 6; IQR: 5-6), which offer good cosmetic outcomes after treatment and minor adverse events (median: 6; IQR: 4-6) but higher recurrence could be expected (median: 6; IQR: 4.5-6.5).

Surgical approaches are better than non-surgical ones, and laser vaporization techniques could be used for treating multicentric or diffuse AC lesions with mild epithelial dysplasia (median: 6; IQR: 4-7). Besides, AC lesions with moderate/severe epithelial dysplasia (median: 6; IQR: 5-7), and particularly those with diffuse presentations and with severe epithelial dysplasia are best treated by vermiliectomy techniques to prevent the development of a carcinoma (median: 6; IQR: 6-7), table 3.

5.4 DISCUSSION

5.4.1 *Strengths and limitations of the study*

The Delphi methodology was used to address consensus with the objective of contributing to a common effort targeted towards agreement (Boulkedid et al., 2011). A standardized Delphi procedure (Jünger et al., 2017; Diamond et al., 2014) was used on a non-homogeneous consensus group to minimize the impact of individual opinions (Loblaw et al., 2012; Boulkedid et al., 2011). Considering that the composition of the panel influences the rating of Delphi studies (Boulkedid et al., 2011), we have used strict criteria for the recruitment of the 21 experts (6 of them are university full professors). In addition, this methodology permits the identification of any different opinions between specialties (Boulkedid et al., 2011).

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In an attempt to evaluate the agreement level of the answers, a seven-point scale was adopted to be more discriminative (Loblaw et al., 2012; Cramer et al., 2008), together with the selection of quality indicators for the study (number of rounds, reproducible criteria for the selection of experts, and criteria for dropping items). Furthermore, the degree of confidence with each of the answers was incorporated as a subrogated indicator for the quality of the data obtained (Loblaw et al., 2012; Cramer et al., 2008). However, reaching a consensus does not indicate the results of the consensus being factual (Boulkedid et al., 2011), and it does not substitute for established clinical guidelines based on strong scientific evidence. Unfortunately, existing evidence on diagnosis and treatment of AC is scarce and disconcordant and a guideline development by professional societies may be very useful supporting future clinical practice.

5.4.2 *Taxonomy and potential for malignant transformation*

Although "*solar cheilosis*" has been recommended as an appropriate term for labelling this entity (inflammation is not the starting event, and "solar" is more accurate than "actinic", that includes other types of radiation as aetiological agent (Jadotte et al., 2012), "actinic cheilitis" is the most frequently term used in the literature (de Santana et al., 2014) and the best valued by the panel of experts.

Classically, the terms "*potentially malignant*", "*premalignant*", and "*precancerous*" have been used interchangeably (van Der Waal, 2015; Lai et al., 2019), although "*potentially malignant disorder*" has been the term adopted by the WHO Collaborating Centre in 2007 (Warnakulasuriya, 2020; Warnakulasuriya, 2018). This concept combines both precancerous lesions and conditions separately grouped in

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the earlier WHO definitions (Warnakulasuriya et al., 2007). Potentially malignant is more suited as not all ACs will eventually develop into lip cancer (van Der Waal, 2014; Warnakulasuriya, 2020; Warnakulasuriya, 2018). This nomenclature has reached the highest level of consensus in the current study, although some authors (Menta et al., 2007; Heaphy et al., 2000) still consider AC as micro-invasive, superficial, incipient malignant epithelial neoplasia.

The chances for a patient with actinic changes and lip carcinoma to develop a second primary lip cancer is 2.5 times higher than for a patient without AC (Cataldo et al., 1981). In addition, 95% of squamous cell carcinomas of the lip occur on a background of AC (Rodriguez Blanco et al., 2018; Dancyger et al., 2018; Camara et al., 2016). Conversely, and taking into account that more than two decades of chronic sun exposure is needed for AC to turn into lip cancer (Cataldo et al., 1981), and with the limited long-term, prospective, observational studies on non-treated AC, it is difficult to know the actual rate of malignant transformation of this entity (Dancyger et al., 2018). It is not surprising that the moderate rate of malignant transformation, 3%, reported in a recent systematic review (Dancyger et al., 2018). supported by a single study (Markopoulos et al., 2004) has not reached a consensus of experts.

AC may present with a wide range of clinical features which do not unveil the severity of the lesion (Menta et al., 2007; Camara et al., 2016). Thus, the presence of ulceration, severe inflammation, blurring of the vermilion, nodularity, atrophy, friability, and chronic bleeding is indicative of epithelial dysplasia or presence of malignization (Jadotte et al., 2012; Dufresne et al., 1997). Besides, the panel has agreed the presence of hyperkeratotic lesions, paleness, and heterogeneous lesions as additional clinical predictors for malignant transformation in AC. Although histopathological changes are not sufficient

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for predicting the risk for malignization of AC (Warnakulasuriya, 2018) because malignant transformation can also occur in non-dysplastic epithelia (Diamond et al., 2014), epithelial dysplasia becomes relevant with AC progression (Santana et al., 2020). In addition, different biomarkers (including overexpression of p53) have proved able to identify early events in lip carcinogenesis but the precise contribution of bimarkers to clinical practice is very limited (Neto Pimentel et al., 2006; Santana et al., 2020).

5.4.3 *Diagnosis of actinic cheilitis*

Considering AC being a clinical term (van Der Waal, 2014) and obtaining a biopsy would be particularly recommended from clinically suspicious sites (Kaugars et al., 1999; Onofre et al., 1997), clinicians should avoid treating AC relying on clinical findings alone (Jadotte et al., 2012). Considering the divergence between the clinical appearance of the lesion and its histopathological characteristics, combined with the difficulties for a single biopsy to represent the whole lesion, the use of multiple incisional biopsies (Cavalcante et al., 2008) or other procedures to enhance diagnostic accuracy (Walsh et al., 2015) would be desirable. Autofluorescence imaging and staining with toluidine blue combined with clinical examination may well be one of the adjunctive chair-side procedures (Walsh et al., 2015) for detecting high risk zones and selecting a biopsy site. However, the panel of experts considered this option as of minor importance and the item was removed after 2nd round because of the low level of confidence expressed in the responses (median:4; IQR: 2-7).

Besides, pre-treatment diagnosis has usually been based upon the information obtained from incisional biopsies although the nature of AC as a non-homogeneous, multifocal lesion (Menta et al., 2007) is

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a well-known limitation for this approach. This can result in the underdiagnosis of dysplastic lesions and masking of non-contiguous foci of squamous cell carcinoma, even in diffuse and poorly demarcated lesions (Menta et al., 2007; Cavalcante et al., 2008). The chances for this to occur seem to be supported by the frequently reported carcinomas in vermilionectomy surgical specimens (excisional biopsies) from patients with clinical diagnosis of AC (Robinson et al., 1989) even in those cases with a previous incisional biopsy confirming no invasion (Menta et al., 2007).

5.4.4 *Therapeutic approaches*

Different reports acknowledge a lack of consensus (Varela Centelles et al., 2020; de Vasconcelos et al., 2018; Salgueiro et al., 2019; Lai et al., 2019) and insufficient evidence for recommending a particular treatment option (Lai et al., 2019). This circumstance reflects the absence of both controlled clinical trials and methodological standardization of AC studies (Varela Centelles et al., 2020; de Vasconcelos et al., 2018; Salgueiro et al., 2019; Lai et al., 2019). This heterogeneity is favoured by the unclear clinic-histopathological definition of AC. Several studies have defined their AC cases before treatment relying only on clinical criteria (Hohenleutner et al., 1999; Rossoe et al., 2011; Castiñeiras et al., 2010), and even on restrictive clinical criteria: “refractory cases”, “only severe diffuse damages cases”, or “non-ulcerated AC” (Sotirou et al., 2010). Conversely, certain reports defined AC according to Kaugrar’s criteria, which requires the presence of epithelial dysplasia (Laws et al., 2000; Berking et al., 2007; Kim et al., 2013; Chaves et al., 2017).

Notwithstanding, four different systematic reviews have proved the superiority of surgical treatments over of non-surgical op-

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tions in terms of histopathological control of this disorder [15-19], particularly partial surgery and laser therapy (CO2, Er-Yag, Thulium) alone or in combination with photodynamic therapy elicited the best clinical response [18], followed by 5% imiquimod - topical chemotherapy drug - (once a day, 3 to 5 days per week for 4 to 6 weeks) and photodynamic therapy (Salgueiro et al., 2019; Lai et al., 2019). In any case, other non-surgical measures such as regular follow-up after treatment and intense photoprotection should also be implemented especially for high-risk patients (Varela-Centelles et al., 2020; de Vasconcelos et al., 2018; Mello et al., 2019).

5.4.5 *Implications for clinical practice and research*

The choice of therapy should be based upon the clinical and histopathological features of the lesion. Thus, mild- or non-dysplastic lesions can benefit from non-surgical approaches (Varela- Centelles et al., 2020; Menta et al., 2007) and surgical treatments should be prioritized for those cases with severe epithelial dysplasia, particularly vermilionectomy with serial analysis of the vermillion which could be the best option for diffuse and severe dysplastic lesions.

New strategies should be implemented to reach a consensus for the non-consensual statements identified in the current study, especially about the definition of a case and on the significance of epithelial dysplasia as a diagnostic criterion. More randomized clinical trials incorporating pre-treatment histopathological information and considering among their outcomes variables such as the remission rate, clinical response, changes to dysplasia grade, adverse events, cosmetic outcomes, healing time, and recurrence rate, as well as follow-up, or malignization rate after treatment are needed to ease evidence-based decision-making. In addition, multicentric, longitudinal (prospective

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cohort design) studies are required to identify biomarkers able to predict malignant transformation in AC (Santana et al., 2020).

5.5 CONCLUSIONS

AC is a potentially malignant disorder about which there is a significant lack of agreement on diagnostic criteria and procedures and about biopsy indications and the importance of ancillary techniques to assist in biopsy. However, and despite the insufficient scientific evidence, a consensus was reached on the nomenclature and about the therapeutical management of this disorder, which can be useful to the clinicians in their decision-making processes.

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5.6 FIGURE CAPTIONS, TABLES, FIGURES AND SCHEMES

Table 1. Level of agreement (1-3: Disagreement; 5-7: Agreement) about nomenclature, taxonomy and potential for malignant transformation of actinic cheilitis in both rounds. IQR: Interquartile rage; VC: Variation coefficient.

	Round I				Round II			
	Score 1-3 (%)	Score 5-7 (%)	Median (IQR)	VC	Score 1-3 (%)	Score 5-7 (%)	Median (IQR)	VC
Nomenclature								
Actinic cheilitis	(-)	90.4	7 (6-7)	18.78		95.2	7 (6-7)	21.33
Actinic cheilosis	57.1	(-)	3 (1-5)	68.96	66.6	(-)	2 (1-4)	72.85
Solar cheilitis	52.4	(-)	3 (1-4)	56.42	57.1	(-)	3 (1.5-4)	50.80
Solar cheilosis	66.7	(-)	2 (1-4)	73.15	85.7	(-)	1 (1-3)	63.00
Taxonomy								
Preinvasive malignant lesion	81.0	(-)	2 (1-3)	61.94	71.4	(-)	2 (1-4.5)	67.3
Precancerous lip lesion	57.1	(-)	4 (1.5-6.5)	61.53	52.4	(-)	4 (1.5-6)	58.00
Potentially malignant disorder	(-)	71.5	6 (3-7)	40.07	(-)	85.6	7 (5.5-7)	34.31
Potentially malignant lesion	(-)	70.0	6 (3-7)	45.45	(-)	66.7	6 (3-7)	45.51
Precursor lesion	52.4	(-)	2 (1-4.5)	67.14	90.5	(-)	3 (1-3)	56.22
AC potential for malignant transformation								
AC, good clinico-pathological correspondence	60.0	(-)	4 (3-6)	36.97	57.2	(-)	4 (4-6)	28.88
AC, has a moderate rate (3%) of malignant transformation.	(-)	47.7	4 (3-7)	45.07	(-)	47.6	4 (3.5-6.5)	41.84
AC, there are no prospective studies reporting malignant transformation.	(-)	75.0	6 (5-7)	29.38	(-)	80.9	7 (5-7)	27.25
Pathological findings are not sufficient to predict malignant transformation.	(-)	61.9	5 (4-6)	34.08	(-)	66.7	6 (4-6)	32.72
Lip cancer can develop on non-dysplastic lip epithelia.	(-)	80.9	6 (5-6.5)	26.38	(-)	90.5	6 (5-7)	21.87

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Table 2. Level of agreement (1-3: Disagreement; 5-7: Agreement) on diagnostic aspects of actinic cheilitis in both rounds. IQR: Interquartile range; VC: Variation coefficient.

Diagnostic Features of Actinic Cheilitis.	Round I				Round II			
	Score 1-3 (%)	Score 5-7 (%)	Median (IQR)	VC	Score 1-3 (%)	Score 5-7 (%)	Median (IQR)	VC
AC is clinical term, not a pathological one.	(-)	56.6	7 (3.5-7)	38.39		66-70	7 (4-7)	38.92
AC displays a wide histological variation, but it always includes some degree epithelial dysplasia.	52.4	(-)	3 (1-6)	67.54	(-)	61.30	3 (1-4)	72.75
Solar elastosis is a constant histological finding in actinic cheilitis.	(-)	85.7	7 (5.5-7)	28.81	(-)	85.7	7 (5-7)	22.83
AC can be diagnosed without any observable pathological alteration in the lip epithelium.	61.9	(-)	2 (1.5-5.5)	64.08	61.9	(-)	3 (1.5-5.5)	62.53
A definitive diagnosis of AC can be established with clinical criteria alone.	(-)	71.4	6 (4-6.5)	41.40	(-)	80.9	6 (5-7)	37.36
Histological confirmation is mandatory for a definitive diagnosis of AC.	(-)	57.1	5 (2.5-7)	44.48	(-)	52.4	5 (2.5-7)	45.41
Biopsy is also indicated in AC homogeneous lesion	(-)	55.0	5 (3-6)	39.09	(-)	61.9	5 (3.5-6)	35.08
Pretreatment biopsy is not required when vermilionectomy is intended	66.7	(-)	2 (1-6)	76.00	71.4	(-)	2 (1-6)	79.29
How important are tissue autofluorescence procedures as assistant to biopsy	65.0	(-)	2.5 (1-4)	53.58	85.7	(-)	2 (1-3)	48.85
AC Diagnosis (Certainty)								
Certainty 1 (C1): Inspection and palpation permit a definitive clinical diagnosis.	(-)	57.1	5 (4-6)	30.47	(-)	61.8	5 (4-6)	21.61
Certainty 2 (C2): Clinical diagnosis supplemented by pretreatment incisional biopsy permits a definitive clinical-pathological diagnosis.	(-)	95.2	7 (6-7)	11.34	(-)	100	7 (6-7)	6.85
Certainty 3 (C3): Surgical excision after incisional biopsy (Pathological assessment of whole specimen) permits a definitive diagnosis with maximum level of certainty.	(-)	90.5	7 (7-7)	16.28	(-)	90.5	7 (6.5-7)	15.87

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Table 3. Level of agreement (1-3: Disagreement; 5-7: Agreement) on approaches for treating actinic cheilitis in both rounds. IQR: Interquartile range; VC: Variation coefficient.

Therapeutic approaches	Round I				Round II			
	Score 1-3 (%)	Score 5-7 (%)	Median (IQR)	VC (DS/X)	Score 1-3 (%)	Score 5-7 (%)	Median (IQR)	VC
An adequate histological diagnosis may be critical for selecting therapeutic approach.	(-)	71.4	6 (4-7)	35.98	(-)	66.7	6 (3.5-7)	33.52
For mild AC cases, treatment can be started after clinical diagnosis without pathological confirmation.	(-)	66.6	6 (3-7)	42.8	(-)	71.4	5 (2.5-7)	39.30
Non-dysplastic AC lesions - either circumscribed or diffuse- may be treated using drugs and/ or photodynamic therapy	(-)	85.0	6 (5-6)	26.54	(-)	85.7	6 (5-6)	23.79
Non-surgical treatments offer clear advantages (good cosmetic outcomes, low cost, and minor adverse events).	(-)	61.90	5 (3.5-6)	35.71	(-)	80.9	6 (4-6)	31.35
Surgical approaches are better than non-surgical ones for removing AC with moderate/severe dysplasia.	(-)	85.0	7 (5-7)	26.77	(-)	85.3	7 (5-7)	26.21
Non-surgical treatments result in a higher rate of recurrences than surgical ones.	(-)	66.7	6 (4-6)	22.87	(-)	76.2	6 (4.5-6.5)	19.78
Vermiliectomy techniques may be reserved for diffuse AC with severe dysplasia	(-)	95.2	6 (6-7)	13.46	(-)	95.2	6 (6-7)	13.84
Laser vaporization techniques may be used in diffuse or multicentric lesion with mild dysplasia (When high diagnostic certainty is achieved)	(-)	66.6	6 (3.5-7)	40.46	(-)	71.5	6 (4-7)	35.87

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Table 4. Level of agreement with close-ended questions about actinic cheilitis. (N.A: no answers)

Actinic Cheilitis	Yes (n; %)	No (n; %)	N.A. (n;%)
1- Actinic cheilitis is a primarily malignant lesion	5 (23.80)	12 (57.14)	4 (19.04)
2-Clinical diagnosis of actinic cheilitis should be supplemented with information about the degree of dysplasia (if any), or about the presence of lip carcinoma.	13 (61.90)	2 (9.52)	6 (28.57)
3-Assessing p53 expression together with clinic-pathological findings may contribute to predict the risk for malignant transformation.	8 (38.09)	7 (33.33)	6 (28.57)
4-The presence of erosions or ulcerations, white patches, and heterogeneous lesions are the best clinical predictors for malignant transformation in actinic cheilitis.	16 (76.19)	2 (9.25)	3 (14.28)
5-The presence of crusts, hyperkeratotic lesions, paleness, and blurring of the vermillion border can also indicate a clinical suspicion for malignant transformation.	17 (80.95)	2 (9.52)	2 (9.52)
6-About when to undertake an incisional biopsy in AC: It should be taken when a clinical diagnosis is established and also when clinical changes or signs of suspicion for malignant transformation are detected during follow-up.	20 (95.23)	(-)	1 (4.76)
7-In my experience, the follow-up protocol for AC should include control visits every 3 or 6 months depending on the clinical presentation and on the severity of the dysplasia.	18 (85.71)	1 (4.76)	2 (9.52)
8- Treatment for AC should be stated when the diagnosis is established (preventive measures, etc…)	17 (80.95)	(-)	4 (19.04)
8-Follow-up visits after treatment: The first visits should be adapted to the severity of the lesion and the type of treatment. Later, they should be scheduled every 3 or 6 months depending on clinico-pathological criteria and on the response to treatment.	19 (90.47)	1 (4.76)	1 (4.76)

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5.7 PAPER´S CONTENTS

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6 CAPITULO II.
THERAPEUTIC APPROACHES FOR ACTINIC CHEILITIS: A SYSTEMATIC REVIEW ON THERAPEUTIC EFFICACY AND MALIGNANT TRANSFORMATION AFTER TREATMENT

Abstract

Actinic cheilitis (AC) is a sun-induced premalignant lesion. AC is a clinical term housing a wide pathological spectrum ranging from hyperkeratosis to invasive squamous cell carcinoma. The aim of this systematic review was to examine the therapeutic efficacy of different approaches in clinical, histological, and cosmetic terms, and the malignization rate after treatment. A systematic search was undertaken in October 2016 and updated in April 2019 at MEDLINE (from 1966), EMBASE (from 1980), and Proceedings Web of Science (Conference Proceedings Citation Index-Science (CPCI-S) from 1990) databases. The search strategy was ((“actinic” or “solar”) AND (“cheilitis”)) using both medical subject headings (MeSH) and freetext. A total of 392 potentially eligible reports were identified. After the selection procedure, 20 articles were included. It is concluded that surgical treatment is the first line of treatment for AC and has proved useful for the clinical and pathological control of the disorder. However, there is no evidence of effective treatment in preventing malignant transformations. Non-surgical procedures have shown less consistent results, although drug therapy may improve the results obtained by other therapeutic approaches.

Keywords: actinic cheilitis, treatment, malignization, squamous cell carcinoma, systematic review.

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6.1 INTRODUCTION

Actinic cheilitis (AC), also known as “solar cheilosis” (SC), is a sun-induced premalignant lesion whose main clinical features include variations in the colour of the lips, blurred limits between the vermilion border and the skin, and often atrophic areas, scaly lesions, and pronounced folds together with white spots (Cavalcante et al., 2008; Shah et al., 2010). Ulcerations and crust-making lesions may be also present in the lower lip Cavalcante et al., 2008; Shah et al., 2010; Menta et al., 2007; Markopoulos et al., 2004).

AC is a clinical term housing a wide pathological spectrum ranging from hyperkeratosis -with or without epithelial dysplasia-, carcinoma *in situ*, or superficially-invasive squamous cell carcinoma, to an openly invasive squamous cell carcinoma (Menta et al., 2007; Markopoulos et al., 2004). Anyhow, and as the clinical aspect of AC gives no hint of its pathological severity, an adequate pretreatment histological diagnosis may well be critical for selecting the most suitable therapeutic approach (Menta et al., 2007; Markopoulos et al., 2004).

The precancerous nature of AC (Menta et al., 2007) has been based on the coexistence of AC and squamous cell carcinoma (SCC) and also on the results of retrospective longitudinal studies (Markopoulos et al., 2004). Yet, the rate of malignant transformation of AC remains unknown due to the lack of observational studies following untreated AC lesions in the long run (Dancyger et al., 2018).

AC can be topically treated using imiquimod, 5-fluorouracil, and diclofenac; by photodynamic therapy; or by surgical procedures such as vermilionectomy, CO_2 laser ablation, cryosurgery, including Mohs micrographic surgery (Shah et al., 2010; Dancyger et al., 2018). Other approaches like dermabrasion or chemical peels can also be

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used (de Vasconcelos et al., 2018; Salgueiro et al., 2019). However, the evidence supporting these latter treatments is scarce and it is mainly based upon retrospective case series and experimental studies without a control group (Shah et a., 2010; Dancyger et al., 2018).

Several reviews have focused on therapeutic approaches to AC (Shah et al., 2010; de Vasconcelos et al., 2018; Salgueiro et al., 2019); Jadotte et al., 2012; Dufresne et al., 1997). However, the pertinence of our investigation is based upon the flaws observed in the two systematic reviews on this topic published so far: one provides not trustworthy evidence and does not consider all important outcomes (de Vasconcelos et al., 2018; Brignardello et al., 2019), and the other (Salgueiro et al., 2019) does not include recurrence and malignization rates after treatment among its outcomes despite recognizing the AC potential for malignant transformation. These flaws severely limit the usefulness of the information provided to make adequate treatment choices (Salgueiro et al., 2019). All these attempts have chosen as outcomes the degree of clinical and/or histological resolution of the lesion, morbidity, and aesthetic results of the treatments (Shah et al., 2010; de Vasconcelos et al., 2018; salgueiro et al., 2019; Jadotte et al., 2012; Dufresne et al., 1997). Besides, bearing in mind AC is one of oral potentially malignant disorders (OPMD), the main therapeutic objective should be to avoid malignant transformations (later expected lip cancer incidence), so improvements in clinical and histological parameters should not be considered robust outcomes.

Therefore, the aim of this systematic review was to examine the therapeutic efficacy of different approaches in clinical, histological, side effects and cosmetic terms, as well as the rate of AC malignant transformation after treatment.

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6.2 MATERIAL AND METHODS.

The review protocol was established in advance and agreed by all authors before being registered in the International Prospective Register of Systematic Reviews (PROSPERO, 2016; CRD420160500323) (Seoane J et al., 2016). This systematic review was performed according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analysis) guidelines and following the outlines of PICO: i) Population: subjects clinically and/or pathologically diagnosed of AC; ii) Intervention: Any treatment undertaken under curative intention; iii) Comparisons: patients treated by non-surgical approaches (topical treatment or photodynamic therapy) *vs*. subjects treated by surgical approaches (including LASER devices); iv) Outcomes: *primary outcome*: clinical and histological outcomes after treatment and adverse effects. *Secondary outcomes*: malignant transformation after treatment.

6.2.1 Systematic search

A systematic search was undertaken in October 2016 (updated April 2019) at MEDLINE (from 1966), EMBASE (from 1980), and Proceedings Web of Science (Conference Proceedings Citation Index-Science (CPCI-S) from 1990) databases. The search strategy was ((“actinic” or “solar”) AND (“cheilitis”)) using both medical subject headings (MeSH) and freetext.

6.2.2 Eligibility criteria

Inclusion criteria: All studies reporting original data from AC case series (≥10 patients), with a pathological diagnosis, treated either

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by surgical or non-surgical procedures considering the clinical and/or pathological response as their outcome.

Exclusion criteria: Cross-sectional studies with no follow-up after treatment.

6.2.3 Data collection and extraction

Two researchers (YL and JS) independently extracted the data in an unblinded manner and entered into a custom-made form following a standardized procedure. Disagreements were solved by a third researcher, blinded to the study hypothesis. Inter-observer concordance was calculated by means of the Epidat 3.1 statistical package (Programa para Análisis Epidemiológico de Datos Tabulados. Xunta de Galicia. Santiago de Compostela. Spain).

6.2.4 Quality assessment

The methodological quality of the selected studies was assessed using the Downs and Black checklist which included five main domains: *reporting* (10 items), *external validity* (3 items), *bias* (7 items), *confounding* (6 items) and *power* (1 item). Each item was given one point when the criterion was fulfilled, except for item #5 (principal confounders) in the reporting sub-scale -which scored 0 to 2-, summing up to a maximum of 28 points per study (Downs et al., 1998). According to their score, studies were allocated a grade of "*excellent*" (24-28 points), "*good*" (19-23 points), "*fair*" (14-18 points), or "*poor*" (<14 points) (O´Connor et al., 2015). Quality was independently assessed by two authors (YL and JS), who solved disagreements by discussion until a consensus was reached.

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6.3 RESULTS

A total of 392 potentially eligible reports were identified and 323 of them were discarded after assessing both titles and abstracts because they did not deal with treatment and follow-up of AC (k=0.903).

Another 49 papers did not meet the inclusion criteria Finally, 20 articles reporting on AC were included in the qualitiative synthesis (fig.1), and their relevant information is summarized in tables 1 to 3.

6.3.1 Surgical approaches

Ten papers published between 1986 and 2011 in USA (n=6), Israel (n=1), Brazil (n=2), and Germany (n=1), reporting on surgical treatments for 227 patients were identified (Table 1) (Whitaker, 1987; Dufresne et al., 1988; Robinson, 1989; Zelickson et al., 1990; Never et al., 1992; Johnson et al., 1992; Hohenleutner S., 1999; Laws et al., 2000; de Godoy et al., 2009;Rossoe et al., 2011). The quality of these papers was moderate (4 good/fair and 6 poor), and reported mainly on retrospective/prospective interventional case series, and only four were comparative in nature (randomized trials) and met the eligibility criteria (Robinson, 1989; Laws et al., 2000; de Godoy et al., 2009; Rossoe et al., 2011).

Both vermiliectomy with cold blade/CO_2 laser or vaporization with CO_2 laser have demonstrated excellent clinical outcomes with complete resolution of the lesion (Whitaker et al., 1987; Robinson et al., 1989; Laws et al., 2000; de Godoy et al., 2009; Rossoe et al., 2011) and functional preservation of the lip (Whitaker, 1987; Zelickson et al., 1990; Rossoe et al., 2011)). Besides, 3 studies including pre- and postoperative biopsies have proved that vermiliectomy by

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cold blade[16] and CO_2 laser vaporization (Dufresne et al., 1988; Johnson et al., 1992) can completely eliminate epithelial dysplasia, whereas low-morbidity, CO_2 laser vaporization one-pass protocols only solves about 53.8 % to 61.6% of dysplastic cheilitis (de Godoy et al., 2009).

Lip dysaesthesias have been reported as the most frequent adverse effect linked to these techniques, ranging from 0% (Whitaker, 1987;Zelickson et al., 1990; Johnson et al., 1992; Laws et al., 2000) to 33% (Rossoe et al., 2011), being more common after vermilionectomy with cold blade when compared to laser vaporization. Prevalence is also higher among those treated by w-plasty vermilionectomy compared to classic vermilionectomy (Rossoe et al., 2011).

Eight reports analysed the recurrence rate of AC after treatment and mainly reported either no recurrences (Whitaker, 1987; Robinson, 1989; Johnson et al., 1992) or low rates (Hohenleutner et al., 1999; Laws et al., 2000). However, the highest reported recurrence rates were observed in patients treated by one-pass protocols for CO_2 laser vaporization (12.5%) (Rossoe et al., 2011).

6.3.2 Non-surgical approach

Another 11 studies have investigated non-surgical approaches for AC treatment (tables 2 and 3). Nine of them assessed photodynamic therapy (Berking et al., 2007; Sotirou et al., 2008; Sotirou et al., 2010; Sotirou et al., 2011; Ribeiro et al., 2012;Kim et al., 2013; Choi et al., 2015; Suárez-Pérez et al., 2015; Chaves et al., 2017) and the other one evaluated different topical pharmacological approaches: imiquimod (Smith et al., 2002). An additional prospective study comparing the outcomes of surgical and non-surgical therapies (fluoroura-

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cil and trichloroacetic acid) also fulfilled the inclusion criteria (Robinson, 1989).

Information on the therapeutic efficacy of photodynamic therapy was obtained from papers reporting on 187 AC patients diagnosed using clinical and pathological criteria (Berking et al., 2007; Sotirou et al., 2008; Sotirou et al., 2010; Sotirou et al., 2011; Ribeiro et al., 2012;Kim et al., 2013; Choi et al., 2015; Suárez-Pérez et al., 2015; Chaves et al., 2017). (table 2) The protocols of these studies include a series of sensitizing agents, such as MAL (methyl aminolaevulinate) (Sotirou et al., 2011; Ribeiro et al., 2012;Kim et al., 2013; Choi et al., 2015; Suárez-Pérez et al., 2015; Chaves et al., 2017), ALA (5-aminolevulinic acid) (Sotirou et al., 2008; Sotirou et al., 2010), and MAOP (methylaminoxipentanoate) (Berking et al., 2007) activated either by red, or day lights, as well as by Er:YAG laser at a 37-40 J/cm^2 irradiation dose (Berking et al., 2007; Sotirou et al., 2008; Sotirou et al., 2010; Sotirou et al., 2011; Ribeiro et al., 2012;Kim et al., 2013; Choi et al., 2015; Suárez-Pérez et al., 2015; Chaves et al., 2017).

This procedure has elicited excellent cosmetic outcomes (Sotirou et al., 2008; Sotirou et al., 2010; Choi et al., 2015; Suárez-Pérez et al., 2015) and a moderate clinical therapeutic efficacy, with complete healing percentages ranging from 30% after 45 minutes (Kim et al., 2013), to >70% after 12 minutes (Sotirou et al., 2008) . In this sense, the relevant outcomes have been obtained by protocols using combinations of imiquimod and red light: 40 J/cm^2/MAL (Sotirou et al., 2008). However, phothodynamic therapy has also shown high recurrence rates[30] and persistence of epithelial dysplasia after treatment (Berking et al., 2007; Ribeiro et al., 2012; Chaves et al., 2017).

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Pain was the most frequently reported unwanted effect, which disappeared after a short period of time after photodynamic therapy (Sotirou et al., 2010; Sotirou et al., 2011; Ribeiro et al., 2012; Choi et al., 2015; Suárez-Pérez et al., 2015).

Articles on AQ medical treatments gather 35 USA patients (Robinson, 1989; Smith et al., 2002), all of them with a previous pathological diagnosis. Despite trichloroacetic-acid-treated lesions have been reported to show the highest recurrence rates (Robinson, 1989), 5% imiquimod cream has shown a high clinical effectiveness, but investigations on malignant transformations after treatment are scarce (Robinson et al., 1989; Smith et al., 2002). In addition, pathological studies undertaken after medical treatment have shown these drugs are unable to eliminate epithelial dysplasias (Robinson, 1989). (table 3).

6.3.3 Malignant transformation after treatment

Malignant transformation rates after surgical treatments have been assessed in 6 longitudinal studies, and four of them did not report any case of malignization (Whitaker, 1987; Robinson, 1989; Hohenleutner et al., 1999; de Godoy et al., 2009), whereas studies using different protocols for CO_2 laser vaporization reported low rates of malignant transformations in the treated areas: 1/43 (Zelickson et al., 1990),Case series with smaller sample size have also reported malignizations after CO_2 vermilionectomy (1/14) (Laws et al., 2000) (table 1). On the other hand, none of the other two reports (Robinson, 1989; Sotirou et al., 2008) which had undertaken non-surgical treatment for AQ with long follow-up periods (18 to 50 months) could identify a single case of malignization after treatment.

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6.4 DISCUSSION

6.4.1 Limitations of this systematic review

Certain limitations inherent to the moderate quality of the individual studies considered, together with the potential selection biases, should be taken into account despite the fact that only patients with a pathological diagnosis of AC have been included in this SR. Data on malignant transformations of AC should also be interpreted with caution because of the reduced number of studies investigating this variable (Whitaker, 1987; Robinson, 1989; Zelickson et al., 1990; Hohenleeutner et al., Laws et al., 2000; de Godoy et al., 2009; Sotirou et al., 2008), and also because it can behave as a censored observation due to a hypothetical insufficient observation time for malignization to occur, thus resulting in an underestimation of its frequency.

Besides, pre-treatment diagnosis has mainly been established upon incisional biopsies despite the well-known non-homogenous, multifocal nature of AC (Menta et al., 2007). Thus, incisional biopsies may result in underdiagnosis of dysplastic lesions and also masking non-contiguous foci of squamous cell carcinomas, even in diffuse and poorly demarcated lesions (Cavalcante et al., 2008; Menta et al., 2007). These possibilities are somehow reinforced by the widely reported findings of squamous cell carcinomas in surgical specimens obtained by vermilionectomy (excisional biopsy for AC diagnosis) from patients with clinical diagnosis of AC or who had undergone previous incisional biopsies (Menta et al., 2007; Laws et al., 2000; Castiñeiras et al., 2010).

The papers included in this review are mainly retrospective/prospective observational case series and prospective quasi-experimental studies. However, 7 studies were categorised as of good

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or fair quality (supplementary file) (Robinson, 1989; Laws et al., 2000; de Godoy et al., 2009; Rossoe et al., 2011; Sotirou et al., 2010; Ribeiro et al., 2012; Choi et al., 2015), which offer a moderate level of evidence. Besides, the current investigation is the first systematic review comparing different therapeutic approaches to AC which includes studies with adverse events, patient follow-up and post-treatment malignization outcomes.

6.4.2 Surgical vs. non-surgical treatments for AC.

Vermilionectomy with cold blade (Robinson, 1989; Rossoe et al., 2011) and CO_2 laser with secondary intention healing allow an adequate clinical-pathological control of the lesion (Neder et al., 1992). Regarding non-surgical approaches, photodynamic therapies provided not very effective clearance rates (Ozog et al., 2016) and poorly consistent results (Kim et al., 2013). However, daylight photodynamic therapy with MAL has proved better tolerated than the conventional one (Dufresne et al., 1988) and it may be specifically indicated for AC cases associated to multiple actinic keratoses of the face.

Topical drug therapies are poorly studied (Robinson, 1989; Smith et al., 2002) and seem to provide acceptable clinical results. Moreover, may help in controlling the cancerization field and its association with other therapeutic approaches may increase their clinical efficacy (Shah et al., 2010; Jadotte et al., 2012; Dufresne et al., 1997).

6.4.3 AC malignization after treatment

The main therapeutic intention when dealing with pre-malignant oral lesions is to reduce the risk of oral cancer in the affected area in the future. Most squamous cell carcinoma cases were re-

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ported in series using clinical diagnostic criteria exclusively (Castiñeiras et al., 2010; Ozog et al., 2016; Satorres et al., 2001), (where diagnostic uncertainty is higher), and were not considered in this SR. In these cases, the lip carcinoma may well have been already there before the treatment was started. The other case series (Zelickson et al., 1990; Laws et al., 2000) reporting malignant transformations of AC lesions have selected patients with epithelial dysplasia, particularly those moderate and severe (Zelickson et al., 1990).

This can be explained because the presence and severity of epithelial dysplasia condition the potential for malignization (Hsue et al., 2007; Takahama et al., 2013). In any case, well-designed clinical trials considering malignization rate among their outcomes are required to render strong evidence on the different treatment options, particularly for non-invasive procedures (Takahama et al., 2013).

6.4.4 Clinical implications

Therapy selection should be made on an individual basis and be guided by the pathological findings and the potential for malignant transformation taking into account the side effect profiles (Jadotte et al., 2012), the patient cosmetic wishes, and the available scientific evidence (Shah et al., 2010;Jadotte et al., 2012). Therefore, vermilionectomy techniques may be reserved for diffuse AC with severe dysplasia whereas laser vaporization techniques may be used in diffuse or multicentric lesions with mild dysplasia provided a high preoperative diagnostic certainty is achieved. AC circumscribed lesions suspicious for malignancy should be removed by excision or vaporization if moderate/severe dysplasia is detected, or under oncological criteria if squamous cell carcinoma is diagnosed in the previous (one or more) incisional biopsies. Despite photodynamic therapy continues

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with unclear indication of use, non-dysplastic AC lesions -either circumscribed or diffuse- may be treated using drug therapy (Robinson, 1989; Smith et al., 2002), avoiding the recommendation of 5-fluorouracil and imiquimod for treating clinically suspicious areas (>0.5 cm) with mild to severe dysplasia, or when dealing with diffuse lesions, leukoplakia, or atrophy, with mild to moderate dysplasia.

In any case, preventive measures and regular follow-up after treatment are mandatory.

It is concluded that surgical treatment is the first line of treatment for AC and has proved useful for the clinical and pathological control of the disorder. However, there is no evidence of effective treatment in preventing malignant transformations. Non-surgical procedures have shown less consistent results, although drug therapy may improve the results obtained by other therapeutic approaches.

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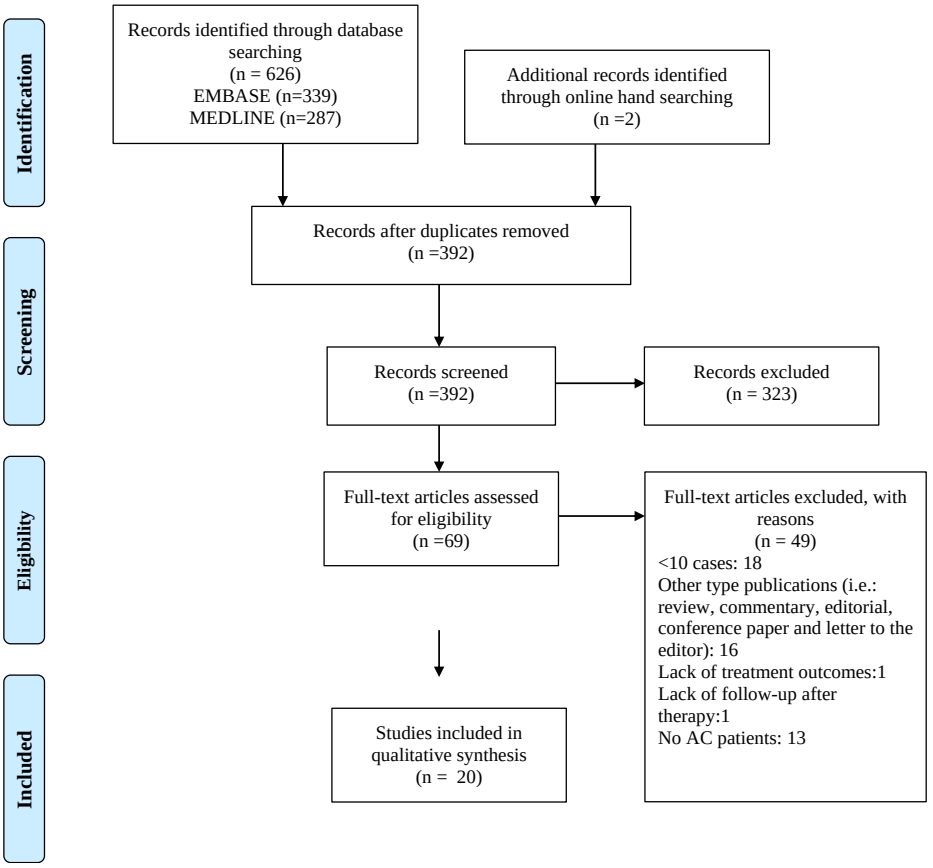
PRISMA 2009 Flow Diagram



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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PRISMA-2009-Checklist

Section/ topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1-2
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2-4
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	1-2
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	4
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4-5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4-5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4

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Section/ topic	#	Checklist item	Reported on page #
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4-5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Not applicable
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Not applicable
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	5
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Not applicable
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	5 (fig.1)
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	5-7
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	5-7
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	5-7
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Not aplicable
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	5-7

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Section/ topic	#	Checklist item	Reported on page #
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Not applicable
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	8-10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	7-8
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	9-10
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Not funding

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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Table 1. Summary of surgical treatments for actinic cheilitis.

1st Author, year Country	Patients (M/F)	Diagnostic criteria for AC	Surgical treatment	Clinical outcomes Clinical AC after treatment	Histological outcomes (after surgery on follow-up)	Cosmetic outcomes	Adverse events	Follow up	Recurrence rate	Malignization (lip cancer)	QS
Whitaker[14] 1987 USA	n=16 (1/15)	Clinical & histological**	CO_2 laser ablation (4-8W)	CR at 2 w. No sensitivity or function changes	3-6m after treatment: ND	No hypertrophic scarring	No adverse events (except 1 patient)	24 m	1 recurrence (at 14-m)	None	6
Dufresne [15] 1988 USA	n=13 (8/5)	Histological* (8 ED, 1SCC)	CO_2 laser vaporization (3-5W)	No functional restrictions	CR at 4-w	Focal scarring (n=3)	4-7 d: minor pain=3 Dysesthesia=1	11 m	No recurrences	None stated	7
Robinson[16] 1989 USA	1. n=10 2. n=10	Histological (ED)	1.Vermilionectomy 2. CO_2 laser vaporization (5W)	2. Blurred appearance	1 &2: No dysplasia	Not assessed	1. Paraesthesias=1; Hematoma=1	1.54 m 2.50 m	1. No recurrences 2. No recurrences	1. None 2. None	14
Zelickson[17] 1990 USA	n=43 (38/5)	Histological (ED)	CO_2 laser Vaporization (5-7Wt)	Function: Improved=18 Unchanged=22 Worse=1	Not assessed	Improved=26 Unchanged=16 Worse=1	No pain, Mild postoperative swelling (n=3)	31 m	Recurrences (n=3) leukoplakia (n=1)	SCC (n=1)	7
Neder[18] 1992 Israel	n=16	Histological*	CO_2 laser vermilionectomy with (8W)	No scars, more elastic lip	CR at 4-w	Similar lip configuration as prior to surgery	Minimal discomfort	1 y	None stated	None stated	5
Johnson [19] 1992 USA	n=14 (12/2)	Clinical & histological (ED)	CO_2 laser vaporization (2-3 W)	CR after 2-4 w	100% CR	No evidence of scars	No pain after 2-3 w	12 m	No recurrences	None stated	6
Hohenleutne[20] 1999 Germany	n=19	Clinical & histological	CO_2 laser vaporization	Erosion 2 m post-treatment (n=1)	Not assessed	Excelent cosmetic results	Minor scarring (n=1)	16 m	Recurrence (n=1)	None	5

1st Author, year Country	Patients (M/F)	Diagnostic criteria for AC	Surgical treatment	Clinical outcomes Clinical AC after treatment	Histological out-comes (after surgery on follow-up)	Cosmetic out-comes	Adverse events	Follow up	Recurrence rate	Malignization (lip cancer)	QS
Laws, [21] 2000 USA	n=14 (13/1)	Histological (ED)	1.E (5W-4.3W) 2.CO_2 Laser (18W;360mj/cm^2)	Improved all patients (ND)	N=5. 3-m follow-up biopsy	ND	Minimal pain (ND)	3 m	Recurrence (n=1)	1 SCC (1 LSCC 3-m previous)	16
de Godoy[22] 2009 Brazil	n=40 (36/4)	Histological (ED)	1. CO_2 laser 250 mJ, 5 W 2. CO_2 laser 350 mJ, 3.5 W	CR: 35	1. ND=53.8% 2. ND =61.5%	No visible scarring	N=12 moerate pain.	6-30 m	Recurrences 1.(12.5%) 2. (12.5%)	None	22
Rossoe[23] 2011 Brazil	n=32 (13/19)	Clinical & histological (ED)	1.Classic vermil-ionectomy 2.W-plasty vermil-ionectomy	No function abnor-mality	Surgical specimen: 2 SCC 1 BCC	Association be-tween no scar retraction and W-plasty procedure	1.Paraesthesia=23 .5% 2.Paraesthesia=33 .3%	19-m	Non stated	None stated	17

AC: actinic cheilitis; ***** incisional biopsy; ******excisional biopsy or surgical specimen after incisional diagnostic biopsy; **CR**: complete re-epitelization; **d**: days; **w**: weeks; **m**: months; **y**: years; **Er:YAG**: erbium:ythrium-aluminium-garnet; **NCR**: non-complete re-epithelialization; **SCC**: squamous cell carcinoma; **M:** male; **F:** female; **ED:** epithelial dysplasia.

Table 2. Summary of studies reporting on photodynamic therapy.

3	Pa-tients (M/F)	AC diagnos-tic criteria (*)	PDT Light/Lase r	Photosensiti-zing agent	Treat-ment Protocol. Light dose	Clinical out-comes Clinical AC after treat-ment: CR vs non-CR	Histological outcomes (after treatment on follow-up) (**) (***)	Cosmetic outcomes	Adverse events	Follow up	Maligniza-tion rate (lip cancer)	QS
Berking[24] 2007 Ger-many	n=15 (9/6)	Histological (ED)	Red light	MAOP	37 J/cm²	CR: 47% after 3 m	CR: 38% *** (ED=8)	Very good: 33%	All resolved within 4 d	3 m	None stated	13
Sotiriou [25] 2008 Greece	n=10 (10/0)	Histological (ED)	Red light	ALA	40 J/cm²	CR: 90% after 3 m	CR: 80%	Excellent: 80-60%	All resolved within 13 d	3 m	None stated	6
Sotiriou[26] 2010 Greece	n=40 (40/0)	Histological (ED)	Red light	ALA	40 J/cm²	CR: 22/26 after 18 m	CR: 17/22 after 18 m	Excellert: 81.8% (18/22)	Pain & burning (Mild to moderate)	18 m	None	14
Sotiriou[27] 2011 Greece	n=34 (33/1)	Histological	Red light +Imiquimo d	MAL	40 J/cm²	CR: 90% after 3 m	CR: 73% after 12 m	Not assessed	Mild-moderate All resolved within 8 d	12 m	None stated	8
Ribeiro[28] 2012 Brazil	n=19 (10/9)	Histological (ED)	Red light (LED)	MAL	37 J/cm²	CR: 47% to 68%	CR: 16% ED: 84%	85% satisfac-tion	Moderate pain. All resolved within 7 d	51 to 94 d	None stated	14
Kim [29] 2013 Korea	n=10 (6/4)	HIstological	Red light	MAL	37 J/cm²	CR: about 30% after 45 m (n=2 recurrences)	Not stated	Not assessed	Well tolera-ted	45 m	None stated	6
Choi[30] 2015 Korea	n=33 1: n=14 (9/5) 2: n=19 (11/8)	Histological	1: Er:YAG AFL+ red diode light 2: red diode light	MAL	37 J/cm²	1: CR=12 after 3 m 2: CR=10 after 3 m (p=0.040) Still significat after 12 m	12 m recurrences 1: 8% 2: 50%	Excellent or good 1: 73% 2: 60%	Mild/modera te pain. All resolved within 7 d)	12 m	None stated	23

3	Pa-tients (M/F)	AC diagnos-tic criteria (*)	PDT Light/Lase r	Photosensiti-zing agent	Treat-ment Protocol. Light dose	Clinical out-comes Clinical AC after treat-ment: CR vs non-CR	Histological outcomes (after treatment on follow-up) (**) (***)	Cosmetic outcomes	Adverse events	Follow up	Malignizа-tion rate (lip cancer)	QS
Suárez-Pérez [31] 2015 Spain	n=10 (8/2)	Histological	Red LED light	MAL	20 J/cm² + 80 J/cm²	CR= 8 after 3 m 2 recurrences	AC=5 after 3 m ***	Excellent or good 80%	Minor: n=7 Moderate: n=3 All resolved within 14 d	1 m	None stated	8
Chaves[32] 2016 Brazil	n=16 (10/6)	Histological	Red LED light	MAL	37 J/cm²	CR=10 (62.5%)	Persistence of dysplasia after treatment	Not assessed	Erythema & Oedema. Herpes labialis (n=1)	3 m	None stated	12

:** incisional biopsy; *:** excisional biopsy or surgical specimen after incisional diagnostic biopsy; *****:** incisional biopsy after treatment; **CR:** completly curation; **M:** male; **F:** female; **PDT:** photodynamic therapy; **QS:** qualitative score; **MAL:** methyl aminlevulinate; **h:** hour; **d:**days; **m:** month; **ALA:** 5-aminolevulinic acid; **MAOP:** methylanomoxopentanoate; **Er:YAG**: erbi-um:ythrium-aluminium-garnet laser; **ED:** epithelial dysplasia.

Table 3. Summary of papers reporting on topical drug therapy.

1st Author Year Country	Patients (M/F)	Criteria for AC diagnosis (*)	Drug employed	Protocol	Lenght of treatment	Clinical outcomes Clinical AC after treatment	Histological outcomes (after treatment on follow-up) (**) (***)	Cosmetic outcomes	Adverse events	Follow up	Recurrences	Malignization (lip cancer) n(%)	QS
Robinson,[16] 1989 USA	1. n=10 2. n=10	AC pathological diagnosis (ED)	1. FU 5% solution 2. TCA (50%)	1. 3 times a day for 14 days	1. 14 d 2. chemical peeling (twice)	Not assessed	Biopsy after 1 y 1. ED (n=5) 2. ED (n=10)	Not assessed	1. Irritation & Sore 2. No	1. 50-m 2. 49-m	1. n=5 2. n= 7	None	14
Smith,[33] 2002 EEUU	n=15 (12/3)	Clinical & histological	5% imiquimod cream,	3 times weekly	4 to 6 weeks	All CR; 11 patients requiered 2 aditional weeks	Not assessed	Not assessed	Moderate - marked inflammation	n=15, 4weeks n=7, 1 y	No recurrences	Not stated	11

(*) incisional biopsy; (**)= excisional biopsy after treatment; (CR= completely curation; PR= Partial curation); **FU**: fluorouracil; **TCA**: trichloroacetic acid; **y**: year; **m**: month; **ED:** epithelial dysplasia.

6.5 PAPER´S CONTENTS

Varela-Centelles P, **Seoane-Romero J**, García-Pola MJ, Leira-Feijoo Y, Seoane-Romero JM. Therapeutic approaches for actinic cheilitis: therapeutic efficacy and malignant transformation after treatment. Int J Oral Maxillofac Surg. 2020 Oct;49(10):1343-1350. doi: 10.1016/j.ijom.2020.02.014.

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7 CAPITULO III. IMMUNOHISTOCHEMICAL ANALYSIS OF EPITHELIUM ADJACENT TO LIP CANCER. A META-ANALYSIS

Abstract

Objectives: To identify the immunohistochemical pattern of non-tumoral epithelium adjacent to lip cancer (ANTE) to unveil molecular alterations and potential biomarkers in lip cancer patients. Materials and Methods: A systematic search at MEDLINE, EMBASE, and Proceedings Web of Science, OpenGrey, and WorldCat. Population: lip cancer patients; Intervention: immunostaining with biomarkers in surgical specimens; Comparisons: ANTE *vs* LC; Outcomes: Primary: to identify the immunohistochemical pattern of peritumoral epithelium. Secondary: to assess the immunoreactivity of LC epithelium; Study design: cross-sectional, case-control, and cohort studies. Results: A total of 339 records were identified. Seven studies reporting on ANTE and LCs were included in the qualitative synthesis and 4 of them (p53 immunostaining) were selected for meta-analysis. In the ANTE group, the pooled prevalence of p53 was 0.74 (95%CI: 0.66 – 0.82) while in the lip squamous cell carcinoma group this prevalence was 0.83 (95% CI: 0.75 – 0.89). Conclusions: Similar immunoexpression was found in lip epithelium adjacent to lip carcinoma, even in epithelia with normal appearance or mild histological alterations. The role of biomarkers in the follow-up of actinic cheilitis patients deserves additional clinical assessment.

Keywords: actinic cheilitis; lip neoplasms; biomarkers; meta-analysis

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7.1 INTRODUCTION

The estimated world incidence rate for lip cancer (LC) in 2012 was 0.3 cases per 100,000 habitants, mainly males (2.5/1) in their sixties, with wide geographical variations (Shield et al, 2017). Most incident cases (about 20%) occurred in Central and Eastern Europe (Shield et al, 2017) but, when countries are considered, Papua New Guinea (8.9/100,000) and Australia (2.6/100,000) gathered the highest age-standardized incidence rate of LC cases. In Southern Europe, Spain and Portugal show the highest age-standardized rate for males (Diz et al., 2017). These cases are mostly attributed to sunlight exposure, whereas those in Central Europe (Serbia and Ukraine) and United States have tobacco as a remarkable risk factor (Shield et al., 2017; Duet al., 2020).

Apart from exposure to ultraviolet radiation (UVA wavelength 320-400 nm, and largely UVB wavelength 290-320 nm) and tobacco smoking (by a cumulative effect through keeping the cigarette on the lip), other risk factors such as alcohol intake, precursor lesions, viral infections, skin reaction to sun exposure, genetic predisposition, and immunosuppression together with employment and socioeconomic status have been identified (de Visscher et al., 1998; Perea-Milla López et al., 2003; Rodríguez-Blanco et al., 2018).

Despite the stable or decreasing trends observed for incidence rates in high-income European regions in the last 15 years (Miranda-Filho et al., 2020) and its good overall prognosis (Pietersma et al., 2015),-with 5-year survival rates ranging between 55% and 97%- lip cancer can still be considered a significant health concern because of its metastatic potential (20%) and absolute incidence (Abreu, et al., 2006; Wang et al., 2018; Dancyger et al., 2018).

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Most lip squamous cell carcinomas are preceded by actinic cheilitis (AC), a potentially malignant disorder (Savage et al., 2010; Lopes et al, 2019; Warnakulasuriya, 2020) with a moderate (about 3%) rate of malignant transformation (Dancyger et al, 2018; Varela-Centelles, et al., 2020). As AC progresses, epithelial dysplasia becomes more relevant (Santana et al., 2020) but pathological findings are not sufficient to predict the risk for malignization of this disorder (Warnakulasuriya, 2020). In addition, a number of LCs arise on clinically and pathologically normal lip vermilions (Abreu et al, 2006). In this vein, there may be a chance for the lip to develop cancer on "pre-malignant fields" without evident clinical lesions, as occurs at other intraoral sites. Thus, the quest for molecular markers on predisposed subjects (chronically exposed to sunlight or heavy smokers) could contribute to assess the risk for lip cancer in the absence of noticeable AC.

A recent systematic review on AC has considered 27 papers on immunohistochemical biomarkers potentially involved in lip carcinogenesis in order to disclose differences among degrees of AC and between AC and lip squamous cell carcinomas (Santana et al., 2020). Unfortunately, this systematic review failed to provide prognostic information, as samples of ACs and LCs were obtained from different patients.

Bearing in mind the aforementioned issues, and to contribute to disclose the process of lip carcinogenesis, the current systematic review is aimed at identifying the immunohistochemical pattern of non-tumoral epithelium adjacent to lip cancer (ANTE) to unveil molecular alterations and potential biomarkers in lip cancer patients.

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7.2 METHODS

The review protocol was established in advance and agreed by all authors. This protocol was submitted to the PROSPERO register (Prospective Register Of Systematic Reviews; www.crd.york.ac.uk/PROSPERO) to diminish the risk of bias and to increase transparency (Supplementary material #1).

The results obtained from this review are reported according to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analysis) guidelines (Moher et al.,2009), and the PICOS outline (Population: lip cancer patients; Intervention: immunostaining with biomarkers in surgical specimens; Comparisons: ANTE *vs* LC; Outcomes: Primary: to identify the immunohistochemical pattern of peritumoral epithelium. Secondary: to assess the immunoreactivity of LC epithelium; Study design: cross-sectional, case-control, and cohort studies).

7.2.1 *Systematic search*

A systematic search was undertaken in March 2020 (updated July 2020) at MEDLINE (from 1966), EMBASE (from 1980), and Proceedings Web of Science (Conference Proceedings Citation Index-Science (CPCI-S) from 1990) databases using both descriptors (MeSH) and freetext. Other databases, such as OpenGrey (www.opengrey.eu) literature and WorldCat (www.wordlcat.org) were used to identify grey literature and dissertations and thesis respectively. A hand search was also performed.

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7.2.2 The following search strategies were used:

((lip cancer OR lip neoplasm OR oral cancer OR oral neoplasm) and (non-tumor epithelium OR adjacent epithelium OR surrounding epithelium OR surrounding dysplasia) and (immunohistochemistry)). (Supplementary material #2)

("lip neoplasms"[MeSH Terms] OR ("lip"[All Fields] AND "neoplasms"[All Fields]) OR "lip neoplasms"[All Fields] OR ("lip"[All Fields] AND "cancer"[All Fields]) OR "lip cancer"[All Fields]) OR ("lip neoplasms"[MeSH Terms] OR ("lip"[All Fields] AND "neoplasms"[All Fields]) OR "lip neoplasms"[All Fields] OR ("lip"[All Fields] AND "neoplasm"[All Fields]) OR "lip neoplasm"[All Fields]) OR ("mouth neoplasms"[MeSH Terms] OR ("mouth"[All Fields] AND "neoplasms"[All Fields]) OR "mouth neoplasms"[All Fields] OR ("oral"[All Fields] AND "cancer"[All Fields]) OR "oral cancer"[All Fields]) OR ("mouth neoplasms"[MeSH Terms] OR ("mouth"[All Fields] AND "neoplasms"[All Fields]) OR "mouth neoplasms"[All Fields] OR ("oral"[All Fields] AND "neoplasm"[All Fields]) OR "oral neoplasm"[All Fields]) AND ((non-tumor[All Fields] AND ("epithelium"[MeSH Terms] OR "epithelium"[All Fields])) OR (adjacent[All Fields] AND ("epithelium"[MeSH Terms] OR "epithelium"[All Fields])) OR (surrounding[All Fields] AND ("epithelium"[MeSH Terms] OR "epithelium"[All Fields])) OR (surrounding[All Fields] AND dysplasia[All Fields])) AND ("immunohistochemistry"[MeSH Terms] OR "immunohistochemistry"[All Fields])

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7.2.3 Eligibility criteria

Inclusion criteria: all investigations reporting original data from LSCC patients with results of immunohistochemical studies on neoplastic and non-neoplastic epithelia adjacent to lip cancer (ANTE). Only reports providing quantitative or semiquantitative data on staining of nucleus, cytoplasm, or membrane were considered.

Exclusion criteria: Investigation reporting histological studies without immunohistochemical analysis. Reports whose samples of ANTE and LSCC were obtained from different patients. Letters to the editor, case reports, and narrative or systematic reviews.

Two researchers (YL and JS) independently applied the eligibility criteria, without language restrictions, to select the reports by their titles and abstracts (inter-observer concordance k= 0.86) for further full-text assessment.

7.2.4 Data collection and extraction

YL and JS independently extracted the data in an unblinded manner and entered them into a custom-made form following a standardised procedure. Disagreements were solved by a third researcher, blinded for the study hypothesis.

7.2.5 Quality assessment

The methodological quality of the included studies was assessed using the Downs & Black checklist (1998). Criteria for qualitative assessment comprised five main domains: reporting (10 items), external validity (3 items), bias (7 items), confounding (6 items), and power (1 item). All items were given one point when the respective

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criterion was fulfilled, except for one item in the reporting subscale, which scored 0 to 2, summing up to a maximum of 28 points per study. Each study was assigned a grade of "excellent" (24-28 points), "good" (19-23 points), "fair" (14-18 points), or "poor" (<14 points).

7.2.6 Meta-analysis

We calculated the pooled prevalence estimate for each type of tissue (ANTE and LSCC) using the arcsine transformation, as recommended by Barendregt, Doi, Lee, Norman, & Vos (2013). All calculations and graphs were carried out using MetaXL software 5.3, available from https://www.epigear.com/index_files/metaxl.html

7.3 RESULTS

7.3.1 Study selection

A total of 339 records were identified and, after removing duplicate records, 315 potentially eligible documents remained. After assessing both titles and abstracts, 241 were discarded because they did not deal with biomarkers in ANTE. Another 67 papers did not meet the inclusion criteria after full-text assessment. Finally, 7 studies reporting on ANTE and LCs were included in the qualitative synthesis and 4 of them (p53 immunostaining) were selected for meta-analysis (figure 1). Relevant data are summarized in table 1.

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7.3.2 Study characteristics

Seven quasi-experimental studies met the eligibility criteria (Nagata et al., 2018; Chrun, et al., 2017; García et al, 2016; Neto Pimentel et al., 2006; Pannone et al., 2004; Sulkowska et al., 2001; Berner et al.,1993), three of them from Brazil (Nagata et al, 2018; Chrun et al, 2017; García et al, 2016), one from Italy (Pannone et al, 2004), one from Poland (Sulkowska et al, 2001), and another from Norway (Berner et al., 1993). An additional report assessed samples from Spain and Brazil (García et al., 2016). As a result, 193 lip cancer samples were considered in this systematic review. These studies were published between 1993 and 2018 and analyzed immunoexpression of 10 different immunoantibodies, enzymes linked to acetylation (Chrun et al, 2017), cyclins and catenins (García et al, 2016), makers of cell proliferation(García et al, 2016), proteins with pro-inflammatory activity (Panone et al, 2004), and anti-apoptosis proteins (Sulkowska et al, 2001), together with different p53 clones (Nagata et al, 2018; Neto Pimentel et al, 2006; Sulkowska et al, 2001; Berner et al, 1993). These four reports assessing p53 showed no heterogeneity thus permitting a qualitative synthesis of the prevalence of the immunoexpression of this protein in ANTE. Six studies provided histological information about the epithelium adjacent to the tumours (Nagata et al, 2018; Chrun et al, 2017; García et al, 2016; Neto et al, 2006; Panone et al, 2004; Sulkowska et al, 2001) but only one reported data on close and distant ANTE (García et al, 2016). Histological differentiation and tumour extension were reported in 2 papers (García et al, 2016; Neto et al, 2006; Pannone et al, 2004), while three articles only describe the degree of differentiation of the tumours (Chrun et al, 2017; Neto Pimentel et al, 2006; Berner et al, 1993). The remaining two reports did not provide sufficient information to characterize LC cases (Nagata et al, 2018; Sulkowska et al, 2001).

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Only one paper evaluated the association of biomarkers with histological, clinical and prognostic parameters of LC (García et al, 2016). Four studies considered only pathological parameters (Chrun et al, 2017; Neto Pimentel et al, 2006; Sulkowska et al, 2001). The remaining two documents reported on the association of immunoreactivity with histopathological and demographical data (Nagata et al, 2018) and on the link between histology and prognosis (Pannone et al, 2004) respectively.

The ANTE has shown different degrees of dysplasia, epithelial hyperplasia, or histological normalcy (Nagata et al, 2018; García et al, 2016; Pannone et al, 2004; Sulkowska et al, 2001; Berner et al, 1993). However, even this latter possibility revealed molecular alterations (high expression of cyclin D1 and Ki67 at the basal layer) and positivity (García et al, 2016) for COX-2 in superficial layers (Berner et al, 1993) when compared to normal epithelium not adjacent to a tumour.

7.3.3 Risk of bias

The quality assessment of the original reports disclosed their limitations, as only one of them (García et al, 2016) scored good quality values, and the remaining six were classified as of "fair quality", with some flaws that do not interfere with the aim of the current systematic review (Supplementary material ~~#2~~ #3) (Nagata et al, 2018; Neto Pimentel et al, 2006; Pannone et al, 2004; Sulkowska et al, 2001; Bener et al, 1993).

All reports included in this systematic review are cross-sectional in nature, with a retrospective design based on convenience samples recruited from pathology services and limited in size (10-45 cases) (García et al, 2016; Sulkowska et al, 2001). Several terms have been used to describe ANTE (Neto Pimentel et al, 2006): "adjacent

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tumour-free epithelium" (Nagata et al 2018), "peripheral lip cancer epithelium" (Chrun et al, 2017), and "surrounding tumour tissues" (Pannone et al, 2004). Definition criteria are also heterogeneous: "adjacent free margins" (Neto Pimentel et al, 2006; Pannone et al, 2004), "normal-appearing and dysplastic surrounding tumour epithelium" (Pannone et al, 2004), and "vicinity of LSCC, about 0.5-1 cm." (Sulkowska et al, 2001). Only one study defined clearly criteria for close and distant ANTE (about 1 cm from the invasion point or carcinoma *in situ* border (García et al, 2016).

Most immunohistochemical studies have been undertaken with positive and negative controls for immunoreactivity (Nagata et al, 2018; Chrun et al, 2017; García et al, 2016; Neto Pimentel et al, 2006; Sulkowska et al, 2001; Berner et al, 1993) and two reports have also used samples of lip epithelium adjacent to mucoceles as a control group (Chrun et al, 2017; García et al, 2016).

Five documents included patients' demographical information (Chrun et al, 2017; García et al, 2016; Neto Pimentel et al, 2006; Pannone et al, 2004; Sulkowska et al, 2001), two of them detailed ethnicity (Chrun et al, 2017; Neto Pimentel et al, 2006), and only one described risk factors linked to lip carcinogenesis (Chrun et al, 2017).

Apart from the different antibodies used in the investigations, the procedures for assessment and criteria for classification of positivity in a sample were particularly heterogeneous. Both quantitative (Pannone et al, 2004) and semiquantitative (Chrun et al, 2017) procedures were used: four papers give their results as percentages of positivity of marked cells (Chrun et al, 2017; García et al, 2016; Neto Pimentel et al, 2006; Sulkowska et al, 2001), and another paper used a dichotomous response (positive/negative) with an unclear cut-off point (Chrun et al, 2017).

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7.3.4 Meta-analysis

Only four documents testing the same hypothesis (prevalence of p53 expression in ANTE and lip cancer) were included in the quantitative synthesis (Nagata et al, 2018; Neto Pimentel et al, 2006; Sulkowska et al, 2001; Berner et al, 1993). Neither the analysis of ANTE, nor that of LSCC harboured any substantial amount of heterogeneity (I^2=0% for ANTE and I^2=25% for LSCC). We then present the results of the fixed effects model. In the ANTE group, the pooled prevalence is 0.74 (95%CI: 0.66 – 0.82) while in the LSCC group this prevalence is 0.83 (95% CI: 0.75 – 0.89). This information is summarized in the forest plots (figure 2).

7.4 DISCUSSION

A large number of studies (over 75) have investigated the behaviour of immunomarkers in lip carcinogenesis (Santana et al, 2020) but only a few have focused on the tissues surrounding the tumour, despite it has been suggested that a careful and comprehensive pathological analysis of this epithelium could bring prognostic information about lip cancer (Lopes et al, 2019). The current systematic review is not directed towards a diagnostic objective but to a prognostic one through an assessment of immunohistochemical biomarkers in lip epithelium at risk for malignization, which can be potentially useful for disclosing patients susceptible for either tumour recurrence or secondary primary tumours after treatment (Varela-Centelles et al, 2020; García et al, 2016; González-Moles et al, 2013).

Among the limitations of the studies included in this systematic review, it should be considered their cross-sectional design and the

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reduced number of reports meeting the inclusion criteria. However, they have proved homogeneity for the prevalence analysis of p53 in ANTE and a high control of the immunohistochemical analysis.

Potential sources of variability could be the lack of demographical and ethnic data, as the origin of the patients could be related to aetiological factors (sun exposure) with 3 in 7 studies reporting on Brazilian samples (Nagata et al, 2018; Chrun et al, 2017; Neto Pimentel et al, 2006). No information is reported on tobacco consumption, despite that overexpression of COX-2 in ANTE and LC could be partially due to pro-inflammatory factors, such as smoking (Berner et al, 1993). In this vein, differences in the expression of p53 in tumour-adjacent epithelia could be identified between Brazilian and European studies despite the fact that p53 expression in Brazilian lip cancer reports (pooled prevalence 0.84 (0.75-0.92) -where sunlight is expected to be the cause of the lip cancers- was similar to the one found for European studies (pooled prevalence: 0.81 (0.67-0.92) whose lip cancers should be associated with tobacco consumption.

The pooled prevalence of p53 expression in ANTE for Brazilian studies was 0.79 (0.68-0.87), much higher than found for European studies (pooled prevalence: 0.66 (0.50-0.81), and this difference was close to statistical signification. This could be explained by an overexpression of p53 in epithelia with chronic actinic damage and also by the fact that p53 mutations induced by ultraviolet light constitute an early event in development of lip carcinoma associated to sunlight exposure (Crosthwaite et al., 1996).

It has also been proved the association of p53 overexpression with smoking and heavy drinking (Field et al.,1992) eliciting a particular spectrum of mutations. In this sense, the analysis of p53 spectrum may aid in identifying the aetiological factors (Ostwald et al, 2000).

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Another potential source of heterogeneity could be the lack of clarity of clinical and histological definitions (Smith et al., 2009). Although AC is clinical term housing a spectrum ranging from hyperkeratosis (with or without dysplasia), carcinoma in situ, or superficially invasive lip squamous cell carcinomas, to an openly invasive one (Varela-Centelles et al, 2020; Menta et al., 2007; Markopoulos et al., 2004); two reports (Nagata et al, 2018; Neto Pimentel et al, 2006) have only considered AC fulfilling Kaugars' criteria (Neto Pimentel et al, 2006) -which require the presence of epithelial dysplasia,- but malignant transformations can also occur in non-dysplastic epithelia (Abreu et al, 2006; Neto Pimentel et al, 2006; Sulkowska et al, 2001) in up to 25% to 37% of all LC (Abreu et al, 2006; García et al, 2016).

Two different p53 antibody clones (Nagata et al, 2018; Neto Pimentel et al, 2006; Sulkowska et al, 2001; Berner et al, 1993) have been used, each of them specific for a single conformation of this protein (Danks et al., 1998). The DO-07 clone is not associated to the degree of AC dysplasia and it has shown the same immunoreactivity for AC linked or not to LC (Neto Pimentel et al, 2006), which suggests a limited value for predicting malignant transformation (Smith et al., 2009). Nevertheless, another report has demonstrated a high expression of mutant p53 (Pab240) in mild dysplasia (11/15;73.3%), even higher than the one showed by the group with severe dysplasia (5/7;71.4%), and may indicate that there is no need for progression in the degree of dysplasia to reach malignization (Nagata et al, 2018). This circumstance may weaken the protocol for making decisions about AC treatment based on pathological criteria only (Varela-Centelles et al, 2020). In this sense, mutant p53 protein can be found in cells with chronic actinic damage (Brash et al, 1996) and their positivity indicates risk for malignant transformation (Gannon et al.,

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1990). Thus, its predictive reliability in AC should be explored in future longitudinal studies.

Five studies reported on very similar immunoexpressions for p53, COX-2, HDAC1, HDAC2 and HAT1 in ANTE and LSCC (Nagata et al, 2018; Chrun et al, 2017; Neto Pimentel et al, 2006; Pannone et al, 2004; Sulkowska et al, 2001). This seems to suggest these proteins are involved in early events in lip carcinogenesis, reinforcing the idea of molecular alterations existing before histological changes become evident (Garcia et al, 2016; Pannone et al, 2004; Berner et al, 1993), and indicates they may well behave as very early markers for lip cancer risk. Histone deacetilase (HDAC2), in particular, has shown similar values in ANTE and LC that were markedly different from those of normal epithelium and AC not associated to tumour (Chrun et al, 2017). These findings disclosed epigenetic changes related to early stage of LC (Chrun et al, 2017) that could be useful as predictive markers for malignization of lip epithelium. In addition, one paper (García et al, 2016) reported on the high expression of cyclin D1 and Ki67 in the basal layer of ANTE and on the loss of the asymmetrical proliferative pattern, both linked to lip carcinogenesis.

As regards the meta-analysis, the presence of p53 in ANTE (primary outcome) has shown an excellent homogeneity (I^2=0%) but the results about LSCC have demonstrated a wide variability. This heterogeneity may well be due to a sampling error, as two studies have not reported on tumour characteristics (Nagata et al, 2018; Sulkowska et al, 2001) and another one did not consider undifferentiated LC (Neto Pimentel et al, 2006). As in any meta-analysis, it is highly desirable to carry out an analysis aimed at detecting publication bias. Standard analysis is based on assessing the asymmetry of the funnel plot first through visual inspection and then through regression models as a confirmatory analysis (Egger et al., 1997). Unfortunately,

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only 4 studies fulfilled the inclusion criteria of our meta-analysis. With such a reduced sample size, it was impossible to observe any pattern in the graph. We then declined to carry out any assessment of publication bias.

7.4.1 Implications for practice and research

Different markers have proved valuable for identifying molecular alterations previous to lip cancer. The potential development of this neoplasia on non- or mild-dysplastic epithelia seems to make a follow-up mandatory for patients with unambiguous risk factors for AC, where the use of biomarkers may have a prognostic/predictive significance and could orientate treatment planning (surgical vs non-surgical approach). In our opinion, these makers offer the additional advantages of being detected by cheap, simple, routine immunohistochemical techniques available at most pathology laboratories.

All investigations included in the current systematic review are cross-sectional studies with small samples that provide no clinical data and suffer from methodological flaws that have to be addressed in future research. In this sense, and to increase the internal validity of these studies and to report on a time sequence, additional longitudinal studies (preferably prospective cohort designs) are needed for making causal inferences. The p53 protein has demonstrated very consistent results in ANTE meta-analysis and should be included in these future investigations.

Prospective, multi-centre studies stratifying patients by treatment have also been suggested for obtaining quality follow-up data (Moher et al, 2009; Smith et al, 2009). The definition of case should also be clearly established, as well as the differentiation and standardization of clinical and histological criteria (Santana et al, 2020; Smith

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et al, 2009; Menta Simonsen et al, 2007; Markopoulos et al, 2009). Bearing in mind the variety of aetiological factors and lip carcinogenesis, future investigations should also include information on risk factors and patient susceptibility (working outdoors, tobacco/alcohol, Fitzpartic's skin phototypes, and immunosuppression) (Shield et al, 2017; Rodríguez-Blanco et al, 2018). Besides, and to increase replicability of biomarkers studies, immunoreactivity should be described for the different epithelial strata (Neto Pimentel et al, 2006) and the counting process detailed clearly. Strategies to minimize inter-observer variability (although nuclear immunostains may be less affected by this bias) taking into account the different intracellular topographical sites where each protein is expressed should also be implemented. The use of image software has also been recommended to reduce examiner's observation variability (Santana et al, 2020). The inclusion of a control group (e.g.: normal lip epithelium) in studies with biomarkers may offer additional data about the process of lip carcinogenesis (Santana et al, 2020; Chrun et al, 2017; García et al, 2016).

7.5 CONCLUSIONS

Several studies have proved a limited clinical usefulness of biomarkers for distinguishing different degrees of dysplasia. Furthermore, similar immunoexpression was found in lip epithelium adjacent to lip carcinoma, even in epithelia with normal appearance of mild histological alterations. The use of biomarkers in the follow-up of actinic cheilitis patients to disclose malignant transformations should be validated by further clinical, methodologically sound studies.

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7.6 FIGURE LEGENDS:

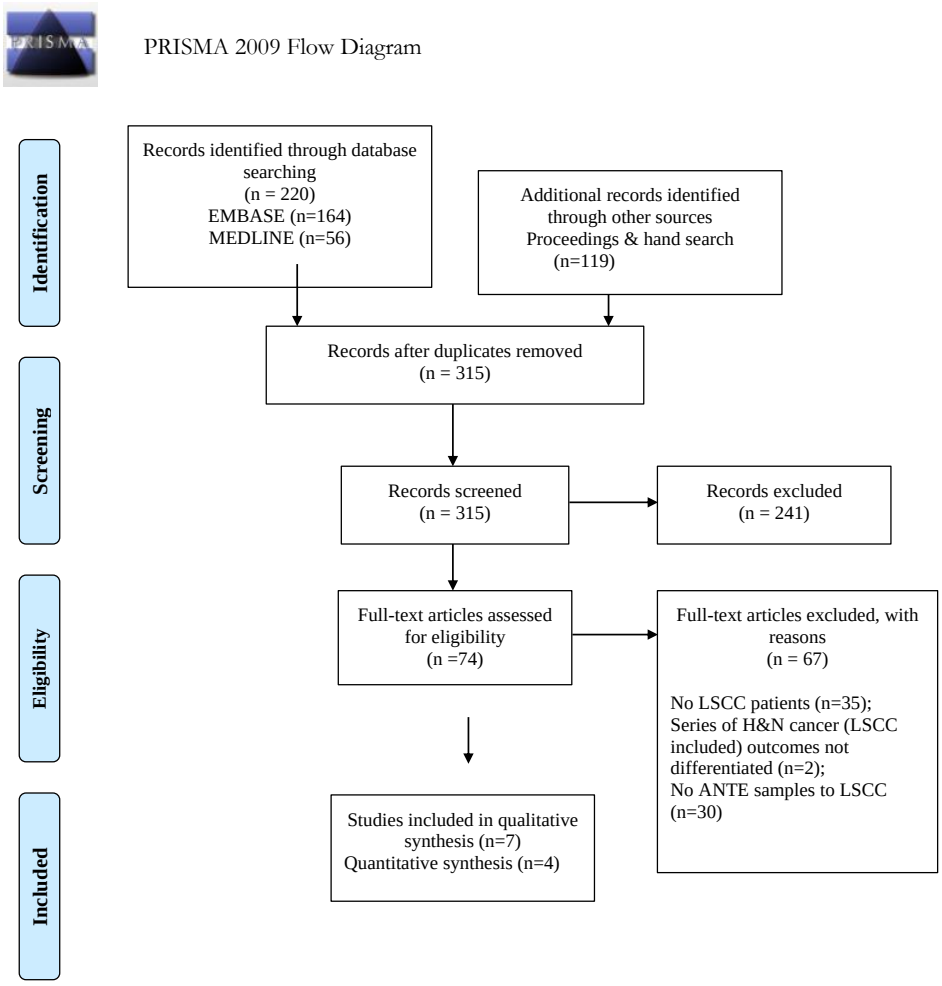
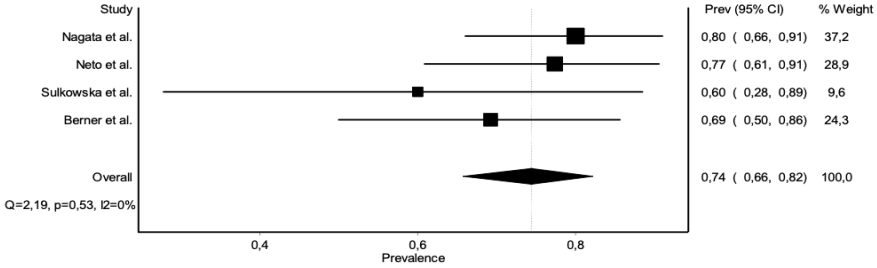


Fig. 1: Flow chart for study selection.

P53 prevalence meta-analysis in adjacent non-tumour epithelium studies (ANTE)



P53 prevalence meta-analysis in lip squamous cell carcinoma studies

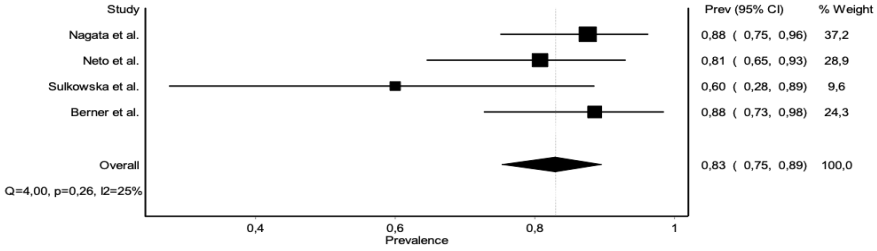


Fig. 2: Forest plots for studies on P53 prevalence.

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Table 1. Immunohistochemical features of epithelium adjacent to lip cancer.

Author/ country	Sample LSCC Cases (n)	Age/ Gender	ANTE characteristics	LSCC characteris-tics	Histological/ Immunohistochem-istry assessment	Antibodies	(ANTE) Non-dysplastic epithelium	(ANTE) DEAT Mild	(ANTE) DEAT Moderate	(ANTE) DEAT Severe	AC	LSCC
Nagata et al, 2018; Brazil	LSCC n=40	31M/9F	Epithelial dysplasia	NR	WHO criteria & binary system. p53 nuclear/ cytoplasmic staining	p53 (PAb240 clone)	n=0	15/37.5%	18/45%	7/17.5%	n= 40	P53+ n=35 (87.5%)
							p53+ (80% ANTE)					
Chrun et al, 2016; Brazil	LSCC n=30	59.4 yr./ 21M/9F	Peripheral cancer epitheli-um	Histological Grading	% positive cells/total counted cells (nuclear+)	Anti-HDAC1 Anti-HDAC2 Anti-HAT	n=30 (ANTE histology not reported) Similar immunodepression to LSCC (HDAC1/HDAC2/HAT1)				NR.	HDAC2 (46%)
García et al, 2016; Brazil/Spain	n=45 LSCC=30 CIS= 15	12-92 yr./ 79M/11F	Clouse ANTE Dysplasia/ Hyperplasia	Histological grading TNM-Stage	Mean % expression (Basal; Suprabasal; Medium third; Upper third)	Clone b-Catenin-1 Cyclin D1 (EP12) Ki-67 (Mib-1)	NE =9/20% EH =8/17.8%	26 /57.8%	2/4.4%	(-)	n= 15	Cyclin D1 Suprabasal Worse prognosis
								Cyclin D1,Ki-67 expression basal layer Cyclin D1 is higher in severe DE **				
Neto-P et al; 2006; Brazil	LSCC=31	NR.	Actinic Cheilitis	Histological Grading	WHO criteria % p53 + (nuclei stained brown)	Anti-human p53 (Clone D0-7)	n=0	n=31/100%; cases p53+ =24/77.4% media %p53+ =22% % p53 is not related to the degree of dysplasia			n= 31	P53+=80%; Media% P53+=36.3
Pannone et al; 2004 Italy	(LSCC=11)	63 yr/31M 65 yr/14 F	Normal Epitheli-um/ Dysplasia	Histological Grading TNM-Stage	COX-2 + cells (out of 1000 cells evaluat-ed)	Polyclonal anti-human COX-2	NE Cox2+=7/63.6% Superficial layer	n=11; Cox-2+ = 7/63.6% Intensity Cox-2 moderate to strong			NR.	COX-2+ 7/11 (63.6%)
Sulkowska et al,2001; Poland	LSCC=10	63.3 yr. 10M.	Vicinity of LSCC (about 0.5-1cm.)	NR.	% positive cells (p53) out of 500 cells evaluated	p 53 (DO-7) Bcl-2	P53+ 6/10 (60%) Bcl-2 + 5/10 (50%)				L.	P53+ 6/10 (60%)
Berner et al, 1993; Norway	LSCC=26	70yr (49-90)	Adjacent epithe-lium (normal & atypical)	Histological grading	Semiquantitative Score	Anti-p53	"Normal" epithelium p53 += 64%; DE p53=4/4 (100%) Adjacent non-tumor epithelium p53+=18/26 (69.2%)					P53+ 23/26 (88.4%)

(LSCC= Lip squamous cell carcinoma; ANTE= Adjacent non-tumour epithelium; AC= Actinic Cheilitis; DEAT= dysplastic epithelium associated tumours; NEAT: Normal epithelium associated tumours; NR= not reported; O&OP: oral and oropharyngeal cancer; CK= cytokeratins; DE=Dysplastic epithelium ; CIS=carcinoma in situ; NE=Normal epithelium; EH=Epithelial hyperplasia; A= Acanthosis; H=hyperkeratosis; L= Leukoplakia)

Table 1 (Supplementary information). Downs and Black checklist for quality assessment: † "good quality"; ‡ "fair quality";

Study (year)	Reporting										External validity			Internal validity - bias							Internal validity - confounding						Power	Sum
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
Nagata (2018)	1	1	1	1	1	1	0	0	0	1	1	1	1	0	0	0	1	1	1	1	1	1	0	0	0	0	0	16‡
Chrun (2016)	1	1	1	1	1	1	0	0	0	1	1	1	1	0	0	0	1	1	1	1	1	1	0	0	0	0	1	17‡
García (2016)	1	1	1	1	1	1	0	0	0	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	0	0	1	19†
Neto Pimentel (2006)	1	1	1	1	1	1	0	0	0	1	1	1	1	0	0	0	1	1	1	1	1	1	0	0	0	0	0	16‡
Pannone (2004)	1	1	1	1	1	1	0	0	0	0	1	1	1	0	0	0	1	1	1	1	1	1	0	0	0	0	0	15‡
Sulkowska (2001)	1	1	1	1	1	1	0	0	0	0	1	1	1	0	0	0	1	1	1	1	1	1	0	0	0	0	0	15‡
Berner (1993)	1	1	1	1	1	1	0	0	0	0	1	1	1	0	0	0	1	0	1	1	1	1	0	0	0	0	0	14‡

Table 2 (Supplementary information). Dows & Black's checklist for assessment of methodological quality

Item	Description	Grading
1	REPORTING Is the hypothesis/aim/objective of the study clearly described?	0 = no 1 = yes
2	REPORTING Are the main outcomes to be measured clearly described in the Introduction or Methods section?	0 = no 1 = yes
3	REPORTING Are the characteristics of the patients included in the study clearly described?	0 = no 1 = yes
4	REPORTING Are interventions of interest clearly described?	0 = no 1 = yes
5	REPORTING Are the distributions of principal confounders in each group of subjects to be compared clearly described?	0 = no 1 = partially 2 = yes
6	REPORTING Are the main findings of the study clearly described?	0 = no 1 = yes
7	REPORTING Does the study provide estimates of the random variability in the data for the main outcomes?	0 = no 1 = yes
8	REPORTING Have all important adverse events that may be a consequence of the intervention been reported?	0 = no 1 = yes
9	REPORTING Have the characteristics of patients lost to follow-up been described?	0 = no 1 = yes
10	REPORTING Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	0 = no 1 = yes
11	EXTERNAL VALIDITY Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	0 = no 0 = unable to determine 1 = yes
12	EXTERNAL VALIDITY Were the subjects who were prepared to participate representative of the entire population from which they were recruited?	0 = no 0 = unable to determine 1 = yes
13	EXTERNAL VALIDITY Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	0 = no 0 = unable to determine 1 = yes

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Item	Description	Grading
14	INTERNAL VALIDITY – Bias Was an attempt made to blind study subjects to the intervention they have received?	0 = no 0 = unable to determine 1 = yes
15	INTERNAL VALIDITY– Bias Was an attempt made to blind those measuring the main outcomes of the intervention?	0 = no 0 = unable to determine 1 = yes
16	INTERNAL VALIDITY– Bias If any of the results of the study were based on "data dredging", was this made clear?	0 = no 0 = unable to determine 1 = yes
17	INTERNAL VALIDITY– Bias In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	0 = no 0 = unable to determine 1 = yes
18	INTERNAL VALIDITY– Bias Were the statistical tests used to assess the main outcomes appropriate?	0 = no 0 = unable to determine 1 = yes
19	INTERNAL VALIDITY– Bias Was compliance with the intervention/s reliable?	0 = no 0 = unable to determine 1 = yes
20	INTERNAL VALIDITY– Bias Were the main outcome measures used accurate (valid and reliable)?	0 = no 0 = unable to determine 1 = yes
21	INTERNAL VALIDITY – Confounding (selection bias) Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	0 = no 0 = unable to determine 1 = yes
22	INTERNAL VALIDITY– Confounding (selection bias) Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	0 = no 0 = unable to determine 1 = yes
23	INTERNAL VALIDITY– Confounding (selection bias) Were study subjects randomised to intervention groups?	0 = no 0 = unable to determine 1 = yes
24	INTERNAL VALIDITY– Confounding (selection bias) Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?	0 = no 0 = unable to determine 1 = yes

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Item	Description	Grading
25	INTERNAL VALIDITY- Confounding (selection bias) Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	0 = no 0 = unable to determine 1 = yes
26	INTERNAL VALIDITY- Confounding (selection bias) Were losses of patients to follow-up taken into account?	0 = no 0 = unable to determine 1 = yes
27	POWER Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?	0 = no 1 = yes

7.7 PAPER´S CONTENTS

Varela-Centelles P, Gonzalez-Moles MÁ, **Seoane-Romero J**, Leira-Feijoo Y, Takkouche B, Seoane-Romero JM Immunohistochemical analysis of epithelium adjacent to lip cancer: A meta-analysis. Oral Dis. 2020 Jan;28 (1):57-65.

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8 DISCUSSION

This PhD dissertation gathers three studies; the first one is a systematic review aimed at examining the therapeutic efficacy of different approaches in clinical, histological, side effects and cosmetic terms, as well as the rate of AC malignant transformation after treatment

The papers included in this review are mainly retrospective/prospective observational case series and prospective quasi-experimental studies. However, 7 studies were categorised as of good or fair quality (supplementary file) (Robinson, 1989; Laws et al., 2000; de Godoy et al., 2009; Rossoe et al., 2011; Sotirou et al., 2010; Ribeiro et al., 2012; Choi et al., 2015), which offer a moderate level of evidence. Besides, the current investigation is the first systematic review comparing different therapeutic approaches to AC which includes studies with adverse events, patient follow-up and post-treatment malignization outcomes.

Vermilionectomy with cold blade (Robinson, 1989; Rossoe et al., 2011) and CO_2 laser with secondary intention healing allow an adequate clinical-pathological control of the lesion (Neder et al., 1992). Regarding non-surgical approaches, photodynamic therapies provided not very effective clearance rates (Ozog et al., 2016) and poorly consistent results (Kim et al., 2013). However, daylight photodynamic therapy with MAL has proved better tolerated than the conventional one (Dufresne et al., 1988) and it may be specifically

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indicated for AC cases associated to multiple actinic keratoses of the face.

Topical drug therapies are poorly studied (Robinson, 1989; Smith et al., 2002) and seem to provide acceptable clinical results. Moreover, may help in controlling the cancerization field and its association with other therapeutic approaches may increase their clinical efficacy (Shah et al., 2010; Jadotte et al., 2012; Dufresne et al., 1997).

The main therapeutic intention when dealing with pre-malignant oral lesions is to reduce the risk of oral cancer in the affected area in the future. Most squamous cell carcinoma cases were reported in series using clinical diagnostic criteria exclusively (Castiñeiras et al., 2010; Ozog et al., 2016; Satorres et al., 2001), (where diagnostic uncertainty is higher), and were not considered in this SR. In these cases, the lip carcinoma may well have been already there before the treatment was started. The other case series (Zelickson et al., 1990; Laws et al., 2000) reporting malignant transformations of AC lesions have selected patients with epithelial dysplasia, particularly those moderate and severe (Zelickson et al., 1990).

This can be explained because the presence and severity of epithelial dysplasia condition the potential for malignization (Hsue et al., 2007; Takahama et al., 2013). In any case, well-designed clinical trials considering malignization rate among their outcomes are required to render strong evidence on the different treatment options, particularly for non-invasive procedures (Takahama et al., 2013).

Therapy selection should be made on an individual basis and be guided by the pathological findings and the potential for malignant transformation taking into account the side effect profiles (Jadotte et

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al., 2012), the patient cosmetic wishes, and the available scientific evidence (Shah et al., 2010; Jadotte et al., 2012). In any case, preventive measures and regular follow-up after treatment are mandatory.

Bearing in mind the disconcordant criteria on terminology, diagnosis, and therapeutical approaches to actinic cheilitis, the second study in this dissertation was designed for assembling an experts' consensus on these topics. The Delphi methodology was used to address consensus with the objective of contributing to a common effort targeted towards agreement (Boulkedid et al., 2011). Although "*solar cheilosis*" has been recommended as an appropriate term for labelling this entity, "actinic cheilitis" is the most frequently term used in the literature (de Santana et al., 2014) and the best valued by the panel of experts.

Classically, the terms "*potentially malignant*", "*premalignant*", and "*precancerous*" have been used interchangeably (van Der Waal, 2015; Lai et al., 2019), although "*potentially malignant disorder*" has been the term adopted by the WHO Collaborating Centre in 2007 (Warnakulasuriya, 2020; Warnakulasuriya, 2018). This concept combines both precancerous lesions and conditions separately grouped in the earlier WHO definitions (Warnakulasuriya et al., 2007). Potentially malignant is more suited as not all ACs will eventually develop into lip cancer (van Der Waal, 2014; Warnakulasuriya, 2020; Warnakulasuriya, 2018). This nomenclature has reached the highest level of consensus in the current study, although some authors (Menta et al., 2007; Heaphy et al., 2000) still consider AC as micro-invasive, superficial, incipient malignant epithelial neoplasia.

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8.1 DIAGNOSIS OF ACTINIC CHEILITIS

Considering AC being a clinical term (van Der Waal, 2014) and obtaining a biopsy would be particularly recommended from clinically suspicious sites (Kaugars et al., 1999; Onofre et al., 1997), clinicians should avoid treating AC relying on clinical findings alone (Jadotte et al., 2012). Considering the divergence between the clinical appearance of the lesion and its histopathological characteristics, combined with the difficulties for a single biopsy to represent the whole lesion, the use of multiple incisional biopsies (Cavalcante et al., 2008) or other procedures to enhance diagnostic accuracy (Walsh et al., 2015) would be desirable. Autofluorescence imaging and staining with toluidine blue combined with clinical examination may well be one of the adjunctive chair-side procedures (Walsh et al., 2015) for detecting high risk zones and selecting a biopsy site. However, the panel of experts considered this option as of minor importance and the item was removed after 2nd round because of the low level of confidence expressed in the responses (median:4; IQR: 2-7).

Besides, pre-treatment diagnosis has usually been based upon the information obtained from incisional biopsies although the nature of AC as a non-homogeneous, multifocal lesion (Menta et al., 2007) is a well-known limitation for this approach. This can result in the underdiagnosis of dysplastic lesions and masking of non-contiguous foci of squamous cell carcinoma, even in diffuse and poorly demarcated lesions (Menta et al., 2007; Cavalcante et al., 2008).

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8.2 THERAPEUTIC APPROACHES

Different reports acknowledge a lack of consensus (Varela Centelles et al., 2020; de Vasconcelos et al., 2018; Salgueiro et al., 2019; Lai et al., 2019) and insufficient evidence for recommending a particular treatment option (Lai et al., 2019). This circumstance reflects the absence of both controlled clinical trials and methodological standardization of AC studies (Varela Centelles et al., 2020; de Vasconcelos et al., 2018; Salgueiro et al., 2019; Lai et al., 2019). This heterogeneity is favoured by the unclear clinic-histopathological definition of AC. Several studies have defined their AC cases before treatment relying only on clinical criteria (Hohenleutner et al., 1999; Rossoe et al., 2011; Castiñeiras et al., 2010), and even on restrictive clinical criteria: “refractory cases”, “only severe diffuse damages cases”, or “non-ulcerated AC” (Sotirou et al., 2010). Conversely, certain reports defined AC according to Kaugar’s criteria, which requires the presence of epithelial dysplasia (Laws et al., 2000; Berking et al., 2007; Kim et al., 2013; Chaves et al., 2017).

Notwithstanding, four different systematic reviews have proved the superiority of surgical treatments over of non-surgical options in terms of histopathological control of this disorder [15-19], particularly partial surgery and laser therapy (CO2, Er-Yag, Thulium) alone or in combination with photodynamic therapy elicited the best clinical response [18], followed by 5% imiquimod - topical chemotherapy drug - (once a day, 3 to 5 days per week for 4 to 6 weeks) and photodynamic therapy (Salgueiro et al., 2019; Lai et al., 2019). In any case, other non-surgical measures such as regular follow-up after treatment and intense photoprotection should also be implemented especially for high-risk patients (Varela-Centelles et al., 2020; de Vasconcelos et al., 2018; Mello et al., 2019).

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The choice of therapy should be based upon the clinical and histopathological features of the lesion. Thus, mild- or non-dysplastic lesions can benefit from non-surgical approaches (Varela- Centelles et al., 2020; Menta et al., 2007) and surgical treatments should be prioritized for those cases with severe epithelial dysplasia, particularly vermilionectomy with serial analysis of the vermillion which could be the best option for diffuse and severe dysplastic lesions.

New strategies should be implemented to reach a consensus for the non-consensual statements identified in the current study, especially about the definition of a case and on the significance of epithelial dysplasia as a diagnostic criterion. More randomized clinical trials incorporating pre-treatment histopathological information and considering among their outcomes variables such as the remission rate, clinical response, changes to dysplasia grade, adverse events, cosmetic outcomes, healing time, and recurrence rate, as well as follow-up, or malignization rate after treatment are needed to ease evidence-based decision-making. In addition, multicentric, longitudinal (prospective cohort design) studies are required to identify biomarkers able to predict malignant transformation in AC (Santana et al., 2020).

Different biomarkers (including overexpression of p53) have proved able to identify early events in lip carcinogenesis but the precise contribution of biomarkers to clinical practice is very limited (Neto Pimentel et al., 2006; Santana et al., 2020). In this vein, the third investigation included in this dissertation focused on unveiling molecular alterations and potential biomarkers in lip cancer patients by means of a systematic review aimed at identifying the immunohistochemical pattern of non-tumour epithelium adjacent to lip cancer (ANTE).

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A large number of studies (over 75) have investigated the behaviour of immunomarkers in lip carcinogenesis (Santana et al, 2020) but only a few have focused on the tissues surrounding the tumour, despite it has been suggested that a careful and comprehensive pathological analysis of this epithelium could bring prognostic information about lip cancer (Lopes et al, 2019). The current systematic review is not directed towards a diagnostic objective but to a prognostic one through an assessment of immunohistochemical biomarkers in lip epithelium at risk for malignization, which can be potentially useful for disclosing patients susceptible for either tumour recurrence or secondary primary tumours after treatment (Varela-Centelles et al, 2020; García et al, 2016; González-Moles et al, 2013).

Among the limitations of the studies included in this systematic review, it should be considered their cross-sectional design and the reduced number of reports meeting the inclusion criteria. However, they have proved homogeneity for the prevalence analysis of p53 in ANTE and a high control of the immunohistochemical analysis.

Potential sources of variability could be the lack of demographical and ethnic data, as the origin of the patients could be related to aetiological factors (sun exposure) with 3 in 7 studies reporting on Brazilian samples (Nagata et al, 2018; Chrun et al, 2017; Neto Pimentel et al, 2006). Another potential source of heterogeneity could be the lack of clarity of clinical and histological definitions (Smith et al., 2009). Although AC is clinical term housing a spectrum ranging from hyperkeratosis (with or without dysplasia), carcinoma in situ, or superficially invasive lip squamous cell carcinomas, to an openly invasive one (Varela-Centelles et al, 2020; Menta et al., 2007; Markopoulos et al., 2004); two reports (Nagata et al, 2018; Neto Pimentel et al, 2006) have only considered AC fulfilling Kaugars' criteria (Neto Pimentel et al, 2006) -which require the presence of epithelial dyspla-

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sia,- but malignant transformations can also occur in non-dysplastic epithelia (Abreu et al, 2006; Neto Pimentel et al, 2006; Sulkowska et al, 2001) in up to 25% to 37% of all LC (Abreu et al, 2006; García et al, 2016).

Five studies reported on very similar immunoexpressions for p53, COX-2, HDAC1, HDAC2 and HAT1 in ANTE and LSCC (Nagata et al, 2018; Chrun et al, 2017; Neto Pimentel et al, 2006; Pannone et al, 2004; Sulkowska et al, 2001). This seems to suggest these proteins are involved in early events in lip carcinogenesis, reinforcing the idea of molecular alterations existing before histological changes become evident (Garcia et al, 2016; Pannone et al, 2004; Berner et al, 1993), and indicates they may well behave as very early markers for lip cancer risk. As regards the meta-analysis, the presence of p53 in ANTE (primary outcome) has shown an excellent homogeneity (I^2=0%) but the results about LSCC have demonstrated a wide variability. This heterogeneity may well be due to a sampling error, as two studies have not reported on tumour characteristics (Nagata et al, 2018; Sulkowska et al, 2001) and another one did not consider undifferentiated LC (Neto Pimentel et al, 2006).

Different markers have proved valuable for identifying molecular alterations previous to lip cancer. The potential development of this neoplasia on non- or mild-dysplastic epithelia seems to make a follow-up mandatory for patients with unambiguous risk factors for AC, where the use of biomarkers may have a prognostic/predictive significance and could orientate treatment planning (surgical vs non-surgical approach). In our opinion, these makers offer the additional advantages of being detected by cheap, simple, routine immunohistochemical techniques available at most pathology laboratories.

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Bearing in mind the variety of aetiological factors and lip carcinogenesis, future investigations should also include information on risk factors and patient susceptibility (working outdoors, tobacco/alcohol, Fitzpartic's skin phototypes, and immunosuppression) (Shield et al, 2017; Rodríguez-Blanco et al, 2018). Besides, and to increase replicability of biomarkers studies, immunoreactivity should be described for the different epithelial strata (Neto Pimentel et al, 2006) and the counting process detailed clearly. Strategies to minimize inter-observer variability (although nuclear immunostains may be less affected by this bias) taking into account the different intracellular topographical sites where each protein is expressed should also be implemented.

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9 CONCLUSIONS

9.1 FIRST:

Actinic cheilitis is a potentially malignant disorder for which there is a significant lack of agreement on the diagnostic criteria and procedures, indications for biopsies, and relevance of auxiliary procedures among the scientific community. Nonetheless, despite the scarce scientific evidence, greater consensus has been achieved on the therapeutic management of this entity. Clinicians may use this consensus to help them in their decision-making.

9.2 SECOND:

Based on studies of poor quality, surgical treatment seems to be the first therapeutic choice for AC, as it has shown to be useful for the clinical and pathological control of this entity. However, there is no evidence on an effective treatment to prevent malignant transformation associated to actinic cheilitis. Non-surgical procedures show less consistent results in the histological control of the lesion, although pharmacological therapy may optimize the results obtained by other therapeutic approaches.

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9.3 THIRD:

Immunohistochemical markers have shown to have a limited ability to distinguish between different degrees of dysplasia in the epithelium adjacent to lip cancer. In addition, there seems to be similar p53 immune expression in peritumoral and tumoral epithelia, even in epithelium with normal appearance or mild histological changes. To use these biomarkers in the follow-up of patients with actinic cheilitis as alert for potential malignancy or for therapeutic decision-making, requires that additional studies with the appropriate methodology and in clinical settings are conducted.

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11 ANEXO. QUALITY INDICATIONS OF THE CONTRIBUTIONS FOR MONOGRAPHIC THESES WITH REPRODUCTION OF PUBLICATIONS

11.1 QUALITY INDICATIONS OF THE CONTRIBUTIONS FOR THE THESIS

1. Seoane J, Warnakulasuriya S, Bagán JV, Aguirre-Urizar JM, López-Jornet P, Hernández-Vallejo G, González-Moles MÁ, Pereiro-Ferreiros M, **Seoane-Romero J,** Varela-Centelles P. Assembling a consensus on actinic cheilitis. A Delphi study. J Oral Pathol Med. 2021 Nov;50(10):962-970. doi: 10.1111/jop.13200.

   (Q1)

   Nivel de Contribución:

   He participado substancialmente en el diseño adquisición de los datos, análisis e interpretación de los datos, redacción del artículo, revisión crítica del mismo y aprobación final de la versión publicada.

2. Varela-Centelles P, **Seoane-Romero J**, García-Pola MJ, Leira-Feijoo Y, Seoane-Romero JM. Therapeutic approaches for actinic cheilitis: therapeutic efficacy and malignant transformation after treatment. Int J Oral Maxillofac Surg. 2020 Oct;49(10):1343-1350. doi: 10.1016/j.ijom.2020.02.014.

   (Q2)

   Nivel de Contribución:

   He participado substancialmente en el diseño, adquisición de los datos, análisis e interpretación de los datos, redacción del artículo, revisión crítica del mismo y aprobación final de la versión publicada.

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3. Varela-Centelles P, Gonzalez-Moles MÁ, **Seoane-Romero J**, Leira-Feijoo Y, Takkouche B, Seoane-Romero JM Immunohistochemical analysis of epithelium adjacent to lip cancer: A meta-analysis. Oral Dis. 2020 Jan;28 (1):57-65. doi: 10.1111/odi.13643. **(Q1)**

   Nivel de Contribución:

   He participado substancialmente en el diseño adquisición de los datos, análisis e interpretación de los datos, redacción del artículo, revisión crítica del mismo y aprobación final de la versión publicada.

4. **Seoane Romero J**, García Caballero L, Pereiro-Ferreiros M. Queilitis Actínica. Modalidades Terapéuticas, en Cáncer oral para dentistas. Edita Fundación Dental Española. Consejo General de Dentistas de España. 2019. Primera edición. ISBN: 978-84-948717-9-5; Depósito Legal: C 296-2019.

   Nivel de Contribución:

   He participado substancialmente en el diseño adquisición de los datos, análisis e interpretación de los datos, redacción del capítulo de libro, revisión crítica del mismo y aprobación final de la versión publicada.

Contributions	Impact Factor	Ranking JCR (Dentistry)	Quartile
Immunohistochemical analysis of epithelium adjacent to lip cancer: A meta-analysis..Oral Dis. 2020 Sep 18. doi: 10.1111/odi.13643.	3,511	22/92	Q1
Assembling a consensus on actinic cheilitis. A Delphi study. J Oral Pathol Med. 2021 May 16. doi: 10.1111/jop.13200.	4,253	10/92	Q1
Therapeutic approaches for actinic cheilitis: therapeutic efficacy and malignant transformation after treatment. Int J Oral Maxillofac Surg. 2020 Oct;49(10):1343-1350. doi: 10.1016/j.ijom.2020.02.014.	2,789	34/92	Q2

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11.2 AUTORIZACIÓN DE LA REVISTA/EDITORIAL PARA EL USO DE LAS PUBLICACIONES EN LA TESIS

El autor de la Tesis cuenta con la autorización de las editoriales para el uso de las publicaciones en la presente Tesis Doctoral

1. Seoane J, Warnakulasuriya S, Bagán JV, Aguirre-Urizar JM, López-Jornet P, Hernández-Vallejo G, González-Moles MÁ, Pereiro-Ferreiros M, **Seoane-Romero J,** Varela-Centelles P. Assembling a consensus on actinic cheilitis. A Delphi study. J Oral Pathol Med. 2021 Nov;50(10):962-970. doi: 10.1111/jop.13200.

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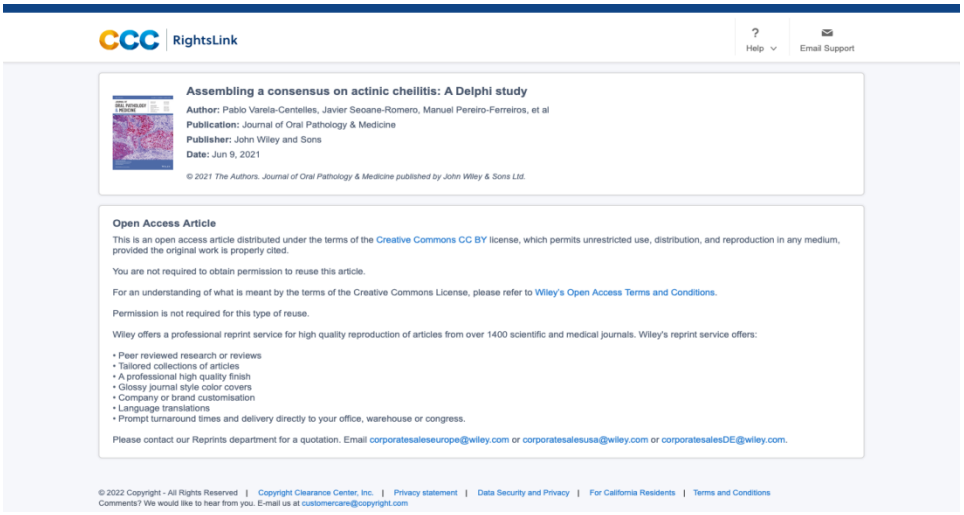
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Assembling a consensus on actinic cheilitis: A Delphi study

Author: Pablo Varela-Centelles, Javier Seoane-Romero, Manuel Pereiro-Ferreiros, et al

Publication: Journal of Oral Pathology & Medicine

Publisher: John Wiley and Sons

Date: Jun 9, 2021

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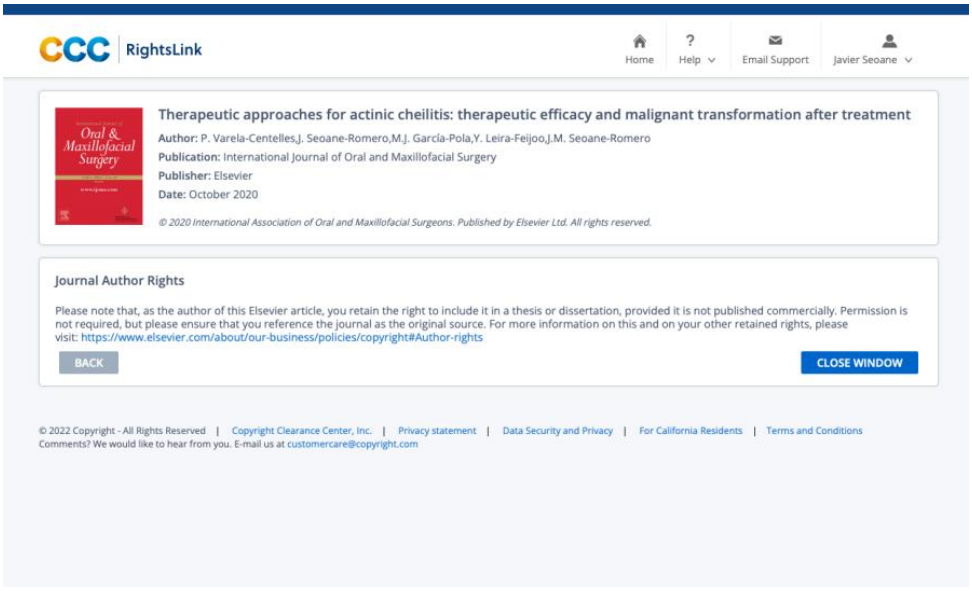

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La Queilitis Actínica es un trastorno oral potencialmente maligno, para el que existe una considerable falta de acuerdo respecto a los criterios diagnósticos, indicaciones de biopsia o sobre la relevancia de los procedimientos auxiliares en su diagnóstico. Sin embargo, mediante el método Delphi, hemos conseguido alcanzar acuerdo sobre su manejo terapéutico. Este consenso puede ayudar a los clínicos en la toma de decisiones. Además, en base a una revisión sistemática, se propone la terapia quirúrgica para el control clínico-patológico de esta entidad. Sin embargo, no se ha podido identificar ningún tratamiento que pueda prevenir la transformación maligna de la Queilitis Actínica. Tampoco, en un estudio de revisión sistemática con meta-análisis hemos podido identificar biomarcadores, incluyendo el p53, que permitan diferenciar el grado de displasia en el epitelio peri-tumoral o que permitan predecir la malignización de la Queilitis Actínica.