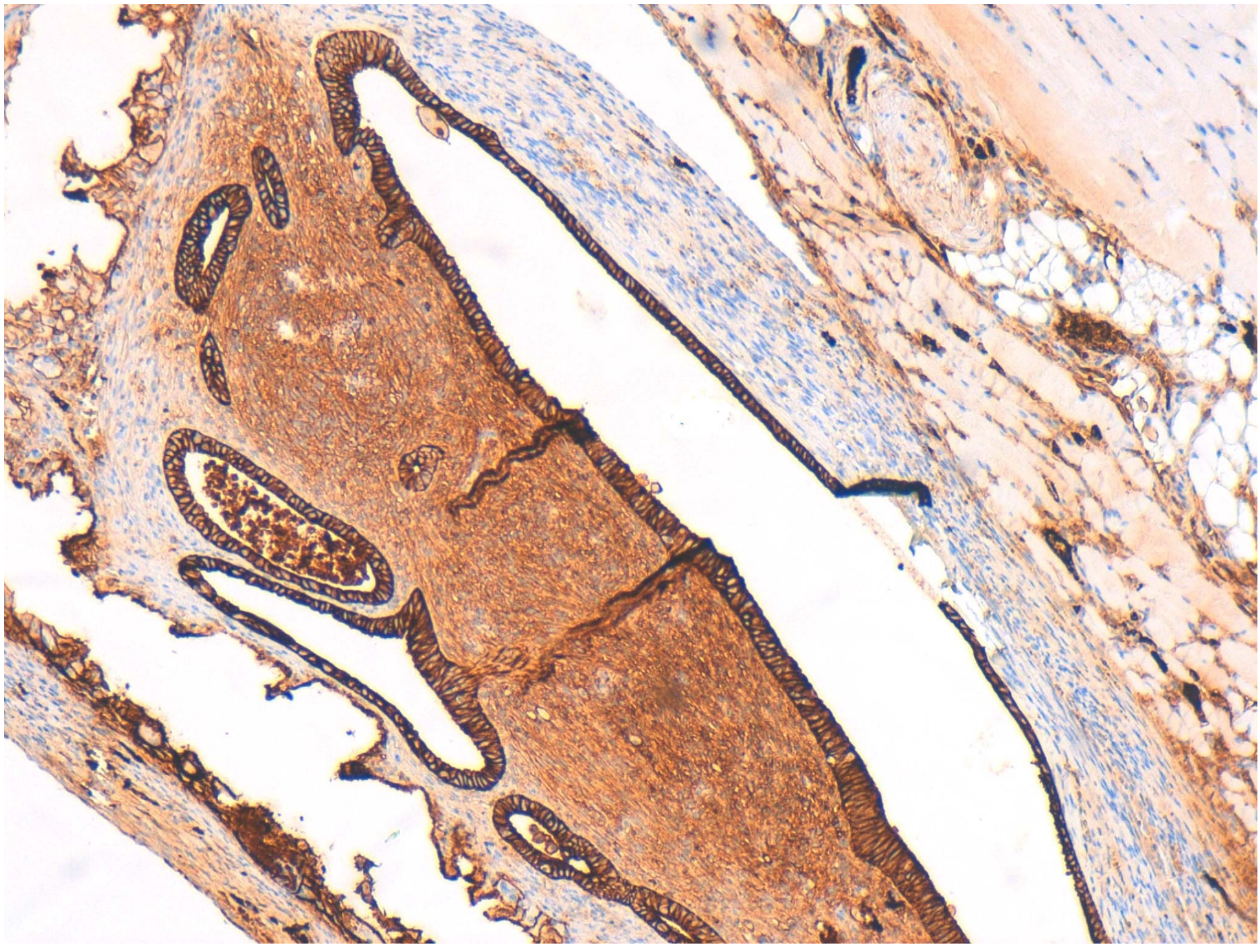
UNIVERSITAT DE BARCELONA

Nous marcadors clínics i tractament immunològic de l'endometriosi

Maria Francesca Perelló Serra



NOUS MARCADORS CLÍNICS I TRACTAMENT IMMUNOLÒGIC DE L'ENDOMETRIOSI

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Tesi Doctoral 2017 Universitat de Barcelona

Departament d'Obstetrícia, Ginecologia, Pediatria, Radiologia i Anatomia

Facultat de Medicina

NOUS MARCADORS CLÍNICS I TRACTAMENT IMMUNOLÒGIC DE L'ENDOMETRIOSI

Memòria presentada per

Maria Francesca Perelló Serra

per optar al grau de Doctor

per la Universitat de Barcelona

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Línia de recerca del grup investigador:

FETGE, SISTEMA DIGESTIU I METABOLISME:

ENDOCRINOLOGIA GINECOLÒGICA I REPRODUCCIÓ HUMANA

PRESENTACIÓ

La present tesi doctoral està estructurada seguint les directrius de la normativa per a la presentació de tesis doctorals com un compendi de publicacions, aprovada per la Comissió de Doctorat de la Facultat de Medicina el 28 de març del 2014.

Els estudis inclosos en aquesta tesi doctoral pertanyen a una mateixa línia d'investigació. Els resultats obtinguts gràcies a aquests estudis han aportat informació rellevant i innovadora sobre el tema i han estat recollits en 3 articles originals, publicats en diverses revistes d'àmplia difusió internacional.

PUBLICACIONS INTERNACIONALS INCLOSES A LA TESI DOCTORAL I PREMIS OBTINGUTS COM A CONSEQÜÈNCIA DEL TREBALL D'INVESTIGACIÓ

PUBLICACIONS

1. "MARKERS OF DEEP INFILTRATING ENDOMETRIOSIS IN PATIENTS WITH OVARIAN ENDOMETRIOMA: A PREDICTIVE MODEL" *Perelló M, Martínez-Zamora MA, Torres X, Munrós J, Llecha S, De Lazzari E, Balasch J, Carmona F. Eur J Obstet Gynecol Reprod Biol. 2015 Nov 26. doi: 10.1016/j.ejogrb.2015.11.024. [Epub ahead of print]*

   Factor Impacte: 1.695. Quartil: 3er quartil Obstetrics & Gynecology

2. "ENDOMETRIOTIC PAIN IS ASSOCIATED WITH ADENOMYOSIS, BUT NOT WITH THE COMPARTMENTS AFFECTED BY DEEP INFILTRATING ENDOMETRIOSIS" *Perelló M, Martínez-Zamora MA, Torres X, Munrós J, Balasch J, Carmona F. Gynecol Obstet Invest. 2016 Oct 7. [Epub ahead of print]*

   Factor Impacte: 1.672. Quartil: 3er quartil Obstetrics & Gynecology

3. "ORAL ADMINISTRATION OF PENTOXIFYLLINE REDUCES ENDOMETRIOSIS-LIKE LESIONS IN A NUDE MOUSE MODEL" *Perelló M, González-Foruria I, Castillo P, Martínez-Florensa M, Lozano F, Balasch J, Carmona F. Reprod Sci. 2016 Oct 12. pii: 1933719116673198. [Epub ahead of print]*

Factor Impacte: 2.429. Quartil: 2on quartil Obstetrics & Gynecology

PREMIS

Premi Fi de Residència Emili Letang concedit per l'Hospital Clínic de Barcelona l'any 2013 pel treball d'investigació: "EFECTO DE LA PENTOXIFILINA SOBRE LOS IMPLANTES DE ENDOMETRIOSIS INDUCIDOS EN UN MODELO ANIMAL DE RATÓN".

ABREVIATURES UTILITZADES EN AQUESTA TESI DOCTORAL

AMP: *Adenosine Monophosphate*

ASRM: *American Society of Reproductive Medicine*

CD: *Cluster of Differentiation*

CK: *Cytokeratine*

DIU: Dispositiu Intrauterí

EAPP: *Endometriotic Associted Pelvic Pain*

EO: Endometriosi Ovàrica

EP: Endometriosi Profunda

ESP: Endometriosi Superficial Peritoneal

GnRH: *Gonadotropin Releasing Hormone*

IC: Interval de Confiança

IL: Interleucina

LP: Líquid Peritoneal

mAb: *Mouse Monoclonal Antibody*

NK: *Natural Killer*

OR: *Odds Ratio*

RANTES: *Regulated upon Activation Normal T cell Expressed and Secreted*

RMN: Ressonància Magnètica Nuclear

TNFalfa: *Tumor Necrosis Factor* alfa

VEGF: *Vascular Endothelial Growth Factor*

ÍNDEX

1. INTRODUCCIÓ GENERAL, PLANTEJAMENT DEL PROBLEMA I JUSTIFICACIÓ DE LA TESI

1.1. INTRODUCCIÓ A LA MALALTIA

L'endometriosi és una malaltia crònica estrogen-dependent caracteritzada per la presència de teixit endometrial fora de la cavitat uterina, que afecta principalment els òrgans pèlvics, però també a altres òrgans a distància. Trobem tres formes diferents de presentació de la malaltia: endometriosi superficial peritoneal (ESP), endometriosi ovàrica (EO) i endometriosi profunda (EP). L'EP és el tipus d´endometriosi que sol tenir una presentació més agressiva, de manera que penetra més de 5 mm sota la superfície peritoneal. Es tracta d'una malaltia que sovint és multifocal i que afecta principalment la zona posterior de la cavitat abdominal i, amb freqüència, involucra els lligaments uterosacres i la zona retrouterina, així com la paret vaginal posterior i la paret rectal anterior. A més, la bufeta i el sistema urinari, entre altres òrgans, també es poden veure afectats (1,2). L'adenomiosi es defineix per la presència de glàndules endometrials i estroma dins el miometri. Alguns la consideren una entitat diferent a l'endometriosi, però en general s'accepta que comparteixen alguns símptomes i mecanismes patogènics i es presenten molt freqüentment de forma associada (3).

L'endometriosi és una malaltia ginecològica molt freqüent que afecta entre el 10-20% de les dones en edat reproductiva (4). S'estima que es dóna en el 26-39% de pacients estèrils primàries, del 4-65% en les dones amb àlgies pèlviques cròniques, del 7-32% en les pacients que consulten per dismenorrea i del 10-35% en pacients amb quists d'ovari

(5). Per tant cal destacar la importància sociosanitària que té, atès que molt freqüentment interfereix en la qualitat de vida de les pacients per la dismenorrea, dolor pèlvic crònic, disparèunia i fracàs reproductiu que la malaltia ocasiona, com a manifestacions clíniques més habituals.

Tot i la seva prevalença, l'etiopatogènia de la malaltia segueix sent poc clara. S´han suggerit diferents hipòtesis sobre el desenvolupament de l´endometriosi, sent la teoria de la menstruació retrògrada, descrita per Sampson en 1927, la més acceptada. Aquesta teoria proposa la disseminació de les cèl·lules endometrials a la cavitat peritoneal com un causa principal d'aparició de la malaltia (6).

El diagnòstic de la malaltia resulta difícil per la gran variabilitat de presentació dels símptomes, cosa que en ocasions representa un retard d'anys entre l'inici d'aquests i el diagnòstic. Tot i que el diagnòstic histològic és considerat el diagnòstic definitiu, els actuals avenços en les tècniques diagnòstiques ha permès que amb l'ús de l'ecografia transvaginal i de la ressonància magnètica nuclear (RMN) sigui suficient per dur a terme la detecció de la malaltia en la pràctica clínica (7,8).

Com que el ventall terapèutic mèdic disponible és limitat, rares vegades és un tractament definitiu, i els fàrmacs utilitzats es basen en tractaments hormonals que alteren el cicle ovàric interferint en la fertilitat, la cirurgia amb l'excisió dels implants endometrials és en molts casos el tractament a realitzar en pacients amb EP, tot i que quan la cirurgia conserva la funció ovàrica, la recidiva de la malaltia és freqüent (9). La cirurgia radical que inclou l'extirpació de tots els focus d´endometriosi juntament amb l´exèresi dels ovaris, representaria el tractament definitiu, motiu pel qual es considera

una malaltia altament frustrant per a la dona en edat reproductiva. A més, la malaltia té importants repercussions en la qualitat de vida familiar, social i professional de la pacient, i juntament amb el retard en el diagnòstic de la mateixa, ha portat a la creació d'associacions de dones afectades per endometriosi, tant a nivell mundial com a nivell espanyol, per donar suport a les pacients i als seus familiars.

Per tot això, el diagnòstic de la malaltia i la seva extensió prequirúrgica, juntament amb la investigació de noves línies de tractament que permetin preservar la fertilitat i evitar la cirurgia, representen actualment els dos reptes més importants de l'estudi de la malaltia.

1.2. ETIOPATOGÈNIA

Encara que la relació de l'endometriosi amb l'activitat hormonal ovàrica de la dona és àmpliament coneguda, l'etiologia i la patogènesi de la malaltia és encara controvertida.

Durant el segle passat es van desenvolupar diverses teories sobre aquest tema. La més acceptada és la teoria de la menstruació retrògrada amb la subsegüent implantació de les cèl·lules endometrials en la cavitat pelviana. Diferents tipus d'evidència donen suport a aquesta teoria, com el fet que en el 90% de les dones amb trompes permeables es pot trobar sang en el líquid peritoneal quan es fa una laparoscòpia durant la menstruació o com el fet que les cèl·lules endometrials que arriben a la cavitat peritoneal mantenen la seva viabilitat i capacitat d'implantació (10). No obstant això, altres defensen com a causa més probable l'origen *in situ* de l'endometriosi a partir de canvis metaplàsics de l'epiteli peritoneal o de l'epiteli germinal ovàric o a partir de la diferenciació de restes embrionàries, bé per estímuls hormonals o per estímuls químics

derivats de cèl·lules endometrials degenerades o fins i tot per factors tòxics ambientals (11).

Independentment de l'origen metaplàsic o implantatori de les cèl·lules endometrials ectòpiques, hi ha un interès creixent a conèixer els factors afavoridors de l'inici i del desenvolupament de la malaltia, ja que entre el 10 i 20% de les dones la pateixen (4). Múltiples factors (genètics, ambientals, inflamatoris i immunològics) han estat assenyalats com elements que predisposen algunes dones a patir endometriosi.

Dins d'aquests factors inductors o moduladors destaquen els factors immunològics, que han estat motiu d'estudi en els últims anys ja que juguen un paper essencial tant en l'etiopatogènia de l'endometriosi en si mateixa, com en l'esterilitat associada que comporta, fins al punt que alguns autors consideren l'endometriosi com una veritable malaltia de caràcter immunològic i plantegen tractaments específics amb immunomoduladors i moduladors de la inflamació (12-14). Per altra banda, el fet que existeixin estudis que demostren una alta concordança d´autoimmunitat (lupus eritematós sistèmic, artritis reumatoide, síndrome de Sjögren, malaltia tiroïdal autoimmune) i malaltia atòpica (al·lèrgies, asma i èczema) en dones afectades, dóna suport a la idea de l'existència d'un sistema immune fonamentalment alterat en aquestes pacients (15).

1.3. ASPECTES INMUNOLÒGICS DE L´EP

En condicions normals, el teixit endometrial que arriba a la cavitat peritoneal per reflux s'elimina pel sistema immune i, per tant, la desregulació d'aquest mecanisme

d'eliminació ha estat relacionada amb la predisposició a la implantació i el creixement de les cèl·lules endometrials ectòpiques (10).

Entre els diferents fenotips de presentació que té l'endometriosi (ESP, EO i EP), el mecanisme patogènic agressiu de l'EP es diferencia dels mecanismes de l'ESP i l'EO per presentar una destacada disminució de l'apoptosi i un augment en l'activitat proliferativa, aspectes que advoquen per l'existència d'una entitat patogènica específica independent, més agressiva (16).

L'EP es desenvolupa a través d'un procés d'inflamació pèlvica que implica cèl·lules relacionades amb el sistema immune i els seus productes de secreció (citocines) que presenten una funció alterada dins de la cavitat peritoneal. La resposta inflamatòria, la reparació del teixit inflamatori i la neovascularització depenen de les cèl·lules i citocines del líquid peritoneal (LP). Per tant, una alteració en la seva funció pot induir aberracions immunològiques i afavorir la implantació de les cèl·lules (17).

Dins de les cèl·lules immunes implicades destaquen els macròfags, per ser les cèl·lules més representatives dintre del LP, però també es veuen afectats els mastòcits, les cèl·lules Natural Killer (NK) i els limfòcits B i T. De la mateixa forma, les proteïnes (citocines o interleucines (IL)) IL-1beta, IL-2, IL-6, IL-10, TNFalfa (*Tumor Necrosis Factor* alfa) i RANTES (*Regulated upon Activation Normal T cell Expressed and Secreted*), han estat implicades en la patogènesi de la malaltia i es troben elevades en el LP. Finalment, l'establiment del subministrament de sang és essencial per a la supervivència de l'endometri i s'ha observat un augment de la densitat dels microvasos en el teixit

endometrial amb potencial proliferatiu, així com alts nivells de VEGF (*Vascular Endothelial Growth Factor*) en el LP de pacients amb endometriosi (17,18).

Tot i que la funció de tots aquests factors ha estat analitzada en molts estudis previs, existeixen encara alguns resultats controvertits i, per tant, són fonamentals més estudis que expliquin el paper essencial de la immunitat i les citocines en el desenvolupament de la malaltia, que ajudaran a millorar la comprensió de la mateixa i permetran avançar en la recerca de noves teràpies immunològiques per a l'endometriosi.

1.4. DIAGNÒSTIC

Encara que el diagnòstic definitiu ve donat per la confirmació histològica de la malaltia, disposem d'altres mètodes que ens permeten tenir una alta sospita diagnòstica. Així com l'avaluació de l'EO resulta fàcilment assequible a través de l'ecografia transvaginal, la detecció d´EP és més dificultosa i requereix de l'ecografia transvaginal realitzada per un ecografista expert en detecció d´EP com a primera línia i de vegades la posterior RMN (7,8). No obstant això, aquesta última és una tècnica cara i es necessiten radiòlegs experts i amb determinades habilitats per a la correcta interpretació de les imatges. Malgrat tot, l'ús de l'ecografia transvaginal en l´actualitat és molt útil i amb una curta corba d'aprenentatge (19, 20) per a la detecció de lesions d´EP, de manera que es converteix, així, en la prova d'elecció pel fet de presentar avantatges tant econòmiques com tècniques.

Cal destacar que l'avaluació prequirúrgica és fonamental per a la planificació correcta de la cirurgia. Així doncs, el fet que l'avaluació preoperatòria pugui resultar dificultosa i

requereixi una formació específica, implica que no és infreqüent trobar de forma inesperada EP intraoperatòriament en pacients programades per dur a terme una cirurgia d'EO, la qual cosa incrementa els riscos de la pacient i genera cirurgies incompletes i innecessàries (21, 22). Aquest increment en els riscos quirúrgic ve donat pel fet d'haver de realitzar tècniques quirúrgiques no planificades, sense informar a la pacient d´aquests riscos, a banda que la cirurgia de l'EP per si sola és més complexa que la cirurgia en altres tipus d'endometriosi i sovint requereix un enfocament multidisciplinari per les diverses localitzacions que pot presentar. Per tant, és necessària una estadificació prèvia a la cirurgia, que possibiliti esbrinar la total extensió de la malaltia i permeti alhora planificar una cirurgia adient individualitzada per a cada pacient.

Cal tenir en compte que el fet de no treure tot el teixit endometrial durant el procediment quirúrgic pot ser la causa de la persistència de la malaltia i de la persistència o reaparició dels símptomes a curt termini (22). També implicarà la necessitat de dur a terme cirurgies repetides en casos de subestimació de l'extensió de la malaltia. A més, s'ha demostrat que el propi mecanisme de trauma sobre la superfície peritoneal durant la cirurgia i les adhesions que es formen postoperatòriament són factors afavoridors de la proliferació i la invasió i, per tant, predisposants d'EP (23), fet que accentuarà encara més la presència de la malaltia en aquelles pacients sotmeses a intervencions successives.

D'aquí sorgeix la importància de millorar el diagnòstic de l'EP prequirúrgicament, de forma que es pugui planificar la cirurgia adequada o, el que és més important, detectar

quan és necessari derivar la pacient a un centre de referència, atès que és altament recomanable dur a terme aquest tipus de cirurgia en centres amb alta experiència i amb equips multidisciplinaris (21).

A banda de les tècniques d'imatge diagnòstiques de què es disposa, s'han desenvolupat prèviament en la literatura múltiples intents no invasius de predicció d'EP. Alguns autors han suggerit que diverses circumstàncies que es presenten durant l'adolescència poden predir-la (24, 25). A més, la presència de certes interleucines s'ha associat amb una major gravetat de la malaltia (24-28). Recentment, el dolor pèlvic sever preoperatori s'ha associat amb EP en les dones que presenten EO (29). A més, Chapron *et al*. (30) i Lafay Pillet *et al*. (31) van crear models basats en l'anamnesi específica del dolor dissenyats específicament per identificar EP posterior. Tot i així, la sospita i el diagnòstic prequirúrgic de la malaltia continua sent subòptim i estudis recents han suggerit la necessitat de desenvolupar millors eines de diagnòstic per predir l'EP (32). *D'aquí sorgeix l'interès de crear un nou model de predicció que sigui senzill i de fàcil aplicabilitat.*

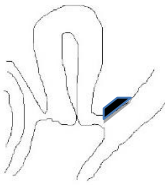
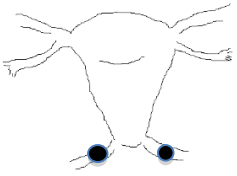
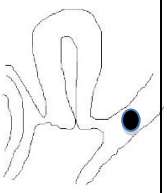
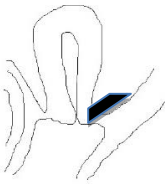
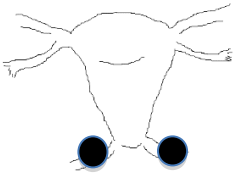
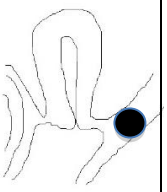
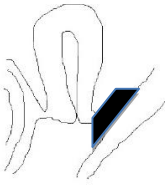
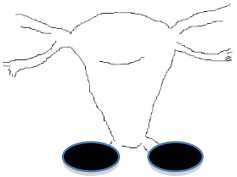
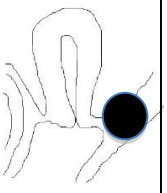
De la mateixa forma, s'ha intentat en múltiples estudis previs, predir l'extensió i la localització de la malaltia per poder enfocar les proves diagnòstiques a realitzar de forma dirigida segons la sospita i planificar una cirurgia adient en cada cas. Alguns estudis han trobat una associació significativa entre el tipus de dolor i una localització anatòmica específica de les lesions endometrials (33-35). No obstant això, aquests estudis han reportat resultats no concloents, per diverses raons. En primer lloc, tot i que la relació entre el dolor i l'endometriosi s'ha descrit moltes vegades, no existeix

consens en la definició del dolor pèlvic crònic. La definició àmplia inclou dismenorrea severa, disparèunia profunda, i altres símptomes dolorosos de la pelvis (34). Igualment, no existeix un mètode estàndard per a la classificació dels símptomes de dolor i aquests poden estar presents també en altres patologies. Com a resultat, els metges podrien confondre els signes i símptomes amb altres afeccions freqüents ginecològiques o no ginecològiques, com per exemple l'adenomiosi, que es caracteritza per la invasió de les glàndules endometrials i l'estroma al miometri (36) i sovint també representa un diagnòstic difícil (37). Per altra banda, la majoria d'aquests estudis van utilitzar la classificació de l'endometriosi de la *American Society of Reproductive Medicine* (ASRM) revisada, que tot i ser la classificació actual de referència, no té en compte la participació de les estructures retroperitoneals afectades per l'EP, ni l'adenomiosi (38). *L'ús d'una eina de classificació d'endometriosi més acurada, que tingui en compte tant la participació de les estructures retroperitoneals i de l'adenomiosi, és necessària per poder crear proves diagnòstiques i de sospita universals.* En aquest sentit, la classificació Enzian (Imatge 1) compleix aquests requeriments ja que divideix l'àrea pèlvica en tres compartiments d'acord amb les estructures retroperitoneals. El compartiment A conté l'envà rectovaginal i la vagina; el compartiment B s'estén des dels lligaments sacrouterins a la paret pèlvica, i el compartiment C conté el recte i el còlon sigmoide. Els casos d'invasió profunda d'òrgans (afectació de la bufeta o urèter, malaltia intestinal cranial a la unió recte-sigmoïdal, i altres llocs) i la presència d'adenomiosi es classifiquen com "altres" (39).

En conclusió, l'ús d'una definició de dolor pèlvic crònic consensuada i un acord en l'eina de classificació del dolor a utilitzar, equivaldria a un llenguatge universal que ajudaria en el

diagnòstic de la malaltia i en els avenços en la recerca al voltant de l'endometriosi, en general.

Imatge 1. Classificació Enzian

	COMPARTIMENT				
GRAU		A Septe rectovaginal i vagina	B/BB* Lligaments sacrouterins i paret pèlvica	C Recte i Còlon sigmoide	ALTRES
	GRAU 1 <1cm				Bufeta Urèter Intestí cranial a la unió recte-sigmoïdal Altres llocs
	GRAU 2 1-3 cm				
	GRAU 3 >3 cm				

BB*= Bilateral

Imatge adaptada de *Haas D, Chvatal R, Habelsberger A, Schimetta W, Wayand W, Shamiyeh A, Oppelt P. Preoperative planning of surgery for deeply infiltrating endometriosis using the ENZIAN classification. Eur J Obstet Gynecol Reprod Biol. 2013;166(1):99–103.*

1.5. TRACTAMENT

Les opcions terapèutiques actuals es basen en la cirurgia i els tractaments hormonals supressors de l'ovulació, que conseqüentment impedeix la gestació. Com s'ha comentat anteriorment, la cirurgia radical amb extirpació dels ovaris representaria el tractament definitiu, però implicaria la impossibilitat de gestació en aquestes pacients. Les teràpies no quirúrgiques poden resultar no satisfactòries en algunes pacients, perquè es centren en el tractament dels símptomes en comptes d'interferir en les causes de la malaltia, el seu efecte és reversible i, a més, l'ús a llarg termini d'algunes d'aquestes teràpies està condicionat pels freqüents efectes secundaris que ocasionen (9). Malgrat això, el tractament mèdic de l'endometriosi, a banda dels analgèsics, és efectiu en el tractament del dolor i en la prevenció de recurrències després de la cirurgia. En aquest sentit, els anticonceptius orals administrats de forma continuada, es consideren un tractament vàlid i són els més freqüentment utilitzats, atès que provoquen pocs efectes secundaris i juntament amb alguns progestàgens i el DIU (Dispositiu Intrauterí) alliberador de levonorgestrel, constitueixen les primeres línies de tractament. Altres progestàgens, que poden causar patrons de sagnat irregular, o els anàlegs de la GnRH (*Gonadotropin Releasing Hormone*) que produeixen fogots, mals de cap, sequedat vaginal i pèrdua de la densitat mineral òssia amb el seu ús prolongat, són teràpies que es reserven per a segones i terceres línies de tractament. A més, cap d'aquests

tractaments és compatible amb el desig gestacional. Tanmateix, no existeixen actualment tractaments aprovats que millorin la fertilitat en aquelles pacients que pateixen una infertilitat associada a l'endometriosi (40). ***Per tant, hi ha una clara necessitat de desenvolupar noves alternatives terapèutiques per proporcionar solucions específiques més eficaces per eliminar les lesions d'endometriosi, prevenir les recurrències i no interferir amb la fertilitat de les pacients (41).***

Amb aquests objectius, i donades les alteracions immunitàries presents a la malaltia comentades anteriorment, es planteja en l'actualitat la recerca de teràpies d'índole immunològica que inclourien antiTNFalfa, interferó 2 beta recombinant i antagonistes dels receptors dels leucotriens, entre d'altres. Aquests nous fàrmacs a desenvolupar oferirien una opció potencial per combatre aquesta malaltia i alleujar el dolor i la infertilitat sense inhibir la ovulació (42).

En aquest sentit, la pentoxifil·lina s'assenyala en la literatura com un fàrmac de potencial utilitat en base a estudis tant experimentals com clínics encara molt limitats però esperançadors. El seu mecanisme molecular d'acció principal comprèn la inactivació de fosfodiesterases i també és capaç de reduir la producció i l'acció de citocines com ara el TNFalfa a través de l'elevació dels nivells d'AMP (*Adenosine Monophosphate*) cíclic intracel·lular. Es tracta d'un fàrmac antioxidant i immunomodulador que no altera el cicle ovàric i permet la possibilitat d'embaràs en les pacients tractades. S'ha utilitzat durant molts anys per modificar la viscositat de la sang i millorar el lliurament d'oxigen tissular en la malaltia cerebrovascular i vascular perifèrica així com en altres condicions amb microcirculació defectuosa. També s'ha demostrat

que millora la motilitat de l'esperma i augmenta la taxa d´èxit de la fecundació in vitro en infertilitats causades per factors masculins (42).

La pentoxifil·lina és un fàrmac econòmic, amb absorció ràpida i pràcticament completa per via oral. Els efectes adversos que presenta solen estar associats amb l'ús a altes dosis; els més freqüents són fogots i trastorns gastrointestinals (opressió gàstrica, nàusees, vòmits, diarrees, sensació de plenitud). Altres efectes secundaris com trastorns cardíacs (arítmies), afectacions del sistema nerviós (vertigen, cefalees, agitació, alteració de la son) i de la pell (prurit, eritema, urticària) apareixen només de forma ocasional (43). La incidència d'efectes adversos reportada és del 3,1% (44).

De forma més específica, la pentoxifil·lina altera el sistema immunitari mitjançant la inhibició de la fagocitosis i la generació d'espècies reactives de l'oxigen i enzims proteolítics pels macròfags i granulòcits *in vitro* i *in vivo*, la inhibició de la producció del factor de necrosi tumoral *in vitro*, i la reducció de l'acció inflamatòria de TNF i la interleucina-1 en els granulòcits *in vitro* (45). Aquests efectes no estan directament relacionats amb els limfòcits T que són les cèl·lules deficients en ratolins immunodeprimits. La majoria de les citocines són secretades localment per altres cèl·lules com ara monòcits, macròfags, cèl·lules endotelials i fibroblasts. Per tant, l'ús d'un model de ratolins immunodeprimits és factible per estudiar l'efecte de la pentoxifil·lina sobre l'endometriosi.

Com a conclusió, existeixen diversos arguments afavoridors per considerar l'ús de la pentoxifil·lina en la recerca bàsica del tractament de l'endometriosi.

1.6. MODELS ANIMALS EN ENDOMETRIOSI

La manca de tractaments actuals adequats respon, almenys parcialment, a les limitacions que presenta la investigació de la malaltia en humans. Així, algunes de les limitacions que presenten els estudis en endometriosi inclouen diferents aspectes ètics, la impossibilitat de monitoratge de la progressió de la malaltia que requeriria laparoscòpies repetides i la dificultat de quantificar l'efecte dels fàrmacs sobre la mida de les lesions.

L'experimentació animal és una bona alternativa que permetria evitar aquestes restriccions. No obstant això, l'aparició de la malaltia de forma espontània es presenta sols en humans o en alguns primats superiors, ja que requereix la presència de cicle menstrual retrògrad, però l'ús d'aquests últims com a model animal també planteja limitacions ètiques i està associada a grans costos. Per aquest motiu s'han establert models d'endometriosi validats en animals petits de laboratori, com el ratolí, mitjançant l'implant de teixit endometrial a localitzacions ectòpiques. Aquests estudis es poden classificar en dos tipus depenent de l'origen de l'endometri trasplantat: homòlegs, si s'implanta endometri de ratolí en un ratolí receptor immunocompetent, o heteròlegs, si s'implanta endometri humà en un ratolí receptor immunosuprimit per evitar el rebuig. Aquest últim model té l'avantatge d'evitar les possibles diferències fisiològiques existents amb l'endometri de ratolí que podrien emmascarar els resultats i impedir la seva aplicabilitat posterior. El model animal basat en el ratolí, a més de l'avantatge de ser de baix cost, permet treballar sobre animals genèticament similars, l'estandardització de les condicions experimentals i la realització de diferents anàlisis de

forma senzilla, així com l'obtenció de material histològic per al seu estudi posterior (41,46). El fet que es tracti de ratolins immunodeprimits és bàsic per evitar el rebuig si volem utilitzar teixit humà, com hem comentat anteriorment, i aquest s'ha convertit en un mètode estandarditzat d'estudi de la malaltia en animals, tal i com s'ha reflectit en els estudis previs realitzats per altres investigadors (47-49).

1.7. ÚS DE LA PENTOXIFIL·LINA COM A TRACTAMENT DE L´ENDOMETRIOSI EN MODELS ANIMALS

L'eficàcia de la pentoxifil·lina per al tractament de la infertilitat associada a l'endometriosi s'ha demostrat *in vivo* (50, 51) i alguns assaigs clínics aleatoritzats en humans han avaluat l'eficàcia de la pentoxifil·lina com a tractament postquirúrgic o com a agent de millora de la fertilitat en les dones amb endometriosi (45, 52-55). No obstant això, encara s'està avaluant la capacitat d'aquest medicament per reduir el creixement d'implants d'endometriosi en estudis animals.

En un estudi dut a terme en rates femella amb endometriosi induïda quirúrgicament, l'ús de la pentoxifil·lina va demostrar ser un èxit en la inducció de la regressió de teixit endometrial sense induir un estat hipoestrogènic (56). Resultats similars es van resumir en un estudi més recent, el qual va demostrar que el tractament de 21 dies de les rates femelles amb pentoxifil·lina produïa reduccions en el volum mitjà i el nombre d'implants d'endometriosi (57). Aquests dos estudis van usar models animals homòlegs en els quals es van implantar fragments autòlegs de teixit endometrial, i en tots dos estudis es va administrar pentoxifil·lina a través d'injeccions subcutànies. Per tant, l'ús de la

pentoxifil·lina en un model heteròleg de ratolí, administrant el fàrmac per via oral i amb dosis equivalents a les utilitzades en humans (obtingudes mitjançant una fórmula de conversió), suposaria una novetat respecte als estudis previs, evitaria possibles diferències fisiològiques entre animals i humans, alhora que augmentaria el seu potencial d'aplicabilitat posterior. Tot i utilitzar ratolins immunodeprimits amb aplàsia congènita del timus, i que presenten per tant un sistema de limfòcits T deficients, la majoria de les citocines es poden secretar localment per altres cèl·lules tals com monòcits, macròfags, cèl·lules endotelials i fibroblasts i, de fet, són les cèl·lules que en major mesura secreten els productes regulats per la pentoxifil·lina. Així doncs, l'ús d'un model de ratolins immunodeprimits és factible per estudiar l'efecte de la pentoxifil·lina sobre l'endometriosi.

En base a tot l'esmentat anteriorment, sorgeixen les diferents hipòtesis de treball i es desprenen la intencionalitat i els objectius d'aquesta tesi doctoral, tal i com s'exposen a continuació.

2. HIPÒTESIS DE TREBALL

Com hem esmentat prèviament, la cirurgia amb l'excisió dels implants endometrials representa en molts casos l'únic tractament eficaç per a l'EP, però suposa una cirurgia extensa i agressiva, amb l'exèresi sovint dels òrgans reproductors, a banda de no assegurar la desaparició completa de la malaltia, que pot reaparèixer amb el temps. Mentre la cirurgia representi el tractament principal de la malaltia, **el diagnòstic de la mateixa i la seva extensió prequirúrgica serà un dels pilars més importants en la investigació de l'EP**. Pel mateix motiu, **la recerca futura de noves línies de tractament que permetin preservar la fertilitat i evitar la cirurgia, representarà el segon pilar essencial en l'estudi de la malaltia**. De tot el que s'ha exposat anteriorment, sorgeixen les següents **hipòtesis de treball**:

1. Diagnòstic:
    - 1.1. L´EO, però no l´EP, és de fàcil diagnòstic ecogràfic. L´Anamnesi de la pacient pot ajudar a sospitar EP en pacients amb EO. Hauria de ser possible crear models predictius de fàcil ús a la pràctica clínica habitual que permetin diferenciar a les pacients amb EO aïllada o amb EP associada. L'exploració de diferents variables clíniques combinades ens haurien de permetre crear un model de predicció.
    - 1.2. El dolor és un símptoma-guia que podria permetre l´estadificació de l´EP i sospitar la seva localització, així com sospitar la presència d´adenomiosi associada.

2. Tractament: La pentoxifil·lina, un fàrmac immunomodulador que no altera el cicle ovàric i per tant no interfereix en la fertilitat, podria ajudar a la reducció de les lesions d'endometriosi així com a modular la resposta immunològica i l´angiogènesi produïda en les lesions endometrials en un model heteròleg de ratolins immunodeprimits.

3. OBJECTIUS

D'aquí sorgeix l'interès i la intencionalitat del nostre treball, que inclou investigació clínica i investigació bàsica, i que té com a objectiu aprofundir en els dos pilars esmentats anteriorment:

1. Facilitar el diagnòstic prequirúrgic de l'EP, permetent la sospita de la malaltia en pacients amb dolor i la sospita de la seva extensió de forma no invasiva, de tal manera que ens orienti en el grau d'infiltració i extensió de la malaltia (òrgans afectats) i la conseqüent necessitat de derivació a centres especialitzats on es dugui a terme les proves d'imatge necessàries per poder programar una cirurgia *ad hoc* per a cada pacient. Els objectius específics d'aquesta investigació clínica inclouran:

1.1. La creació d'un model de predicció d'EP en pacients amb EO.

1.2. L'estudi del dolor en pacients amb EP com a marcador d'extensió de la malaltia amb la utilització de la classificació Enzian.

2. Investigar l'ús de la pentoxifil·lina, un fàrmac immunomodulador que no altera el cicle ovàric i per tant no interfereix en la fertilitat, com a nova línia de tractament no quirúrgic sobre l'endometriosi induïda en un model animal de ratolí, seguint l'enfocament actual en el qual es considera l'EP com a malaltia d´índole immunològica. D'aquesta forma es pretén aprofundir en el coneixement del caràcter immunològic de la malaltia i crear una base racional per al futur tractament en humans. Els objectius específics de la investigació bàsica seran:

2.1. Avaluar l'efecte del fàrmac sobre la mida dels implants endometrials en ratolins.

2.2. Avaluar el comportament dels marcadors de proliferació vascular i els nivells de citocines implicades en la malaltia en el líquid peritoneal dels ratolins.

4. INVESTIGACIONS REALITZADES, MATERIALS I MÈTODES

La descripció de les pacients i els animals inclosos en els estudis d'investigació clínica i bàsica, així com la metodologia utilitzada en cada cas per aconseguir els objectius plantejats anteriorment, es troben detalladament exposats a la secció de "Materials i Mètodes" de cada un dels tres articles que constitueixen el cos doctrinal de la present tesi doctoral. Els esmentats articles s'adjunten a continuació.

ESTUDI 1

MARKERS OF DEEP INFILTRATING ENDOMETRIOSIS IN PATIENTS WITH OVARIAN ENDOMETRIOMA: A PREDICTIVE MODEL

Article publicat a *European Journal of Obstetrics & Gynecology and Reproductive Biology*.

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El nostre article forma part d'una edició especial dedicada exclusivament a Endometriosi.

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Markers of deep infiltrating endometriosis in patients with ovarian endometrioma: a predictive model

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ABSTRACT

Objective: The purpose of the study was to develop an easily applicable predictive model to predict deep infiltrating endometriosis in patients with ovarian endometrioma.
Study design: We performed a retrospective analysis of 178 consecutive women with ovarian endometrioma who underwent surgery, with histological confirmation and complete removal of endometriosis in the Hospital Clinic of Barcelona. Several markers were prospectively obtained and compared between the group of patients presenting deep infiltrating endometriosis associated with ovarian endometrioma and women with only ovarian endometrioma. Multiple logistic regression analysis was performed to create a model to predict the presence of deep infiltrating endometriosis and internal validation was later performed.
Results: Of the 178 patients studied, 80 (45%) were classified in the ovarian endometrioma group and 98 (55%) in the group of patients presenting deep infiltrating endometriosis associated with ovarian endometrioma. The independent variables to predict deep infiltrating endometriosis were: at least one previous pregnancy, a past history of surgery for endometriosis and the mean endometriosis-associated pelvic pain score. The area under the ROC curve was 0.91 (95% confidence interval: 0.86–0.94), with an optimal cut-off of the predicted probability of 0.54. The sensitivity of the model was 80% and the specificity 84%.
Conclusions: This model predicts the development of deep infiltrating endometriosis in patients with ovarian endometriomas allowing prioritization of women for referral to specialized centers.

Introduction

Endometriosis is a chronic disease characterized by the presence of endometrial tissue outside the uterine cavity, causing pain and reproductive failure [1].

The two most important manifestations of endometriosis are ovarian endometrioma (OE) and deep infiltrating endometriosis (DIE), being OE the most common (17–44% of patients with endometriosis) [2]. DIE has the most aggressive presentation, penetrating to more than 5 mm under the peritoneal surface. It is a multifocal disease primarily affecting the posterior area, and frequently involves the uterosacral ligaments and the torus uterinus, as well as the posterior vaginal and anterior rectal walls. Furthermore, the bladder and urinary system may also be affected [3,4].

Surgery in DIE is usually more complex than in other types of endometriosis and requires a multidisciplinary approach. Thus, failure to remove all of the endometriotic tissue may result in recurrence and persistent symptoms, and thus, referral of patients with DIE to a center with the necessary expertise is strongly recommended [5]. However, the preoperative assessment is complex and therefore it is not rare to find unexpected DIE in patients with OE undergoing scheduled surgery [6,7]. The presence of DIE significantly increases the difficulty of the procedure, thereby making preoperative suspicion of DIE in patients with OE very important to avoid underdiagnosis and undertreatment [8]. DIE associated with OE may be detected by transvaginal ultrasound as a first-line approach but also by transrectal

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ultrasound and magnetic resonance imaging (MRI) [9,10]. However, this is an expensive technique, and skilled radiologists are needed.

Some authors have suggested that several circumstances presented during adolescence may predict DIE [11,12]. Additionally, the presence of certain interleukins has been associated with higher severity of the disease [13–15]. Recently, preoperatively assessed severe pelvic pain has been associated with DIE in women presenting OE [16]. Nevertheless, despite previous attempts to develop noninvasive predictive models [17,18], the presurgical diagnosis of DIE remains suboptimal. Indeed, Chapron et al. [19] and Lafay Pillet et al. [20] created a standardized questionnaire specifically designed to identify posterior DIE. However, recent reports have suggested the need to develop better diagnostic tools for predicting DIE [21]. Therefore, the aim of the present study was to evaluate the predictive value of a novel equation developed with three easily obtainable markers in order to identify among patients diagnosed with OE those with a high risk of associated DIE.

Materials and methods

We assessed a total of 196 consecutive patients undergoing surgery for complete removal of endometriosis in the Service of Gynecology of the Hospital Clínic of Barcelona, from January 2011 to December 2013. The indication for surgery was: infertility in 58 patients (33%), pain and infertility in 32 patients (18%) and isolated pain in 88 (49%) patients (Fig. 1).

The exclusion criteria were: the impossibility to perform complete removal of the lesions and lack of histological confirmation of endometriosis. This study finally included 178 patients.

All the patients underwent an extensive preoperative work-up fully described elsewhere [9], including clinical examination, MRI and transvaginal sonography. Surgery was performed by two experienced surgeons (MAMZ, FC) and all the patients underwent a complete surgical exploration in order to confirm or exclude DIE. Based on the results of the surgical exploration, the women were then distributed into two groups: patients with isolated OE (OE-only group) and patients with DIE associated with OE (OE-DIE group), and thereafter a retrospective analysis was performed. According to the local regulations the Institutional Ethics Committee of our hospital approved this study. All participants provided written informed consent for data collection.

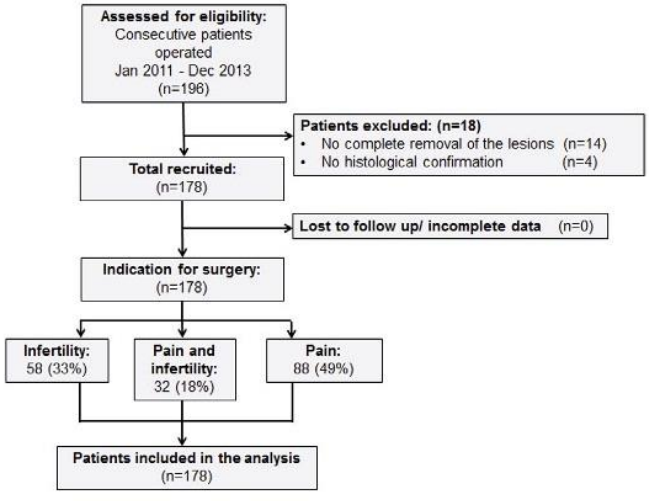

Fig. 1. Flow chart of the patients included.

The clinical data were prospectively recorded for each patient from surgical and pathological medical reports, and included the age at first visit, body mass index (BMI), previous pregnancies, past history of surgical treatment for endometriosis and the use of hormone treatment. Histological confirmation of all endometriotic lesions was obtained and a description of the location of the DIE lesions was recorded. Pain symptoms including dysmenorrhea, dyschezia, dyspareunia and pelvic pain were assessed using a VAS before surgery, regardless of the indication of the surgery (i.e. pain, infertility), and were also classified by each patient as the presence or absence of each pain. Women graded their feeling for each type of pain on a 10-cm line, from 0 "no pain" to 10 "unbearable pain". The mean VAS value for all previous types of pain was calculated for each patient and defined as the endometriosis-associated pelvic pain (EAPP) score. Disease-related data included the presence or absence of associated DIE, laterality of OE, multiplicity, size of the OE or sum of the sizes of OEs in the case of multiplicity. The origin of the patients was also recorded as follows: "out of area" women, in cases of referral from other gynecological centers because of the severity of the disease, and "in area" patients attended directly in our department as our hospital acts as a primary care center for some districts of Barcelona.

Descriptive analysis of the qualitative variables was performed using frequencies and percentages and the quantitative variables using mean and standard deviation (SD) or median and interquartile range (IQR). Categorical characteristics were compared between DIE and non-DIE patients using the χ^2 tests, and quantitative variables were compared using the *t*-test or the Wilcoxon rank-sum test.

In order to identify a logistic predictive regression model of DIE, clinical and statistical judgment led to the assessment of the following characteristics: age, BMI, clinical examination, previous pregnancies (yes/no), past history of surgical treatment of endometriosis (yes/no), dysmenorrhea (yes/no), dyschezia (yes/no), dyspareunia (yes/no), non cyclic pelvic pain (yes/no) and mean EAPP, hormone treatment (yes/no), laterality (unilateral/bilateral), multiplicity (single/multiple) and the sum of the sizes of OE. The log-likelihood ratio test and the Akaike's and Schwarz's Bayesian Information Criteria (AIC and SBIC, respectively) were used to choose among different logistic regression models allowing the identification of the variables included in the final multiple model (those with the lowest AIC and SBIC value) [22,23]. The goodness-of-fit model was assessed using the Hosmer–Lemeshow test along with an observed vs. predicted probabilities graph with a Lowess smoothing curve. The performance of the model was based on its discrimination ability and calibration. The area under receiver operating characteristic (AUC) curve was estimated. In order to estimate the optimal cutpoint, two methods were used: the Liu method that maximizes the product of the sensitivity and specificity, and the nearest to (0,1) method that finds the cutpoint on the ROC curve closest to (0,1). The positive (LR+) and negative likelihood ratios (LR) were calculated as a measure of the extent to which pre-model odds were altered by the model result [24].

The model was developed of the whole dataset, and its performance was internally validated choosing the AUC as the prognostic indicator. By means of the non-parametric bootstrap technique, the bias-corrected confidence interval (CI) of the AUC was estimated. One thousand bootstrap replications were performed drawing with replacement samples of 178 patients from the initial sample, and the final predictive model was plotted using a nomogram.

To further validate the model, we split the sample into two groups defined by the origin of the patients and we re-assessed our model in the "in area" sub-sample.

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Table 1
Clinical characteristics of the overall study sample.

	OE-control	OE-DIE	*p*-Value
	N = 80	*N* = 98	
Age, mean (±SD)	35 ± 6	34 ± 5	0.29[a]
BMI, mean (IQR)	21.4 (3.8)	22.2 (5.0)	0.13[b]
Previous pregnancies, *N* (%)	25 (31)	20 (20)	0.10[c]
Previous endometriosis surgery, *N* (%)	10 (13)	52 (53)	<0.01[c]
Previous hormone treatment, *N* (%)	12 (15)	31 (32)	0.01[c]
Unilateral endometrioma, *N* (%)	57 (71)	53 (54)	0.02[c]
Single endometrioma, *N* (%)	52 (65)	43 (44)	0.01[c]
Sum of sizes of OE, median (IQR)	68.5 (38.5)	68.5 (60)	0.82[b]
EAPP score, median (IQR)	1.1 (1.2)	3.6 (2.6)	<0.01[b]

Abbreviations: IQR: interquartile range; EAPP, endometriosis associated pelvic pain; SD, standard deviation.
EAPP: The score was calculated by the average of the results of the patient self-reported 10-cm VAS for dysmenorrhea, dyschezia, dyspareunia and pelvic pain. IQR: interquartile range.
[a] *t*-Test.
[b] Wilcoxon Rank Sum test.
[c] Chi-squared test.

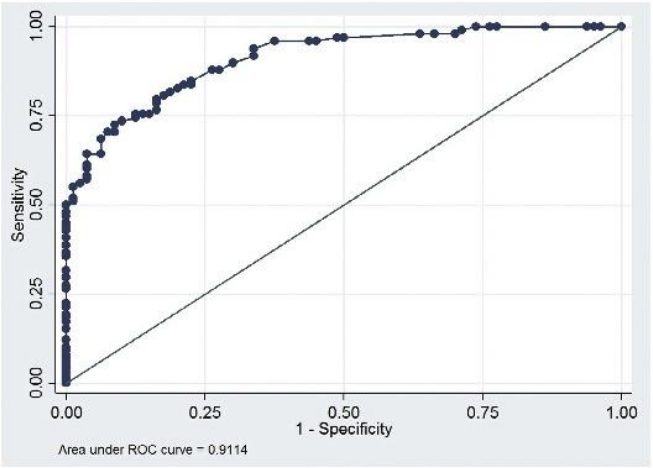

Fig. 3. Area under the ROC (AUC) curve of the predictive model.

All tests were two-tailed and the CI was set at 95%. The analyses were done using Stata (StataCorp. 2013. Stata: Release 13. Statistical Software. College Station, TX: StataCorp LP.).

Results

A total of 178 consecutive patients were included in the study, 80 (45.0%) in the OE-only group and 98 (55.0%) in the OE-DIE group. The description of the location of the DIE lesions was as follows: uterosacral ligament involvement in 23 (29%) patients, vaginal fundus in 16 (20%), broad ligament in 10 (13%), round ligament in 3 (4%), retrocervical in 25 (31%), retrouterine in 12 (15%), tube in 14 (18%), abdominal wall 8 (10%), bladder in 8 (10%), ureteral involvement in 12 (15%) and intestinal involvement in 26 (33%); a total of 149 lesions. Table 1 shows the parameters obtained from the clinical reports of the patients regarding the characteristics, history and symptoms. Compared to the OE-only group the patients in the OE-DIE group showed a significantly higher rate of past history of surgery for endometriosis (53% vs. 13%, respectively; $p < 0.01$) and a significantly higher mean EAPP score (3.6 vs. 1.1, respectively; $p < 0.01$).

According to the estimation using the adjusted odds ratio (OR), having previous pregnancies (0.25; 95%CI: 0.09–0.72; $p < 0.01$), a history of surgical treatment for endometriosis (10.17; 95% CI: 3.62–28.53; $p < 0.01$) and a unit increase in the mean EAPP score (3.49; 95% CI: 2.37–5.15; $p < 0.01$) were significantly associated with the presence of DIE. OR was just adjusted for the variables included in the model.

The equation model to predict the risk of DIE is described in Fig. 2. The predictive ability of this model was very good (AUC: 0.91; 95% CI: 0.86–0.95; Fig. 3). The optimal cut-off in the predicted probability was 0.538, with a sensitivity of 80% for the diagnosis of DIE in OE patients and a specificity of 84%, with 81% being correctly classified. The pre-model OR of DIE was 1.23 and the LR+ was 4.90, and thus, the post-model odds ratio was 6.03, providing a post-model probability of 0.86. The LR was 0.24, and hence the negative post-model odds ratio was 0.13, with a post-model probability of 0.12. Additionally, the probability of DIE was easily interpreted on presentation of the variables in a nomographic chart (Fig. 4).

Risk score = (-3.00) + (-1.38 x previous pregnancies) + (2.32 x previous surgery) + (1.25 x EAPP score)

The predicted risk of DIE = 1 / (1 + exp[-risk score])

Fig. 2. The equation model to predict the risk of deep infiltrating endometrioma in the ovarian endometriosis population. EAPP: The score was calculated by the average of the results of the patient self-reported 10 cm VAS for dysmenorrhea, dyschezia, dyspareunia and pelvic pain.

The bootstrap technique based on data reuse resulted in good reliability for the model, with a lower limit of 0.860 and an upper limit of 0.942. The subsample for "in area" patients differed from "out of area" women on presenting a lower history of surgical treatment for endometriosis; however there were no differences in the mean EAPP score and previous pregnancies (Table 2). The final model was retested in "in area" patients, and the AUC obtained was 0.91 (95% CI: 0.83–0.96). Table 3 shows the distribution of the different types of endometriosis depending on the origin of the patients while Table 4 describes the clinical characteristics of the patients.

Comment

We have developed an easy model to reliably predict associated DIE in women with OE with the use of three clinical markers. In this situation, the practitioner should perform an appropriate preoperative imaging evaluation in order to plan the optimal surgical approach or refer the patient to a specialized center.

Many previous studies have attempted to predict endometriosis. Chapron et al. [16] evaluated the intensity of pelvic pain in a population of patients presenting with OE and found a significant association with deep infiltrating lesions. In another previous investigation, these authors designed a diagnostic model based on symptoms and clinical history [19] specifically developed to predict posterior DIE among women with chronic pelvic pain, with a sensitivity of 74.5% and a specificity of 68.7%. The prediction of DIE was also analyzed in another study using an equation developed with clinical indicators and the plasma CA-125 assay during the follicular phase [18]. The sensitivity and specificity for this model were acceptable at 83% and 87%, respectively. However, the model was limited due to the small sample of women with DIE ($N = 13$). In a study by Eskenazi et al. [17], the prediction of OE was accurately achieved by transvaginal ultrasonography, signs and symptomatology, but only 38% of nonovarian endometriosis was predicted. In a multicenter study, a model based on menstrual dyschezia and a history of benign ovarian cysts accurately predicted stage III and IV endometriosis (sensitivity of 82.3% and specificity 75.8% at an optimal cut-off of 0.24) [25].

The methodology to build a diagnostic score of DIE described in the recent case control study by Lafay Pillet et al. is probably the

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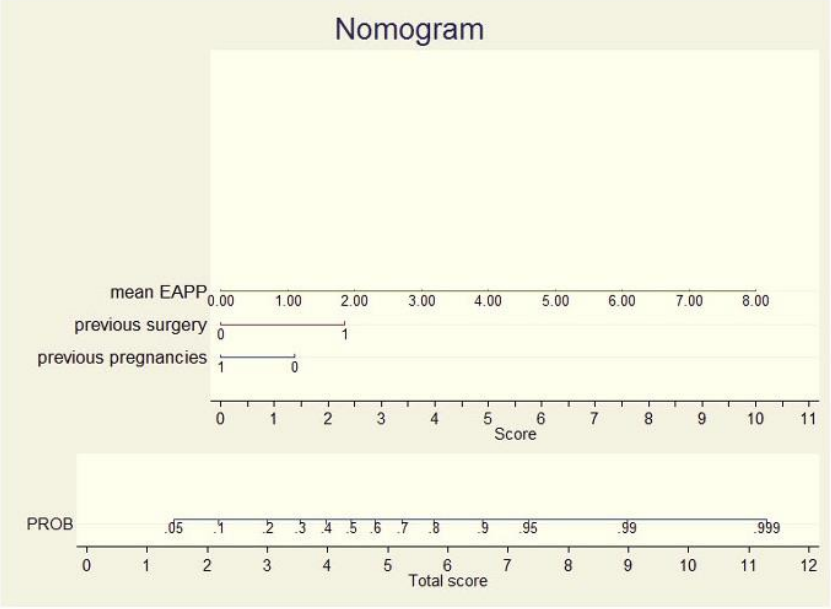

Fig. 4. Nomogram for predicting deep infiltrating endometriomas in patients with ovarian endometriomas. A nomogram is a graphical representation of a mathematical formula to predict an endpoint with one or more covariables. Its utility lies in that it maps the predicted probability as points. To obtain the predicted probability of DIE, locate patient values at each axis corresponding to variables. Draw a vertical line at the 'Score' axis to determine how many points are attributed to each variable value. Sum the points for all the variables. Locate the sum on the 'Total Score' line to be able to assess the individual probability of DIE upgrading on the 'PROB'.

Table 2
Parameters of the predictive equation model in the overall study sample categorized according to "in area" and "out of area" patients.

	"In area"	"Out of area"	*p*-Value
	N = 81	*N* = 97	
Previous pregnancies, *N* (%)	23 (28)	22 (23)	0.38[a]
Previous surgery for endometriosis, *N* (%)	15 (19)	47 (48)	<0.01[a]
EAPP score, median (IQR)	1.8 (2.6)	2.8 (2.4)	0.078[b]

Abbreviations: IQR: interquartile range; EAPP, endometriosis associated pelvic pain; SD, standard deviation.
EAPP: The score was calculated by the average of the results of the patient self-reported 10-cm VAS for dysmenorrhea, dyschezia, dyspareunia and pelvic pain.
"In area" patients are those attended directly in our department because our hospital acts as a primary care center for some districts of Barcelona.
"Out of area" patients are those referred of referral from other gynecological centers because of the severity of the disease.
[a] Chi-squared test.
[b] Wilcoxon Rank Sum test.

most similar to ours [20]. This was based on a standardized preoperative questionnaire. In a study including 326 women they obtained a low sensitivity, albeit with a high specificity (51% and 94%, respectively in patients with scores >35). On comparison with our study, it is worthy to note the different results concerning sensitivity and specificity. Despite having a small sample, with a cut-off of 0.538 we obtained a sensitivity of 80% and a specificity of 84% for the diagnosis of DIE in OE patients, with 81% of the patients correctly classified. Nonetheless, strict comparison among different studies is difficult due to differences in methodology or in the variable intended to predict. This cut-off can be used to classify patients at low or high risk of having DIE, but we leave the decision as to when to refer the patient to clinical discretion since this may differ among centers depending on their experience.

Our work also registered results from patient self-evaluation using a 10-cm VAS for the different types of pain. We used the VAS for the EAPP score, which integrates in a single variable all types of pain commonly presented in endometriosis. This tool is easy to use

Table 3
Distribution of different types of endometriosis depending on the origin of the patients.

		Type of endometriosis		Total
		OE-control *N* (%)	OE-DIE *N* (%)	
Origin	"Out of area" *N* (%)	37 (46)	60 (61)	97 (54)
	"In area" *N* (%)	43 (54)	38 (39)	81 (46)
Total		80 (100)	98 (100)	178 (100)

Abbreviations: DIE, deep infiltrating endometriosis; OE, Ovarian endometrioma.
"In area" patients are those attended directly in our department because our hospital acts as a primary care center for some districts of Barcelona.
"Out of area" patients are those referred of referral from other gynecological centers because of the severity of the disease.

Table 4
Clinical characteristics of the patients depending on the origin.

	"Out of area"	"In area"	*p*-Value
	N = 97	*N* = 81	
Age, mean (±SD)	35 ± 6	34 ± 6	0.20[a]
BMI, mean (IQR)	22 (5)	22 (4)	0.59[b]
Previous pregnancies, *N* (%)	22 (23)	23 (28)	0.38[c]
Previous endometriosis surgery, *N* (%)	47 (49)	15 (19)	<0.01[c]
Previous hormone treatment, *N* (%)	28 (30)	15 (19)	0.07[c]
Unilateral endometrioma, *N* (%)	57 (59)	53 (65)	0.36[c]
Single endometrioma, *N* (%)	44 (45)	51 (63)	0.02[c]
Sum of sizes of OE, median (IQR)	69 (58)	68 (43)	0.65[b]
EAPP score, median (IQR)	2.8 (2.4)	1.8 (2.6)	<0.08[b]

Abbreviations: BMI, body mass index; DIE, deep infiltrating endometriosis; IQR: interquartile range; EAPP, endometriosis associated pelvic pain; OE, Ovarian endometrioma; SD, standard deviation.
EAPP: The score was calculated by the average of the results of the patient self-reported 10-cm VAS for dysmenorrhea, dyschezia, dyspareunia and pelvic pain.
"In area" patients are those attended directly in our department because our hospital acts as a primary care center for some districts of Barcelona.
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and has demonstrated a good correlation with treatment satisfaction, probably because patients can easily describe the overall amount of pain [26]. We worked with the mean EAPP because we think that all types of pain should have the same influence on the final VAS value, and despite the many publications related to this issue, no consensus has yet been established. The mean EAPP score often shows low mean scores (as zeros are common in the calculation), as in our study, despite the presentation of severe disease as can be deduced by the high rates of bilaterality, multiplicity and the previous use of hormone therapy. However, small variations are clinically relevant. Indeed, differences of 10 mm have recently been defined as the minimal clinically important difference for EAPP measured in a VAS for endometriosis [26]. On the other hand, screening between specific types and intensities of pain may be useful for differentiating endometrial implants inside or outside the ovary. In the study by Chapron et al. [16], uterosacral ligament involvement was associated with very severe chronic pelvic pain and deep dyspareunia while, vaginal involvement was related to intensive lower urinary tract symptoms and intestinal endometrioma was associated with increased severity of dysmenorrhea and gastrointestinal symptoms. Nevertheless, assessing the location of nonovarian endometrioma was not the objective of the model in our study, and similar to Chapron et al. [16] we found that more severely painful OE was significantly associated with a higher risk of DIE lesions.

Another indicator of DIE involvement was observed in patients with previous surgery for endometriosis. This is probably a consequence of an underestimation of the extent of the disease during the previous intervention, in line with previous reports [16,19] in addition to the mechanism of trauma to the peritoneal surfaces and post-operative adhesions from previous surgery, which may provoke endometriosis prolifereation/invasion, and thus, DIE [27]. Interestingly, we found a statistical association with previous pregnancies (identified as a protective factor) and DIE, although this has not previously been reported in the literature [19]. This is consistent with the fact that DIE has traditionally been associated with infertility and thus, this variable should be considered in the model from the statistical and clinical point of view even if it is not significant in univariate analysis. Indeed, this may occasionally happen but not does necessarily invalidate its use in the final model. Nonetheless, further studies are needed. We did not record the percentage of spontaneous or after IVF pregnancies, but spontaneous pregnancy probably acts as a higher protective factor.

Our model was found to be effective in identifying DIE in women with OE as shown by the increase in the odds of DIE from 1.23 (pre-model) to 6.03 (post-model). Moreover, the performance of the equation underwent internal cross-validation through a bootstrapping method, in order to assess the stability of the model and avoid overfitting of data [28]. We chose to do the bootstrapping on AUC as it represents the discrimination ability of our model. Thus, the lower limit of the CI represents the worst scenario of discrimination. This was previously performed by Chapron et al. with the jackknife method, with 70.9% of their sample being correctly classified [19]. Additionally, predictive models are often neglected at the time of clinical decision making because of the difficulty of output calculation. We used a logistic regression nomogram, a tool which has been widely adopted in biomedical research and is intended to help clinicians in the daily application of this model. Furthermore, surgery was always performed by two experienced surgeons and histological confirmation of the disease was obtained in all cases.

This study should be considered as a preliminary study and is probably small from a biometric point of view. Nonetheless, it was intended to stimulate future larger investigations to address this issue and provide a greater basis to help clarify the predictive value of this approach. However, our results need to be confirmed in an external data set.

On the other hand, the predictive capacity of models developed in secondary care is usually reduced when extrapolated to a primary care setting [24] and may be biased by some factors (e.g. the selection of patients) which may be a weakness in our study. Therefore, in an effort to add further value to our model, we reassessed our model in an "in area" subsample and confirmed good performance for the prediction of DIE.

To conclude, the predictive equation described in this study is based on three simple, accessible and noninvasive indicators that may serve to guide practitioners to determine the probability of patients with OE having DIE associated. The present model showed good value for predicting DIE and was internally validated with the bootstrap technique as well as in a data set defined as "in area" patients. Additionally, the use of the nomogram should facilitate application by general physicians or less skilled gynecological centers in their daily practice. Women found to be at high risk may, however, require more invasive exploration in order to undergo a one-step surgical approach for complete removal of endometrial implants. Nonetheless, additional studies are needed to further validate our predictive model in other populations.

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Conflict of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with interest in or conflict with the subject matter discussed in the manuscript.

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ESTUDI 2

ENDOMETRIOTIC PAIN IS ASSOCIATED WITH ADENOMYOSIS, BUT NOT WITH THE COMPARTMENTS AFFECTED BY DEEP INFILTRATING ENDOMETRIOSIS

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Endometriotic Pain Is Associated with Adenomyosis but Not with the Compartments Affected by Deep Infiltrating Endometriosis

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Key Words
Endometriosis · Pain · Enzian classification · Marker · Adenomyosis

Abstract
Background: The identification of presurgical clinical markers may be helpful to allow the staging of endometriosis severity. It has been suggested that pain characteristics orientate the gynecologist about the anatomical involvement of endometriosis. The study was performed to analyze the correlation between pain symptoms and the anatomical location of endometriosis. ***Methods:*** One hundred fifty-five consecutive patients with a complete removal of deep infiltrating endometriosis (DIE) were included. Prior to surgery, data on patient and disease characteristics were obtained. The intensity of the pain symptoms was registered using a Visual Analogue Scale. The endometriotic lesions were categorized according to the Enzian morphological classification. Correlation and multivariate analysis were performed to assess the potential associations between pain characteristics (dysmenorrhea, pelvic pain, dyschezia, dyspareunia or dysuria) and the location of endometriosis or other disease-related characteristics (hematuria, rectal bleeding or adenomyosis). ***Results:*** Pelvic pain was significantly associated with the presence of adenomyosis. Dyschezia was correlated with rectal bleeding and dysuria with the presence of hematuria. No relationship was found between other kinds of pain and the morphological location of endometriosis or other disease-related characteristics. ***Conclusion:*** Our data suggest that pelvic pain is correlated with the presence of adenomyosis in women with DIE. Further studies are required.

Introduction

Endometriosis affects 10–20% of women at the reproductive age [1]. This disorder is characterized by the ectopic endometrial implants that can occur as superficial peritoneal (or ovarian) endometriosis, endometriotic cysts of the ovaries or ovarian endometrioma (OE) and deep infiltrating endometriosis (DIE). The cause of this disorder remains unknown to date despite substantial research efforts [1–3].

Surgical treatment of DIE is difficult; it presents substantial risks and requires a high level of surgical skill. One of the challenges at present time is the preoperative staging of the disease. Unfortunately, clinical examination is insufficient to identify DIE lesions, especially in higher pelvic locations [4]. For the non-invasive diagnosis of endometriosis, accurate imaging techniques such as transvaginal ultrasound (TVS) or MRI are necessary [5].

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Recently, considerable effort has been made to use pain as a marker of endometriosis. Some studies have focused on pain as a clinical predictive tool to correctly identify patients with DIE [6]. Other studies have found a relevant association between the type of pain and a specific anatomic location of endometrial lesions [7–9]. However, these studies have reported inconclusive outcomes for various reasons. Although the relationship between pain and endometriosis has been described many times, the definition of chronic pelvic pain has not been consistently defined. The broad definition includes severe dysmenorrhea, deep dyspareunia, and other painful symptoms in the pelvis [8]. Moreover, there is no standard method for the classification of pain symptoms. As a result, clinicians might confound the signs and symptoms with those of other gynecological conditions. For example, non-explored adenomyosis is characterized by the invasion of the endometrial glands and stroma in the myometrium [10, 11], and also often presents a challenging diagnosis. Finally, most of these studies used the revised American Society for Reproductive Medicine (rASRM) score, which does not take into account the involvement of retroperitoneal structures with DIE [12].

The aim of the present study was to analyze the usefulness of different types of pain as predictive markers to indicate the morphological location of endometriosis. We used the Enzian classification that takes into account both the involvement of retroperitoneal structures and adenomyosis [13].

Material and Methods

The study included 155 consecutive women with histologically confirmed DIE, who underwent surgery for complete removal of endometriosis after an exhaustive exploration of the abdominal cavity. The surgical procedures were performed in the Clinical Institute of Gynecology, Obstetrics and Neonatology of the Hospital Clinic of Barcelona, Spain, from January 2013 to December 2014. This study was conducted following the principles of the Declaration of Helsinki for Medical Research Involving Human Subjects and was approved by the Institutional Ethics Committee according to the local regulations. All participants had provided written informed consent before the data collection.

Information was prospectively registered for each patient. Baseline characteristics were obtained during the first visit. We recorded age, body mass index, previous pregnancies and the history of surgical treatment for endometriosis. Before the surgery, pain symptoms including dysmenorrhea, dyschezia, dyspareunia, dysuria and pelvic pain (defined as pain located in the pelvis and not otherwise described) were identified. Pain intensity was classified using the Visual Analogue Scale (VAS), being one of the most frequently used pain scale and the best adapted for endometriosis pain measurement [14]. The women marked a point on the 10-cm line corresponding to the amount of pain they experienced; the scale was from 0 (no pain) to 10 (unbearable pain). The mean VAS score for each type of pain was registered. The mean VAS value for all the types of pain was calculated and defined as the mean endometriosis-associated pelvic pain (mEAPP) score for each patient. Additionally, the maximum VAS value for all the types of pain measured was given as the maximum endometriosis-associated pelvic pain (maxEAPP) score [15]. Scores of 4–6 were considered as moderate pain and those ≥7 as severe pain.

An extensive preoperative evaluation was performed in all the patients, including clinical examination, MRI and transvaginal sonography, as previously described [16]. The morphological description of DIE was conducted using the Enzian classification during surgery and confirmed by histological analysis [13]. This classification divides pelvic area into 3 compartments according to the retroperitoneal structures. Compartment A contains the rectovaginal septum and vagina, compartment B extends from sacrouterine ligament to the pelvic wall and compartment C contains the rectum and sigmoid colon. The cases of deep invasion of organs were separately registered as 'others': adenomyosis, the involvement of the bladder, ureter, bowel disease cranial to the rectosigmoid junction and other locations. Adenomyosis was diagnosed before surgery using MRI or transvaginal sonography, a 2- and 3-dimensional TVS examination with specific features predefined according to the work of Pinzauti et al. [17]. Moreover, adenomyosis was histologically confirmed in cases in which a hysterectomy was performed. Disease-related data included presence of OE, laterality of OE (uni- or bilaterality), multiplicity, size of the largest OE and the sum of the sizes of OEs in cases of multiplicity, number of DIE implants, the size of the largest implant, the sum of all implant sizes and their specific localization and the presence or absence of hematuria and rectal bleeding.

Statistical Analysis

The results of the descriptive analysis of qualitative variables were expressed as frequencies and percentages, and those of the analysis of quantitative variables as means and SDs. The Pearson correlation coefficient was used for analyzing the relationship between the type of pain and the extent of the disease (including location, disease-related characteristics and presence of hematuria, rectal bleeding or adenomyosis). Finally, a multivariate logistic regression model was used to identify independent factors that could most accurately predict pain. All the tests were 2-tailed and the confidence level (CI) was set at 95%. A p value <0.05 was considered significant. The analyses were performed using IBM SPSS statistics 20.0 (IBM, Armonk, New York, N.Y., USA).

Results

A total of 155 consecutive women with histologically confirmed endometriosis were included in the study. The baseline characteristics of the patients and the distribution of pain intensity are shown in table 1. The most common symptoms were dysmenorrhea in 148 (95.5%) patients and pelvic pain in 98 (63.2%). The maxEAPP was severe (8.1 ± 1.8), as expected in this population. The

Table 1. Baseline characteristics of the women included in the study and the intensity of the different types of pain measured by a 10-cm VAS and other disease-related characteristics (n = 155)

	Mean ± SD	Number	%
Age, years	34.5±5.6		
BMI, kg/m^2	22.9±3.7		
Previous pregnancies	0.4±0.8		
Previous endometriosis surgery		78	50.3
Dysmenorrhea	7.26±2.5	148	95.5
Pelvic pain	7.27±2.0	98	63.2
Dyschezia	7.23±2.3	67	43.2
Dyspareunia	6.50±1.9	65	41.9
Dysuria	7.32±2.5	23	14.8
mEAPP	3.7±1.8		
maxEAPP	8.1±1.8		
Hematuria		5	3.2
Rectal bleeding		19	12.3
Adenomyosis		33	21.3

BMI = Body mass index.

In the 10-cm VAS, the women marked a point on a 10-cm line corresponding to the intensity of the pain experienced, from 0 (no pain) to 10 (unbearable pain).

Scores of 4–6 were considered as moderate pain and those ≥7 as severe pain.

Table 2. Intensity of the different types of pain measured by a 10-cm VAS according to endometriosis location (mean ± SD). Note that these results were calculated taking into account the value of 0 in the absence of a specific type of pain

	Compartment A (n = 94)	Compartment B (n = 74)	Compartment C (n = 46)	Others (n = 72)
Dysmenorrhea	6.70±2.9	7.24±2.5	7.13±2.6	6.93±3.0
Pelvic pain	4.48±3.8	4.86±4.0	4.33±4.0	4.58±4.0
Dyschezia	3.36±3.9	3.2±4.0	2.30±3.5	2.83±3.8
Dyspareunia	2.82±3.9	2.82±3.6	2.33±3.3	2.13±3.1
Dysuria	0.95±2.5	0.89±2.6	0.80±2.3	1.33±2.9
mEAPP	3.67±1.8	3.79±1.9	3.38±1.4	3.56±1.8
maxEAPP	8.06±1.7	8.20±1.6	8.09±1.8	8.09±2.1

In the 10-cm VAS, the women marked a point on a 10-cm line corresponding to the intensity of the pain experienced, from 0 (no pain) to 10 (unbearable pain).

Scores of 4–6 were considered as moderate pain and those ≥7 as severe pain.

mEAPP was 3.7 ± 1.8 (note that these results were calculated by taking into account the value of 0 in the absence of a specific type of pain). When each type of pain was separately evaluated, the mean VAS score was elevated, indicating that, overall, the women had severe pain. Table 2 shows the intensity of the different types of pain according to the compartment involved. Adenomyosis was diagnosed by MRI in 33 patients (21.3%) and confirmed by histology in all cases in which hysterectomy was performed (10 patients, 30.3%). In the absence of MRI suspicion adenomyosis was not, in any case, histologically diagnosed in the hysterectomy specimen (data not shown).

Endometriosis was characterized during pelvic surgery; the clinical data for the different compartments are presented in table 3. The distribution of lesions categorized according to the Enzian classification in the different compartments is shown in figure 1. The most common area involved was compartment A (60.6%). DIE was identified in the vagina in 20 (12.9%) women, in retrocervical nodule

Table 3. Clinical description of OE and DIE nodules after pelvic surgery according to the different anatomical compartments affected (n = 155)

	Compartment A (n = 94)	Compartment B (n = 74)	Compartment C (n = 46)	Others (n = 72)	Overall (n = 155)
DIE					
Number of DIE implants, n (%)	94 (100.0)	74 (100.0)	46 (100.0)	72 (100.0)	155 (100.0)
1 implant	29 (30.9)	7 (9.5)	6 (13.0)	17 (23.6)	55 (35.5)
2 implants	32 (34.0)	27 (36.5)	9 (19.6)	20 (27.8)	49 (31.3)
3 implants	14 (14.9)	20 (27.0)	9 (19.6)	14 (19.4)	25 (16.1)
4 implants	9 (9.6)	8 (10.8)	11 (23.9)	9 (12.5)	13 (8.4)
5 or more implants	10 (10.6)	12 (16.2)	11 (23.9)	12 (16.7)	13 (8.4)
Size of the largest implant, n (%)					
Unknown	5 (5.3)	3 (4.1)	0 (0)	0 (0)	6 (3.9)
<10 mm	1 (1.1)	2 (2.7)	0 (0)	3 (4.2)	4 (2.6)
>10 mm	15 (16.0)	12 (16.2)	5 (10.9)	10 (13.8)	28 (18.1)
>20 mm	24 (25.5)	20 (27.0)	7 (15.2)	18 (25.0)	38 (24.5)
>30 mm	13 (13.8)	13 (17.6)	6 (13.0)	10 (13.8)	24 (15.5)
>40 mm	16 (17.0)	14 (18.9)	11 (23.9)	11 (15.3)	24 (15.5)
≥50 mm	20 (21.3)	10 (13.5)	17 (37.0)	20 (27.8)	31 (20.0)
Sum of implant sizes, mm, median	54.7±41.8	54.0±38.7	75.2±52.3	67.0±53.7	52.8±42.9
OE					
Laterality endometrioma, n (%)	56 (59.6)	45 (60.8)	25 (54.3)	50 (69.4)	96 (61.9)
Unilateral	26 (46.4)	24 (53.3)	10 (40.0)	28 (56.0)	50 (32.3)
Bilateral	30 (53.6)	21 (46.7)	15 (60.0)	22 (44.0)	46 (29.7)
Multiplicity	36 (64.3)	28 (62.2)	16 (64.0)	25 (50.0)	56 (36.1)
Size of the largest OE, mm, mean ± SD	50.8±25.6	45.9±27.3	51.8±26.0	52.5±28.5	51.3±27.2
Sum of sizes of OE, mm, mean ± SD	73.0±36.3	66.8±40.0	79.4±38.8	70.9±42.4	71.9±38.8

The Enzian classification categorizes DIE morphologically into 3 compartments depending on the retroperitoneal structures involved: compartment A, the rectovaginal septum and vagina; compartment B, sacrouterine ligament to pelvic wall; compartment C, the rectum and sigmoid colon. The group named 'others' comprises adenomyosis, involvement of the bladder, ureter, bowel disease cranial to the rectosigmoid junction and other locations.

in 62 (40.0%), in retrouterine in 19 (12.3%) and in the uterine fundus in 3 individuals (1.9%). The lesions were also specifically located in the following areas of compartment B: the uterosacral ligament in 53 (34.2%) patients, broad ligament in 20 (13.0%) and round ligament in 6 (3.8%). In compartment C, the rectal area was affected in 13 (8.4%) patients and the sigma in 24 (15.5%). We also found (in 'other' compartments) lesions in the abdominal wall peritoneum in 21 (13.6%), bladder peritoneum in 26 (16.8%), ureter in 16 (10.3%), ileum in 5 (3.2%), cecum in 4 (2.6%) and appendix in 8 (5.2%) women.

All the variables included in the correlation analysis are shown in table 4. A significant positive association was found between pelvic pain and the presence of adenomyosis (r = 0.468; p = 0.037), between dyschezia and rectal bleeding (r = 0.461; p = 0.046), and dysuria and hematuria (r = 0.532; p = 0.004). Moreover, a clear associative trend was observed between dysmenorrhea and the presence of adenomyosis (r = 0.418; p = 0.080). The mEAPP and maxEAPP values were also positively correlated with the presence of rectal bleeding (r = 0.511; p = 0.008 and r = 0.518; p = 0.007, respectively). There was no correlation between the specific compartments affected (Enzian classification) and the pain characteristics. Other clinical features studied related to DIE, OE or other conditions were not significantly associated with any of the pain outcomes. Table 5 shows the multivariate logistic regression analysis performed in order to predict variables associated with pelvic pain in our series. Among the variables studied, only adenomyosis showed a significant association with pelvic pain (OR 0.32; 95% CI 0.14–0.20; p = 0.02).

Discussion

We found some correlations between pelvic pain and adenomyosis, dyschezia and rectal bleeding, and dysuria and hematuria. We also observed a tendency to an asso-

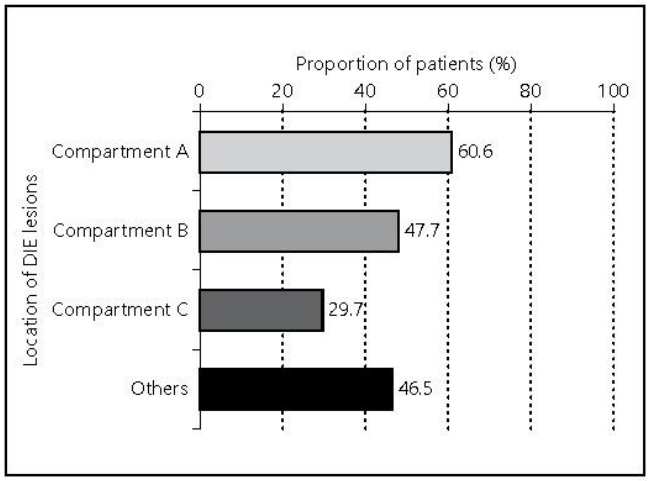

Fig. 1. Distribution of patients according to the morphological classification of DIE by the Enzian classification (n = 155). The Enzian classification allows morphologically locating DIE into 3 compartments according to the retroperitoneal structures: compartment A, rectovaginal septum and vagina; compartment B, sacrouterine ligament to pelvic wall; and compartment C, rectum and sigmoid colon. The group named 'others' comprises adenomyosis, involvement of the bladder, ureter, bowel disease cranial to the rectosigmoid junction and other locations.

ciation between dysmenorrhea and adenomyosis. Interestingly, an absence of correlation between endometriotic pain markers and specific compartments affected was also found.

Several features of this study are worth emphasizing. We used the Enzian classification of endometriosis, which is considered the best rating tool for the morphological categorization of DIE lesions [18]. This classification method allows registering the extent to which the retroperitoneal structures are affected and takes adenomyosis into account. Therefore, according to the objectives of this study, this classification was chosen for use in the present study. Furthermore, this method is useful for the description of not only histologically confirmed endometriosis but also of clinically suspected lesions. In contrast, although the rASRM score is currently the best known, most widely used and the reference classification for endometriosis, it does not take into account either the involvement of retroperitoneal structures in DIE or adenomyosis [12, 13, 18]. In our study, the rectovaginal septum and vagina (compartment A) were primarily affected, followed by the sacrouterine ligament to the pelvic wall (compartment B) and the rectum and sigmoid colon (compartment C). Other compartments were affected in 46.5% of patients, and 21.3% of all women had adeno-

Table 4. The relationship between the characteristics of pain and morphological location and clinical data for OE, DIE and other conditions

	Pain characteristics													
	dysmenorrhea		pelvic pain		dyschezia		dyspareunia		dysuria		mEAPP		maxEAPP	
	r value	p value	r value	p value	r value	p value	r value	p value	r value	p value	r value	p value	r value	p value
Compartment A	–0.312	0.167	–0.212	0.884	0.349	0.064	0.210	0.904	0.202	0.981	–0.213	0.872	–0.218	0.825
Compartment B	0.346	0.071	0.206	0.944	0.225	0.754	–0.226	0.751	–0.236	0.660	0.258	0.476	0.259	0.469
Compartment C	0.273	0.365	–0.232	0.695	–0.294	0.244	–0.311	0.170	–0.233	0.685	–0.315	0.156	–0.201	0.988
Other compartments	–0.247	0.565	–0.241	0.614	–0.258	0.477	–0.460	0.087	0.321	0.135	–0.265	0.419	0.204	0.965
Presence of endometrioma	–0.307	0.187	–0.285	0.220	0.247	0.562	0.240	0.618	–0.209	0.910	–0.268	0.403	0.318	0.142
Laterality endometrioma	–0.211	0.917	–0.237	0.722	–0.289	0.387	0.342	0.166	0.335	0.188	0.237	0.719	0.393	0.059
Multiplicity	–0.244	0.673	–0.264	0.536	–0.306	0.302	0.324	0.229	0.250	0.629	–0.240	0.700	0.315	0.264
Size of the largest OE	–0.202	0.982	–0.221	0.842	0.357	0.128	–0.249	0.638	0.258	0.572	0.270	0.500	0.362	0.114
Sum of sizes of OE	–0.253	0.610	–0.213	0.901	0.245	0.660	0.216	0.876	0.292	0.371	0.245	0.660	0.376	0.086
Number of DIE localizations	0.289	0.270	–0.234	0.678	0.224	0.764	–0.327	0.116	0.241	0.614	–0.226	0.747	0.231	0.706
Size of the largest implant	0.257	0.481	–0.381	0.074	0.235	0.661	–0.240	0.625	–0.283	0.307	–0.334	0.095	0.000	0.999
Sum of implant sizes	0.240	0.629	–0.275	0.364	0.220	0.807	–0.246	0.581	–0.208	0.928	–0.234	0.682	0.276	0.362
Presence of hematuria	0.240	0.624	–0.212	0.880	–0.207	0.930	–0.212	0.883	**0.532**	**0.004**	0.341	0.079	0.350	0.062
Presence of rectal bleeding	0.281	0.315	–0.282	0.310	0.461	**0.046**	–0.208	0.917	0.265	0.419	**0.511**	**0.008**	**0.518**	**0.007**
Presence of adenomyosis	0.418	0.080	**0.468**	**0.037**	–0.259	0.468	0.223	0.773	0.293	0.093	0.299	0.222	0.209	0.914

r = Correlation coefficient.

Table 5. Multivariate logistic regression analysis for independent variables that could most accurately predict pain

	Pelvic pain		
	OR	95% CI	p value
Involvement of compartment A	–0.13	–0.40 to 0.12	0.30
Involvement of compartment B	0.07	–0.23 to 0.39	0.62
Involvement of compartment C	0.02	–0.31 to 0.36	0.87
Involvement of other compartments	–0.14	–0.41 to 0.12	0.28
Presence of endometrioma	0.03	–0.35 to 0.42	0.85
Laterality of endometrioma	–0.10	–0.33 to 0.27	0.31
Multiplicity of endometrioma	–0.19	–0.62 to 0.23	0.36
Size of the largest OE	–0.30	–0.01 to 0.10	0.27
Sum of OE sizes	–0.29	–0.10 to 0.05	0.35
Number of DIE localizations	–0.17	–0.22 to 0.09	0.40
Size of the largest DIE implant	–0.07	–0.22 to 0.45	0.50
Sum of DIE implant sizes	0.11	–0.48 to 0.20	0.48
Presence of hematuria	–0.01	–0.84 to 0.72	0.87
Presence of rectal bleeding	–0.06	–0.37 to 0.38	0.96
Presence of adenomyosis	0.32	0.14 to 0.20	0.02

myosis. It is clear, however, that there was an overlap between different locations, since the majority of women had lesions in more than one location. This is not unusual in clinical practice, and the Enzian classification was expected to reduce bias by gathering lesions in the 3 compartments described.

The second important feature of the study was the inclusion of adenomyosis. It is well-defined that there is an overlap in the pathogenesis of endometriosis and adenomyosis, which is particularly important in DIE [19–21]. Gonzales et al. [22] found a correlation between uterine adenomyosis and DIE, particularly in the rectosigmoid. Although 30–50% of women with adenomyosis are asymptomatic [11, 23–25], the severe form of the disease often shows symptoms correlated with the intensity and depth of the lesions [22]. We observed a statistically significant correlation between pelvic pain and the presence of adenomyosis, in agreement with the previously reported results [23, 26, 27]. Dysmenorrhea is a risk factor in the deep adenomyotic process with a high density of endometrial glands in the myometrium [11, 23, 25, 28, 29]. We observed a clear trend associating the presence of dysmenorrhea with adenomyosis, although this relationship was not statistically significant. Among the patients reporting these symptoms, 95.5% described the intensity as severe. In contrast, dyspareunia and dysuria were self-reported as moderate to severe (when the types of pain were individually evaluated by our patients) and not related to involvement of the myometrium. These results are in agreement with previous findings [23, 30]. Another study conducted by Haas et al. [31] showed an association between adenomyosis and dysmenorrhea as well as with dyspareunia. However, adenomyosis was present in only 9.3% of patients, which clearly differs from what was observed in our study and can be a reason for not making a direct comparison. In fact, there is a clear variation in the reported rate of adenomyosis in the literature. Unresolved adenomyosis may consequently lead to pain recurrences after pelvic surgery. Therefore, correct identification of coexisting pathological conditions for DIE and adenomyosis is necessary for the development of effective surgical protocols.

Finally, we histologically confirmed the diagnosis of endometriosis in all the cases included in this study after surgical removal of implants. This may explain the differences observed with other studies. Previous publications have reported an association between pain semiology and the precise anatomical location [7–9]; thus, pain symptoms have been described as location indicating pain [8]. However, the diagnosis of the disease in these reports presented an important limitation; it was established macroscopically. Only in the study conducted by Vercellini et al. [9] was the histological diagnosis available in 72.9% of the patients. Our study does not support the possibility of a relationship between pain and number of the nodules; this feature has been neglected in previous reports with inconsistent results [32, 33]. We found that the presence of endometriomas was not correlated with the intensity of pain. This result was consistent with the conclusions of previous studies in which OE did not contribute to chronic pain [34–36].

The main limitation of our study was the sample size, which was rather small from a biometric point of view. We believe that this work should be considered a preliminary study intended to stimulate future larger, adequately powered investigations including patients and controls, as well as a larger multivariate analysis of the factors predicting DIE and adenomyosis. It is useful to provide the results of well-designed studies, even those that have low power, to encourage other investigators to address this issue and provide a larger basis for eventual review of small series of patients in order to help clarify the relationships between clinics and disease. Other minor limitations were the fact that data related to the presence of adnexal adhesions and the rate of pain recurrence after surgery was only available in a small group of patients.

In conclusion, our study suggests that in women with DIE, pelvic pain is correlated with adenomyosis and, im-

portantly, there is no correlation between endometriotic pain markers and the specific compartments affected. Although we do not know the rate of pelvic pain recurrence in the group of patients with associated adenomyosis because it was not the objective of the study, this presurgical assessment should be taken into account to correctly diagnose the extent of the disease. Therefore, imaging tests such as ultrasound or MRI must continue to be used when planning surgical treatment.

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ESTUDI 3

ORAL ADMINISTRATION OF PENTOXIFYLLINE REDUCES ENDOMETRIOSIS-LIKE LESIONS IN A NUDE MOUSE MODEL

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Oral Administration of Pentoxifylline Reduces Endometriosis-Like Lesions in a Nude Mouse Model

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Abstract

Introduction: Recent reports consider endometriosis to be an immunological disorder, thus suggesting potential efficacy of immunomodulators for its treatment. The aim of this study was to assess the effects of oral administration of pentoxifylline on endometriosis-like lesions in a heterologous mice model. **Study Design:** Human endometrial tissue obtained from women (n = 5) undergoing surgery for benign conditions was implanted in nude female mice (n = 30). The animals were distributed into 3 experimental groups receiving: saline 0.1 mL/d (control, group 1); pentoxifylline 100 mg/kg/d (group 2), and pentoxifylline 200 mg/kg/d (group 3). After 28 days, the number of implants and the total volume of surgically extracted tissue were recorded. Immunohistochemical analysis was performed to assess the area of endometriosis and vascularization of endometriosis-like lesions. Cytokine levels in peritoneal fluid samples were measured. **Results:** Macroscopic quantification showed a trend to dose-dependent reduction in the number of the endometriosis-like lesions after 28 days. The volume was significantly reduced in group 3 versus group 2 and controls (399.10 $\pm$ 120.68 mm^3 vs 276.75 $\pm$ 94.30 mm^3 and 145.33 $\pm$ 38.20 mm^3, respectively; $P = .04$). Similarly, the mean area of endometriosis was significantly lower in group 3 (0.12 $\pm$ 0.08 mm^2) versus group 2 (1.35 $\pm$ 0.43 mm^2) and control (2.84 $\pm$ 0.60 mm^2; $P = .001$). Vascularization and cytokine levels were also reduced posttreatment. **Conclusion:** Our results suggest that the oral administration of pentoxifylline may be an alternative to current therapies for endometriosis. Nonetheless, further studies are required.

Keywords

endometriosis, pentoxifylline, mouse model, dose translational formula, immunomodulation

Introduction

Endometriosis is an estrogen-dependent gynecological disorder characterized by the presence of endometrial glands and stroma outside the uterine cavity. It is associated with chronic pelvic pain among other symptoms.[1-4] Several different hypotheses have been suggested for the development of the disease. The most commonly accepted theory is retrograde menstruation, first described by Sampson in 1927. This theory proposes dissemination of endometrial cells from the uterine endometrium into the peritoneal cavity as a cause of endometriosis.[5] One of the major shortcomings of this theory, however, is that at reproductive age nearly all women exhibit some degree of retrograde menstruation, whereas not all develop this condition.

Recent studies have shown that the increased presence of immune cells in the peritoneal fluid of women with endometriosis potentially favors the development of the disease.

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Immune alterations may facilitate the implantation and development of endometrial foci through the secretion of cytokines and growth factors.[6] Thus, it is now generally accepted that the immune system is involved in the pathogenesis of the disease.[7,8] Therefore, the putative use of inmunomodulatory agents, such as pentoxifylline, which is a methylxanthine derivate that modulates cytokine production, may provide a new therapeutic approach to endometriosis.[9-11]

Pentoxifylline alters the immune system by inhibiting phagocytosis and the generation of toxic oxygen species and proteolytic enzymes by macrophages and granulocytes in vitro and in vivo, inhibiting tumor necrosis factor (TNF) production in vitro, and reduces the inflammatory action of TNF and interleukin (IL)-1 on granulocytes in vitro.[12] Interestingly, these effects are not directly related to T lymphocytes which are deficient cells in immune-compromised mice. Most cytokines are locally secreted by other cells such as monocytes, macrophages, endothelial cells, and fibroblasts. Thus, the use of a nude mice model is feasible to study the effect of pentoxifylline on endometriosis. In this study, we have approached the problem by evaluating the effect of orally administered pentoxifylline, considering that its absorption is fast and almost complete after the oral administration.[13] Human endometrial tissue was implanted in immunodeficient mice; this is a standardized procedure to study this disease in mice when avoiding both tissue rejection and the presence of several differences that there are when using mouse endometrium is needed.[14] Tissue growth and histological characterization of human endometriotic implants and peritoneal cytokine levels were assessed.

Materials and Methods

Ethical Approval

The present experimental study was conducted in the Hospital Clinic of Barcelona, Spain, from January 2014 to January 2015. It was approved by the Ethical Committee of Animal Experimentation of the University of Barcelona on May 28, 2013 (510/13), and by the Ethical Committee of the Hospital Clinic of Barcelona on November 28, 2013 (2013/8838).

Human Endometrial Tissue

Menstrual endometrium was obtained by aspiration using Cornier cannula for endometrial biopsy (Gynétics Medical Products N.V., Lommel, Belgium) during the proliferative phase (from 5 to 10 days) of 5 women undergoing surgery for benign conditions (2 myomas and 3 simple cysts). The endometrium samples were preserved in physiological saline and implanted immediately to prevent cell death. Patients were of reproductive age (18-45 years old) and had not received hormone treatment within 8 weeks prior to sample collection. Medical history of endometriosis or adenomyosis was not allowed, and an extensive preoperative evaluation was performed for all the patients, including clinical exploration, MRI, and transvaginal sonography to exclude both. The absence of endometriotic lesions was confirmed during the laparoscopy conducted for benign disease. All participants signed informed consent forms to participate in the study.

Experimental Model of Endometriosis

Nude (Swiss nu/nu) 4-week-old female mice (n = 30; Charles River Laboratories, Wilmington, Delaware) were used in the study. The animals were housed 6 per cage under specific pathogen-free conditions, with artificial lighting of 12-hour light/dark cycles. The animals were allowed to acclimatize to these conditions for 7 days prior to carrying out any intervention and were fed ad libitum with standard pellet food and tap water. A mixture of ketamine 100 mg/kg (Imalgène 1000, 100 mg/mL, Merial, Lyon, France) and xylazine 10 mg/kg (Rompun 2%, Bayer HealthCare, Kiel, Germany) was injected intraperitoneally (IP) to anesthetize the mice prior to the invasive procedures. Atipamezole hydrochloride 0.2 mg/kg IP (Antisedan, Dr Esteve Laboratory, Barcelona, Spain) was used to reverse the anesthesia and prevent respiratory depression.

On day 8, following the 7-day acclimatization phase, a bilateral oophorectomy was performed by lumbotomy. Estradiol supplementation was applied to provide continuous hormone release and thus eliminate interrodent differences related to the stage of the estrous cycle. The hormone supplementation was based on a 60-day release tablet containing 0.36 mg 17β-estradiol (Cat.# SE-121; Innovative Research of America, Sarasota, Florida), which was inserted at the level of the shoulder of each animal.

On day 15, concurrent with the extraction of the endometrial tissue from patients, all the mice underwent midline laparotomy to implant 4 blocks of 2 mm each of human endometrium into the parietal peritoneum (2 on each side of the incision) using *n*-butyl-ester cyanoacrylate adhesive (Vetbond, 3M, España S.L., Madrid, Spain). The endometrial tissue sample from each patient was implanted into 6 mice, and the animals were then randomized into 1 of the 3 treatment groups (1 control group and 2 experimental groups) to minimize possible bias due to the characteristics of patient's endometrial tissue.

Study Drug

Buprenorphine 0.1 mg/kg subcutaneously (Buprex, Reckitt Benckiser Healthcare, Berkshire, United Kingdom) was used as analgesia during surgery and every 12 hours during the following 2 days after surgery to minimize suffering.

Following implantation of endometrial tissue, pentoxifylline (Hemovás 300 mg, 15 mL vial, Robert S.A. Laboratory, Barcelona, Spain) was administered by oral gavage. The drug doses were calculated using a translation formula based on a body surface area normalization method to establish the dose equivalence between humans and experimental animals.[15] The animals were divided into 3 groups (n = 10 animals each): group 1 (control) received saline 0.1 mL/d; group 2, pentoxifylline 100 mg/kg/d; and group 3, pentoxifylline 200 mg/kg/d.

Figure 1. Necropsy of the animal, visual inspection, and identification of the implants. Identification of 4 implants of endometriosis; 2 on each side of the midline laparotomy in a mouse from the control group (group 1).

Macroscopic Quantification of Endometriosis-Like Lesions

After 28 days of treatment with oral pentoxifylline, the animals were sacrificed by cervical dislocation. The peritoneum was opened and the lesions identified, counted, and excised, together with the adjacent peritoneum, by a single surgeon (M.P.; Figure 1). In cases in which macroscopical lesions were not identified, all the parietal peritoneum where the endometrial tissue had been implanted was removed. Another investigator (P.C.), who was blinded to the treatment group, used a caliper to measure the total volume of tissue extracted from mice according to 3 perpendicular diameters (width, length, and height).

Immunohistochemical Morphometric Analysis of Endometriosis-Like Lesions

For this analysis, we used the methodology previously described by Defrère et al.[16] Paraffin-embedded blocks of tissue were cut into semiserials sections of 5 μm. The presence of viable endometriosis was confirmed by hematoxylin and eosin (H&E) staining of every fifth slide. From these H&E stained slides, those with the largest endometriotic lesion surface area were selected for histological quantification analysis. The following 2 serial sections from the selected slides were used to confirm the initial diagnosis of endometriosis observed in the H&E stained slides. These sections underwent immunohistochemical analysis with mouse monoclonal antibodies (mAb) to human CD10 (Clone 56C6, Ready-to-Use, Dako Omnis, Dako Denmark A/S, Denmark) to identify stroma and to cytokeratin 7 (CK7; Clone OV-TL 12/30, Ready-to-Use, Dako Autostainer/Autostainer Plus, Dako Omnis, Dako Denmark A/S) to identify glandular tissue,[17-19] according to the manufacturer's instructions. The next serial section was used as a marker of vascular proliferation and labeled with mAb to human CD31 (Clone JC70A, Ready-to-Use, Dako Autostainer/Autostainer Plus, Dako Omnis, Dako Denmark A/S).[20]

The H&E-, CD10-, CK7-, and CD31-stained slides were examined under an Olympus BX51 light microscope. The area of endometriosis was calculated by the extent of a 2-dimensional shadow on the slide with H&E staining under a Leica DMD108 microscope (Leica Microsistemas S.L.U., Barcelona, Spain). Quantification of the vascular proliferation marker was expressed as the percentage of microvessels stained with CD31 identified in the area of endometriosis (%CD31).

Cytokine Quantification

After sacrifice and prior to opening of the abdomen of each animal, the mice were fixed on a platform in supine position and peritoneal fluid was collected by irrigating the abdominal cavity with 2 mL of saline.

Peritoneal fluid samples were centrifuged at 2000 rpm for 5 minutes at 4°C. Cytokine levels were determined using the Mouse Cytokine 20-Plex Panel (Invitrogen, LMC0006) and the Mouse regulated on activation, normal T-expressed, and secreted (RANTES) Singleplex Bead Kit (Invitrogen, LMC1031, Waltham, MA, USA) following the manufacturer's instructions and using a Luminex's xMAP technology luminometer (Luminex B.V., MV 's-Hertogenbosch, Netherlands).

Statistical Analysis

Results are expressed as mean ± standard error of the mean (SEM; Table 1). The characteristics of endometriosis-like lesions (number of lesions, volume, area of endometriosis, and %CD31) and the quantification of cytokines of the study groups were compared using the Kruskal-Wallis test. Statistical significance was set at a P value of $<.05$. The statistical analyses were performed using the IBM SPSS Statistics 20.0 package (IBM, Armonk, New York).

Results

Three of 30 animals initially included in the study died: 2 from cardiac arrest during surgery (group 1), and the other due to heavy bleeding caused by accidental injury during the administration of oral medication (group 3).

Table 1. Macroscopic Results of Endometriosis-Like Lesions and Mice Weight According to Treatment Groups.[a]

Measurements	Group 1 (Saline)	Group 2, mg/kg/d	Group 3, mg/kg/d	*P* Value
# of implants (n)	2.40 ± 0.50	1.63 ± 0.42	1.11 ± 0.48	.065
Tissue volume (mm^3)	399.10 ± 120.68	276.75 ± 94.30	145.33 ± 38.20	.040
Initial body weight (g)	18.20 ± 0.50	18.10 ± 1.20	17.20 ± 0.90	.095
Final body weight (g)	20.10 ± 0.70	21.10 ± 0.50	19.30 ± 1.30	.135

Abbreviation: SEM, standard error of the mean.
[a]Mean ± SEM.

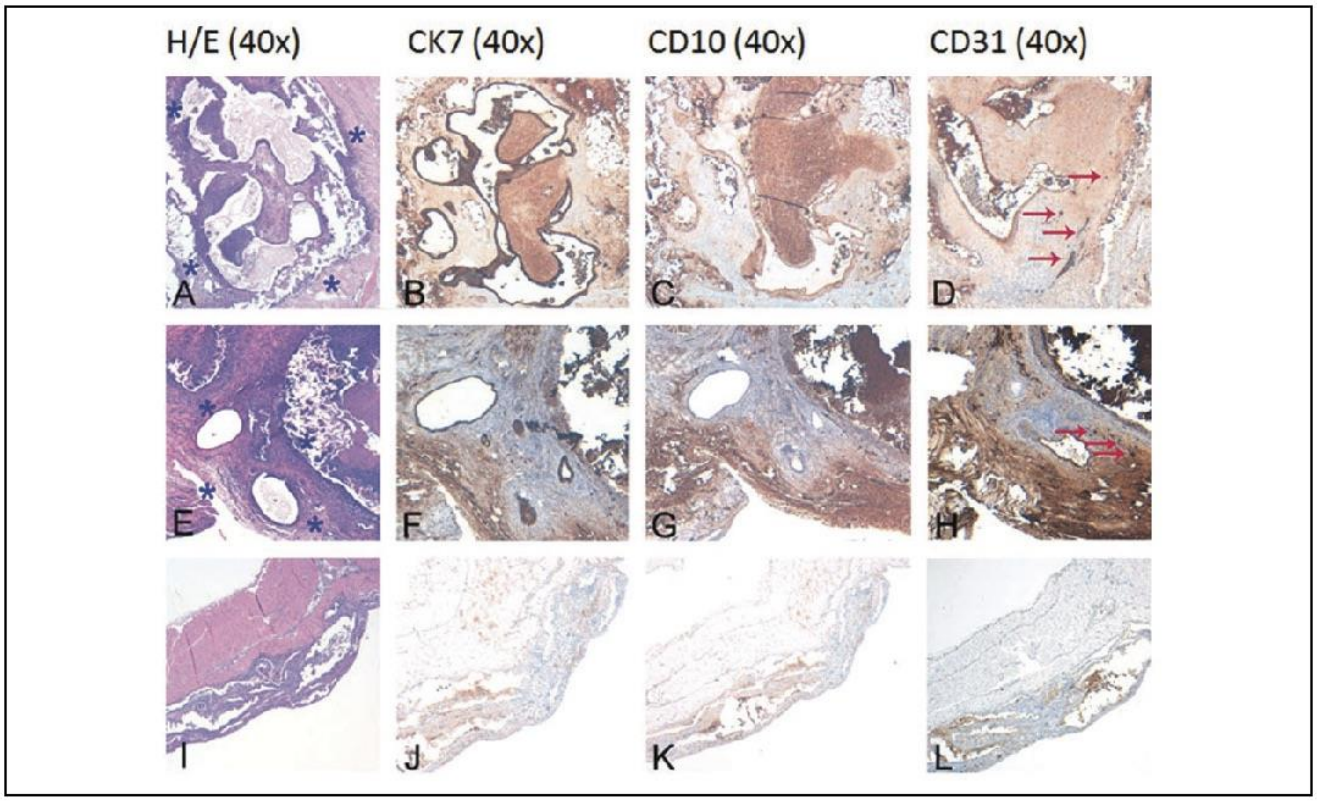

Figure 2. Effects of pentoxifylline on endometriotic lesion histology. Visual representation of immunohistochemical results. A to D, Control mouse not treated with pentoxifylline (group 1): (A) large implant of endometriosis; (B) strong glandular epithelium staining; (C) strong stromal staining; and (D) high number of microvessels. E to H, Mouse treated with 100 mg/kg/d of pentoxifylline (group 2): (E) moderate implant of endometriosis; (F) strong glandular epithelium staining; (G) strong stromal staining; and (H) medium number of microvessels. I to L, Mouse treated with 200 mg/kg/d of pentoxifylline (group 3): (I) inflammatory reaction without implants of endometriosis; (J) absence of endometrial epithelium; (K) absence of endometrial stroma; and (L) absence of microvessels. Blue stars: area considered as endometrial implant. Red arrows: microvessels.

Macroscopic Quantification of Endometriosis-Like Lesions

After 28 days, the human endometrial implants were excised. In 7 animals (2 in group 2 and 5 in group 3), all the parietal peritoneum in which the endometrial tissue had been implanted was finally extracted because macroscopical lesions were not identified. The results of the observations are listed in Table 1. A dose-dependent decrease in the number of lesions was observed, although the differences between groups did not reach the statistical significance ($P = .065$). The mean volume of the tissue extracted was significantly reduced ($P = .040$). No differences in the final body weight were observed among the 3 groups (Table 1).

Immunohistochemical Morphometric Analysis of Endometriosis-Like Lesions

The histological quantification of H&E-stained sections (Figure 2A) revealed a significant decrease in the mean area

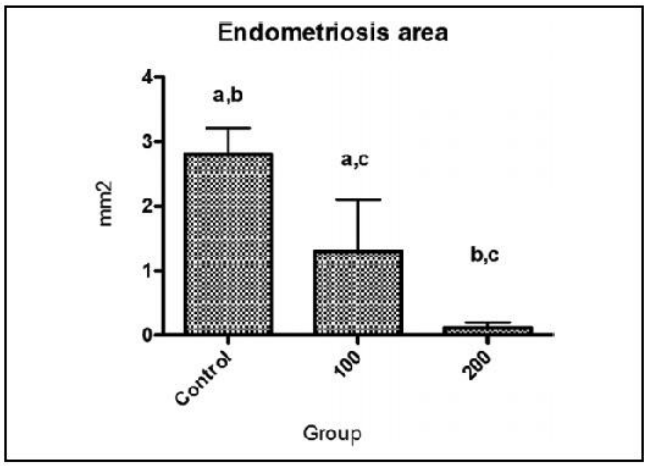

Figure 3. Comparison of the mean area of endometriosis among the 3 study groups. The mean area was calculated for a 2-dimensional shadow in the hematoxylin and eosin-stained slides under a microscope. Statistically significant differences are indicated with common superscripts, all *P* values <.05.

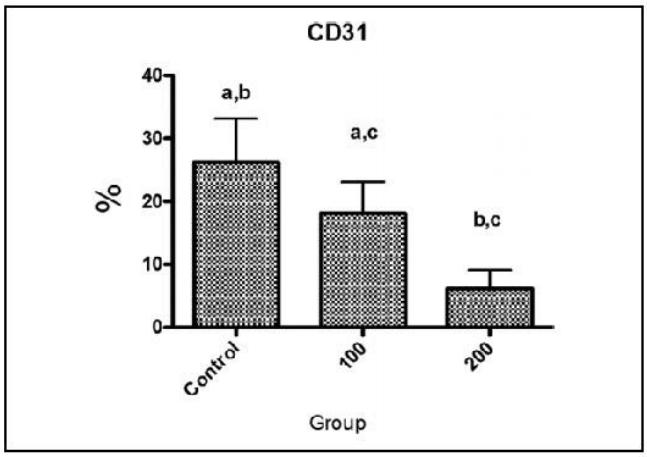

Figure 4. Comparison of the mean percentage of vascularization determined by antihuman CD31 among the 3 study groups. The vascular proliferation marker was expressed as the percentage of each slide that was labeled with the monoclonal mouse anti-human CD31 endothelial cell. Statistically significant differences are indicated with common superscripts; all *P* values <.05.

of endometriosis between groups, with the largest mean area being observed in group 1 (2.84 $\pm$ 0.60 mm^2), followed by group 2 (1.35 $\pm$ 0.43 mm^2), and the smallest in group 3 (0.12 $\pm$ 0.08 mm^2; $P = .001$; Figure 3). Staining with mAb to human CK7 and CD10 confirmed the endometriotic origin of these lesions (Figure 2B and C, respectively).

Treatment with pentoxifylline significantly reduced vascularization of the endometrium implants (Figure 2D). The density of microvessels calculated by the %CD31 detected in groups 2 and 3 (18.13 $\pm$ 5.09 and 6.22 $\pm$ 2.91) was significantly lower than in group 1 (26.20 $\pm$ 6.94; $P = .030$) at day 28 (Figure 4).

Cytokine Quantification

A statistically significant reduction in peritoneal levels was observed for the proinflammatory cytokines IL-1β, IL-6, TNF-α, and vascular endothelial growth factor (VEGF) by comparing group 1 with groups 2 and 3 (Table 2). Nonstatistically significant changes were also observed for the proinflammatory and anti-inflammatory cytokines RANTES and IL-10, respectively (Table 2).

Discussion

This study demonstrates the efficacy of the oral administration of pentoxifylline as a good alternative to current therapies for endometriosis. Pentoxifylline does not interfere with fertility, thereby favoring its potential as a good therapeutic option for patients with gestational desire. The current standard options for managing endometriosis are almost exclusively based on surgery and anovulatory hormonal therapies. These treatments are associated with a number of side effects and nullify the possibility of pregnancy.[21,22] However, there are few studies on possible endometriosis medical therapies that could allow pain control and search of pregnancy.[23] The use of immunomodulators, such as pentoxifylline, has been suggested and evaluated as an option[7,9,24] because it can reduce the production and action of cytokines. Women with endometriosis appear to exhibit increased macrophage activation, and the suppression of this macrophage response induced by pentoxifylline seemed to work as a cellular proliferation inhibitor for endometriosis.[12]

Table 2. Analysis of Cytokine Levels in pg/mL.[a,b]

Cytokines	Group 1 (Saline)	Group 2, mg/kg/d	Group 3, mg/kg/d	*P* Value
IL-1β	7.9 $\pm$ 2.7	0	0	.034
IL-6	2.6 $\pm$ 1.9	0	0	.029
IL-10	210.1 $\pm$ 21.5	237.6 $\pm$ 27.3	248.2 $\pm$ 31.6	.071
RANTES	98.4 $\pm$ 26.2	83.4 $\pm$ 20.9	62.9 $\pm$ 18.3	.060
VEGF	3.7 $\pm$ 1.2	1.3 $\pm$ 0.6	0.3 $\pm$ 0.09	.045
TNF-α	5.6 $\pm$ 2.9	0	0	.040

Abbreviations: IL, interleukin; RANTES, regulated on activation, normal T-expressed, and presumably secreted; SEM, standard error of the mean; TNF-α, tumor necrosis factor α; VEGF, vascular endothelial growth factor.
[a]Mean $\pm$ SEM.
[b]Values expressed as 0 represent that cytokine levels were not detected.

Although pentoxifylline has been evaluated in fertility in the past,[11,25-29] the ability of this drug to reduce endometriotic implant growth is still under investigation. In a study of female rats with surgically induced endometriosis, the use of pentoxifylline successfully produced regression of endometriosis tissue without inducing a hypoestrogenic state.[30] Similar results were reported in a more recent study, which demonstrated that the 21-day treatment of female rats with pentoxifylline led to reductions in the mean volume and mean number of endometriotic implants per animal.[31] However, both these studies used homologous rat models in which autologous fragments of

endometriotic tissue were implanted in the animals, and pentoxifylline was administered via subcutaneous injections.

The advantage of a xenogeneic model, which is generally used when specific effects or functions of drugs on human endometrium are evaluated in terms of assessing mechanistic insights as in the present study, is that its design compensates for the numerous physiological differences between mouse endometrium and its human counterpart.[32] This has been stressed by other researchers who are using this model,[33-35] and it has become a standardized procedure to study this disease. Although nude mice have congenital thymus aplasia resulting in a deficient T lymphocyte system, certain related cytokine secretion (eg, IL-4, IL-5, and interferon [IFN]-γ) and the role of T lymphocyte response on the proliferation of endometriotic implants in mice are not the principal objectives of this study. Most cytokines can be locally secreted by other cells. The main end points of this study are focused on these locally secreted cytokines, including IL-1β, IL-6, and TNF-α, which are typically involved in endometriosis.

With regard to the use of this model, as well as the dose and route of administration chosen for this study, this is the first time that the human dose translation formula has been applied in an animal model for endometriosis to establish an oral dose equivalence for this agent between humans and experimental animals. The oral administration is the route of choice in humans, making our findings much more transferable to clinical trials. Furthermore, the inclusion of a control group facilitates comparisons.

Our results suggest that treating nude mice with pentoxifylline leads to a significant reduction in endometriosis area and vascularization in the animals. Similar to the results reported in syngeneic mouse models of endometriosis, the number of lesions decreased in a dose-dependent manner, even though the differences among the groups were not statistically significant. Quantification of the total volume of the extracted tissue, however, showed a significant reduction between the pentoxifylline-treated and control groups. The results were confirmed by immunohistochemical analysis. Microscopy showed a significant reduction in endometriosis area in the H&E-stained tissue sections among the groups with the greatest reduction being detected in the group treated with 200 mg/kg/d pentoxifylline dose (group 3). Additionally, the morphometric analysis of the CD10 and CK7 markers used to establish the stromal and glandular structures of lesions, respectively, confirmed the endometriosis origin of the tissue evaluated. Evaluation of the %CD31 showed a significant reduction in vascularization in groups 2 and 3 treated with pentoxifylline compared to group 1, which is in agreement with previous reports showing that the establishment and development of endometriosis lesions depend on the formation of blood vessels to guarantee oxygen and essential nutrient supply.[36,37] This suggests that the effect of pentoxifylline on vascularization may be essential to reduce the size of the nodules, as previously hypothesized in many human cancers.[38]

It is known that the presence of endometriosis lesions alters the peritoneal environment, suggesting the importance of these secretory products in the pathogenesis of the disease. Increased levels of many cytokines have been implicated in the onset of endometriosis, mainly IL-1, IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ, and RANTES.[39,40] On one hand, our results showing a reduction in the proinflammatory cytokines (IL-1β, IL-6, and TNF-α) are in accordance with the effects previously described for pentoxifylline.[10,41] Moreover, we found other proinflammatory proteins to be reduced (RANTES, VEGF). Indeed, we found that the greater the cytokine values the stronger the presence of endometriosis, similar to previous reports.[42] On the other hand, the levels of the anti-inflammatory cytokine IL-10 were elevated after treatment, which might be explained by its role in the control of inflammation.[43] Overall, the immunomodulatory effects of pentoxifylline were clearly demonstrated.

Although our study provides valuable data suggesting a beneficial effect of pentoxifylline on the reduction of endometriosis, there are some drawbacks. First, all animal experimentation has a major limitation based on the differences between the physiology of mice and humans, although the use of a heterologous model may reduce these differences. Additionally, mice do not spontaneously develop endometriosis, and the adaptive (but not innate) immune system of these animals was compromised to avoid tissue rejection. Finally, the different tissues used from patients may be a limitation; however, this is the only way to obtain a large sample, and mice implanted with tissue from the same patient were randomized to minimize the possible bias due to patient characteristics.

In conclusion, the results in our study suggest that the immunomodulatory properties of pentoxifylline can significantly reduce the number and size of endometriosis-like lesions, as well as their vascularization, in a xenogeneic model of endometriosis. The cytokine pattern observed in this study confirms the importance of the innate immune system and the involvement of several cytokines in the pathogenesis of the disease, although their specific roles need to be further explored. This is the first study in which pentoxifylline was orally administered to the animals. On confirmation of the present results in larger studies, pentoxifylline could be an effective and economic therapeutic option for endometriosis, replacing anovulatory drugs incompatible with gestation. Further studies are guaranteed to make it transferable to humans.

Authors' Note

This work was conducted at the Clinical Institute of Gynecology, Obstetrics, and Neonatology, in Hospital Clinic, Universitat de Barcelona, and at the CCiTUB, Animal facilities of the Universitat de Barcelona, Faculty of Medicine, Barcelona, Spain.

Declaration of Conflicting Interests

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5. RESUM GLOBAL DELS RESULTATS I DISCUSSIÓ

Malgrat la millora en els coneixements sobre l´endometriosi en els darrers anys continua essent fonamental el desenvolupament d´eines que ajudin a millorar el diagnòstic i l'estadificació de la malaltia, així com la creació de noves línies de tractament que permetin preservar la fertilitat durant el seu ús. Els treballs realitzats en la present tesi doctoral sobre aquesta temàtica han mostrat, en resum, els resultats següents que es discuteixen a continuació.

La cirurgia de l´EP és complexa i sovint requereix un equip multidisciplinari i per això és molt important sospitar prequirúrgicament l´EP i estadificar correctament la malaltia. El diagnòstic de l´EO mitjançant ecografia ginecològica és molt fàcil i comú, però la sospita i diagnòstic ecogràfic de l´EP no està tant estesa ni és tan coneguda. Això sovint genera un infradiagnòstic i retard en el diagnòstic de l´EP. S´ha postulat que l´anamnesi i l´ús de qüestionaris dirigits poden ajudar en la sospita i diagnòstic de l´EP (30,31). Per tant, existeix una necessitat de desenvolupar millors eines diagnòstiques per predir l'EP. Els EO poden estar presents amb molta freqüència en pacients amb EP. Amb la intenció de poder discriminar mitjançant l´anamnesi aquelles pacients amb EO coneguda que presentaven EP associada es va realitzar el primer estudi d'aquesta tesi doctoral. En aquest primer estudi (ESTUDI 1) es va realitzar un model de predicció d'EP en pacients amb EO amb l'ús de tres marcadors clínics. Sobre una mostra de 178 pacients, d'acord amb l'estimació feta amb el *Odds Ratio* (OR), tenir gestacions anteriors (0,25; IC (Interval de Confiança) del 95%: 0,09-0,72; p <0,01), història prèvia de tractament

quirúrgic de l'endometriosi (10,17; IC del 95%: 3,62-28,53; p <0,01) i un augment d'una unitat en la puntuació mitja del EAPP (*Endometriotic Associted Pelvic Pain)* (3,49; IC del 95%: 2,37-5,15; p <0,01) es van associar significativament amb la presència d´EP.

La capacitat predictiva observada d'aquest model va ser molt alta (Àrea sota la corba ROC: 0,91; IC del 95%: 0,86-0,95). El punt de tall òptim era 0,538; amb una sensibilitat del 80% per al diagnòstic d´EP en pacients EO i una especificitat del 84%, amb un 81% de pacients correctament classificades.

El model aplicat al subgrup de pacients "de la població general" va mostrar igualment bona capacitat predictiva (AUC: 0,91; IC dels 95%: 0,83-0,96).

Molts estudis anteriors han intentat predir l'endometriosi. Entre aquests, destaca el realitzat per Chapron *et al*. (29) en el qual van avaluar la intensitat del dolor pèlvic en una població de pacients que presentaven EO i van trobar una associació significativa amb les lesions infiltrants en profunditat. En una altra investigació, aquests autors van dissenyar un model de diagnòstic basat en símptomes i història clínica (30), desenvolupat específicament per predir EP posterior entre les dones amb dolor pèlvic crònic, amb una sensibilitat del 74,5% i una especificitat del 68,7%. En l'estudi de casos i controls realitzat per Lafay Pillet *et al.*, es descriu la metodologia per construir una puntuació suggestiva de diagnòstic d'EP i és probablement la metodologia més similar a la nostra (31). Aquest es va basar en un qüestionari preoperatori estandarditzat. És un estudi que va incloure 326 dones i va obtenir una sensibilitat baixa, encara que amb una alta especificitat (51% i 94%, respectivament, en les pacients amb puntuacions > 35). En comparació amb el nostre estudi, destaquen els diferents resultats pel que fa a

sensibilitat i especificitat. Tot i que el nostre estudi té una mostra petita, hem obtingut una sensibilitat del 80% i una especificitat del 84% per al diagnòstic de pacients amb EO, amb un 81% dels pacients classificat correctament. No obstant això, la comparació estricta entre els diferents estudis és difícil a causa de les diferències en la metodologia o les variables utilitzades.

Les tres variables incloses en el model predictiu de l´Estudi 1 són variables que han estat avaluades en estudis anteriors:

- Trobem una associació estadística amb gestacions anteriors (identificat com un factor protector) i EP, encara que això no havia estat prèviament reportat en la literatura (30). Això és consistent amb el fet que l'EP ha estat tradicionalment associada amb la infertilitat.
- El motiu que un factor predictor sigui la història prèvia de tractament quirúrgic de l'endometriosi és probablement una conseqüència d'una subestimació de l'extensió de la malaltia durant la intervenció, d'acord amb la literatura (29,30), a més del mecanisme de trauma sobre el peritoneu superficial i les adherències postoperatòries, que poden provocar la proliferació i la invasió endometrial i, per tant, EP (23).
- El dolor havia estat postulat com a bon predictor de la presència d'EP en pacients amb EO (29) i això ha estat confirmat en el nostre Estudi 1. Es va treballar amb l'EAPP mitjà perquè tots els tipus de dolor estiguessin representats en el valor final de VAS. Va ser el primer cop que aquesta variable era utilitzada en un model de predicció, de forma que s'integrava en un sol valor tots els tipus de dolor que es presenten habitualment en la malaltia.

A més, el nostre model va ser eficaç en la identificació d'EP en dones amb EO.

Per altra banda, els models predictius són sovint poc pràctics en el moment de la presa de decisions clíniques a causa de la dificultat de càlcul que comporten. En aquest àmbit, la creació d'un nomograma permet la fàcil aplicabilitat de l'equació per part dels professionals sanitaris, sense la necessitat de realitzar un càlcul matemàtic complex. El nomograma és una representació gràfica d'una fórmula matemàtica que serveix per predir una determinada situació a partir d'una o més covariables, i és una eina que ha estat àmpliament adoptada en investigació biomèdica amb l'objectiu d'ajudar els metges en l'aplicació diària d'aquest model. D'altra banda, la capacitat predictiva de models desenvolupats en hospitals de tercer nivell es redueix en general quan s'extrapola a un àmbit d'atenció primària (58) i pot estar esbiaixat per alguns factors (per exemple, la selecció dels pacients). Per tant, en un esforç per afegir més valor al nostre model, la revaluació del model en la submostra "de la població general" confirma un bon rendiment per a la predicció de l'EP en tots els àmbits. Per altra part, aquest estudi ha de ser considerat com un estudi preliminar i és probablement petit des d'un punt de vista biomètric. No obstant això, l'objectiu és estimular futures investigacions més àmplies per obtenir una base més sòlida que ajudi a aclarir el valor predictiu d'aquest enfocament.

Una altra problemàtica associada al diagnòstic de sospita de l'EP i la seva estadificació és la difícil interpretació del dolor que presenten les pacients amb aquesta i altres patologies. S´han proposat símptomes-guies d´empitjorament catamenial que poden ajudar al diagnòstic i a la localització anatòmica de l´EP (33-35). En relació amb aquesta

problemàtica, en el segon estudi (ESTUDI 2) es va avaluar la possible relació entre dolor i la localització anatòmica de l'endometriosi. Per a dur a terme l'estudi, les lesions van ser categoritzades segons la classificació Enzian.

En l´Estudi 2 es va trobar una associació positiva significativa entre el dolor pèlvic i la presència d'adenomiosi (r = 0,468; p = 0,037), entre disquèzia i hemorràgia rectal (r = 0,461; p = 0,046), i disúria i hematúria (r = 0,532; p = 0,004). D'altra banda, es va observar una clara tendència d'associació entre la dismenorrea i la presència d'adenomiosi (r = 0,418; P = 0,080). Per altra banda, també es va trobar una absència de correlació entre els marcadors de dolor i els compartiments específics afectats. A més, es va dur a terme una anàlisi de regressió logística multivariant realitzat per tal de predir variables associades amb el dolor en la nostra sèrie. Entre les variables estudiades, només l'adenomiosi va mostrar una associació significativa amb el dolor pèlvic (OR 0,32; IC del 95%: 0,14-0,20; p: 0,02).

Gonzales *et al*. van trobar una correlació entre l'adenomiosi i l'EP, sobretot en el recte i còlon sigmoide (59). Encara que del 30% al 50% de dones amb adenomiosi són asimptomàtiques (38, 60-62), la forma greu de la malaltia sovint mostra símptomes correlacionats amb la intensitat i la profunditat de les lesions (59). En el nostre estudi, vam observar una correlació estadísticament significativa entre el dolor pèlvic i la presència d'adenomiosi, d'acord amb els resultats prèviament reportats (60, 63, 64).

En l´Estudi 2 vam confirmar histològicament el diagnòstic de l'endometriosi en tots els casos inclosos després de l'extirpació quirúrgica dels implants. Això pot explicar les diferències observades amb altres estudis. Publicacions anteriors han reportat una associació entre la semiologia del dolor i la localització anatòmica precisa (33-35); per

tant, els símptomes de dolor s'han descrit com *dolor que indica la ubicació* (34). No obstant això, el diagnòstic de la malaltia en aquests estudis presenta la gran limitació de no haver-se confirmat histològicament. Només en l'estudi realitzat per Vercellini *et al*. presentava diagnòstic histològic en el 72,9% del les pacients (35). Per altra banda, el nostre Estudi 2 va descartar la possibilitat d'una relació entre el dolor i el nombre dels nòduls de la malaltia; aquesta possibilitat ha estat avaluada en estudis anteriors amb resultats inconsistents (65, 66). Finalment, el nostre estudi va veure que la presència d'EO no es correlacionava amb la intensitat del dolor. Aquest resultat és coherent amb les conclusions d'estudis anteriors en els quals l'EO no va contribuir al dolor crònic (67-69).

La principal limitació del nostre Estudi 2 va ser la mida de la mostra, que era petita des d'un punt de vista biomètric. Creiem, de nou, que aquest treball hauria de ser considerat com un estudi preliminar destinat a estimular futurs treballs amb potència adequada així com majors anàlisis multivariades que incloguin més factors predictors d'EP i adenomiosi. Es considera útil proporcionar els resultats d'estudis ben dissenyats, fins i tot els que tenen baixa potència, per encoratjar a altres investigadors a abordar aquesta qüestió i proporcionar una base més gran per a una eventual revisió de petites sèries de pacients per tal d'ajudar a clarificar les relacions entre la clínica i la malaltia. Una altra limitació de menor importància va ser el fet que les dades relacionades amb la presència d'adherències annexials i la taxa de recurrència del dolor després de la cirurgia només estaven disponible en un petit grup de pacients.

Tot i que el diagnòstic de sospita de la malaltia representa reptes importants, el seu tractament comporta grans limitacions, i la recerca de nous fàrmacs que permetin

evitar cirurgies o recidives és fonamental. Un fàrmac immunomodulador molt prometedor pel tractament de l´endometriosi és la pentoxifil·lina que és un derivat de la metilxantina que modula la producció de citocines. Les dones amb endometriosi presenten un augment de l'activació de macròfags, i la supressió d'aquesta resposta de macròfags induïda per la pentoxifil·lina sembla funcionar com un inhibidor de la proliferació cel·lular per l'endometriosi (45). Per tot això, en l'últim treball de recerca (ESTUDI 3) es van avaluar els efectes de l'administració oral de pentoxifil·lina sobre les lesions d'endometriosi induïdes en un model heteròleg de ratolins.

Es va observar una disminució dependent de la dosi en el nombre de lesions, encara que les diferències entre els grups no van assolir significació estadística (p = 0,065). El volum mitjà del teixit extret es va reduir significativament (p = 0,040). La quantificació histològica mitjançant la tinció de hematoxilina & eosina va revelar una disminució significativa en l'àrea mitjana de l'endometriosi entre els grups (Group 1, control; Group 2, pentoxifil·lina 100 mg/kg/dia; Group 3, pentoxifil·lina 200 mg/kg/dia.), sent observada una major àrea mitjana en el Grup 1 (2,84 ± 0,60 mm^2), seguit pel Grup 2 (1,35 ± 0,43 mm^2), i essent la més petita en el Grup 3 (0,12 ± 0,08 mm^2; p = 0,001). La tinció amb mAb (*Mouse Monoclonal Antibody)* de CD10 (*Cluster of Differentiation* 10) humana i CK7 (*Cytokeratine* 7) va confirmar l'origen endometrial d'aquestes lesions. El tractament amb pentoxifil·lina va reduir significativament la vascularització dels implants d'endometriosi. La densitat de microvasos calculats pel % de CD31 detectat en els Grups 2 i 3 (18,13 ± 5,09 i 6,22 ± 2,91) va ser significativament menor que en el Grup 1 (26,20 ± 6,94; p = 0,030) en el dia 28. Es va observar una reducció estadísticament significativa en els nivells peritoneals de les citocines proinflamatòries IL-1β, IL-6, TNF-a,

i VEGF al comparar del Grup 1 amb els Grups 2 i 3. També es van observar diferències no estadísticament significatives per les citocines pro i antiinflamatòries RANTES i IL-10, respectivament.

Aquest estudi va demostrar l'eficàcia de l'administració oral de pentoxifil·lina com una bona alternativa a les teràpies actuals per l'endometriosi. La pentoxifil·lina no interfereix amb la fertilitat, d'aquesta manera afavoreix el seu potencial com una bona opció terapèutica per les pacients amb desig gestacional.

La pentoxifil·lina és una metilxantina, que actua com a inhibidor de la fosfodiesterasa. Aquest medicament també pot reduir la producció i l'acció de citocines com ara TNF-α, per l'elevació dels nivells d'AMP cíclic intracel·lular. Les dones amb endometriosi semblen exhibir un augment de la resposta humoral, l'activació de macròfags, i la disminució de la immunitat cel·lular, amb una disminució de cèl·lules T i la capacitat de resposta de cèl·lules NK. La supressió de la resposta de macròfags induïda per la pentoxifil·lina sembla funcionar com un inhibidor de la proliferació cel·lular per l'endometriosi (45).

La capacitat d'aquest fàrmac per reduir el creixement d'implants d'endometriosi està encara sota investigació. Hi ha dos estudis realitzat en models homòlegs de rates, en els quals s'ha observat reducció de l'endometriosi amb l'ús de la pentoxifil·lina. En aquests models, els fragments autòlegs de teixit endometrial es van implantar en els animals i la pentoxifil·lina es va administrar a través d'injeccions subcutànies (56, 57).

L'avantatge d'un model heteròleg, és que el seu disseny compensa les nombroses diferències fisiològiques entre l'endometri de ratolí i el seu homòleg humà (46). Tot i que els ratolins *nude* tenen aplàsia tímica congènita que resulta en un sistema de

limfòcits T deficient, la secreció de citocines relacionades amb aquests (per exemple, IL4, IL-5, IFN gamma) i la funció de resposta dels limfòcits T sobre la proliferació dels implants d'endometriosi no van ser els objectius principals d'aquest estudi. A més, la majoria de les citocines es poden secretar a nivell local per altres cèl·lules. Els principals objectius de valoració d'aquest estudi es van centrar en aquestes citocines secretades localment, incloent IL-1β, IL-6 i TNF-α, que normalment estan implicades en endometriosi.

Cal destacar que en l´Estudi 3 va ser la primera vegada que es va utilitzar una fórmula de translació de dosi aplicada en un model animal d'endometriosi per establir una equivalència de dosi oral d'aquest agent entre els éssers humans i els animals experimentals. L'administració oral és la via d'elecció en els éssers humans, per aquest motiu, l'ús d'aquesta via en el nostre estudi el fa molt més transferible a possibles futurs assajos clínics.

Alguns estudis previs mostren que l'establiment i desenvolupament de les lesions d'endometriosi depenen de la formació de vasos sanguinis per garantir el subministrament d'oxigen i nutrients essencials (70, 71). En concordança a aquests estudis, els nostres resultats de l´Estudi 3 van suggerir que l'efecte de la pentoxifil·lina sobre la vascularització pot ser essencial per reduir la mida dels nòduls, com anteriorment s'ha postulat en molts estudis de càncer en humans (72).

Finalment, nivells elevats de moltes citocines han estat implicats en l'aparició de l'endometriosi, principalment IL-1, IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ i RANTES (73, 74). Els resultats del nostre Estudi 3, d'una banda van mostrar una reducció de les citocines proinflamatòries (IL-1β, IL-6, TNF-α) i està en concordança amb els efectes descrits

anteriorment per la pentoxifil·lina (42, 75). D'altra banda, es va observar la reducció d'altres proteïnes proinflamatòries (RANTES, VEGF). De fet, es va observar que valors alts de citocines estaven relacionats amb la presència d'endometriosi, de forma similar al que es va observar en estudis anteriors (18). Per altra part, els nivells de la citocina antiinflamatòria IL-10 van ser elevats després del tractament; aquest fet podria ser explicat pel seu paper en el control de la inflamació (76). En general, els efectes immunomoduladors de la pentoxifil·lina van ser demostrats.

Si bé el nostre Estudi 3 proporciona dades valuoses que suggereixen un efecte beneficiós de la pentoxifil·lina, hi ha alguns inconvenients. En primer lloc, tota l'experimentació animal té una limitació important en base a les diferències entre la fisiologia dels ratolins i els éssers humans, encara que l'ús d'un model heteròleg pot reduir aquestes diferències. A més, els ratolins no desenvolupen espontàniament endometriosi i el sistema immune adaptatiu (però no innat) d'aquests animals està compromès per evitar el rebuig de teixits. Finalment, els diferents teixits endometrials que provenen de diferents pacients poden ser una limitació, però aquesta és l'única manera d'obtenir una mostra àmplia. Per tal de minimitzar aquesta limitació, els ratolins amb teixit procedent de la mateixa pacient van ser assignats a l'atzar als diferents grups de tractament, disminuint d´aquesta manera el possible biaix produït per les característiques específiques de cada pacient.

Els nostres estudis ens permeten arribar a les conclusions que s´exposen a continuació i que es corresponen amb els 4 objectius proposats en l´apartat corresponent d´aquesta tesi doctoral (veure pàgines 29 i 30).

6. CONCLUSIONS I IMPLICACIONS CLÍNIQUES

1. Després de la investigació realitzada, hem obtingut una equació de predicció d'EP en pacients amb EO, basada en tres indicadors simples, accessibles i no invasius que poden orientar als professionals a determinar la probabilitat d'EP. A més, l'ús del nomograma ha de facilitar l'aplicació per part dels metges generals o ginecòlegs menys qualificats en la seva pràctica diària. Les dones que presentin alt risc requeriran una exploració més invasiva per tal de poder establir l'estadificació de la malaltia i poder realitzar un abordatge quirúrgic d'un sol pas per a l'eliminació completa de la malaltia i per tant s'haurà de valorar la seva derivació a centres especialitzats.

2. S'han obtingut més eines a l'hora d'orientar el diagnòstic i l'extensió de la malaltia. Els resultats obtinguts en el segon estudi ens indiquen que en determinats casos és necessari valorar una possible adenomiosi associada i aquesta pot ser sospitada prequirúrgicament per les característiques del dolor que presenten les pacients. Per altra banda, el tipus de dolor ens proporciona poca informació per orientar la localització anatòmica de la malaltia. Per tant, s'haurà de valorar de forma acurada i individualitzada, la necessitat de determinades proves diagnòstiques.

3. S'ha demostrat la possibilitat d'ús dels immunomoduladors, en aquest cas la pentoxifil·lina, en el tractament de la malaltia per reduir el nombre d'implants i la

seva mida en un model animal. La pentoxifil·lina suposa una possible alternativa als medicaments anovulatoris incompatibles amb la gestació. Cal destacar dos aspectes importants del nostre estudi que augmenten la probabilitat de transferibilitat a la clínica i marquen una diferència respecte a estudis previs: l'ús d'un model heteròleg on disminuïm les possibles diferències fisiològiques entre l'endometri humà i el de ratolí que hi ha present en els models homòlegs, donat que usem directament endometri humà; i el fet d'haver escollit la via oral com a via d'administració.

4. A més, amb l'efecte de la pentoxifil·lina, s'ha avaluat i reforçat el paper important de les citocines en el desenvolupament de la malaltia. Finalment, l'efecte observat sobre la reducció de la vascularització, suggereix que aquesta també juga un paper important en la reducció dels implants endometrials.

Com a conseqüència de tot el que hem exposat i a manera de conclusió general d'interès clínic i pràctic, podem afirmar que, en global, hem contribuït a la millora en el diagnòstic de la malaltia, hem permès la sospita de forma prequirúrgica d'EP en pacients amb EO i hem posat de manifest la importància de la sospita d'adenomiosi associada en pacients amb endometriosi amb dolor. Per altra banda, hem obert nous horitzons en el tractament de la mateixa i hem postulat la possibilitat d'ús de la pentoxifil·lina com a fàrmac no anovulatori per al tractament de la malaltia i per aconseguir reduir la mida dels implants.

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