UNIVERSIDAD DE GRANADA

FACULTAD DE MEDICINA

TESIS DOCTORAL

"EXPRESIÓN DE LA ESMOTELINA EN LA PIEL NORMAL Y TUMORAL"

JOSÉ ANEIROS FERNÁNDEZ

DIRECTORES:

PROF. DR. JOSE ANEIROS CACHAZA

PROF. DR. FRANCISCO NOGALES FERNÁNDEZ

El trabajo de investigación que se expone en la siguiente Tesis, Titulada "Expresión de la esmotelina en la piel normal y tumoral", ha sido realizado bajo nuestra dirección por Don Jose Aneiros Fernández.

Una vez aceptados para publicación los artículos correspondientes y redactados la presente memoria, ésta ha sido revisada y es adecuada para ser presentada y permitir al doctorando aspirar al título de Doctor ante el tribunal que en su día se designe.

El doctorando José Aneiros Fernández y los directores de la tesis Francisco Nogales Fernández y José Aneiros Cachaza, Garantizamos, al firmar esta tesis doctoral, que el trabajo ha sido realizado por el doctorando bajo la dirección de los directores de la tesis y hasta donde nuestro conocimiento alcanza, en la realización del trabajo, se han respetado los derechos de otros autores a ser citados, cuando se han utilizado sus resultados o publicaciones.

En Granada 29 de Octubre del 2015

Director/es de la Tesis:

Fdo.: Francisco Nogales Fernández

Fdo.:Jose Aneiros Cachaza

Doctorando:

Fdo.: Jose Aneiros Fernandez

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TESIS COMO COMPENDIO

TESIS COMO COMPENDIO DE TRABAJOS PUBLICADOS

La presente tesis doctoral, de acuerdo con el informe correspondiente, autorizado por los Directores de Tesis y el órgano responsable del Programa de Doctorado, se presenta como un compendio de trabajos publicados. Las referencias completas de los artículos que constituyen el cuerpo de la tesis y que se adjuntan al final de esta memoria son los siguientes:

1. **Aneiros-Fernández J,** Husein-ElAhmed H, Arias-Santiago S, Campos A, Carriel V, Sánchez-Montesinos I, Garcia del Moral R, Sánchez G, O'Valle F, Aneiros J. Expression of smoothelin and smooth muscle actin in the skin. Histol Histopathol. 2011 Jun;26(6):673-8.
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2. **Aneiros-Fernández J,** Retamero JA, Husein-Elahmed H, Ovalle F, Aneiros-Cachaza J. Primary cutaneous and subcutaneous leiomiosarcomas: review of their evolution and prognostic factors. Eur J Dermatol Res 2015 Aceptado 7 de Octubre (In press).
   Factor Impacto: 1.990. Quartil 2.

3. Carriel V, **Aneiros-Fernández J**, Ruyffelaert M, Arias-Santiago S, Riady V, Izquierdo-Martínez F, Roda O, Cornelissen M, Campos A, Alaminos M. Histological and immunohistochemical study of an unusual type of gallbladder duplication. Histol Histopathol. 2014 Jul;29(7):957-64.
   Factor Impacto: 2.096. Quartil 2

4. Espiñeira-Carmona MJ, **Aneiros-Fernández J**, Girón Prieto MS, Carriel V, Antonia, Fernandez M, Buendía-Eisman A, Campos A, Alaminos

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5. **Aneiros-Fernandez J,** Nicolae A, Preda O. Smoothelin in bladder and gastrointestinal tract again. Histopathology. 2011 Jun;58(7):1173. Factor Impacto; 3.453. Quartil 1.

6. **Aneiros-Fernandez J**, Retamero JA, Husein-Elahmed H, Carriel V, Ovalle F, Aneiros-Cachaza J. Smoothelin and WT-1 Expresisión in glomus tumors and glomuvenous malformations. Histol Histopathol. 2015 Aceptado con revisión menor 29 de Octubre.
   Factor Impact: 2.096. Quartil 2.

7. **Aneiros-Fernandez J,** Retamero JA, Husein-ElAhmed H, Carriel V, Ovalle F, Aneiros-Cachaza J. Smoothelin expression in skin leiomyomas and leiomiosarcomas. Histopathology. 2015 (En revisión).

8. **Aneiros-Fernandez J**, Husein-ElAhmed H, Lopez Peña C, Lopez Caballero JJ, Ovalle F, Aneiros-Cachaza J. Dermal leiomiosarcoma with polypoid growth arising in a leiomyoma. Dermatopathol. 2015 (Revisión).

9. **Aneiros-Fernández J,** Husein-ElAhmed H, Retamero JA, Carriel V, Aneiros-Cachaza J. Estudio inmunohistoquímico de marcadores de diferenciación muscular lisa en los tumores y malformaciones vasculares de la piel. Dermatology online Journal. (Revisión).

RESUMEN

INTRODUCCIÓN

La esmotelina que no ha sido estudiada en la piel, podría ser un marcador que permitiría determinar células musculares lisas con capacidad contráctil. Por otra parte, la esmotelina no ha sido valorada en los tumores musculares lisos, lesiones perivasculares y anomalías vasculares cutáneas.

PROPÓSITO

El objetivo principal de la presente tesis es establecer el fenotipo inmunohistoquímico de la piel normal y en situaciones patológicas, con especial referencia a la esmotelina. Partiendo de las estructuras musculares cutáneas que son positivas para la esmotelina, estudiaremos aquellos procesos tumorales o malformativos que reproducen a dichas estructuras. Para ello se aplicará un protocolo inmunohistoquímico que permita caracterizar los distintos procesos.

MATERIAL, MÉTODOS Y RESULTADOS

El desarrollo de nuestros planteamientos nos ha permitido realizar los siguientes trabajos:

1. **Aneiros-Fernández J,** Husein-ElAhmed H, Arias-Santiago S, Campos A, Carriel V, Sánchez-Montesinos I, Garcia del Moral R, Sánchez G, O'Valle F, Aneiros J. Expression of smoothelin and smooth muscle actin in the skin. Histol Histopathol. 2011 Jun;26(6):673-8.
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CONCLUSIONES

La expresión de la esmotelina en la piel permite diferenciar el plexo vascular profundo del plexo vascular superficial. Hecho que no lo diferencian otros marcadores como la alfa actina de músculo liso.

La expresión nuclear de las esmotelina ayuda a distinguir leiomiomas de leiomiosarcoma. En los leiomiosarcomas la correlación estadística entre la esmotelina nuclear, la positividad para ki67 y la actividad mitótica sugiere que la expresión nuclear de la esmotelina acontece en las células que por lo general están en el ciclo celular. La expresión de la esmotelina a diferencia de otros marcadores de diferenciación muscular, permite precisar el difícil diagnóstico de la transformación maligna de un leiomioma a leiomiosarcoma. El término de neoplasia atípica intradermal del músculo liso que se aplica a los leiomiosarcoma dermales es incorrecta en base a hechos morfológicos y evolutivos.

La esmotelina se debería utilizar como un marcador de células glómicas tanto en los tumores como en las malformaciones glomovenosas. La ausencia

de expresión de WT1 en los endotelios de las estructuras vasculares de las malformaciones es una característica diferencial con los tumores glómicos.

Los tumores vasculares cutáneos benignos no demuestran células musculares lisas con capacidad contráctil. Sin embargo, en las malformaciones arterio-venosas y malformaciones venosas existen estructuras vasculares con capa muscular positiva para esmotelina.

INTRODUCCIÓN

INTRODUCCIÓN

La piel está constituida por tres capas: la epidermis, la dermis y el tejido subcutáneo (hipodermis). La epidermis es la capa superficial que contacta con el ambiente externo. Las invaginaciones de la epidermis originan las glándulas sudoríparas y folículos pilosos. La dermis es la capa media formada por un estroma elástico y colágeno producido por los fibroblastos que contienen vasos sanguíneos y estructuras nerviosas. El tejido subcutáneo es la capa más profunda que varía de espesor, dependiendo de la localización (gruesa en la planta del pie y palma de la mano, y fina en el brazo, etc) y que está formado fundamentalmente por tejido adiposo, estructuras vasculares y nerviosas. El tejido subcutáneo contiene el plexo vascular constituido por arterias y venas, dando lugar al plexo vascular profundo de la dermis. Dicho plexo emite ramas a los anejos cutáneos y finalizan en una red superficial de pequeñas vénulas y arteriolas que constituyen el plexo superficial o subpapilar (Braverman and Yen, 1977; Braverman and Yen, 1977). Las papilas dérmicas aparecen ocupadas por capilares sanguíneos.

Existe un trayecto arterial que asciende verticalmente hacia la papila dérmica y un trayecto venoso descendente. Este trayecto desemboca progresivamente en vénulas de mayor calibre, que a su vez drenan en vénulas de mayor grosor del plexo venoso profundo en el límite dermo-hipodérmico.

Las arterias del plexo vascular profundo y arteriolas de la dermis poseen una pared constituida por tres capas: íntima, media y adventicia. La íntima está constituida por células endoteliales y una lámina elástica interna. La capa media está formada principalmente por células musculares lisas y limitada externamente por una membrana elástica. La adventicia contiene fibroblastos,

colágeno tipo III y fibras elásticas. Las arteriolas más pequeñas de la dermis superficial están compuestas por una hilera de células endoteliales y por células musculares lisas. Los capilares sanguíneos están constituidos por una capa de células endoteliales rodeada por una hilera discontinua de pericitos.

La dermis presenta canales anastomóticos arteriovenosos que pueden estar muy especializados dando lugar a los cuerpos glómicos más abundantes en los extremos distales de los dedos. Un componente mesénquimal inespecífico de la piel es el músculo erector del pelo que fija la posición del folículo y tronco piloso. Dicha estructura nace en la vaina fibrocolágena (vaina dérmica) que circunda al folículo piloso y discurre oblicuamente hacia la dermis superficial.

En la piel existe una abundante patología que puede afectar a las diferentes capas. Una de ellas es la derivada de las células musculares lisas que se localizan en el músculo erector del pelo, capa media de los vasos arteriales y venosos, y en el cuerpo glómico. De esta manera, se pueden producir tumores que se originan en las células musculares lisas de la piel o malformaciones que reproducen a dichas células, dando lugar a leiomiomas, leiomiosarcomas, hamartomas del músculo liso, tumor glómicos sólidos, glomangiomiomas, glomangiomas (malformación glomovenosa). Otros procesos como las anomalías vasculares, que aunque no derivan de las células musculares lisas pueden estar presentes en la pared de los tumores o malformaciones vasculares.

HAMARTOMA DE MÚSCULO LISO DE LA PIEL

El hamartoma de músculo liso es una proliferación de fibras musculares lisas infrecuente que esta normalmente localizada en la dermis, el cual puede ser congénito, y menos frecuentemente adquirido (Gerdsen et al., 2000; Oiso et al., 2005). Se han descrito cuatro variantes clínicas congénitas, siendo la variante congénita localizada la más frecuente.

Esta lesión aparece más comúnmente en varones y se localiza preferentemente en el tronco y parte proximal de las extremidades (Goldman et al., 1987;Johnson and Jacobs, 1989). Las lesiones congénitas pueden asociarse a hiperpigmentación e hipertricosis (Zarineh et al., 2008). La presentación típica consiste en una placa solitaria de tamaño variable con medidas comprendidas entre 1 y 10 cm. No obstante, la lesión puede aumentar de tamaño en relación con el crecimiento del paciente. La variante adquirida no se asocia con hiperpigmentación ni hipertricosis (Darling et al., 1993). Sin embargo, existen estudios que han planteado la posibilidad de que el hamartoma de músculo liso adquirido pueda ser un leiomioma pilar difuso (Mitra et al., 2009).

Histopatologicamente consiste en agrupaciones de células musculares lisas separadas por bandas de fibras de colágena, que pueden comprometer a todo el espesor de la dermis e incluso al tejido celular subcutáneo (Zvulunov et al., 1990). Por lo general, los haces musculares aparecen retraídos del colágeno circundante. También se ha descrito que la células musculares lisas pueden conectar con los folículos pilosos (Johnson and Jacobs, 1989). El estudio inmunohistoquímico evidencia positividad para la actina músculo específica, alfa actina músculo liso y desmina (Sbano et al., 2005). Además, se

han descrito células CD34 positivas en el estroma que delimita el componente muscular hamartomatoso (Koizumi et al., 1999).

LEIOMIOMAS CUTÁNEOS

Los leiomiomas de la piel constituyen el 3-5% de todos los leiomiomas. Estos tumores se dividen en dos subtipos, teniendo en cuenta el origen. Del músculo erector del pelo se originaría el leiomioma pilar, de la capa media de los vasos sanguíneos se produciría el angioleiomioma y los derivados del músculo liso del escroto, de la vulva y de la areola, darían lugar a los leiomiomas genitales.

LEIOMIOMA PILAR

Los leiomiomas pilares son lesiones infrecuentes que pueden ser solitarias y múltiples, siendo esta última la más frecuente. Las formas solitarias se localizan por lo general en el tronco y extremidades, mientras que las formas múltiples aparecen en la cara, espalda y en superficies extensoras de las extremidades. Las lesiones solitarias tienen predilección por el sexo femenino. El leiomioma pilar solitario se presenta como una pápula o nódulo que por lo general es menos doloroso que el leiomioma piloso múltiple. La presentación clínica puede ser poco específica y confundirse con otras lesiones como angiolipoma, dermatofibroma, espiroadenoma ecrino, lipoma, tumor glómico, neurofibroma, hamartoma de músculo liso, etc (Raj et al., 1997).

El 75% de los leiomiomas pilares múltiples tienen una incidencia familiar con una herencia autosómica dominante, presentando en las mujeres asociación con leiomiomas uterinos. Dicha asociación se denomina síndrome

de Reed. El responsable de este proceso es una mutación en la línea germinal del FH (gen de la fumarasa hidratasa) que se localiza en el cromosoma 1q42.3-q43. (Martinez-Mir et al., 2002; Chuang et al., 2006). El gen FH se comporta como un gen supresor tumoral y su mutación justifica el desarrollo de los leiomiomas pilares y uterinos múltiples. En algunos casos este síndrome cursa con carcinomas papilares de riñón tipo 2 y de los conductos colectores, denominándose leiomiomatosis hereditaria con cáncer de células renales (Rothman et al., 2006). Para el diagnóstico de este síndrome se han establecido criterios mayores y menores (Smith et al., 2011). El criterio mayor consiste en la confirmación histopatológica de leiomiomas pilares múltiples. Los criterios menores comprenden: 1. Diagnóstico histopatológico de leiomiomas uterinos antes de los 40 años. 2. Diagnóstico histopatológico de carcinoma de células renales papilar tipo 2 antes de los 40 años. 3. Presencia de un miembro familiar de primer grado con alguno de los criterios anteriormente mencionados. No obstante, se han descrito mutaciones del gen FH en carcinoma de células renales con ausencia de leiomiomas pilares (Kuwada et al., 2014).

Histopatologicamente los leiomiomas pilares se localizan en la dermis como lesiones parcialmente delimitadas que están constituidas por fascículos de células fusocelulares organizadas en diferentes direcciones. Las células proliferantes tienen núcleos monomórficos y citoplasma alargado y eosinófilo. Entre los fascículos se advierten fibras de colágeno, que por lo general son menos abundantes que en el hamartoma de músculo liso. En ocasiones, los leiomiomas pilares tienen algunas irregularidades nucleares que se ha considerado como un fenómeno degenerativo en relación con el tiempo de

evolución del tumor. No obstante, se han descrito leiomiomas atípicos o simplásticos que tienen núcleos irregulares que presentan escasa actividad mitótica que pueden plantear el diagnóstico diferencial con leiomiosarcomas (Usmani et al., 2008). Esta dificultad diagnóstica es mayor en los casos de leiomiosarcomas que puedan originarse en los leiomiomas pilares simplásticos (Fons et al., 2011). Se han descrito variantes infrecuentes de leiomiomas pilares, tales como células granulares, con metaplasia ósea y de células claras (Mentzel et al., 1994; Massi et al., 1998; Xu et al., 2005)

Para establecer el diagnóstico de leiomioma pilar hay que realizar técnicas de inmunohistoquímica, utilizando marcadores que demuestren diferenciación muscular lisa. De esta manera se ha evidenciado positividad para alfa actina músculo liso, calponina y h-caldesmon (Mentzel et al., 1994).

ANGIOLEIOMIOMA

Los angioleiomiomas corresponden al 3-4% de todos los tumores benignos de partes blandas. Estos tumores se localizan por lo general en la extremidad inferior, tronco y cabeza e incidiendo más frecuentemente en mujeres, aunque los tumores localizados en la extremidad superior y cabeza aparece más frecuentemente en hombres (Hachisuga et al., 1984). El 50% de los angioleiomiomas pueden cursar con dolor. Macroscópicamente se presentan como lesiones nodulares que tienen por lo general menos de 2 cm de diámetro mayor. Morfológicamente los angioleiomiomas corresponde a una formación bien delimitada y localizada en el tejido subcutáneo y más raramente en la dermis. Dicha formación esta constituida por células fusocelular dispuestas en fascículos con núcleos monomórficas y citoplasma eosinófilo. La

presencia de algunos núcleos irregulares y actividad mitótica es muy infrecuente.

Los angioleiomiomas se han dividido en tres tipos según el patrón histológico predominante: sólido, venoso y cavernoso. El tipo sólido que corresponde al 66% de los casos presenta abundantes estructuras vasculares de finas paredes. El tipo venoso que corresponde al 23% de los casos tienen estructuras venosas de gruesas paredes. El tipo cavernoso que es el 11% de los casos muestra vasos sanguíneos dilatados con paredes finas. No obstante, debido a que estos tres tipos pueden coexistir, esta clasificación ha sido poco utilizada. En los angioleiomiomas pueden tener zonas de hialinización, calcificaciones, hemorragia, cambios mixoides y escasas agrupaciones de adipocitos (Hachisuga et al., 1984). Asimismo, se han descrito angioleiomiomas asociados a virus de Epstein Barr en pacientes inmunodeprimidos los cuales pueden tener algunos cambios atípicos. (Deyrup et al., 2006). En raras ocasiones los angioleiomiomas pueden transformarse en leiomiosarcoma cutáneo (White and Macdonald, 1981). Los angiomiolipomas desde punto de vista inmunohistoquímico expresan actina de músculo liso, actina de músculo específica, desmina, calponina y h-caldesmon.

LEIOMIOSARCOMA

Los leiomiosarcomas de la piel pueden ser primarios o secundarios. Los leiomiosarcoma primarios se pueden originar en el músculo erector del pelo y en la paredes de los vasos sanguíneos, dando lugar a leiomiosarcomas de localización dérmica o cutánea y subcutánea. También pueden originarse en el músculo dartos, en el músculo de la dermis vulvar y areola, dando lugar a los

leiomiosarcoma genitales. Los leiomiosarcoma se originan de novo y muy raramente sobre leiomiomas previos (White and Macdonald, 1981). Los leiomiosarcoma primarios de la piel se localizan más frecuentemente en las extremidades inferiores, cuero cabelludo y cara (Jensen et al., 1996; Kaddu et al, 1997). También se han descrito en otras localizaciones incluido el escroto, vulva y areola mamaria (Newman and Fletcher, 1991). Los leiomiosarcoma aparecen preferentemente en edades avanzadas, siendo más frecuente en hombres que en mujeres. Clínicamente los leiomiosarcoma son lesiones exofíticas o de apariencia nodular. Los leiomiosarcomas tienen dos patrones de crecimiento difuso y nodular (Kaddu et al., 1997). Los leiomiosarcomas difusos se localizan en la dermis, aunque pueden tener repercusión el tejido subcutáneo y tienden a mostrar márgenes irregulares. Mientras que en los leiomiosarcomas nodulares suelen ser subcutáneos y con márgenes de apariencia menos infiltrativa. Histopatologicamente los leiomiosarcomas están constituido por células y núcleo y citoplasma alargado. Los núcleos son irregulares e hipercromáticos con proporción variable de pleomorfismo. Se ha considerado que la variante nodular tiende a mostrar más atípia que los que tienen crecimiento con patrón difuso (Kaddu et al., 1997). En los leiomiosarcomas pueden existir zonas con diferente grado de diferenciación y para establecer el grado hay que valorar las áreas más indiferenciadas junto con la presencia o no de necrosis. En los leiomiosarcomas se advierte actividad mitótica y se considera que debe existir más de una mitosis /10 campos de gran aumento. En las zonas más indiferenciadas, la actividad mitótica es más frecuente, siendo en estas donde se deben valorar las mitosis. Las células tumorales se disponen en fascículos que tienden a ser más compactos en el

patrón nodular que en el difuso. En este último, los fascículos de células tumorales tienen evidentes fibras de colágeno en el estroma. Esto es poco llamativo en los leiomiosarcomas con patrón nodular. Se han descrito diferentes variantes de leiomiosarcomas: epitelioide, de células pequeñas, de células multinucleadas simulando osteoclastos, de células granulares, desmoplásico, inflamatorio, mixoide y pleomórfico (Mentzel et al., 2002; Diaz-Cascajo et al., 2002; Merchant et al., 2002). Las variantes mixoides y pleomórficas se consideran las más frecuentes. Para poder precisar el diagnóstico de los leiomiosarcomas de la piel es necesario realizar estudio inmunohistoquímico, demostrándose positividad para la alfa actina de músculo liso, actina de músculo especifica, desmina y h-caldesmon. Se ha considerado que la expresión de estos marcadores es mayor en los leiomiosarcomas dérmicos que en los leiomiosarcomas subcutaneos que son más indiferenciados (Oliver et al., 1991; Watanabe et al, 1999). Existen estudios que valora la expresión de marcadores de diferenciación muscular lisa en los leiomiosarcomas dérmicos, evidenciándose positividad para la alfa actina músculo liso en el 77,8% de los casos y de h-caldesmon en el 75% de los casos (Massi et al., 2010). También se ha estudiado la presencia de P53 demostrandose positividad en el 75% de los leiomiosarcomas y en el 19,35 % de los leiomiomas cutáneos. Se sugiere que la expresión de P53 en alto porcentaje de células puede ayudar a establecer el diagnóstico de leiomiosarcoma y a diferenciarlo de los leiomiomas simplásticos (Fernandez-Flores et al., 2010). Las alteraciones citogenéticas que mas recientemente se detectan en los leiomiosarcomas es la pérdida de la región 13q14q21 (Wang et al., 2003). También se ha descrito perdidas en 10q o ganancias en 5p, en

leiomiosarcomas con un comportamiento más agresivo (Hu et al., 2005). La evolución y los factores pronósticos de los leiomiosarcomas no están bien establecidos ya que existen pocos estudios de series de casos. Algunos estudios indican que los leiomiosarcomas cutáneos y subcutáneos demuestran diferente conducta biológica. Aunque la mayor parte de los estudios no señalan claramente estas diferencias (Jensen et al., 1996). Las metástasis de los leiomiosarcomas dermales habian sido reportadas hasta en el 14% de los casos (Berstein and Roenigk, 1996; Fauth et al., 2010). Mientras que en otros estudios la capacidad metastásica de estos leiomiosarcoma es menor (Hashimoto et al., 1986; Kraft and Fletcher, 2011). No obstante, está en debate si los leiomiosarcoma confinados en la dermis tienen potencial metastático y si la extensión al tejido subcutáneo se correlaciona con peor pronóstico. Los leiomiosarcoma subcutáneos se han considerado que puede recurrir en el 61% de los casos y metástasis en el 62% de los casos. Los leiomiosarcoma subcutáneos son por lo general de mayor tamaño que los leiomiosarcoma que se localizan a nivel dérmico. Este hecho podría explicar por qué los leiomiosarcoma cutáneos se diagnostican más tardiamente. Los leiomiosarcoma de mayor tamaño están asociados con peor pronóstico (Jensen et al., 1996; Kaddu et al., 1997). Los tumores que recurren o están localizados más profundamente muestra mayor actividad mitótica, aunque parece ser que este hecho no influye en el pronostico. El grado histológico basado en la Federation Nationale des Centres de Lutte Contre le Cancer (FNCLCC), se discute si puede influir en el diagnóstico. Posibles factores que pueden estar en relación con mejor pronóstico es la localización en la vulva. Mientras que hay factores que pueden indicar peor pronóstico son: aneuploidia

celular, invasión vascular, expresión de P53, expresión de la migfilina (proteína de adhesión matriz celular) (Papachristou et al., 1991; Newman and Fletcher, 1991). En definitiva en la mayoría de los estudios señalan alto grado de recurrencia y mortalidad en los leiomiosarcomas subcutáneos. Mientras que la conducta biológica de los leiomiosarcomas dérmicos no esta del todo claro. Más recientemente se propuso que los leiomiosarcomas dérmicos se reclasificasen como neoplasias atípicas intradermales de músculo liso debido a que no se observan metástasis en estas lesiones. Asimismo, el tipo de tratamiento de los leiomiosarcomas de la piel puede afectar en el pronóstico, para ello se deberá realizar extirpación quirúrgica con márgenes adecuados (Svarvar et al., 2007). También, la cirugía de Mosh había sido considerada como posible tratamiento de los leiomiosarcomas de la piel (Humphreys et al., 2004).

NEOPLASIA ATÍPICA INTRADERMAL DE MÚSCULO LISO

Se ha realiza un estudio de 84 casos que fueron diagnosticados de leiomiosarcomas dérmicos y que aparecen preferentemente en hombres (4,3:1), con una media de edad de 56 años (rango 6-82 años) (Kraft and Fletcher, 2011). Los tumores midieron 1,3 cm de media, localizándose en el tronco y extremidad inferior. Todas las lesiones estaban ubicadas en la dermis o presentaban extensión superficial o mínima extensión subcutánea.

Los hallazgos histológicos mostraron un patrón de crecimiento infiltrante con formación de fascículos constituidos por células alargadas y de citoplasma eosinófilo. Entre los fascículos se evidenciaran haces de fibras de colágeno. Los tumores mostraban por término medio 4,7 mitosis /10 campos de gran

aumento. Asimismo, los tumores en el 97% de los casos eran grado 1, y solo

en el 3% tenían necrosis (grado 2-3) según la gradación de FNCLCC.

Inmunohistoquimicamente los tumores presentaron positividad en el 100% de

los casos para actina de músculo liso, 98% de los casos para la desmina y en

el 90% para el h-caldesmón. La media del seguimiento de estos tumores fue de

51 meses y en el 18% de los casos presentaron recurrencia local con una

media de intervalo de 43 meses. No obstante, en ninguno de los casos se

evidenció metástasis. Los tumores que recurrieron, presentaron 13,7 mitosis

/10 campos de gran aumento y el 47% fueron grado 1, 35 % grado 2 y 18%

grado 3. En 28 de los casos con recurrencia mostraron necrosis. Los autores

consideran que estos tumores tienen capacidad para recidivar y no

metastatizar, y de esta manera no deberían de ser considerados como

sarcomas, siendo el término mas apropiado el de neoplasia atípica dérmica del

músculo liso. Dicha terminología es utilizada en trabajos mas recientes (Hall et

al., 2013)

LEIOMIOMAS Y LEIOMIOSARCOMAS GENITALES EXTERNOS

En la piel se incluyen tumores musculares lisos que se originan en los

fascículos musculares en la dermis del escroto, pezón, areola y vulva (Newman

et al., 1991). Los leiomiomas genitales son infrecuentes. Los leiomiomas de la

vulva son más frecuentes y se localizan en los labios mayores, pudiendo

confundirse clínicamente con un quiste de la glándula de Bartholino. El

leiomioma de escroto aparece como una pápula o nódulo solitario y

asintomático. El leiomioma de la areola mamaria es más infrecuente,

apareciendo en pacientes más jóvenes y que con lesiones de menor tamaño

que pueden ser dolorosas. Los leiomiomas de la vulva son por lo general bien delimitados y pueden remedar a los leiomiomas uterinos. Los leiomiomas escrotales son menos delimitados que los de localización vulvar. Los leiomiomas del pezón presentan un patrón diferente a los anteriores ya que aparecen constituido por fascículos de células musculares lisas dispersas por la dermis y separados con abundantes fibras de colágeno con fascículos de músculo normal de la areola. El leiomioma del escroto, las células tumorales se agrupan de manera más compacta con escaso estroma. En el leiomioma de vulva pueden tener el estroma zonas de hialinización y cambios mixoides. El estudio inmunohistoquímico muestra expresión para la actina de músculo liso, alfa actina de músculo liso, desmina. Los leiomiomas en varones pueden expresar receptores androgénicos, mientras que los leiomiomas de vulva por lo general expresan receptores de estrógenos y progesterona.

Los leiomiosarcoma genitales son más infrecuentes que los leiomiomas. Las características histopatológicas son similares a otros leiomiosarcoma cutáneos. No obstante, se han considerado que los que se localizan en la vulva por lo general miden más de 5 cm y tienen más de 5 mitosis por campo de gran aumento.

TUMOR GLÓMICO Y MALFORMACIONES GLOMOVENOSAS

Los tumores glómicos son neoplasias benignas que proceden de las células glómicas de los canales de Succquet-Hoyer que corresponden a anastomosis arteriovenosas especializadas en la termorregulación y denominadas cuerpo glómico (Landthaler et al., 1990; Glick et al., 1995; Schiefer et al., 2006). Las células glómicas en un principio fueron consideradas

pericitos, no obstante tras la realización de estudios ultraestructurales observaron que dichas células estaban rodeadas por una gruesa membrana basal en cuyo citoplasma se identificaban múltiples filamentos citoplasmáticos de actina que se condensaban formando cuerpos densos. Debido a estos hallazgos se consideró que la células glómicas eran células musculares lisas modificadas. Posteriormente se realizaron numerosos estudios inmunohistoquímicos que evidenciaron positividad para marcadores de diferenciación muscular como son, actina de músculo liso, desmina y h-caldesmon (Bertalot et al., 1994; Watanabe et al., 1999).

La localización anatómica más frecuente son las zonas distales (subungueal, lateral de los dedos y palma de la mano) *(Gombos and Zhang, 2008)* que normalmente son lesiones únicas, siendo los tumores glómicos múltiples extradigitales. La sintomatología más frecuente de los tumores glómicos son dolor, punto de sensibilidad, compresión y sensibilidad térmica.

Las formas solitarias aparecen con mayor frecuencia en edades comprendiadas entre 20-40 años, localizándose con mayor periodicidad en zonas distales, aunque existen lesiones múltiples a nivel subungueal que están asociados a neurofibromatosis tipo 1. La formas múltiples se denominan malformaciones glomovenosas que son menos comunes y se heredan de forma autosómica dominante. En función del incremento de las células glómicas, de las estructuras vasculares o del componente muscular liso, las anomalías glómicas muestran diferentes tipos histológicos como son tumor glómico sólido (clásico), glomangioma (malformación glomovenosa) y glomangiomioma.

El tumor glómico sólido es una neoformación bien delimitada que fue descrita por primera vez por Masson en 1924. Su incidencia es poco frecuente siendo < 2% de los tumores de partes blandas (Schiefer et al., 2006), con loalización más frecuente en extremidades superiores, sobretodo en zona subungueal ,extremidades inferiores, cabeza y cuello y tronco. Estas lesiones muestran un claro predominio en mujeres y con un rango de edad entre 20-50 años. Clínicamente se identifican como una lesión azulada con un tamaño < 1 cm, observándose en dermis, dermo-hipodermis o hipodermis. Histológicamente el tumor glómico sólido está constituido por una sábana de células monomórficas similares a las células glómicas normales, mostrando un núcleo redondo con un citoplasma anfófilo o eosinófilo con la tinción de hematoxilina eosina. Dentro de esta variante sólida, existen formas con dilataciones vasculares que pueden en ocasiones simular una malformación glomovenosa. Se han descrito variantes de tumor glómico sólido que son infrecuentes, y que comprende el tipo oncocítico, simplástico, con apariencia hemangiopericitoma like, con cambios mixoides y epitelioide. *(Yanagi and Matsumura, 2006; Mentzel et al., 2002; Tse and Chan, 2002; Kamarashev et al., 2009).*

Debido a su localización y a su abundante componente celular, pueden confundirse con tumores anexiales como los hidroadenomas, espiroadenomas o con lesiones névicas benignas.

El glomangioma (malformación glomovenosa) clásicamente se ha considerado una variante del tumor glómico, pero recientemente se han realizado estudios genéticos donde se ha comprobado que existen mutaciones en el gen glomulina/TIE2 que esta localizado en el cromosoma 1q21—22

(Brouillard et al., 2002). La malformación glomovenosa representa el 20% de las lesiones glómicas, afectando más frecuentemente a la infancia con un ligero predominio en varones. Se localizan con mayor frecuencia en extremidades superiores en forma de placa. Histológicamente son diferentes de los tumores glómicos sólidos, ya que están constituidos por ectasias vasculares rodeadas por una o dos capas de células glómicas con un límite mal definido .

El glomangiomioma es una lesión glómica que muestra diferenciación a músculo liso originada sobre un tumor glómico sólido o sobre una malformación glomovenosa. Cuando se originan sobre un tumor glómico sólido, aparece una transición de las células glómicas a células fusocelulares con citoplasma eosinófilo que adquieren características parciales de musculo liso (Wollstein et al., 2012). Si se desarrolla sobre una malformación glomovenosa, las células glómicas se entremezclan con las células fusocelulares y las dilataciones vasculares (Kim et al., 2010). La diferenciación a musculo liso ocurre de manera focal en ambas entidades.

A nivel inmunohistoquímico las células glómicas del tumor glómico y malformación glomovenosa son positivas para vimentina, AML y un 20% de los casos son focalmente positivas para CD34. La laminina y el colágeno tipo IV rodean normalmente el tumor con un patrón en forma de red. La expresión de h-caldesmon y calponina es variable, siendo muy infrecuente la expresión de desmina (Miettinen et al., 2002; Mentzel et al., 2002; Wong et al., 2014).

TUMORES Y MALFORMACIONES VASCULARES

La terminología de las anomalías vasculares desde su origen ha sido utilizada de manera confusa. Esto ha dado lugar a diagnósticos incorrectos,

tratamientos inadecuados y posteriormente una investigación mal dirigida. En 1982 se introdujo la primera clasificación en base a estudios que correlacionaban los hallazgos físicos, evolución y las características morfológicas, aclarando las mayor parte de los procesos vasculares y dando lugar a dos categorías: tumores vasculares y malformaciones vasculares (Mulliken and Glowacki,1982).

El término de hemangioma se utiliza comúnmente para nombrar diferentes tipos de tumores vasculares, así como malformaciones vasculares, a pesar de tener distinta evolución natural, diferente origen y tratamiento. Por tanto, es necesario manejar una nomenclatura unificada para hacer un diagnóstico correcto (Hassanein et al., 2011).

El hemangioma se ha utilizado para referirse al hemangioma infantil, la neoplasia más frecuente en la infancia, existiendo también hemangiomas fetales. El hemangioma infantil suele aparecer a las dos semanas después del nacimiento teniendo un crecimiento rápido y llegando a tener su máximo tamaño al noveno mes de vida, que posteriormente va involucionando durante la infancia. A diferencia de las malformaciones que están presentes al nacer, aunque en muchas ocasiones no son muy evidentes y nunca regresan. La mala utilización del término hemangioma por parte de los clínicos también fue influenciado por la localización de las lesiones (Enjolras and Mulliken, 1997).

Para evitar toda esta nomenclatura utilizada de forma errónea, la Sociedad Internacional para el estudio de las anomalías vasculares (ISSVA) realizo una clasificación en 1996 (Enjolras and Mulliken., 1997). La clasificación de 1996 (ISSVA) se basa en la separación de lesiones con componente proliferativo (tumores vasculares), de las lesiones estáticas (malformaciones

vasculares). Las malformaciones vasculares que se deben a errores de la

morfogénesis vascular, se clasifican en función de su extirpe: capilar, venosa,

linfática, arterial o malformaciones combinadas.

Posteriormente a esta clasificación, han sido reclasificadas muchas

malformaciones, asociados a trastornos genéticos. Existen otras clasificaciones

que se realizaron con posterioridad como la de WHO de tumores de piel que se

corresponde a una lista jerárquica de una serie de anomalías vasculares con

independencia de si es tumor, malformación o naturaleza infecciosa (LeBoit et

al., 2006). También, la clasificación de la WHO de los tumores de partes

blandas usa la palabra de hemangioma para describir un tumor o una

malformación, dando lugar a aún mas a confundir la terminología (Fletcher et

al., 2013). Esto hace que la clasificación de la WHO sea engañosa y confusa.

Más tarde se observó que las malformaciones tronculares no

presentaban el mismo comportamiento biológico que las malformaciones extra-

tronculares, dando lugar a una nueva clasificación en 2014 (ISSVA) (Wassef et

al., 2015).

ISSVA classification of vascular tumors ©

Benign vascular tumors	
Infantile hemangioma / Hemangioma of infancy	see details
Congenital hemangioma	
Rapidly involuting (RICH) *	
Non-involuting (NICH)	
Partially involuting (PICH)	
Tufted angioma * °	
Spindle-cell hemangioma	
Epithelioid hemangioma	
Pyogenic granuloma (aka lobular capillary hemangioma)	
Others	
Locally aggressive or borderline vascular tumors	
Kaposiform hemangioendothelioma * °	
Retiform hemangioendothelioma	
Papillary intralymphatic angioendothelioma (PILA), Dabska tumor	
Composite hemangioendothelioma	
Kaposi sarcoma	
Others	
Malignant vascular tumors	
Angiosarcoma	
Epithelioid hemangioendothelioma	
Others	

ISSVA classification for vascular anomalies ©

Simple vascular malformations I	
Capillary malformations (CM)	
Cutaneous and/or mucosal CM (aka "port-wine" stain)	G
CM with bone and/or soft tissue overgrowth	
CM with CNS and/or ocular anomalies (Sturge-Weber syndrome)	
CM of CM-AVM	G
CM of MICCAP (microcephaly-capillary malformation)	
CM of MCAP (megalencephaly-capillary malformation-polymicrogyria)	
Telangiectasia	
Hereditary hemorrhagic telangiectasia (HHT) (different types)	G
Others	
Cutis marmorata telangiectatica congenita (CMTC)	
Nevus simplex / Salmon patch / "angel kiss", "stork bite"	
Others	

Simple vascular malformations II	
Lymphatic malformations (LM)	
Common (cystic) LM	
Macrocystic LM	
Microcystic LM	
Mixed cystic LM	
Generalized lymphatic anomaly (GLA)	
LM in Gorham-Stout disease	
Channel type LM	
Primary lymphedema (different types)	G
Others	

Simple vascular malformations IIb	
Primary lymphedema	
Nonne-Milroy syndrome	G
Primary hereditary lymphedema G	
Lymphedema-distichiasis	G
Hypotrichosis-lymphedema-telangiectasia G	
Primary lymphedema with myelodysplasia G	
Primary generalized lymphatic anomaly (Hennekam lymphangiectasia-lymphedema syndrome)	G
Microcephaly with or without chorioretinopathy, lymphedema, or mental retardation syndrome	G
Lymphedema-choanal atresia	G

Simple vascular malformations III	
Venous malformations (VM)	
Common VM	G
Familial VM cutaneo-mucosal (VMCM)	G
Blue rubber bleb nevus (Bean) syndrome VM	
Glomuvenous malformation (GVM)	G
Cerebral cavernous malformation (CCM) (different types)	G
Others	

Simple vascular malformations III	
Venous malformations (VM)	
Common VM	G
Familial VM cutaneo-mucosal (VMCM)	G
Blue rubber bleb nevus (Bean) syndrome VM	
Glomuvenous malformation (GVM)	G
Cerebral cavernous malformation (CCM) (different types)	G
Others	

Simple vascular malformations IV	
Arteriovenous malformations (AVM)	
Sporadic	
In HHT	G
In CM-AVM	G
Others	
Arteriovenous fistula (AVF) (congenital)	
Sporadic	
In HHT	G
In CM-AVM	G
Others	

En nuestro medio los tumores vasculares benignos y malformaciones más frecuentes son granuloma piógeno, hemangioma infantil, angioma en penacho, malformación arteriovenosa, malformación venosa.

GRANULOMA PIOGENO

La mayoría de los autores consideran el granuloma piógeno como un proceso hiperplásico. Esta lesión presenta un crecimiento rápido en los sitios donde se produce un trauma superficial, aunque algunas lesiones se asocian a alteraciones endocrinas o medicamentosas. Otros autores, sin embargo consideran incluir esta entidad en un grupo de neoplasias vasculares (Wassef et al., 2015). Entre los argumentos utilizados para apoyar la naturaleza neoplásica del granuloma piógeno es la presencia de una arquitectura lobular, similar a la observada en otros procesos neoplásicos, incluyendo el angioma en pencho. Mills et al señalo que un patrón lobular es un hallazgo que se repite en algunas etapas del desarrollo de todas las variantes, incluyendo la subcutánea. Estos autores acuñaron el término hemangioma capilar lobular para tales lesiones y considerandolas como neoplasias benignas, en función de su morfología. Sin embargo, los capilares dispuestos en un patrón lobular se pueden ver en diferentes proliferaciones vasculares, tanto en hiperplasias como neoplasias (Mills et al., 1980) (LeBoit et al., 1989). Otros autores consideran

que son hiperplasias en lugar de neoplasias por las siguientes razones: la lesión aparece a menudo como una respuesta a un trauma, factores hormonales, o terapia con retinoides (Jafarzadeh et al., 2006). Las lesiones generalizadas aparecen de forma eruptiva, pero por lo general se resuelven espontáneamente en pocos meses (Netchiporouk et al., 2015); y las lesiones en las mujeres embarazadas, así como lesiones secundarias a las píldoras anticonceptivas orales o terapia retinoide suelen disminuir después del parto o de la retirada del fármaco responsable (Fernandez et al., 2014). El granuloma piógeno es uno de los tumores más frecuentes en niños por encima de los tres años de edad (Pagliai et al., 2004). Se caracteriza por una pápula vascular friable localizada más frecuentemente en cabeza y cuello. Son menos frecuentes en otras localizaciones como acrales, mucosa y conjuntiva (Gordón-Nuñez et al., 2010) (Shields et al., 2011). El tamaño varía entre 0,1 y 1 cm (Shields et al., 2011). En general, se manifiesta mas frecuentemente en varones. El pico de incidencia de granuloma piógeno ocurre en la segunda década de la vida. Generalmente se desarrollan en el sitio de una lesión preexistente, en el que evolucionan rápidamente durante un período de semanas y luego involucionan siendo reemplazados por tejido fibroso finalmente desaparece en unos pocos meses.

Microscópicamente, el granuloma piógeno es una lesión exofítica, que a menudo se ulcera, y esta caracterizada por una proliferación lobulada de vasos capilares de tamaño variable rodeado de un estroma ligeramente edematoso. Cada lóbulo está formado por agrupaciones de capilares y vénulas revestidos por células endoteliales. El epitelio de superficie forma unas crestas que rodean parcialmente la proliferación vascular dando lugar a un collarete epidérmico. La

ulceración y la inflamación aguda es frecuente que aparezca, dando lugar a un endotelio reactivo con presencia de mitosis.

HEMANGIOMA INFANTIL

Los hemangiomas infantiles están definidos por una proliferación vascular que está presente con mayor frecuencia en la infancia. Se caracteriza por una fase inicial proliferativa rápida, seguida de una fase de estancamiento y por último una fase de involución. Durante años los hemangiomas infantiles fueron clasificados de manera errónea como capilar, cavernoso o con componente mixto. De esta manera, la lesión que se presentaba en la dermis mostraba una proliferación vascular de tipo capilar, mientras que las lesiones localizadas más profundamente, mostraban capilares con vasos dilatados de apariencia cavernosa (Mulliken and Glowacki, 1982).

Clínicamente debido a que los hemangiomas infantiles de tipo capilar se encuentran en la dermis muestran un color carmesí intenso mientras que los hemangiomas localizados en al dermis profunda muestran una coloración azulada. Durante la infancia es difícil distinguir los hemangiomas de las malformaciones vasculares. Una de las características fundamentales, es que los hemangiomas raramente aparecen antes de la 2-3 semana de vida, presentando un crecimiento rápido en las primeras semanas. Sin embargo, las malformaciones son evidentes desde el nacimiento, mostrando un crecimiento progresivo al crecimiento del niño (Mulliken and Glowacki, 1982). Para complicar mas la diferencia entre las malformaciones y hemangiomas, existe un hemangioma congénito que no involuciona que está presente al nacer y no

muestra la fase rápida proliferativa creciendo progresivamente con el niño y no evidenciando regresión (Lee et al., 2014).

Los hemangiomas infantiles se presentan en 1-3% de los recién nacidos y un 10% de los recién nacidos en el primer año de vida (Redondo et al., 2007). Pueden aparecer en cualquier parte de la piel, aunque son más frecuentes en la cabeza y en el cuello, normalmente como lesión solitaria.

Las características histológicas de los hemangiomas varía con la edad de las lesiones. En fases iniciales los hemangiomas son altamente celulares y se caracterizan por espacios vasculares pequeños con células endoteliales. Cuando las lesiones son maduras las células endoteliales se aplanan y la luz de las estructuras vasculares se agrandan, mostrando una apariencia cavernosa, la cual puede confundirse con una malformación venosa. La fase de regresión se representa por una fibrosis intersticial y metaplasia adiposa.

ANGIOMA EN PENACHO

El angioma en penacho es una neoplasia que ha mantenido diferentes nombres como hemangioma capilar progresivo y angioblastoma (Macmillan and Champion, 1971; Mittal and Tripathy, 2013). Esta lesión afecta más frecuentemente a los niños, adultos jóvenes, describiéndose casos de inicio congénito o muy tardíos (Wang et al., 2013; Colmenero et al., 2014). Las lesiones tienen predilección por el cuello, pecho, espalda y hombros, aunque se han reportado casos en la cabeza, extremidades y mucosa oral (Colmenero et al., 2014). El tumor presenta un crecimiento a lo largo de los años, pero existe una ligera tendencia de regresión espontánea (Ishikawa et al., 2005).

Las características histológicas del angioma en penacho muestra
múltiples lóbulos vasculares individuales dentro de la dermis y en ocasiones
dentro de la hipodermis. Cada lóbulo está compuesto por células endoteliales y
pericitos concéntricas alrededor de un plexo vascular preexistente, mostrando
algunas luces dentro de esos agregados de células endoteliales.

MALFORMACION ARTERIOVENOSA

Las malformaciones arteriovenosas son malformaciones congénitas
constituidas por lechos capilares anómalos, que con frecuencia son mal
diagnosticados al nacer como otras lesiones vasculares. La pubertad y el
trauma desencadenan el crecimiento de la lesión y la manifestación
sintomatológica. Son lesiones infiltrativas, causando destrucción local, y en
ocasiones pueden dar lugar a hemorragias masivas. El origen y la patogenia no
está clara. La mayoría de las malformaciones arteriovenosas están presentes
desde el nacimiento y otras asociadas a traumatismo (Richter and Friedman,
2012). Se han aislado receptores de progesterona que explicarían la capacidad
de aumentar de tamaño durante la pubertad (Liu et al., 1999).

Clínicamente se presenta como una lesión hipervascular que puede
manifestarse como un ligero rubor desde el nacimiento con un crecimiento
paralelo al desarrollo del niño. La localización más frecuente es la cara, cavidad
oral, y las extremidades (Richter and Friedman, 2012).

Histologicamente, la malformación arteriovenosa consiste en una
proliferación bien circunscrita de vasos sanguíneos que contienen paredes
gruesas, bordeadas por una capa de células endoteliales que afecta
normalmente la dermis papilar y reticular. Entre ellas pueden existir algunos

vasos dilatados con paredes finas. Las paredes de los vasos gruesos simulan a las arterias, pero no contienen membrana elástica interna (Koutlas and Jessurun, 1994).

MALFORMACIÓN VENOSA

Las malformaciones venosas (VM) son el tipo más común de malformación vascular. Se componen de canales de paredes delgadas, dilatadas, esponjosas de tamaño variable y espesor de la pared aumentada con revestimiento endotelial normal y músculo liso inmaduro. En general, son azuladas que tienden a expandirse lentamente con el tiempo. Se producen principalmente en la piel y los tejidos subcutáneos, pero también pueden afectar al músculo, vísceras, estructuras articulares, y el sistema nervioso central. La mayoría de las malformaciones son solitarias, aunque pueden existir múltiples lesiones cutáneas y viscerales (Richter and Friedman, 2012). Las formas multifocales pueden ser hereditarias. Las malformaciones venosas crecen con el niño, y pueden aumentar de tamaño rápidamente con formación de trombos. También, puede asociarse a múltiples síndromes (Marler and Mulliken, 2005).

Histopatológicamente las malformaciones venosas consisten en vasos sanguíneos dilatados y forma irregular que afecta a la dermis profunda y a la hipodermis. Algunos de los vasos sanguíneos implicados muestran paredes delgadas, mientras que otros presentan una gruesa capa de músculo liso en sus paredes.

INMUNOHISTOQUÍMICA

ALFA ACTINA DE MÚSCULO LISO

Las actinas citoplásmicas que pertenecen al sistema de microfilamentos de las proteinas citoesqueléticas, son algunas de las proteínas eucarióticas más conservadas que se expresan en mamíferos y aves. La proteína actina está compuesta por seis isoformas con diversas secuencias de aminoácidos, pero todas poseen la misma masa molecular de 42 kDa. Existen distintas isoformas alfa específicas para los tejidos musculares, es decir, alfa músculo esquelético, alfa músculo cardíaco y alfa músculo liso, respectivamente (Schurch et al., 1987). Las isoformas gamma y alfa se identifican en los tejidos con diferenciación miogénica, pero también se han demostrado en células con características miofibroblásticas y mioepiteliales. Debido a que existen problemas en la detección de proteínas heteropoliméricas a nivel de la inmunohistoquimica hace que no sea específico para diferenciación miogénica. Esto ocurre especialmente con la clona 1A4 conocida como la alfa-actina de músculo liso, que se expresa en distintas células de músculo liso, incluyendo miofibroblastos, células mioepiteliales (Jones et al., 1990). De hecho, cualquier neoplásia que muestra células fusocelulares podría evidenciar expresión para alfa actina de músculo específico. Sin embargo, la alfa actina de musculo liso no se expresa en músculo esquelético normal (Skalli et al., 1988).

H-CALDESMON

El h-caldesmon es una proteína que se encuentra ampliamente distribuida en el músculo liso y en las células no musculares. Se han caracterizado dos clases el h-caldesmon (120-150 kDa) que es expresada por las células musculares lisas, mientras que l-caldesmon (70-80 kDa) se localiza

en el tejido no muscular, no identificando en ningún momento expresión en músculo esquelético (Sobue et al., 1991). El h-caldesmon es una proteína fijadora localizada en los filamentos del citoesqueleto y parece jugar un papel en la regulación de la integración de actina miosina, ya que se fija de forma específica con gran afinidad por el extremo N-terminal. La fijación de h-caldesmon a f-actina produce una inhibición de actividad ATPasa el músculo liso. El h-caldesmon junto con la tropomiosina es un factor mediador de la inhibición de Ca2+ dependiente de la contracción del músculo liso (Sobue et al., 1991). Este anticuerpo ha demostrado que es un marcador específico de las células musculares lisas (Watanabe et al., 1999), y ha sido reportado como útil para diferenciar tumores de músculo liso de los tumores con diferenciación miofibroblástica, sarcoma del estroma endometrial, rabdomiosarcoma, tumor de la vaina nerviosa periférica y osteosarcomas (Watanabe et al., 1999; Ceballos et al., 2000; Rush et al., 2001). La expresión de h-caldesmon es específica pero variable en los leiomiosarcoma (Hisaoka et al., 2001). Además, se encontraron positividad en las células mioepiteliales normales, pero negatividad en las células mioepiteliales neoplásicas de los tumores mixtos de la piel y glándulas salivares (Watanabe et al., 1999). Se han realizado estudios de serie de casos de tumores fusocelulares de la piel, encontrando que el h-caldesmon sirvió como un marcador específico del músculo liso totalmente diferenciado y que podría ayudar a distinguir tumores de células musculares lisas de la piel de los tumores de origen fibroblástico o miofibroblástica como son fascitis nodular, fibromatosis y el sarcoma miofibroblástico. (D'Addario et al., 2002; Sakamoto et al., 2002).

MIOSINA MUSCULO LISO

La miosina músculo lisa es una proteína estructural citoplásmática, que forma parte del mecanismo contráctil de las células musculares lisas. La composición de la cadena pesada de la miosina del musculo liso humano ha sido investigada por electroforesis. La expresión de la miosina muscular lisa se regula con el desarrollo, observándose en fases tempranas en el musculo liso. Se han descrito dos isoformas de cadenas pesadas de miosina que son MHC-1 (205 kDa) y MHC-2 (200 kDa) (Borrione et al., 1989). La miosina se ha considerado marcador para células mioepiteliales, células musculares lisas y escasa expresión en células miofibroblásticas (Perez-Montiel et al., 2006).

DESMINA

La desmina es una proteína citoplasmática que se encuentra típicamente en las células musculares y en las neoplasias con diferenciación miogénica (Rangdaeng and Troung, 1991). En las células de músculo liso la desmina se identifica como cuerpos densos citoplasmáticos y en las células del músculo estriado los filamentos de desmina están vinculadas a los discos Z sarcoméricas. En ambos tipos de músculo la desmina ayuda a los miofilamentos a unirse formando haces. La desmina presenta un peso molecular de 53 kDa, que esta constituida por una zona inicial N-terminal y una zona final C-terminal. En general, la desmina es un marcador específico para tumores de diferenciación miogénica (leiomioma, rabdomiosarcoma, leiomiosarcoma, rabdomioma). Aunque en la mayoría de los leiomiomas expresan desmina, el porcentaje de positividades es menor en los leiomiosarcomas (Schurch et al., 1987).

ESMOTELINA

La esmotelina es una proteína del citoesqueleto que se expresa
específicamente en células musculares lisas diferenciadas, y que está asociado
con los filamentos de actina (Van del Loop et al., 1996). Este hecho, se ha
considerado tiene importancia en la modulación de las propiedades contráctiles
de las células musculares lisas. Se han identificado una Isoforma larga y corta,
la cual pierde los aminoácidos 1-544 de la región amino terminal de la isoforma
larga. La esmotelina corta (A) de 59 kDa aparece en las células musculares
lisas de los órganos. La esmotelina corta (B) de 110 kdA se observa en la capa
muscular de los vasos sanguíneos (Kramer, 1999, 2001). El gen que codifica a
esta proteína se localiza en el cromosoma 22q12.3 (Rensen et al., 2002). La
pérdida de la expresión de la esmotelina está asociada con la disminución del
potencial contráctil de las células musculares lisa. Hecho que ha sido valorado
experimentalmente y en situaciones patológicas como la aterosclerosis y
reestenosis (Niessen et al., 2005) se ha considerado que la esmotelina se
expresa tardíamente en el desarrollo, cuando las células musculares lisas
adquieren capacidad contráctil. Asimismo, la expresión de la esmotelina es
específica no evidenciándose positividad en las células mioepiteliales,
musculares estriadas ni en miofibroblastos. La esmotelina ha sido estudiada en
el tracto gastrointestinal y vejiga normal, junto con otros marcadores de
diferenciación lisa (alfa actina de músculo liso, h-caldesmon etc). Mientras que,
estos últimos presentaban intensa positividad en la capa muscular propia y en
la muscularis mucosae. Sin embargo, la esmotelina fue intensamente positiva
en la capa muscular propia y menos evidente en la muscularis mucosae

(Montani et al., 2010; Poletajew et al., 2015). Se ha establecido que la esmotelina podría ayudar a valorar los plexos vasculares de la vejiga y del tracto gastrointestinal (Aneiros-fernandez et al., 2011). También se ha valorado la esmotelina en los vasos sanguíneos endometriales en mujeres con menorragias, demostrándose incremento de la expresión de la esmotelina en las menorragias con respecto al endometrio normal (Biswas Shivhare et al., 2014). Teniendo en cuenta que la esmotelina es una proteína contráctil músculo liso específica que se expresa en células completamente diferenciadas de la capa muscular propia del tracto gastrointestinal y de la vejiga y no en células musculares lisas no contráctiles de la muscularis mucosae. Este hallazgo se ha utilizado para diferenciar estas estructuras que tienen importancia para precisar la invasión de los tumores (Bovio et al., 2010; Hodges et al., 2010). También se ha valorado la esmotelina en los ganglliones evidenciándose células h-caldesmon, actina músculo liso y calponina positivas que posiblemente corresponden a células musculares lisas en fase de diferenciación temprana o a miofibroblastos, dichas células fueron negativas para la esmotelina (O´Valle et al., 2013).

La esmotelina ha sido estudiada en los tumores musculares lisos del tracto gastrointestinal considerándola como un marcador específico para el diagnóstico de estos tumores (Coco et al., 2009). En este estudio se evidencia positividad citoplasmática y nuclear en los leiomiomas, leiomiosarcomas y en la capa muscular propia del tracto gastrointestinal. Posteriormente se ha valorado la expresión de la esmotelina en los leiomiomas gastrointestinales, evidenciándose positividad únicamente en el citoplasma (Wong et al., 2009). Sin embargo, en los leiomiosarcomas retroperitoneales y gastrointestinales la

positividad de la esmotelina fue en el citoplasma y menos frecuentemente en el núcleo (Wong et al., 2009; Yamamoto et al, 2013). Se ha considerado que estas diferencias de expresión pueden estar en relación con el protocolo inmunohistoquímico realizado (Wong et al., 2009).

TUMOR DE WILMS 1

WT1 es un gen involucrado en la inducción del tumor de Wilms, una enfermedad maligna renal pediátrica. El gen WT1, ubicado en el cromosoma 11p13, está inactivado en un 5 a 10% de los tumores de Wilms esporádicos, y en cerca del 100% de pacientes con Denys-Drash, síndrome asociado a anormalidades genitourinarias y tumor de Wilms. WT1 codifica un factor de transcripción de dedo de zinc que reconoce la secuencia consenso de respuesta de crecimiento temprano (EGR-1) localizada en promotores de genes de factor de crecimiento. La proteína codificada por el gen WT1 regula la transcripción de otros genes y puede funcionar como activador y como represor de la transcripción. Se ha demostrado que la WT1 ha reprimido la transcripción de diversos genes relacionados con el crecimiento, como la cadena A de factor de crecimiento derivada de plaquetas (PDGF-A), factor de crecimiento similar a la insulina (IGF) (Coppes MJ et al., 1993; Lee and Haber, 2001). La actividad de transcripción de esta proteína sin embargo, puede modularse mediante interacciones entre WT1 y p53. En ausencia del tipo no mutante p53, se ha observado que WT1 actúa como activadora de transcripción del promotor EGR-1 en ensayos de transfección (Maheswaran et al., 1993). La expresión de este marcador es a nivel del núcleo de la célula, aunque se ha observado expresión citoplasmática en células neoplásicas de diferentes tumores malignos o

benignos. Además, se han descrito casos de expresión citoplasmática en fibromatosis infantil, miofibroma, miofibromatosis y lipofibromatosis. La diferente localización celular de WT1 nuclear, citoplásmica o núcleo-citoplasmática, en diferentes tumores benignos y malignos apoya la hipótesis de que este factor de transcripción juega un papel complejo en la tumorigénesis, probablemente como un funcionamiento proteína camaleón, ya sea como un gen supresor de tumor o un Oncogene, dependiendo del contexto celular (Magro et al., 2014). La expresión de WT1 a nivel cutáneo ha sido publicado en relación a proliferaciones vasculares, tumores y malformaciones, valorando la expresión citoplasmática en las células endoteliales. Dicha expresión es observada en relación con procesos de tipo tumoral y no con malformaciones, excluyendo la malformación arteriovenosa (Lawley et al., 2005; Trindade et al., 2011).

HIPÓTESIS

HIPOTESIS

Se desconoce en la piel normal que estructuras con células musculares lisas tienen capacidad contráctil. Mediante la aplicación de marcadores inmunohistoquímicos en la piel normal podemos establecer el fenotipo haciendo especial referencia al músculo erector del pelo, estructuras musculares de los vasos sanguíneos, y del cuerpo glómico. De esta manera, nos permitiría comparar el fenotipo muscular liso de la piel normal con su contrapartida tumoral y malformativa.

OBJETIVOS

OBJETIVOS GENERALES

1. Establecer la expresión en la piel normal y en aquellos procesos que reproduce a las estructuras cutáneas donde la esmotelina es positiva. Asímismo, comparar la expresión de la esmotelina con otros marcadores de diferenciación lisa.

OBJETIVOS ESPECÍFICOS

1. Describir el fenotipo muscular liso de la piel normal aplicando un panel de anticuerpos.

2. Valorar el perfil inmunohistoquímico de las malformaciones y de los tumores musculares lisos que permitan favorecer el diagnóstico diferencial.

3. Precisar las características inmunohistoquímicas de los tumores glómicos y malformaciones glomovenosas encaminadas a establecer diferencias.

4. Analizar problemas de diagnóstico y de terminología para ciertas lesiones cutáneas de fenotipo muscular liso.

5. Determinar la expresión inmunohistoquímica del componente muscular de los tumores y malformaciones vasculares que puedan ayudar a definir patrones diagnósticos.

6. Aplicar un panel inmunohistoquímico de anticuerpos en una lesión extra cutánea con especial referencia a la expresión de la isoforma corta (A) de la esmotelina en estructuras musculares lisas no vasculares.

MATERIAL, MÉTODOS Y RESULTADOS

MATERIAL, MÉTODOS Y RESULTADOS

El desarrollo de nuestros planteamientos nos ha permitido realizar los siguientes trabajos:

1. **Aneiros-Fernández J,** Husein-ElAhmed H, Arias-Santiago S, Campos A, Carriel V, Sánchez-Montesinos I, Garcia del Moral R, Sánchez G, O'Valle F, Aneiros J. Expression of smoothelin and smooth muscle actin in the skin. Histol Histopathol. 2011 Jun;26(6):673-8. Factor Impacto: 2.096. Quartil 2.

2. **Aneiros-Fernandez J,** Retamero JA, Husein-ElAhmed H, Carriel V, Ovalle F, Aneiros-Cachaza J. Smoothelin expression in skin leiomyomas and leiomiosarcomas. Histopathology. 2015 (En revisión).

3. **Aneiros-Fernández J,** Retamero JA, Husein-Elahmed H, Ovalle F, Aneiros-Cachaza J. Primary cutaneous and subcutaneous leiomiosarcomas: review of their evolution and prognostic factors. Eur J Dermatol. 2015 Aceptado 7 de Octubre (In press). Factor Impacto: 1.990. Quartil 2.

4. **Aneiros-Fernandez J**, Husein-ElAhmed H, Lopez Peña C, Lopez Caballero JJ, Ovalle F, Aneiros-Cachaza J. Dermal leiomiosarcoma with polypoid growth arising in a leiomyoma. Dermatopathol. 2015 (En revisión).

5. Espiñeira-Carmona MJ, **Aneiros-Fernández J**, Girón Prieto MS, Carriel V, Antonia, Fernandez M, Buendía-Eisman A, Campos A, Alaminos

Mingorance M, Arias-Santiago S. Smoothelin, a new marker for smooth muscle hamartoma. Eur J Dermatol. 2014 Jul-Aug;22(4):549-50.

Factor Impacto: 1.990. Quartil 2.

6. **Aneiros-Fernandez J**, Retamero JA, Husein-Elahmed H, Carriel V, Ovalle F, Aneiros-Cachaza J. Smoothelin and WT-1 Expresisión in glomus tumors and glomuvenous malformations. Histol Histopathol. 2015 Aceptado con revisión menor 29 de Octubre.

Factor Impact: 2.096. Quartil 2.

7. Carriel V, **Aneiros-Fernández J**, Ruyffelaert M, Arias-Santiago S, Riady V, Izquierdo-Martínez F, Roda O, Cornelissen M, Campos A, Alaminos M. Histological and immunohistochemical study of an unusual type of gallbladder duplication. Histol Histopathol. 2014 Jul;29(7):957-64.

Factor Impacto: 2.096. Quartil 2

8. **Aneiros-Fernandez J,** Nicolae A, Preda O. Smoothelin in bladder and gastrointestinal tract again. Histopathology. 2011 Jun;58(7):1173.

Factor Impacto; 3.453. Quartil 1.

9. **Aneiros-Fernández J,** Husein-ElAhmed H, Retamero JA, Carriel V, Aneiros-Cachaza J. Estudio inmunohistoquímico de marcadores de diferenciación muscular lisa en los tumores y malformaciones vasculares de la piel. Dermatology online Journal. (Revisión).

Expression of smoothelin
and smooth muscle actin in the skin

José Aneiros-Fernández[1], Husein Husein-ElAhmed[2],
Salvador Arias-Santiago[2], Antonio Campos[3], V. Carriel[3], Indalecio Sánchez-Montesinos[4],
Raimundo Garcia del Moral[1], Guillermo Sánchez[1], Francisco O'Valle[1] and Jose Aneiros[1]

[1]Department of Pathology, [2]Department of Dermatology, San Cecilio University Hospital, [3]Tissue Engineering Group, Department of
Histology and [4]Department of Human Anatomy and Embriology, Faculty of Medicine, University of Granada, Granada, Spain

Summary. Introduction: Smoothelin is a cytoskeletal
protein of differentiated smooth muscle cells with
contractile capacity, distinguishing it from other smooth
muscle proteins, such as smooth muscle actin (SMA).

Objective: To evaluate the expression of smoothelin
and SMA in the skin in order to establish specific
localizations of smoothelin in smooth muscle cells with
high contractile capacity and in the epithelial component
of cutaneous adnexal structures. Methods:
Immunohistochemical analysis (smoothelin and SMA)
was performed in 18 patients with normal skin.

Results: SMA was expressed by the vascular
structures of superficial, deep, intermediate and
adventitial plexuses, whereas smoothelin was
specifically expressed in the cytoplasm of smooth
muscle cells of the deepest vascular plexus and in no
other plexus of the dermis. The hair erector muscle
showed intense expression of smoothelin and SMA.
Cells with nuclear expression of smoothelin and
cytoplasmic expression of SMA were observed in the
outer root sheath of the inferior portion of the hair
follicles and intense cytoplasmic expression in cells of
the dermal sheath to SMA.

Conclusions: We report the first study of smoothelin
expression in normal skin, which differentiates the
superficial vascular plexus from the deep. The deep
plexus comprises vessels with high contractile capacity,
which is important for understanding dermal
hemodynamics in normal skin and pathological
processes. We suggest that the function of smoothelin in
the outer root sheath may be to enhance the function of
SMA, which has been related to mechanical stress.

Smoothelin has not been studied in cutaneous pathology;
however we believe it may be a marker specific for the
diagnosis of leiomyomas and leiomyosarcomas of the
skin. Also, smoothelin could differentiate arteriovenous
malformations of cavernous hemangioma of the skin.

Key words: Immunohistochemical, Smoothelin, Smooth
muscle actin, Hair follicle, Vessels

Introduction

Smoothelin has two tissue-specific isoforms: the
short 59-kDa isoform (A), found in smooth muscle cells
of the organs; and the long 110-kDa isoform (B), in
smooth muscle cells of vascular structures (Krämer et
al., 2001; Rensen et al., 2002). Smoothelin contains an
actin-binding domain and is considered to be a
cytoskeleton protein exclusively expressed by
differentiated smooth muscle cells with contractile
capacity (Krämer et al., 1999, 2001).

Different grades of differentiation of muscle cells in
vessels are determined by expression of phenotypic
features such as smoothelin, myosin and actin of smooth
muscle. Depending on the grade of differentiation of
these cells, vessels have an important role in physiologic
processes, such as thermoregulation with vaso-
constriction and vasodilatation as a response to
environmental temperature changes, and in
physiopathologic processes such as atherogenesis and
restenosis after angioplasty and other surgical techniques
(Holifield et al., 1996; Hungerford and Little, 1999), as
well as Raynaud phenomenon and erythromelalgia: two
cutaneous microvascular disorders whose
pathophysiological features are poorly understood
(Greenstein et al., 1995; Davis et al., 2000).

Immunohistochemical studies have detected smoothelin expression in smooth muscle cells of the esophagus, stomach, gut, prostate, uterus, and bladder, and in leiomyoma, leiomyosarcomas, and gastro-intestinal stromal tumors (Van der Loop et al., 1996; Council and Hameed, 2000; Niessen et al., 2005; Wedel et al., 2006; Amiot et al., 2009; Coco et al., 2009; Bovio et al., 2010). We have found only one published study on the cell localization of smoothelin expression, which evaluated its cytoplasmatic and nuclear expression in normal and tumor smooth muscle cells (Coco et al., 2009).

The objective of this study was to investigate the expression of smoothelin and smooth muscle actin (SMA) in the skin in order to establish specific localizations of smoothelin in smooth muscle cells with high contractile capacity and in the epithelial component of cutaneous adnexal structures.

Materials and methods

Patient samples

We studied 18 biopsies of normal skin that included the epidermis, dermis, and part of the hypodermis. They derived from flaps removed for grafting purposes from the limb or scalp of 10 females and 8 males aged between 25 and 48 yrs. Written informed consent was
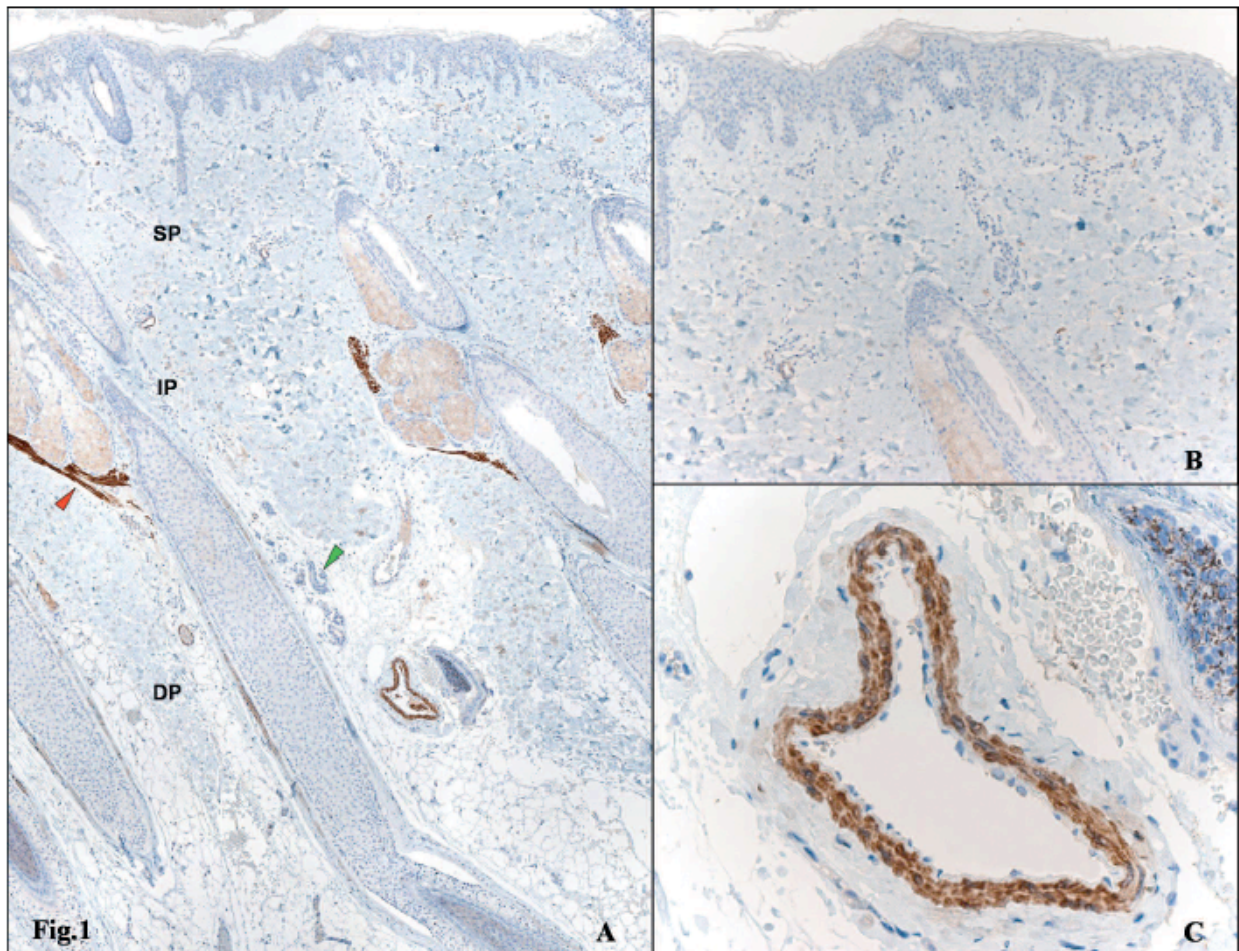
Fig. 1. A. Smoothelin expression is positive only in the vessel walls of deep plexus (DP), with no expression in the superficial plexus (SP). Some vessels of the intermediate plexus (IP) are also positive for smoothelin. Intense expression is found on hair erector muscle (Red arrow), however ecrine sweat glands shows no expression (Green arrow). **B.** On higher magnification, vessels of SP are negative for smoothelin. **C.** Vessel walls of DP are positive for smoothelin. A, x 100; B, x 200; C, x 400

obtained from all subjects.

Immunohistochemical analysis

Samples were fixed in 10% buffered formalin for 24 hrs and embedded in paraffin. Paraffin-embedded 4-μm sections were dewaxed, hydrated, and heat-treated at 95°C for 20 min in 1 mM EDTA buffer pH 8 for antigenic unmasking. Sections were incubated for 30 min at room temperature with smoothelin (prediluted monoclonal antibody, clone R4A) or SMA (prediluted clone 1A4) (Master Diagnóstica, Granada, Spain). The immunohistochemical study was done on an automatic immunostainer (Autostainer 480, LabVision Fremont, CA) by indirect polymer-peroxidase-based method followed by development with diaminobenzidine (Masvision, Master Diagnóstica). The intensity of cytoplasmic and/or nuclear expression was graded as weak, moderate, or strong.

Results

Smoothelin and SMA expression in the vascular structures of the dermis and dermal-hypodermal interface, comprising superficial, deep, intermediate (communicating vessels), and adventitial plexuses

All biopsies showed intense cytoplasmic expression of smoothelin in smooth muscle cells of arteries and moderate cytoplasmic expression in veins of the deep vascular plexus at the dermal-hypodermal interface. No expression was found in superficial or adventitial plexuses (Fig. 1). All biopsies showed intense SMA expression in the vessel walls of deep, intermediate, superficial, and adventitial plexuses (Fig. 2).
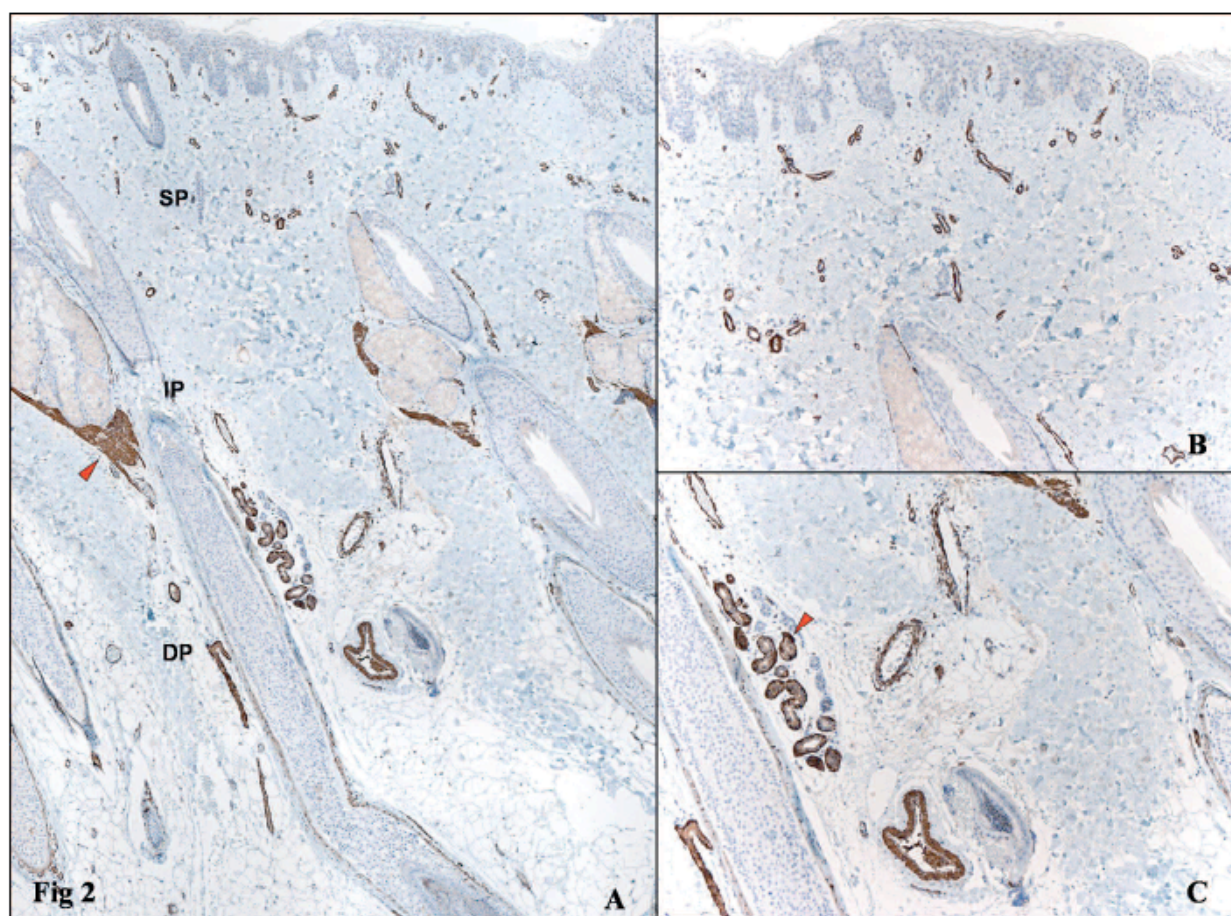
Fig. 2. A. Smooth muscle actin expression is noted in the vessel walls of deep plexus (DP), intermediate plexus (IP) and superficial plexus (SP). Intense expression is found on hair erector muscle (Arrows). **B.** On higher magnification, IP and SP show intense expression for smooth muscle actin. **C.** Vessels on the DP are positive for smooth muscle actin (Arrows). A, x 100; B, C, x 200
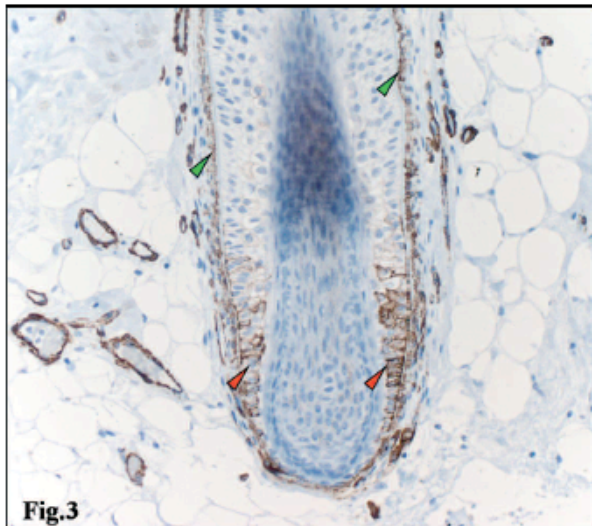
Fig. 3. Smooth muscle actin expression is positive in the dermal sheath (green arrows) and in the bottom area of the outer radicular sheath (red arrows). x 200

Fig. 4. Nuclear expression of smoothelin is noted in the cells of the outer radicular sheath (red arrows). x 400

Smoothelin and SMA expression in hair erector muscle
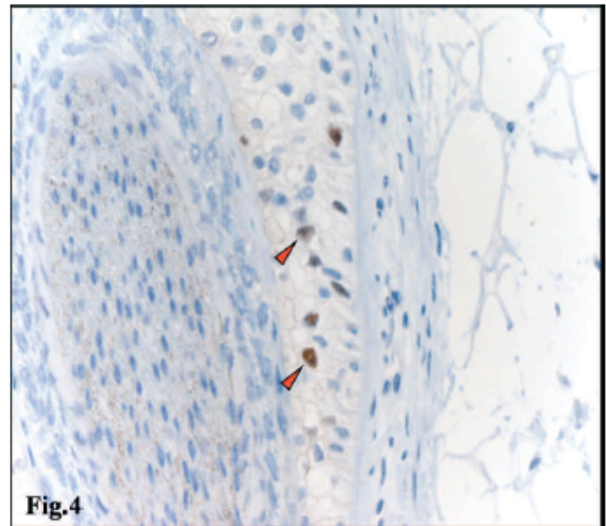
All biopsies showed intense cytoplasmic expression of smoothelin and SMA in smooth muscle cells of the hair erector muscle (Figs. 1A, 2A).

Smoothelin and SMA expression in hair follicle

All biopsies showed moderate cytoplasmic expression of SMA in cells of the inferior portion of the outer root sheath and intense cytoplasmic expression in cells of the dermal sheath (Fig. 3).

An intense nuclear expression of smoothelin was found in some cells of the inferior portion of the outer root sheath of all hair follicles in anagen (Fig. 4), while no expression was observed in the remaining layers of the hair structure.

Smoothelin and SMA expression in sweat glands (eccrine and apocrine) and sebaceous glands

No smoothelin expression was detected in sweat or sebaceous glands (Fig. 1A).

In all biopsies, an intense cytoplasmic expression of SMA was found in myoepithelial cells of the secretory portion of eccrine and apocrine glands (Fig. 2A), while no SMA expression was detected in the excretory portion or sebaceous glands.

No smoothelin or SMA expression was detected in other components, such as epidermis, dermal fibroblasts, hypodermal adipocytes, or nerve fibers.

Discussion

This study of normal skin biopsies found a higher expression of smoothelin in cells of the hair erector muscle than in muscle cells of deep plexus veins, reflecting the elevated contractile capacity of the hair erector muscle and suggesting that it may serve as a good positive control in smoothelin assays. An important finding was that SMA was identified all vascular structures of the dermal plexuses, but smoothelin expression allowed differentiation of the deep vascular plexus from the other structures, which may facilitate research into the homodynamic of the cutaneous structure normal and pathological dermatological processes, such as dermatitis, stasis, alterations caused by heat and cold which can present vasodilation and / or vasoconstriction. We also believe that smoothelin may help to understand the pathophysiological mechanisms involved in the different stages of rosacea. Thus the assessed value of smoothelin could help explain the vascular changes that occur in different processes in rosacea. Most blood vessels in the middle and superficial dermis have no substantial contractile capacity and showed no smoothelin expression.

Smoothelin expression in cutaneous vascular tumours has not been studied previously, although there is a work about the differential expression of smoothelin in brain vascular lesions, in which a positive expression in arteriovenous malformations and a negative expression in cavernous hemangioma were demonstrated (Uranishi et al., 2001). Since smoothelin is expressed in

well-differenciated muscle cells with contractile capacity from the deep vascular plexus, we think this expression can be used to distinguish arteriovenous malformations which consist of malformed vessels with thick walls, and muscle cells with contractile capacity which are smoothelin-positive. However, no well-differenciated muscle cells can be found in cavernous hemangioma, therefore these lesions are negative for smoothelin.

SMA expression in cells of the hair follicle dermal sheath cells has been implicated in contractile processes that may control hair follicle shortening (Thibaut et al., 2005), and play a role in curly hair follicle morphology. Cytoplasmic expression of SMA in cells of the outer root sheath has been related to stress mechanisms (Baltenneck et al., 2000; Thibaut et al., 2005). We report for the first time the nuclear expression of smoothelin in some cells of the outer root sheath. The only published report on the nuclear and cytoplasmic expression of smoothelin in (normal and tumor) smooth muscle cells offered no explanation of its nuclear expression (Coco et al., 2009). The nuclear expression of smoothelin in the hair outer root sheath can be helpful in the diagnosis of skin adnexal tumors whose origin is the outer root sheath, such as trichilemmoma and inverted follicular keratosis (Kurokawa et al., 2003).

Smoothelin isoforms are homologous with other cytoskeletal smooth muscle proteins and contain an actin-binding domain. Treatment of cells with α-amanitin induced the formation of an actin bundle network in the nucleus (Baltenneck et al., 2000; Zhu et al., 2004), and it is known that some types of stress (e.g., heart shock and dimethylsulfoxide treatment) can induce the nuclear translocation of actin in various eukaryotic cells (Iida et al., 1992; Wada et al., 1998). Hence, the nuclear expression of smoothelin may be attributable to a translocation mechanism, especially in tumor disease (e.g., leiomyosarcoma, lymphoma) (Abd et al., 2007; Coco et al., 2009). Based on these data, it can be proposed that smoothelin may act to enhance the role of SMA in the hair follicle.

In the present study, the nuclear expression of smoothelin and the cytoplasmic expression of SMA in epithelial cells of the hair follicle outer root sheath may be related to multipotential cell elements, which would explain the expression of the two muscle markers. It has long been known that the multipotent capacity of epithelial cells of the follicle outer root sheath affords them a critical role in the regeneration of damaged interfollicular epidermis (Jahoda et al., 1993), explaining the expression of a muscle marker in the outer epithelial sheath of the follicle. Likewise, epithelial-mesenchymal interaction is essential for hair follicle development (Tobin et al., 2003a,b). Various studies have reported the multipotent capacity of epithelial and mesenchymal cells, demonstrating that cells of the dermal papilla and outer hair follicle dermal sheath can differentiate into adipocytes and express bone differentiation markers such as alkaline phosphatase (Jahoda et al., 2003; McElwee et al., 2003). Multipotent stem cells have been

described in the outer root sheath (Webb et al., 2004; Raposio et al., 2007), which under certain circumstances may give rise to epithelial cells that express smoothelin and SMA.

Smoothelin is expressed in non-cutaneous muscle tumors (Coco et al., 2009), and in our study the hair erector muscle and the walls of vessels from the deep vascular plexus are smoothelin-positive, therefore this expression can be implemented in the diagnosis of benign and malignant muscle tumors of the skin, e.g. leiomyoma and leiomyosarcomas.

This is the first report of the positive expression of smoothelin in the outer root sheath of the hair follicle and its possible functional link to SMA. Moreover, smoothelin allows the deep vascular plexus to be distinguished from remaining vascular structures of the dermis.

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Histological and immunohistochemical study of an unusual type of gallbladder duplication

Víctor Carriel[1,2,3], José Aneiros-Fernández[4], Muriel Ruyffelaert[5], Salvador Arias-Santiago[1,6,7], Vadir Riady[3], Francisco Izquierdo-Martínez[3], Olga Roda[8], Maria Cornelissen[2], Antonio Campos[1] and Miguel Alaminos[1]

[1]Department of Histology (Tissue Engineering Group), University of Granada, Granada, Spain, [2]Department of Basic Medical Sciences, Faculty of Medicine, Ghent University, Ghent, Belgium, [3]Pathology Unit, San Camilo Hospital, University of Valparaíso, San Felipe, Chile, [4]Department of Pathology, University of Granada, Granada, Spain, [5]Preventive Youth Health Care, Center for Student Care of the Flemish Community Commission, Brussels, Belgium, [6] San Cecilio University Hospital, Granada, Spain, [7]Baza General Hospital, Granada, Spain and [8]Department of Human Anatomy and Embryology, University of Granada, Spain

Summary. Gallbladder duplication is a rare congenital anomaly, with an incidence of 1 in 3,800 autopsies. The correct diagnosis and treatment of this type of entity is important in clinical practice, because it may cause some clinical and surgical problems. In this report, we present the clinical case of a 28-year-old female with abdominal pain. Ultrasound of the upper abdomen showed a distended gallbladder with the presence of a septum that could suggest a congenital anomaly of the extrahepatic biliary system. During surgery, a distended and inflamed gallbladder with a lithiasis was found. In addition, a complete septum and double cystic duct were observed. The gross and histopathological evaluation of the surgical specimen allowed us to confirm the diagnosis of a Y- shaped type gallbladder duplication according to Boyden's classification. In conclusion, in presence of an atypical imaging of the gallbladder, diagnosis of this group of congenital anomalies should be considered in order to adequately plan surgical intervention if necessary.

Key words: Gallbladder duplication, Cholelithiasis, Cholecystectomy, Muscular differentiation, Immuno-histochemistry

Offprint requests to: Dr. Víctor Carriel, Department of Histology (Tissue Engineering Group), University of Granada, Avenida de Madrid 11, 18012 Granada, Spain, e-mail: vcarriel@ugr.es

Introduction

The gallbladder is one of the most common surgical specimens in pathology. The layers of the gallbladder include mucosa (surface epithelium and lamina propria), smooth muscle, perimuscular or subserosal connective tissue, and serosa. The muscularis mucosae and submucosa are not present (Mills, 2007). The structure and function of the gallbladder can be affected by several pathological conditions and congenital abnormalities. Duplication of the gallbladder is one of the most rare congenital anomalies, having an incidence of 1 in 3,800 autopsies (Boyden, 1926). The variable anatomy of this organ is well documented (Boyden, 1926; Harlaftis et al., 1977; Lamah et al., 2001; Singh et al., 2006; Causey et al., 2010). The duplication occurs because of outpouchings from the normal extrahepatic biliary system during the fifth and sixth week of gestation. These outpouchings typically regress; however, their persistence may result in the formation of an accessory gallbladder (Harlaftis et al., 1977; Causey et al., 2010).

Gallbladder duplication does not cause specific symptoms, and surgical treatment is indicated only when patients become symptomatic (Silvis et al., 1996; Khandelwal et al., 2010). Despite the advances in diagnostic techniques, a gallbladder duplication may be discovered during surgery or may even be missed intra-operatively, particularly when it has an intra-hepatic location (Singh et al., 2006). Preoperative diagnosis of this type of anomaly is especially important to prevent

possible surgical complications and second interventions (Singh et al., 2006; Hekimoglu et al., 2010). Several entities should be considered in the differential diagnosis, including folded gallbladder, choledochal cyst, Phrygian cap, pericholecystic fluid, gallbladder diverticulum, bilobed gallbladder and focal adenomyomatosis (Singh et al., 2006; Hekimoglu et al., 2010).

In this study, we report an incidental preoperative diagnosis, treatment and histopathological evaluation of one case of true Y-shaped gallbladder duplication and we discuss the diagnostic alternatives of this group of congenital anomalies from a morphological and histological standpoint.

Materials and methods

Case history

A 28-year-old woman with occasional abdominal pain for three days reported to the emergency department of the San Camilo Hospital (University of Valparaíso, San Felipe, Chile). The pain she experienced was associated with the intake of fatty food. The patient described right upper quadrant abdominal pain associated with bilious vomiting. She did not report to have had fever, diarrhea or any other symptoms.

Abdominal palpation was painful and Murphy's sign was negative. Sodium metamizole and meperidine were prescribed. However, the patient did not respond to this treatment. Extensive laboratory testing and abdominal ultrasound were performed. The results of the laboratory testing, including full blood cell count, coagulation test, C-reactive protein and biochemistry panel test were within normal limits. Ultrasound of the upper abdomen showed a distended gallbladder with the presence of a septum that could suggest congenital anomaly of the extrahepatic biliary system. In addition, a single calculus with a diameter of 1.8 cm was identified in one of the lumens (Fig. 1).

Due to the intensity of the abdominal pain and its resistance to treatment, an open cholecystectomy was performed. During the open surgery, a distended gallbladder with signs of inflammation, the presence of an external constriction along the organ and two cystic ducts with one unique cystic artery were identified. Both cystic ducts converged into the common hepatic duct forming the common bile duct. A calculus with a diameter of 1.8 cm was observed and removed from the distended and inflamed gallbladder. The surgical specimen was referred to the pathology unit for gross and both histological and immunohistochemical analysis.

Procedures

After surgery, the surgical specimen of the duplicated gallbladder was routinely fixed in 10% neutral buffered formalin. Subsequently, gross analysis

was performed and the duplicated gallbladder was sectioned transversally from the fundus to the neck (cystic area) to realize a complete histological evaluation. All paraffin-embedded samples were cut in 5 μm thick sections for the histological and immuno-histochemical analysis. The histopathological analysis was evaluated using haematoxylin-eosin and picrosirius stain at light microscopy.

The identification of the blood and nerve supply was determined by immunohistochemistry using the following antibodies: anti-laminin clone LAM-89 (Sigma-Aldrich, Steinheim, Germany), and prediluted anti-CD31 clone JC/ 70A (Master Diagnóstica, Granada, Spain).

The smooth muscle layer was evaluated by immunohistochemistry using the following muscular differentiation markers: prediluted anti-smooth muscle actin clone 1A4, prediluted anti-H caldesmon clone H-Cald, prediluted anti-desmin clone D33, prediluted anti-myosin clone SM-M10, and prediluted anti-smoothelin clone R4A (Master Diagnostica, Granada, Spain).

The immunohistochemical study of laminin was performed as previously described (Carriel et al., 2013). The antibodies CD 31 and all the muscular differentiation markers were performed using an automatic immunostainer (Autostainer 480, LabVision Fremont, CA) by an indirect polymer-peroxidase-based method followed by development with diamino-benzidine (Masvision, Master Diagnostica) as previously described (Aneiros-Fernandez et al., 2011).

Results

Gross and histopathological findings

Gross analysis of the surgical specimen revealed that

Fig. 1. Preoperative ultrasound image of the right upper abdominal quadrant. Note the septum (white arrow) that divides the gallbladder in two ovoid and anechoic structures, and a stone with a diameter of 1,8 cm.

PICROSIRIUS STAIN
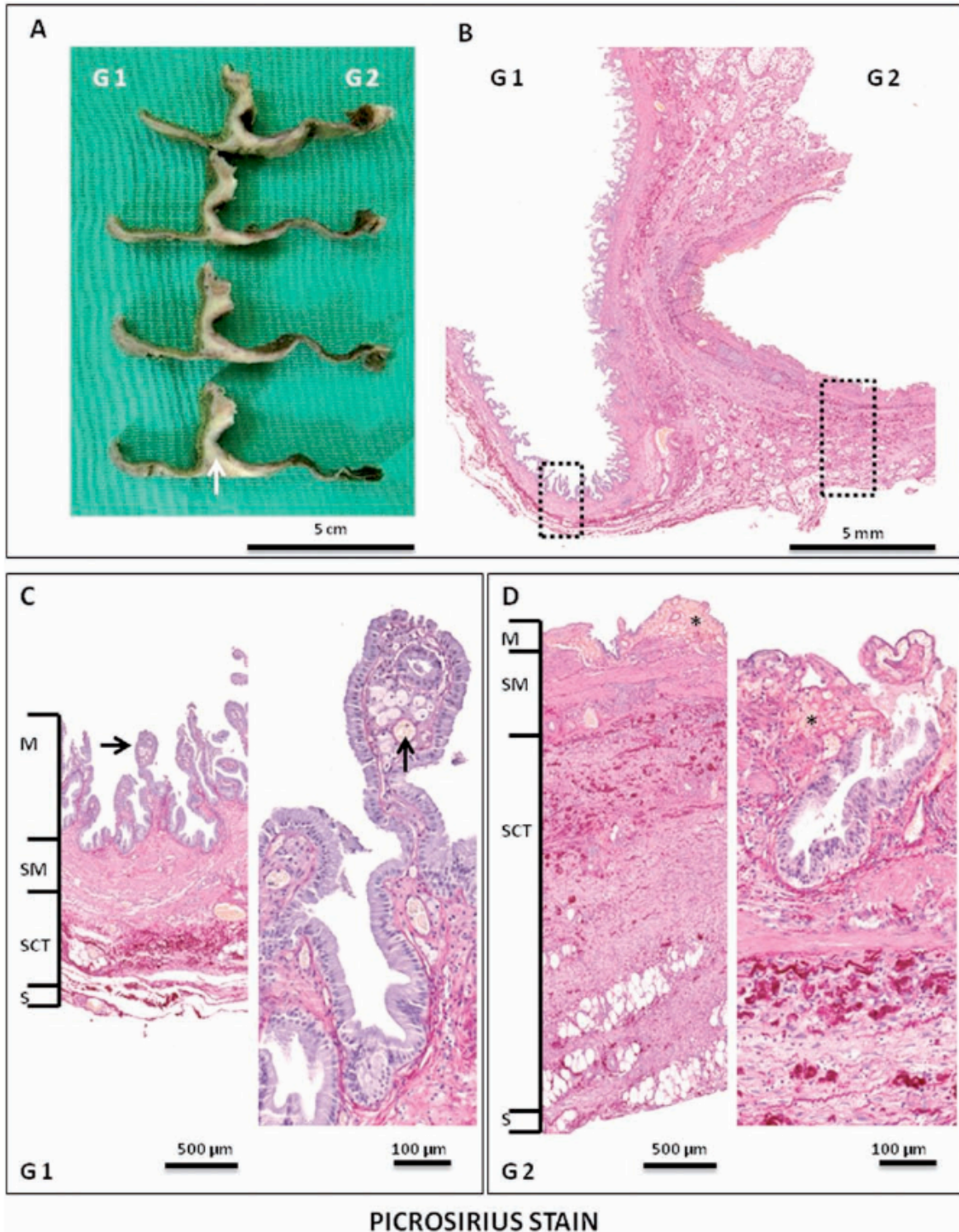
Fig. 2. A. Gross analysis of the surgical specimens, where it is possible to observe two partial gallbladders (G 1, G 2) separated by a complete septum (white arrow) coated at both surfaces by mucosa. **B.** Histological section of both gallbladders at low magnification. **C.** Histological analysis of the G 1, with evident cholesterolosis in the lamina propria (black arrow). **D.** Histological image of the G 2, where it is possible to observe the evident signs of inflammation and acute hemorrhage in the lamina propria (asterisk). M: mucosa; SM: smooth muscle; SCT: subserosal connective tissue; S: serosa.
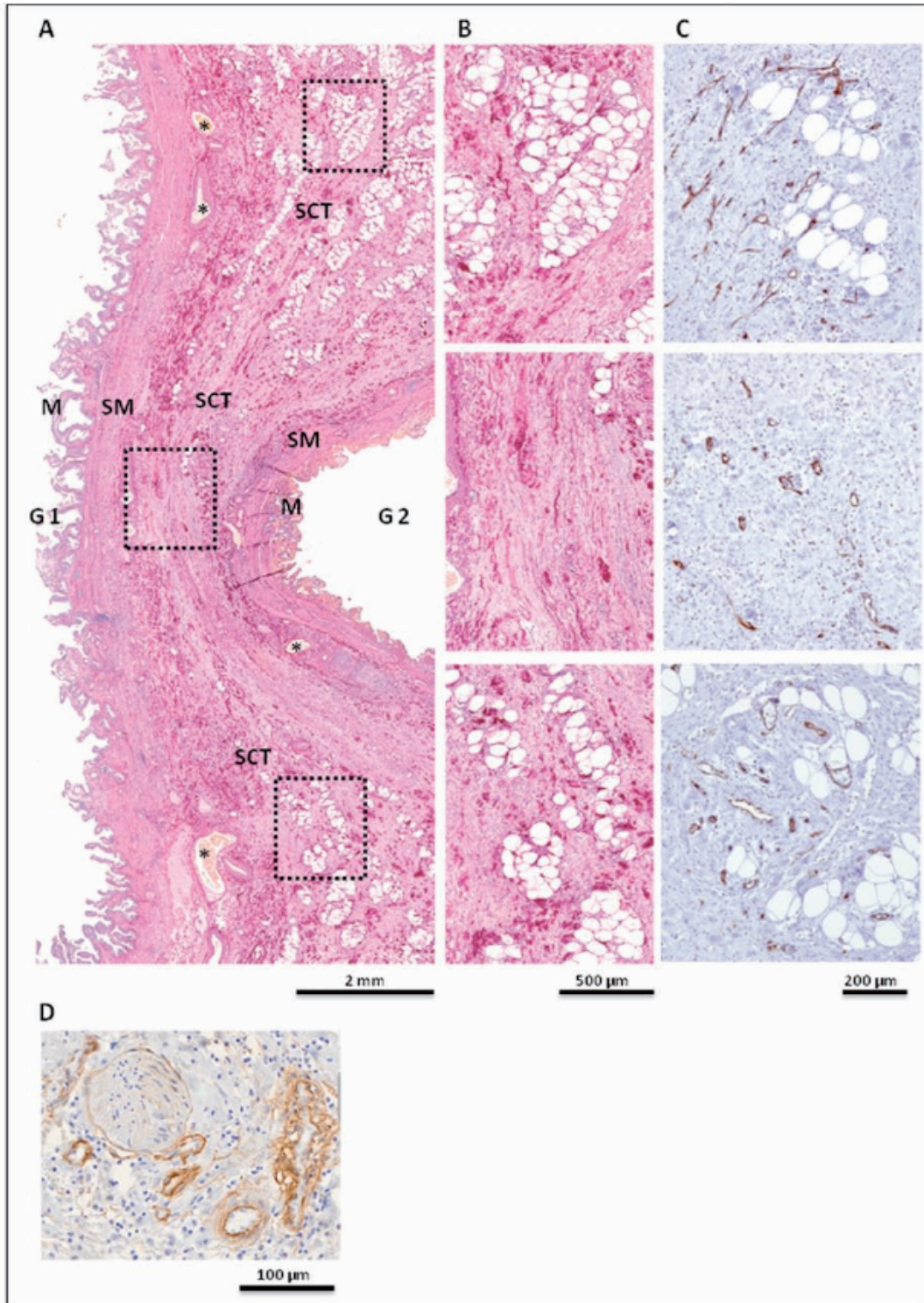
Fig. 3. Histological analysis of the longitudinal septum stained with picrosirius. A. Histological image at low magnification of the septum, note the independent histological layers of both gallbladders fused by the subserosal connective tissue (SCT). B. Magnification analysis of the SCT of the septum at different regions. C. Distribution of blood vessels in the SCT analyzed by CD31 immunohistochemistry. D. Laminin immunohistochemistry in the wall of blood vessels and peripheral nerves of the SCT. Asterisk: muscular arteries; M: mucosa; SM: smooth muscle; SCT: subserosal connective tissue; S: serosa.

the length of one gallbladder (G1) was 6 cm and that the width was 4 cm at the main perimeter. The second gallbladder (G2) had 7 cm of length and 4.5 cm of width at the main perimeter. Sectioned gallbladders showed the presence of a complete longitudinal septum coated by mucosa at both surfaces separating both gallbladders. In addition, two cystic ducts were identified. The G1 showed yellow spots on its mucosal surface (due to the accumulation of lipid in the lamina propria) without signs of inflammation. However, evident signs of inflammation in the mucosal surface were observed in the G2 (Fig. 2 A).

Histological analysis confirmed the gallbladder duplication with the presence of a septum coated by mucosa (Fig. 2B). The G1 showed a mucosa without signs of inflammation, and with the presence of macrophages with lipid content that confirms the diagnosis of cholesterolosis of the gallbladder (Fig. 2C). The histological analysis of G2 revealed the loss of epithelial surface, signs of a recent hemorrhage in the lamina propria and the presence of inflammatory infiltrate and edema. All of these findings confirm the histological diagnosis of chronic cholecystitis with acute hemorrhage of the G2 (Fig. 2D).

The histological study allows us to confirm that both gallbladders were completely independent, containing mucosa (surface epithelium and lamina propria), a

discontinuous layer of smooth muscle, perimuscular or subserosal connective tissue, and serosa. The histological evaluation of the longitudinal septum showed that the two gallbladders were fused only by the subserosal connective tissue rich in collagen fibers and adipose tissue (Fig. 3). In relation with the blood and nerve supply, we observed the presence of muscular arteries and nerves in the lamina propria and perimuscular connective tissue of each gallbladder, and we identified by immunohistochemistry a few small blood vessels and nerve branches in the shared subserosal connective tissue (Fig. 3C,D). We observed that the muscular layer of the G2 was hypertrophic and thicker than the muscular layer of the G1. However, the immunohistochemical analysis revealed that smooth muscle layers of both gallbladders were positive for all the markers of muscular differentiation confirming that both gallbladders had fully developed muscle layers with contractile function (Fig. 4).

Finally, gross and histopathological evaluation of the surgical specimen confirmed the diagnosis of unusual Y-shaped gallbladder duplication. This duplicated gallbladder had two independent cystic ducts which became confluent close to their union with the common bile duct (Y-shaped type), and both gallbladder bodies were fused lengthwise by the subserosal connective tissue.
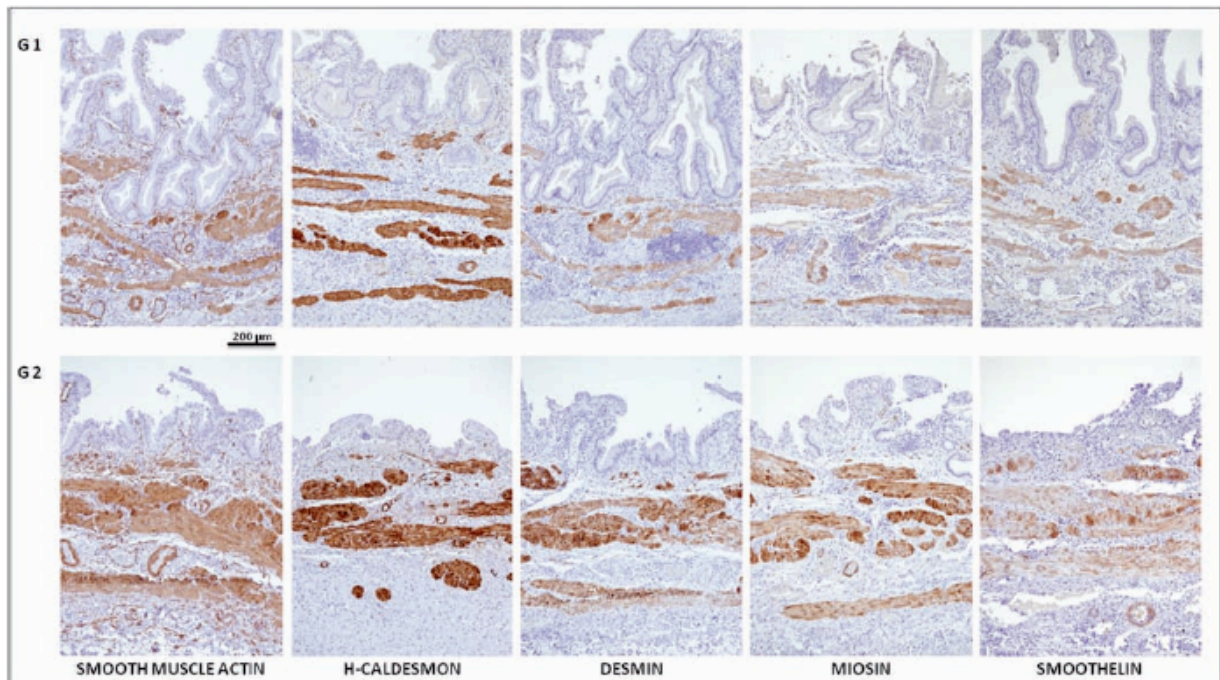

Fig. 4. Immunohistochemical analysis of the smooth muscle layers of the G1 and the G2.
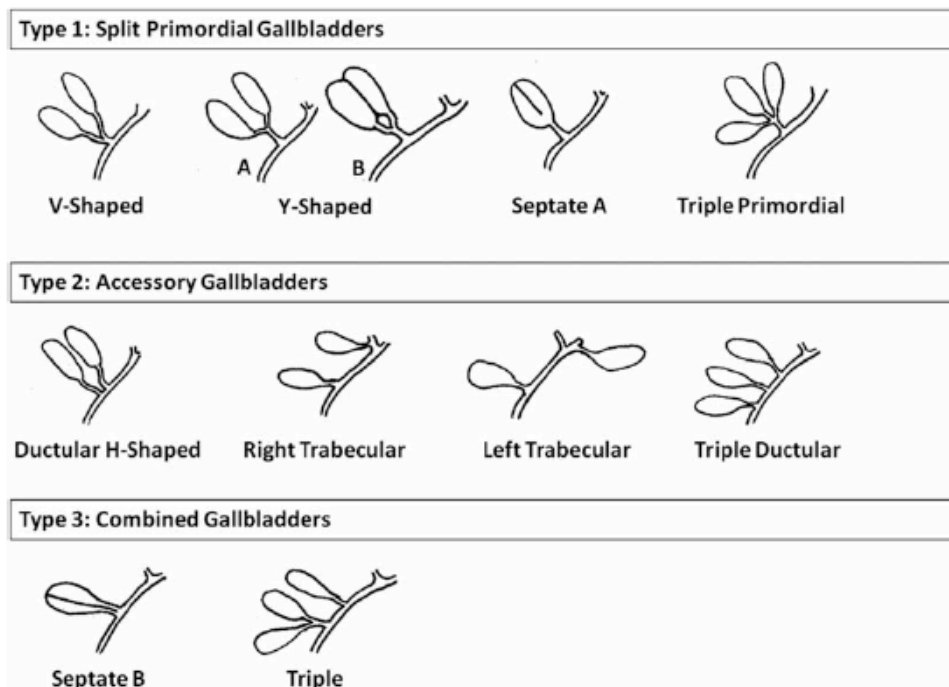
Discussion

Gallbladder duplication is an extremely rare congenital anomaly of the extrahepatic biliary system, and the earliest review of this group of anomalies was published by Boyden in 1926 (Boyden, 1926). In multiple gallbladder anatomy, each gallbladder must have valves at the neck, a tunica muscularis, and the capacity to concentrate bile (Causey et al., 2010).

Several classifications have been proposed according to anatomic or embryological development of the gallbladder (Boyden, 1926; Harlaftis et al., 1977; Causey et al., 2010). The first classification was performed by Boyden in 1926, and according to this classification, congenital abnormalities of the gallbladder include *"vesica fellea divisa"* or bilobed gallbladder and *"vesica fellea duplex"* or true gallbladder duplication (Boyden, 1926; Rabinovitch et al., 1958). The true duplication is subdivided into the Y-shaped type (two cystic ducts united before entering into the common bile duct, unusually both gallbladders are adherent and occupy the same fossa) and the H-shaped type or ductular type (two separated gallbladders and cystic ducts entering separately into the common bile duct). The accessory gallbladder of the ductular type can be localized in the gallbladder fossa, the intrahepatic region, the subhepatic region or within the gastrohepatic ligament (Khandelwal et al., 2010). True gallbladder duplication is more common and occurs due to bifurcation of the gallbladder primordium during the fifth and the sixth week of embryonic life (Khandelwal et al., 2010). Harlaftis et al. (1977) classified gallbladder duplication in two main groups based upon embryogenesis. These were the type 1 or split primordial group which included the septate gallbladder, the V-shaped and the Y-shaped gallbladders. When the cystic primordium splits during embryogenesis, both gallbladders share a common cystic duct. Type 1 septated duplicated gallbladder occurs when there is a single cystic duct and both gallbladders are separated by a septum. Type 2 describes accessory gallbladders that are ductular or trabecular, meaning that they arise from a separate primordium from the biliary tree and have individual cystic ducts (Harlaftis et al., 1977; Causey et al., 2010). Recently, Causey et al. proposed a unified classification of multiple gallbladders based on Harlaftis's classification. In this unified classification, the authors incorporate a third group called combined gallbladders (Causey et al., 2010).

The gross and histopathological evaluations of the surgical specimen demonstrate that both gallbladders were fully developed and only fused lengthwise by the subserosal connective tissue. In accordance with previous works, the positive expression of the muscular differentiation markers, especially smoothelin suggests that both gallbladders were functional with a contractile capacity of the smooth muscle layer (Raparia et al., 2010; Aneiros-Fernandez et al., 2011). The differences

Type 1: Split Primordial Gallbladders

V-Shaped Y-Shaped Septate A Triple Primordial

Type 2: Accessory Gallbladders

Ductular H-Shaped Right Trabecular Left Trabecular Triple Ductular

Type 3: Combined Gallbladders

Septate B Triple

Fig. 5. Schematic representation of the different types of gallbladder duplication (modified from Causey et al., 2010).

on the intensity of the markers of muscular differentiation could be explained due to the inflammation and muscular hypertrophy of the G2 associated to the presence of a calculus. The histological analysis allowed us to confirm that our patient had true Y-shaped gallbladder duplication according to the classical Boyden's classification. However, our case differs with the classification published by Singh, 2006, where the author considers a Y-shaped gallbladder as two completely separate gallbladders, with two Y-shaped cystic ducts (Harlaftis et al., 1977; Singh et al., 2006; Causey et al., 2010). Due to the fact that our case is a true Y-shaped gallbladder duplication with gallbladder bodies fused lengthwise by subserosal connective tissue, we suggest the incorporation of our case in the most recent unified classification described by Causey, and we classify our gallbladder duplication as type 1 Y-shaped B (with fused bodies) (Fig. 5).

Patients with gallbladder duplication usually do not have any specific symptoms, and this group of anomalies is rarely diagnosed in the preoperative period (Ozmen et al., 2003). For surgeons and radiologists, it is very important to recognize these anomalies as a possible confusing issue, and to prevent iatrogenic injuries during surgery (Causey et al., 2010).

The diagnosis of gallbladder duplication is difficult, and successful preoperative diagnosis is noted in only half of the cases (Kim et al., 2009). In this sense, preoperative imaging is crucial in the study of this group of anomalies. However, these imaging methods are limited by the type of aberrant anatomy (Causey et al., 2010). Ultrasonography is definitively the initial imaging modality that can help in the diagnosis of these gallbladder anomalies (Senecail et al., 2000). Nevertheless, Magnetic Resonance Cholangio-pancreatography (MRCP) has better diagnostic capability than ultrasound, and retrograde Cholangio-pancreatography is considered the gold standard for diagnosis of these types of anomalies (Kim et al., 2009; Causey et al., 2010). Finally, the definitive diagnosis of true gallbladder duplication could be established during open surgery when there is an evident type 2 or 3 gallbladder anomaly or after surgery during gross and histopathological analysis when there is a type 1 with fused bodies.

In relation with the treatment of gallbladder duplication, surgery should be the treatment of choice only in symptomatic patients (Silvis et al., 1996; Ozmen et al., 2003; Causey et al., 2010; Khandelwal et al., 2010; Walbolt and Lalezarzadeh, 2011). Overall, patients with aberrant anatomy are more likely to undergo open surgery or laparoscopic surgery (Desolneux et al., 2009; Causey et al., 2010). Some authors recommended open cholecystectomy for these patients, because an additional anatomical anomaly can exist (Silvis et al., 1996). Open surgery gives the opportunity to the surgeon to palpate and explore the entire gallbladder fossa and the adjacent area. This enables the surgeon to diagnose cases of congenital anomalies of the gallbladder (Singh

et al., 2006). Laparoscopic resection is a reasonable alternative and it is a very well described procedure in the literature (Ozmen et al., 2003; Kim et al., 2009; Causey et al., 2010; Khandelwal et al., 2010; Walbolt and Lalezarzadeh, 2011).

In general, the majority of authors suggest removing both gallbladders to avoid cholecystitis and symptomatic lithiasis in the remaining organ (Hekimoglu et al., 2010). The complete removal of gallbladders prevents a second surgical intervention in these patients (Silvis et al., 1996; Ozmen et al., 2003; Causey et al., 2010; Khandelwal et al., 2010; Walbolt and Lalezarzadeh, 2011). However, if its presence is not known before surgery, the second gallbladder could be missed during surgery, particularly when it has an intra-hepatic location (Gigot et al., 1997; Horattas, 1998; Hekimoglu et al., 2010).

In conclusion, gallbladder duplication is a rare and uncommon congenital anomaly of the gallbladder and extrahepatic biliary system, and knowledge about its existence is important in medical and surgical practice. With the presence of atypical imaging, preoperative diagnosis of this congenital anomaly should be considered in order to plan appropriate surgical intervention if necessary.

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Correspondence

Smoothelin in bladder and gastrointestinal tract again

Sir: We read with great interest the papers by Montani *et al.*[1] and Bovio *et al.*[2] on smoothelin expression in gastrointestinal tract and bladder, demonstrating its usefulness for distinguishing muscularis mucosae from the muscular propia layer. However, neither paper mentioned that, although smoothelin is a cytoplasmatic protein, its nuclear expression has been reported in smooth muscle cells of the gastrointestinal tract and in muscle tumours.[3]

The R4A clone used in both studies identifies two isoforms: smoothelin A for differentiated contractile smooth muscle cells of organs with muscle layers, and smoothelin B for those of blood vessels.[4] Figures in the papers show vascular structures with slight-to-moderate smoothelin positivity, i.e. structures with incomplete differentiation and contractile capacity. These findings, which were not reported by Montani *et al.*[1] and were noted only briefly by Bovio *et al.*,[2] warrant wider interpretation. In this regard, the use of smoothelin to evaluate vascular plexuses in the skin allowed our group to differentiate between superficial (smoothelin-negative) and deep (smoothelin-positive) vascular plexuses.[5] We believe that the determination of smoothelin expression would allow vascular plexuses in the bladder and gastrointestinal tract to be characterized.

Jose Aneiros-Fernandez
Alina Nicolae
Ovidiu Preda

Department of Pathology, University Hospital, Granada, Spain

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Human epidermal growth factor receptor 2 overexpression and amplification in mucinous tumours of ovary

Sir: Han *et al.*[1] reported on human epidermal growth factor receptor 2 (HER2) amplification and overexpression in four mucinous carcinomas of the ovary. We would like to draw your readers' attention to our study from 2009, in which we examined HER2 protein expression and gene amplification in 33 mucinous carcinomas and 16 mucinous borderline tumours of the ovary, finding overexpression/amplification in 18% of these cases (a frequency similar to that seen in adenocarcinomas of the gastro-oesophageal junction).[2] HER2 overexpression/amplification was not of prognostic significance in this series, but because of the small sample size we are now studying a larger series of cases. In this study we saw a clinical response to trastuzumab therapy in a patient with platinum-resistant disease, and another patient experienced an isolated cerebral recurrence while on trastuzumab therapy (an unusual pattern of recurrence also seen in HER2-positive breast cancer and thought to be due to poor penetration of the blood–brain barrier by trastuzumab, which is only effective against the disease outside the central nervous system[3]). Thus, targeted therapy against HER2 in mucinous carcinomas of the ovary is very much an option in the minority of patients who have HER2 overexpression/amplification, although it should be noted that most patients with mucinous carcinoma of the ovary will have stage Ia disease and have such a favourable prognosis that no adjuvant therapy is indicated.[4]

Cyril B Gilks[1,2]
Jessica McAlpine[3]

[1]*Department of Pathology, Vancouver General Hospital, Vancouver,*
[2]*Laboratory Medicine, Vancouver General Hospital and University of British Columbia, Vancouver, and*
[3]*Department of Obstetrics and Gynecology, Vancouver General Hospital and University of British Columbia, Vancouver, BC, Canada*

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Smoothelin, a new marker for smooth muscle hamartoma

Smoothelin is a cytoskeleton protein of differentiated smooth muscle cells with contractile capacity, distinguishing it from other smooth muscle proteins, such as smooth muscle actin (SMA). Smoothelin has two tissue-specific isoforms: the short 59-kDa isoform (A), found in smooth muscle cells of the organs; and the long 110-kDa isoform (B), in smooth muscle cells of vascular structures [1, 2]. Smoothelin has not been studied in cutaneous pathology; however, it could behave as a specific marker for the diagnosis of congenital smooth muscle hamartoma.

A 9-year-old healthy girl was referred for a localized pigmented lesion in the submandibular region, present at birth. Physical examination revealed a 6 × 4 cm plaque of uniform brown color with well defined borders and increased hair growth on the surface (*figure 1A- B*). The dermatoscopic examination showed areas of homogenous brown pigment with hypopigmented dots without pigmented network. Histological examination (*figures 1C-F*) showed variably-oriented small bundles of smooth muscle in superficial and middle portions of the reticular dermis, separated by bands of normal collagen. The epidermis was middle hyperplastic with acanthosis, papillomatosis and basal layer hyperpigmentation. Immunohistochemistry was performed with smooth muscle actin (clone 1A4, Master Diagnostica, Granada, Spain) and smoothelin (clone R4A, Master Diagnostica, Granada, Spain); both were positive, smoothelin was more specific for the detection of smooth muscle fibers in the hamartoma (*figures 1C-F*).

Smooth muscle hamartoma (SMH) is a rare hyperplasia of piloerector skin muscles within the reticular dermis [3]. It has a more frequent congenital variant (CSMH) and an acquired variant (ASMH). The most common presentation of CSMH is a slightly hyperpigmented plaque, usually located on the trunk, especially in the lumbosacral area and proximal extremities [4]. The friction of the plaque can cause transient piloerection or induration, known as the pseudo-Darier sign [5].

Clinical diagnosis is difficult, although it should be suspected in any congenital lesion with hypertrichosis, especially on the lower back [3]. In congenital localized forms the clinical differential diagnosis includes dysraphism of the spine and connective or elastic tissue hamartoma. Pigmented forms should be distinguished from melanocytic nevi and *café au lait* spots. For ASMH, Becker nevus, especially in case of hypertrichosis and onset in adolescence, should be considered.

Histological examination is essential for the diagnosis and it is characterized by markedly increased bundles of smooth muscle fibers in the reticular and deep dermis, not necessarily attached to hair follicles. Histochemical study with Masson trichrome and immunohistochemical stains with specific smooth muscle actin or desmin are used to confirm the nature of the smooth muscle proliferation.

However, these histological findings are not specific, since smooth muscle hyperplasia can be found in Becker's nevus. Several authors consider SMH and Becker's nevus as part of a spectrum of hamartomatous lesions showing increased smooth muscle cells with hypertrichosis and hyperpigmentation. On the one hand SMH, with mesenchymal predominance and, on the other, Becker nevus with epidermal predominance. Other authors consider them as independent entities [6].

Here, we highlight the use of smoothelin for the diagnosis of SMH. The hair erector muscle and vessel walls of deep vascular plexus are smoothelin-positive, so this expression can be applied in the diagnosis of benign and malignant muscle tumors of the skin, such as smooth muscle hamartoma.

In conclusion, although smoothelin presents a more specific immunohistochemical marker for the diagnosis of smooth muscle hamartoma than smooth muscle actin, improving its ability to detect subtle lesions and distinguish other structures that are positive for smooth muscle actin, such
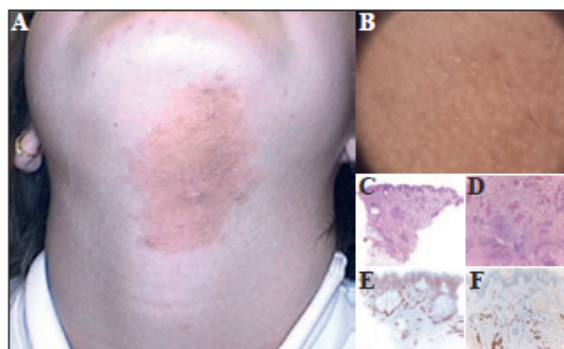
Figure 1. A) Pigmented lesion in the submandibular region with hypertrichosis. **B)** The dermatoscopic examination showed areas of homogenous brown pigment with hypopigmented dots without a pigmented network. **C, D)** Variable oriented small bundles of smooth muscle in superficial and middle portions of the reticular dermis separated by bands of normal collagen (H&E). **E, F)** Smooth muscle actin (E) and smoothelin (F) were positive, but smoothelin was more specific for the detection of smooth muscle fibers in the hamartoma.

as myofibroblasts or superficial vessels, several cases of smooth muscle tumors need to be comparatively studied for the expression of both smoothelin and SMA. ∎

[1] Dermatology Unit,
[2] Pathology Unit,
San Cecilio University Hospital,
Av Dr. Oloriz 16,
Granada 18012, Spain
[3] Metropolitan District, Granada,
Spain
[4] Histology Department,
School of Medicine, Granada, Spain
[5] Dermatology Unit,
Baza General Hospital,
Baza, Spain
<salvadorarias@hotmail.es>

María Jose ESPIÑEIRA-
CARMONA[1]
José ANEIROS-
FERNÁNDEZ[2]
María-Sierra GIRÓN
PRIETO[3]
Victor CARRIEL[4]
María ANTONIA
FERNANDEZ[1]
Augustín BUENDÍA-
EISMAN[5]
Antonio CAMPOS[4]
Miguel ALAMINOS
MINGORANCE[4]
Salvador ARIAS-
SANTIAGO[1,4,5]

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Sarcoidosis associated with Kaposi's sarcoma: description of four cases

Sarcoidosis is a granulomatous disease that affects mainly the lungs and skin [1]. Kaposi's sarcoma (KS) is a proliferation of endothelial precursor cells driven toward a lymphatic lineage by human herpes virus 8 (HHV8). We report 4 patients who had concomitant KS and sarcoidosis or sarcoidosis granulomas, an uncommon association, with only 6 cases reported in the literature [1-4].

Case 1
A 47-year-old man, born in the Congo, seronegative for HIV, presented facial papules for 4 years and an ulcerated lesion on the fourth toe for one year. A toe skin biopsy showed a vascular proliferation with HHV8 positivity, consistent with KS. The neck lesion histology showed an epithelioid granuloma without signs of
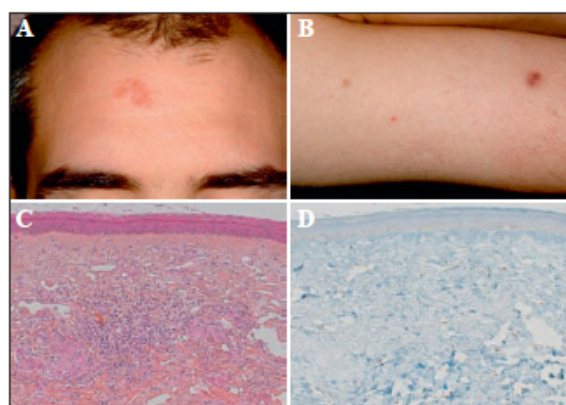
Figure 1. Patient 2.
A) Violaceous macular and lupoid lesion of the forehead histologically consistent with sarcoidosis. B) Purple lesion on the left arm with histological features of Kaposi's sarcoma.
Patient 4
C) Histology of purple papules of the inguinal region. Epithelioid granulomas without necrosis, and proliferation of lymphatic-like vessels in the superficial dermis forming cuffs (Hematoxylin and Eosin staining. Original magnification ×200). D) HHV8 positivity at immunostaining (Original magnification ×200).

mycobacterial infection, consistent with sarcoidosis. Polyclonal hypergammaglobulinemia and CD4 lymphopenia (154/mm^3) was noted. Chest radiography showed hilar noncompressive lymph node enlargement. Bone radiography showed condensing lesions at the iliac crest and rachis. Bone histology showed sarcoidosis. No specific treatment was prescribed for KS. The sarcoidosis facial skin lesions were treated with clobetasol 0.05% cream with partial improvement.

Case 2
A 31-year-old Caucasian man, seronegative for HIV, presented with a purple macular lesion on the forehead and skin papules on the arms. He had a history of sarcoidosis with rhinitis and pulmonary stage II involvement, treated with prednisone 1 mg/kg/day (stopped nine years ago). The forehead macular lesion biopsy (*figure 1A*) showed sarcoidosis. Ziehl staining was negative. The left arm erythematous and purple lesion biopsy showed KS with HHV8 positivity. A new biopsy of a purple patch (*figure 1B*) of the right arm showed sarcoidosis. Clobetasol cream 0.05% associated with hydroxychloroquine was started for the forehead lesion with partial improvement.

Case 3
A 61-year-old man, born in Morocco and seronegative for HIV, had a 4-year history of skin KS (immunostaining positive for HHV8), with copper-colored patches on the thighs and the right knee. A year ago, a lesion appeared on the hand, for which biopsy confirmed KS. Non-compressive mediastinal lymph node biopsies showed sarcoidosis. Mycobacterial culture and QuantiFERON® tuberculosis tests were negative. KS and sarcoidosis required no specific treatment.

Case 4
A 70-year-old man, born in Morocco and seronegative for HIV, presented papules on the left thigh, which spread to the

SMOOTHELIN EXPRESSION IN SKIN LEIOMYOMAS AND LEIOMYOSARCOMAS

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TITLE: SMOOTHELIN EXPRESSION IN SKIN LEIOMYOMAS AND LEIOMYOSARCOMAS

Running head: smoothelin in skin leiomyomas and leiomyosarcomas

Key words: WT-1; Smoothelin; leiomyoma; leiomyosarcomas; immunohistochemical; skin.

Abbreviations: LM, leiomyoma; LMS, leiomyosarcoma; SMA. alfa-smooth muscle actin: SMM, smooth muscle myosin; HPF, high-power field; FNCLCC, Federation Nationale des Centres de Lutte Contre le Cancer

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Authors:

Jose Aneiros-Fernandez

Juan Antonio Retamero

Husein Husein-ElAhmed [a]

Victor Carriel [b]

Francisco O'Valle [c]

Jose Aneiros-Cachaza [c]

Department of Pathology. Complejo Universitario de Granada. Granada. Spain.

[a]Department of Dermatology. Hospital de Baza. Granada. Spain

[b]Tissue Engineering Group. Faculty of Medicine, University of Granada and Instituto de Investigación Biosanitaria Ibs. Granada. Spain.

[c]Medicine Faculty. University of Granada. Spain.

Corresponding Author:

Dr. Jose Aneiros-Fernandez

Address: Departament of Pathology. University Hospital. Granada. Spain. Avd.
Madrid

S/N. CP: 18012. Email: janeirosf@hotmail.com

The authors have no conflict of interest to declare.

All the authors approve the submission

All authors have participated sufficiently to take public responsibility for
appropriate portions of the work

(i) informed, written consent has been obtained;
(ii) studies have been performed according to the Declaration of Helsinki;
(iii) the procedures have been approved by a local ethics committee. Please
include the date, approval number/code and name of the ethics approving
committee.

Authors' contributions

JAF and wrote the initial draft of and helped revise the manuscript. JAR, HHE,
VC and FO obtained consent from the patients and helped revise the
manuscript. JAC assisted with manuscript revision. All authors read approved
the final manuscript.

ABSTRACT

Background:Smoothelin is a cytoskeletal protein specifically expressed in differentiated smooth muscle cells. This protein has not been studied in smooth muscle skin tumors.

Objective:Smoothelin expression was studied in 46 cases of smooth muscle tumors of the skin, and compared with the expression of other markers of myoid differentiation.

Methods:We studied 28 leiomyomas (LM), 18 angioleiomyomas, 6 pilar-LM and 4 LM of the external genitalia and 18 leiomyosarcomas (LMS), 13 cutaneous and 5 subcutaneous. We evaluated the immunohistochemical profile for smooth muscle actin, smooth muscle myosin, desmin, h-caldesmon and smoothelin.

Results:The immunohistochemical study shows that the expression of nuclear smoothelin (p = 0.000) and the percentage of Ki-67 positive cells (p = 0.001) were statistically different when comparing LM and LMS (Mann Whitney U-test). In LMS, nuclear expression of smoothelin has a high positive correlation with Ki-67 expression (rho = 0.791. P = 0.000, Spearman correlation) and the number of mitosis per 10 high power field (HPF) (rho = 0.754, p = 0.000, Spearman correlation). These facts are not seen in the LM. In all cases studied, smoothelin has a cytoplasmic expression similar to

other myoid markers used. However, LMS show both cytoplasmic and nuclear expression of smoothelin.

Conclusions:The nuclear expression of smoothelin helps differentiate LM from LMS. The statistical correlation between nuclear smoothelin and positivity for Ki-67 and mitotic activity suggests that the nuclear expression of smoothelin may occur in cells that are in cycle. We provided the first study concerning the expression of smooth muscle smoothelin in skin tumors.

INTRODUCTION

Smooth muscle tumors of the skin are rare. These tumors can originate in the erector muscle of hair, vascular structures and, less frequently, in dartoic, vulvar and mammillary muscle (1,2,3). Thus, pilar LM, angioleiomyomas, and LMS may arise, and they that can

adopt a diffuse or nodular pattern. Although very rarely, LMS can originate on LM (4). Also, LM and LMS of the skin can be cutaneous (dermal), subcutaneous and present in unusual locations such as vulva, scrotum or nipple.

,The presence of cellular atypia, mitosis (> 1 per 10 high-power fields) (HPF), necrosis and the demonstration of smooth muscle phenotype help differentiate LMS from LM (5,6). The more commonly used immunohistochemical markers used to show smooth muscle differentiation are alfa-smooth muscle actin (SMA), smooth muscle myosin (SMM), desmin and h-caldesmon. SMA is considered the most sensitive myogenic marker (6, 7,8). However, none of these markers differentiate between LM and LMS. Smoothelin is a marker of smooth muscle differentiation that has been studied in smooth muscle tumors of the gastrointestinal tract and in some retroperitoneal neoplasms (9,10,11) but its expression in smooth muscle tumors of the skin is not known. We reported smoothelin expression in normal skin compared with SMA expression (12). We found that, unlike SMA, smoothelin is positive only in smooth muscle structures with contractile capability (erector muscle of the hair and deep vascular plexus). We have also reported intense smoothelin expression in smooth muscle hamartoma (13).

The aim of this study is to evaluate the expression of smoothelin in skin LM and LMS and to compare it with other markers of smooth muscle differentiation, in order to determine its potential value in the differential diagnosis of LM and LMS

MATERIAL AND METHODS

A total of 28 cases of LM and 18 cases of LMS were obtained from the routine caseload of the Provincial Clinical Intercenter Management Unit of Pathology, Granada, Spain.

For histopathological analysis, skin samples were fixed in 10% buffered formalin for 24 hours, dehydrated with alcohol, embedded in paraffin in an automatic tissue processor Excelsior ES (Thermo Scientific, CA, USA). 4 micron sections were stained with hematoxylin and eosin (H&E). The area of a single HPF was 0,196 mm2 (14). Histopathological changes were graded on a 0-2 scale in a blinded manner.

For Immunohistochemical analysis, sections were dewaxed, hydrated, and heat-treated in 1 mM EDTA pH 8 for antigenic retrieval using a PT module (Thermo Fisher Scientific Inc., Waltham, MA) at 95°C for 20 minutes. These sections were incubated for 10 min at room temperature with prediluted monoclonal antibodies

against alfa-smooth muscle actin (SMA) (1A4), h-caldesmon (H-CD)
smooth muscle myosin (SMMS-1), desmin (D33), smoothelin (R4 A)
and KI67 (sp6). All antibodies used were supplied by Master
Diagnostica, Granada, Spain. An appropriate isotype for each
antibody was used as negative control.

The immunohistochemical staining was conducted in an automatic
immunostainer (Autostainer 480, LabVision Fremont, CA) using the
micropolymer-peroxidase-based method (Ultravision Quanto, Master
Diagnostica, Granada, Spain), followed by development by
diaminobenzidine. The degree of immunoreactivity for SMA, SMM,
desmin, h-caldesmon and smoothelin in tumor cells was assessed in
a iquantitative manner, using a scale of 0 to 3 (0 [absence], 1 [<25%
positive cells], 2 [25-75% positive cells], 3 [>75% positive cells]).
The percentage of positivity for Ki67 was valued per 10/HPF.

The statistical software package SPSS 20.0 (IBM Inc., Chicago, IL)
was used for the statistical analysis. The Kruskal-Wallis and Mann
Whitney U-test were applied for the analysis of non-parametric
variables to analyze the differences in morphological and
immunohistochemical variables between LM and LMS. The
Spearman coefficient (rho) test was used to analyze the correlation
between variables. A P-value threshold of 0.05 was set.

RESULTS

Study of leiomyomas

Clinical Findings

We studied 28 cases of skin LM (18 angioleiomyomas, 6 pilar LM and 4 LM of unusual areas such as vulva, scrotum and nipple. The average age was 58.77 +/- 13.01 (range 21-79 years) and were more frequent in women (21/28 cases), in the lower limb (17/28) and frequently appeared in the foot (7/28).

Pathology Findings

The clinical and morphology features are summarized in Table 1.The average tumor size was 1.06 cm +/- 0.72 (range. 0.4-3 cm). The histological localization of skin LM was dermal (11 cases) including the genital LM, dermal-subcutaneous (3 cases) and subcutaneous (14 cases). The proliferating cells were spindled and arranged in fascicles with scarce atypia in 5 cases and absence of mitotic activity . Angioleiomyomas are characterized by be well circumscribed and present vascular structures (Figs 1A-B). While that, pilar LM genital LM had imprecise margens with variable proportion of collagen bundles among fascicles of tumor cells (Figs 2A-B).

Immunohistochemical analysis

The immunohistochemical results are summarised in Table 2. SMA, SMM and h-caldesmon were positive in all cases of LM (Figs 1C and 2C). Desmin was negative in 3 cases. Also, smoothelin was positive in the cytoplasm in all cases (Figs 1D and 2D), but in 2 cases the expression was less intense. Ki67 had an average positivity of 0.25% (range 0.1-1%).

Leiomyosarcoma study

Clinical Findings

We studied 18 cases of skin LMS. Average patient age was 57.82 +/- 15.33 years (range 38-88 years), were more frequent in women (10/18), and were more commonly located the lower extremity (10/18).

Pathology Findings

The clinical and hitopathologic features are summarized in Table 3.The average tumor size was 1.38 +/- 0.84 cm (range, 0.4 to 4.2 cm). The histological localization was dermis or only minimal subcutaneous involvement (13/18) or subcutaneous (5/18). The lesions presented a nodular (12 cases) or diffuse pattern (6 cases) (Figs 3A and 4B). The tumor cells were spindled with atypia and some were markedly pleomorphic (3 cases including one in the vulva) (Figs 3B and 4B). Myxoid changes were present in 1 case . Mitotic activity was on average 4.25 +/- 4.13 mitosis per 10 HPF

(range 3-15 mitoses per 10 HPF) (Fig 3C). In 11 cases necrosis was evident (Fig 3D). The FNCLCC grades were grade 1 in 6/7 cases of cutaneous LMS, grade 2 in 6/8 cases of cutaneous LMS, and grade 3 in 2/3 cases of cutaneous LMS. Vascular invasion (1 case) and perineural invasion (1 case) were also observed. In a case cutaneous LMS, recurrence occurred after 9 years. In 8/18 cases there is no follow-up information and in 9/18 cases the follow up did not exceed two years.

Immunohistochemical analysis

The immunohistochemical result are summarized in Table 4. The immunohistochemical study of LMS showed positivity in all cases for SMA, SMM and h-caldesmon (Figs 4D and 5A). Desmin was negative in 2 cases. Smoothelin showed cytoplasmic expression in all cases, but less marked in 5 cases. Also, smoothelin showed nuclear positivity in all cases, which on average was 7.85 +/- 8.63 (range 3-33) (Figs 5 B,C and D)). Ki-67 had an average positivity of 10.55 +/-10.31 (range 8-37) (Fig 4C).

Statistical analysis

Immunohistochemical study showed that only the nuclear smoothelin expression (p = 0.000) and the percentage of Ki-67 positive cells (p = 0.001) were statistically different when comparing LM and LMS (Mann Whitney U-test). The expression of nuclear

smoothelin in LMS keeps a high positive correlation with the expression of Ki-67 (rho = 0.791, p = 0.000, Spearman correlation) and the number of mitosis per 10 HPF (rho = 0.754, p = 0.000, Spearman correlation). Also, LMS showed no statistical differences regarding their smoothelin nuclear expression or percentage of Ki-67 positivity and mitoses in relation to their histological localization or their diffuse or nodular pattern.

DISCUSSION

Smoothelin is a specific cytoskeletal protein present in differentiated smooth muscle cells with contractile capability. This protein appears to be expressed later in the development, when the smooth muscle cells acquire contractile capacity. Smoothelin has two tissue specific isoforms: a short 59-kDs isoform (A), located in smooth muscle cells of organs, and a long 110-kDs isoform (B) present in smooth muscle cells of vascular structures (15, 16,17). These isoforms have been shown to colocalize with alpha SMA. This fact seems involved in the modulation of contractile properties of differentiated smooth muscle cells (16). Loss of smoothelin expression involves the absence of contractility (18).

In a previous study we evaluated the expression of smoothelin in normal skin using the clone R4A, that identifies both isoforms. We

observed intense cytoplasmic positivity for smoothelin in the hair

erector muscle (isoform A) that could serve as a positive control,

and in vascular structures, mainly in the deep vascular plexus

(isoform B) (12). The study showed that smoothelin is more specific

than SMA, which was positive in other cells (such as pericytes and

myoepithelial cells). The A isoform was also identified in the

cytoplasm of proliferating cells of the smooth muscle hamartoma of

the skin (13). It has been considered that, compared to other smooth

muscle markers (SMA, SMM, desmin and h-caldesmon), smoothelin

is more specific (9, 17). In all smooth muscle tumors in this study,

the expression of SMA, SMM, h-caldesmon and smoothelin were

similar, except for desmin, that was negative in 3 LM cases and 2

LMS cases . A striking difference was the nuclear expression of

smoothelin in the LMS, that was not present in LM. The statistical

study shows a high correlation between the nuclear expression of

smoothelin and Ki-67 expression and mitotic activity in LMS, a fact

not observed in LM. Therefore, nuclear positivity for smoothelin and

the Ki-67 index differentiate LMS from LM. Few studies have

evaluated the expression of Ki-67 in LM and LMS, and Ki-67

proliferative index has shown a statistically significant correlation

with a diagnosis of LMS (19).

Pilar leiomyomas with atypia and mitotic activity have been described, as well as atypical/symplastic pilar leiomyomas and cutaneous leiomyosarcoma originating in a symplastic pilar leiomyoma (4,20,21,22,23). In these cases, the nuclear expression of smoothelin and Ki-67 could be helpful in the differential diagnosis with LMS. In our study we did not observe statistically significant differences in nuclear smoothelin expression, Ki-67 index or mitotic activity between cutaneous (dermal) and subcutaneous LMS. We therefore believe that the term atypical intradermal smooth muscle neoplasm (24) used to refer to the cutaneous (dermal) LMS is not fully justified, since the morphological and immunohistochemical characteristics are typical of LMS. These authors argue that the dermal LMS can recur, but not metastasize. However, a recent study reports a metastatic rate of 12% for cutaneous LMS (25).

Previous studies show smoothelin cytoplasmic and nuclear expression in smooth muscle tumors of the digestive tract and retroperitoneum (9,10,11). Coco et al demonstrate cytoplasmic and nuclear smoothelin expression of LM, LMS and in smooth gastrointestinal tract muscle (9). Subsequently, cytoplasmic and nuclear positivity was reported in gastrointestinal LMS without evidence of nuclear expression in LM (10). These differences may

be explained due to the the immunohistochemical protocol used, since no antigen retrieval was performed, and an inappropriate dilution of the primary antibody may have been carried out (10). The explanation behind the nuclear smoothelin expression in skin LMS is unknown. Although the smoothelin gene is known to be located in the chromosome 22q12 (26) it is not known whether smoothelin exist in the nucleus, as occurs with actin and myosin (27). However, we observed the nuclear expression of smoothelin in the outer root sheath of the inferior portion of the hair follicle and the cytoplasmic expression of SMA in the dermal sheath cells in normal skin (12). In this study we consider that the nuclear smoothelin presence could intervene with SMA in hair morphology and contraction.

In pathological situations such as ACT-1 gene mutation, heat shock, dimethyl sulfoxide and alpha amanitin treatment, actin occurs in the nucleus (28,29,30) Also, the nuclear expression of SMA in certain tumors such as diffuse large B cell lymphoma (31) has been described. Therefore an explanation for the nuclear smoothelin expression could be related to a mutation of the smoothelin gene. There could also be a translocation process whereby smoothelin would move to the nucleus. The statistical correlation observed in

our study suggests that the nuclear expression of smoothelin is related to the positivity of Ki-67 and mitotic activity. Therefore an aberrant nuclear smoothelin expression in tumor LMS cells could occur in cells in cycle. Moreover, the possibility that this expression is due to a cross-reaction is unlikely, since it would appear both in cells in cycle as well as in G0.

In summary, we provide the first report of smoothelin expression in smooth muscle skin tumors, that due to its specificity as a smooth muscle differentiation marker should be used in routine diagnosis. We also propose that the nuclear expression of smoothelin helps differentiate between LM and LMS. However, more extensive studies involving larger case series may be needed to further support this observation.

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FIGURES:

Fig 1. Angioleiomyoma. A. Well circumscribed tumor (H-E, x1

original magnification). B. Proliferation of spindle cells with vascular

structures (HE, x 10 original magnification). C. Strong and diffuse

cytoplasmic immuhistochemical staining for h-caldesmon (h-

caldesmon, x 10 original magnification). D. Marked cytoplasmic

positivity for smoothelin (smoothelin, x 40 original magnification).

Fig 2. Pilar leiomyoma. A. Non-encapsulated tumor in the dermis (H-E, x1 original magnification). B. Spindle cells with fascicular pattern and without atypia (H-E, x 10 original magnification). C. Immunohistochemical staining with positivity for SMA (SMA, x1 original magnification). D. Tumor cells with cytoplasmic expression for smoothelin (smoothelin, x 40 original magnification).

Fig 3. Leiomyosarcoma. A. Tumor with nodular pattern (H-E, x1 original magnification). B. Pleomorphic proliferation of spindle cells (H.E, x 40 original magnification). C. Atypical cells with mitotic figures (H.E, x 20 original magnification). D. Area of necrosis (H.E, x 4 original magnification).

Fig 4.Leiomyosarcoma. A. Tumor with diffuse pattern (H-E, x1 original magnification). B. Spindle cells with atypical changes (H-E. x 10 original magnification). C. Ki-67 immunostain with positive nuclei (ki-67. x 40 original magnification). D. SMA immunostain with positive cytoplasmatic (SMA, x1 original magnification).

Fig 5. Leiomyosarcoma. A. Tumor with nodular pattern and expression for H-caldesmon (H-caldesmon, x1 original magnification). B. Fascicles of spindle cells with positive

immunophenotype for smoothelin (smoothelin, x 20 original

magnification). C. Cytoplasmic and nuclear immunuhistochemical

staining for smoothelin (smoothelin, x 20 original magnification). D.

Smoothelin stain shows evident positive nuclear (smoothelin, x 20

original magnification).

immunophenotype for smoothelin (smoothelin, x 20 original

Table 1. Skin leiomyoma: Clinical and histopathologic features and KI67 stain.

Case	Diagnosis	Age (y)	Sex	Location	Size	Atypia	Mitoses/10hpf	Ki 67 (%)
1	AL	39	F	LE	0,4	-	-	0,3%
2	AL	70	F	LE	0,5	-	-	0,1%
3	AL	56	M	LE	1,7	-	-	0,2%
4	AL	51	M	UE	1,1	-	-	0,3%
5	AL	45	F	UE	0,9	-	-	0,1%
6	AL	44	F	LE	1,1	-	-	0,2%
7	AL	58	F	LE	1	-	-	0,5%
8	AL	37	F	UE	0,9	-	-	0,6%
9	AL	60	F	LE	0,7	-	-	0,1%
10	PL	54	F	TRUNK	0,8	Mild	-	0,1%
11	PL	36	F	LE	1	-	-	0,1%
12	AL	71	M	UE	0,7	Mild	-	0,2%
13	PL	49	M	UE	0,5	-	-	0,5%
14	AL	47	F	LE	3	-	-	0,1%
15	PL	50	M	LE	0,7	-	-	0,1%
16	AL	72	F	UE	0,6	-	-	0,3%
17	AL	39	F	LE	0,5	-	-	0,2%
18	AL	47	M	HN	0,3	-	-	0,1%
19	AL	70	F	LE	1	Mild	-	1%
20	AL	51	F	LE	1,1	Mild	-	0,1%
21	PL	79	F	LE	0,5	-	-	0,1%
22	AL	67	F	LE	0,9	-	-	0,1%
23	AL	57	F	LE	1,3	-	-	0,1%
24	AL	58	F	LE	1	Mild	-	0,4%
25	GL	55	F	VULVA	2,2	-	-	0,2%
26	GL	48	F	VULVA	1,9	Mild	-	0,3%
27	GL	74	F	NIPPLE	0,4	-	-	0,2%
28	GL	21	M	SCROTUM	0,3	-	-	0,1%

AL, Angioleimyoma; PL, Pilar leiomyoma; GL, Genital Leiomyoma; M, male; F, female; UE, Upper extremity; LE, Lower extremity; HN, Head and neck; hpf, high-power-field.

Tabla 2. Skin leimyoma: Immunohistochemical features.

Case	Diagnosis	SMA	SMM	Desmin	h-caldesmon	Smoothelin (citoplasmatic)	Smoothelin (nuclear)
1	AL	+++	+++	+++	+++	+++	-
2	AL	+++	+++	+++	+++	+++	-
3	AL	+++	+++	+++	+++	+++	-
4	AL	+++	+++	+++	+++	+++	-
5	AL	+++	+++	+++	+++	+++	-
6	AL	+++	+++	+++	+++	+++	-
7	AL	+++	+++	+++	+++	+++	-
8	AL	+++	+++	+++	+++	+	-
9	AL	+++	+++	-	+++	+++	-
10	PL	+++	+++	+++	+++	+++	-
11	PL	+++	+++	+++	+++	+++	-
12	AL	+++	+++	-	+++	++	-
13	PL	+++	+++	+++	+++	+++	-
14	AL	+++	+++	+++	+++	+++	-
15	PL	+++	+++	+++	+++	+++	-
16	AL	+++	+++	+++	+++	+++	-
17	AL	+++	+++	+++	+++	+++	-
18	AL	+++	+++	+++	+++	+++	-
19	AL	+++	+++	-	+++	+++	-
20	AL	+++	+++	+++	+++	+++	-
21	PL	+++	+++	+++	+++	+++	-
22	AL	+++	+++	+++	+++	++	-
23	AL	+++	+++	+	+++	+++	-
24	AL	+++	+++	+++	+++	+++	-
25	GL	+++	+++	+++	+++	+++	-
26	GL	+++	+++	+++	+++	+++	-
27	GL	+++	+++	+++	+++	+++	-
28	GL	+++	+++	+++	+++	+++	-

AL, Angioleiomyoma; PL, Pilar leimyoma; GL, Genital leiomyoma. , SMA, Smooth muscle actin; SMM, smooth muscle myosin.

Table 3. Skin leiomyosarcomas: Clinical and histopathologic features and Ki67 stain.

CASE	Diagnosis	AGE (y)	SEX	LOCATION	SIZE (cm)	TYPE	ATYPIA	NECROSIS	MITOSES/10hpf	KI67 (%)
1	CLMS	38	M	Trunk	1,9	Diffuse	mild	-	4	8,4%
2	CLMS	77	M	Trunk	1,3	Nodular	Moderate	-	6	9%
3	CLMS	57	M	UE	1,9	Difuse	Moderate	< 50%	6	10%
4	CLMS	69	F	LE	1,2	Nodular	Severe	> 50%	3	12%
5	CLMS	56	M	Trunk	0,4	Nodular	Mild	-	9	16%
6	CLMS	76	F	LE	2,2	Diffuse	Moderate	-	10	20%
7	SLMS	79	F	LE	1	Nodular	Moderate	< 50%	4	7%
8	SLMS	57	M	LE	2,8	Nodular	Moderate	< 50%	4	15%
9	SLMS	58	F	LE	1,7	Nodular	Severe	< 50%	6	12%
10	SLMS	62	F	Vulva	1,2	Nodular	Severe	< 50%	15	37%
11	SLMS	43	M	LE	1,9	Nodular	Mild	-	3	7%
12	CLMS	51	F	Trunk	0,8	Nodular	Moderate	< 50%	4	12%
13	CLMS	75	F	LE	1,5	Diffuse	Mild	-	4	19%
14	CLMS	47	F	LE	2,5	Nodular	Mild	< 50%	10	21%
15	CLMS	68	M	LE	1,5	Diffuse	Moderate	<50%	7	32%
16	CLMS	79	F	LE	1,5	Nodular	Severe	> 50%	11	15%
17	CLMS	88	M	Trunk	2,1	Nodular	Moderate	< 50%	7	12%
18	CLMS	54	F	Trunk	0,5	Diffuse	Mild	-	6	19%

CLMS, Cutaneous leiomyosarcoma; SLMS, Subcutaneous Leiomyosarcoma, M, male; F, female; UE, Upper extremity; LE, Lower extremity; hpf, high-power-field

Tabla 4. Skin leiomyosarcoma: Immunohistochemical features.

Case	Diagnosis	SMA	SMM	Desmin	h-caldesmon	Smoothelin (cytoplasmic)	Smoothelin (nuclear %)
1	CLMS	+++	+++	+++	+++	+++	10%
2	CLMS	+++	+++	+++	+++	+++	6%
3	CLMS	+++	+++	+++	+++	++	12%
4	CLMS	+++	+++	+++	+++	+	5%
5	CLMS	+++	+++	+++	+++	+++	12%
6	CLMS	+++	+++	+++	+++	+++	14%
7	SLMS	+++	+++	++	+++	+++	4%
8	SLMS	+++	+++	++	+++	+++	16%
9	SLMS	+++	+++	++	+++	++	12%
10	SLMS	+++	+++	-	+++	++	3%
11	SLMS	+++	+++	+++	+++	+++	10%
12	CLMS	+++	++	+++	+++	+++	16%
13	CLMS	+++	+++	+++	+++	++	13%
14	CLMS	+++	+++	+++	+++	+++	6%
15	CLMS	+++	+++	+++	+++	+++	29%
16	CLMS	+++	+++	+++	+++	+++	35%
17	CLMS	+++	+++	+++	+++	+++	10%
18	CLMS	+++	+++	+++	+++	+++	9%

CLMS, Cutaneous leiomyosarcoma; SLMS, Subcutaneous leiomyosarcoma; SMA, Smooth muscle actin; SMM, smooth muscle myosin.
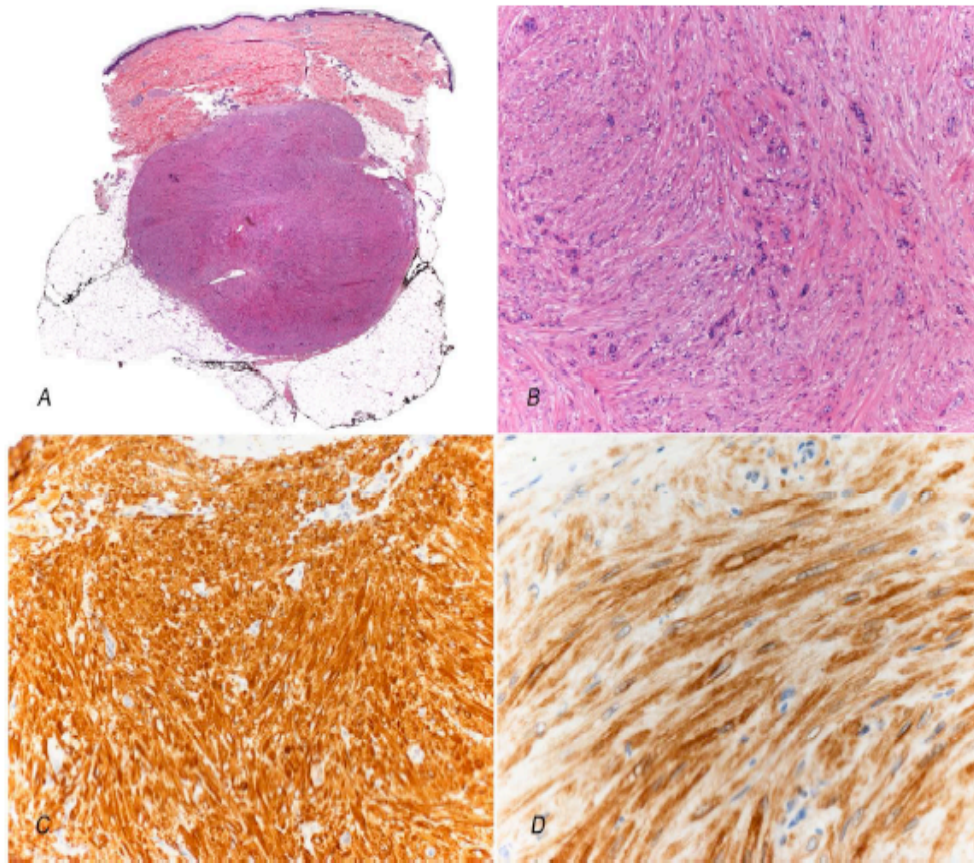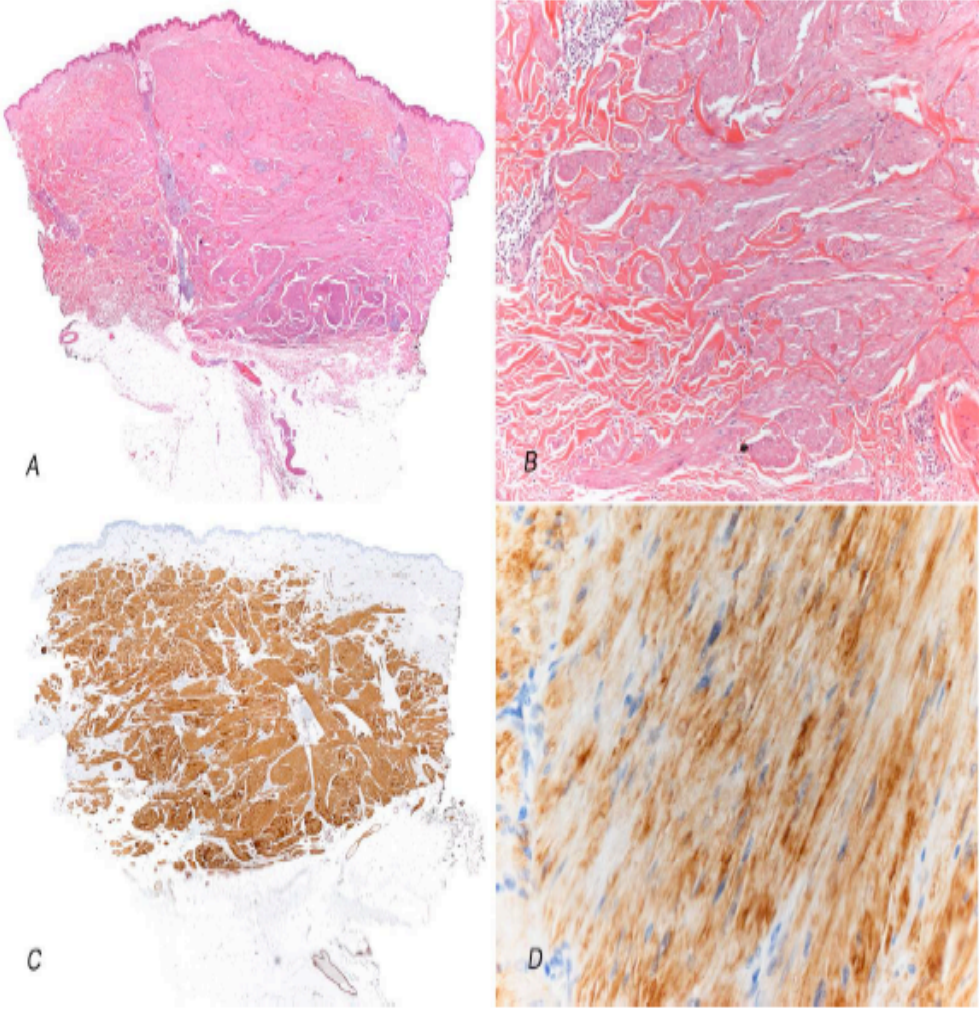
Figure 1
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Figure 2
170x124mm (300 x 300 DPI)

Figure 3
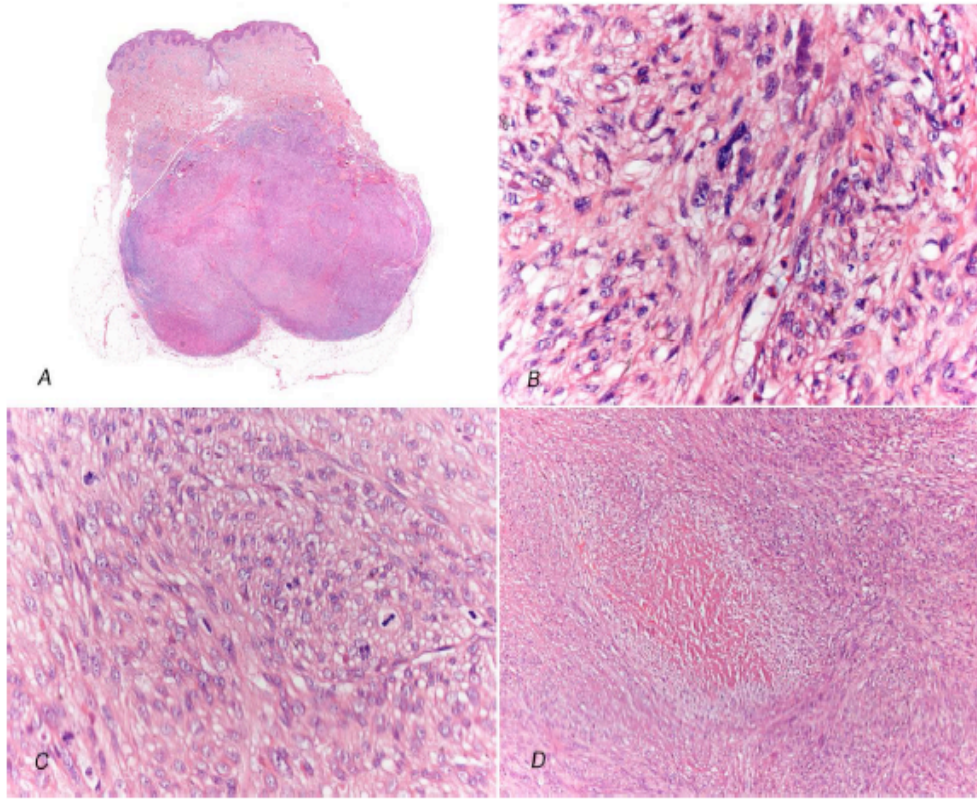170x124mm (300 x 300 DPI)
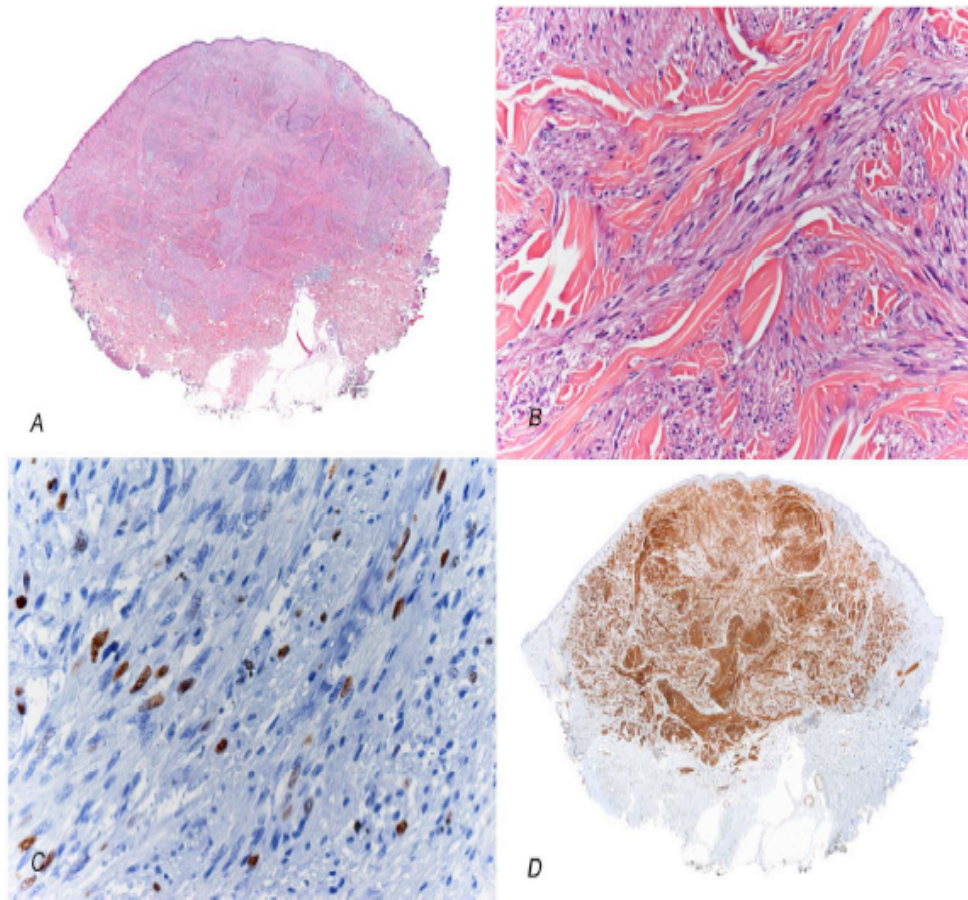
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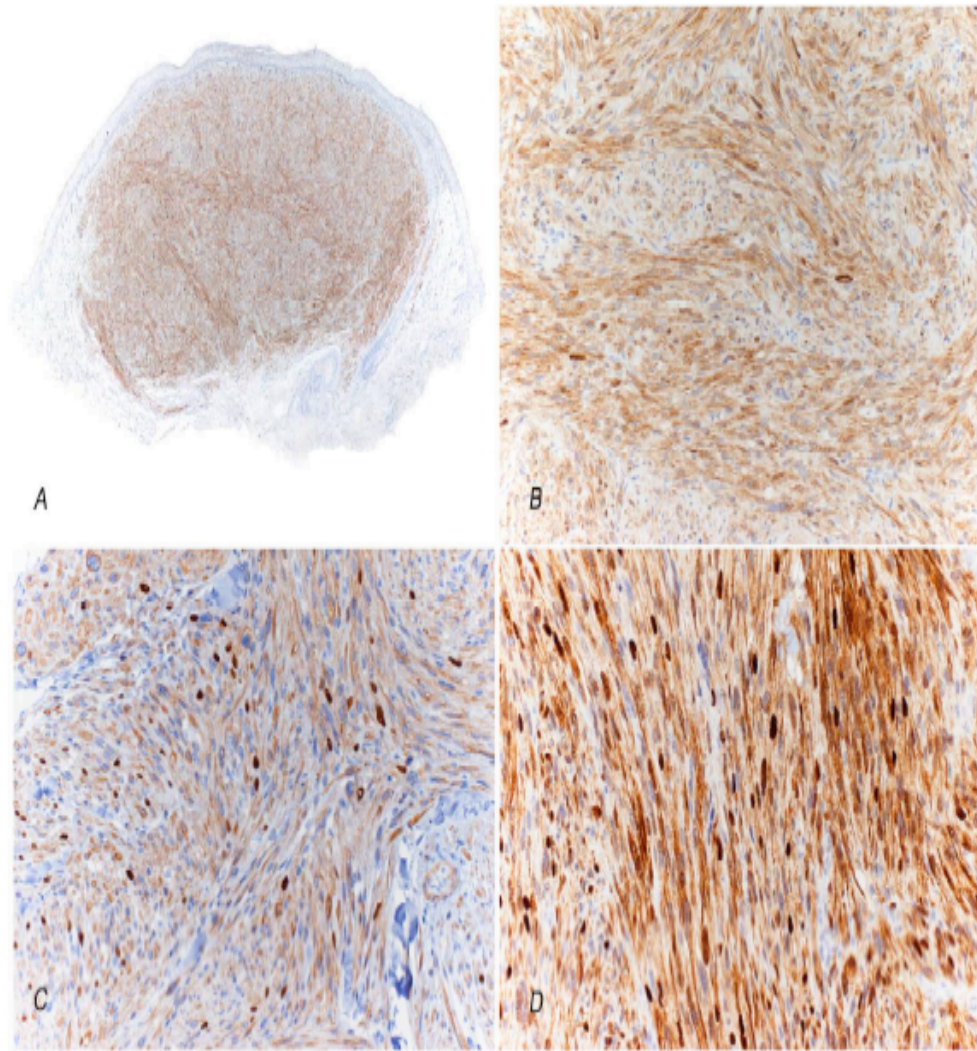
Figure 5
170x124mm (300 x 300 DPI)

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De: **dpa@karger.com**
Enviado: lunes, 01 de octubre de 2015 10:48:18
Para: janeirosf@hotmail.com

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t +4161 306 1437
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TITLE: DERMAL LEIOMYOSARCOMA WITH POLYPOID GROWTH ARISING IN A LEIOMYOMA

KEYWORDS: cutaneous leiomyosarcoma, cutaneous leiomyoma, malignant transformation, immunohistochemical stains, smoothelin.

SHORT TITLE: MALIGNANT TRANSFORMATION OF LEIOMYOMA

AUTHORS

Jose Aneiros-Fernandez. MD

Husein Husein-ElAhmed. MD [1]

Carmen Lopez Peña. MD [2]

Jose Javier Lopez Caballero. MD PhD

Raimundo García del Moral. MD PhD

Francisco Ovalle. MD PhD

Jose Aneiros-Cachaza. MD PhD

Department of Pathology. Hospital Universitario Clinico San Cecilio. Granada. Spain

Department of Dermatology[1]. Hospital Baza. Granada. Spain.

Departmenr of Pathology[2]. Hospital Universitario "Santa Lucía". Cartagena (Murcia).

Corresponding Author:

Dr. Jose Aneiros-Fernandez

Address: Departament of Pathology. University Hospital. Granada. Spain. Avd. Madrid S/N. CP: 18012. Email: janeirosf@hotmail.com

The authors have no conflict of interest to declare.

All the authors approve the submission

ABSTRACT

We report a case of polypoid cutaneous leiomyosarcoma arising on leiomyoma. An 82-yr-old man presented with an exophytic and pedunculated lesion on the dorsal area that had existed for several years but had grown larger over the previous 6 months. The leiomyosarcomatous component comprised spindle-shaped cells with moderate nuclear pleomorphism, 7 mitoses per 10 /high-power fields and necrotic foci. The cells showed intense cytoplasmic expression of SMA, h-caldesmon and smoothelin but no expression of desmin; smoothelin was also positive in 9% of nuclei, and Ki-67 was positive in 12% of cells. The leiomyomatous component comprised fascicles of spindle-shaped cells with no atypia or mitotic activity with collagen fiber bundles. Cytoplasmic expression of SMA, h-caldesmon, smoothelin, and desmin was observed, but no nuclei were smoothelin-positive, and < 1% of cells were Ki-67-positive. The morphological and immunohistochemical characteristics of the two components permitted their differentiation and established the diagnosis of leiomyosarcoma arising in leiomyoma. This is the first report on smoothelin expression in a cutaneous smooth muscle tumor.

Key Words: cutaneous leiomyosarcoma, cutaneous leiomyoma, malignant transformation, immunohistochemical stains, smoothelin

INTRODUCTION

Cutaneous smooth muscle tumors are uncommon, especially leiomyosarcomas. Dermal leiomyosarcomas have been designated as atypical intradermal smooth muscle neoplasms [1] because there appears to be little risk of metastasization, unlike subcutaneous leiomyosarcomas. Cutaneous leiomyosarcomas can be diffuse or fascicular and can develop *de novo* in piloerector muscle or vascular plexus. However, there been only a few reports of cutaneous leiomyosarcomas arising in existing lesions, including leiomyomas [2,3], leiomyoma scar [4], and *nevus sebaceous* [5].

We report the clinical, morphological, and immunohistochemical characteristics of a dermal leiomyosarcoma arising in a leiomyomatous lesion.

CASE REPORT

An 82-yr-old man presented with a painful elevated cutaneous lesion of polypoid appearance on his back that had existed for several years but had grown larger over the previous 6 months. Clinical findings suggested a sarcoma. Macroscopically, the lesion was exophytic and pedunculated, measuring 2.1 x 1.8 cm and extending to the surgical. Microscopically, the dermis showed a proliferation of spindle-shaped cells arranged in fascicles, with moderate nuclear pleomorphism and 7 mitosis/10 high-power fields (HPF) (Fig 1); foci of necrosis were also observed (<50%). In the immunohistochemical study, 12% of tumor cells were positive for Ki-67. The cells showed intense cytoplasmic positivity for SMA, h-caldesmon, and smoothelin but negativity for desmin (Fig 2); nuclei were positive for smoothelin in 9% of tumor cells. The diagnosis was grade 2 dermal leiomyosarcoma with fascicular pattern. The base of the tumor showed a well-identified area formed by fascicles of spindle-

shaped cells separated by collagen bands with scarce atypia and no mitotic activity. These cells evidenced intense cytoplasmic positivity for desmin, smooth muscle actin, h-caldesmon, and smoothelin, with no nuclear expression of smoothelin (Fig 3). Likewise, Ki 67 was positive in <1% of cells. These findings are consistent with a pilar leiomyoma that is largely occupied by the leiomyosarcoma, suggesting the possible transformation of a leiomyoma into a leiomyosarcoma. A widening of the surgical margin revealed a leiomyomatous lesion with no sarcomatous component. No recurrence has been observed at 7 months since surgical excision.

DISCUSSION

The differential morphological diagnosis between cutaneous leiomyoma and leiomyosarcoma is based on the increased cellularity, nuclear pleomorphism, mitotic activity, and necrosis in the latter. Nevertheless, it has been reported that the mitotic rate can be <1 mitosis per 10 HPF in 28% of pilar leiomyomas [6, 7]. Mitosis can be observed in the uncommon atypical/symplastic variant of leiomyoma, but there have been no reports of cases with > 2 mitoses per 10 HPF [8-10]; focal nuclear hyperchromasia and pleomorphic cells are also observed in these leiomyomas.

The diagnosis of a smooth muscle tumor requires immunohistochemical studies to demonstrate smooth muscle differentiation. Leiomyomas are generally positive for SMA, desmin, and h-caldesmon. However, the results for this widely used antibody panel have been more variable for cutaneous leiomyosarcomas, especially in the case of desmin (positivity ranging from 50% to 98%) and h-caldesmon (positivity ranging from 75% to 92%) [1, 11, 12].

Although SMA is positive in most cutaneous smooth muscle tumors, it is not specific for smooth muscle differentiation. Some recent studies have used only SMA and desmin for this purpose, without considering the expression of h-caldesmon, a more specific marker [3]. In our experience, smoothelin is a good specific marker for smooth muscle differentiation. We propose the use of smoothelin and h-caldesmon alone for the differentiation of smooth muscle. Ki67 has been used in only a few smooth muscle tumor series, with results ranging from 10 - 50% with a mean of 24.9% /10 HPF in leiomyosarcomas and a range of 0.2 – 3% with a mean of 0.83%/10 HPF in leiomyomas [13].

In the present study ,the leiomyosarcoma was negative for desmin, except for an area with a morphologic pattern of pilar leiomyoma that showed no mitosis or pleomorphism and Ki 67-positity in <1% of cells, consistent with the reported features of cutaneous leiomyomas [13]. We believe that Ki- 67 should be considered in the panel for differentiation between leiomyosarcomas and leiomyomas.

The leiomyomatous component was at the base of the present lesion, whereas it was localized on the surface in a recent report [3]. This would be explained by polypoid growth of the leiomyosarcoma towards the base of the leiomyomatous lesion. The diagnosis of leiomyosarcoma arising in a leiomyoma should not be confused with hyperplastic or hamartomatous arrector pili muscle. Although the leiomyomatous component was not predominant in the present case, the morphological and immunohistochemical data support the diagnosis of a malignant pilar leiomyoma that is largely occupied by a leiomyosarcoma. The possibility that the leiomyomatous lesion might be a well-differentiated area of the leiomyosarcoma was ruled out by the absence of mitosis, scarce Ki67-

assessed proliferative activity, and intense positivity for desmin. Although desmin is not specific for smooth muscle differentiation, it assisted differentiation between the benign and malign component in our case.

Smoothelin has two tissue-specific isoforms: A, found in visceral smooth muscle cells; and B, in vascular smooth muscle cells [14, 15]. The present study used clone R4A, which detects both isoforms, at 1/20 dilution. Smoothelin is a marker of smooth muscle cells with contractile capacity and was previously studied by our group in normal skin, evidencing cytoplasmic positivity in arrector pili muscle and the deep vascular plexus and in intercommunicating vessels [16]. Smoothelin has previously been used in a cutaneous smooth muscle hamartoma [17] but not in a cutaneous smooth muscle tumor. However, cytoplasmic and nuclear positivity for smoothelin has been reported in smooth muscle tumors of the gastrointestinal tract [18,19]. The significance of nuclear expression of smoothelin is unknown, but it has been considered an aberrant expression probably related to the antibody dilution [19]. Based on the experience of our group, we consider smoothelin to be a specific marker for smooth muscle differentiation that may help to distinguish leiomyomas from leiomyosarcomas and may therefore be of value in the diagnosis of leiomyosarcomas arising in cutaneous leiomyomas. However, further studies are required to confirm its usefulness in these cases.

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FIGURE LEGENDS
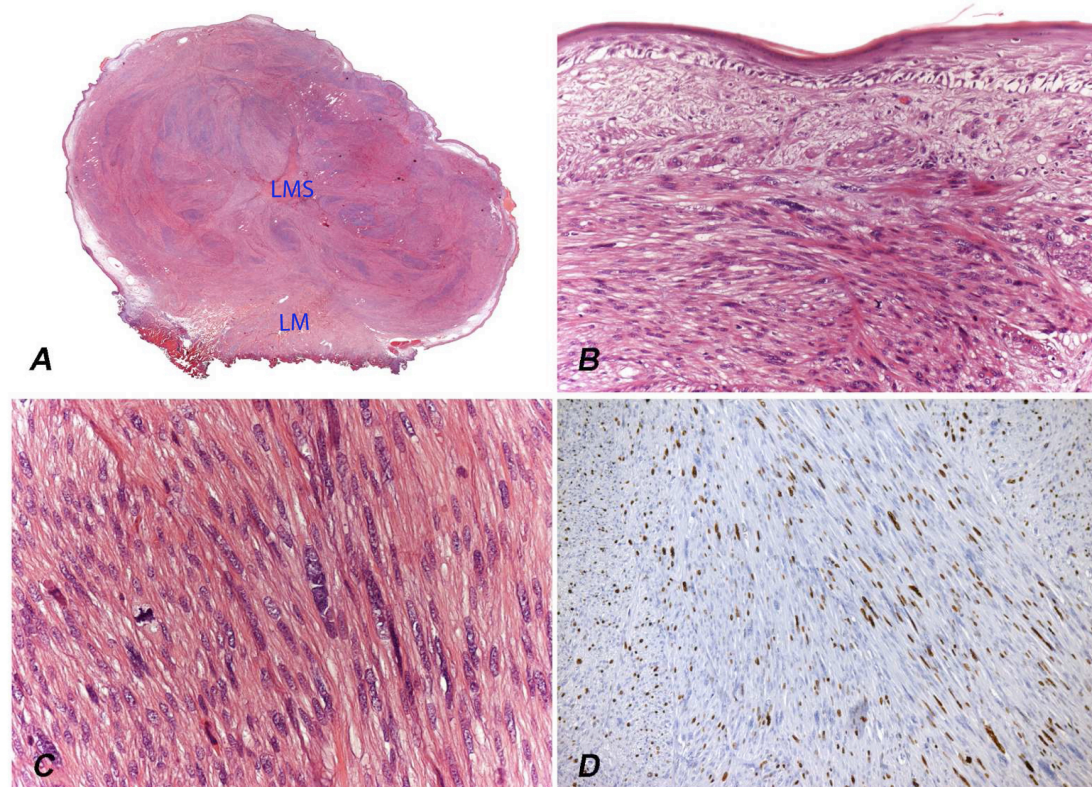
Fig 1: A. Leiomyosarcoma (LMS) with growing polypoid basal area corresponding to leiomyoma (LM) (H&E, panoramic). B. Fascicles of atypical spindle cells (H&E, original magnification x10). C. Mitotic figures (H&E, x original magnification x20). D. Ki-67 immunostaining in approximately 14 % of cells (original magnification x10)
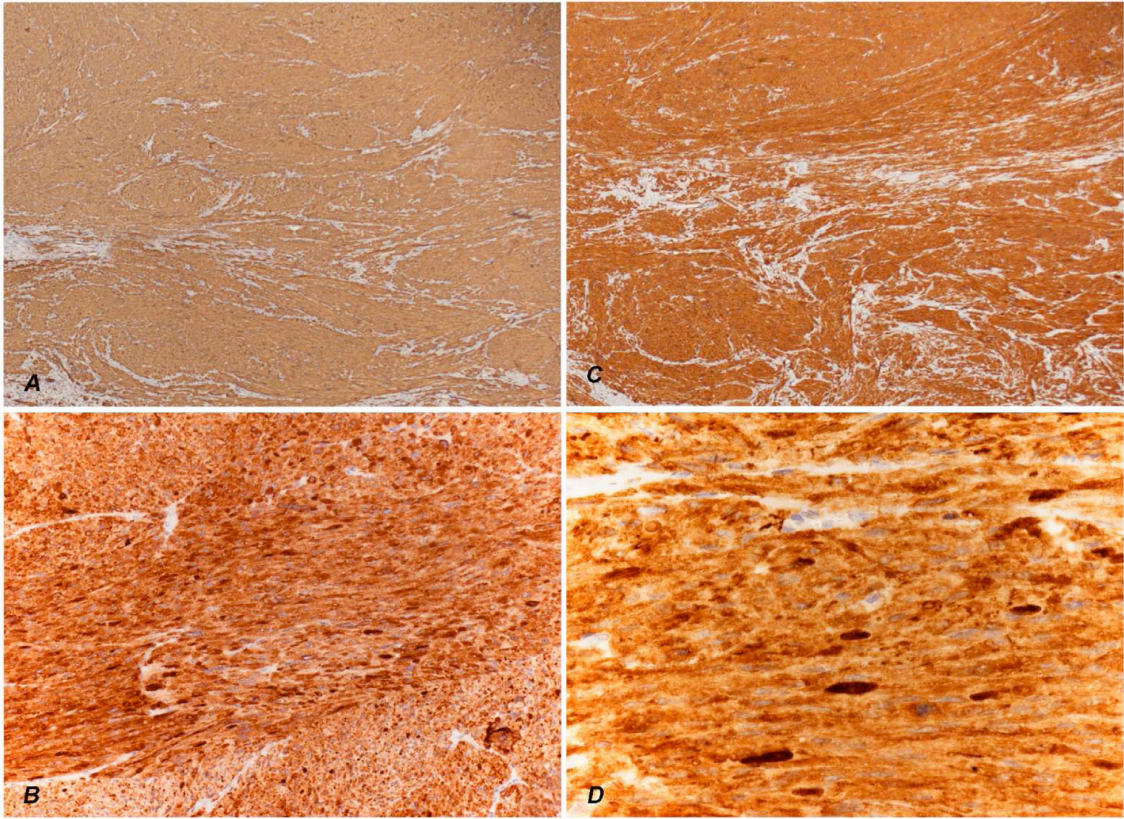
Fig 2: A. Leiomyosarcoma with diffuse cytoplasmic immunohistochemical staining for SMA (original magnification x4), h-caldesmon (original magnification x4) (B), and smoothelin (original magnification x20) (C). D. Nuclear positivity in immunohistochemical staining for smoothelin (original magnification x40).
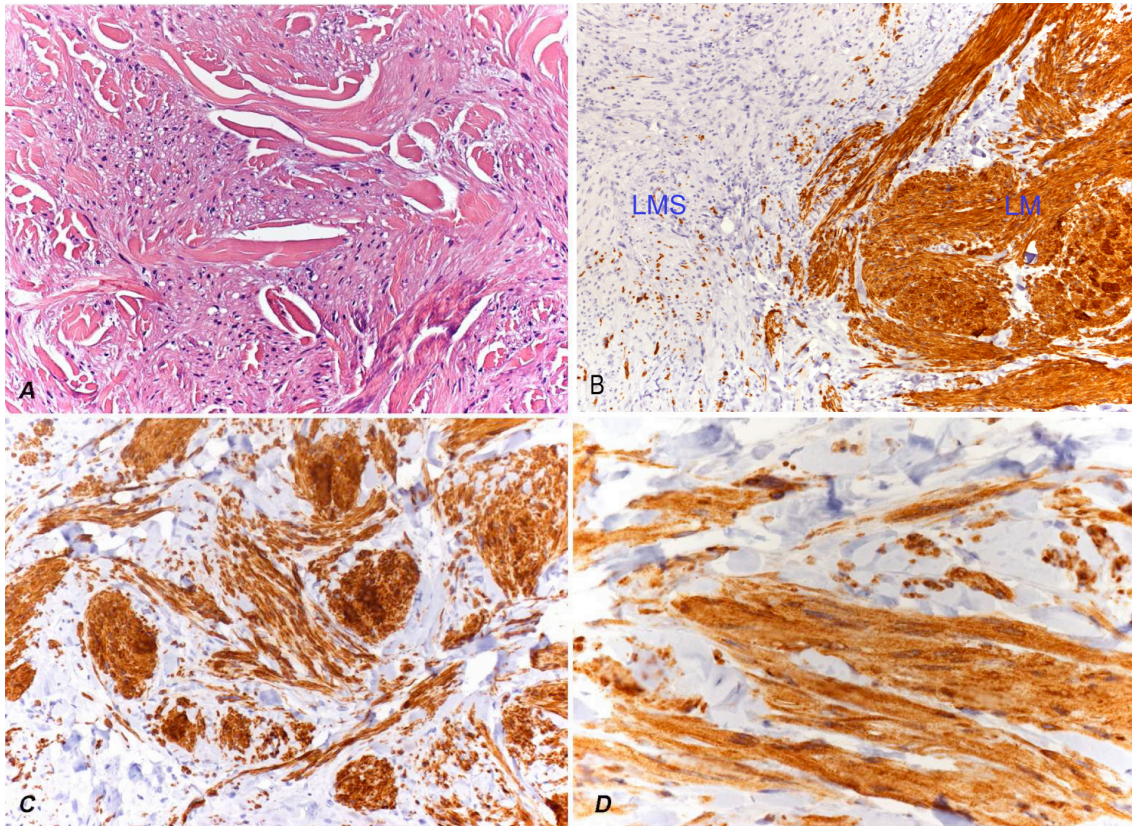
Fig 3: A. Leiomyoma with spindle-shaped cells showing no atypia or mitotic activity (H&E, original magnification x10). B Leiomyoma with diffuse cytoplasmic immunohistochemical staining for desmin (LM), and leiomyosarcoma with negative staining for desmin (LMS) (original magnification x10); C) Leiomyoma with positivity for h-caldesmon (original magnification x20). D. Leiomyoma with cytoplasmic positivity for smoothelin, with no nuclear staining (original magnification x40).

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Ejd150359 review article

Primary cutaneous and subcutaneous leiomyosarcomas: review of their evolution and prognostic factors

Primary skin leiomyosarcomas

Jose ANEIROS-FERNANDEZ

Juan Antonio RETAMERO

Husein HUSEIN-ELAHMED

Francisco OVALLE

Jose ANEIROS-CACHAZA

Department of Pathology,

Complejos Universitario Hospitalario de Granada,

Avd. Madrid S/N,

18012 Granada, Spain

Reprints
J Aneiros-Fernandez
janeirosf@hotmail.com

ABSTRACT:

Cutaneous and subcutaneous leiomyosarcomas (LMS) are uncommon neoplasms. We reviewed the MEDLINE database to assess their rates of recurrence and metastasis, mortality and recommended follow-up period. Other prognostic factors were also studied. This review included 112 subcutaneous LMS and 313 cutaneous LMS. In subcutaneous LMS, we observed that rates of recurrence, metastasis and mortality were

36.63%, 43.23% and 37.82%, respectively, after a median follow-up period of 4.40 years, while in cutaneous LMS those figures were 24.40%, 4.22% and 3.33%, respectively, after a median follow-up period of 3.45 years. Although subcutaneous and cutaneous LMS show similar morphologic features, the latter show less tendency to recur and metastasize; in certain cases they both may be cause of death. For these reasons we suggest avoiding the term "atypical intradermal smooth muscle neoplasm". Location, size and histologic grade are essential prognostic factors for superficial LMS. Recurrence after incomplete excision can be avoided when performed with a surgical margin of at least 1 cm. Follow-up should be at least 5 years.

Primary skin leiomyosarcoma (LMS), also called primary superficial leiomyosarcoma, is a rare tumor and corresponds to 2-3% of all sarcomas, with an overall incidence of 0.04% [1-4]. This tumor can originate either in the hair erector muscle and be located in the dermis (cutaneous LMS), or arise from vascular structures in the subcutaneous cellular tissue (subcutaneous LMS) [5-7]. Cutaneous and subcutaneous LMS show the same morphologic features, such as the presence of spindled cells with smooth muscle phenotype, variable rate of nuclear pleomorphism, presence of mitosis (> 1 mitosis per high-power field) and absence or presence of necrosis *(Figs 1a, b and c)*. Knowledge of whether the surgical margins are free of disease or not is essential in order to establish the likelihood of future recurrence. Therefore, all the pathology reports should include this data as well as the distance to the nearer surgical margin.

Cutaneous LMS may recur but rarely metastasize [7-11]. It has been proposed that, due to its scant metastatic potential, it should be called atypical intradermal smooth muscle neoplasm [12]. In contrast, subcutaneous LMS is more likely to recur (50-70%), metastasize (30-60%), and cause death (30-40%) [4, 7, 13-15].

The objective of this study was to review the published data regarding superficial LMS in order to assess their rates of recurrence and metastasis, mortality, recommended follow-up period and prognostic factors. The inclusion of studies for this review was conducted by two independent reviewers who searched the MEDLINE database for articles written in English up to February 2015. For the PubMed library, a combination of MeSH and non-MeSH terms was used. MeSH terms were skin, leiomyosarcoma, smooth muscle tumor, sarcoma, neoplasm primary, recurrence, mortality, surgery, radiotherapy, pathology, immunohistochemistry, subcutaneous adipose tissue, prognosis, treatment outcome and follow-up. Non-MeSH terms were cutaneous, subcutaneous, prognostic factor, treatment and clinicopathologic. Articles were included in this systematic review if they met the following inclusion criteria: prospective or retrospective, randomized or not, case series involving human patients with superficial LMS. Case reports or case series with fewer than 10 patients were excluded. An initial screening yielded a total of 494 articles, of which 18 potentially relevant articles were selected after evaluation of their titles and abstract. Full text articles were obtained and thoroughly evaluated. Of these, only 11 articles [6, 7, 12, 16-23] fulfilled the inclusion criteria and were subsequently included in the systematic review. However, some evident limitations found in the qualitative assessment for included articles.

 Due to the absence of large case series of superficial LMS, its rate of recurrence and metastasis, management and recommended follow-up periods have not been sufficiently evaluated. We obtained 112 cases of subcutaneous LMS and 313 cases of cutaneous LMS [6, 7, 12, 16-23] (HALL) *(Tables 1, 2)*. In this review we conclude that 36.63% of subcutaneous LMS recur, 43.23% metastasize, with a mortality rate of 37.82% after a mean follow up of 4.40 years. Cutaneous LMS has a recurrence rate of 24.40%, a metastasis rate of 4.22%, and shows a mortality rate of 3.33% after a mean follow-up of 3.45 years. So that, in cutaneous LMS, rates of recurrence, metastasis and mortality were 14.28-52.98, 0-14.28% and 0-23.52% respectively, while in subcutaneous LMS, they were 18.28-50%, 27.27-66.66% and 9.09-70% respectively.

In a recent study of 71 cases of superficial LMS [16], the mean time to first recurrence was 8.9 years (range, 7.5 months to 34 years) for cutaneous LMS. For subcutaneous LMS, it was 4.6 years (range, 2 months to 20 years). The 5-year overall metastasis rates for cutaneous and subcutaneous LMS were 12% and 51% respectively. However,

cutaneous LMS metastases have been described 15 years after the original resection [20] and after 20 years in subcutaneous LMS [16]. Also, the metastases were located in the skin in 76% of cases [16].

The data we obtained pertaining cutaneous LMS are in disagreement with a study that reviewed 84 cases of dermal or focal subcutaneous LMS, classified as atypical intradermal smooth muscle neoplasm [12]. These lesions showed a mean size of 1.3 cm, cellular atypia, a mitotic rate of 4.7 mitosis per high-power field, a specific smooth muscle phenotype and occasional necrosis. They followed-up 52 of their 84 cases for 4.25 years and had 18 recurrence cases but no evidence of metastasis. The authors argued that the term sarcoma was therefore inappropriate since these lesions did not appear to carry any evident risk of metastasis. Despite this, the morphological features of the atypical intradermal smooth muscle neoplasms corresponded to high and low grade LMS. We believe, however, that, in the light of our review, LMS located in the dermis are indeed malignant lesions and therefore the term atypical intradermal smooth muscle neoplasm is not completely justified.

The rates of recurrence and metastasis of superficial LMS are associated to poor prognostic factors. Tumor size and cutaneous or subcutaneous location have been related to prognosis [6, 19, 22]. Cutaneous LMS averages 1.59 cm and is smaller than subcutaneous LMS, which has a mean size of 3.86 cm [5, 7, 12, 17, 19]. These facts may justify the worse prognosis for subcutaneous LMS. In a multivariate analysis of prognostic factors for superficial LMS, such as size, depth of invasion, histological grade and proliferative activity (Ki67), only tumor size was shown to be an independent prognostic factor in relation to decreased survival [19]. In this study and in a univariate analysis, a tumor size equal to or greater than 5 cm, deep location with fascia involvement and a high histological grade have been correlated with decreased survival. Additionally, in subsequent studies, histological grade II and III (according to the Fédération Nationale des Centres de Lutte le Cancer, or FNCLCC grading system) have been associated with recurrence and metastasis [17]. Although those superficial LMS that recur or that have a deeper location tend to have higher mitotic activity, the assessment of number of mitoses does not appear to be associated to prognosis [7, 17, 19, 24]. Also, there are a few studies which consider that the aneuploid DNA pattern could be a likely predictor of metastasis [25].

Recurrence is an important predictive factor of mortality from the disease, and is related to proper treatment [2, 4, 6, 25]. The treatment of choice for superficial LMS is wide local excision with margins ranging from 3 to 5 cm [26, 27], although more recent studies suggest that a minimum margin of 1 cm shows an increase in disease free survival [16, 21]. Also, micrographic Mohs surgery has shown a reduction in recurrence rates to 13%, whereas with conventional surgery they were 30-45% [27-29]. Mohs micrographic surgery may therefore be a reasonable treatment option in selected cases [16, 30]. In a revision encompassing 164 cases, radiotherapy was administered to 18 cases (10.97%), and chemotherapy to an even smaller percentage of participants [16, 17, 20, 21]. In general terms, the dosage and response to these treatments was not fully detailed. Due to the scarcity of studies and the paucity of information they report, the efficacy of radiotherapy and chemotherapy has not been fully established.

There is no unanimous agreement regarding the most suitable follow-up period for superficial LMS, since there are few studies with follow-ups longer than 5 years. In our study, the mean follow-up period for cutaneous LMS was 3.45 years (range, 2 months - 30 years), while in subcutaneous LMS it was 4.40 years (range, 1 months -16.5 years). According to our findings, it might be justified to monitor the superficial LMS for at least 5 years.

In summary, this literature review provides rates of recurrence, metastasis and mortality and follow-up periods in superficial LMS. We conclude that cutaneous LMS have a potential risk of metastasizing and in certain cases they may be the cause of death. The data collected suggest that the term "atypical intradermal smooth neoplasm" should be avoided to identify dermal LMS. Recurrence after incomplete excision, location (dermal *vs* subcutaneous), tumor size and histologic grade are the most relevant prognostic factors. We recommend that the follow-up for these lesions should be at least 5 years.

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FIGURES

Figure 1**a**. Nodular lesion of cutaneous leiomyosarcoma **b**. Cutaneous leiomyosarcoma with complete excision. (Hematoxilin & Eosin, x1 original magnification) **c.**

Subcutaneous leiomiosarcoma side to the surgical margin. (Hematoxilin & Eosin, x2 original magnification)
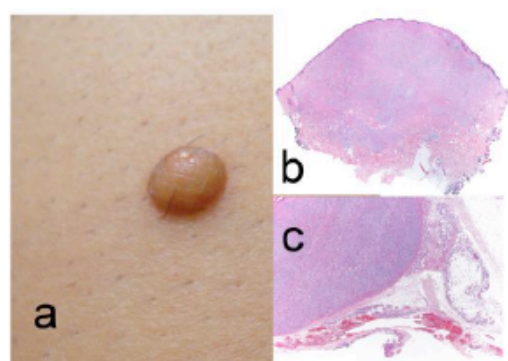
Table 1. Cutaneous leiomyosarcomas.

Authors	Nº of cases	Recurrence	Metastasis	Mortality	Follow-up (mean)
Winchester et al (2014)	48	18% Mean 8.9 y Range 7.5 m – 34 y	12% Mean 5.6 y Range 1 m – 26 y	6% NR NR	Mean 8 y
Deneve et al (2013)	33	33% NR NR	0%	0%	Mean 1.29 y Range 2 m – 4.08 y
Hall et al (2013)	19/20	10.52% NR NR	0%	0%	Mean 2.91 y Range 1 m – 10.33 y
Kraft et al (2011)	52/84	34.61% Mean 3.58 y Range 8m – 10 y	0%	0%	Mean 4.25 y
Massi et al (2010)	27/36	11.11% NR	3.70% NR	0%	Mean 3.41 y Range 2 m – 16 y

		Range 22 m – 7 y	NR		
Fauth et al (2010)	14	21.42% NR Range 1 m – 8 y	14.30 % NR NR	7.14% NR NR	NR NR
Kaddu et al (1997)	16/19	26.31% NR Range 8 m – 3 y	0 %	0 %	Mean 4 y Range 13 m – 9 y
Bernstein et al (1996)	21	14.28% NR NR	4.76% NR NR	0%	Mean 5.28 y Range 3.96 m – 10 y
Jensen et al (1996)	7	14.28% NR NR	0%	0%	Mean 3 y Range 5 m – 10 y
Fields et al (1981)	59/65	32% NR NR	0%	0%	Mean 3.90 y Range 2 m – 30 y
Dahl et al (1974)	17	52.94% NR NR	11.76% NR NR	23.50% NR NR	Mean 6 y Range 1 m – 16.5 y

NR: Not reported; y: years; m: month.

Table 2. Subcutaneous leiomyosarcomas.

Authors	N° of cases	Recurrence	Metastasis	Mortality	Follow-up (Mean)
Winchester et al (2014)	23	28% Mean 4.6 y Range 2 m –20 y	51% Mean 5.6 y Range 1	40% NR NR	Mean 8 y NR

			m – 26 y		
Fauth et al (2010)	11	18.18% NR Range 1 m – 8 y	27.27% NR NR	9.09% NR NR	NR NR
Bernstein et al (1996)	12/13	58.33% NR NR	66.66% NR NR	41.66% NR NR	Mean 4.33 y Range 1 m – 10 y
Jensen et al (1996)	34	35.29% NR NR	41.17% NR NR	41.17% Mean 4 y Range 4 m – 10 y	Mean 6 y Range 6 m – 10 y
Fields et al (1981)	12/15	50% NR NR	33.33% NR NR	25% NR NR	Mean 2.10 y Range 5 m – 7 y
Dahl et al (1974)	20	30% NR NR	40% NR NR	70% NR NR	Mean 6 y Range 1 m – 16.5 y

NR: Not reported; y: years; m: month.

Profs. F. Hernández and J.F. Madrid, Editors
School of Medicine
University of Murcia
30100 Espinardo – MURCIA
SPAIN   Fax: 34-868887508 / 34-868884150

Histology and
Histopathology
http://www.hh.um.es

e-mail: jfmadrid@um.es

Our reference: B-5778
Jose Aneiros-Fernandez
Departament of Pathology
University Hospital
Avd. Madrid S/N
18012 Granada
Email: janeirosf@hotmail.com

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Dear Dr. Aneiros-Fernandez,

*We are pleased to informe you that the paper entitled "Smoothelin and WT-1 expression
in glomus tumors and glomuvenous malformations" (authors: J.Aneiros-Fernandez,
JA.Retamero, H.Husein-ElAhmed, V.Carriel, F.OValle, J.Aneiros-Cachaza) is accepta-
ble for publication in histology and histopathology, but it is neccesary that you prepare
the paper according to the editor´s comments.*

Yours sincerely,

Prof. F. Hernández, Editor

TITLE: SMOOTHELIN AND WT-1 EXPRESSION IN GLOMUS TUMORS AND GLOMUVENOUS MALFORMATIONS

Running head: Expression of smoothelin and WT-1 in glomus lesions

Key words: WT-1; Smoothelin; glomus tumors; glomuvenous malformation; immunohistochemical.

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Authors:
Jose Aneiros-Fernandez MD
Juan Antonio Retamero MD
Husein Husein-ElAhmed MD[a]
Victor Carriel MD, PhD[b]
Francisco O'Valle MD PhD[c]
Jose Aneiros-Cachaza MD PhD[c]

Department of Pathology. Complejo Universitario de Granada. Granada. Spain.

[a]Department of Dermatology. Hospital de Baza. Granada. Spain

[b]Tissue Engineering Group. Faculty of Medicine, University of Granada and Instituto de Investigación Biosanitaria Ibs. Granada. Spain.

[c]Medicine Faculty. University of Granada. Spain.

Corresponding Author:

Dr. Jose Aneiros-Fernandez

Address: Departament of Pathology. University Hospital. Granada. Spain. Avd. Madrid S/N. CP: 18012. Email: janeirosf@hotmail.com

ABSTRACT

BACKGROUND: Smoothelin is a specific marker of contractile smooth muscle cells that has not been studied neither in glomus tumors nor in glomuvenous malformation. Also, Wilms tumor 1 (WT1), that is expressed in different tumors, has not been assessed in glomus lesions.

OBJECTIVE: We studied the significance of immunohistochemical expression of smoothelin and WT1 in 25 glomus lesions.

METHODS: We assessed 9 cases of solid glomus tumors (SGT), 8 cases of glomus tumors with vascular ectasia (VEGT), 2 cases of glomangiomyomas (GMM) and 6 cases of glomuvenous malformation (GM). Immunohistochemistry was performed, evaluating the expression of WT1, smoothelin, smooth muscle actin (SMA), smooth muscle myosin (SMM), h-caldesmon and desmin.

RESULTS: We have modified the results section of the abstract as follows: "Glomic cells showed cytoplasmic positivity for WT1 and smoothelin expression was present in all studied cases. SGT showed WT1 positivity in all endothelia. However, in VEGT and GMM, WT1 endothelial expression was positive in some areas, but not in others. GM did not show endothelial cell positivity for WT1.

CONCLUSIONS: Smoothelin expression in glomic cells indicates that they are contractile smooth muscle cells, and thus its role in routine diagnosis should be considered. The absence of WT1 expression in the endothelium of the vascular structures of the GM is a differential characteristic between SGT, VEGT and GMM.

INTRODUCTION

Glomus lesions that reproduce the neuromyoarterial glomus (glomus body), comprise three variants: solid glomus tumor (SGT), glomuvenous malformation/glomangioma (GM) and glomangiomyoma (GMM) (Gomos et al., 2008)."The World Health Organization (WHO) includes glomangioma as a variant of the typical glomus tumor. However, we believe this is misleading, given that glomangioma is in fact a glomovenous malformation, and this is better reflected in the classification proposed by the International Society for the Study of Vascular Anomalies (ISSVA) (Fletcher CDM et al., 2013; Wassef M et al., 2015).

The glomus tumor is a benign lesion that appears in young adults as a nodule or red-blue papule, usually located in the distal portion of the extremities, and may be painful in response to changes in pressure or temperature (Tomak Y et al., 2007). The glomuvenous malformation usually appears in children and adolescents, tends to present as bluish-red nodules or multiple papules that may be scattered or forming converging plates (Iqbal A et al., 1998; Requena et al., 1998; Monteagudo et al., 2007).

 GM that can be inherited in an autosomal dominant pattern with incomplete penetrance and variable expressivity, associated to a mutation of the GLMN gene, located in chromosome 1p21-p22 (Bomm et al., 2004; Brouillard et al., 2005). Glomulin, its gene product, is expressed in vascular smooth muscle cells and seems to be implicated in there late–stage maturation.

GMM are glomus lesions with smooth muscle differentiation that have been considered as a variant of SGT or GM (Ezinguer and Weiss, 2014).

The glomus tumors and malformations present immunoreactivity for smooth muscle differentiation markers, being the most used smooth muscle

actin (SMA), muscle specific actin and h-caldesmon (Boon et al., 2004; Gombos et al., 2008; Mravic et al., 2015). However, few studies exist assessing cytoplasmic expression of smoothelin and Wilms tumor 1 (WT1) in glomus cells (Wong et al., 2014; Galfione et al., 2014; Borroni et al., 2014). Smoothelin is a protein specifically expressed in smooth muscle cells, and is used as a marker to assess contractile function. It has two tissue-specific isoforms; the long isoform is exclusive to vascular muscle cells (Krämer et al., 1999; Krämer et al., 2001;Niessen et al., 2005). However, the expression of smoothelin in smooth muscle tumors is little known (Coco et al., 2009; Wong et al., 2014).

WT1 was originally described as a tumor suppressor gene based on its mutational inactivation in a subset of Wilms tumor, with a traditional nuclear staining pattern (Haber et al., 1990; Anuchapreeda et al., 2006). More recently, the cytoplasmic expression of WT1 was demonstrated in a variety of tumors of the gastrointestinal tract, lung, breast, bladder, soft tissues, etc (Nakatsuka et al., 2006; Trindade et al., 2011; Galfione et al., 2014;).

The aim of this study is to assess the expression of WT1 and smoothelin in SGT, GMM and GM, and its potential diagnostic role.

MATERIAL AND METHODS

A total of 25 cases of glomus tumor and malformations, were selected and included in our study. The cases were obtained from the routine caseload of the Provincial Clinical Intercenter Management Unit of Pathology, Granada, Spain.

Histopathological and immunohistochemical studies

For histopathological analysis, skin samples fixed in 10% buffered formalin for 24 hours, dehydrated with alcohol, embedded in paraffin in an automatic tissue processor Excelsior ES (Thermo Scientific, CA, USA). 4 micron sections were stained with hematoxylin and eosin (H&E). Histopathological changes were graded on a 0-2 scale in a blinded manner.

For Immunohistochemical analysis, sections were dewaxed, hydrated, and heat-treated in 1 mM EDTA pH 8 for antigenic retrieval using a PT module (Thermo Fisher Scientific Inc., Waltham, MA) at 95°C for 20 minutes. These sections were incubated for 10 min at room temperature with prediluted monoclonal antibodies against smooth muscle actin (SMA) (1A4), h-caldesmon (H-CD) and smooth muscle myosin (SMM) (SMMS-1), desmin (D33) WT1 (6F-H2) and smoothelin (R4A). All antibodies used were supplied by Master Diagnostica, Granada, Spain. An appropriate isotype for each antibody was used as negative control.

The immunohistochemical staining was conducted in an automatic immunostainer (Autostainer 480, LabVision Fremont, CA) using the micropolymer-peroxidase-based method (Ultravision Quanto, Master Diagnostica, Granada, Spain), followed by development by diaminobenzidine. The degree of immunoreactivity for SMA, SMM, h-caldesmon, desmin, smoothelin and WT1 in the glomus cells was assessed in a semiquantitative manner, using a scale of 0 to 3: 0 [absence], 1 [<25% positive cells], 2 [25-75% positive cells], 3 [>75% positive cells]. The level of immunoreactivity for WT1 in the endothelial cells was also assessed in a semiquantitative manner, but using a scale of - to ++: - [Negativity in all endotelial cells], + [Positive and negative endothelial cells], ++ [Positivity in all endothelial cells]

Statistical analyses

A statistical software package SPSS 20.0 (IBM Inc., Chicago, IL) was used for the statistical analysis. Kruskal-Wallis and Mann Whitney U-test for the analysis of non-parametric variables to analyze the differences in morphological and immunohistochemical variables between different glomus lesions were used. Spearman coefficient (rho) test to analyze the correlation between variables was also used. A P-value of 0.05 was accepted for statistical significance threshold.

RESULTS

Clinical Findings

Glomic tumor patients had a mean age of 61 ± 12 years with an age range between 32 to 77 years, showing a male gender predominance (13M/4F). The lesions were more frequently located in the upper limb (11/17). Patients with glomovenous malformation had an average age of 11,16 ± 2, with a male gender predominance (5M/1F), and the lesions were also more frequently located in the upper limb (5/6) (Table 1).

Pathology Analysis

The lesions studied had a mean size of 0.5 ± 0.21 cm and ranged from 0.20 to 1 cm. They were solitary in all cases and located in the dermis (48%), dermoepidermal junction (28%) and hypodermis (24%). Histopathological diagnosis was made according to the amount and distribution of glomus cells, the presence of vascular channels and the typical smooth muscle differentiation. Thus the diagnosis of SGT was made when the lesions were well delimited with diffuse pattern and showed vascular structures that, when

ecstatic, were classified as glomus tumors with vascular ectasia (VEGT) (Figure 1A and 1B). One case of SGT showed myxoid component. The GMM had the same morphological features as VEGT but had smooth muscle areas that connected with vascular structures (Figure 1D). GM had irregular margins and showed vascular channels that were surrounded by several layers of glomus cells (Figure 1C). We thus classified our 25 lesions in four histopathological groups: SGT (9/25), VEGT (8/25), GMM (2/25) and GM (6/25).

Immunohistochemical analysis

The immunohistochemistry results are displayed in Table 1. All cases showed intense cytoplasmic positivity for SMA, SMM and h-caldesmon (Figure 2A). Smoothelin also showed cytoplasmic positivity though less intensely in GM (Figure 2B-D). Desmin showed only focal positivity in those areas with the typical smooth muscle differentiation of GMM. In all cases, WT1 is expressed in the cytoplasm of the glomus cells, with lesser expression in GM (Figure 3). Positive staining for WT1 in endothelial cells of the vascular structures of SGT was present in all cases. (Figure 3A). WT1 expression in EVGT and GMM was observed in the majority of endothelial cells, but in some of them was negative (Figure 3B,C). In GM, endothelial WT1 expression was negative in all instances (Figure 3D).

Statistical analyses

Of all the markers studied, only the cytoplasmic positivity for WT1 (p=0.010), smoothelin (p=0.049) and endothelial cytoplasmic positivity for WT1 (p=0.000) were statistically significant (Kruskall-Wallis test). GM had lesser cytoplasmic expression of WT1 and smoothelin (Figure 4). Also, no endothelial WT1 positivity was observed in GM. Cytoplasmic expression in glomics cells of

WT1 was positively correlated with the endothelial expression of WT1

(Spearman's rank correlation coefficient, rho=0.475) and smoothelin expression

(rho=0.476) (p = 0.016).

DISCUSSION

We assessed smoothelin expression in normal skin, and found

cytoplasmic positivity in the smooth muscle cells of the deep vascular plexus

and the erector muscle of hair, not appreciating neuromyoarterial glomus in the

skin biopsies studied (Aneiros-Fernandez et al., 2011). We previously

discussed smoothelin positivity in cutaneous hamartoma (Espiñeira-Carmona et

al., 2012). There is only one previous report describing weak cytoplasmic

reactivity for smoothelin in four of the seven glomic tumors studied (Wong et al.,

2014). However, all lesions of our case series presented variable cytoplasmic

positivity for smoothelin, being this reactivity more evident in those glomic cells

closer to the vessels in GM. This positivity indicates that they are cells with

contractile smooth muscle phenotype. SMA, SMM and h-caldesmon were more

intense than smoothelin. However, in our experience, smoothelin is the most

specific smooth muscle differentiation marker, so It could be routinely used in

the diagnosis of glomus lesions. Desmin was negative in the glomus cells of all

cases studied, although presented only focal positivity in areas with typical

smooth muscle differentiation of GMM. The expression of desmin in this study is

consistent with previous reports (Dervan et al., 1989; Mentzel et al., 2002).

Some authors have recently described GM associated with prominent smooth

muscle component and eccrine glands (Borroni et al., 2014). However, these

finding shave not been observed in our work.

Two previous studies have reported WT1 expression in glomic tumors, showing focal weak staining in 2/8 cases and 2/2 cases of GM, but this finding has not been fully explained. (Galfione et al., 2014; Borroni et al., 2014).

In the present study, we show cytoplasmic positivity for WT1 in glomus cells in all of our cases. WT1 has a pattern of nuclear staining that is usually seen in Wilms tumor, acute leukemia, ovarian and urothelial carcinoma, desmoplastic small round cell tumor, leiomyosarcoma, etc. It shows a cytoplasmic pattern in different carcinomas, such as lung, breast, kidney etc., as well as soft tissues tumors (rhabdomyosarcoma, angiosarcoma, angiomas, etc.) (Anuchapreeda et al., 2006; Nakatsuka et al., 2006; Lee et al., 2009; Trindade et al., 2011; Galfione et al., 2014). An explanation for the cytoplasmic WT1 staining may lay in its presence as a major component of polysomes as a cytoplasmic translational regulator (Galfione et al., 2014). The importance of WT1 in the SGT, VEGT, GMM and GM may be due to the fact that WT1 plays an important role in angiogenesis, regulating vascular endothelial growth factor, angioproteins and nestin (Mokry et al., 2004; Cohen et la., 2006; Small et al., 2006; Hanson et al., 2007). It is also considered that WT1 may be involved in vascular smooth muscle proliferation (Small et al., 2006). We have also observed cytoplasmic endothelial expression of WT1 in the tumors studied. However, in VEGT and GMM, endothelial WT1 was negative in some vascular structures. Also, immunostaining for WT1 in the endothelium of the vascular channels of the GM was negative in all cases. There are few studies of endothelial WT1 expression in benign vascular tumors and vascular malformations showing endothelial positivity in tumors and negativity in malformations, except for arteriovenous malformations that were positive, and

vascular malformations with re-endothelialized neovessels within thrombi (Lawley et al., 2005; Al Dhaybi et al., 2010; Trindade et al., 2011). The positivity for WT1 in arteriovenous malformations could be considered in relation to the proliferative stage of the malformation. Thus, endothelial positivity for WT1 is related to the ability of endothelial cells to remodel and proliferate, while negativity for WT1 would indicate that endothelial cells are static and hence do not have the capacity to proliferate, as would occur in malformations. The inhibition or loss of the endothelial ability to proliferate could happen in some VEGT or GMM endothelial cells that present some vessels with WT1 negative endothelia. Taking these facts into account, the assessment of WT1 expression in vascular endothelial cells of the glomus lesions may help in the differential diagnosis of glomus tumors and malformations.

The classification of glomus tumors in the literature is controversial, WHO include glomangioma as a type of glomus tumor, whereas ISSVA consider glomangioma to be a glomangiovenous malformation (Fletcher CDM *et al.,* 2013; Wassef M *et al.,* 2015).

To avoid confusion, the term glomangioma should not be used as a synonym for glomangiovenous malformation. We propose that the term glomangioma should apply to those lesions similar to the solid glomus tumor with vascular ectasia and with WT1 expression in the majority of the endothelial component. Conversely, the term glomuvenous malformation would correspond to glomus lesions with WT1 negativity in endothelial cells of the vascular channels.

In summary, in this study we describe that smoothelin expression indicates that glomic cells are smooth mucle cells with contractile capability, and

should be used in the routine diagnosis. We also demonstrate the presence of WT1 in glomus cells, that may be implicated in cell proliferation and angiogenesis of glomus lesions. Furthermore, we show that the absence of WT1 expression in endothelial cells differentiates malformations from glomus tumors. Thus, the expression of smoothelin and WT1 help to better define the diagnosis of glomus lesions.

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Table 1. Clinical features and results of immunohistochemical assessed on glomus tumors and glomuvenous malformation.

Cases	Age (year)	Sex	Location	Clinical Lesions	Family History	Pain	Clinical appearance	Diagnosis	SMA	SMM	h-caldesmon	Desmin	Smoothelin	WT1	WT1 (Endothelial cells)
1	56	M	Thigh	Solitary	-	+	GT	SGT	3*	3	3	0	1	3	++
2	77	F	Forearm	Solitary	-	+	GT	SGT	3	2	3	0	1	3	++
3	60	M	Leg	Solitary	-	+	Leimyoma	SGT	3	3	3	0	1	3	++
4	61	F	Hand	Solitary	-	+	GT	SGT	3	3	3	0	1	2	++
5	77	F	elbow	Solitary	-	+	Keratosis	SGT	3	3	3	0	2	1	++
6	64	M	Hand	Solitary	-	NR	GT	SGT	3	3	3	0	2	3	++
7	37	M	Shoulder	Solitary	-	+	Leiomyoma	SGT	3	3	3	0	1	3	++
8	54	M	Leg	Solitary	-	+	Leiomyoma	SGT	3	3	3	0	3	3	++
9	58	M	Forearm	Solitary	-	+	GT	SGT	3	3	3	0	1	2	++
10	56	M	Arm	Solitary	-	+	GT	VEGT	3	3	3	0	2	3	+
11	62	M	Thigh	Solitary	-	+	Hemangioma	VEGT	3	3	3	0	2	3	+
12	74	F	Forearm	Solitary	-	+	GT	VEGT	3	3	3	0	1	3	+
13	56	M	Forearm	Solitary	-	+	GT	VEGT	3	3	3	0	3	3	+
14	37	M	Thigh	Solitary	-	NR	GT	VEGT	3	3	3	0	3	3	+
15	46	M	Hand	Solitary	-	+	Leimyoma	VEGT	3	3	3	0	3	3	+
16	50	M	Hand	Solitary	-	NR	Hemangioma	VEGT	3	3	3	0	2	3	+
17	32	M	Forearm	Solitary	-	+	GT	VEGT	3	3	3	0	1	1	+
18	37	M	Thigh	Solitary	-	+	GT	GMM	3	3	3	1	1	3	+
19	69	M	Forearm	Solitary	-	NR	Hemangioma	GMM	3	3	3	1	3	3	+
20	13	M	Forearm	Multiple	+	NR	Malformation	GM	3	3	3	0	1	1	-
21	11	F	Arm	Plaque-like	-	-	GM	GM	2	2	2	0	1	2	-
22	1	M	Forearm	Multiple	-	-	VM	GM	3	3	3	0	1	2	-
23	16	M	Thigh	Plaque-like	-	+	VM	GM	3	3	3	0	1	2	-
24	8	M	Arm	Plaque-like	+	-	GM	GM	3	3	3	0	1	1	-
25	18	M	Arm	Plaque-like	-	+	NR	GM	3	3	3	0	1	1	-

M, Male; F, Female; SGT, Glomus Tumor with solid pattern; VEGT, Vascular Ectasia Glomus Tumor; GMM, Glomangiomyoma; GM, glomuvenous malformation; VM, Venous Malformation; NR, Not report; SMA: Smooth muscle actin; SMM: Smooth muscle myosin WT1: Wilms Tumor
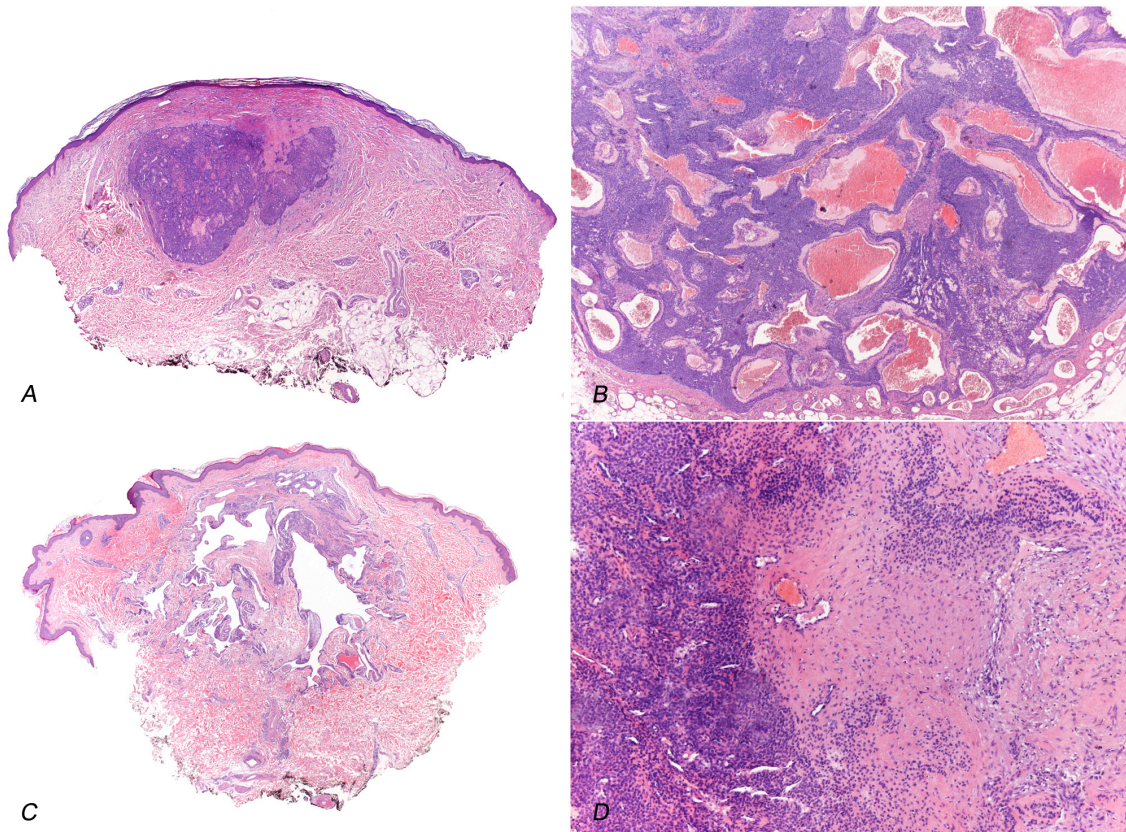
Figure 1. A. Solid glomus tumor with diffuse pattern located in the dermis (Hematoxilin & Eosin, Original magnification: x 1). B. Glomus tumor with vascular ectasia (Hematoxilin & Eosin, Original magnification: X 2). C. Glomuvenous malformation in dermis with vascular channels (Hematoxilin & Eosin, Original magnification: x 1). D. Glomangiomyoma with proliferation of smooth muscle cells and glomus cells (Hematoxilin & Eosin, Original magnification: x4).
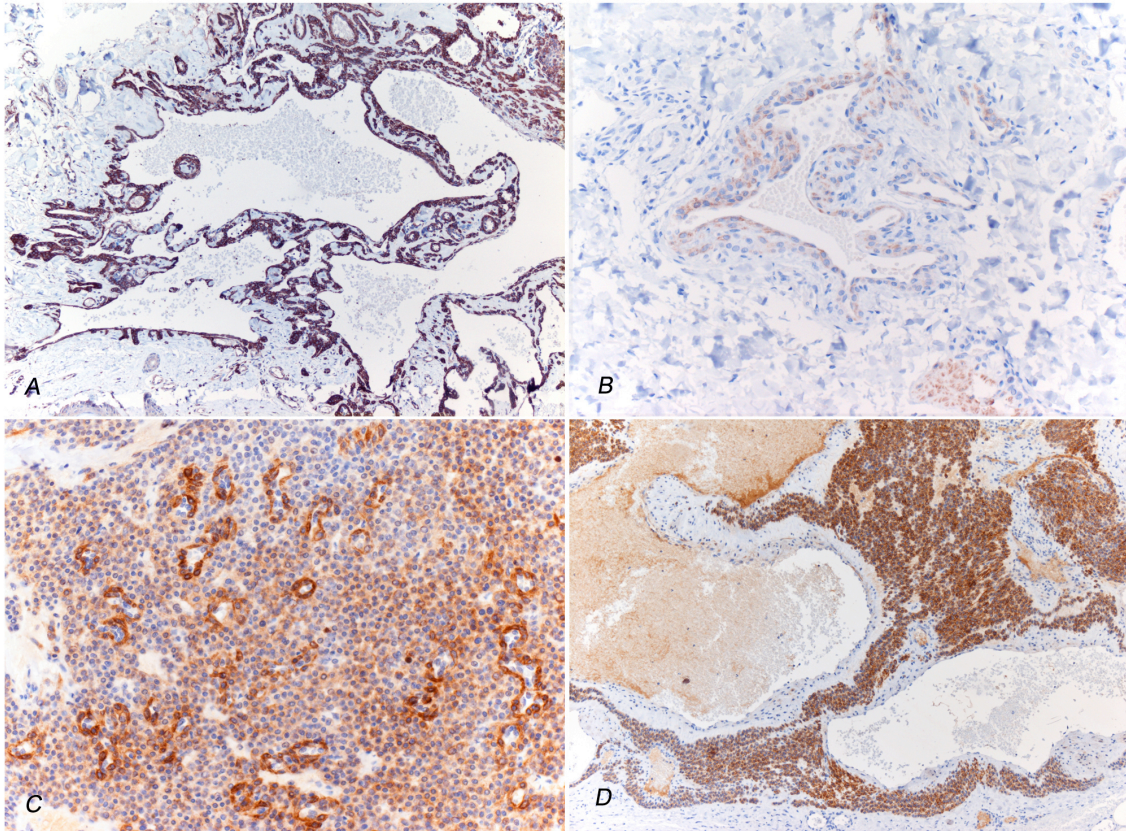
Figure 2. A. Glomuvenous malformation positive for H-caldesmon in glomus cells (H-Caldesmon, Original magnification: x2). B. Glomuvenous malformation with low positive smoothelin in glomus cells (Smoothelin, Original magnification: x10). C. Solid glomus tumor with smoothelin positive in glomus cells (Smoothelin, Original magnification: x10). D. Glomus tumor with vascular ectasia with smoothelin expression in glomus cells (Smoothelin, Original magnification: x 4).
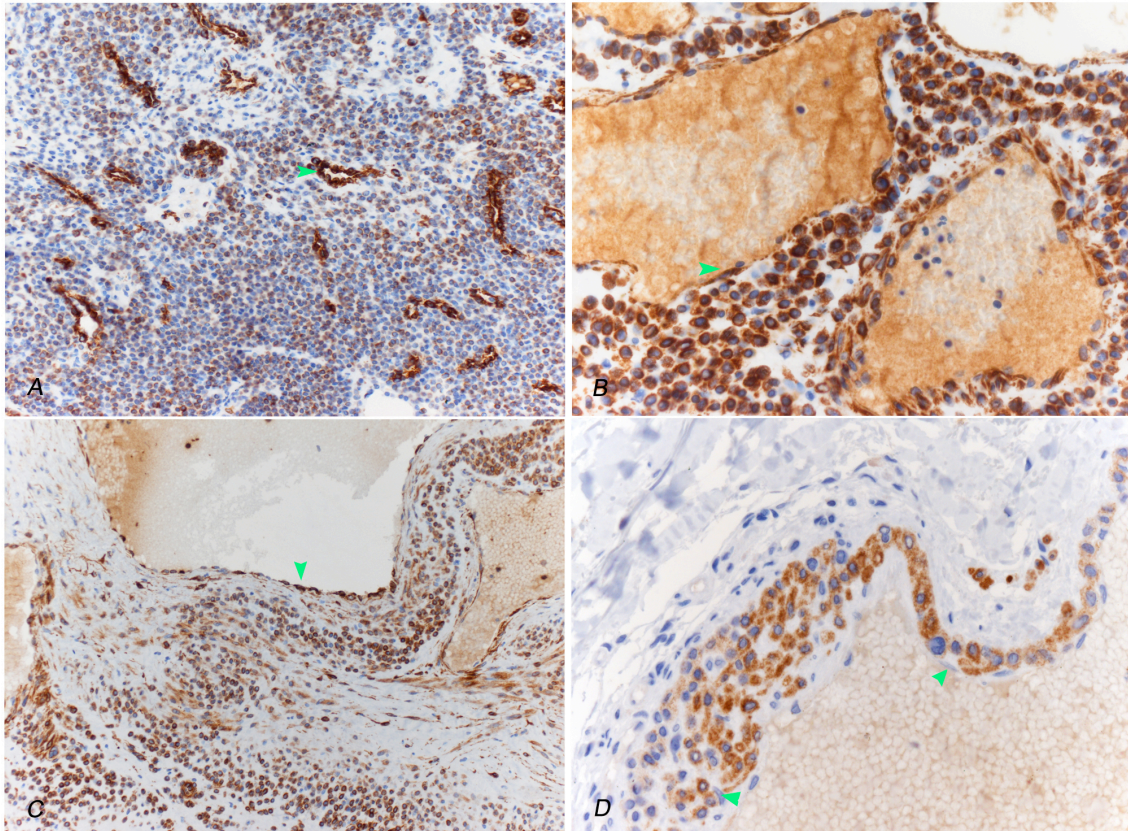
Figure 3. A. Solid glomus tumors with WT-1 expression in glomus cell and endotelial cells (Green arrow) (WT-1, original magnification: x10). B. Glomus tumor with vascular ectasia with WT-1 positivity in glomus and endotelial cells (Green arrow) (WT-1, original magnification: x20). C. Glomangiomyoma with expression in glomus cells, smooth muscle cells and endotelial cells (Grees arrow) (WT1-1, Original magnification: x10). D. Glomuvenous malformation positive for WT-1 in the glomus cells and negative for endotelial cells (Green arrow) (WT1, Original magnification: x20).
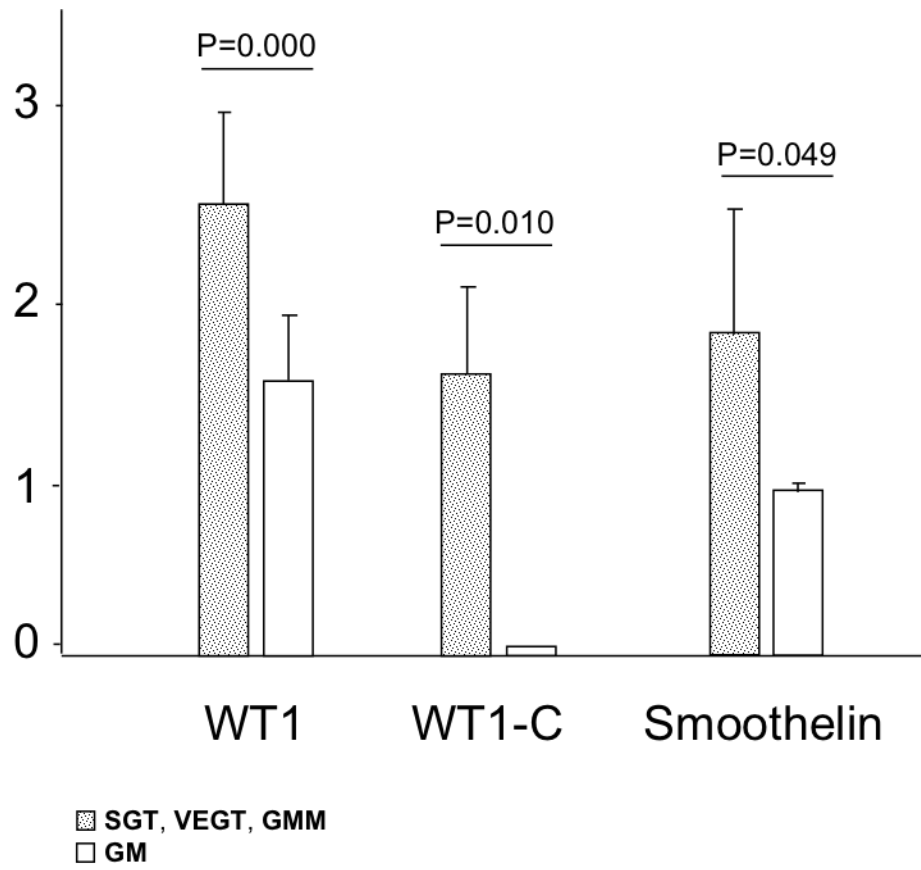
Figure 4. Comparison between the semiquantitative assessment of the

expression of GM vs SGT, GMM, VEGT (Mann-Whitney).

FW: Submission Confirmation for Estudio inmunohistoquímico de marcadores de diferenciación muscular lisa en los tumores y malformaciones vasculares de la piel

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To: janeirosf@hotmail.com
Date: Sat, 26 Sep 2015 08:29:41 -0400
Subject: Submission Confirmation
Dear Dr. Aneiros-Fernandez,

Your submission entitled " Estudio inmunohistoquímico de marcadores de diferenciación muscular lisa en los tumores y malformaciones vasculares de la piel "

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TÍTULO: ESTUDIO INMUNOHISTOQUÍMICO DE MARCADORES DE

DIFERENCIACIÓN MUSCULAR LISA EN LOS TUMORES Y

MALFORMACIONES VASCULARES DE LA PIEL

Authors:
Jose Aneiros-Fernandez MD
Juan Antonio Retamero MD
Husein Husein-ElAhmed MD[a]
Victor Carriel MD, PhD[b]
Francisco O'Valle MD PhD[c]
Jose Aneiros-Cachaza MD PhD[c]

Department of Pathology. Complejo Universitario de Granada. Granada. Spain.

[a]Department of Dermatology. Hospital de Baza. Granada. Spain

[b]Tissue Engineering Group. Faculty of Medicine, University of Granada and Instituto de Investigación Biosanitaria Ibs. Granada. Spain.

[c]Medicine Faculty. University of Granada. Spain.

Corresponding Author:

Dr. Jose Aneiros-Fernandez

Address: Departament of Pathology. University Hospital. Granada. Spain. Avd. Madrid S/N. CP: 18012. Email: janeirosf@hotmail.com

INTRODUCCIÓN

En la piel normal pueden observarse anomalías vasculares tumorales y malformaciones. El diagnóstico diferencial morfológico no siempre es idóneo y exige para ello una buena correlación clínico patológica. Los estudios inmunohistoquímicos que se han realizado de estos procesos van encaminados a demostrar marcadores que ayudan en el diagnóstico, como la expresión de Glut-1 y WT-1 etc (1,2). Sin embargo, los estudios de marcadores de diferenciación muscular lisa en los procesos vasculares son escasos. De esta manera, la expresión de alfa actina músculo liso se ha utilizado para valorar pericitos y no células musculares lisas (3,4). La esmotelina que demuestra células musculares lisas bien diferenciadas y con capacidad contráctil ha sido estudiada en malformaciones vasculares cerebrales pero no en anomalias vasculares de la piel (5).

En el presente trabajo se pretende determinar el fenotipo muscular liso en los tumores y malformaciones vasculares de la piel, con el objeto de demostrar en cuales persiste la capacidad contráctil.

MATERIAL Y METODOS

Se ha realizado una revisión de 25 casos de lesiones vasculares procedentes de la Unidad de Gestíon Clínica de Anatomía Patológica de Granada (España). Todos los casos se diagnosticaron, siguiendo las directrices de la clasificación de la Sociedad Internacional del Estudio de Anomalías Vasculares (2014)(6) De esta manera, agrupamos nuestros casos en hemangioma infantiles (4), granulomas piógenos (6), angiomas en penacho (4), malformaciones arteriovenosas (6) y malformaciones venosas (5).

Las muestras de los casos estudiados se fijaron en formol al 10%, siendo procesadas rutinariamente e incluidas en parafina. Asimismo, se realizaron secciones de 4 micras que se tiñeron con HE, Para el estudio inmunohistoquímico, las secciones del tejido en parafina fueron incubados con anticuerpos para actina músculo liso (AML) (1A4), miosina músculo liso (MML) (SMMS-1), desmina (D33), h-caldesmon (H.CD), WT1 (6F-H2) y esmotelina R4A). Todos los anticuerpos utlizados proceden de Master Diagnóstica, Granada, España. Asimismo se utiliza el sistema de inmunotinción autostainer 480, Labvisions Fremont, Ca. La valoración fue semicuantitativa y de la siguiente manera: negativo (-) y positivo (+)   en los vasos sanguíneo difuso y focal. Para WT1 se establece una escala de negativo (-) y positivo focal o difuso (+) en las células endoteliales.

RESULTADOS

Los resultados de la inmunohistoquímica quedan detallados en la tabla 1. La expresión de WT1 es positiva difusamente en las células endoteliales de los tumores (figura 1B), y las malformaciones arteriovenosas, siendo negativa en la malformación venosa. Los hemangiomas infantiles y los granulomas piógenos son positivos para AML y MML (figura 1A). El h-caldesmon se expresa focalmente en el granuloma piógeno (Figura 1C). Los angiomas en penacho fueron positivos únicamente para AML (figura 1D). En las malformaciones arteriovenosas y venosas se evidenciaron capa muscular positiva para AML, MML y h-caldesmon (Figura 2 A y C), mientras que la esmotelina mostró positividad focal (Figura 2B y D).

DISCUSIÓN

Los procesos vasculares estudiados los agrupamos en tumores y malformaciones vasculares, teniendo en cuenta la expresión de WT1 en endotelio de las estructuras vasculares como se había descrito en estudios previos (2). WT1 es un marcador de células endoteliales proliferativas y de endotelios normales. La negatividad de WT1 en endotelio vascular de las malformaciones indicaría ausencia de proliferación. No obstante, en la malformación arteriovenosa ya se ha descrito positividad para WT1, al igual que nuestro estudio (2). Sin embargo, positiva posiblemente está en relación con el estadio evolutivo de la malformación (estadio II). Se desconoce la expresión de WT1 en estadio I (2).

La esmotelina es una proteína del citoesqueleto que es específica de las células musculares lisas diferenciadas. Esta proteína tiene una isoforma A que se expresa en las células musculares lisas de los órganos, y una isoforma B que se expresa en las células musculares lisas de los vasos sanguineas (7,8). Se ha descrito que la pérdida de positividad para esmotelina se asocia con disminución de la capacidad contráctil de las células musculares lisas. También se ha establecido que esta proteína aparece tardíamente el desarrollo cuando las células musculares lisas adquieren capacidad contráctil (9). El presente estudio evidenciamos algunas estructuras vasculares con células musculares lisas positivas para esmotelina y que corresponden a malformaciones arteriovenosas y venosas. No obstante, se observan vasos sanguíneos con células musculares lisas que fueron negativas para la esmotelina y positivas para AML, MML y h-caldesmon. Este hecho indica que la esmotelina no se expresa en las células musculares lisas inmaduras y que no han adquirido capacidad contráctil.

La desmina que fue positiva solo en 2 de las 5 malformaciones venosas se puede justificar por el hecho de que la desmina no es un marcador totalmente especifico de diferenciación muscular lisa, ya que se aprecia en células musculares estriadas, etc. En los procesos tumorales (hemangioma infantiles y granuloma biogénico) la AML y MML también son expresadas en pericitos y posiblemente en miopericitos y en células musculares inmaduras. No obstante, en los angiomas en penacho se evidenció positividad únicamente para AML que se expresa de manera específica en los pericitos. Dicha expresión habia sido previamente descrita (10). Sin embargo no se ha valorado en la literatura en la expresión de otros marcadores MML, que en nuestros casos fue negativa y que se considera es positiva en los pericitos.

Existe un estudio que valora la expresión de marcadores de diferenciación muscular lisa incluida la esmotelina en las malformaciones arteriovenosas y venosas intracraneales (5),. De esta manera la expresión de la esmotelina fue menor que la expresión de AML y MML. La positividad de la esmotelina era menos intensa en la malformación arteriovenosa que en las estructuras vasculares cerebrales normales, no evidenciandose expresión en la malformación cavernosa cerebral. Este hecho podría sugerir, que la pérdida de la propiedad contráctil podría estar asociada con modificaciones hemodinámicas. Nosotros consideramos que esto podría suceder en las malformaciones vasculares cutáneas.

En resumen, se aporta el estudio de 25 pacientes con tumores y malformaciones cutáneas, demostrándose presencia de células musculares lisas con capacidad contráctil únicamente en las malformaciones arteriovenosas y en menor proporción en las malformaciones venosas. Por el

contrario, las células musculares lisas de las estructuras vasculares que se observaban en los tumores, tenían pérdida de la capacidad contráctil.

Tabla 1. Estudio inmunohistoquímico de marcadores de músculo liso en los tumores y malformaciones vasculares cutáneas.

Diagnóstico/Nº Casos	AML Pericitos / CML	MML Pericitos / CML	Desmina Pericitos / CML	h-caldesmon Pericitos / CML	Esmotelina Pericitos / CML
Hemangioma infantil /4	+ / +	+ / -	- / -	- / -	- / -
Granuloma piógeno / 6	+ / +	+ / +	- / -	- / + (Focal)	- / -
Angioma en penacho / 4	+ / -	- / -	- / -	- / -	- / -
Malformación arteriovenosa / 6	+ / +	+ / +	- / -	- / +	- / + (focal)
Malformación venosa / 5	+ / +	+ / +	- / + (2/5)	- / +	- / + (Focal)

AML, actina de músculo lisa; MML, miosina de músculo lisa; CML, células musculares lisas.

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issues). Adv Dermatol. 1997;13:375-423.

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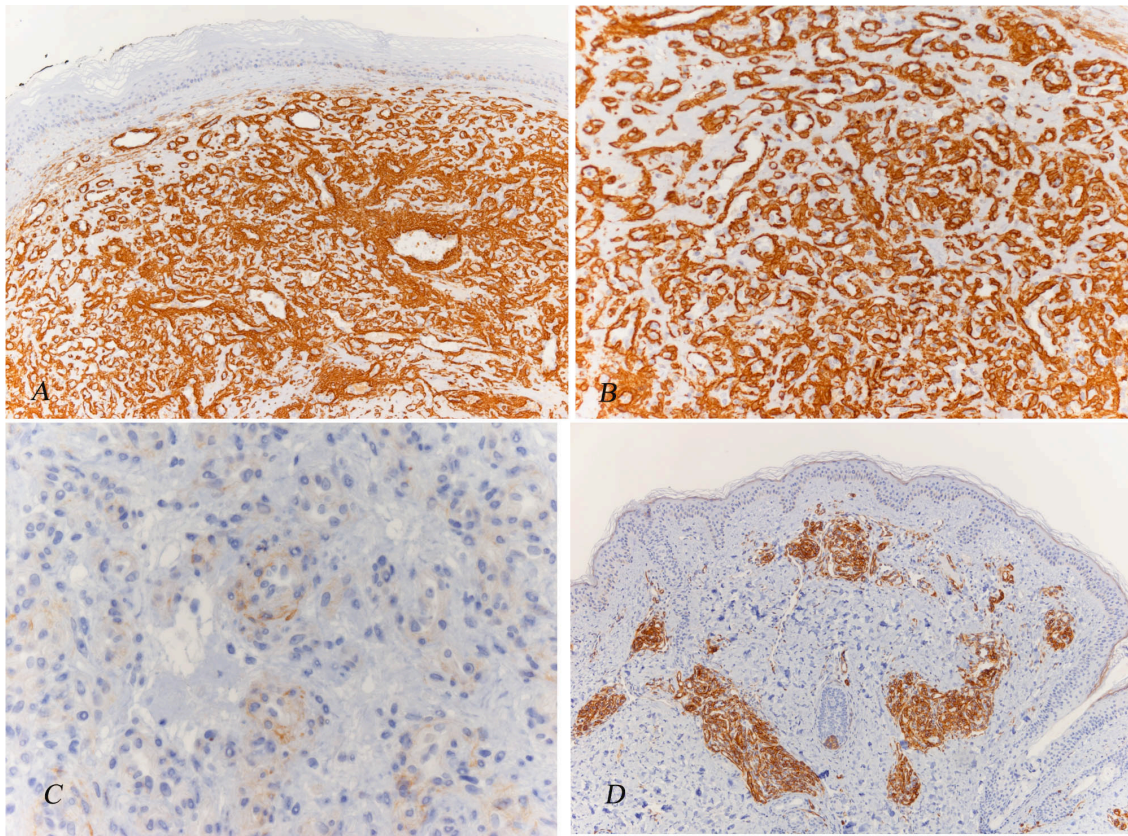
FIGURAS.

Figura 1. A. Granuloma piógeno con expresión de AML (X 4 aumento), y de WT1 (B) (x10 aumento). C. Granuloma piógeno con positividad focal para h-caldesmon (x20 aumento). D. Angioma en penacho con positividad para AML en los pericitos.
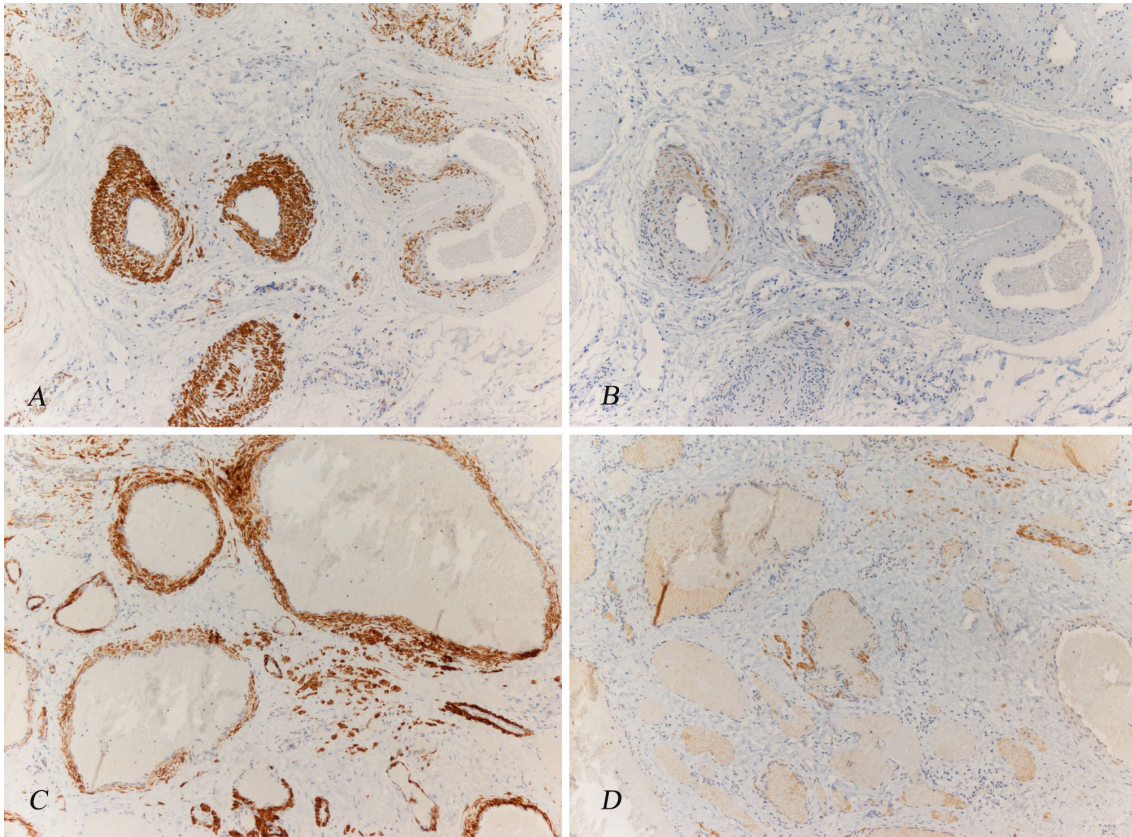
Figura 2. A. Malformación arteriovenosa con gruesa capa muscular positiva para h-caldesmon (x10 aumento) y con positividad focal para esmotelina (B) (x10 aumento). C. Malformación venosa con células musculares positivas para h-caldesmon y esmotelina (D) (x10 aumento).

CONCLUSIONES

CONCLUSIONES

1. La esmotelina en la piel normal a diferencia de otros marcadores de diferenciación muscular lisa, permite diferenciar el plexo vascular profundo del superficial.

2. La valoración de la expresión en estructuras musculares no vasculares extracutáneas permite concluir que el anticuerpo R4A demuestra células musculares lisas con capacidad contráctil en las estructuras musculares vasculares y extravasculares.

3. El concepto de neoplasia atípica intradérmica de músculo liso consideramos que es un término incorrecto para referirse a los leiomiosarcomas dérmicos.

4. La expresión nuclear de la esmotelina en los leiomiosarcomas es un hecho diferencial entre leiomiosarcomas y leiomiomas.

5. La ausencia de expresión de WT1 en los endotelios de las malformaciones vasculares permite establecer la diferencia con los tumores glómicos en general, y en particular a los que pueden simular a las malformaciones.

6. De las anomalías vasculares estudiadas, únicamente las malformaciones arteriovenosas y venosas cutáneas tienen células musculares lisas con capacidad contráctil. Este hecho podría estar en relación con cambios hemodinámicos fundamentalmente en la malformación arteriovenosa

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