Universitat Autònoma de Barcelona

CONDILOMA, DISPLASIA E INFECCIÓN POR EL VIRUS DEL PAPILOMA HUMANO EN EL CANAL ANAL EN PACIENTES INFECTADOS POR EL VIRUS DE LA INMUNODEFICIENCIA HUMANA

Sandra Vela Bernal

Universitat Autònoma de Barcelona

FACULTAD DE MEDICINA

Departamento de Cirugía General y Digestiva

CONDILOMA, DISPLASIA E INFECCIÓN POR EL VIRUS DEL PAPILOMA HUMANO EN EL CANAL ANAL EN PACIENTES INFECTADOS POR EL VIRUS DE LA INMUNODEFICIENCIA HUMANA

Memoria de tesis presentada en la modalidad de compendio de publicaciones realizadas por

Sandra Vela Bernal

para optar al grado de Doctor en Medicina y Cirugía

Tesis realizada bajo la dirección de los doctores:

Sebastián Videla Cés

Francesc García-Cuyás

Jaume Fernández-Llamazares Rodríguez

y del tutor:

Francesc García-Cuyás

Abril – 2019

CONDILOMA, DISPLASIA E INFECCIÓN POR EL VIRUS DEL PAPILOMA HUMANO EN EL CANAL ANAL EN PACIENTES INFECTADOS POR EL VIRUS DE LA INMUNODEFICIENCIA HUMANA

Programa de Doctorado de Cirugía y Ciencias Morfológicas

Tesis realizada por:

Sandra Vela Bernal

Directores:

Dr.Sebastián Videla Cés

Dr.Francesc García-Cuyàs

Dr. Jaime Fernández-Llamazares

Tutor:

Dr. Francesc García-Cuyàs

AGRADECIMIENTOS

Al Dr. Sebastià Videla per la seva gran empenta en el mon de la recerca i per la seva dedicació en aquest treball sense límit horari. Gràcies Sebas per haver-me dedicat molt més que temps.

Al Dr. Francesc García-Cuyàs per tenir la mà estesa en tot moment, pel temps compartit i per la seva implicació en aquest treball, sempre positiu i constructiu.

Al Dr. Jaime Fernández- Llamazares por haber creído en mi desde el primer encuentro, por haberme apoyado siempre y no dejarme perder jamás la ilusión en todos estos años de trabajo.

Al Dr. Joan Francesc Julián, mi jefe de servicio, por apoyarme siempre y animarme a continuar caminando con una sonrisa aunque hubiera piedras en el camino.

A la Dra . Marta Piñol per la seva motivació i per tots els coneixements que m'ha transmès al llarg d'aquests anys.

A tot l'equip de Fundació Lluita Contra la Sida per haver-me ajudat sempre i fer-me més fàcil aquesta feina.

A tots els companys del Servei de Cirurgia General i Digestiva de l'Hospital de Can Ruti, el meu servei, pel granet de sorra que sens dubte tots han aportat en la meva vida aquests anys.

Al meu pare; *Papa, ja l'he acabat.*

ÍNDICE

ABREVIATURAS UTILIZADAS EN ESTA MEMORIA

AAR	Anoscopia de Alta Resolución (vocablo inglés, "**HRA**: high-resolution anoscopy")
CEA	Carcinoma Escamoso de Ano
ETS	Enfermedad de Transmisión Sexual
LEI	Lesión escamosa intraepitelial (vocablo inglés, "**SIL**: squamous intraepithelial lesion")
LEI-B	Lesión escamosa intraepitelial de bajo grado oncogénico (vocablo inglés, "**LSIL**: low squamous intraepithelial lesion")
LEI-A	Lesión escamosa intraepitelial de alto grado oncogénico (vocablo inglés, "**HSIL**: high squamous intraepithelial lesion")
HSH	Hombres que practican Sexo con Hombres
HSM	Hombres que practican Sexo con Mujeres
NIA	neoplasia intraepitelial anal
IR	Infrarrojos
SIDA	Síndrome de Inmunodeficiencia Humana
TAR	Terapia Antiretroviral
VIH	Virus de la Inmunodeficiencia Humana
VIH+	paciente infectado por el Virus de la Inmunodeficiencia Humana
VPH	Virus del Papiloma Humano

Introducción

La esperanza de vida de las personas infectadas por el virus de la inmunodeficiencia humana (VIH) ha cambiado radicalmente desde la introducción del tratamiento antirretroviral (TAR) a finales del siglo pasado. En la actualidad, la infección por el VIH es una enfermedad crónica. Este hecho ha conllevado que las personas infectadas por el VIH presenten un mayor riesgo de desarrollar otras patologías como las enfermedades relacionadas con la infección por el virus del papiloma humano (VPH).

La infección por el VPH es la enfermedad de transmisión sexual más frecuente en el mundo. Los VPH con capacidad para infectar las mucosas han sido clasificados en dos grupos según el riesgo de producir cáncer de cérvix: genotipos de alto y de bajo riesgo oncogénico. Los genotipos de bajo riesgo oncogénico son el agente etiológico de patologías más banales como verrugas, condilomas; y los genotipos de alto riesgo oncogénico son los relacionados con los carcinomas escamosos (cérvix, ano, pene...).

Entre las personas infectadas por el VIH, la prevalencia e incidencia de patología relacionada con el VPH está aumentada respecto a las personas no infectadas por el VIH.

En relación con los genotipos de bajo riesgo oncogénico, la incidencia de nuevos casos de condiloma en el canal anal está aumentada entre las personas infectadas por el VIH respecto a la población general, aunque no se dispone de datos actualizados que ilustren esta afirmación. En relación con los genotipos de alto riesgo oncogénico, la incidencia de nuevos casos de carcinoma escamoso de ano (CEA) en la población general es de 1,8 casos por 100000 persona-años en EEUU y de ≈0.9 casos por 100000 persona-años en España, representando el 2.0-2.5% de todos los tumores del tubo digestivo. Entre la población masculina infectada por el VIH, la incidencia de nuevos casos de CEA es entre 35 y 70 veces mayor que la población general (70 casos por 100000 persona-años).

Los condilomas en el canal anal es una patología prevalente, sin un tratamiento consensuado y con una tasa de recidiva alta especialmente entre las personas infectadas por el VIH. Esta patología, a priori banal, es una necesidad médica no cubierta. En la actualidad, uno de los retos que tienen los profesionales al cuidado de esta patología es encontrar un tratamiento efectivo que la erradique y prevenga la recidiva. Entre los diferentes tratamientos que se disponen se encuentran los farmacológicos y los de ablación (exéresis quirúrgica, infrarrojos, crioterapia). Es importante destacar que la existencia de condiloma/s en el canal anal en personas infectadas por el VIH se asocia a la presencia de lesiones escamosas intraepiteliales (lesiones displásicas) causadas por diferentes genotipos del VPH de alto riesgo oncogénico.

Esta tesis doctoral, enmarcada dentro de la línea de investigación "Infección por el VPH y patologías asociadas: condiloma y displasia del canal anal en pacientes infectados por el VIH", está basada en 4 artículos originales publicados en revistas de habla inglesa y de revisión por pares (2 artículos son la base de esta memoria y los otros dos son de soporte de la misma), tiene como objetivo principal aportar evidencias actualizadas en pacientes infectados por el VIH sobre:

1. la prevalencia de condiloma/s en el canal canal,
2. la prevalencia de lesiones escamosas intraepiteliales en el canal anal,
3. la prevalencia de la coexistencia de codiloma/s y lesiones escamosas intraepiteliales en el canal anal,
4. qué genotipos del VPH están presentes en el canal anal cuando existe una lesión,
5. la efectividad de los diferentes tratamientos usados en el manejo de los condiloma/s del canal anal en la práctica clínica.

Todas las investigaciones realizadas y presentadas en esta memoria se han llevado a cabo de acuerdo a las normas de buena práctica clínica y a la legislación vigente.

1. Infección por el Virus de la Inmunodeficiencia Humana

1.1 Epidemiología

El Síndrome de la Inmunodeficiencia Humana (SIDA) se describió por primera vez en Los Ángeles en junio de 1981 en Hombres que practican Sexo con Hombres (HSH) como una curiosidad epidemiológica. Sin embargo, el origen del VIH-1 precede probablemente en unas cinco décadas a su identificación como agente causal de la enfermedad [1].

Aunque el primer caso documentado de infección por el VIH-1 data de 1959, detectado en territorio de la actual República del Congo, análisis recientes basados en las distancias genéticas en árboles filogenéticos, indican que el antecesor común responsable de la inmensa mayoría de infecciones por el VIH a escala global es un virus propio de los chimpancés localizado en África central [1]. Este virus propio de los chimpancés, pasó a seres humanos posiblemente a través de contactos con sangre de monos cazados para su consumo. La diversificación de este virus y la aparición de formas recombinantes parece haber tenido lugar en África central en la década de los sesenta, momento en el cual la infección por el VIH en el ser humano fue capaz de desarrollar el SIDA [1].

Hoy en día, el impacto de la infección por el VIH y sus consecuencias en nuestro medio es considerable. Desde 1981, cuando se detectaron los primeros casos, más de 37 millones de personas se han infectado por el VIH y, actualmente, es una de las causas más importantes de muerte en todo el mundo; tanto por la propia enfermedad como por las enfermedades asociadas a esta infección. Se calcula que al año mueren 1.2 millones de personas en el mundo por causas relacionadas con la infección por el VIH [1].

La epidemia ha ido creciendo de forma exponencial en muchos países y se ha ido extendiendo a todas las áreas del planeta, por lo que es posible hablar de pandemia del VIH. En el año 2017 se infectaron unos 2 millones de personas en todo el mundo [1].

La epidemia del VIH/SIDA ha evolucionado de forma diferente y con distinta intensidad en las diversas áreas geográficas del planeta, en función de los factores sociodemográficos, culturales, económicos y políticos de cada zona. Por ello, el conocimiento y la monitorización de la distribución de esta epidemia en una comunidad determinada, constituye una herramienta básica para consensuar y establecer las intervenciones preventivas necesarias.

1.1.1 Transmisión

Según el Plan Nacional de SIDA en España, hasta el año 2017 la tasa de SIDA ha sido de 1,2 casos por 100.000 habitantes. Se estima que en España hay unas 150.000 personas infectadas por el VIH [1]. Asimismo, se estima que entre un 25 y un 30% de los pacientes infectados por el VIH desconocen que están infectados. En el año 2017, se diagnosticaron en España 3.381 nuevos casos de infección por el VIH, lo que supone 7.26 nuevos casos por cada 100.000 habitantes. En nuestro entorno, la infección por el VIH es una enfermedad característica de la población joven. El 70% de los nuevos casos se han diagnosticado entre los 25 y 39 años de edad. Este hecho puede estar relacionado con la forma de transmisión.

El principal mecanismo de trasmisión del VIH en nuestra área es a través del contacto sexual, representando algo más de un 80% de los nuevos casos de infección por el VIH, de los cuales, el 68% corresponde a HSH y el 32% a hombre que tienen sexo con mujeres (HSM). El resto de mecanismos de trasmisión del VIH concierne al uso de drogas por vía parenteral (3.1% de los nuevos casos), trasmisión vertical (2%), transfusión de componentes sanguíneos (<1%) [1].

Es importante destacar que estudios recientes han aportado evidencias de un aumento importante de la incidencia de enfermedades de transmisión sexual (ETS) entre HSH [1, 2]. Los principales factores relacionados con este aumento son:

- Ausencia de métodos barrera en las relaciones sexuales
- Elevado número de contactos sexuales (promiscuidad sexual)
- Relaciones anales, principalmente receptivas
- Irrigaciones anales antes del contacto sexual
- Coexistencia de otras ETS
- Uso de drogas

1.1.2 Historia natural de la infección por el VIH

Tras la inoculación del virus del VIH en el organismo, la infección es un proceso muy rápido. En unas horas se produce la infección de las células linfoides de la submucosa vaginal y/o rectal, y en 7 días la infección se ha propagado a ganglios linfáticos sistémicos en los que se alcanza un nivel de carga viral similar al de una infección crónica [1].

Una vez la infección está establecida, se pueden diferenciar distintas fases o estadios evolutivos relativamente bien definidos de acuerdo a las categorías clínicas preestablecidas (tabla 1). La duración de cada uno de estos estadios es variable y dependerá de distintos factores relacionados tanto con el virus del VIH como con el huésped.

Las distintas fases en que se puede dividir la enfermedad son:

- **Fase aguda:** desde que se produce la infección hasta la seroconversión.
- **Fase crónica:** en la que empezaran a aparecer patologías asociadas al estado de inmunosupresión y/o a la infección por el VIH.
- **Fase final:** a partir del diagnóstico de SIDA.

Tabla 1: **Clasificación de la Infección por el VIH y criterios de definición de SIDA para adultos**

Categoría según cifra de linfocitos CD4+ (T4)		Categorías clínicas		
		A	**B**	**C (SIDA)**
≥ 500 / mm³	(> 29%)	A1	B1	C1
200 – 499 / mm³	(14-28%)	A2	B2	C2
≤ 199 / mm³	(<14%)	A3	B3	C3

En la categoría clínica A, los pacientes no suelen presentar patología específica asociada. En la categoría clínica B, además del descenso de linfocitos CD4+, aparecen enfermedades oportunistas propias de la inmunodepresión. Y en la categoría clínica C o fase de SIDA, la patología intercurrente asociada es la propia de un estado de inmunosupresión severo, independientemente del número de linfocitos, aunque su recuento se utilice para establecer tres fases terminales [1].

La infección por el VIH origina una inmunodepresión celular al causar una disminución crónica y progresiva del número de linfocitos CD4, dando lugar a la aparición de múltiples infecciones oportunistas a medida que avanza la inmunosupresión. El origen de las infecciones oportunistas puede ser consecuencia de

- Reactivación de una infección latente adquirida tiempo atrás (meses o años).
- Infección exógena: las mismas que en población inmunocompetente pero con una evolución diferente debido al estado de inmunodepresión.
- Proliferación de microorganismos saprófitos en piel y mucosas.

1.2 Tratamiento: Era 'TAR'

La introducción del tratamiento antirretroviral (también llamado en el pasado Terapia Antiretroviral de Gran Actividad o TARGA; en inglés "Highly Active Antiretroviral Therapy" o HAART) en el año 1996 ha modificado la historia natural de la infección por el VIH. A partir de la introducción del TAR se ha asistido a un aumento espectacular de la supervivencia de los pacientes infectados por el VIH y a una drástica reducción de las enfermedades oportunistas y neoplasias asociadas al SIDA [1].

El principio fundamental del tratamiento antirretroviral consiste en suprimir al máximo la replicación del VIH con la finalidad de permitir que el sistema inmune, de forma espontánea, no sólo no siga deteriorándose sino que se recupere [1]. Con ello se consigue evitar la progresión clínica (disminución en la incidencia de infecciones oportunistas y neoplasias asociadas al SIDA) y aumentar la supervivencia. Inicialmente se pensó que el TAR conseguiría la erradicación del VIH. Si bien, el TAR consigue el control de la replicación viral y la recuperación de buena parte del sistema inmunológico, disminuyendo así la morbi-mortalidad derivada de la infección por el VIH, actualmente se sabe que este tratamiento no consigue erradicar este virus.

En la década de los 80 y 90 del siglo pasado, la baja expectativa de vida de los pacientes con SIDA hizo que se restara importancia a algunas patologías que se asociaban a la infección por el VIH. Con la introducción del TAR, el futuro de esta infección cambió, convirtiéndose en una enfermedad crónica. En consecuencia, se abrió así un área nueva de trabajo para los profesionales quienes deben enfrentarse a un conjunto de patologías propias y específicas de la infección crónica por el VIH [1].

2. Infección por el Virus del Papiloma Humano

2.1 Epidemiología

La familia Papillomaviridae está formada por un grupo numeroso de virus sin envoltura, que poseen un DNA de doble cadena y una nucleocápside proteica de simetría icosaédrica de unos 60 nm de diámetro. Los papilomavirus se replican exclusivamente en tejidos superficiales, piel y mucosas, ya que explotan el ciclo vital del epitelio estratificado y su diferenciación para reproducirse [2]. Su estudio detallado no ha sido posible hasta hace relativamente pocos años gracias técnicas basadas en biología molecular: la hibridación, el 'Southern blot' y la reacción en cadena de la polimerasa (RCP ó PCR) [2].

En el año 1933 se describió por primera vez el virus del papiloma como agente causal del papiloma de los conejos, siendo descrito con posterioridad en el ser humano: virus del papiloma humano (VPH) [2]. Los papilomavirus infectan muchas especies diferentes de animales, a las cuales están muy adaptados. Además de la adaptación a la especie, en función del tipo, muestran un tropismo por superficies corporales particulares, en especial, el área anogenital.

Se calcula que actualmente en nuestro país un 30-40% de la población menor de 25 años y sexualmente activa está infectada o se ha infectado por el VPH [2]. Si bien, la prevalencia de la infección por el VPH se va modificando cuando se analiza por grupos de edad. Ésta disminuye en grupos de edad más avanzada [2]. Del mismo modo, la prevalencia de esta infección varía entre grupos según sus prácticas sexuales, siendo la más alta en el grupo de HSH [2].

Las enfermedades causadas por la infección por el VPH se pueden clasificar de acuerdo a si infectan o no las mucosas:

- enfermedades de mucosa anogenital,
- enfermedades de mucosa no-anogenital,
- enfermedades específicas tipo epidermodisplasia verruciforme,

o de acuerdo a su localización (tabla 2): enfermedades de la piel, enfermedades de la región anogenital y enfermedades del tracto respiratorio.

Tabla 2: **Patología asociada a la infección por el VPH de acuerdo a su localización**

Localización	Patología
enfermedades de la piel	Lesiones plantares Verrugas comunes Verrugas en mosaico Verrugas planas Verrugas periungueales
enfermedades de la región anogenital	Verrugas anogenitales Papulosis Bowenoide Tumor de Buschke-Löwenstein Neoplasia intraepitelial perianal, vulvar y cervical Carcinoma de vulva Carcinoma de cérvix Carcinoma de ano Carcinoma de pene
enfermedades del tracto respiratorio	Papilomatosis laríngea Carcinoma escamoso de laringe Carcinoma escamoso de senos Carcinoma escamoso de pulmón

2.1.1 Transmisión

La principal vía de transmisión del VPH se produce durante las relaciones sexuales principalmente. La vía de transmisión no sexual es menos frecuente. Se han descrito casos de transmisión vertical en el momento del parto y de transmisión por contacto directo con lesiones infectadas por el VPH. Por tanto, la infección por el VPH es ante todo una ETS [2]. Entre los factores de riesgo descritos para infectarse por el VPH en mujeres, se encuentra el número de parejas sexuales, el tiempo transcurrido entre las relaciones sexuales y la presencia de lesiones genitales en dichas parejas [2]. Entre los factores de riesgo descritos en los hombres está el número de parejas ocasionales, el no uso de preservativos y la ausencia de circuncisión [2].

2.1.2 Historia natural de la infección por el VPH

El VPH necesita un mínimo de lesión del epitelio de los tejidos para que pueda penetrar e infectar. La gran mayoría de las lesiones que utiliza el VPH para causar una infección son asintomáticas. Este virus puede causar una infección hacia las 3 semanas y 8 meses tras la exposición [2]. La persistencia de la infección por el HPV en el área anogenital es probablemente el factor de riesgo más importante para que la infección progrese a lesión [2,3]. La persistencia de la infección está relacionada con el estado de inmunocompetencia del huésped [2-4]. Los pacientes trasplantados de órganos sólidos y los pacientes infectados por el VIH son grupos de riesgo para la infección por el VPH [2-4].

Se han identificado más de 120 genotipos del VPH, diferenciándose por la proteína L1 de la cápside [2-4]. De éstos, unos 40 genotipos pueden infectar superficies mucosas, específicamente, el área genital. En la mayoría de los casos se trata de una infección subclínica, asintomática, hecho que dificulta aún más su estudio.

2.2 Diagnóstico

El diagnóstico de la infección por el VPH se puede realizar por métodos directos como son las técnicas de biología molecular antes mencionadas o por métodos indirectos como es la citología o histología. Entre ellos, la citología (cervical y anal) es el método más extendido en la práctica clínica para el diagnóstico de la patología causada por el VPH [3, 5]. Este método se basa en las alteraciones morfológicas que la infección produce a nivel celular. La exploración de los pacientes mediante colposcopia de cérvix en el caso de las mujeres o anoscopia del canal anal complementa el diagnóstico de la patología causada por el VPH en la práctica clínica [6-9].

Las técnicas de biología molecular (hibridación *in situ*, PCR) informan de la infección del VPH haya o no lesión. La PCR también nos informa del genotipo de VPH implicado en la infección [3].

El VPH infecta el epitelio escamoso de la piel y las membranas mucosas. Por tanto, el área anogenital es una diana perfecta para el VPH, dado que combina los dos tipos de tejido donde es capaz de reproducirse: piel y mucosa [2-4]. Es importante mencionar que el tropismo por los diferentes tejidos depende del genotipo infectante del VPH. En la tabla 3 se presenta los genotipos más frecuentes detectados en diferentes patologías del área genital [10].

Tabla 3: **Genotipos del VPH aislados en patología anogenital**

Lesiones	Genotipos del VPH
Lesiones genitales	6,11
Neoplasia intraepitelial cervical y Carcinoma invasivo	16,18,31,33,35,39,42,43,44,45,51,52,56
Papulosis Bowenoide	16,34,37,42
Tumor de Buschke-Löwenstein	6,11
Neoplasia intraepitelial y carcinoma vulvar	16
Neoplasia intraepitelial y carcinoma pene	16,18
Neoplasia intraepitelial y carcinoma de ano	16 (raramente 18,33)

2.3 Patología anal benigna relacionada con la infección por el VPH

Los genotipos más comúnmente asociados a lesiones benignas anales son el 6 y el 11 [10]. Las lesiones anales benignas más frecuentes ocasionadas por el VPH en el área anal son los condilomas (11). En la siguiente imagen (imagen 1) se muestra unos condilomas intranales.

Imagen 1. Condilomas intranales.

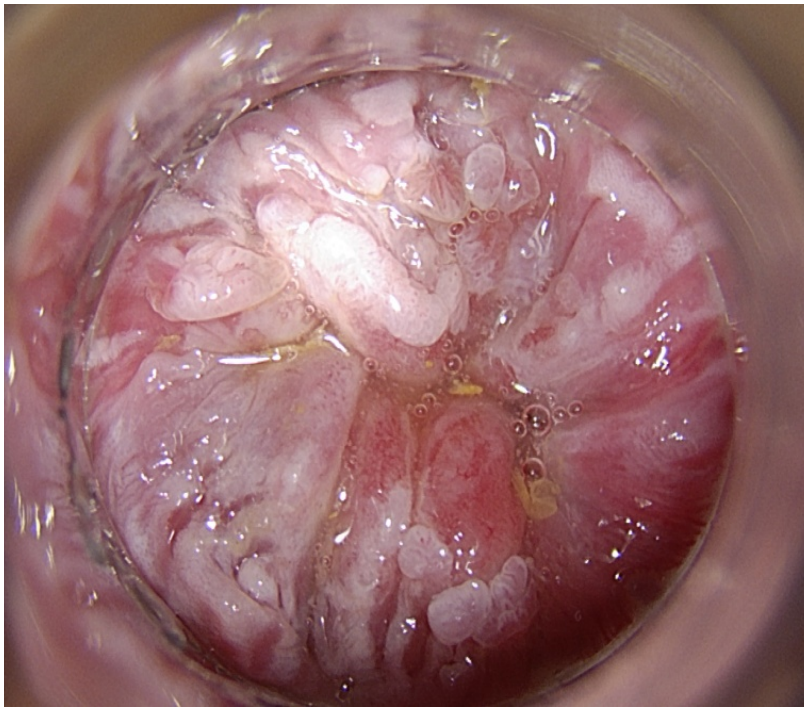


Foto decida por el Dr. Javier Corral

Una variante poco usual de estas lesiones, es el llamado Tumor de Buschke-Lowenstein, cuya presentación es en forma de un condiloma perianal gigante (imagen 2).

Imagen 2. Tumor de Lowenstein.

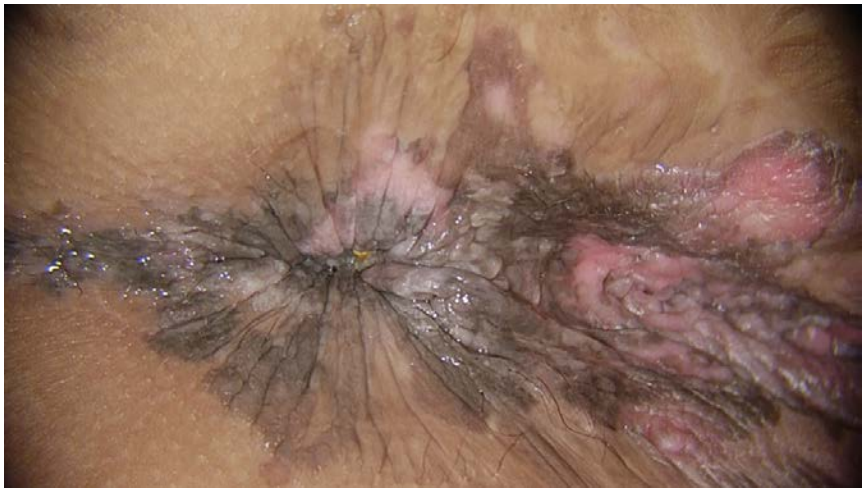


Foto cedida por el Dr. Javier Corral

Es importante destacar que aunque estas lesiones (condilomas) sean benignas, no se debe excluir la presencia o no de otras lesiones, como pueden ser la displasia o el carcinoma anal.

El objetivo del tratamiento de los condilomas es aliviar los síntomas que esta patología causa y evitar que recidiva. El tratamiento de los condilomas no es sinónimo de erradicar la infección por el VPH.

En los pacientes inmunocompetentes estas lesiones tienen un tratamiento definido [12-14] y aunque son frecuentes las recidivas (se han descrito tasas de recurrencia entre el 4 y el 29% [2,3], el sólo hecho de evitar nuevos contactos con el VPH puede conllevar que las lesiones desaparezcan en el plazo de un año, aproximadamente [15, 16].

2.4 Patología anal premaligna y maligna relacionada con la infección por el VPH: lesiones displásicas y carcinoma escamoso de ano

Aunque la mayoría de lesiones provocadas por el VPH son benignas, numerosos estudios han aportado evidencias de la relación entre la infección por el VPH y algunos cánceres [4, 17-20]. Entre ellos el más estudiado y conocido es el cáncer de cérvix, si bien el CEA es una neoplasia con una gran actividad en investigación en la última década [4, 20]

En el 80% de los casos de CEA se ha detectado ADN del VPH. El genotipo 16 es el más comúnmente detectado en este tipo de cáncer [21]. Previo al desarrollo de un CEA, diferentes genotipos del VPH pueden causar cambios anormales a nivel de la mucosa anal denominados displasia anal [22]. La displasia anal se clasifica según el grado de modificaciones que produce a nivel celular y la profundidad en el epitelio de las mismas [6, 23, 24].

De acuerdo a los hallazgos citológicos hablamos de "lesión escamosa intraepitelial" (LEI) y de acuerdo a los hallazgos histológicos hablamos de "neoplasia intraepitelial anal" (NIA) [25]. La tabla 4 presenta la concordancia entre los hallazgos citológicos e histológicos a nivel anal.

Tabla 4: Concordancia entre los hallazgos citológicos e histológicos

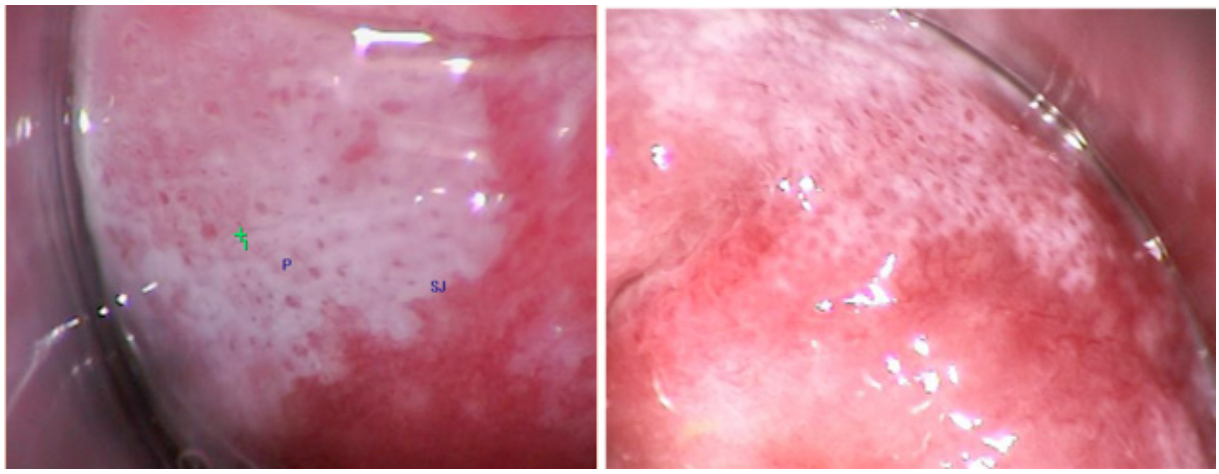
Citológicos	Histológicos	
ASCUS (Atypical Squamous Cells of Unknown Significance)		se trata de células anormales inespecíficas. No se puede establecer ningún diagnóstico
LEI-B (lesión escamosa intraepitelial de bajo grado)	NIA I (neoplasia intraepitelial anal grado I)	la displasia está confinada al tercio inferior del epitelio
LEI-A (lesión escamosa intraepitelial de alto grado)	NIA II (neoplasia intraepitelial anal grado II)	la displasia afecta a dos tercios del espesor del epitelio
LEI-A (lesión escamosa intraepitelial de alto grado)	NIA III (neoplasia intraepitelial anal grado III)	la displasia afecta entre los dos tercios y la totalidad del espesor del epitelio

Aunque la lesión de NIA-III es la única aceptada como un marcador precursor de CEA, numerosos estudios sugieren que las otras lesiones displásicas anales también puede ser precursororas del CEA [4, 9, 17-21, 26-28].

La displasia anal suele localizarse en la zona alta del canal anal (a nivel del epitelio de transición), y en la zona perianal (en la unión cutáneo-mucosa). Suele ser una lesión multifocal en la mayoría de los casos, por lo que se requiere la toma de varias muestras para establecer su diagnóstico [5, 8, 26]. En la mayoría de pacientes, la presencia de una displasia anal es asintomática. En algunos casos, los síntomas son inespecíficos como irritación, prurito o bien eritema a la exploración. En los casos sintomáticos, los pacientes pueden referir aparición de lesiones escamosas o úlceras. Ante este escenario de síntomas escasos, es habitual que el diagnóstico de displasia anal se efectúe en los programas de cribado [7, 25]. La anoscopia de alta resolución (AAR) es un procedimiento muy valioso para el cribado de la patología causada por el VPH en el canal anal [5]. La AAR consiste en explorar el canal anal con un anoscopio por el que se visualiza el canal anal a través de un microscopio. Durante la exploración, se localiza la lesión y se biopsia bajo anestesia local.

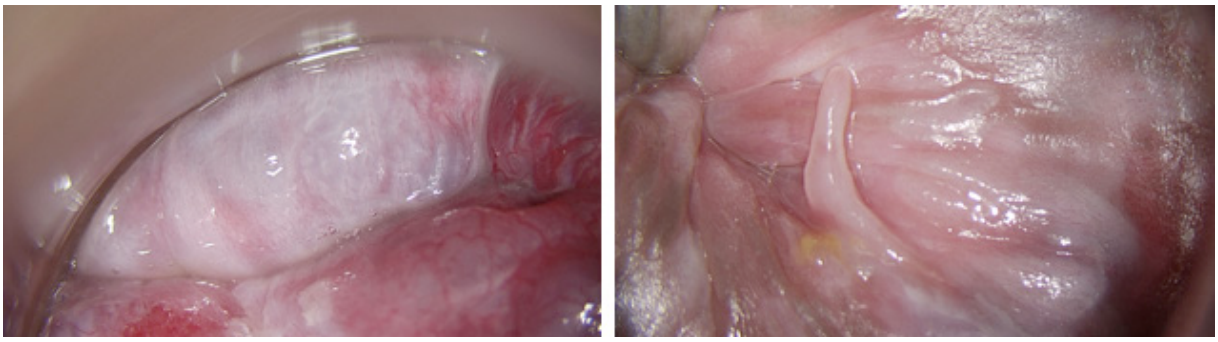
La imagen 3 presenta lesiones del canal anal tipo LEI-B / NIA I y la imagen 4 lesiones del canal anal tipo LEI-A / NIA III.

Imagen 3. Lesión del canal anal tipo LEI-B / NIA I.



Fotos: Dra. Sandra Vela Bernal

Imagen 4. Lesión del canal anal tipo LEI-A / NIA III.



Fotos: Dra. Sandra Vela Bernal

Una vez realizado el diagnóstico de displasia anal, el objetivo es que esta lesión no progrese a CEA. Para ello, se dispone de diferentes tratamientos como el electrocauterio, el láser o la fulguración con infrarojos [29-31]. Dado que en la mayoría de casos los pacientes presentan lesiones multifocales y/o extensas [19], suele ser necesarias varias sesiones de tratamiento. A su vez, se requiere un control estricto en el seguimiento post-tratamiento por el elevado riesgo de recidiva. Aun así, es difícil diferenciar entra enfermedad residual y recidiva [13, 14, 29,32].

A pesar de la elevada incidencia de infección anal por el VPH, el CEA es una patología muy poco frecuente en la población general. La incidencia de CEA en la población general es de 1,8 casos por 100000 persona-años en EEUU y de ≈0.9 casos por 100000 persona-años en España, representando el 2.0-2.5% de todos los tumores del tubo digestivo. Estar infectado por el VPH no es suficiente para desarrollar un CEA. Existen otros cofactores implicados en la evolución hacia CEA como el tabaco, la actividad sexual, la asociación con otras ETS y/o el estado inmunitario del paciente, que parecen tener un papel muy importante en la fisiopatología de este tipo de cáncer. Por ejemplo, entre la población de HSH, la incidencia

del CEA es de 35 casos por 100000 persona-años en personas no infectadas por el VIH y de 70 casos por 100000 persona-años en personas infectadas por el VIH. Ello hace que el grupo de población de pacientes VIH+, y especialmente los HSH VIH+, son candidatos a estar incluidos en programas de cribado [7, 25].

La imagen 5 muestra un carcinoma escamoso perianal y la imagen 6 un Carcinoma escamoso del canal anal.

Imagen 5. Carcinoma escamoso perianal.

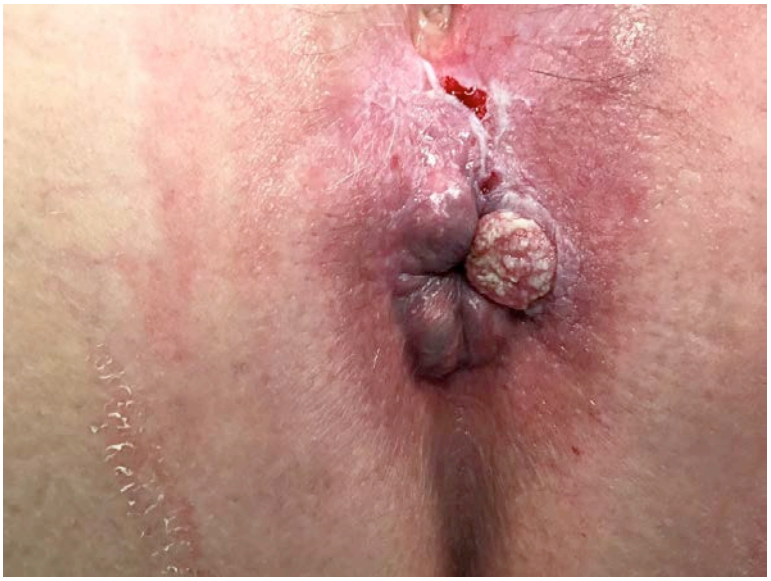


Foto: Dra. Sandra Vela Bernal

Imagen 6. Carcinoma escamoso del canal anal.

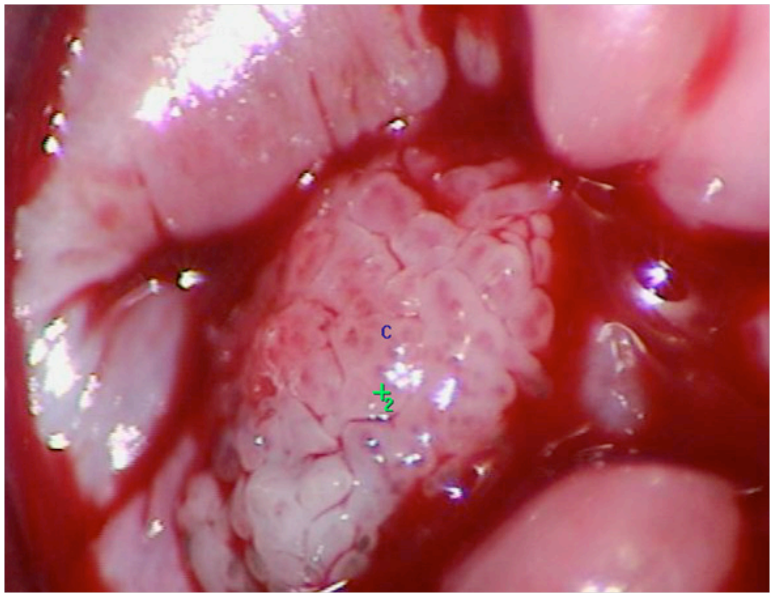


Foto: Dra. Sandra Vela Bernal

3. Patología anal en pacientes varones infectados por el virus de la inmunodeficiencia humana

3.1 Patología anal en varones infectados por el VIH

Como en otras partes del organismo, los pacientes infectados por el VIH presentan patología anal específica asociada, por un lado, a la inmunosupresión que condiciona la propia infección por el VIH, y por otro lado, a las prácticas sexuales que llevan a cabo un gran número de la población infectada por el VIH, en concreto los HSH.

Los HSH presentan una incidencia de ETS superior a la de los HSM [1]. Esta mayor incidencia está relacionada con su conducta sexual: parejas sexuales múltiples y sexo anónimo. Ambas prácticas son factores de riesgo para la adquisición y transmisión de ETS entre los HSH. Además, el sexo genital-anal sin protección, práctica sexual habitual en el colectivo HSH, es una práctica de riesgo para la transmisión de múltiples infecciones, ya que facilita la transferencia de microorganismos como el VIH y el VPH [1].

El grupo de pacientes HSH VIH+ no sólo pueden presentar la patología proctológica clásica: hemorroides, fisuras y fístulas; sino que presentan también una mayor incidencia de ETS como la sífilis, la gonorrea, la infección por clamidia y los condilomas respecto a los HSH no infectados por el VIH. Los **condilomas** son la patología anal más frecuente objetivada en los HSH VIH+ [11, 15]. Existe una serie de patologías muy específicas de este grupo, que en algunos casos es el primer síntoma que lleva al diagnóstico de infección por el VIH. Entre estas están la infección por Citomegalovirus, por herpes simple, por cándida, la úlcera idiopática y el CEA relacionado con la infección por el VPH [1].

Al igual que las ETS, la incidencia de lesiones displásicas en el canal anal y CEA es más elevada en la población infectada por el VIH que en la población general (o no infectada por el VIH), siendo los HSH VIH+ los que presentan la mayor incidencia de estas patologías. Tanto las displasias del canal anal como los CEA suelen ser unas patologías asintomáticas [2]. De hecho, la displasia del canal anal suele ser detectada o en programas de cribado o bien como hallazgo casual en el estudio de piezas quirúrgicas.

Entre los diferentes tipos de neoplasias que se pueden localizar en la zona perianal, se diferencian básicamente tres:

- Carcinoma escamoso / epidermoide de ano (CEA): representa un 80% aproximadamente de los tumores malignos en esta zona. Suele localizarse en el margen del canal anal y su origen se localiza en el epitelio de transición. Este tipo de tumor se asocia estrechamente con la coinfección con el VPH [4].

- Adenocarcinoma anal: representa el 5% y se origina en las glándulas mucosas. Una variedad del adenocarcinoma anal es la enfermedad de Paget. Su origen está en las glándulas mucosas apocrinas. No es extraño que sea una forma de presentación en pacientes VIH+.
- Melanoma anal: representa menos del 1% de las neoplasias del ano. Aunque el ano no es una zona expuesta al sol, en pacientes inmunodeprimidos puede aparecer esta lesión localizada en el margen cutáneo anal.

Dado que la población HSH VIH+ presenta una prevalencia más elevada que el resto de la población a estas patologías, y dado que en la actualidad la infección por el VIH es una enfermedad crónica, exige a la clase médica a elaborar programas de cribado para el diagnóstico precoz de estas patologías específicas y su correcto tratamiento.

3.2 Patología anal benigna relacionada con la infección por el VPH en varones infectados por el VIH: condilomas

Tal como se ha mencionado, las lesiones benignas relacionadas con la infección por el VPH son los condilomas.

Los pacientes infectados por el VIH presentan diferencias de esta patología respecto a la población general. El número de condilomas suele ser mayor, especialmente entre los HSH VIH+ [30]. El estado de inmunosupresión no facilita eliminar con facilidad el VPH aunque no exista un nuevo contacto con el mismo [16, 17, 33]. Asimismo, los pacientes VIH+ con condilomas en el canal anal presentan una infección por otros genotipos del VPH en el canal anal. Todo ello dificulta su tratamiento y la prevención de recidivas [16, 23]. Aunque no existe consenso respecto cuál es el mejor tratamiento para estas lesiones, lo que sí que es necesario es llevar a cabo un tratamiento específico en estos pacientes inmunodeprimidos [1].

3.3 Patología anal premaligna y maligna relacionada con la infección por el VPH en varones infectados por el VIH: lesiones displásicas y carcinoma escamoso de ano

Tal como se ha mencionado, las lesiones premalignas y malignas relacionadas con la infección por el VPH son las lesiones displásicas y el carcinoma escamoso de ano.

Los pacientes infectados por el VIH, presentan una mayor prevalencia de infección por el VPH en el canal, una mayor prevalencia de lesiones displásica en el canal anal y una mayor incidencia de CEA en comparación con la población general [11, 34, 35]. Aunque entre los varones VIH+, los HSH son los que presentan un mayor riesgo de desarrollar un CEA (131 nuevos casos por 100000 persona-años), los HSM VIH+ también presentan un riesgo casi unas 25 veces mayor (46 nuevos casos por 100000 persona-años) que la población general (2 nuevos casos por 100000 persona-años) [36]. Esto hace que la población de pacientes infectados por el VIH sea un colectivo candidato a estar incluido en programas de cribado.

4. Cohorte CARH·MEN

CARH·MEN es el acrónimo de "cohorte de Can Ruti de hombres infectados por el VIH". Se trata de una cohorte de hombres infectados por el VIH, de 18 años o mayores, sin antecedentes o sintomatología de patología relacionada con el VPH, reclutados en un solo centro: consultas externas de la Fundación LLuita contra la SIDA del hospital Germans Trias i Pujol (también conocido como Can Ruti), entre enero de 2005 y mayo de 2009. El objetivo de esta cohorte es el cribado preventivo para la detección precoz de cánceres escamosos de canal anal, de pene y de boca.

Respecto al canal anal, el método de cribado consistió en la inspección visual y tacto rectal realizado por los profesionales de la sección de proctología de la unidad del VIH. La doctoranda es uno de estos médicos. Además, se obtuvieron muestras del canal anal introduciendo un cepillo para citología ("cytobrush"). Este se introduce unos 3 cm en el canal anal, girándolo suavemente durante unos 30 a 45 segundos. Una vez finalizada esta maniobra, el cepillo es introducido en 20 mL de líquido de preservación (ThinPrep) y agitado durante 30 segundos. Esta muestra de células del canal anal se utilizó para el estudio citológico (Papanicolaou) y para la detección del VPH (PCR múltiple), y en caso de detección positiva del VPH se procedió a identificar el/los genotipos.

Si la citología dio un resultado anormal (patológico): ASCUS (células escamosas atípicas de importancia no determinada), LEI-B (lesión escamosa intraepitelial de grado bajo) o LEI-A (lesión escamosa intraepitelial de grado alto), se procedió a realizar un AAR para identificar la lesión en el canal anal y biopsiarla.

Los **condiloma/s** en el canal anal se suelen diagnosticar durante la inspección visual y tacto rectal, o en el momento de la AAR. Si se diagnosticó un condiloma/s en el canal, posteriormente se procedió a su tratamiento. Tanto el diagnóstico como el tratamiento fueron llevados a cabo por los profesionales de la sección de proctología de la unidad del VIH [37].

5. Motivación de la tesis

Dos hechos, que coincidieron en el tiempo, fueron el motivo para que esta tesis doctoral se llevara a cabo:

1. Mi implicación como cirujana de la sección de Proctología de la Fundació LLuita contra la SIDA desde su inicio en el año 2005, participando en la elaboración del programa de cribado de patología anal en pacientes infectados por el VIH.

2. Mi participación en la elaboración del protocolo asistencial de tratamiento de los condilomas en personas infectadas por el VIH, me hizo percatar de la falta de evidencias sobre la eficacia y seguridad de los diferentes tratamientos utilizados en el manejo de los condilomas del canal anal. Es importante mencionar que participé activamente en la realización de este protocolo asistencial.

Todo ello fue el estímulo suficiente para llevar a cabo todos los trabajos de investigación clínica que componen esta tesis doctoral.

Hipótesis

Las hipótesis planteadas en la presente tesis doctoral son las siguientes:

HIPÓTESIS 1: Los condiloma/s en el canal anal en personas infectadas por el VIH, especialmente entre los hombres que practican sexo con hombre, es una patología prevalente.

HIPÓTESIS 2: Las personas infectadas por el VIH con un diagnóstico de condiloma/s en el canal anal, presentan una coinfección por más de un genotipo del VPH en el canal anal.

HIPÓTESIS 3: La presencia de condiloma/s en el canal anal se asocia a una alta prevalencia de lesiones escamosas intraepiteliales del canal anal en personas infectadas por el VIH. Por tanto, la coexistencia de dos patologías relacionadas con el VPH el canal anal: condiloma/s y lesiones escamosas intraepiteliales, es prevalente en personas infectadas por el VIH.

HIPÓTESIS 4: Los diferentes tratamientos usados en el manejo de los condiloma/s del canal anal en la práctica clínica presentan una alta tasa de recidiva entre las en personas infectadas por el VIH.

Objetivos

Los objetivos planteados en esta tesis doctoral son los siguientes:

OBJETIVO 1: Estimar la prevalencia condiloma/s en el canal anal en hombres infectados por el VIH.

Estudio 1: "Condylomata, cytological abnormalities and human papillomavirus infection in the anal canal in HIV-infected men".
HIV Med 2012; Oct;13(9):549-57. doi: 10.1111/j.1468-1293.2012.01013.x. Epub 2012 Mar 21.

OBJETIVO 2: Aportar evidencias sobre la infección por el VPH y la distribución de genotipos del VPH en el canal anal entre los hombres infectados por el VIH.

Estudio 1: "Condylomata, cytological abnormalities and human papillomavirus infection in the anal canal in HIV-infected men".
HIV Med 2012; Oct;13(9):549-57. doi: 10.1111/j.1468-1293.2012.01013.x..

Estudio 2: "Effectiveness of physically ablative and pharmacological treatments for anal condyloma in HIV-infected men",
PLoS One. 2018 Aug 1;13(8):e0199033. doi: 10.1371/journal.pone.0199033

Estudio 3: "Distribution of Human Papillomavirus Genotypes in Anal Cytological and Histological Specimens from HIV Infected Men Who Have Sex with Men and Men Who Have Sex with Women".
Dis Colon Rectum. 2013 Sep;56(9):1043-1052.

OBJETIVO 3: Estimar la prevalencia de lesiones escamosas intraepiteliales del canal anal en hombres infectados por el VIH diagnosticadas de condiloma/s en el canal anal.

Estudio 1: "Condylomata, cytological abnormalities and human papillomavirus infection in the anal canal in HIV-infected men".
HIV Med 2012; Oct;13(9):549-57. doi: 10.1111/j.1468-1293.2012.01013.x.

Estudio 3: "Distribution of Human Papillomavirus Genotypes in Anal Cytological and Histological Specimens from HIV Infected Men Who Have Sex with Men and Men Who Have Sex with Women".
Dis Colon Rectum. 2013 Sep;56(9):1043-1052.

OBJETIVO 4: Aportar evidencias sobre la efectividad de los diferentes tratamientos usados en la práctica clínica en el manejo de los condiloma/s del canal anal en los hombres infectados por el VIH.

Estudio 2: "Effectiveness of physically ablative and pharmacological treatments for anal condyloma in HIV-infected men",
PLoS One. 2018 Aug 1;13(8):e0199033. doi: 10.1371/journal.pone.0199033

Estudio 4: "Long-term effectiveness of infrared coagulation for anal intraepithelial neoplasia 2 and 3 in HIV-infected males and females".
AIDS 2013, 27:951–959.

Publicaciones
base de la tesis doctoral

Estudio 1: "Condylomata, cytological abnormalities and human papillomavirus infection in the anal canal in HIV-infected men"
HIV Med 2012; Oct;13(9):549-57. doi: 10.1111/j.1468-1293.2012.01013.x. Epub 2012 Mar 21.

"CONDILOMAS, ALTERACIONES CITOLÓGICAS E INFECCIÓN POR EL VIRUS DEL PAPILOMA HUMANO EN EL CANAL ANAL EN HOMBRES VIH POSITIVOS"

ANTECENDENTES. Las infecciones genitales por genotipos de virus del papiloma humano (VPH) de bajo y de alto riesgo se asocian con condilomas anogenitales y cáncer de células escamosas. Las patologías relacionadas con el VPH en los hombres infectados por el VIH son una preocupación. En este estudio, se estimó la prevalencia de condilomas y su asociación con anomalías citológicas y la infección por el VPH en el canal anal en hombres infectados por el VIH [hombres que tienen sexo con hombres (HSH) y heterosexuales].

MÉTODOS. Se trata de un estudio transversal basado en las primeras visitas de la cohorte de hombres infectados por el VIH de nuestro hospital (cohorte CARH·MEN: "Can Ruti hombres VIH+"). Los condilomas anales fueron diagnosticados durante la exploración clínica y en el examen proctológico. Se recolectaron muestras del canal anal para la determinación del genotipo del VPH y el diagnóstico citológico.

RESULTADOS. Un total de 640 hombres infectados por el VIH (473 HSH y 167 heterosexuales) se incluyeron en el estudio. La prevalencia global de condilomas anales fue del 25% [157 de 640; Intervalo de confianza del 95% (IC95%): 21-28%]; en HSH fue del 28% y en heterosexuales fue del 15% [odds ratio (OR) 2.2; IC95%: 1.4–3.5]. En los pacientes con condilomas anales, la infección por el VPH en el canal anal fue más prevalente (92% versus 67% en aquellos sin condilomas anales; OR 8,5; IC 95%: 3,2-22). Esta mayor prevalencia de infección por el VPH involucró al menos dos genotipos del VPH (OR: 4.0; IC 95%: 2.2-7.1), principalmente genotipos de alto riesgo oncogénico (OR: 3.3; IC 95%: 1.7-6.4). Del mismo modo, la prevalencia acumulada de VPH-6 y VPH -11 fue mayor en pacientes con condilomas anales (63% vs. 19% en aquellos sin condilomas anales). Tener un condiloma anal se asoció a una mayor prevalencia de anomalías citológicas (83% vs.32% en aquellos sin condilomas anales; OR: 6.9; IC95%: 3.8-12.7) y lesiones escamosas intraepiteliales de alto grado (LEI-A) (9% vs 3% en aquellos sin condilomas anales; OR 9.0; IC95%: 2.9–28.4) en el canal anal.

CONCLUSIONES. Los hombres infectados por el VIH con condilomas anales presentan un mayor riesgo de presentar una lesión LEI-A y de albergar Infecciones por múltiples genotipos del VPH en el canal anal. Aunque los HSH presentaron la mayor prevalencia de condilomas en el canal anal, los hombres heterosexuales también tuvieron una prevalencia clínicamente importante. Nuestros hallazgos enfatizan la importancia de la detección y el seguimiento de los condilomas en el canal anal en hombres infectados por el VIH.

DOI: 10.1111/j.1468-1293.2012.01013.x

HIV Medicine (2012), **13**, 549–557

ORIGINAL RESEARCH

Condylomata, cytological abnormalities and human papillomavirus infection in the anal canal in HIV-infected men

L Darwich,[1,2] MP Cañadas,[1,3] S Videla,[4] J Coll,[1,4] M Piñol,[5] P Cobarsi,[4] RA Molina-López,[2] S Vela,[5] F García-Cuyás,[5] M Llatjos,[6] G Sirera[4,7] and B Clotet[1,4,7] on behalf of the HIV-HPV Can Ruti Team

[1]Retrovirology Laboratory IrsiCaixa Foundation, [2]Department of Sanitat i Anatomia Animal, Universitat Autònoma de Barcelona (UAB), [3]Labco.GeneralLab, Barcelona, Spain, [4]Lluita Contra La SIDA Foundation, [5]Department of Surgery, [6]Department of Pathology and [7]HIV Clinical Unit, Department of Medicine, University Hospital Germans Trias i Pujol, UAB, Badalona, Spain

Background

Genital infections with low-risk (LR) and high-risk (HR) human papillomavirus (HPV) genotypes are associated with ano-genital condylomata and anal squamous cell cancer. HPV-related pathologies in HIV-infected men are a serious concern. In this study, the prevalence of anal condylomata and their association with cytological abnormalities and HPV infection in the anal canal in HIV-infected men [men who have sex with men (MSM) and heterosexuals] were estimated.

Methods

This was a cross-sectional study based on the first visits of patients in the Can Ruti HIV-positive Men (CARH·MEN) cohort. Anal condylomata were assessed by clinical and proctological examination. Samples from the anal canal were collected for HPV genotyping and cytological diagnoses.

Results

A total of 640 HIV-infected men (473 MSM and 167 heterosexuals) were included in the study. The overall prevalence of anal condylomata was 25% [157 of 640; 95% confidence interval (CI) 21–28%]; in MSM it was 28% and in heterosexuals it was 15% [odds ratio (OR) 2.2; 95% CI 1.4–3.5]. In patients with anal condylomata, HPV infection in the anal canal was more prevalent (92% *vs*. 67% in those without anal condylomata; OR 8.5; 95% CI 3.2–22). This higher HPV prevalence involved at least two HPV genotypes (OR 4.0; 95% CI 2.2–7.1), mainly HR genotypes (OR 3.3; 95% CI 1.7–6.4). Similarly, the cumulative prevalence of HPV-6 and HPV-11 was higher in patients with anal condylomata (63% *vs*. 19% in those without anal condylomata). Having anal condylomata was associated with higher prevalences of cytological abnormalities (83% *vs*. 32% in those without anal condylomata; OR 6.9; 95% CI 3.8–12.7) and high-grade squamous intraepithelial lesions (HSILs) (9% *vs*. 3% in those without anal condylomata; OR 9.0; 95% CI 2.9–28.4) in the anal canal.

Conclusions

HIV-infected men with anal condylomata were at risk of presenting HSILs and harbouring multiple HR HPV infections in the anal canal. Although MSM presented the highest prevalence of anal condylomata, heterosexual men also had a clinically important prevalence. Our findings emphasize the importance of screening and follow-up for condylomata in the anal canal in HIV-infected men.

Keywords: anal condylomata, human papillomavirus anal infection, HIV-positive men, anal cytology

Accepted 15 February 2012

Correspondence: Dr Laila Darwich, Infectious Diseases and Epidemology, DSAA, Universitat Autonoma de Barcelona, Spain. Tel: +34 93 581 10 46; fax: +34 93 581 32 97; e-mail: laila.darwich@uab.cat

Introduction

Human papillomavirus (HPV) types that infect the ano-genital tract can be divided into low-risk (LR) HPV types, which are associated with the development of ano-genital condylomata, and high-risk (HR) HPV types, which are implicated in the evolution of anal squamous cell cancer and its putative precursor, high-grade anal intraepithelial neoplasia (AIN) [1–3]. The association between genital warts and the presence of HPV infection has been widely described [4,5]. Genital condylomata are benign lesions usually resulting from infection by HPV-6 or HPV-11. In contrast, HPV-16, HPV-18 and HPV-31 are associated with the development of high-grade dysplasia or carcinoma [5–7].

Cervical cytology is the most appropriate means of screening for precancerous lesions and cervical HPV-related cancer [8]. Currently, anal cytology is used as a screening tool for anal squamous lesions to detect AIN or anal cancer at an earlier stage [9,10]. It is known that HIV-associated immunosuppression may increase the likelihood of development of both low-grade and high-grade HPV-related lesions. In addition, the longer life expectancies of the HIV-positive population as a result of highly active antiretroviral therapy may permit established high-risk HPV infections to progress to anal cancer [11].

The transmission of HPV depends on sexual behaviour, and HPV infection is strongly related to the lifetime number of sexual partners as well as to the practice of receptive anal intercourse (RAI) in men having sex with men (MSM) [12]. The HIV-positive population, and in particular MSM, have a high risk of developing anal condylomata and precancerous lesions or ano-genital neoplasia [13]. Most condylomata lesions will spontaneously resolve in the immunocompetent population, but immune-compromised patients with condylomata (especially HIV-infected patients) generally require therapy that is painful and expensive, and also have a high risk of recurrence [14–16]. Because of these issues, several research teams have studied the cost-effectiveness of administering the quadrivalent HPV vaccine (containing HPV types 16, 18, 6 and 11) to the HIV-negative male population [17–19], but its use has not yet been established, particularly in HIV-positive men. Thus, the study of HPV genotypes coexisting in the anal canal is of high relevance in HIV-infected men, in order to establish further preventive protocols in this specific population at risk.

The aim of this work was to assess the prevalence of anal condylomata and their association with HPV genotype-specific infection and cytological abnormalities in the anal canal in HIV-infected men (MSM and heterosexuals).

Patients and methods

Study design

A cross-sectional analysis based on the first (baseline) visit of patients in the Can Ruti HIV-positive Men (CARH·MEN) cohort was performed (University Hospital Germans Trias i Pujol, Badalona, Spain). This cohort was a prospective, single-centre of out-patient HIV-positive men who were annually assessed for HPV infection in the anus, penis and mouth. The protocol, amendments and other materials were approved by the hospital's independent ethics committee.

Consecutive patient recruitment among out-patients who attended their clinical routine control was carried out by one staff care provider from 2005 to 2007 and since 2008 has been carried out by two staff care providers. The patients were informed about the study and invited to visit the Clinical Proctology HIV Unit which was created *ad hoc* (two afternoons per week). If they agreed to participate, written informed consent was obtained.

HIV-positive men $\geq$ 18 years old, without a history of (or current) anal cancer, were included in the study. The following data were collected: date of birth, date of HIV-positive diagnosis (time of HIV infection in years), baseline CD4 cell count (the closest value obtained during the participants' usual clinical follow-up visits in the HIV Unit before the cytological sample collection), CD4 count nadir (the lowest CD4 value for each patient abstracted from medical records), HIV viral load (the closest value obtained before the sample collection), highly active antiretroviral therapy (HAART) previous to inclusion (yes/no) and time on HAART, history of sexually transmitted infections (STIs), alcohol and smoking history, sexual behaviour and number of sexual partners. Baseline CD4 count and CD4 count nadir were determined by flow cytometry, and HIV viral load by Nuclisens (detection limit 80 HIV-1 RNA copies/mL; bioMerieux, Inc., Durham, NC).

Clinical examination and anal sample collection

A clinical examination (visual inspection) and a digital rectal examination were performed at the baseline visit of patients in the CARH·MEN cohort. Samples from the anal canal were collected for detection of HPV infection [multiplex polymerase chain reaction (PCR)]. The anal canal sample was also used to carry out the cytology analysis (Pap test). If the anal cytology result showed a pathological finding, the patient was contacted and informed, and a high-resolution anoscopy (with topical application of 2 minutes of duration with 3% acetic acid to the anal canal) was scheduled. If anal warts were diagnosed during the

clinical examination, either by means of the digital rectal examination or the anoscopy, the patient was referred to a surgeon for treatment.

Anal samples were obtained by introducing a cytobrush (Eurogine SL, Sant Boi del Llobregat, Spain) into the anal canal to a depth of 3 cm and gently rotating for 30–45 seconds. The cytobrush was included and shaken into a solution of 20 mL of PreservCyt/ThinPrep Pap test (Cytyc Iberia SL, Barcelona, Spain) for 30 seconds and the solution was stored at −20°C until analysis.

HPV detection and typing

DNA was extracted from cell suspensions (in ThinPrep Pap solution) using the Qiamp Viral DNA kit (Qiagen, Hilden, Germany). HPV detection and typing were performed on all samples using a commercial In Vitro Diagnostics with the CE mark certification (IVD-CE) marked assay: the Multiplex Fluorescent-PCR Kit for Human Papilloma Virus Genotyping (F-HPV typing™; Molgentix SL, Barcelona, Spain) in accordance with the manufacturer's instructions [20]. The kit allows the detection of 13 HR HPV genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68; and the two most frequent LR HPV genotypes: 6 and 11. A human short tandem repeat (STR) sequence included in the same multiplex reaction was amplified as an internal control for DNA integrity and absence of PCR inhibitors. Products were analysed by capillary electrophoresis on an ABI 3130 XL genetic analyser and using GeneMapper 4.0 software (Applied Biosystems, City Foster, CA). Each PCR run included HPV-positive and -negative controls. Particular care was taken to prevent carry-over contamination by separating pre- and post-PCR areas in the laboratory.

Anal cytological assessment

Cytological changes were classified according to the Bethesda System: atypical squamous cells of uncertain significance (ASCUS) and low- and high-grade squamous intraepithelial lesions (LSILs and HSILs, respectively). Samples were independently assessed by two expert cytopathologists.

Statistical analysis

Baseline characteristics were summarized with standard descriptive statistics and a descriptive analysis was carried out. The prevalences of overall anal HPV infection, HPV type-specific infection, and cytological diagnoses were estimated. Differences between groups were evaluated using the χ^2 test for qualitative variables, and odds ratios (ORs) and their 95% confidence intervals (95% CIs) were calculated. Bivariate and multivariate logistic (for HPV infection) and nominal (for cytological changes) regression models were used where appropriate. For the multivariate analyses, factors with $P < 0.2$ in the bivariate model or clinical relevance were used to assess interactions in the multivariate regression model. A P-value ≤ 0.05 was considered statistically significant. Data analysis was carried out using SPSS version 15.0 statistical software (SPSS, Chicago, IL).

Results

Patient characteristics

The CARH·MEN cohort comprised 733 patients recruited from January 2005 to May 2009. Nine-three patients were not included in the analysis of this study because they had been treated in the past for anal condylomata; thus, 640 HIV-infected men with no history of anal condylomata remained in the study (Fig. 1).

Descriptive baseline characteristics by presence or absence of anal condylomata are shown in Table 1. The most relevant differences between patients were that a higher percentage of patients with condylomata had a history of STIs (46%) and were MSM (84%) compared with patients without condylomata (27% had a history of STIs and 71% were MSM). Accordingly, the percentage of patients practising RAI was also higher in patients with anal condylomata than in those without them (76% *vs*. 58%, respectively).

The overall prevalence of anal condylomata in HIV-infected men was 25% (157 of 640; 95% CI 21–28%). According to sexual behaviour, the prevalence was 28% (132 of 473) in MSM and 15% (25 of 167) in heterosexual HIV-infected men (OR 2.2; 95% CI 1.4–3.5). Condylomatous anal lesions were located in the internal region in 111 of 157 patients (71%), in the perianal area in 13 of 157 patients (8%) and in both locations in 33 of 157 patients (21%) (Fig. 1).

HPV infection

The overall prevalence of anal canal HPV infection was 73% (469 of 640; 95% CI 70–77%). The prevalence in patients with anal condylomata was 92% (145 of 157; 95% CI 86–96%) [95.5% (126 of 132) for MSM and 76% (19 of 25) for heterosexuals; $\chi^2 = 11.3$; $P = 0.001$] and that in patients without anal condylomata was 67% (324 of 483; 95% CI 63–71%) [80% (273 of 341) for MSM and 36% (51 of 142) for heterosexuals; $\chi^2 = 88.5$; $P < 0.001$] (with/without anal condylomata, $P < 0.001$). Moreover, the prevalence of LR HPV genotypes (63% *vs*. 19%, respectively; $P < 0.001$) and that of HR HPV genotypes (83% *vs*.

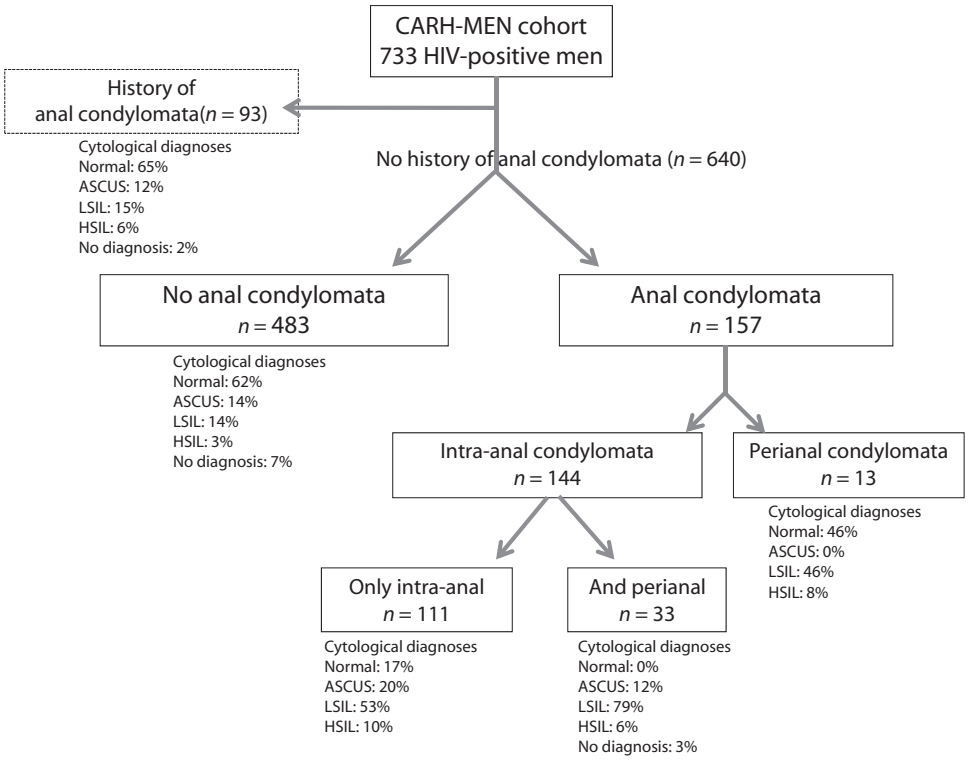


Fig. 1 Flow chart for the Can Ruti HIV-positive Men (CARH·MEN) cohort according to the cytological diagnoses of anal condylomata and human papillomavirus (HPV)-related pathologies. ASCUS, atypical squamous cells of uncertain significance; LSIL, low-grade squamous intraepithelial lesion; high-grade squamous intraepithelial lesion.

62%, respectively; $P < 0.001$) were considerably higher in the anal canals of HIV-infected men with condylomatous lesions than in those without (Table 2). A higher prevalence of presenting any HPV genotype in the anal canal was associated with having anal condylomata (adjusted OR 8.5; 95% CI 3.2–22).

The overall prevalence of single HPV genotype infection was 23% (146 of 640; 95% CI 20–26%). Similar prevalences of single HPV genotype infection were observed in patients with and without condylomata [18% *vs.* 24%, respectively; unadjusted OR 0.7; 95% CI 0.4–1.1: in those with condylomata, 14% (19 of 132) for MSM and 36% (nine of 25) for heterosexuals ($\chi^2 = 6.69$; $P = 0.008$); in those without condylomata, 26% (88 of 341) for MSM and 21% (30 of 142) for heterosexuals ($\chi^2 = 1.19$; $P = 0.275$)]. By contrast, the prevalence of HPV infection involving at least two genotypes differed by condylomata status, and this difference was statistically significant: 75% (117 of 157; 95% CI 70–81%) for patients with condylomata [81% (107 of 132) for MSM and 40% (10 of 25) for heterosexuals; $\chi^2 = 18.6$; $P < 0.001$] and 43% (206 of 483; 95% CI 38–47%) for those without condylomata [54% (185 of 341) for MSM and 15% (21 of 142) for heterosexuals; $\chi^2 = 63.8$; $P < 0.001$] (with/without condylomata, adjusted OR 4.0; 95% CI 2.2–7.1).

Specific HPV genotype infection

The most frequent HPV types identified in the anal canals of HIV-infected men with and without anal condylomata were as follows: HPV-16 (42% *vs.* 23%, respectively), HPV-6 (41% *vs.* 13%), HPV-11 (35% *vs.* 6%), HPV-33 (21% *vs.* 16%), HPV-51 (21% *vs.* 13%) and HPV-58 (21% *vs.* 13%) (Table 3). The prevalence of HPV-18 was 11% in patients with condylomata and 6% in patients without condylomata (OR 1.8; 95% CI 0.9–3.3).

DNA from HPV-6 and/or HPV-11 (alone or in association with each other) was found in 63% of patients (99 of 157) with anal condylomatous lesions, and in 19% of patients (90 of 483) without anal condylomata ($P < 0.001$). Similarly, DNA from HPV-16 and/or HPV-18 (alone or in association) was found in 45% of patients (71 of 157) with anal condylomata and in 27% of patients (128 of 483) without condylomata ($P < 0.001$).

Table 1 Baseline characteristics of HIV-infected men by the presence of anal condylomata

Baseline characteristics	Condylomata ($n = 157$)	No condylomata ($n = 483$)	*P*-value
Age (years)			
Mean ± SD	39.3 (9)	42.7 (9.3)	< 0.001
Median (range)	39 (20–67)	42 (20–77)	
Duration of known HIV infection (years)			
Median (range*)	9 (0–24)	9 (0–26)	0.318
AIDS diagnosis (yes) [*n* (%)]	25 (16)	76 (16)	0.955
HIV plasma load < 50 HIV RNA copies/mL [*n* (%)]	88 (57)	330 (69)	0.005
CD4 count at baseline [*n* (%)]			
< 200 cells/uL	11 (7)	30 (6)	0.662
200–500 cells/uL	71 (46)	203 (43)	
> 500 cells/uL	73 (47)	245 (51)	
CD4 count nadir			
< 200 cells/uL [*n* (%)]	66 (42)	182 (38)	0.867
HAART previous to inclusion (yes)			
n (%)	122 (78)	407 (84)	0.060
Duration of HAART (years)			
Median (25th–75th percentiles)	7 (1–9)	7 (2–9)	0.517
History of STIs (yes)			
n (%)	72 (46)	133 (27)	< 0.001
Alcohol history† [*n* (%)]			
Never	34 (23)	118 (26)	0.699
Current	85 (59)	247 (55)	
Past	26 (18)	87 (19)	
Smoking history† [*n* (%)]			
Never	31 (21)	110 (24)	0.428
Current	85 (59)	237 (53)	
Past	29 (20)	105 (23)	
Injecting drug user† [*n* (%)]			
Never	122 (84)	371 (82)	0.715
Current	1 (1)	7 (2)	
Past	23 (15)	74 (16)	
Number of sexual partners† [*n* (%)]			
1	0	8 (2)	0.144
2–9	19 (15)	67 (17)	
10–25	8 (6)	47 (12)	
> 25	99 (79)	274 (69)	
Men having sex with men [*n* (%)]	132 (84)	341 (71)	0.001
History of RAI (yes) [*n* (%)]	99 (76)	242 (58)	< 0.001

HAART, highly active antiretroviral therapy; RAI, receptive anal intercourse; SD, standard deviation; STI, sexually transmitted infection.
*Range (maximum–minimum values). †Data available for a total of 598 patients. ‡Data available for a total of 522 patients.

Table 2 Frequencies of anal human papillomavirus (HPV) infection and associations between anal HPV infection and the presence of condylomatous lesions in HIV-infected men

	Prevalence (%)		Bivariate model	Multivariate model*
	Group with condylomata ($n = 157$)	Group without condylomata ($n = 483$)	OR (95% CI)	OR (95% CI)
Anal HPV infection				
Any HPV type (all HPV infections)	145 (92)	324 (67)	5.9 (3.2–11.0)	8.5 (3.2–22)
Low-risk HPV types†	99 (63)	90 (19)	7.5 (5.0–11.1)	17.8 (3.6–88)
High-risk HPV types‡	130 (83)	299 (62)	3.0 (1.9–4.7)	3.3 (1.7–6.4)
Single HPV infection (only one genotype)	28 (18)	118 (24)	0.7 (0.4–1.1)	NS
Multiple HPV infections (at least two genotypes)	117 (75)	206 (43)	3.9 (2.6–5.9)	4.0 (2.2–7.1)

Odds ratios (ORs) and 95% confidence intervals (CIs) for bivariate and multivariate regression analyses are shown.
NS, not significant.
*Adjusted for covariables with $P < 0.2$ in the bivariate regression model: age, duration of HIV diagnosis, HIV plasma load, nadir CD4 count, time on highly active antiretroviral therapy, sexual behaviour (men who have sex with men), receptive anal intercourse, sexually transmitted diseases, number of sexual partners and HPV infection (for cytological diagnoses). †Low-risk HPV types included HPV-11 and HPV-6. ‡High-risk HPV types included HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68.

Table 3 Prevalence of specific human papillomavirus (HPV) genotypes and statistical comparisons between 157 patients with anal condylomata and 483 without condylomata at the baseline exploration

	Number (%) of cases					
	Single anal HPV infection			Any anal HPV infection (multiple or single)		
	HIV-positive men			HIV-positive men		
HPV genotype	With condylomata	Without condylomata	OR (95% CI)*	With condylomata	Without condylomata	OR (95% CI)*
HPV-6	8 (5)	18 (3.7)	1.4 (0.6–3.2)	65 (41.4)	62 (12.8)	**4.7 (3.2–7.3)**
HPV-11	5 (3.2)	6 (1.2)	2.6 (0.8–8.7)	55 (35)	31 (6.4)	**7.9 (4.8–12.8)**
HPV-16	5 (3.2)	21 (4.3)	0.7 (0.3–1.9)	66 (42)	109 (22.6)	**2.5 (1.7–3.6)**
HPV-18	0	3 (0.1)	–	17 (10.8)	31 (6.4)	1.8 (0.9–3.3)
HPV-31	0	6 (1.2)	–	5 (3.2)	21 (4.3)	0.7 (0.3–1.9)
HPV-33	1 (0)	10 (2)	0.3 (0.0–2.4)	33 (21)	76 (15.7)	1.4 (0.9–2.2)
HPV-35	1 (0)	5 (1)	0.6 (0.1–5.3)	16 (10.2)	40 (8.3)	1.3 (0.7–2.3)
HPV-39	3 (1.9)	6 (1.2)	1.5 (0.4–6.3)	28 (17.8)	48 (9.9)	**2.0 (1.2–3.3)**
HPV-45	1 (4)	5 (1)	0.6 (0.1–5.3)	12 (7.6)	31 (6.4)	1.2 (0.6–2.4)
HPV-51	0	10 (2)	–	33 (21)	62 (12.8)	**1.8 (1.1–2.9)**
HPV-52	0	8 (1.6)	–	28 (17.8)	55 (11.4)	**1.7 (1.0–2.8)**
HPV-56	0	4 (0.1)	–	16 (10.2)	28 (5.8)	1.8 (0.9–3.5)
HPV-58	3 (1.9)	6 (5)	1.5 (0.4–6.3)	33 (21)	64 (13.2)	**1.7 (1.1–2.8)**
HPV-59	0	4 (0.1)	–	27 (17.2)	53 (11)	**1.7 (1.0–2.8)**
HPV-68	1 (0)	6 (1.2)	0.5 (0.1–4.3)	19 (12.1)	33 (6.8)	**1.9 (1.0–3.4)**
HPV negative	–	–	–	12 (8)	159 (33)	**0.2 (0.1–0.3)**

Data in bold show statistically significant differences ($p<0.05$). CI, confidence interval; OR, odds ratio.

Anal cytological diagnosis

It was possible to analyse 607 (95%) of 640 smears at baseline (Fig. 1). Thirty-three smears (5%) were acellular or showed poor cellularity and were designated as no evaluated cytology in the study. Of the subjects whose smears were analysed, 322 (50%; 95% CI 46–54%) had a normal cytological report, and 96 (15%; 95% CI 12–18%) were diagnosed as having ASCUS, 159 (25%; 95% CI 22–27%) as having LSILs and 30 (5%; 95% CI 3–7%) as having HSILs.

Only 16% (25 of 157) of patients with anal condylomata had normal cytological diagnoses for the anal canal *vs.* 61% (297 of 483) of patients without condylomata ($P < 0.001$). The distribution of cytological abnormalities was as follows: in patients with anal condylomata, 17% (26 of 157) had ASCUS, 58% (91 of 157) had LSILs and 9% (14 of 157) had HSILs, whereas in patients without anal condylomata, 14% (70 of 483) had ASCUS, 14% (68 of 483) had LSILs and 3% (16 of 483) had HSILs.

As regards sexual behaviour, 86% (114 of 132) of MSM and 68% (17 of 25) of heterosexuals with condylomata also presented anal cytological abnormalities and the distribution was as follows: in MSM, 17% (22 of 132) had ASCUS, 60% (79 of 132) had LSILs and 10% (13 of 132) had HSILs, and in heterosexuals, 16% (four of 25) had ASCUS, 48% (12 of 25) had LSILs and 4% (one of 25) had HSILs. In patients without anal condylomata, 37.5% (128 of 341) of MSM and 18% (26 of 142) of heterosexuals also showed anal pathology, as follows: in MSM, 15% (50 of 341) had ASCUS, 19% (64 of 341) had LSILs and 4% (14 of 341) had HSILs, and heterosexuals, 14% (20 of 142) had ASCUS, 3% (four of 142) had LSILs and 1% (two of 142) had HSILs. Thus, having anal condylomata was associated with a higher prevalence of cytological abnormalities in the anal canal [OR 6.9; 95% CI 3.8–12.7; 83% (131 of 157) in HIV-infected patients with anal condylomata and 32% (154 of 483) in those without condylomata]. In particular, in the multivariate analysis, the presence of anal condylomata was associated with a high risk of presenting LSILs (OR 9.0; 95% CI 4.6–18) or HSILs (OR 9.0; 95% CI 2.9–28.4) compared with presenting a normal cytology.

The analyses of HPV genotypes with regard to cytological diagnoses (Table 4) showed a similar pattern of viral type distribution by condylomata status. The most important HPV types associated with low- and high-grade squamous anal lesions were HPV-6 and HPV-16. However, in the patients with condylomata, other HR HPV types (HPV-52 and HPV-58) were the most frequently found in the high-grade anal lesions.

Discussion

The present cross-sectional study reports interesting data on the prevalence of anal condylomata and their association with cytological abnormalities and HPV genotype-specific infection in the anal canal in HIV-infected men

Table 4 Prevalence and distribution of human papillomavirus (HPV) genotypes in relation to anal cytological results at baseline

Cytological results at baseline (*n*, %)	High-risk HPV genotypes [*n* (%)]											Low-risk HPV genotypes [*n* (%)]	
	HPV-16	HPV-18	HPV-31	HPV-33	HPV-35	HPV-39	HPV-45	HPV-51	HPV-52	HPV-56	HPV-58	HPV-59	HPV-68	HPV-6	HPV-11
Patient group															
With condylomata ($n = 157$)															
Normal (25, 16%)	6 (24)	1 (4)	1 (4)	6 (24)	3 (12)	5 (20)	0	3 (12)	5 (20)	4 (16)	5 (20)	1 (4)	1 (4)	5 (20)	6 (24)
ASCUS (26, 17%)	9 (35)	2 (8)	1 (4)	4 (15)	1 (4)	4 (15)	2 (8)	4 (15)	6 (23)	4 (15)	7 (27)	6 (23)	1 (4)	11 (42)	6 (23)
LSIL (91, 58%)	47 (52)	11 (12)	2 (2)	20 (22)	10 (11)	18 (20)	6 (7)	22 (24)	12 (13)	8 (9)	16 (17)	16 (17)	15 (16)	43 (47)	38 (42)
HSIL (14, 9%)	4 (28)	3 (21)	1 (7)	3 (21)	2 (14)	1 (7)	4 (28)	4 (28)	5 (35)	0	5 (35)	4 (28)	2 (14)	6 (42)	5 (35)
Not evaluated* (1, 0%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Without condylomata ($n = 483$)															
Normal (297, 62%)	48 (16)	10 (3)	11 (4)	32 (11)	21 (7)	26 (9)	16 (5)	31 (10)	29 (10)	16 (5)	29 (10)	21 (7)	21 (7)	37 (12)	15 (5)
ASCUS (70, 14%)	22 (31)	2 (3)	1 (1)	14 (20)	7 (10)	8 (11)	5 (7)	9 (13)	9 (13)	4 (6)	7 (10)	10 (14)	5 (7)	9 (13)	1 (1)
LSIL (68, 14%)	26 (38)	16 (23)	8 (12)	20 (29)	10 (15)	10 (15)	6 (9)	12 (18)	15 (22)	6 (9)	22 (32)	15 (22)	5 (7)	11 (16)	11 (16)
HSIL (16, 3%)	9 (56)	2 (12)	0	8 (50)	1 (6)	2 (12)	3 (19)	7 (44)	2 (12)	1 (6)	6 (37)	5 (31)	1 (6)	4 (25)	2 (12)
Not evaluated* (32, 7%)	4 (12)	1 (3)	1 (3)	2 (6)	1 (3)	2 (6)	1 (3)	3 (9)	0	1 (3)	0	2 (6)	1 (3)	1 (3)	2 (6)

ASCUS, atypical squamous cells of uncertain significance; LSIL, low-grade squamous intraepithelial lesion; high-grade squamous intraepithelial lesion.
*Samples with poor cellularity or quality without cytological diagnoses.

without a history of HPV-related pathology (anal condylomata and anal squamous cell cancer). HIV-infected men with anal condylomata presented high-grade squamous intraepithelial lesions in the anal canal. These findings are critical for future clinical approaches, as more stringent monitoring seems to be indicated in HIV-infected men, principally if they already present anal condylomata.

The higher prevalence of anal condylomata (25%) found in this study in comparison with a previous study by Abramowitz *et al.* (10%) [12] may be attributable to the characteristics of the screened population. While 42% of Abramowitz *et al.*'s study population were MSM (the remainder being heterosexual men and women), 74% of our study population were MSM. Nevertheless, although MSM had the highest prevalence of anal condylomata (28%), the prevalence in heterosexual men was also high (15%).

Epidemiological studies have been conducted to assess the cost-effectiveness of HPV vaccination in the general male population (HIV-seronegative) [21,22]. However, to our knowledge, little information has been published on the association between HPV type-specific infection in HIV-positive men and the presence of anal condylomata. Determining the prevalence of specific HPV genotypes in the HIV-infected male population is the first step to preventing further HPV-related pathologies in these immunocompromised patients. We found that a higher prevalence of HPV infection (any HPV genotype) in the anal canal in HIV-positive men was associated with having anal condylomata. A possible explanation for this finding is that it is a consequence of including male populations with at-risk behaviours in the study. For example, having multiple sexual partners and practising RAI are common in MSM and have been shown to be associated with an elevated risk of recurrence of condylomata [12,16,23]. In the group of patients with anal condylomata described here, there was a higher proportion of MSM and more cases of STIs (mainly syphilis and gonorrhoea) than in the population without anal condylomata, which suggests at-risk sexual behaviours in these subjects. Although being MSM was associated with multiple and HR-type HPV infections and with the presence of anal condylomata in univariate analysis, being MSM was not statistically significant in the adjusted multivariable regression model for LR HPV infections.

The data for the overall prevalence of HPV in this study revealed that HIV-positive men with anal condylomatous lesions had a higher probability of presenting with HPV infection in the anal canal (92%) than men without condylomatous lesions (67%). However, we found that 8% of patients with condylomatous lesions had a negative PCR result for HPV infection in the anal canal. Nevertheless, we have to take into account that our study did not specifically test the wart tissue for HPV DNA, so the prevalence of HPV

type-specific infection in the wart remains unknown. Other authors reported an HPV prevalence of 99% in a French cohort of women and men with external ano-genital acuminate condylomata [19]. This difference in HPV prevalence could be related to gender differences between the populations tested or to the LR HPV types identified using the genotyping technique. In relation to this last point, the previous study included up to 11 different LR HPV types, although HPV-6 and HPV-11 represented the most common types of single and multiple HPV infections, in agreement with our study. In fact, the percentage of single infections attributable to other LR HPV types was relatively low in the French study (< 5% of all single HPV infections) [19].

The analysis of HPV type-specific prevalence provides data on the distribution of HPV genotypes in the anal canals of HIV-positive men. Our results provide evidence that the most prevalent types were HPV-6 (41% in HIV-positive men with condylomata and 13% in HIV-positive men without condylomata) and HPV-16 (42% in HIV-positive men with condylomata and 23% in HIV-positive men without condylomata), in agreement with other published works [3,16,19]. Moreover, HR HPV genotypes were detected in a higher proportion of HIV-positive men presenting with anal condylomata (83%) than HIV-positive men without condylomata (62%). It is important to note the high anal canal prevalence of HPV-16 in HIV-positive men. In fact, the prevalence of HPV-16 in the anal canal in HIV-infected men without anal condylomata was very high compared with that previously reported in HIV-negative men (23% *vs.* 9%, respectively) [19]. Similarly, HPV-18 infection was notably more frequent in the anal canals of HIV-positive men (11% in the group with condylomata and 6% in the group without condylomata), compared with the frequency reported in the HIV-negative population (3%) [19]. The most prevalent viral genotypes found in the CARH·MEN cohort are included in the quadrivalent HPV vaccine, suggesting the potential use of vaccination as an alternative strategy for prevention of HPV-related pathology. However, other HR HPV types, such as HPV-33, 51, 58, 39, 52 or 59, with a significant predominance in HIV-infected men with anal condylomata lesions should be taken into account for their potential impact on the development of high-grade precancerous lesions.

LR and HR HPV genotypes share a common route of transmission and the presence of condylomatous lesions indicates HPV exposure and a risk of exposure to HR HPV types too. In fact, most of the CARH·MEN patients with anal condylomata harboured HR HPV DNA in the anal canal and thus had a high probability of developing anal intraepithelial precancerous lesions. It is known that external acuminate condylomata are typically benign lesions with a low risk of progression to invasive cancer but represent a clinical marker of the risk of developing an HR HPV genotype-related malignant lesion in the anal canal [19]. In fact, in our study of HIV-infected men without a known history of anal condylomata, having anal condylomata was associated with a higher prevalence of high-grade cytological lesions in the anal canal. This finding is in agreement with that of another study [9]. Furthermore, results from a study of a large urban population of MSM with condylomata requiring surgical excision showed that these patients had a high frequency of occult high-grade anal intraepithelial neoplasia or anal squamous cell cancer [24]. These results highlight the need for regular monitoring and screening of these at-risk patients.

Two important limitations need to be considered in the interpretation of the results of this cross-sectional study. Firstly, because the results were based on anal cytological diagnosis, there could be an underestimation or overestimation of the actual pathology. Secondly, there were a low number of patients (13 out of 157) presenting anal condylomata exclusively in the perianal area. This means that it is difficult to establish with certainty an association between the development of high-grade lesions and the anatomical location of the condylomatous lesion.

In summary, MSM presented the highest risk of anal condylomata, although the prevalence of anal condylomata in heterosexual men was also high. HIV-infected men with anal condylomatous lesions were at high risk of having high-grade squamous intraepithelial lesions and harbouring multiple HPV infections involving HR HPV types in the anal canal in comparison to HIV-infected men without condylomata. These data emphasize the importance of screening and follow-up of condylomata in the anal canal in HIV-infected men.

Acknowledgements

We would especially like to thank the male patients attending our HIV Unit, and the HIV-HPV Study Group: Ms I. Fernández (Department of Proctology), Drs E. Castella and M. LLatjós (Department of Pathology), Dr A. Bonjoch, Dr P. Echevarría, Dr M. Jabaloyas, Dr A. Jou, Dr J. M. Llibre, Dr J. Moltó, Dr E. Negredo, Ms N. Pérez-Alvarez, Dr C. Rey-Joly, Dr J. Romeu, Dr J. R. Santos and Dr C. Tural (HIV Clinical Unit and Internal Medicine Department), and Ms C. Alcalde, Ms R. Guerola and Ms A. Salas (nurses of the HIV Clinical Unit), University Hospital Germans Trias i Pujol, Badalona (Barcelona), Autonomous University of Barcelona, Barcelona; Ms I. Castilla, Dr V. Cirigliano, Dr M. Ejarque, Ms E. López, Ms E. Ordóñez and Ms L. Rueda, General Lab, Department of Molecular Biology, Barcelona, Spain.

Funding: This work was supported by grants from the Lluita Contra La SIDA Foundation, Red de Investigación en SIDA (RIS), the ART AIDS Foundation, Gilead Sciences and Obra Social Caixa Sabadell.

Conflicts of interest: SV has received honoraria for collaborating with Laboratorios Dr Esteve, and BC for serving on the advisory boards of Abbott, Bristol-Myers Squibb, Boehringer-Ingelheim, Gilead Sciences, GlaxoSmithKline, Pfizer, Merck and Janssen-Tibotec. The other authors have no conflict of interest to declare.

Author contributions: SV, GS and BC designed and wrote the study protocol. GS and JC visited and interviewed the patients; MPC and LD performed HPV detection and genotyping; PC, FG-C, MP and SV collected specimens; ML analysed the anal cytology samples; LD, MPC, SV, JC and BC wrote the manuscript; and the statistical analysis was performed by RM-L. All the authors read and approved the final version of the manuscript.

References

1 Chin-Hong PV, Palefsky JM. Natural history and clinical management of anal human papillomavirus disease in men and women infected with human immunodeficiency virus. *Clin Infect Dis* 2002; **35**: 1127–1134.

2 Johnson LG, Madeleine MM, Newcomer LM, Schwartz SM, Daling JR. Anal cancer incidence and survival: the surveillance, epidemiology, and end results experience, 1973–2000. *Cancer* 2004; **101**: 281–288.

3 Kreuter A, Brockmeyer NH, Hochdorfer B *et al.* Clinical spectrum and virologic characteristics of anal intraepithelial neoplasia in HIV infection. *J Am Acad Dermatol* 2005; **52**: 603–608.

4 Chiao EY, Giordano TP, Palefsky JM, Tyring S, El Serag H. Screening HIV-infected individuals for anal cancer precursor lesions: a systematic review. *Clin Infect Dis* 2006; **43**: 223–233.

5 Dupin N. Genital warts. *Clin Dermatol* 2004; **22**: 481–486.

6 Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. *Vaccine* 2006; **24**: S1–15.

7 Kreuter A, Wieland U. Human papillomavirus-associated diseases in HIV-infected men who have sex with men. *Curr Opin Infect Dis* 2009; **22**: 109–114. Review.

8 Cain JM, Howett MK. Preventing cervical cancer. *Science* 2000; **288**: 1753–1754.

9 Etienney I, Vuong S, Daniel F *et al.* Prevalence of anal cytologic abnormalities in a French referral population: a prospective study with special emphasis on HIV, HPV, and smoking. *Dis Colon Rectum* 2008; **51**: 67–72.

10 Scott H, Khoury J, Moore BA, Weissman S. Routine anal cytology screening for anal squamous intraepithelial lesions in an urban HIV clinic. *Sex Transm Dis* 2008; **35**: 197–202.

11 Bower M, Powles T, Newsom-Davis T *et al.* HIV-associated anal cancer: has highly active antiretroviral therapy reduced the incidence or improved the outcome? *J Acquir Immune Defic Syndr* 2004; **37**: 1563–1565.

12 Abramowitz L, Benabderrahmane D, Ravaud P *et al.* Anal squamous intraepithelial lesions and condyloma in HIV-infected heterosexual men, homosexual men and women: prevalence and associated factors. *AIDS* 2007; **21**: 1457–1465.

13 Chin-Hong PV, Vittinghoff E, Cranston RD *et al.* Age-related prevalence of anal cancer precursors in homosexual men: the EXPLORE study. *J Natl Cancer Inst* 2005; **97**: 896–905.

14 Alam M, Stiller M. Direct medical costs for surgical and medical treatment of condylomata acuminata. *Arch Dermatol* 2001; **137**: 337–341.

15 Wiley D, Masongsong E. Human papillomavirus: the burden of infection. *Obstet Gynecol Surv* 2006; **61**: S3–14. Review.

16 D'Ambrogio A, Yerly S, Sahli R *et al.* Human papilloma virus type and recurrence rate after surgical clearance of anal condylomata acuminata. *Sex Transm Dis* 2009; **36**: 536–540.

17 Villa LL, Costa RL, Petta CA *et al.* Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. *Lancet Oncol* 2005; **6**: 271–278.

18 Villa LL, Costa RL, Petta CA *et al.* High sustained efficacy of a prophylactic quadrivalent human papillomavirus types 6/11/16/18 L1 virus-like particle vaccine through 5 years of follow-up. *Br J Cancer* 2006; **95**: 1459–1466.

19 Aubin F, Prétet JL, Jacquard AC *et al.* EDiTH Study Group. Human papillomavirus genotype distribution in external acuminata condylomata: a Large French National Study (EDiTH IV). *Clin Infect Dis* 2008; **47**: 610–615.

20 Videla S, Darwich L, Cañadas MP *et al.* HIV-HPV Study Group. Epidemiological data of different human papillomavirus genotypes in cervical specimens of HIV-1-infected women without history of cervical pathology. *J Acquir Immune Defic Syndr* 2009; **50**: 168–175.

21 Kim JJ. Targeted human papillomavirus vaccination of men who have sex with men in the USA: a cost-effectiveness modelling analysis. *Lancet Infect Dis* 2010; **10**: 845–852.

22 Giuliano AR, Lee JH, Fulp W *et al.* Incidence and clearance of genital human papillomavirus infection in men (HIM): a cohort study. *Lancet* 2011; **377**: 932–940.

23 Palefsky JM. Human papillomavirus-related disease in men: not just a women's issue. *J Adolesc Health* 2010; **46**: S12–S19. Review. Erratum in: J Adolesc Health 2010; 46:614.

24 Schlecht HP, Fugelso DK, Murphy RK *et al.* Frequency of occult high-grade squamous intraepithelial neoplasia and invasive cancer within anal condylomata in men who have sex with men. *Clin Infect Dis* 2010; **51**: 107–110.

Estudio 2: "Effectiveness of physically ablative and pharmacological treatments for anal condyloma in HIV-infected men"[0]
PLoS One. 2018 Aug 1;13(8):e0199033. doi: 10.1371/journal.pone.0199033

"EFECTIVIDAD DE LOS TRATAMIENTOS ABLATIVOS Y FARMACOLÓGICOS PARA EL MANEJO DE LOS CONDILOMAS EN EL CANAL ANAL EN HOMBRES INFECTADOS POR EL VIH"

ANTECEDENTES. La información sobre la efectividad de los tratamientos disponibles para el manejo de los condilomas anales en hombres infectados por VIH-1 es limitada.

OBJETIVO. Proporcionar datos sobre la efectividad de la escisión quirúrgica, la coagulación con infrarrojos y el tratamiento farmacológico (imiquimod) para el condiloma acuminado en canal anal (perianal y/o intra-anal) en hombres infectados por el VIH-1 de acuerdo a la práctica clínica.

MÉTODOS. Análisis descriptivo, retrospectivo, unicéntrico llevado a cabo en de hombres infectados por el VIH-1, mayores de 18 años.

Se ofrecieron los siguientes tratamientos: tratamientos estándar o escisión quirúrgica, coagulación con infrarrojos e imiquimod tópico. La efectividad de los tratamientos se evaluó mediante la tasa de recurrencia al año del tratamiento. La recurrencia se definió como cualquier condiloma en el canal anal diagnosticado después de 3 meses de haber iniciado el tratamiento, y tras haber conseguido la erradicación del condiloma. También se estudió la citología en el canal anal y la infección el virus del papiloma humano (VPH).

RESULTADOS. Entre enero de 2005 y mayo de 2009, 101 hombres fueron tratados por condiloma en el canal anal: 65 (64%) con cirugía, 27 (27%) con coagulación con infrarrojos y 9 (9%) con imiquimod. Al año del tratamiento, la tasa de recurrencia acumulada fue del 8% (4/65, IC95%: 2-15%) con cirugía, 11% (3/27, IC95%: 4-28%) con coagulación con infrarrojos y 11% (1/9, IC95%: 2-44%) con tratamiento con imiquimod. No se asociaron factores predictivos con la recurrencia.

Una infección en el canal anal por los genotipos del VPH 6 y 11 fue detectable en 98 (97%) pacientes y todos estaban también infectados por genotipos de alto riesgo oncogénico, y 89 (88%) pacientes tuvieron una citología anormal del canal anal.

LIMITACIONES. Ser un análisis descriptivo retrospectivo; limitado a un solo centro; no se puede saber si la recurrencia está relacionado con una nueva infección.

CONCLUSIÓN. La recurrencia de condiloma anal después de cualquier tratamiento fue común. Citología anormal e infección por el VPH de alto riesgo oncogénico fue altamente prevalente en nuestra población. Por tanto, los hombres infectados por el VIH-1 presentan un riesgo alto de desarrollar un cáncer en el canal anal, por lo que son merecedores de un seguimiento cuidadoso.

PLOS ONE

RESEARCH ARTICLE

Effectiveness of physically ablative and pharmacological treatments for anal condyloma in HIV-infected men

Sandra Vela[1,2☯], Sebastian Videla[2,3☯*], Arelly Ornelas[2], Boris Revollo[2,4], Bonaventura Clotet[2,4,5], Guillem Sirera[2,4], Marta Piñol[1,2], Francesc García-Cuyás[1,2]

1 Department of Surgery, University Hospital Germans Trias i Pujol, Badalona, Catalonia, Spain, **2** Lluita Contra La SIDA Foundation, University Hospital Germans Trias i Pujol, Badalona, Catalonia, Spain, **3** Department of Clinical Pharmacology, University Hospital Bellvitge / IDIBELL / Barcelona University, Hospitalet de Llobregat, Barcelona, Catalonia, Spain, **4** HIV Clinical Unit, Department of Medicine, University Hospital Germans Trias i Pujol, Badalona, Catalonia, Spain, **5** Retrovirology Laboratory IrsiCaixa Foundation, Badalona, Catalonia, Spain

☯ These authors contributed equally to this work.
* svidela@flsida.org, svidelaces@gmail.com, svidela@bellvitgehospital.cat

OPEN ACCESS

Citation: Vela S, Videla S, Ornelas A, Revollo B, Clotet B, Sirera G, et al. (2018) Effectiveness of physically ablative and pharmacological treatments for anal condyloma in HIV-infected men. PLoS ONE 13(8): e0199033. https://doi.org/10.1371/journal.pone.0199033

Editor: Magdalena Grce, Rudjer Boskovic Institute, CROATIA

Received: December 4, 2017

Accepted: May 30, 2018

Published: August 1, 2018

Data Availability Statement: All relevant data are within the paper and its Supporting Information files. Likewise, the raw data will be available in Dryad doi:10.5061/dryad.fn74266.

Funding: This study was funded by grants from the Lluita Contra La SIDA Foundation and FIS P15/00492. The study's funder was not involved in study design, neither data interpretation, nor revision of the final report. The corresponding author had full access to all study data and had final responsibility for the decision to submit the

Abstract

Background

There is limited information on the effectiveness of available treatments for anal condyloma acuminata in HIV-1-infected men.

Aim

To provide data on the effectiveness of electrosurgical excision, infrared coagulation and pharmacological (imiquimod) treatments for anal condyloma acuminata (peri-anal and/or intra-anal) in HIV-1-infected men based on authors' practice.

Methods

Single-center, retrospective descriptive analysis of HIV-1-infected men, 18 years or older treated for anal condyloma acuminata. Standard treatments were offered: electrosurgery excision, infrared coagulation and topical imiquimod. Effectiveness was evaluated by the recurrence rate at 1 year after treatment. Recurrence was defined as any anal condyloma acuminata diagnosed after 3 months of condyloma-free survival post-treatment. Anal cytology and human-papillomavirus-infection (HPV) was assessed.

Results

Between January 2005 and May 2009, 101 men were treated for anal condyloma acuminata: 65 (64%) with electrosurgery, 27 (27%) with infrared coagulation and 9 (9%) with imiquimod. At 1 year after treatment, the cumulative recurrence rate was 8% (4/65, 95%CI: 2–15%) with electrosurgery excision, 11% (3/27, 95%CI: 4–28%) with infrared coagulation and 11% (1/9, 95%CI: 2–44%) with imiquimod treatment. No predictive factors were associated with recurrence.

paper for publication. B.C. has received honoraria for speaking and participating in advisory boards from Abbott, Bristol-Myers Squibb, Boehringer-Ingelheim, Gilead Sciences, GlaxoSmithKline, Pfizer, Merck, and Janssen-Tibotec. These funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: B.C. has received honoraria for speaking and participating in advisory boards from Abbott, Bristol-Myers Squibb, Boehringer-Ingelheim, Gilead Sciences, GlaxoSmithKline, Pfizer, Merck, and Janssen-Tibotec. This does not alter our adherence to PLOS ONE policies on sharing data and materials. There are no patents, products in development or marketed products to declare. The remaining authors have no conflict of interest.

Anal HPV-6 or HPV-11 was detectable in 98 (97%) patients and all had high-risk HPV genotypes, and 89 (88%) patients had abnormal anal canal cytology. Limitations: this was a retrospective descriptive analysis; limited to a single center; it cannot know if the recurrence is related to new infection.

Conclusion

Recurrence of anal condyloma after any treatment was common. Abnormal anal cytology and high-risk HPV-infection were highly prevalent in this population, therefore at high-risk of anal cancer, and warrants careful follow-up.

Introduction

Anal condyloma acuminata (CA) are frequently associated with human papillomavirus (HPV) types 6 and 11 [1,2]. CA may be located peri-anally or intra-anally and patients commonly present for medical treatment due to feeling 'bumps' when washing or more infrequently after findings on routine medical examinations such as colonoscopy, or more rarely with symptoms such as itch, wetness or pain. Global incidence of anogenital warts ranges from 160 to 289 cases per 100 000 person-years [3,4]. The histology of anogenital warts typically shows benign characteristics, although intraepithelial or invasive squamous cell carcinomas can coexist [5].

There is limited scientific literature available on the effectiveness and safety of treatments used in clinical practice for anal CA [6]. Moreover, there is no consensus on the best treatment to manage these lesions. In clinical practice, there are various treatment modalities including physical ablation [electrosurgery excision, cryotherapy, infrared coagulation (IRC), laser ablation], and pharmacological treatments (imiquimod, podophyllotoxin, tricloracetic acid, sinecatechins) [7–9]. Generally, the election of the treatment depends on localization, size, number of the CAs and on the doctor's experience. For example, physically ablative treatments are used at peri- and intra-anal area, meanwhile pharmacological treatments are indicated when the lesions exclusively affect the perianal area. A high regression rate in the first year after diagnosis of genital warts can be common among HIV-1-infected women (on 60%) and in general population (80%) [10]. However, despite this regression rate, the main challenge in clinical practice for any treatment is the high percentage of recurrence, after a long period of follow-up, independent of the type of treatment used [7,11–13]. Albeit these treatments can result in resolution of the wart, removing the lesion is not synonymous with eradicating the HPV infection, and this may explain the high recurrence rate, regardless of the treatment modality employed.

Therefore, the aim of this study was simple, to provide data on the effectiveness and safety of electrosurgery excision, IRC and imiquimod treatments for anal CA in HIV-1-infected men, based on authors' clinical practice.

It is noteworthy that this study does not aim to compare the effectiveness among treatments, and is a descriptive analysis. Likewise, the results of the present study are complementary to data of previous published works: data on the prevalence of anal CA [14] from the CARH·MEN cohort [15].

Patients and methods

Study design

The study was a single-center, retrospective analysis using data from a prospectively compiled database of outpatients included in CARH·MEN cohort [15] and to whom anal CA was

diagnosed. In brief, CARH·MEN cohort is a screening program for detection and treatment of anal intraepithelial neoplasia.

The study was approved by the Hospital Investigational Review Board (University Hospital Germans Trias i Pujol, Badalona, Catalonia, Spain). Data confidentiality was ensured according to Spanish legislation on the protection of personal data (LOPD 15/1999).

The starting point of this study was January 1st 2005, when the Clinical Proctology HIV Section was created and CARH·MEN cohort started. Hence, this study embraces the first 10 years of our outpatients Clinical Proctology HIV Section of the Germans Trias i Pujol University Hospital (Badalona, Spain).

Study population

The patients included had to fulfill the following criteria: age $\geq$18 years, men from CARH·MEN cohort at first visit, no medical history of anal CAs, a visual diagnosis of a CA at peri-anal and/or intra-anal (including at high-resolution anoscopy (HRA), treated in our hospital and with at least a year of follow-up after treatment.

The period of follow-up was defined as the time between the baseline (corresponding to baseline visit of CARH·MEN cohort and when the CA was diagnosed) and last available documented visit (up to the diagnosis of anal CA recurrence or up to the last documented visit).

The following data were gathered at baseline visit (CA diagnosis): date of birth, date of HIV diagnosis (time of known HIV), date of the visit, CD4 T cell count (the most recent value before the wart diagnosis visit), nadir CD4 T count, plasma HIV-1 viral load, antiretroviral therapy (ART) before inclusion (Yes/No), date of starting the ART (time on ART), anal canal cytology (normal, atypical squamous cells of unknown significance [ASCUS], low-grade squamous intraepithelial lesion [LSIL], or high-grade squamous intraepithelial lesion [HSIL]) results, HPV DNA testing and typing at anal canal sample. The following data during the follow-up was also gathered: type of treatment for wart performed, recurrence (YES/NO), date of recurrence and adverse events reported by the patients and related to the procedures.

Anal CA diagnosis

The diagnosis was based on visual inspection or HRA. Each visit of CARH·MEN cohort includes a clinical examination (visual inspection) including a digital rectal examination and to obtain a sample from the anal canal for cytological examination. If the cytological result at baseline was abnormal (ASCUS, LSIL, or HSIL) a HRA was performed.

Treatments available

A guidance clinical protocol for the treatment of anal CA, based on the number of condyloma, size of affected area (diameter, cm^2) location and characteristics of the patients, was available in the Clinical Proctology HIV Clinic. A brief summary of the treatments offered in our proctology unit:

a. Electrosurgery excision under anesthesia. Electrosurgery treatment was proposed when more than 1 CA greater than 0.5 cm^2 with each one affecting the peri-anal and/or intra-anal areas (circumferential or near-circumferential area affected), or to patients with more than 5 small CAs smaller than 0.5 cm^2 each one localized at peri-anal and/or intra-anal areas, or when at least a single CA greater than 1 cm^2 was diagnosed, or when the patients had a poor tolerance to HRA. This treatment consists on the excision of all visible lesions by electrocautery (electric scalpel). In some cases, several interventions could be required to eliminate all lesions to avoid the risk of anal stenosis.

b. Ablation with IRC (Redfield IRC-2100; Redfield-Corporation, Rochelle Park, New Jersey, USA). IRC was offered to patients with a single CA of anal canal smaller than 1 cm^2, or to patients with up to 5 small CAs smaller than 0.5 cm^2 each one localized at peri-anal and/or intra-anal areas or when a surgical excision could be very traumatic according the treating surgeon. The anal condylomata were treated with in-office IRC ablation in the same facilities of HIV proctology service. Before performing ablation with IRC directed by HRA, anal condyloma was identified and local anesthesia (mepivacaine, Scandinibsa®, INIBSA-HOSPITAL, S.L.U., Lliçà de Vall, Barcelona, Spain) was used. IRC delivers short pulses of a narrow beam of visible infrared light through a small contact tip applicator that was applied directly to the condyloma. It results in thermal coagulation and tissue necrosis. The scab was removed, and the process was repeated with 1.5-second pulses until the submucosal vessels were coagulated;

c. Pharmacologic treatment. Topical imiquimod (Aldara 5% cream®, Meda-AB, Solna, Sweden) three times per week, at night before going to the bed, and a minimum of 12 weeks or until the lesions disappear was offered to patients with peri-anal small wart (lower than 0.5 cm^2).

The abovementioned treatments (electrosurgery excision, IRC and imiquimod) were managed for clinicians from the Clinical Proctology HIV Clinic. Treatment modality was selected based on clinician recommendation and patient preference. That is to say, the different treatments available were explained to the patients, the doctor in charge based on his/her experience advised, but the decision to select one was agreed with the patient. After being treated, the patients were reviewed every 3 months in the first year (including HRA at first control visit), and annually thereafter.

Anal canal samples: Cytology and HPV detection

Anal canal cytological procedure. Anal canal sample for cytological examination was obtained, according previously described [16]. This sample was used to carry out the cytology analysis (Papanicolaou test). Anal canal cytological changes were classified according to the Bethesda System as normal, ASCUS, LSIL and HSIL.

HPV detection. Detection of HPV Infection was performed in the anal cytological sample according previously described [16]. HPV was detected and typed using the F-HPV typing™ kit (Molgentix, Spain) for HPV-6,-11,-16,-18,-31,-33,-35,-39,-45,-51,-52,-56,-58,-59,-and -68.

Statistical analysis

Sample size. The sample size was defined as the number of patients from CARH·MEN cohort who fulfilled the inclusion criteria.

Definitions. Recurrence of anal condyloma was defined as the reappearance of any evidence of anal condyloma after 3 months of condyloma-free survival post-treatment, assessed by physical examination for perianal-CA and by HRA for intra-anal-CA. The effectiveness of treatments was assessed by means of the recurrence rate.

Statistical procedures. A descriptive analysis was performed for baseline population characteristics. Recurrence (number of patients) was estimated and its 95% confidence intervals (95%CI) was calculated.

Bivariate and multivariate logistic analyses and Cox proportional hazard regression models were performed, when appropriate, to determine potential factors associated with the recurrence. Recurrence rates were also calculated based on person-time denominator (100 person-years).

The association between potential explanatory variables and anal condyloma was tested in 2 steps. First, a bivariate regression model was performed to obtain an indication of the relevance of the explanatory variables in the risk of HIV-1 infection. Second, factors showing a P value less than 0.3 were used to perform a multiple regression model using a stepwise selection of variables. Hazard ratios (HRs) for incidence were estimated as well as their corresponding 95%CI. A P value of 0.05 or less was considered statistically significant. Data analysis was performed using SPSS version 15.0 (SPSS Inc. Chicago, IL) and R version 3.3.1 (2016-06-21).

Results

Patient characteristics

One hundred and fifty-seven patients from CARH·MEN cohort were diagnosed with peri-anal and/or intra-anal condyloma [14,15]. Of them, 103 were treated in our hospital, and had at least 1 year of follow-up after the wart treatment. Given that the descriptive nature of this work, two patients treated with cryotherapy in our hospital but out of HIV facilities were also included. This treatment was managed in dermatology department of our hospital. The two patients treated with cryotherapy presented with a peri-anal small wart (lower than 0.5 cm^2). This treatment consists on the application of liquid nitrogen to freeze and destroy the CA as well as the immediate surrounds area. Therefore, 65 (63%) patients were treated with electrosurgery excision, 27 (26%) with IRC, 9 (9%) with pharmacological treatment and 2 (2%) with cryotherapy. Table 1 shows the baseline characteristics. The group of patients treated with IRC were slightly older, with a lower nadir CD4 and with greater time of HIV-1-infection with respect to other treatments groups.

Effectiveness of treatments for CA

All patients achieved a primary clearance at 3 months after treatment, no recurrence (assessed by physical examination for peri-CA and by standard or HRA for intra-anal CAs). The cumulative recurrence rate at 1 year after treatment was 8% (4/65, 95%CI: 2–15%) with electrosurgery excision, 11% (3/27, 95%CI: 4–28%) with infrared and 11% (1/9, 95%CI: 2–44%) with

Table 1. Baseline characteristic.

Baseline characteristic		All n = 103	Electrosurgery excision n = 65	Infrared coagulation n = 27	Pharmacologic n = 9	Cryotherapy n = 2
Age	median (IQR)	38.8 (32.4–44.2)	38.4 (31.0–42.5)	42.3 (38.7–48.6)	30.3 (26.1–43.3)	38.2 (37.6–38.8)
Sexual behavior:						
MSM	n (%)	91 (88)	59 (91)	24 (89)	6 (67)	2 (100)
MSW	n (%)	12 (12)	6 (9)	3 (11)	3 (33)	—
Time of known HIV (years)	median (IQR)	6.9 (0.6–14.2)	5.7 (0.5–13.6)	10.2 (1.1–17.4)	2.5 (0.2–13.5)	8.2 (0.4–15.9)
ART (yes)	n (%)	73 (71)	47 (72)	19 (70)	6 (67)	1 (50)
Time on ART HIV (years)	median (IQR)	7.3 (0.0–9.9)	5.1 (0.3–9.6)	9.1 (5.4–10.6)	2.7 (0.1–8.6)	9.9 (9.8–10.0)
HIV-1 RNA plasma load (copies/mL)	median (IQR)	40 (40–40)	40 (40–40)	40 (40–80)	40 (40–142)	350020 (40–700000)
HIV plasma load, <80 copies/mL	n (%)	91 (88.3)	62(95.4)	21 (77.8)	7 (77.8)	1 (50)
CD4 nadir cells/uL	median (IQR)	269 (121–410)	282 (155–415)	204 (91–299)	316 (34–579)	346 (324–368)
CD4 nadir cells/uL (<200) (yes)	n (%)	37 (36)	21 (32)	13 (48)	3 (33)	0 (0)
CD4 cells/uL	median (IQR)	705 (482–900)	753 (499–953)	548 (426–731)	612 (278–1162)	1269 (880–1657)
CD4 cells/uL (<200) (yes)	n (%)	6 (6)	2 (3)	2 (7)	2 (22)	0 (0)

MSM: men who have sex with men; MSW: men who have sex with women; ART: antiretroviral therapy; IQR: interquartile range.

https://doi.org/10.1371/journal.pone.0199033.t001

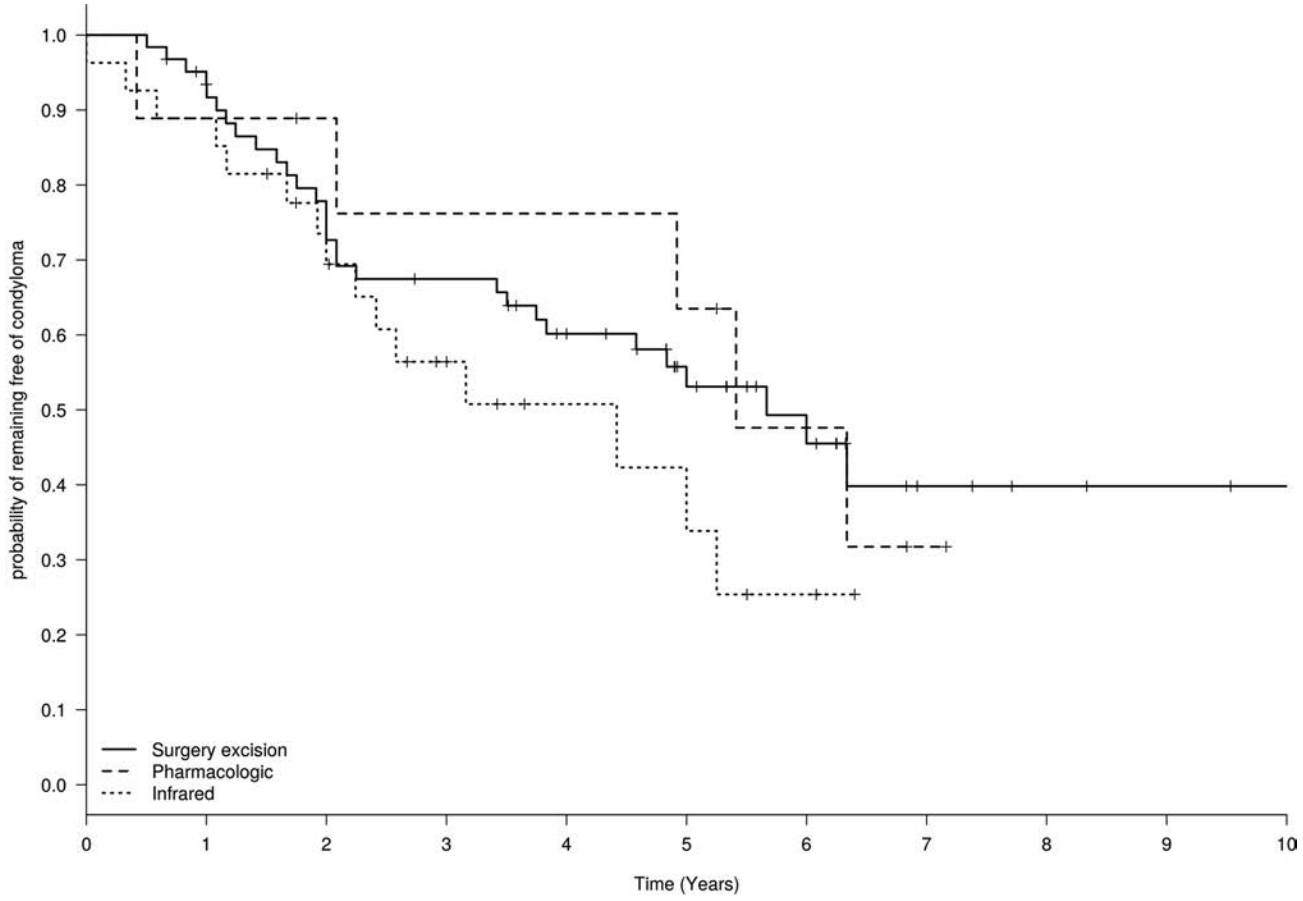


Fig 1. Actuarial probability (Kaplan–Meier curve) of remaining free of anal condylomata in HIV-infected men according to the different treatments performed: Electrosurgery excision (65 patients), infrared (27 patients) and imiquimod (9 patients).

https://doi.org/10.1371/journal.pone.0199033.g001

pharmacologic treatment. No predictive factors associated with the recurrence of anal CA were found.

After up to 10 years of follow-up, the overall cumulative recurrence rate was 49.5% (51 recurrence in 103 patients treated, 95%CI: 40–59%). The rate (range) of recurrence was 13.6 (10.2; 17.9) per 100 person-years. By treatments, the cumulative recurrence rate was 46% (30 patients, 95%CI: 35–58%) with electrosurgery excision, 56% (15 patients, 95%CI: 37–72%) with IRC, 56% (5 patients, 95%CI: 27–81%) with pharmacologic treatment and 50% (1 patient, 95%CI: 1–91%) with cryotherapy. The actuarial probability (Kaplan-Meier curve) of remaining free of anal CA by the different treatments is shown in Fig 1. The median time of recurrence was 4.0, 2.6, 5.3 and 5.7 years for electrosurgery excision, IRC, pharmacologic treatment and cryotherapy, respectively. A *post-hoc* analysis stratified by sexual behavior (men who have sex with men–MSM- or men who have sex with women–MSW-) was performed in patients who suffered either electrosurgery excision or infrared coagulation. No differences were observed.

Table 2 shows the anal canal cytological results at the diagnosis of anal CA. Respect to SIL in anal canal, similar results were found between MSM and MSW. Fig 2 depicts the HPV genotypes distribution involved at CA diagnosis. All patients with anal CA had an HPV infection at anal canal with 2 or more HPV genotypes. The most predominant genotypes were HPV-6

Table 2. Anal canal cytological findings of HIV-infected men at the visit of condyloma diagnosis.

Anal canal cytology					
		Normal	ASCUS	LSIL	HSIL
WHOLE POPULATION (n = 103)					
Electrosurgery excision	n = 65	6 (9%)	11 (17%)	40 (62%)	8 (12%)
Infrared	n = 27	3 (11%)	4 (15%)	16 (59%)	4 (15%)
Pharmacologic	n = 9	4 (44%)	1 (11%)	4 (44%)	—
Cryotherapy	n = 2	1 (50%)	—	1 (50%)	—
	total	14 (14%)	16 (16%)	61 (59%)	12 (12%)
MSM (n = 91)					
Electrosurgery excision	n = 59	6 (10%)	11 (19%)	35 (59%)	7 (12%)
Infrared	n = 24	3 (13%)	3 (13%)	14 (58%)	4 (17%)
Pharmacologic	n = 6	2 (33%)	1 (17%)	3 (50%)	—
Cryotherapy	n = 2	1 (50%)	—	1 (50%)	—
	total	12 (13%)	15 (17%)	53 (58%)	11 (12%)
MSW (n = 12)					
Electrosurgery excision	n = 6	—	—	5 (83%)	1 (17%)
Infrared	n = 3	—	1 (33%)	2 (67%)	—
Pharmacologic	n = 3	2 (67%)	—	1 (33%)	—
Cryotherapy	n = 0	—	—	—	—
	total	2 (17%)	1 (8%)	8 (67%)	1 (8%)

https://doi.org/10.1371/journal.pone.0199033.t002

(52%, 53/103, 95%CI: 42–61%), HPV-11 (46%, 47/103, 95%CI: 36–55%) and HPV-16 (48%, 49/103, 95%CI: 38–57%). The cumulative prevalence of genotypes 6 and 11 was 97% (100/103, 95%CI: 92–99%) in cytological samples from anal canal previous to receive the treatment for anal condylomata, therefore, HPV-6 or HPV-11 was not detected in only 3 patients. Likewise, it is noteworthy that cumulative prevalence of genotypes 16 and 18 was 61% (63/103, 95%CI: 52–70%), and the cumulative prevalence taking into account all high-risk HPV genotypes was of 100%. Respect to the other HPV genotypes involved in the new vaccine, the prevalence were 14% HPV-18 (14/103, 95%CI: 8–22%), 5% HPV-31 (5/103, 95%CI: 2–11%), 23% HPV-33 (24/103, 95%CI: 16–32%), 7% HPV-45 (7/103, 95%CI: 3–13%), 20% HPV-52 (21/103, 95%CI: 14–29%), and 19% HPV-58 (20/103, 95%CI: 13–28%).

Adverse events related to procedures

Pain was the main adverse event reported by patients and related to treatments [7 (11%) patients after surgical excision, 2 (7%) patients after IRC and 1 (11%) after imiquimod]. Three (5%) patients had a stenosis develop in anal canal and 1 (2%) had an abscess in the surgical scar area after the electrosurgical excision. All patients treated with imiquimod had skin irritation or a burning sensation. No adverse event related with the obtaining of sample from anal canal for cytology and HPV assessment was gathered.

Discussion

The aim of this study was not to compare the effectiveness of different treatments for anal condyloma in HIV-1-infected men, as treatment depended on factors such as the number of condyloma, size of affected area, location and characteristics of the patients. Nevertheless, to our knowledge, this is the first time that the effectiveness of different treatments (electrosurgery, infrared coagulation and imiquimod) for the treatment of anal CA in HIV-1-infected men

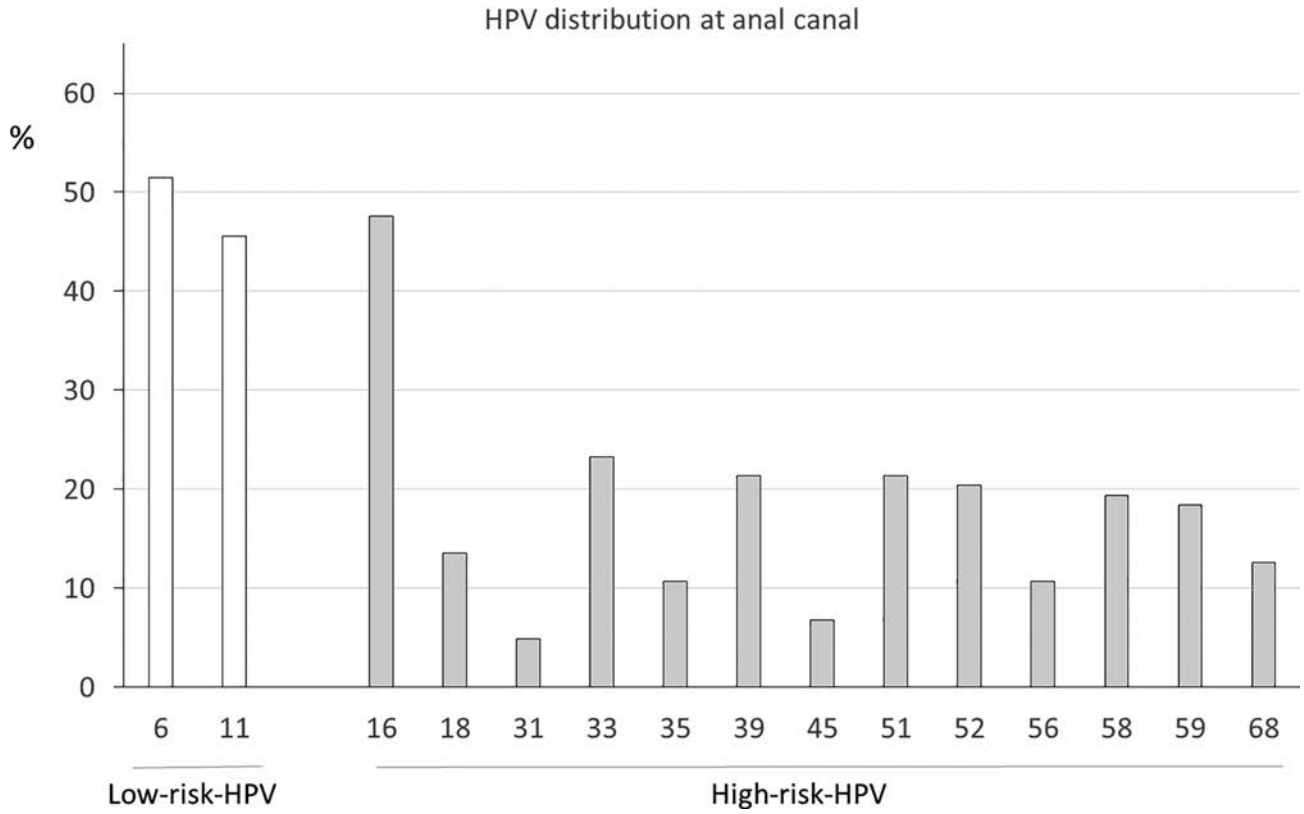


Fig 2. HPV genotypes distribution at the visit of anal condylomata diagnosis.

https://doi.org/10.1371/journal.pone.0199033.g002

(MSW and MSM) is evaluated during a long period of time. In spite of an excellent observed effectiveness at 3 months (no recurrence) and at one year after treatment (on 10% of recurrence), a high rate of CA recurrence was found when the follow-up was up to 10 years treatment (on 50% of recurrence). These recurrence rates were unrelated to treatment type. However, our descriptive results suggest that HIV-1-infected men treated for anal CA are a population with a high probability of suffering a recurrence, independent of the treatment used.

The decision to propose one of the treatments available in our hospital was mainly based on the characteristics of CAs (number, area affected. . .) and doctor's experience [17]. Surgical excision was performed in over 60% of the patients. This data reflects that large condylomata, affecting internal and external area of anal canal, were the frequent among our HIV-1-infected men. Although complications of surgical excision were low, these were not exhaustively collected in our study. Consequently, they should not to be ignored. Ablation with IRC and pharmacological treatment (imiquimod) were offered to patients with smaller anal condylomata in comparison to patients treated with electrosurgery excision. Although the effectiveness of treatments should not be compared, the recurrence rate during the follow-up in each group approximated 50% of the treated patients. Moreover, no predictive factors (such as HPV infection) were associated with the recurrence of anal condyloma. In front of the lack of efficacy clinical trials [6], our results could suggest that an ideal treatment for all types of anal CA is currently not available, and to personalize the treatment, that is, to select the treatment based on number, localization, area affected and surgeon's experience could be an acceptable option.

Anal CA often harbor high-grade intraepithelial neoplasia and squamous cell cancer, mainly in MSM [5,18]. Similarly, data on histopathological findings in anogenital condylomata showed a high rate (greater than 35% in MSM) of the coexistence the high-grade anal intraepithelial neoplasia within surgically excised warts [19]. We found that the coexistence of both pathologies, anal condyloma and anal canal cytological LSIL and HSIL (obtained prior to be treating the CA) was 70%. This figure was 86% if ASCUS is considered. The co-existence of an apparently benign lesions and a pre-cancerous lesion is unsettling. No patient evolved to cancer during the 10 years of study period. An explanation to this finding could be that these patients are involved in a screening program for anal cancer (CARH·MEN cohort) and this screening program for detection and treatment of anal intraepithelial neoplasia has demonstrated to be effective (S. Videla, unpublished data).

Low-risk HPV genotypes 6 and 11 are associated with the development of anogenital condylomata [20–22]. These genotypes were detected in practically in all patients. However, given that the long median time of recurrence (over 3 years), recurrence of anal CA may be due to a new infection, recurrence at the same site, or development of a metachronous lesion. In HIV-1-infected patients it is common to have a multiple infections with several HPV types including high-risk types that is independent of immune status [23,24]. In our study, a 70% of patients were infected by HPV-16 or HPV-18. If, besides, it is taken into account the rest of high-oncogenic risk HPV genotypes, all patients of our study were infected for high-risk oncogenic genotypes. In consequence, HIV-1-infected men with CA are a population with a high risk to develop anal squamous cancer. Therefore, a special attention in our clinical practice has to be taken into account with this population, HIV-1-infected men and diagnosis of anal condyloma.

Our study is subject to several limitations. Its observational retrospective design (e.g. the exact number of anal CAs per patient was not systematically gathered in the medical file, and therefore, this information is not provided) and the population analyzed (from a single geographical site and HIV-1-infected patients without a medical history of anal CA) could lead us to underestimate or overestimate the generalizability of the results beyond the population and conditions studied. As above-mentioned, the treatments were offered depending on the characteristics of the lesion/s, which could lead us to underestimate or overestimate the generalizability of the results beyond our clinical guidance protocol (treatment used depend on the characteristics of condyloma). In the case of imiquimod treatment, the compliance and its correct application cannot be guaranteed. The sample size could also lead us to underestimate or overestimate the generalizability of the results. HRA was only performed in patients with an abnormal anal cytology result; hence CA in the anal canal could be underdiagnosed. Likewise, we cannot know if the CA recurrence was related to new infection.

In summary, our results indicate that HIV-1-infected men, along their life, have a high rate of anal CA recurrence after physically ablative (electrosurgery, infrared) and pharmacological treatment (imiquimod). Randomized clinical trials are needed to best understand which the best treatment is. Furthermore, the high rate of the co-existence of CA and dysplasia at anal canal and the high percentage of high-risk HPV reinforce that, in clinical practice, the goal to manage the anal condylomata is to prevent the recurrence and to carry on with careful clinical follow-up of HIV-1-infected men. This population of HIV-1-infected men with CA are at risk of developing an anal cancer related to high-risk HPV genotypes.

Acknowledgments

Dra. Sandra Vela is a PhD candidate at the Universitat Autònoma de Barcelona, Spain.

Author Contributions

Conceptualization: Sebastian Videla, Guillem Sirera, Francesc García-Cuyás.

Data curation: Arelly Ornelas, Marta Piñol.

Formal analysis: Sebastian Videla, Arelly Ornelas.

Funding acquisition: Bonaventura Clotet.

Methodology: Sebastian Videla.

Project administration: Sandra Vela, Guillem Sirera.

Supervision: Sebastian Videla, Boris Revollo.

Validation: Francesc García-Cuyás.

Writing – review & editing: Sandra Vela, Sebastian Videla, Arelly Ornelas, Boris Revollo, Bonaventura Clotet, Guillem Sirera, Marta Piñol, Francesc García-Cuyás.

References

1. Darwich L, Videla S, Cañadas MP, Piñol M, García-Cuyàs F, Vela S, et al. Distribution of human papillomavirus genotypes in anal cytological and histological specimens from HIV-infected men who have sex with men and men who have sex with women. Dis Colon Rectum. 2013; 56:1043–1052. https://doi.org/10.1097/DCR.0b013e31829c654f PMID: 23929013
2. Poynten IM, Waterboer T, Jin F, Templeton DJ, Prestage G, Donovan B, et al. Human papillomavirus types 6 and 11 seropositivity: risk factors and association with ano-genital warts among homosexual men. J Infect. 2013; 66:503–511. https://doi.org/10.1016/j.jinf.2013.03.005 PMID: 23528181
3. Patel H, Wagner M, Singhal P, Kothari S. Systematic review of the incidence and prevalence of genital warts. BMC Infect Dis. 2013; 13:39. https://doi.org/10.1186/1471-2334-13-39 PMID: 23347441
4. Park IU, Introcaso C, Dunne EF. Human Papillomavirus and Genital Warts: A Review of the Evidence for the 2015 Centers for Disease Control and Prevention Sexually Transmitted Diseases Treatment Guidelines. Clin Infect Dis. 2015; 61 Suppl 8:S849–55.
5. Schlecht HP, Fugelso DK, Murphy RK, Wagner KT, Doweiko JP, Proper J, et al. Frequency of occult high-grade squamous intraepithelial neoplasia and invasive cancer within anal condylomata in men who have sex with men. Clin Infect Dis. 2010; 51:107–10. https://doi.org/10.1086/653426 PMID: 20482370
6. Werner RN, Westfechtel L, Dressler C, Nast A. Anogenital warts and other HPV-associated anogenital lesions in the HIV-positive patient: a systematic review and meta-analysis of the efficacy and safety of interventions assessed in controlled clinical trials. Sex Transm Infect. 2017; Jun 21. pii: sextrans-2016-053035.
7. Leszczyszyn J, Łebski I, Łysenko L, Hirnle L, Gerber H. Anal warts (condylomata acuminata)—current issues and treatment modalities. Adv Clin Exp Med. 2014; v23:307–311.
8. Mistrangelo M. Surgical treatment of anal condylomata acuminata. Dis Colon Rectum. 2009; 52:1803; author reply 1803. https://doi.org/10.1007/DCR.0b013e3181ab3185 PMID: 19966620
9. Manzione Tda S, Nadal SR, Calore EE, Nadal LR, Manzione CR. Local control of human papillomavirus infection after anal condylomata acuminata eradication. Rev Col Bras Cir. 2014; 41:87–91. PMID: 24918720
10. Massad LS, Xie X, Darragh T, Minkoff H, Levine AM, Watts DH, et al. Genital warts and vulvar intraepithelial neoplasia: natural history and effects of treatment and human immunodeficiency virus infection. Obstet Gynecol. 2011; 118:831–9. https://doi.org/10.1097/AOG.0b013e31821a0f4d PMID: 21934446
11. Wiley D, Masongsong E. Human papillomavirus: the burden of infection. Obstet Gynecol Surv. 2006; 61(6 Suppl 1):S3–14.
12. Silvera RJ, Smith CK, Swedish KA, Goldstone SE. Anal condyloma treatment and recurrence in HIV-negative men who have sex with men. Dis Colon Rectum. 2014; 57:752–761. https://doi.org/10.1097/DCR.0000000000000080 PMID: 24807601
13. Villa LL, Costa RL, Petta CA, Andrade RP, Ault KA, Giuliano AR, et al. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicenter phase II efficacy trial. Lancet Oncol. 2005; 6:271–278. https://doi.org/10.1016/S1470-2045(05)70101-7 PMID: 15863374

14. Darwich L, Cañadas MP, Videla S, Coll J, Piñol M, Cobarsi P, et al. Condylomata, cytological abnormalities and human papillomavirus infection in the anal canal in HIV-infected men. HIV Med. 2012; 3:549–557.

15. Videla S, Darwich L, Cañadas MP, Coll J, Piñol M, García-Cuyás F, et al. Natural history of human papillomavirus infections involving anal, penile, and oral sites among HIV-positive men. Sex Transm Dis. 2013; 40:3–10. https://doi.org/10.1097/OLQ.0b013e31827e87bd PMID: 23250297

16. Videla S, Darwich L, Cañadas MP, Paredes R, Tarrats A, Castella E, et al. Epidemiological data of different human papillomavirus genotypes in cervical specimens of HIV-1-infected women without history of cervical pathology. J Acquir Immune Defic Syndr. 2009; 50:168–175. https://doi.org/10.1097/QAI.0b013e3181938e63 PMID: 19131892

17. Cong X, Sun R, Zhang X, Wang Y, Wang L, Yu Y. Correlation of human papillomavirus types with clinical features of patients with condyloma acuminatum in China. Int J Dermatol. 2016; 55:775–780. https://doi.org/10.1111/ijd.12964 PMID: 26475783

18. Anderson CA, Boller AM, Richardson CJ, Balcos EG, Zera RT. Anal condyloma: A comparison between HIV positive and negative patients. Am Surg. 2004; 70: 1014–1018. PMID: 15586518

19. McCloskey JC, Metcalf C, French MA, Flexman JP, Burke V, Beilin LJ. (), The frequency of high-grade intraepithelial neoplasia in anal/perianal warts is higher than previously recognized. Int J STD AIDS 2007; 18:538–542. https://doi.org/10.1258/095646207781439694 PMID: 17686215

20. Dupin N. Genital warts. Clin Dermatol. 2004; 22(6):481–486. https://doi.org/10.1016/j.clindermatol.2004.07.003 PMID: 15596319

21. Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. Vaccine. 2006; 24: S1–15. https://doi.org/10.1016/j.vaccine.2005.09.054 PMID: 16406226

22. Aubin F, Prétet JL, Jacquard AC, Saunier M, Carcopino X, Jaroud F, et al. Human papillomavirus genotype distribution in external acuminata condylomata: a Large French National Study (EDiTH IV). Clin Infect Dis. 2008: 47: 610–615. https://doi.org/10.1086/590560 PMID: 18637758

23. Lee JT, Goldberg SM, Madoff RD, Tawadros PS. Immune status does not predict high-risk HPV in anal condyloma. J Surg Res. 2016; 201:166–169. https://doi.org/10.1016/j.jss.2015.10.027 PMID: 26850198

24. Sung JH, Ahn EJ, Oh HK, Park SH. Association of immune status with recurrent anal condylomata in human immunodeficiency virus-positive patients. J Korean Soc Coloproctol. 2012; 28:294–298. https://doi.org/10.3393/jksc.2012.28.6.294 PMID: 23346507

Publicaciones de soporte relacionadas con la tesis doctoral

A continuación se adjuntan 2 estudios de investigación clínica relacionados con los condiloma/s en hombres infectados por el VIH, publicados durante el período de realización de la tesis doctoral. Estas publicaciones de soporte aportan pruebas sobre los objetivos de esta tesis doctoral.

Estudio 3: "Distribution of Human Papillomavirus Genotypes in Anal Cytological and Histological Specimens from HIV Infected Men Who Have Sex with Men and Men Who Have Sex with Women".

Dis Colon Rectum. 2013 Sep;56(9):1043-1052.

"DISTRIBUCIÓN DE LOS DIFERENTES GENOTIPOS DEL VPH EN LAS MUESTRAS CITOLÓGICOS Y HISTOLÓGICAS DE VARONES VIH POSITIVOS QUE TIENEN SEXO CON HOMBRES Y VARONES VIH POSITIVES QUE TIENEN SEXO CON MUJERES"

ANTECEDENTES. El cáncer escamoso del canal anal es causado por el virus del papiloma humano (HPV). Estar infectado por el virus de inmunodeficiencia humana (VIH) es un factor de riesgo adicional para el cáncer escamoso del canal anal. Los protocolos preventivos (protocolos de cribado) para hombres infectados por el VIH deberían incluir la detección de los genotipos de VPH oncogénicos de alto riesgo (HR) en el canal anal. Es importante destacar que la mayoría de los estudios disponibles se han centrado sólo en hombres que tienen sexo con hombres (HSH).

OBJETIVO. Estimar la prevalencia de infección por el VPH y describir su distribución genotípica en muestras de citología e histología del canal anal en hombres infectados por el VIH que tienen sexo con hombres (HSH) y hombres que tienen sexo con mujeres (HSM).

DISEÑO. Estudio transversal basado en la cohorte CARH·MEN: cohorte prospectiva de hombres infectados por el VIH que acuden para su control ambulatorio de su infección por el VIH en el Hospital Germans Trias i Pujol (España), controlándose anualmente la infección por el VPH en el canal anal, en el pene y en la boca.

PACIENTES. Se incluyeron 483 hombres infectados por el VIH (341 HSH, 142 HSM) sin antecedentes de historia de condilomas en el canal anal. Se realizó una detección de los genotipos del HPV (multiplex-PCR), una citología (prueba de Papanicolaou) e histología (biopsia dirigida) del canal anal.

RESULTADOS. Se detectaron anomalías citológicas en el 40% de MSM (129/321; IC95%: 35-46) y 20% de MSW (26/131; IC95%: 13-28) (OR = 2.7; IC95%: 1.7-4.4). Las lesiones escamosas intraepiteliales de alto grado (LEI-A) fueron positivas para VPH de alto riesgo en ambos grupos. La anoscopia de alta resolución fue realizada en 146 pacientes (120 MSM, 26 MSW)

con un diagnóstico citológico anormal. Se visualizaron lesiones en 80 MSM (67%) y 14 MSW (54%) (OR = 1.7 [IC95%: 0.7-4,0]). El diagnóstico histológico fue de neoplasia intraepitelial anal (NIA) grado 1 (NIA-1) en 51 HSH (64%) y 6 HSM (43%), NIA-2 en 9 HSH (11%) y 3 HSM (21%), NIA-3 en 7 HSH (9%) y 1 HSM (7%), y normal en 13 HSHM (16%) y 4 HSM (29%). El VPH-16 fue el genotipo de alto riesgo oncogénico más prevalente.

LIMITACIONES. Su diseño transversal.

CONCLUSIONES. Debe ofrecerse el cribado para la prevención del cáncer del canal anal a todos los hombres infectados por el VIH, independientemente de su orientación sexual.

ORIGINAL CONTRIBUTION

Distribution of Human Papillomavirus Genotypes in Anal Cytological and Histological Specimens from HIV-Infected Men Who Have Sex with Men and Men Who Have Sex with Women

Laila Darwich, D.V.M., Ph.D.[1,2] • Sebastian Videla, M.D., Ph.D.[1] • Mari-Paz Cañadas, Ph.D.[1,3] Marta Piñol, M.D., Ph.D.[4] • Francesc García-Cuyàs, M.D., Ph.D.[4] • Sandra Vela, M.D.[4] Rafael A. Molina-López, D.V.M., Ph.D.[1,2] • Josep Coll, M.D.[1,5] • Guillem Sirera, M.D., Ph.D.[1,6] Bonaventura Clotet, M.D., Ph.D.,[1,5,6] on behalf of the Can Ruti HIV-HPV Team

1 Lluita Contra La SIDA Foundation, Badalona, Spain
2 Departament de Sanitat i Anatomia Animal, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain
3 Labco.General Lab, Barcelona, Spain
4 Department of Surgery, University Hospital Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona, Catalonia, Spain
5 Irsicaixa Foundation, Badalona, Spain
6 HIV Clinical Unit, Department of Medicine, University Hospital Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona, Catalonia, Spain

BACKGROUND: Anal cancer is caused by human papillomavirus (HPV). Moreover, human immunodeficiency virus (HIV) is an additional risk factor for anal cancer. Therefore, when designing preventive protocols for HIV-infected men, it is important to detect high-risk (HR) oncogenic HPV genotypes present in their anal canals. However, most studies have focused only on men who have sex with men (MSM).

OBJECTIVE: To estimate the prevalence of HPV and describe its genotype distribution using anal cytology and histology specimens from HIV-infected populations of MSM and men who have sex with women (MSW).

DESIGN: Crosssectional study of the CARH·MEN cohort.

SETTING: Single-center prospective cohort of HIV-infected men attending the Outpatient HIV Clinic of Hospital Germans Trias i Pujol (Spain), where they undergo annual screening for HPV infection of the anus, penis and mouth.

PATIENTS: Four hundred eighty-three HIV-infected men (341 MSM, 142 MSW) with no current or previous history of anal condylomata.

MAIN OUTCOME MEASURES: HPV genotypes detected (multiplex-PCR), cytology results (Papanicolaou test) and histology results (biopsy-based).

RESULTS: Cytological abnormalities were detected in 40% of MSM (129/321; 95%CI, 35–46) and 20% of MSW (26/131; 95%CI, 13–28) (OR=2.7; 95%CI, 1.7–4.4). All high-grade squamous intraepithelial lesions (HSIL) were positive for HR-HPV in both groups. High-resolution anoscopy was performed in 146 patients (120 MSM, 26 MSW) with abnormal cytological diagnoses. Lesions were visualized in 80 MSM (67%) and 14 MSW (54%) (OR=1.7 [95%CI, 0.7–4.0]). Histological diagnosis was anal intraepithelial neoplasia (AIN)-1 in 51 MSM (64%) and 6 MSW (43%), AIN-2 in 9 MSM (11%) and 3 MSW (21%), AIN-3 in 7 MSM (9%) and 1 MSW (7%), and normal in 13 MSM (16%) and 4 MSW (29%). HPV16 was the most prevalent HR genotype.

Funding/Support: This work was supported by grants from Red de Investigación en SIDA (RIS), the ART AIDS Foundation, Gilead Sciences, Obra Social Caixa Sabadell and Gala contra la SIDA-Barcelona 2011.

Financial Disclosure: S.V. received honoraria from Laboratorios Dr. Esteve for work not related to HPV/HIV. B.C. received honoraria for speaking and participating on advisory boards for Abbott, Bristol-Myers Squibb, Boehringer-Ingelheim, Gilead Sciences, GlaxoSmithKline, Pfizer, Merck, Janssen-Tibotec and Siemens. The remaining authors report no conflicts of interest.

Correspondence: Laila Darwich, D.V.M., Ph.D., Infectious Diseases and Epidemiology, DSAA, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain. E-mail: laila.darwich@uab.cat

Dis Colon Rectum 2013; 56: 1043–1052
DOI: 10.1097/DCR.0b013e31829c654f

LIMITATIONS: Study limitations include its crosssectional design.

CONCLUSIONS: Anal cancer screening should be offered to all HIV-infected men, regardless of their sexual orientation.

KEY WORDS: HPV genotypes; Anal lesions; HIV men.

Anal cancer is caused by human papillomavirus (HPV),[1,2] which is detected in approximately 90% of squamous cell anal cancers and its high-grade precursor lesions (high-grade squamous intraepithelial lesions [HSIL] and high-grade anal intraepithelial neoplasia [HGAIN]).[3–5] Both anal HPV infection and anal cancer, as well as cancer precursor lesions, are more prevalent among men who have sex with men (MSM) than in the general population, regardless of their human immunodeficiency virus (HIV) status.[6,7] Receptive anal intercourse (RAI) is thought to be a risk factor for HPV in MSM,[8,9] although a high prevalence of anal HPV infection and anal cancer precursors has also been reported among HIV-infected individuals who do not have RAI.[10] In fact, HIV infection is an additional risk factor for developing anal cancer, and the incidence of anal cancer among HIV-infected men has increased markedly, despite introduction of highly active antiretroviral therapy (HAART).[11–15]

The longer life expectancy of HIV-positive individuals treated with HAART means that established high-risk (HR) HPV infections have time to progress to anal cancer.[16] In women, cervical cytology is currently the most effective means of screening for precancerous lesions,[17] and anal cytology is similarly used as a screening tool for precancerous anal lesions.[18,19] Earlier studies on the association between anal dysplasia and HPV in HIV-infected men focused primarily on MSM,[20–24] and it is now thought that anal cytology is a cost-effective approach to preventing anal cancer in HIV-infected MSM.[25] On the other hand, comparatively little is known about HIV-infected men who have sex with women (MSW).[10] Furthermore, because the risk of acquiring anal HPV infection and anal precancerous lesions is also high in several male populations with no history of RAI, including renal allograft recipients,[26] injected drug users[10] and nonwhite MSW,[22] research into populations with no history of RAI is also necessary. Therefore, the objectives of this crosssectional study were to use anal cytology and histology results to estimate the prevalence and distribution of HPV type-specific infections in a cohort of HIV-infected men comprised of both MSM and MSW.

PATIENTS AND METHODS

Patients and Study Design

The local independent ethics committee approved this study, which entailed a crosssectional analysis of data collected during the baseline visit of patients in the Can Ruti HIV-positive Men (CARH·MEN) cohort. The CARH·MEN is a single-center prospective cohort of Caucasian, HIV-infected men attending the Outpatient HIV Clinic of Hospital Germans Trias i Pujol.[27] These patients are screened annually for HPV infection of the anus, penis and mouth.

Clinical Examination and Anal Sample Collection

A visual inspection and digital rectal examination were performed during the baseline visit. To test for HPV infection, cytology samples were collected by introducing a cytobrush (Eurogine SL, Sant Boi del Llobregat, Spain) into the anal canal to a depth of 3 cm and gently rotating it for 30 to 45 seconds. The cytobrush was then placed in 20 mL of ThinPrep PreservCyt solution (Cytyc Iberia SL, Barcelona, Spain) and shaken for 30 seconds, after which the solution was stored at –20°C until analysis (multiplex polymerase chain reaction [PCR]). These samples were also used for cytology analysis (Papanicolaou test).

If an anal cytology result was positive – ie, it contained atypical squamous cells of uncertain significance (ASCUS) or low- or high-grade squamous intraepithelial lesions (LSIL or HSIL, respectively) – the patient was contacted and informed, and high-resolution anoscopy (HRA) was subsequently performed. If the HRA revealed a lesion (after topical application of 3% acetic acid to the anal canal for 2 minutes), a biopsy was performed. If anal warts were diagnosed during the clinical examination (digital rectal examination or anoscopy), the patient was referred to a surgeon for treatment.

Cytological and Histological Diagnoses

Cytological diagnoses were made on the basis of Papanicolaou tests. Cytology samples were independently examined by expert cytopathologists, who classified them according to the Bethesda System as ASCUS, LSIL or HSIL. Biopsy-based histology samples were independently examined by 2 expert pathologists, who classified histological changes according to the grade of the anal intraepithelial neoplasia (AIN) as AIN-1, AIN-2 or AIN-3.

HPV Testing and Genotyping

DNA was extracted from cytology samples using a QIAamp Viral DNA kit (QIAGEN, Hilden, Germany). HPV was detected and typed using an IVD-CE Multiplex Fluorescent-PCR Kit (F-HPV typing, Molgentix SL, Barcelona, Spain), which can detect 13 HR genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and 2 low-risk (LR) genotypes (6, 11). A human short tandem repeat sequence included in the multiplex PCR kit was amplified as an internal control to check for DNA integrity and confirm the absence of PCR inhibitors. In addition, each PCR run included HPV-positive and HPV-negative controls,

TABLE 1. Baseline characteristics of the CARH·MEN cohort

Characteristics	*Total Population[a] N = 483*	*Men Who Have Sex With Men n = 341 (71%)*	*Men Who Have Sex With Women n = 142 (29%)*	*p*
Age[b]	42 (37–48)	41 (35–47)	44 (39–48)	0.001
Time of known HIV (years)[b]	9 (2–15)	7 (1–13)	14 (9–18)	<0.001
AIDS diagnosis (Yes), n (%)	76 (16)	47 (14)	29 (20)	0.068
HIV viral load at baseline[b]	50 (50–462)	50 (50–1750)	50 (50–50)	0.385
Undetectable: <50 HIV RNA copies/mL of plasma, n (%)	330 (69)	224 (66)	106 (77)	0.012
CD4 cell count at baseline[b]	505 (357–681)	516 (390–689)	464 (241–665)	0.002
CD4 nadir[b]	252 (116–353)	284 (182–376)	125 (54–275)	<0.001
<200 cells/uL, n (%)	182 (38)	93 (27)	89 (63)	
HAART before inclusion (Yes), n (%)	407 (84)	272 (80)	135 (95)	<0.001
History of STI (Yes), n (%)	133 (28)	102 (30)	31 (22)	0.070
Intravenous drug user[c], n (%)				<0.001
Never	371 (77)	305 (97)	66 (48)	
Current	7 (1)	6 (2)	1 (1)	
Past	74 (15)	5 (1)	69 (51)	
Number of lifetime sexual partners[c], n (%)				<0.001
1	8 (2)	0	8 (5)	
2–9	67 (14)	12 (18)	55 (82)	
10–25	47 (10)	25 (53)	22 (47)	
>25	274 (57)	243 (89)	31 (11)	
History of RAI (Yes), n (%)	242 (58)	242 (71)	0	<0.001

HAART = highly active antiretroviral therapy; STI = sexually transmitted infections; RAI = receptive anal intercourse.
[a]Patients from the CARH·MEN cohort with no history of anal condylomata
[b]Median (Percentiles 25 and 75)
[c]Statistics from the data available

and particular care was taken to prevent carry-over contamination by separating pre and post-PCR areas in the laboratory.

Statistical Analysis

The prevalence of baseline abnormal cytology and the histology findings were assessed for each group. In addition, the prevalence of HPV type-specific infections was assessed based on the grades of the anal cytological and histological lesions. For qualitative variables, differences between the groups were evaluated using the χ^2 test or the Fisher exact probability test. Cohen's κ was used to assess agreement between the cytology and histology findings, and between detectable HPV infection and the histology findings. Values of $p \leq 0.05$ were considered significant. Data were analyzed using SPSS version 15.0 (SPSS, Inc., Chicago, Illinois, USA).

RESULTS

The CARH·MEN cohort consists of 733 patients (538 MSM and 195 MSW). Detailed data on the baseline characteristics of this cohort are available elsewhere.[27] Two hundred fifty patients were excluded because of a current or previous history of anal condylomata.[28] The baseline characteristics of the 483 patients included in the present study (341 MSM and 142 MSW) are shown in Table 1. It is noteworthy that MSW had a poorer disease status than MSM; that is, they had longer times of known HIV infection, lower nadir CD4 counts and lower baseline CD4 counts. Figure 1 shows the pathway taken by CARH·MEN cohort toward a histological diagnosis.

Cytological Diagnosis

Cytology results could not be obtained from 31 of the 483 samples (6%): 20 of 341 samples from the MSM (6%) and 11 of 142 from the MSW (8%) ($p = 0.62$). Figure 2 shows the prevalence of HPV and the genotype distributions among HIV-infected men grouped according to their cytological diagnosis. An abnormal cytological result was detected in 40% of MSM (129/321, 95%CI, 35–46) and 20% of MSW (26/131, 95%CI, 13–28) (Table 2). The probability of exhibiting a lesion of any type was higher in MSM than MWM (OR = 2.7; 95%CI, 1.7–4.4), mainly due to the higher prevalences of LSIL and HSIL among MSM. The prevalence of ASCUS was similar in MSW and MSM.

As expected, most specimens with cytological abnormalities (92% from MSM; 65% from MSW) were positive for HPV (Table 2). Nearly all of the genotypes studied were detected in all grades of cytological lesions in MSM, but not in MSW (Fig. 2). All HSIL were positive for HR-HPV types, irrespective of sexual orientation. In both MSM and MSW, HPV16 was the most prevalent HR-HPV type associated with HSIL and was always seen together with

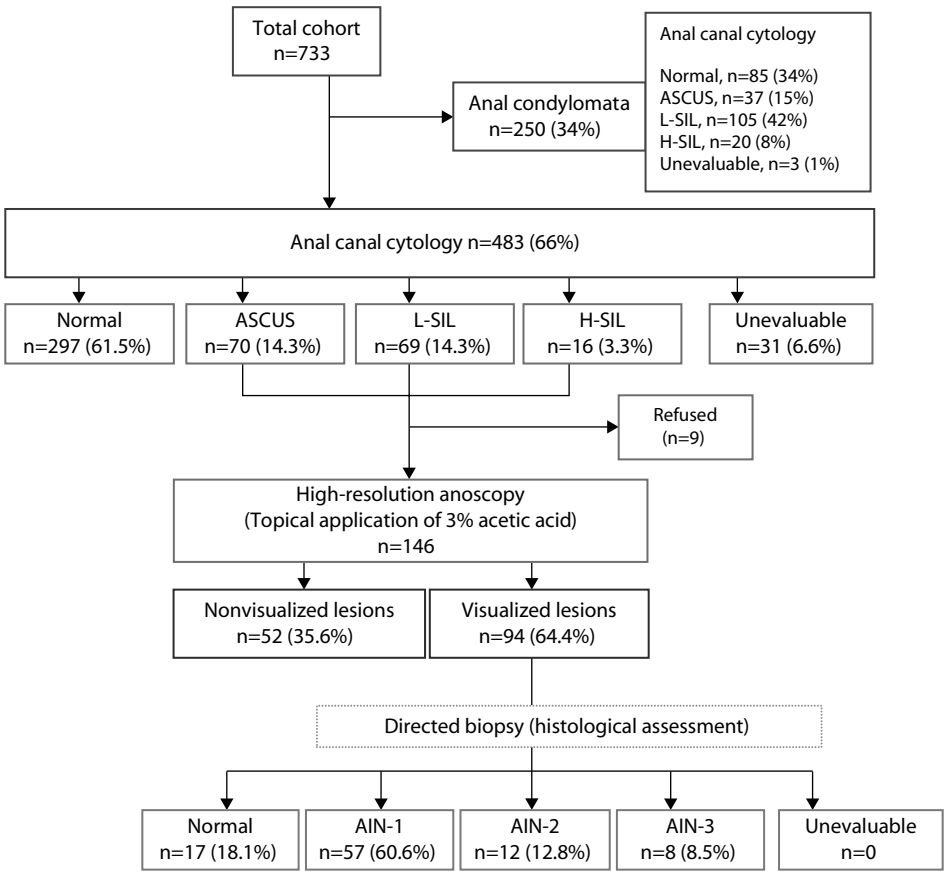


FIGURE 1. Flow chart showing the pathway taken by patients in the CARH·MEN cohort toward a biopsy-proven histological diagnosis. ASCUS = atypical squamous cells of unknown significance; LSIL = low-grade intraepithelial lesions; HSIL = high-grade intraepithelial lesions; AIN = anal intraepithelial neoplasia.

LR-HPV types. Detected somewhat less frequently were HPV-33 in MSM and HPV18, 58 and 59 in MSW (Fig. 2).

Samples positive for abnormal cytology but negative for HPV were obtained more frequently from MSW (35%) than MSM (8%) ($p = 0.22$). In samples diagnosed as being cytologically normal but positive for HPV, all 15 HPV genotypes were detected in MSM, whereas MSW lacked HPV11, 31 and 68.

Histological Diagnosis

Nine of the 155 patients (6%) with an abnormal cytological diagnosis refused to undergo HRA. Therefore, HRA was performed on 146 patients: 120 MSM (82%) and 26 MSW (18%). Lesions were visualized in 94 patients (64%: 80 MSM [67%] and 14 MSW [54%]), with no statistically significant differences between the groups (OR = 1.7; 95%CI, 0.7–4.0).

Table 3 shows the prevalence of AIN stratified according to sexual orientation. All patients with AIN-2 and AIN-3 were infected with HR-HPV genotypes, principally HPV16 (Fig. 3). Interestingly, a high proportion of MSW with AIN-2 were infected with HPV16 alone, whereas HPV33, 58 and 59 were also prevalent in the MSM group.

The selection procedure used in this study is not ideal for assessing the agreement between cytological and histological diagnoses or between HPV infection and histological diagnosis. This is because only patients with abnormal cytological findings were candidates for HRA, and then biopsy if a lesion was found (Fig. 1). Consequently, no conclusions could be drawn; nevertheless, a descriptive and exploratory analysis was performed.

Table 4 summarizes the results of our descriptive analysis of the cytological and histological diagnoses of patients with abnormal cytological findings and a lesion visualized with HRA. We found that there was slight agreement between the cytological and histological diagnoses when the severities of the dysplasias were matched (κ=0.20 [95%CI, –0.03–0.47] when ASCUS was included in the analysis; κ=0.18 [95%CI, −0.11–0.47] when ASCUS was not included in the analysis). However, when the cytological diagnosis was evaluated as only being either normal or abnormal (ASCUS, LSIL or HSIL), and the histological diagnosis was similarly evaluated (abnormal: AIN-1, AIN-2 or AIN-3), in 77 of 94 cases (82%), the sample was diagnosed as being both cytologically and histologically abnormal. Table 4 also shows that there is little or no relationship

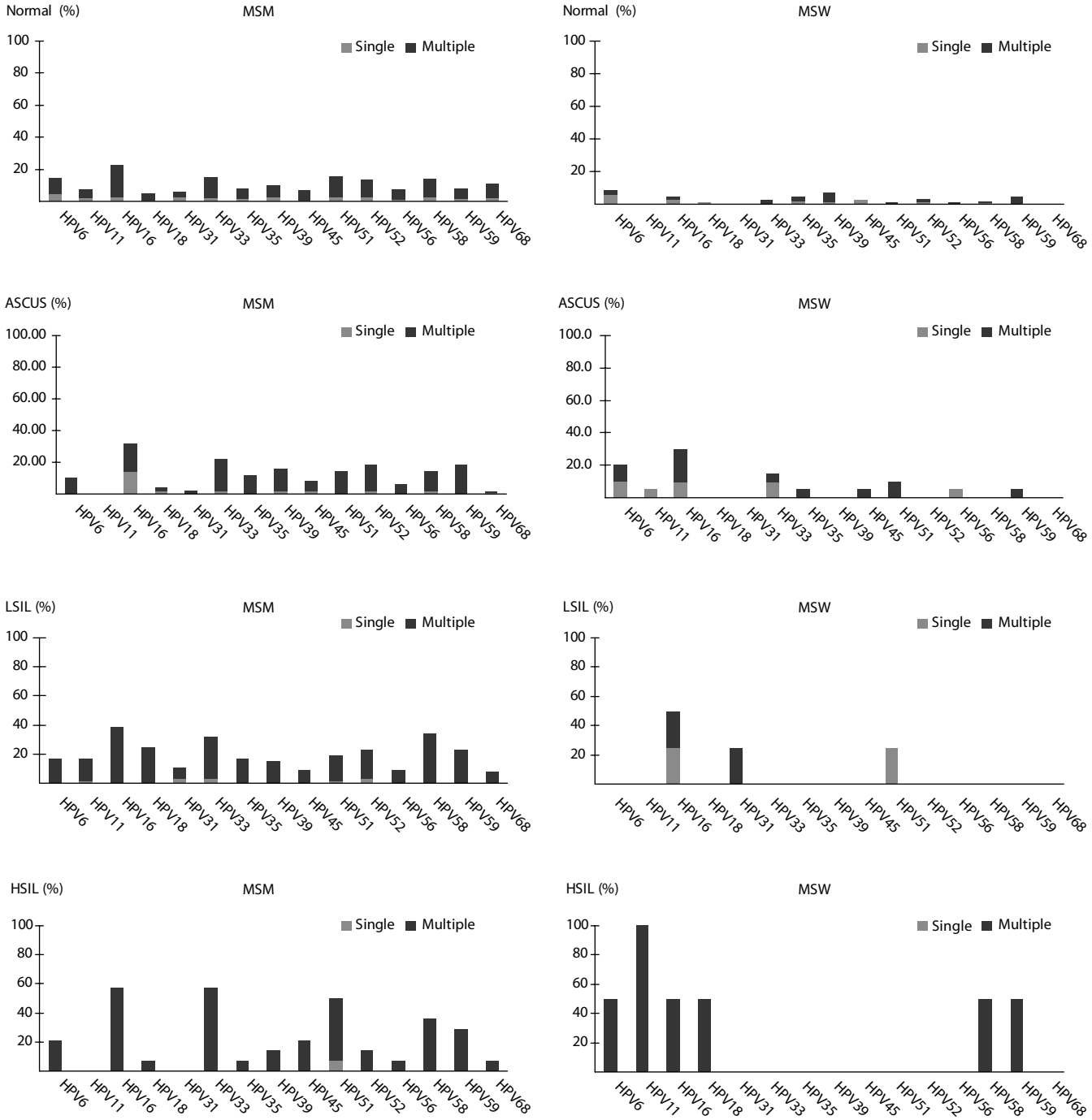


FIGURE 2. Distribution and prevalence of HPV genotypes in HIV-infected men grouped according to cytological diagnosis. ASCUS = atypical squamous cells of unknown significance; LSIL = low-grade intraepithelial lesions; HSIL = high-grade intraepithelial lesions; MSM = men who have sex with men; MSW = men who have sex with women.

between the presence of a detectable HPV infection and the histological diagnosis in patients with abnormal cytological findings (κ=0.02 [95%CI, –0.18–0.22]).

DISCUSSION

In the present crosssectional study, we examined the distribution and prevalence of various HPV genotypes detected in cytology and biopsy samples from the anal canals of HIV-infected MSM and MSW. Using HRA, we found high prevalences of anal lesions in both MSM (24%) and MSW (10%): among patients without anal condylomata, approximately 1 in every 4 MSM and 1 in every 10 MSW undergoing routine HIV screening presented with an anal lesion. This result is particularly important for MSW. Although none of the men in the MSW group had a history

TABLE 2. Proportion of anal cytological abnormalities stratified based on sexual orientation and HPV infection in HIV-infected men

Anal Cytology Status	Men Who Have Sex with Men (n = 321)[a] No. (%)			Men Who Have Sex with Women (n = 131)[a] No. (%)			p^b		
	All samples	HPV-positive	HPV-negative	All samples	HPV-positive	HPV-negative	All samples	HPV-positive	HPV-negative
Normal	192 (60)	147 (76.6)	45 (23.4)	105 (80)	30 (28.6)	75 (71.4)	<0.001	0.176	0.219
Abnormal	129 (40)	119 (92.2)	10 (7.8)	26 (20)	17 (65.4)	9 (34.6)			
ASCUS	50 (15.6)	42 (84)	8 (16)	20 (15.3)	13 (65)	7 (35)	<0.001	0.011	0.453
LSIL	65 (20.2)	63 (96.9)	2 (3.1)	4 (3)	2 (50)	2 (50)			
HSIL	14 (4.4)	14 (100)	0	2 (1.5)	2 (100)	0			

ASCUS = atypical squamous cells of undetermined significance; LSIL = low-grade squamous intraepithelial lesions; HSIL = high-grade squamous intraepithelial lesions.
[a]Total number excluding unevaluable cytological results (n = 20 in MSM and n = 11 in MSW).
[b]Fisher exact probability test.

of anal pathology or RAI, their disease status was much poorer than that of MSM (longer time of known HIV infection, lower nadir CD4 counts and lower baseline CD4 counts), reflecting their greater immunosuppression. Furthermore, MSW were a sexually active group, in that 95% were not monogamous.

HPV DNA can be detected in virtually all patients exhibiting dysplasia of the anal canal,[29] and a high incidence of anal squamous cell carcinoma has been observed in HIV-infected MSM.[15,30] Among that group, the prevalences of cytological and histological abnormalities range from 47% to 81%, while the incidence of anal HPV infection ranges from 61% to 95%.[10,22–24] Although the MSM studied had no current or previous history of anal condylomata, the overall prevalences of AIN (20%), cytological abnormalities (38%) and HPV infection of the anal canal (78%) in this group are consistent with earlier reports and similar to other cohorts of HIV-infected patients.[31,32] On the other hand, our histology results did not show HPV infection in 8 patients with AIN-1 (8.5%: 5 MSM and 3 MSW). One possible explanation for this finding is the involvement of one or more HPV types that cause low-grade lesions but are not detected by multiplex PCR. That said, the PCR technique used is highly sensitive to the HR-HPV[33] and LR-HPV types most frequently related to condylomatous lesions[28,34] and covered by the vaccine. Consistent with the histology results, the cytology findings showed that some low-grade lesions, mainly ASCUS, were HPV-negative (8% in MSM and 35% in MSW). This may reflect the fact that the line between a normal diagnosis and ASCUS is fine and subjective, and that there can sometimes be unconscious overestimation of the cytological lesion.

Although both the MSM and MSW in our study were considered sexually active, the greater diversity of genotypes found in MSM could be explained by the larger number of sexual partners in this group. Although HPV16 was the most frequently identified genotype in both groups, other genotypes, including HPV33, HPV58 and HPV59, were also overrepresented in AIN lesions. This finding is consistent with the hypothesis that, under the same immune conditions, HPV16 is innately better than other HPV types at avoiding immune surveillance. However, in an immunosuppressed population, such as the CARH·MEN cohort, all HPV types may have a similar opportunity to progress to anal cancer. Moreover, our findings also show a high prevalence of anal HPV infection, AIN and anal SIL in men with no history of RAI. Thus RAI is not a necessary factor for anal lesions related to HPV infection.

It is worth noting that there is not yet an optimal screening protocol for anal dysplasia. At present, diagnosis generally hinges on HRA with biopsy confirmation of the findings obtained during the HRA.[35–38] The present study was not designed to investigate the agreement between the cytological and histological diagnoses (after HRA and biopsy confirmation), or between HPV infection and histological diagnosis. Although HPV infection is the major etiological factor for anal squamous cell carcinoma, HPV positivity cannot be considered an ideal

TABLE 3. Proportion of anal histological abnormalities stratified based on sexual orientation and HPV infection in HIV-infected men

Anal Histology Status	Men Who Have Sex with Men (n = 80) No. (%)			Men Who Have Sex with Women (n = 14) No. (%)			p^a		
	All samples	HPV-positive	HPV-negative	All samples	HPV-positive	HPV-negative	All samples	HPV-positive	HPV-negative
Normal	13 (16.2)	12 (92.3)	1 (7.7)	4 (28.6)	3 (75)	1 (25)	0.273	–	–
Abnormal	67 (83.8)	62 (92.5)	5 (7.5)	10 (71.4)	7 (70)	3 (30)			
AIN-1	51 (63.8)	46 (90.2)	5 (9.8)	6 (42.9)	3 (50)	3 (50)	0.414	–	–
AIN-2	9 (11.2)	9 (100)	0	3 (21.4)	3 (100)	0			
AIN-3	7 (8.8)	7 (100)	0	1 (7.1)	1 (100)	0			

AIN = anal intraepithelial neoplasia.
[a]Fisher exact probability test.

Diseases of the Colon & Rectum Volume 56: 9 (2013)

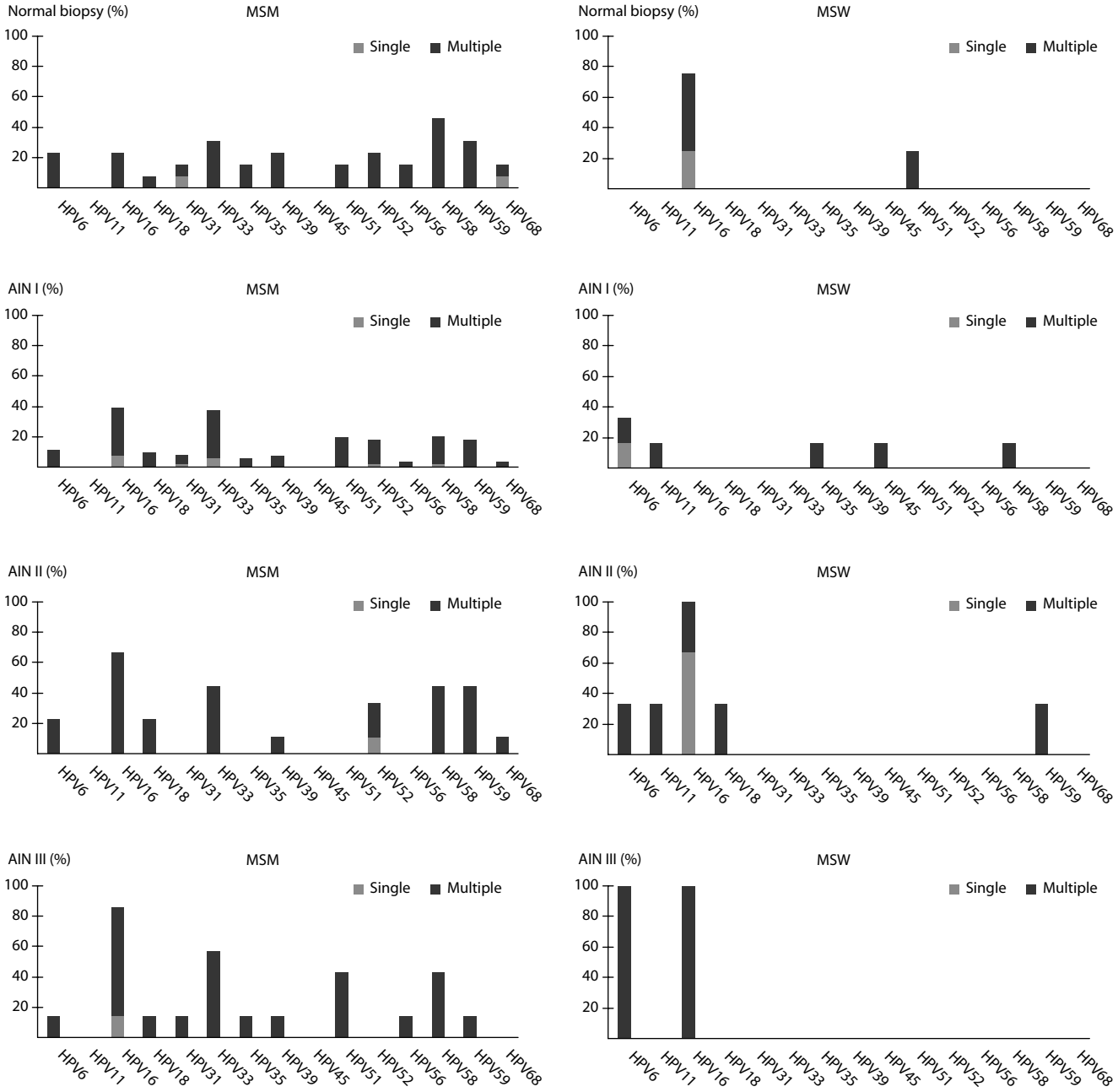


FIGURE 3. Distribution and prevalence of HPV genotypes in HIV-infected men grouped according to histological diagnosis. AIN = anal intraepithelial neoplasia; MSM = men who have sex with men; MSW = men who have sex with women.

biomarker of progression to cancer, given the high prevalence of HPV infection in HIV-infected men. Likewise, the weak agreement between the cytology and histology findings when the different grades of dysplasia were matched is also very concerning. Given the absence of a good biomarker, HRA findings with biopsy confirmation would seem the best alternative. Unfortunately, however, there are not enough trained and proficient providers of HRA to make this a reasonable recommendation. Nonetheless, anal cytology can provide useful information for patient management if one takes into consideration the slow progress from HR-HPV infection to cancer, but the cytology results should be considered as merely suggestive. When we compared samples diagnosed as either cytologically normal and abnormal (independently of the dysplasia grade) with similarly evaluated histological results, the agreement between the 2 diagnostic techniques is greater than 80%; that is, an abnormal cytology result has a high probability of being AIN. This means that in the absence of HRA with biopsy confirmation of the findings, yearly anal cytology screenings are extremely important, despite the poor agreement between

TABLE 4. Number and proportion of histological diagnoses[a] stratified based on cytological diagnosis[b] or presence of detectable HPV infection

			Histology No. (%)			
			NORMAL	*AIN-1*	*AIN-2*	*AIN-3*
Cytology	ASCUS	(n = 33)	7 (21)	21 (64)	2 (6)	3 (9)
	L-SIL	(n = 46)	9 (20)	28 (61)	7 (15)	2 (4)
	H-SIL	(n = 15)	1 (7)	8 (53)	3 (20)	3 (20)
	Undetectable	(n = 10)	2 (20)	8 (80)	0	0
HPV-infection	Detectable	(n = 84)	15 (18)	49 (58)	12 (14)	8 (10)
	Low-risk	(n = 22)	3 (13)	14 (64)	3 (14)	2 (9)
	High-risk	(n = 83)	15 (18)	48 (58)	12 (14)	8 (10)

ASCUS = atypical squamous cells of unknown significance; LSIL = low-grade intraepithelial lesions; HSIL = high-grade intraepithelial lesions; AIN = anal intraepithelial neoplasia.

[a]Based on high-resolution anoscopy findings with biopsy confirmation.

[b]Based on Papanicolaou tests.

the cytology and histology results when different grades of dysplasia were matched.

Notably, 79% of patients with ASCUS had an AIN lesion, and ASCUS appears to have a higher positive predictive value for high-grade disease in the anus among at-risk populations than in the cervix among the general population.[39] Moreover, the prevalence of HSIL was higher in HIV-infected men with condylomata.[28] It is therefore recommended that patients with any kind anal cytological abnormality (including ASCUS), or with anal condylomata, be referred for HRA.[23,28]

Our study is subject to several limitations. Its crosssectional design and the population analyzed (from a single geographical site and HIV-infected patients without condylomata) could lead us to underestimate or overestimate the generalizability of the results beyond the population and conditions studied. Likewise, the sample size could also lead us to underestimate or overestimate the generalizability of the results. HRA after topical application of 3% acetic acid is currently the best procedure for detection of lesions in the anal canal; however, because it is subject to interobserver bias (despite thorough training), primary and recurrent lesions could go undiagnosed. Moreover, because HRA was only performed in patients diagnosed with abnormal cytology, its applicability to patients with normal cytology could be limited. Similarly, because the study included only patients with no evidence of condylomata, its applicability to patients with condylomata could be limited.

The association between HPV infection and anal dysplasia, as well as the elevated risk of anal cancer among HIV-positive men,[40,41] highlights the need for close follow-up of all HIV/HPV-co-infected patients, regardless of their sexual orientation, so as to detect anal cancer early. Consistent with the findings of other authors,[22] our results emphasize the need to offer preventive strategies such as anal cancer screening to all HIV-infected men.

ACKNOWLEDGMENTS

The authors thank the male patients of our HIV Unit.
The Can Ruti HIV-HPV study team consists of:
University Hospital Germans Trias i Pujol, Badalona (Barcelona) and the Autonomous University of Barcelona:

- Department of Proctology: Ms. P. Cobarsi
- Department of Pathology: Dr. E. Castella, Dr. M. Llatjos
- HIV Clinical Unit and Internal Medicine Department: Dr. A. Bonjoch, Dr. P. Echevarría, Dr. M. Jabaloyas, Dr. A. Jou, Dr. J.M. Llibre, Dr. J. Moltó, Dr. E. Negredo, Dr. C. Rey-Joly, Dr. J. Romeu, Dr. J.R. Santos, Dr. C. Tural
- HIV Clinical Unit nurses: Ms. C. Alcalde, Ms. R. Guerola, Ms. A. Salas
- Lluita contra la SIDA Foundation: Ms. S. Gel

Labco.General-Lab, Department of Molecular Biology: Ms. I. Castilla, Dr. V. Cirigliano, Dr. M. Ejarque, Ms. E. López, Ms. E. Ordóñez, Ms. L. Rueda

REFERENCES

1. Melbye M, Sprøgel P. Aetiological parallel between anal cancer and cervical cancer. *Lancet*. 1991;338:657–659.
2. Palefsky JM, Holly EA, Gonzales J, Berline J, Ahn DK, Greenspan JS. Detection of human papillomavirus DNA in anal intraepithelial neoplasia and anal cancer. *Cancer Res*. 1991;51:1014–1019.
3. Daling JR, Madeleine MM, Johnson LG, et al. Human papillomavirus, smoking, and sexual practices in the etiology of anal cancer. *Cancer*. 2004;101:270–280.
4. Melbye M, Frisch M. The role of human papillomaviruses in anogenital cancers. *Semin Cancer Biol*. 1998;8:307–313.
5. Abramowitz L, Jacquard AC, Jaroud F, et al. Human papillomavirus genotype distribution in anal cancer in France: the EDiTH V study. *Int J Cancer*. 2011;129:433–439.
6. Palefsky JM, Holly EA, Ralston ML, Jay N, Berry JM, Darragh TM. High incidence of anal high-grade squamous intra-epithelial lesions among HIV-positive and HIV-negative homosexual and bisexual men. *AIDS*. 1998;12:495–503.

7. Palefsky JM, Holly EA, Ralston ML, et al. Anal squamous intraepithelial lesions in HIV-positive and HIV-negative homosexual and bisexual men: prevalence and risk factors. *J Acquir Immune Defic Syndr Hum Retrovirol.* 1998;17: 320–326.
8. Daling JR, Weiss NS, Hislop TG, et al. Sexual practices, sexually transmitted diseases, and the incidence of anal cancer. *N Engl J Med.* 1987;317:973–977.
9. Holly EA, Whittemore AS, Aston DA, Ahn DK, Nickoloff BJ, Kristiansen JJ. Anal cancer incidence: genital warts, anal fissure or fistula, hemorrhoids, and smoking. *J Natl Cancer Inst.* 1989;81:1726–1731.
10. Piketty C, Darragh TM, Da Costa M, et al. High prevalence of anal human papillomavirus infection and anal cancer precursors among HIV-infected persons in the absence of anal intercourse. *Ann Intern Med.* 2003;138:453–459.
11. Clifford GM, Polesel J, Rickenbach M, et al.; Swiss HIV Cohort. Cancer risk in the Swiss HIV Cohort Study: associations with immunodeficiency, smoking, and highly active antiretroviral therapy. *J Natl Cancer Inst.* 2005;97:425–432.
12. Bower M, Palmieri C, Dhillon T. AIDS-related malignancies: changing epidemiology and the impact of highly active antiretroviral therapy. *Curr Opin Infect Dis.* 2006;19:14–19.
13. Hessol NA, Pipkin S, Schwarcz S, Cress RD, Bacchetti P, Scheer S. The impact of highly active antiretroviral therapy on non-AIDS-defining cancers among adults with AIDS. *Am J Epidemiol.* 2007;165:1143–1153.
14. D'Souza G, Wiley DJ, Li X, et al. Incidence and epidemiology of anal cancer in the multicenter AIDS cohort study. *J Acquir Immune Defic Syndr.* 2008;48:491–499.
15. Piketty C, Selinger-Leneman H, Grabar S, et al.; FHDH-ANRS CO 4. Marked increase in the incidence of invasive anal cancer among HIV-infected patients despite treatment with combination antiretroviral therapy. *AIDS.* 2008;22:1203–1211.
16. Bower M, Powles T, Newsom-Davis T, et al. HIV-associated anal cancer: has highly active antiretroviral therapy reduced the incidence or improved the outcome? *J Acquir Immune Defic Syndr.* 2004;37:1563–1565.
17. Cain JM, Howett MK. Preventing cervical cancer. *Science.* 2000;288:1753–1755.
18. Etienney I, Vuong S, Daniel F, et al. Prevalence of anal cytologic abnormalities in a French referral population: a prospective study with special emphasis on HIV, HPV, and smoking. *Dis Colon Rectum.* 2008;51:67–72.
19. Scott H, Khoury J, Moore BA, Weissman S. Routine anal cytology screening for anal squamous intraepithelial lesions in an urban HIV clinic. *Sex Transm Dis.* 2008;35:197–202.
20. Kiviat NB, Critchlow CW, Holmes KK, et al. Association of anal dysplasia and human papillomavirus with immunosuppression and HIV infection among homosexual men. *AIDS.* 1993;7:43–49.
21. Friedman HB, Saah AJ, Sherman ME, et al. Human papillomavirus, anal squamous intraepithelial lesions, and human immunodeficiency virus in a cohort of gay men. *J Infect Dis.* 1998;178:45–52.
22. Wilkin TJ, Palmer S, Brudney KF, Chiasson MA, Wright TC. Anal intraepithelial neoplasia in heterosexual and homosexual HIV-positive men with access to antiretroviral therapy. *J Infect Dis.* 2004;190:1685–1691.
23. Palefsky JM, Holly EA, Efirdc JT, et al. Anal intraepithelial neoplasia in the highly active antiretroviral therapy era among HIV-positive men who have sex with men. *AIDS.* 2005;19:1407–1414.
24. Damay A, Fabre J, Costes V, et al. Human papillomavirus (HPV) prevalence and type distribution, and HPV-associated cytological abnormalities in anal specimens from men infected with HIV who have sex with men. *J Med Virol.* 2010;82:592–596.
25. Goldie SJ, Kuntz KM, Weinstein MC, Freedberg KA, Palefsky JM. Cost-effectiveness of screening for anal squamous intraepithelial lesions and anal cancer in human immunodeficiency virus-negative homosexual and bisexual men. *Am J Med.* 2000;108:634–641.
26. Ogunbiyi OA, Scholefield JH, Raftery AT, et al. Prevalence of anal human papillomavirus infection and intraepithelial neoplasia in renal allograft recipients. *Br J Surg.* 1994;81:365–367.
27. Videla S, Darwich L, Cañadas MP, et al.; HIV-HPV Study Group. Natural history of human papillomavirus infections involving anal, penile, and oral sites among HIV-positive men. *Sex Transm Dis.* 2013;40:3–10.
28. Darwich L, Cañadas MP, Videla S, et al.; HIV-HPV Can Ruti Team. Condylomata, cytological abnormalities and human papillomavirus infection in the anal canal in HIV-infected men. *HIV Med.* 2012;13:549–557.
29. De Vuyst H, Clifford GM, Nascimento MC, Madeleine MM, Franceschi S. Prevalence and type distribution of human papillomavirus in carcinoma and intraepithelial neoplasia of the vulva, vagina and anus: a meta-analysis. *Int J Cancer.* 2009;124:1626–1636.
30. Patel P, Hanson DL, Sullivan PS, et al.; Adult and Adolescent Spectrum of Disease Project and HIV Outpatient Study Investigators. Incidence of types of cancer among HIV-infected persons compared with the general population in the United States, 1992-2003. *Ann Intern Med.* 2008;148:728–736.
31. Weis SE, Vecino I, Pogoda JM, et al. Prevalence of anal intraepithelial neoplasia defined by anal cytology screening and high-resolution anoscopy in a primary care population of HIV-infected men and women. *Dis Colon Rectum.* 2011;54:433–441.
32. Conley L, Bush T, Darragh TM, et al.; Study to Understand the Natural History of HIV and AIDS in the Era of Effective Therapy (SUN Study) Investigators. Factors associated with prevalent abnormal anal cytology in a large cohort of HIV-infected adults in the United States. *J Infect Dis.* 2010;202:1567–1576.
33. Cañadas MP, Cirigliano V, Darwich L, et al. Comparison of the f-HPV typing™ and Hybrid Capture II® assays for detection of high-risk HPV genotypes in cervical samples. *J Virol Methods.* 2012;183:14–18.
34. Aubin F, Prétet JL, Jacquard AC, et al.; EDiTH Study Group. Human papillomavirus genotype distribution in external acuminata condylomata: a Large French National Study (EDiTH IV). *Clin Infect Dis.* 2008;47:610–615.
35. Lama JMC, Hocha JS, Tinmoutha J, Sano M, Rabouda J, Salita IE. Cost-effectiveness of screening for anal precancers in HIV-positive men. *AIDS.* 2011, 25:635–642.
36. Nahas CS, da Silva Filho EV, Segurado AA, et al. Screening anal dysplasia in HIV-infected patients: is there an agreement between anal pap smear and high-resolution anoscopy-guided biopsy? *Dis Colon Rectum.* 2009;52:1854–1860.

37. Swedish KA, Lee EQ, Goldstone SE. The changing picture of high-grade anal intraepithelial neoplasia in men who have sex with men: the effects of 10 years of experience performing high-resolution anoscopy. *Dis Colon Rectum*. 2011;54:1003–1007.
38. Siekas LL, Aboulafia DM. Establishing an anal dysplasia clinic for HIV-infected men: initial experience. *AIDS Read*. 2009;19:178–186.
39. Palefsky J. Human papillomavirus and anal neoplasia. *Curr HIV/AIDS Rep*. 2008;5:78–85.
40. Chaturvedi AK, Madeleine MM, Biggar RJ, Engels EA. Risk of human papillomavirus-associated cancers among persons with AIDS. *J Natl Cancer Inst*. 2009;101:1120–1130.
41. Palefsky JM. Human papillomavirus-related disease in men: not just a women's issue. *J Adolesc Health*. 2010;46(4 Suppl):S12–S19.

Estudio 4: "Long-term effectiveness of infrared coagulation for anal intraepithelial neoplasia 2 and 3 in HIV-infected males and females". AIDS 2013, 27:951–959.

"EFECTIVIDAD A LARGO PLAZO DE LA COAGULACIÓN CON INFRAROJOS DE LA NEOPLASIA INTRAEPITELIAL ANAL GRADO II Y III EN HOMBRES Y MUJERES VIH+"

OBJETIVOS: Evaluar la efectividad y seguridad de la coagulación con infrarrojos (IR) para el ablación de las neoplasias intraepiteliales anales (NIA) y proporcionar datos sobre la prevalencia de NIA en pacientes infectados por el VIH.

PACIENTES Y MÉTODOS: se realizó un estudio de cohorte retrospectivo de un solo centro basado en los datos recopilados de una base de datos prospectiva de pacientes ambulatorios atendidos en la sección de Proctología de la Unidad de VIH (primera visita). Se estimó la efectividad (citología anal normal después de 12 meses de IR) y la seguridad de la IR.

RESULTADOS: entre enero de 2005 y diciembre de 2011, un total de 69 (5%) pacientes con biopsia comprobada de NIA-2 o NIA-3, de entre 1518 pacientes (1310 hombres; 208 mujeres) atendidos, fueron tratados con IR. La prevalencia de anomalías citológicas fue del 49,5% [751/1518]: células escamosas atípicas de significado desconocido, 14%; lesiones escamosas intraepiteliales de bajo grado, 27,5%; lesiones escamosas intraepiteliales de alto grado, 8%. La anoscopia de alta resolución reveló condilomas intraanales en el 31% de los pacientes (236/751),

RESULTADOS: Siete (13%) pacientes tuvieron una recidiva comprobada por biopsia [media (rango) tiempo de recurrencia, 30 (18-43) meses]. Se detectó una infección por el virus del papiloma humano de alto riesgo en todas las lesiones anales (el genotipo más común fue VPH-16). La concordancia entre los resultados citológicos e histológicos fue pobre.

CONCLUSIÓN: Se encontró una alta prevalencia de NIA en hombres infectados con VIH. Aunque faltan ensayos clínicos con asignación aleatoria, la ablación con IR de las lesiones de NIA-2 y NIA-3 sin condilomas concomitantes, podría ayudar a prevenir el carcinoma escamoso anal.

Long-term effectiveness of infrared coagulation for the treatment of anal intraepithelial neoplasia grades 2 and 3 in HIV-infected men and women

Guillem Sirera[a,b,*], Sebastián Videla[a,*], Marta Piñol[c], Josep Coll[a,d], Francesc García-Cuyás[c], Sandra Vela[c], MariPaz Cañadas[a,e], Laila Darwich[a,f], Núria Pérez[a], Silvia Gel[a], Patricia Cobarsi[a], Bonaventura Clotet[a,b,d], on behalf of the HIV-HPV Study Group

Aims: To assess the effectiveness and safety of infrared coagulation (IRC) for the ablation of anal intraepithelial neoplasia (AIN) and to provide data on the prevalence of AIN in HIV-infected patients.

Patients and methods: We performed a single-center, retrospective cohort study based on data collected from a prospectively compiled database of outpatients attended in the Clinical-Proctology-HIV-Unit (first visit). The effectiveness (normal anal cytology after 12 months of IRC) and safety of IRC were estimated.

Results: Between January 2005 and December 2011, a total of 69 (5%) patients with biopsy-proven AIN-2 or AIN-3 from among 1518 patients (1310 men; 208 women) were treated with IRC. The prevalence of cytological abnormalities was 49.5% [751/1518; (atypical squamous cells of unknown significance, 14%; low-grade squamous intraepithelial lesions, 27.5%; high-grade squamous intraepithelial lesions, 8%)]. High-resolution anoscopy revealed intra-anal condylomata in 31% of patients (236/751), nonvisualized lesions in 30% (227/751), and visualized lesions (from which biopsy specimens were taken) in 38% (288/751). The histological diagnosis was: AIN-1, 52% (151/288); AIN-2, 15% (44/288); AIN-3, 9% (25/288); normal, 19% (56/288); and nonevaluable, 4% (12/288). IRC was applied in-office in 66 patients (three refused to undergo treatment). At 12 months, all patients ($n = 56$) had a normal anal cytology result. Seven (13%) patients had biopsy-proven recurrence [mean (range) time-to-recurrence, 30 (18–43) months]. High-risk-human papilloma virus (HPV) infection was detected in all anal lesions (HPV-16 was the most common genotype). Agreement between cytological and histological results was poor.

Conclusion: A high prevalence of AIN was found in both HIV-infected men and HIV-infected women. Although randomized clinical trials are lacking, IRC ablation of AIN-2 and AIN-3 lesions without concomitant condylomata could help prevent anal squamous cell carcinoma.

AIDS 2013, **27**:951–959

Keywords: anus, HIV-infected persons, human papillomavirus infection, infrared ablation

[a]Lluita Contra La SIDA Foundation, [b]HIV Clinical Unit, Department of Medicine, [c]Department of Surgery, [d]Retrovirology Laboratory IrsiCaixa Foundation; University Hospital Germans Trias i Pujol, Badalona, Universitat Autònoma de Barcelona, Catalonia, [e]Labco.GeneralLab, Barcelona, and [f]Department of Sanitat i Anatomia Animal, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain.

Correspondence to Sebastián Videla, MD, PhD; Fundació Lluita Contra la SIDA; Hospital Universitari Germans Trias i Pujol; 08916 Badalona (Barcelona), Spain.

Tel: +34 93 465 63 74; fax: +34 93 465 39 68; e-mail: svidela@flsida.org/svidela@esteve.es

[*] Guillem Sirera and Sebastián Videla contributed equally to the writing of this article.

Received: 9 August 2012; revised: 9 December 2012; accepted: 17 December 2012.

DOI:10.1097/QAD.0b013e32835e06c1

Introduction

Anal intraepithelial neoplasia (AIN) is the precursor to invasive anal squamous cell carcinoma (SCC) [1]. Its incidence is rising in high-risk groups, particularly individuals infected with HIV [2,3]. Like cervical cancer, anal cancer progresses in a step-wise manner from low-grade dysplasia to high-grade dysplasia, which precedes micro-invasion. Anal SCC develops as a result of infection with oncogenic strains of the human papilloma virus (HPV), mainly HPV-16 and HPV-18 [4]. However, the natural history of AIN is unclear, and management strategies are lacking.

Experience with treatment of cervical cancer shows that removal of intraepithelial neoplasia decreases the incidence of invasive disease. The same could be true of invasive anal SCC [5,6]. The most effective treatment for AIN, excision of the anal squamocolumnar junction, is associated with marked morbidity (anal stenosis, abscess formation, anal spasm, and dyschezia) [7,8]. Treatment of AIN in its early stages could be the best way to modify the natural history of the disease and thus avoid progression to invasive anal SCC. The most common approach to management of AIN during the last decade has largely centered on local ablation of individual lesions using laser therapy, cryotherapy, electrocautery, infrared coagulation (IRC), and topical agents (imiquimod, trichloracetic acid, and 5% 5-fluorouracil cream) [6,8–15].

IRC ablation is an effective treatment for high-grade squamous intraepithelial lesions (HSIL). However, recurrence of squamous intraepithelial lesions (SIL) has to be taken into account. In fact, few studies have been published on the efficacy or effectiveness of IRC [7,13–16]. Four of the five studies published [13–16] focused on HIV-positive men who have sex with men (MSM). Only one study included two HIV-positive women [7], and another only evaluated the long-term efficacy of IRC [13]. Therefore, more data on the effectiveness of IRC are necessary before the real benefits of this treatment become clear. The aim of the present study was to assess the prevalence of AIN and the long-term effectiveness and safety of IRC in HIV-infected patients. We also investigated whether treatment results differed between HIV-infected men and HIV-infected women.

Patients and methods

Study design

The study was a single-center, retrospective cohort study based on data from a prospectively compiled database (electronic medical files) of outpatients who were attended in the Clinical Proctology Section of the HIV Unit of University Hospital Germans Trias i Pujol, Badalona, Spain. All patients were systematically screened for HPV-related anal disease. The study was performed according to the stipulations of the Declaration of Helsinki, and all patients gave their written informed consent for their medical information to be used for purposes of scientific research in accordance with the guidelines of the local ethics committee.

Study population

The patients included in this study had to fulfill the following criteria: age at least 18 years, positive HIV serology results, and biopsy-proven (histological diagnosis) AIN-2 or AIN-3 confirmed by high-resolution anoscopy (HRA).

The inclusion period ran from January 2005, when the Clinical Proctology HIV unit was created and the screening program implemented, through December 2011. The starting point of the study was January 2005. Following our standard operating procedure, all HIV outpatients who were attended in the HIV Unit for the first time from January 2005 onward were informed about the screening program for detection of HPV-related disease of the anal canal.

Patients whose HIV infection was already being managed before January 2005 were informed about this screening program by our reception staff when they came for their routine check-ups. They were also informed about the program by their HIV care providers. It is worth noting that all care providers for HIV-infected patients were reminded at staff meetings (every 2 months during the first 2 years) to inform patients about the program. Consistent with the usual procedure, patients were referred to the Clinical Proctology HIV Unit if their HIV doctor detected an anal condition.

The following data were gathered at baseline: date of birth, date of HIV diagnosis (time with HIV infection in years), date of the visit, CD4 cell count (the most recent value before cytology sample collection), nadir CD4 counts (the lowest CD4 value of each patient taken from medical records), plasma viral load (the most recent value before cytology sample collection), HAART before inclusion (yes/no), and time on HAART. The results of anal examinations were also recorded [anal cytology, HRA (number and localization of the lesions) and histology (biopsy) results], as were data on anal evaluations during follow-up. CD4 cell and nadir CD4 were counted using flow cytometry, and HIV viral load was determined using Nuclisens (detection limit 80 copies/ml; bioMérieux, Inc., Durham, North Carolina, USA).

Clinical examination and anal sample collection

A clinical examination (visual inspection) including a digital rectal examination was performed at the first clinical visit. A sample of tissue from the anal canal was obtained when deemed necessary by introducing a

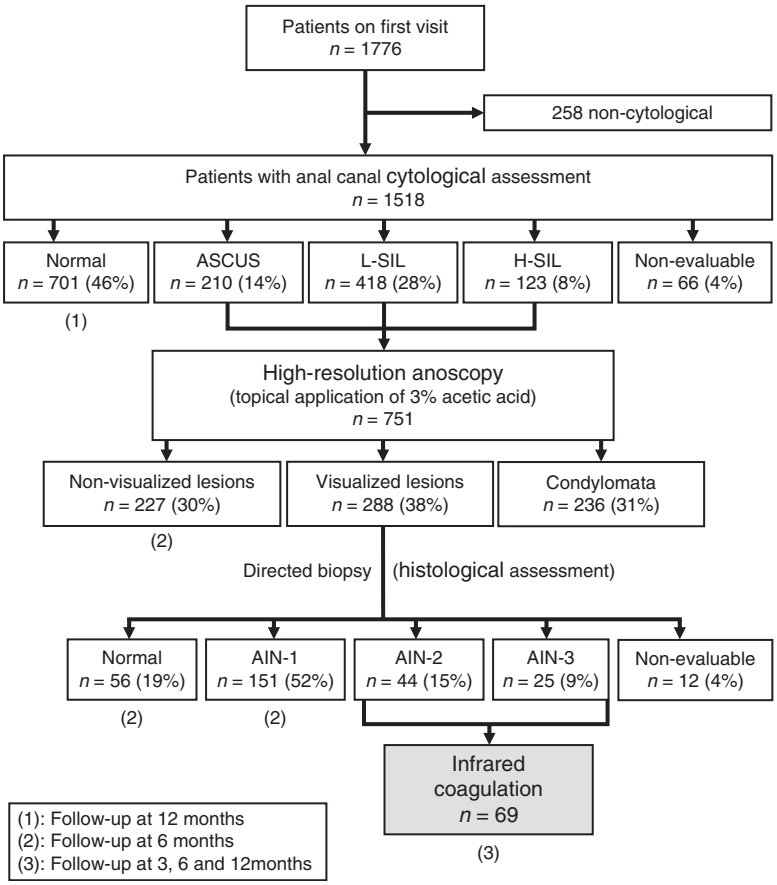


Fig. 1. Flow chart showing progress from the screened population (patients at their first visit to the Clinical Proctology HIV Unit) to the study population (patients diagnosed with AIN-2 or AIN-3). AIN, anal intraepithelial neoplasia; ASCUS; atypical squamous cells of unknown significance; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

cytobrush (Eurogine SL, Sant Boi del Llobregat, Spain) 3 cm into the anal canal and softly rotating for 30–45 s. The cytobrush was introduced into 20 ml of PreservCyt/ThinPrep Pap test solution (Cytyc Iberia SL, Barcelona, Spain) and shaken for 30 s. This sample was used to carry out the cytology analysis (Papanicolaou test). The flow chart for detection of AIN is shown in Fig. 1.

High-resolution anoscopy and infrared coagulation

If the anal cytology result was positive [atypical squamous cells of unknown significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), or HSIL], the patient was informed and HRA was programmed. HRA was performed using a technique described elsewhere [17]. If the HRA (after topical application of 3% acetic acid in the anal canal for 2 min) revealed a lesion, a biopsy was performed. If the result of the biopsy (histology) was AIN-2 or AIN-3, the patient was informed and treatment with IRC was scheduled.

The intra-anal lesions detected with HRA, namely, biopsy-proven AIN-2 or AIN-3, were treated with in-office IRC ablation (Redfield IRC 2100; Redfield Corporation, Rochelle Park, New Jersey, USA). Each lesion was identified, infiltrated with local anesthesia, and repeatedly coagulated with IRC in pulses of 1.5 s. IRC delivers short pulses of a narrow beam of visible infrared light through a small contact tip applicator that is applied directly to the target tissue. It results in thermal coagulation and tissue necrosis. The scab was removed, and the process was repeated until the submucosal vessels were coagulated. The depth of coagulation and tissue necrosis was adjustable. All anal lesions present were treated at the time of ablation. All procedures were performed by trained surgeons (M.P., F.G.-C. and S.Vela).

If anal canal condylomata were diagnosed during clinical examination, either by digital rectal examination or HRA, the patient was referred to the major ambulatory surgery unit for treatment.

Follow-up

After IRC ablation, patients underwent routine evaluations involving visual inspection, digital rectal examination, and anal canal cytology at 3-month to 6-month intervals. Postsurgical complications were also recorded. Patients with abnormal cytology results underwent

another examination with HRA. If HRA revealed a lesion, a biopsy was performed. Patients with biopsy-proven AIN-2 or AIN-3 were advised to undergo further IRC ablation. Patients with AIN-1 were monitored with anal cytology at 3-month to 6-month intervals. Patients with normal cytology results 12 months after IRC were monitored using anal cytology at 12-month intervals. The screening and follow-up algorithm is shown in Fig. 1.

Anal cytological and histological assessment

Cytological changes were classified according to the Bethesda System as ASCUS and LSIL or HSIL. Generally, samples were independently assessed by two expert cytopathologists. Histological changes were classified according to the grade of AIN as AIN-1, AIN-2, and AIN-3. Samples were independently assessed by two expert pathologists.

Human papilloma virus detection and typing

DNA was extracted from cell suspensions (in ThinPrep Pap solution) using the QIAamp Viral DNA kit (QIAGEN, Hilden, Germany). HPV was detected and typed in all samples using a commercial *in vitro* diagnostic CE-marked assay [Multiplex Fluorescent-PCR Kit for HPV Genotyping (F-HPV) typing, Molgentix SL, Barcelona, Spain] in accordance with the manufacturer's instructions [20]. The kit enables the detection of 13 high-risk HPV (HR-HPV) genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and the two most frequent low-risk HPV genotypes (6, 11). A human short tandem repeat sequence included in the same multiplex reaction was amplified as an internal control for DNA integrity and the absence of polymerase chain reaction (PCR) inhibitors. Products were analyzed using capillary electrophoresis on an ABI 3130 XL genetic analyzer and GeneMapper 4.0 Software (Applied Biosystems). Each PCR run included HPV-positive and HPV-negative controls. Particular care was taken to prevent carry-over contamination by separating pre-PCR and post-PCR areas in the laboratory.

Study definitions

Treatment success (percentage of responders) was defined as no evidence of cytology-proven SIL and no evidence of anal lesions on HRA or no evidence of anal lesions on clinical examination (visual inspection and digital rectal examination) if a follow-up HRA was not performed at 12 months after IRC. Overall treatment success was defined as no evidence of cytology-proven SIL and no evidence of anal lesions on HRA or no evidence of anal lesions on clinical examination if a follow-up HRA was not performed at any point during follow-up. Recurrence was defined as cytology-proven SIL or biopsy-proven AIN at the treated site or at a new site at 12 months after treatment. Overall recurrence was defined as identification of cytology-proven SIL or biopsy-proven AIN at any point during follow-up at the treated location or at a new site. Persistence was defined as the existence or recurrence of disease at the treatment site. A metachronous recurrence was defined as biopsy-proven AIN found at a previously untreated site.

Statistical analysis

No formal sample size was calculated. The sample was defined as all HIV-infected patients with biopsy-proven AIN-2 or AIN-3 (histological diagnosis) confirmed by HRA.

Baseline characteristics were summarized using standard descriptive statistics, and a descriptive analysis was carried out. The prevalence of anal canal SIL, AIN, and anal HPV-type specific infection was estimated, and 95% confidence intervals (95% CI) were calculated. The effectiveness of IRC (treatment success) was expressed as a percentage, and overall treatment success was calculated. The mean time to recurrence (range) was analyzed using the Kaplan–Meier method. Cohen's κ was used to assess agreement between the cytological diagnosis (Papanicolaou test) and histological diagnosis (biopsy). Data were analyzed using SPSS version 15.0 statistical software (SPSS, Inc., Chicago, Illinois, USA) and StatXact-8 (Cytel Inc., Cambridge, Massachusetts, USA).

Results

Patient characteristics

We studied 69 HIV-positive patients (55 men and 14 women) with a diagnosis of AIN-2 or AIN-3 confirmed by HRA and biopsy who underwent treatment with IRC. Figure 1 shows the progress of patients through the study, from the screened population (patients at their first visit to the Clinical Proctology HIV Unit) to the study population (patients diagnosed with AIN-2 or AIN-3).

Of the 1776 patients initially screened, a cytological diagnosis was available for 1518 HIV-positive patients (1310 men and 208 women). The number of patients screened represents over 70% of the HIV-positive population attending our HIV unit. The baseline characteristics of the study population are shown in Table 1. Among HIV-positive men ($n = 55$), 51 (93%) were MSM and four (7%) were heterosexual. No patients were vaccinated against HPV. During follow-up, all patients were on HAART, which included at least two nucleoside reverse transcriptase inhibitors in combination with either a protease inhibitor or a nonnucleoside reverse transcriptase inhibitor.

Prevalence of anal disease

Based on the screened population and number of patients with a cytological diagnosis, the prevalence of SIL (low and high grade) was 49.5% (751/1518, 95% CI: 47–52%) (Fig. 1). Based on the histological diagnosis, the

Table 1. Baseline characteristics of the study population ($n = 69$) and the patients treated with infrared coagulation and with a follow-up of greater than 12 months ($n = 56$).

Baseline characteristics	Study population ($n = 69$)	Follow-up ($n = 56$)
Age		
Median years (range[a])	43 (22–58)	42 (22–58)
Sex		
Men (*n*, %)/women (*n*, %)	55 (80%)/14 (20%)	45 (80%)/11 (20%)
Time of known HIV (years)		
Median (range[a])	9 (2–15)	9 (2–14)
AIDS diagnosis (yes) [*n* (%)]	10 (14)	8 (14)
HIV plasma load	54 (78%)	42 (75%)
<50 HIV RNA copies/ml [*n* (%)]		
CD4 cell count [*n* (%)]		
<200 cells/μl	4 (6%)	4 (7%)
200–500 cells/μl	25 (36%)	19 (34%)
>500 cells/μl	40 (58%)	33 (59%)
CD4 nadir		
<200 cells/μl [*n* (%)]	7 (10%)	4 (7%)

[a]Range, (minimum–maximum values).

prevalence of AIN (AIN-1, AIN-2, and AIN-3) was 15% (223/1518, 95% CI: 13%, 17%) (Fig. 1).

The agreement between the cytological diagnosis (Papanicolaou test) and the histological diagnosis (biopsy) was evaluated in 288 patients. Table 2 shows the agreement between the anal cytological and histological results in patients with an abnormal anal cytology result and a lesion visualized using HRA. The agreement between cytological and histological diagnosis was poor [$k = 0.23$ (95% CI: 0.13,0.32), $P < 0.001$, including ASCUS in the analysis; $k = 0.13$ (95% CI: −0.01,0.26), $P = 0.066$, not including ASCUS in the analysis]. Sixty-one percent of patients with a cytological diagnosis of LSIL had a diagnosis of AIN-1, and only 50% of patients with a cytological diagnosis of HSIL had a diagnosis of AIN-2 or AIN-3. Furthermore, 60% of patients with a cytological diagnosis of ASCUS had a histological diagnosis of AIN.

Effectiveness of infrared coagulation

A total of 69 HIV-infected patients with a diagnosis of biopsy-proven AIN-2 or AIN-3 were candidates for treatment with IRC: 68 (99%) patients presented only one confirmed lesion and only one patient (woman) presented two confirmed lesions (total number of lesions treated, 70). Lesions were mainly located at 7 o'clock (12 patients), 11 o'clock (11 patients), 6 o'clock (10 patients), 9 o'clock (8 patients), and 12 o'clock (7 patients). In the patient with two lesions, both were at 3 o'clock.

With regard to sex, the percentage of HIV-positive women candidates for treatment with IRC (7%, 14/208) was slightly higher than that of HIV-positive men [4% (55/1310), $P = 0.103$], MSM [5% (51/996)], and heterosexual men [1% (4/314)].

Three patients (4%) rejected treatment with IRC and 10 (14%) had a follow-up of less than 12 months. Therefore, the effectiveness of IRC was evaluated in 56 patients (45 men, 11 women). No evidence of cytology-proven SIL or anal lesions was detected at 12 months after IRC. Therefore, treatment was successful in all cases. The 10 patients with a follow-up of less than 12 months did not have evidence of cytology-proven SIL or anal lesions after IRC.

The overall success of treatment was 87.5% (49/56), with a mean follow-up of 25 (range: 12–60) months. After the 12 months of post-IRC follow-up, only seven patients [12.5%: two women (18%), five men (11%)] had abnormal cytology findings, and recurrent disease (AIN) was biopsy-proven in all of them (overall recurrence). The mean time to recurrence (range) was 30 (18–43) months. The recurrence of disease was at the treatment site (persistence) in one patient (14%: AIN-1)

Table 2. Agreement between anal cytological (Papanicolau test) and histological (biopsy) results in patients with abnormal anal cytology and visualized lesion by high-resolution anoscopy ($n = 288$).

			Histology				
			Normal	AIN-1	AIN-2	AIN-3	Nonevaluable
Cytology	ASCUS	($n = 79$)	25 (32%)	40 (51%)	4 (5%)	3 (4%)	7 (9%)
	L-SIL	($n = 141$)	23 (16%)	86 (61%)	22 (16%)	6 (4%)	4 (3%)
	H-SIL	($n = 68$)	8 (12%)	25 (37%)	18 (26%)	16 (24%)	1 (1%)

AIN, anal intraepithelial neoplasia; ASCUS; atypical squamous cells of unknown significance; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

and at a previously untreated site (metachronous recurrence) in the remaining six patients (86%: AIN-1, three patients; AIN-2, three patients).

Human papilloma virus infection

Data on HPV infection were available for 58 out of 69 patients with AIN-2 or AIN-3; all 58 patients presented detectable infection by HPV. Single HPV infection was found in only seven patients (12%) and multiple infection (more than one HPV) in 51 (88%). The most prevalent HR-HPV types detected at baseline were HPV-16 (65.5%), HPV-58 (41.4%), HPV-33 (34.5%), HPV-51 (27.6%), and HPV-18 (25.9%). The prevalence of HPV-6 and HPV-11 (HPV related to nonmalignant diseases) was 20.7 and 3.4%, respectively. Figure 2 shows the genotype-specific prevalence of HPV in the anal canal of patients with AIN-2 ($n = 37$) and AIN-3 ($n = 21$). In the group of patients with AIN-2, infection by a single genotype was found in six patients (16%) and by multiple genotypes in 31 patients (84%). However, in the group of patients with AIN-3, only one (5%) patient presented infection by a single genotype; the remaining 20 patients (95%) presented infection by multiple genotypes. Data on HPV information were only available in one out of seven patients with a recurrent lesion. The same HR-HPVs (HPV-33, HPV-58) seen at baseline were detected in the recurrence.

Adverse events

No patients developed serious adverse events (anal stricture, persistent bleeding, significant postoperative hemorrhage, failure to heal, or infection requiring antibiotic therapy) following IRC ablation. Three patients presented mild bleeding during the first few hours after IRC. Pain was the most common post-IRC event until 72 h after the IRC [nine patients (13%) scored greater than 3 in the verbal numerical rating pain intensity scale] and was adequately controlled with mild analgesics. Of note, no patient developed anal SCC during study follow-up.

Discussion

Few data are available on the efficacy or effectiveness of IRC [7,13–16] in high-grade anal dysplasia. Studies have generally focused on HIV-positive MSM, and findings for HIV-infected women are practically nonexistent. The high long-term effectiveness of IRC found in our sample, which included HIV-infected men and women without concomitant condylomata in the anal canal, suggests that IRC might help to prevent anal SCC and progression to invasive disease.

Interrupting the natural history of SCC is challenging. In the last decade, several approaches have been used to manage AIN (cryotherapy, electrocautery, topical agents, IRC), although solid data are scant and randomized clinical trials are lacking [6,8–15]. High rates of persistence and recurrence are reported with most approaches, including IRC. However, our results revealed a low rate of recurrence, possibly because of the characteristics of our population: our patients had only one lesion per person (single lesion without condylomata) and a good immunological status and were receiving HAART during follow-up. Nevertheless, it seems that HAART has not been able to curb rising

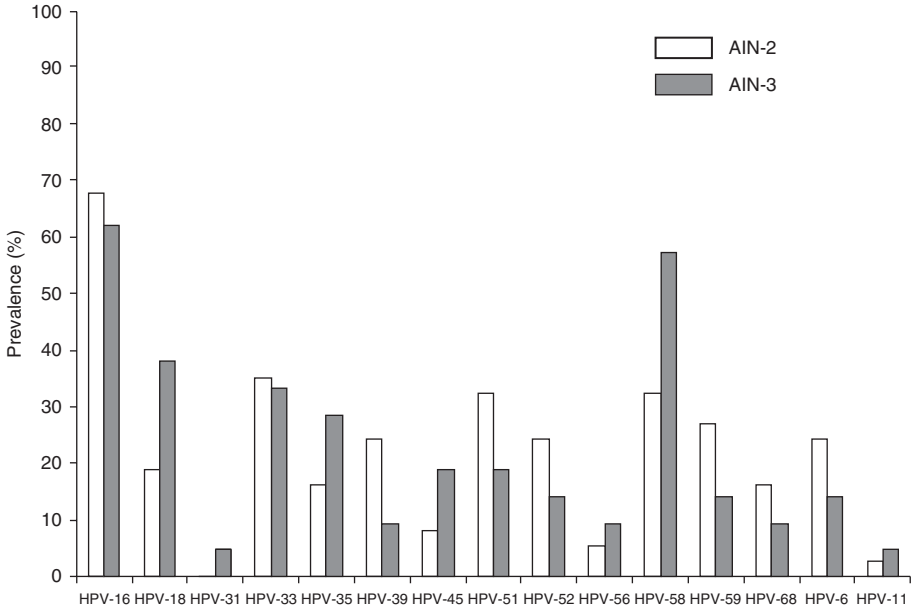


Fig. 2. Prevalence of genotype-specific HPV in the anal canal of patients with AIN-2 ($n = 37$) and AIN-3 ($n = 21$). AIN, anal intraepithelial neoplasia.

incidence [18]. Furthermore, metachronous lesions (i.e. at previously untreated sites) have been considered a major cause of recurrence (82%) in HIV-positive patients after their first IRC treatment [13]. Although the low number of patients with recurrent lesions in our study prevents us from drawing firm conclusions, metachronous lesions recurred in six (86%) out of seven patients. Given the lack of clinical trials and the high rate of persistence or recurrence in HIV-positive patients and the high rate of recurrence due to metachronous lesions, continued surveillance after IRC is necessary until more data become available.

No patient treated with IRC progressed to anal SCC during follow-up. However, three MSM from the screened population were diagnosed with *in situ* anal SCC and treated with surgery. One of them had a recurrence (invasive anal SCC) 18 months after surgery. Among the nonscreened population (~30% of our current population of HIV-positive patients), one case of invasive anal cancer (female IDU in 2006) was diagnosed during the same time period. Furthermore, two more cases of invasive anal cancer (one heterosexual male IDU in 2010 and one transsexual female IDU in 2012) were referred for treatment to our center, which is a referral hospital in our geographic area. From 1983 (the year the hospital opened) to December 2004, six cases of invasive anal SCC in HIV-positive patients were diagnosed and treated [one man in 1999 (no information on sexual practices); three MSM in 1999, 2000, and 2002; and two heterosexual male IDUs in 2004]. The first of these aforementioned 10 cases was diagnosed and treated in 1999 and the last one in 2012, that is, during the HAART era.

Despite the poor agreement between cytological and histological diagnosis of anal dysplasia when different grades of dysplasia are matched, the high prevalence of anal dysplasia (SIL or AIN) alerts us to the high probability of developing anal SCC in HIV-infected patients. Consequently, it is worth considering that around 10% of screened HIV-infected patients presented biopsy-proven AIN-1, and around 5% presented biopsy-proven AIN-2 or AIN-3. In other words, one out of every 20 HIV-infected patients (~1 of every 15 women and ~1 of every 24 men) needed treatment with IRC. These findings imply that physicians should be aware of anal SCC in their approach to HIV-infected patients. HIV-infected MSM are at increased risk of developing anal SCC with an incidence rate of ~131 per 100 000 person-years [19]. However, AIN was also diagnosed among heterosexual HIV-infected men and HIV-infected women. Both HIV-positive heterosexual men (~46 per 100 000 person-years) and HIV-positive women (~30 per 100 000 person-years) also have a clinically relevant incidence rate when they are compared with the general population (1 per 100 000 person-years) [19–21]. Faced with these incidence rates, sexual practices predispose to increased risk but should not be considered the only factor. It is likely that HIV infection itself plays a major role in the natural history of HPV-related diseases.

The poor correlation between cytology and histology findings when the different grades of dysplasia are matched is very concerning, since a number of patients with normal anal cytology results could have had anal intraepithelial disease. We were unable to establish this figure in our study because patients with normal cytology did not undergo HRA. However, our results show that 59% (47/59) of patients with a cytological diagnosis of ASCUS had a histological diagnosis of AIN. Furthermore, 19% (56/288) of patients with abnormal cytology findings (ASCUS, LSIL, or HSIL) had a normal histology result. These findings reinforce the possibility that a number of patients with a normal anal cytology result might have AIN. In fact, 86% (59/69) of patients with a cytological diagnosis of HSIL had a histological diagnosis of AIN. Likewise, 81% (114/141) of patients with a cytological diagnosis of LSIL had a histological diagnosis of AIN. If we compare normal and abnormal cytology results (independently of the grade of dysplasia) with normal and abnormal histology results, agreement between both diagnostic techniques is 81% (232/288). Then, if we take into account that an abnormal cytology result has a high probability of being AIN and that disease progression is slow, the cytological findings could prove sufficiently useful for clinical management. Consequently, the yearly anal cytological assessment could prove to be extremely important, despite the poor agreement between cytology and histology results when the different grades of dysplasia are matched.

HPV infection caused by high-risk genotypes was present in all patients with AIN-2 and AIN-3. Likewise, practically all patients (88%) presented HPV infection caused by more than one genotype. As expected, HPV-16 was the most prevalent. Others, such as HPV-58, HPV-33, and HPV-51, were more prevalent than HPV-18. Low-risk genotypes (HPV-6 and HPV-11) were also detected which are associated with HPV-related nonmalignant diseases (condylomata), although it is noteworthy that patients with condylomata were not included in this study. Given the low number of patients with recurrent lesions, no association can be established between HPV infection and recurrence, even though high-risk HPV-infection was detected in all cases of recurrence of AIN.

Our study is subject to a series of limitations. Its retrospective cohort design, although based on data from a prospectively compiled database (electronic medical files) might underestimate or overestimate the generalizability of the results beyond the population and conditions studied. Likewise, the sample size might underestimate or overestimate the generalizability of the results. HRA (after topical application of 3% acetic acid) is currently the best procedure for the detection of anal canal lesions; however, as it is subject to interobserver bias

(despite thorough training), primary lesions or recurrent lesions could go undiagnosed. In addition, during follow-up, patients with benign lesions according to cytology and negative standard anoscopy results were considered nonrecurrent, thus potentially leading us to further underestimate recurrence rates. Moreover, as the study was only performed in patients with SIL and no evidence of condylomata, its applicability to patients with SIL and condylomata could be limited. Finally, adverse events after IRC were recorded during an interview held at subsequent visits; consequently, patients may have underestimated the number and intensity of the events.

In conclusion, AIN was highly prevalent among HIV-infected men and women. Although randomized clinical trials are lacking, IRC ablation of AIN-2 and AIN-3 lesions without concomitant condylomata in the anal canal could help prevent anal SCC.

Acknowledgements

We are grateful to Thomas O'Boyle for editorial assistance. We thank Eric Goode for the English correction of this article. We are also indebted to the patients of our Clinical Proctology HIV Unit.

Author contributions: S.V., G.S., J.C. B.C. designed and wrote the study protocol. G.S., J.C., F.G.-C., M.P., S.V. and P.C. visited and treated the patients and collected the anal samples. M.P.C. and L.D. performed HPV detection and genotyping using multiplex PCR. S.G. and N.P. were responsible for data management and statistical analysis. S.V., G.S., J.C. and B.C. wrote the article. All the authors read and approved the final version of the article.

Funding: This work was supported by grants from Red de Investigación en SIDA (RIS), ART AIDS Foundation, Gilead Sciences, Obra Social Caixa Sabadell, and Gala contra la SIDA-Barcelona 2011.

HIV-HPV Study Group: University Hospital Germans Trias i Pujol, Badalona (Barcelona), Autonomous University of Barcelona: Department of Pathology: Dr E. Castellà, Dr M. Llatjós

HIV Clinical Unit and Internal Medicine Department: Dr A. Bonjoch, Dr P. Echevarría, Dr A. Jou, Dr JM. Llibre, Dr J. Moltó, Dr E. Negredo, Dr C. Rey-Joly, Dr J. Romeu, Dr JR. Santos, Dr C. Tural

Nurses of HIV Clinical Unit: Ms. C. Alcalde, Ms. R. Guerola, Ms. A. Salas

Conflicts of interest

S.V. has received honoraria for collaborating with Laboratorios Dr Esteve on work unrelated to HPV/HIV. B.C. has received honoraria for speaking and participating on advisory boards from Abbott, Bristol-Myers Squibb, Boehringer-Ingelheim, Gilead Sciences, GlaxoSmithKline, Pfizer, Merck, Janssen-Tibotec, and Siemens. The remaining authors have no conflicts of interest.

References

1. Gervaz P, Hahnloser D, Wolff BG, Anderson SA, Cunningham J, Beart RW Jr, *et al*. **Molecular biology of squamous cell carcinoma of the anus: a comparison of HIV-positive and HIV-negative patients.** *J Gastrointest Surg* 2004; **8**:1024–1031.
2. Chin-Hong PV, Palefsky JM. **Natural history and clinical management of anal human papillomavirus disease in men and women infected with human immunodeficiency virus.** *Clin Infect Dis* 2002; **35**:1127–1134.
3. D'Souza G, Wiley DJ, Li X, Chmiel JS, Margolick JB, Cranston RD, Jacobson LP. **Incidence and epidemiology of anal cancer in the multicenter AIDS cohort study.** *J Acquir Immune Defic Syndr* 2008; **48**:491–499.
4. Welton ML, Sharkey FE, Kahlenberg MS. **The etiology and epidemiology of anal cancer.** *Surg Oncol Clin N Am* 2004; **13**:263–275.
5. Apgar BS, Kittendorf AL, Bettcher CM, Wong J, Kaufman AJ. **Update on ASCCP consensus guidelines for abnormal cervical screening tests and cervical histology.** *Am Fam Physician* 2009; **80**:147–155.
6. Pineda CE, Berry JM, Jay N, Palefsky JM, Welton ML. **High-resolution anoscopy targeted surgical destruction of anal high-grade squamous intraepithelial lesions: a ten-year experience.** *Dis Colon Rectum* 2008; **51**:829–837.
7. Stier EA, Goldstone SE, Berry JM, Panther LA, Jay N, Krown SE, *et al*. **Infrared coagulator treatment of high-grade anal dysplasia in HIV-infected individuals: an AIDS malignancy consortium pilot study.** *J Acquir Immune Defic Syndr* 2008; **47**:56–61.
8. Scholefield JH, Ogunbiyi OA, Smith JH, Rogers K, Sharp F. **Treatment of anal intraepithelial neoplasia.** *Br J Surg* 1994; **81**:1238–1240.
9. Lyons M, Francis N, Allen-Mersh TG. **Treatment of grade 3 anal intraepithelial neoplasia by complete anal mucosal excision without fecal diversion: report of a case.** *Dis Colon Rectum* 1999; **42**:1342–1344.
10. Pehoushek J, Smith KJ. **Imiquimod and 5% fluorouracil therapy for anal and perianal squamous cell carcinoma in situ in an HIV-1-positive man.** *Arch Dermatol* 2001; **137**:14–16.
11. Hamdan KA, Tait IS, Nadeau V, Padgett M, Carey F, Steele RJ. **Treatment of grade III anal intraepithelial neoplasia with photodynamic therapy: report of a case.** *Dis Colon Rectum* 2003; **46**:1555–1559.
12. Lacey CJ. **Therapy for genital human papillomavirus-related disease.** *J Clin Virol* 2005; **32 (Suppl 1)**:S82–S90.
13. Goldstone RN, Goldstone AB, James Russ J, Goldstone SE. **Long-term follow-up of infrared coagulator ablation of anal high-grade dysplasia in men who have sex with men.** *Dis Colon Rectum* 2011; **54**:1284–1292.
14. Goldstone SE, Kawalek AZ, Huyett JW. **Infrared coagulator: a useful tool for treating anal squamous intraepithelial lesions.** *Dis Colon Rectum* 2005; **48**:1042–1054.
15. Goldstone SE, Hundert JS, Huyett JW. **Infrared coagulator ablation of high-grade anal squamous intraepithelial lesions in HIV-negative males who have sex with males.** *Dis Colon Rectum* 2007; **50**:565–575.
16. Cranston RD, Hirschowitz SL, Cortina G, Moe AA. **A retrospective clinical study of the treatment of high-grade anal dysplasia by infrared coagulation in a population of HIV-positive men who have sex with men.** *Int J STD AIDS* 2008; **19**:118–120.
17. Jay N, Berry JM, Hogeboom CJ, Holly EA, Darragh TM, Palefsky JM. **Colposcopic appearance of anal squamous intraepithelial lesions: relationship to histopathology.** *Dis Colon Rectum* 1997; **40**:919–928.

18. Crum-Cianflone NF, Hullsiek KH, Marconi VC, Ganesan A, Weintrob A, Barthel RV, Agan BK. **Anal cancers among HIV-infected persons: HAART is not slowing rising incidence.** *AIDS* 2010; **24**:535–543.
19. Silverberg MJ, Lau B, Justice AC, Engels E, Gill MJ, Goedert JJ, *et al*. **Risk of anal cancer in HIV-infected and HIV-uninfected individuals in North America.** *Clin Infect Dis* 2012; **54**:1026–1034.
20. Moscicki AB, Palefsky JM. **HPV in men: an update.** *J Low Genit Tract Dis* 2011; **15**:231–234.
21. Hartwig S, Syrjänen S, Dominiak-Felden G, Brotons M, Castellsagué X. **Estimation of the epidemiological burden of human papillomavirus-related cancers and nonmalignant diseases in men in Europe: a review.** *BMC Cancer* 2012; **12**:30; doi:10.1186/1471-2407-12-30.

Discusión y resultados

Los artículos presentados en esta tesis doctoral aportan evidencias actualizadas de la prevalencia de condiloma/s en el canal anal, de la prevalencia de infección (por más de un genotipo) del VPH en el canal anal en presencia de condiloma/s, de la coexistencia de dos patologías relacionadas con el VPH el canal anal: condiloma/s y lesiones escamosas intraepiteliales, y de la efectividad de los tratamientos utilizados en la práctica clínica para el manejo de los condiloma/s en el canal anal en los pacientes infectados por el VIH.

Los condilomas/s en el canal anal es una patología banal, pero dada su alta tasa de recidiva hace que sea una entidad de difícil tratamiento. Esto ha sido y es motivo de preocupación para los profesionales encargados de su tratamiento. Es importante destacar que no existen guías de práctica clínica consensuadas que, de acuerdo a las características de los condiloma/s en el canal anal, orienten hacia qué tratamiento se debe aplicar. Asimismo, es escasa la literatura científica sobre esta patología. Ante la falta de consenso y de evidencias, muchos de los procedimientos usados en la práctica clínica para manejar esta entidad clínica se basan en el empirismo y experiencia de los profesionales. Entre éstos están los tratamientos médicos, como son la podofilina, imiquimod y diferentes variedades del ácido acético, el tratamiento quirúrgico y el tratamiento térmico mediante la coagulación con IR.

Durante la elaboración del protocolo asistencial para el tratamiento de los condiloma/s en el canal anal, se constató la falta de ensayos clínicos bien diseñados que evaluaran la eficacia y seguridad de los diferentes tratamientos comentados. Igualmente, se constató la escasa información sobre la epidemiología de los condiloma/s en el canal en pacientes infectados por el VIH. Este hecho significó el punto de partida para la realización de los trabajos de investigación base de esta tesis doctoral.

El primero de los dos trabajos base de esta tesis doctoral, trata de un estudio epidemiológico transversal, que parte de la primera visita de los pacientes incluidos en la cohorte CARH·MEN, titulado "CONDILOMAS, ANORMALIDADES CITOLÓGICAS E INFECCIÓN POR EL VIRUS DEL PAPILOMA HUMANO EN EL CANAL ANAL EN HOMBRES INFECTADOS POR EL VIH", aporta evidencias a los tres primeros objetivos de esta tesis doctoral.

El **PRIMER OBJETIVO** fue estimar la prevalencia de condiloma/s en el canal anal en hombres infectados por el VIH. Nuestros resultados aportan la evidencia de que entre hombres infectados por el VIH, los condiloma/s en el canal anal es una patología prevalente.

Noventa y tres de los 733 (13%, IC 95%: 10-15%) hombres (65 HSH, 28 HSM) estudiados en la cohorte CARH·MEN refirieron haber sido tratados de condiloma/s en el canal anal en el pasado y no fueron incluidos en el análisis. Entre los 640 hombres restantes (473 HSH, 167 HSM), hombres sin antecedentes o sintomatología de patología en el canal anal relacionada con el VPH, la **prevalencia de condilomas** anales fue del **25%** [157 de 640; IC 95%: 21-28%]. Ahora bien, si consideramos el total de pacientes con antecedentes (93) y diagnosticados de

condiloma/s en el canal anal en la visita basal de la cohorte CARH·MEN (157), representa que 250 de 733 hombres infectados por el VIH han tenido o presentan esta patología, es decir, una prevalencia de condilomas en el canal anal del **34%** (IC 95%: 31-38%). En otras palabras, a 1 de cada 3 hombres infectados por el VIH les fue diagnosticado y tratado de un condiloma/s en el canal anal.

Si se estima que la prevalencia de condiloma/s en el canal anal por la orientación sexual de los 640 hombres estudiados, 473 fueron HSH y 167 HSM, fue del **28%** (132 de 473; IC 95%: 24-32%) entre los **HSH** y del **15%** (25 de 167; IC 95%: 10-21%) entre los **HSM**. En consecuencia, el grupo de HSH presenta una mayor riesgo de presentar esta patología [OR: 2.2; IC 95%: 1.4–3.5],

Ante estos datos, y teniendo en cuenta que la prevalencia de condiloma/s en el canal anal en la población general es del 1%-3% [15], los resultados aportados por nuestros trabajos corroboran la primera de las hipótesis de esta tesis doctoral: "**los condiloma/s en el canal anal en pacientes infectadas por el VIH, especialmente entre los hombres que practican sexo con hombre, es una patología prevalente**". Asimismo, no se debe ignorar que los HSM infectados por el VIH también presentan una elevada prevalencia en comparación a la población general.

Los condiloma/s es una patología relacionada con / causada por / el VPH de bajo nivel oncológico como son los genotipos VPH-6 y VPH-11 (15). Esto nos llevó al **SEGUNDO OBJETIVO** de esta memoria, aportar evidencias sobre la prevalencia de infección por el VPH y la distribución por genotipos del VPH en el canal anal entre los hombres infectados por el VIH. Prácticamente a todos los hombres infectados por el VIH y con diagnóstico de condiloma/s en el canal anal se les detectó una infección por varios (más de uno) genotipos del VPH en el canal anal.

Los resultados sobre la prevalencia de infección por el VPH en el canal anal se presentaron en los dos artículos base de esta tesis doctoral titulados "CONDILOMAS, ANORMALIDADES CITOLÓGICAS E INFECCIÓN POR EL VIRUS DEL PAPILOMA HUMANO EN EL CANAL ANAL EN HOMBRES INFECTADOS POR EL VIH" y "EFECTIVIDAD DE LOS TRATAMIENTOS ABLATIVOS Y FARMACOLÓGICOS PARA EL MANEJO DE LOS CONDILOMAS EN EL CANAL ANAL EN HOMBRES INFECTADOS POR EL VIH. En este segundo trabajo se estudiaron 103 hombres (91 HSH, 12 HSM) infectados por el VIH de los 157 diagnosticados de condiloma/s en el canal anal. Estos 103 pacientes fueron tratados de su/s condiloma/s en el canal en la sección de proctología de la unidad de VIH (el resto de pacientes, 54, fueron tratados en otros centros). Los resultados sobre la prevalencia de infección por el VPH en el canal anal se complementan con el tercer trabajo presentado en esta memoria, un trabajo de soporte, titulado "DISTRIBUCIÓN DE LOS GENOTIPOS DEL VRUS DEL PAPILOMA HUMANO EN MUESTRAS

CITOLÓGICAS E HISTOLÓGICAS DEL CANAL ANAL DE HOMBRES INFECTADOS POR EL VIH QUE TIENEN SEXO CON HOMBRES Y HOMBRES QUE TIENEN SEXO CON MUJERES". En este trabajo se estudiaron los 483 (341 HSH, 142 HSM) de los 733 pacientes de la cohorte CARH·MEN que no presentaban ni antecedentes ni se les diagnosticó un condiloma/s en el canal anal en la visita basal de la cohorte CARH·MEN.

La **prevalencia global** de infección por el **VPH** en el canal anal en la primera visita de la cohorte CARH·MEN (733 hombres: 538 HSH, 195 HSM) fue del **73%** (534 de 733, IC 95%: 70-76%): **84%** (453 de 538, IC 95%: 81-87%) entre HSH y **42%** (81 de 195, IC 95%: 35-49%) entre HSM [37]. La población analizada en el primer trabajo de esta memoria (640 pacientes: 473 HSH, 167 HSM) no incluye a los 93 pacientes con antecedentes de condiloma/s en el canal anal; la prevalencia de infección por el VPH en el canal anal fue idéntica: **73%** (469 de 640, IC 95%: 70-77%).

Cuando se estimó la prevalencia de infección por el VPH en el grupo de hombres infectados por VIH **con condiloma/s**, ésta fue del **92%** (145 de 157, IC 95%: 86-96%): del **95.5%** (126 de 132, IC 95%: 90-98%) en **HSH** y del **76%** (19 de 25, IC 95%: 57-89%) en **HSM**. Mientras que la prevalencia de infección por el VPH en el grupo de hombres **sin condiloma/s** fue del **67%** (324 de 483, IC 95%: 63-71%): del **80%** (273 de 341, IC 95%: 75-83%) en **HSH** y del **36%** (51 de 142, IC 95%: 28-44%) en **HSM**. Es importante destacar que los HSM VIH+ con condiloma/s en el canal anal presentan casi el doble de infección por el VPH respecto los HSM VIH+ sin condiloma/s en el canal anal.

En la misma línea que el primer trabajo, los resultados del segundo artículo de esta tesis doctoral, basados en 103 pacientes (91 HSH, 12 HSM) tratados de sus condiloma/s en el canal anal en nuestra sección de proctología de la unidad de VIH, todos (**100%**) estaban infectados por más de un genotipo de VPH en el canal anal.

Respecto a la distribución de genotipos del VPH en el canal anal en pacientes infectados por el VIH y con condiloma/s en el canal anal, la mayoría de infecciones detectadas fueron por múltiples (más de uno) genotipos del VPH.

Los resultados del primer trabajo mostraron que la distribución por genotipos del VPH fue la siguiente de acuerdo a tener un condiloma/s o no tenerlo en el canal anal:

- VPH-16 (42% frente a 23%, respectivamente),
- VPH-6 (41% frente a 13%, respectivamente),
- VPH -11 (35% frente a 6%, respectivamente),
- VPH -33 (21% frente a 16%, respectivamente),
- VPH -51 (21% frente a 13%, respectivamente) y
- VPH -58 (21% frente a 13%, respectivamente).

El VPH-18 fue detectado en el 11% en pacientes con condilomas y en el 6% en pacientes sin condilomas.

Los resultados del segundo trabajo llevado a cabo en los 103 pacientes tratados de condiloma/s en nuestro centro, mostraron la siguiente distribución por genotipos del VPH:

- VPH-6 (52%, 53 de 103, IC 95%: 42-61%),
- VPH-11 (46%, 47 de 103, IC 95%: 36-56%) y
- VPH-16 (48%, 49 de 103, IC 95%: 38-57%).

La prevalencia acumulada de los genotipos 6 y 11 fue del 97% (52%, 100 de 103, IC 95%: 92-99%). Como era de esperar, los genotipos 6 y 11, agentes etiológicos más habituales de los condiloma/s, fueron detectados en el canal anal en prácticamente en todos los pacientes. Sólo en 3 pacientes no se detectó uno de estos 2 genotipos de bajo riesgo oncogénico. Una explicación plausible estaría en que estos 3 pacientes podrían estar infectados por otros genotipos de VPH de bajo riesgo oncogénico no detectables por nuestra técnica diagnóstica (PCR múltiple). Por otro lado, es importante destacar que el genotipo 16 fue detectado en uno de cada 2 pacientes, y este genotipo de alto riesgo oncogénico es el genotipo más frecuentemente detectado entre los cánceres escamosos del canal anal [9, 11, 27, 28].

Por tanto, los resultados aportados por los trabajos publicados en esta memoria, corroboran la segunda de las hipótesis de esta tesis doctoral: "**las personas infectadas por el VIH con un diagnóstico de condiloma/s en el canal anal, presentan una infección por el VPH en el canal anal por más de un genotipo**".

Las lesiones intraepiteliales del canal son consideradas lesiones pre-cancerosas, otra entidad patológica relacionada con la infección por el VPH por genotipos de alto riesgo oncogénico. La alta prevalencia de infección por genotipos de alto riesgo oncogénico en el canal anal nos llevó hacia el **TERCER OBJETIVO** de esta tesis doctoral: estimar la prevalencia de lesiones escamosas intraepiteliales del canal anal en hombres infectados por el VIH diagnosticadas de condiloma/s en el canal anal. Los resultados sobre la prevalencia de lesiones escamosas intraepiteliales del canal anal publicados en el primer trabajo, se complementan con evidencias presentadas en el tercer trabajo de esta memoria, trabajo de soporte.

En los mismos pacientes con una prevalencia alta de condiloma/s en el canal anal, en la que prácticamente a todos se detectó una infección por el VPH en el canal anal por genotipos de alto riesgo oncogénico, se estudió las anomalías citológicas mediante la prueba de Papanicolaou. Los resultados que se discuten a continuación, contribuyeron a demostrar la alta prevalencia de lesiones escamosas intraepiteliales en el canal anal en este grupo de pacientes infectados por el VIH.

La prevalencia de lesiones escamosas intraepiteliales del canal anal en el grupo de los 250 pacientes infectados por el VIH y con antecedentes o diagnóstico de condiloma/s en la visita basal fue, de acuerdo al diagnóstico citológico de:

- Normal: 34% (85 de 250, IC 95%: 28-40%)
- ASCUS: 15% (37 de 250, IC 95%: 11-20%)
- LEI-B: 42% (105 de 250, IC 95%: 36-48%)
- LEI-A: 8% (20 de 250, IC 95%: 5-12%)
- No evaluable: 1% (3 de 250, IC 95%: 0-3%).

Si se estima la prevalencia de lesiones escamosas intraepiteliales del canal anal en el grupo de hombres con antecedentes de condiloma/s y sin condiloma/s en la visita basal (n=93), ésta fue de:

- Normal: 65% (60 de 93, IC 95%: 54-73%)
- ASCUS: 12% (11 de 93, IC 95%: 7-20%)
- LEI-B: 15% (14 de 93, IC 95%: 9-24%)
- LEI-A: 6% (6 de 93, IC 95%: 3-13%)
- No evaluable: 2% (2 de 93, IC 95%: 0-8%).

Si se estima la prevalencia de lesiones escamosas intraepiteliales del canal anal en el grupo de hombres con diagnóstico de condiloma/s en la visita basal (n=157), ésta fue de:

- Normal: 34% (53 de 157, IC 95%: 27-41%)
- ASCUS: 15% (23 de 157, IC 95%: 10-21%)
- LEI-B: 42% (66 de 157, IC 95%: 35-50%)
- LEI-A: 8% (13 de 157, IC 95%: 5-14%)
- No evaluable: 1% (23 de 157, IC 95%: 0-5%).

En este grupo de pacientes con diagnóstico de condiloma/s en la visita basal con respecto a su comportamiento sexual, el 86% (114 de 132) de los HSH y el 68% (17 de 25) de los HSM presentaron anomalías citológicas anales. Entre los HSH la distribución de lesiones intraepiteliales fue la siguiente:

- Normal: 14% (18 de 132, IC 95%: 9-21%)
- ASCUS: 17% (22 de 132, IC 95%: 11-24%)
- LEI-B: 60% (79 de 132, IC 95%: 51-68%)
- LEI-A: 10% (13 de 132, IC 95%: 6-16%),

y entre los HSM:

- Normal: 28% (7 de 25, IC 95%: 14-28%)
- ASCUS: 16% (4 de 25, IC 95%: 6-35%)
- LEI-B: 52% (13 de 25, IC 95%: 34-70%)
- LEI-A: 4% (1 de 25, IC 95%: 1-20%)

En cambio, la prevalencia de lesiones escamosas intraepiteliales del canal anal en el grupo de hombres sin antecedentes de condiloma/s y sin condiloma/s en la visita basal (n=483), ésta fue, de acuerdo al diagnóstico citológico de:

- Normal: 62% (299 de 483, IC 95%: 58-66%)
- ASCUS: 14% (68 de 483, IC 95%: 11-17%)
- LEI-B: 14% (68 de 483, IC 95%: 11-17%)
- LEI-A: 3% (14 de 483, IC 95%: 2-5%)
- No evaluable: 7% (34 de 483, IC 95%: 5-10%)

De acuerdo a su orientación sexual, este grupo de pacientes sin antecedentes de condiloma/s y sin condiloma/s en la visita basal, la distribución de lesiones intraepiteliales entre los HSH fue la siguiente:

- Normal: 56% (192 de 341, IC 95%: 51-61%)
- ASCUS: 15% (50 de 341, IC 95%: 11-19%)
- LEI-B: 19% (65 de 341, IC 95%: 15-24%)
- LEI-A: 4% (14 de 341, IC 95%: 2-7%)
- No evaluable: 6% (20 de 341, IC 95%: 4-9%),

y entre los HSM:

- Normal: 74% (105 de 142, IC 95%: 66-80%)
- ASCUS: 14% (20 de 142, IC 95%: 9-21%)
- LEI-B: 3% (4 de 142, IC 95%: 1-7%)
- LEI-A: 1% (2 de 142, IC 95%: 0-5%)
- No evaluable: 8% (11 de 142, IC 95%: 4-13%).

Nuestros resultados aportan evidencias que los pacientes infectados por el VIH con un diagnóstico de condiloma/s en el canal anal presentan un mayor riesgo de tener lesiones intraepiteliales de tipo LEI-B o LEI-A respecto a los pacientes infectados por el VIH sin antecedentes y/o un diagnóstico de condiloma/s en el canal anal: 50% versus 17%, respectivamente, y entre HSM: 70 versus 23% y entre HSM: 56% versus 4%.

En el primer trabajo base de esta memoria, se reportó la localización de los condiloma/s en el canal anal de los hombres infectados por el VIH. Éstos se localizaron en la **zona interna del canal anal** en un **71%** (111 de 157; IC 95%: 63-77%) de los pacientes, en el **zona perianal** en un

8% (13 de 157; IC 95%: 5-14%) de los pacientes y en **ambas localizaciones** en **21%** (33 de 157; IC 95%: 15-28%) de los pacientes. La localización de los condiloma/s en el canal anal es una de las "variables" a considerar para decidir el tipo de tratamiento a llevar a cabo.

De acuerdo a la localización de los condiloma/s en el canal anal, cuando éstos se localizan sólo en la zona **intra-anal**, la prevalencia de lesiones escamosas intraepiteliales del canal anal fue de:

- Normal: 17% (19 de 111, IC 95%: 11-25%)
- ASCUS: 20% (22 de 111, IC 95%: 13-28%)
- LEI-B: 53% (59 de 111, IC 95%: 44-62%)
- LEI-A: 10% (11 de 111, IC 95%: 6-17%),

cuando se localizan en la zona **intra-anal y perianal**, la prevalencia de lesiones escamosas intraepiteliales del canal anal fue de:

- Normal: 0% (0 de 33, IC 95%: 0-10%)
- ASCUS: 12% (4 de 33, IC 95%: 5-27%)
- LEI-B: 79% (26 de 33, IC 95%: 62-89%)
- LEI-A: 6% (2 de 33, IC 95%: 2-20%)
- No evaluable: 3% (1 de 33, IC 95%: 1-15%),

y cuando se localizan **sólo en el zona perianal**, la prevalencia de lesiones escamosas intraepiteliales del canal anal fue de:

- Normal: 46% (6 de 13, IC 95%: 23-71%)
- ASCUS: 0% (0 de 13, IC 95%: 0-23%)
- LEI-B: 46% (6 de 13, IC 95%: 23-71%)
- LEI-A: 8% (1 de 13, IC 95%: 1-33%).

Estos resultados muestran que la mayor prevalencia de lesiones escamosas intraepiteliales LEI-B y LEI-A del canal anal se observa cuando los condiloma/s se localizan en ambas zonas del canal anal: intra-anal y perianal, es decir, el 85% (IC 95%: 69-93%) de los pacientes infectados por el VIH y con un diagnóstico de condiloma/s en la zona intra-anal y perianal presentan una lesión intraepitelial en el canal anal.

Los resultados sobre la distribución de lesiones intraepiteliales presentados, refuerzan la tercera de las hipótesis de esta tesis doctoral: "**la presencia de condiloma/s en el canal anal se asocia a una alta prevalencia de lesiones escamosas intraepiteliales en pacientes infectados por el VIH**". Por tanto, la coexistencia de dos patologías relacionadas con el VPH en el canal anal, condiloma/s y lesiones escamosas intraepiteliales, es prevalente en personas infectadas por el VIH.

Por último, el segundo trabajo (documento base de esta tesis doctoral) trata de un estudio epidemiológico titulado "EFECTIVIDAD DE LOS TRATAMIENTOS ABLATIVOS Y FARMACOLÓGICOS PARA EL MANEJO DE LOS CONDILOMAS EN EL CANAL ANAL EN HOMBRES INFECTADOS POR EL VIH". Este estudio retrospectivo longitudinal aportó evidencias al **CUARTO OBJETIVO** de esta tesis doctoral: efectividad de diferentes tratamientos para el manejo de los condilomas en el canal anal. Los resultados de este segundo trabajo, se complementan con las evidencias del cuarto trabajo presentado en esta memoria, trabajo de soporte, titulado "EFECTIVIDAD A LARGO PLAZO DE LA FULGURACIÓN CON INFRARROJOS PARA EL TRATAMIENTO DE LA NEOPLASIA INTRAEPITELIAL GRADO 2 Y 3 DEL CANAL ANAL EN HOMBRES Y MUJERES INFECTADAS POR EL VIH". En este trabajo se estudiaron un total de 69 (55 hombres, 19 mujeres) pacientes infectados por el VIH con un diagnóstico histológico de NIA 2 ó 3.

De acuerdo a nuestro protocolo asistencial, los condiloma/s anales en pacientes infectados por el VIH son tratados o con tratamiento farmacológico (imiquimod) o con tratamiento ablativo (coagulación con infrarrojos, escisión quirúrgica o con crioterapia –esporádicamente-). Según las características de los condiloma/s se propone al paciente uno de los tratamientos mencionados. El tratamiento aplicado es decidido entre el cirujano y el paciente. La efectividad de los tratamientos se evaluó mediante la tasa acumulada de recidiva del condiloma al año del tratamiento. Todos los tratamientos consiguieron erradicar los condiloma/s del canal anal a los 3 meses de haber aplicado el tratamiento. En cambio, al año de haber sido tratados, la tasa acumulada de recidiva fue del 8% (4 de 65, IC 95%: 2-15%) con escisión con electrocirugía, 11% (3 de 27, IC 95%: 4-28%) con fulguración con infrarrojos y 11% (1 de 9, IC 95%: 2-44%) con tratamiento farmacológico. A los 10 años después del tratamiento, la tasa acumulada de recidiva global fue del 49,5% (51 recidiva en 103 pacientes tratados, IC 95%: 40-59%). La tasa (rango) de recidiva fue de 13.6 (10.2, 17.9) por cada 100 personas / año. Por tratamientos, la tasa de recidiva acumulada fue del 46% (30 de 65, IC 95%: 35-58%) tras escisión con electrocirugía, 56% (15 de 27, IC 95%: 37-72%) tras coagulación con infrarrojos, 56% (5 de 9, IC 95%: 27-81%) tras tratamiento farmacológico y 50% (1 de 2, IC 95%: 1- 91%) tras crioterapia. Resultados similares han sido reportados por otros autores [16, 33, 38-43].

El dolor fue el principal acontecimiento adverso referido por los pacientes y relacionado con los tratamientos [7 (11%) pacientes después de la escisión quirúrgica, 2 (7%) pacientes después de la fulguración con infrarrojos y 1 (11%) después del tratamiento farmacológico (imiquimod). Tres (5%) pacientes desarrollaron una estenosis en el canal anal y 1 (2%) presentó un absceso en el lecho quirúrgico tras la escisión con electrocirugía. Todos los pacientes tratados con imiquimod tuvieron una irritación de la piel o sensación de ardor. No se recogieron eventos adversos relacionados con la obtención de una muestra del canal anal para el estudio citológico y de la infección por el VPH.

Respecto a la efectividad del tratamiento de las lesiones intraepiteliales grado 2 o 3 del canal anal con coagulación con infrarrojos, 7 (13%) pacientes de 66 (3 pacientes rechazaron ser tratados) presentaron una recurrencia de la lesión. La mediana (rango) de tiempo hasta recurrencia fue de 30 (18-43) meses. A los 3 años el 50% habían presentado una recurrencia de su lesión intraepitelial (datos no publicados).

Los resultados sobre la efectividad de los tratamientos para el manejo de los condiloma/s en el canal anal presentados en esta tesis, corroboran la cuarta de las hipótesis de esta tesis doctoral: "**los diferentes tratamientos usados en el manejo de los condiloma/s del canal anal en la práctica clínica presentan una alta tasa de recidiva entre las en personas infectadas por el VIH**".

Limitaciones

Diferentes limitaciones han de ser consideradas en el momento de establecer las conclusiones:

1. La población estudiada corresponde a pacientes infectados por el VIH sin sintomatología de patología por condiloma/s en el canal anal, es decir, en pacientes asintomáticos o no conscientes de tener esta patología, que aceptaron entrar en el programa de cribado. El condiloma/s en el canal anal fue diagnosticado en la primera visita de la cohorte CARH·MEN. Esto podría sobreestimar o infraestimar la extrapolación de los resultados más allá de los pacientes infectados por el VIH sin sintomatología de patología por condiloma/s en el canal anal.

2. Los resultados de la infección por VPH y de la distribución por genotipos presentados en esta tesis doctoral corresponden a la citología del canal anal en donde estaban asentados los condiloma/s. No se realizó un análisis propiamente de los condiloma/s por lo que no podemos conocer qué genotipo de VPH está relacionado directamente con el condiloma/s. Esto puede sobreestimar o infraestimar los resultados presentados.

3. El bajo número de pacientes infectados por el VIH que presentaron un condiloma/s exclusivamente perianal (13 de 157) puede dificultar establecer con certeza la relación entre las dos patologías causadas por el VPH: condiloma/s y lesiones intraepiteliales.

4. El número exacto de condilomas en el canal anal no fue recogido de manera sistemática en la historia clínica electrónica, y la anoscopia de alta resolución sólo fue realizada en aquellos pacientes con una lesión intraepitelial, Es decir, el número y el diagnóstico de condiloma/s intraanales en pacientes con una citología normal en el canal anal puede estar infraestimada.

5. Una limitación metodológica que debe ser comentada es que en ningún de los cuatro estudios de esta tesis doctoral se realizó un cálculo del tamaño de la muestra formal. Esto podría sobreestimar o infraestimar los resultados obtenidos tanto de las prevalencias estimadas como de la efectividad de los tratamientos.

6. Otra limitación metodológica es el diseño observacional retrospectivo longitudinal llevado a cabo para evaluar la efectividad de los tratamientos utilizados en nuestra práctica clínica (segundo y cuarto estudio de esa tesis doctoral). Consideramos que esta limitación es minimizada por el hecho de que la información fue recogida de una base de datos electrónica cumplimentada de forma prospectiva y por el hecho de que la variable principal, recidiva del condiloma/s en el canal anal, es una variable fácilmente objetivable y evaluable. No obstante, se necesita llevar a cabo ensayos clínicos diseñados para comparar la eficacia entre los tratamientos.

7. Los tratamientos fueron ofrecidos de acuerdo a las características de los condiloma/s siguiendo nuestro protocolo asistencial. Esto podría sobreestimar o infraestimar la extrapolación de los resultados más allá de nuestra guía terapéutica. Asimismo, en el caso del tratamiento farmacológico con imiquimod, no puede ser garantizado la correcta aplicación y seguimiento de este tratamiento.
8. Todos los trabajos presentados en esta memoria corresponden a pacientes infectados por el VIH de una misma área geográfica. Esto podría sobreestimar o infraestimar la extrapolación de nuestros resultados a otra área geográfica.

Trabajos futuros de la línea de investigación

Los resultados obtenidos constituyen los primeros de una línea de investigación viva, englobada dentro de la actividad investigadora del grupo para el estudio de la co-infección por VIH y VPH.

Nuevas dudas, nuevas preguntas han surgido a lo largo de estos años de duro trabajo y tras la interpretación de los resultados. Realizar estudios de investigación encaminados a dar respuesta a las mismas completaría y reforzaría los resultados de esta tesis doctoral. A continuación se enumeran las mismas:

1. La alta prevalencia de la coexistencia de dos patologías relacionadas con el VPH, condiloma/s y lesiones intraepiteliales en el canal anal, nos hace plantear qué factores / características de los pacientes facilitan esta coexistencia.

   Se ha valorado trabajar en modelos matemáticos que aporten información sobre qué combinación (o 'cluster') de genotipos de VPH están favoreciendo que esta patología *a priori* banal (condiloma/s) sea un factor de riesgo para desarrollar un CEA.

2. En paralelo a la propuesta anterior, actualmente, se está trabajando en cultivo con células "sanas" e infectadas con diferentes genotipos del VPH y en cultivo con células infectadas por el VIH e infectadas con diferentes genotipos del VPH, para estudiar qué mecanismos intracelulares pueden estar facilitando que esta patología *a priori* banal (condiloma/s) sea un factor de riesgo para desarrollar un CEA.

3. Dada la alta tasa de recidiva de los condiloma/s en el canal anal en pacientes infectados por el VIH, es necesario llevar a cabo ensayos clínicos que aporten evidencias de la eficacia y seguridad de los tratamientos. Los resultados de esta tesis doctoral son la base curricular para solicitar ayudas competitivas para la realización de estos ensayos clínicos.

4. En la actualidad, los recursos que pueden ser destinados al gasto sanitario que deriva de esta patología, son limitados, por lo que es necesario racionalizar y priorizar la asignación de estos recursos a las opciones que presenten mayores ventajas económicas, siempre y cuando la salud de los pacientes no se vea amenazada.

   La realización de estudios de fármaco-economía aportaría información valiosa para conocer cuál es la eficiencia de los programas de cribado de patología relacionada con el VPH en el canal anal en pacientes infectados por el VIH.

5. En línea a lo mencionado en el párrafo anterior, la realización de estudios de fármaco-economía aportaría información valiosa para conocer cuál es la eficiencia de las diferentes alternativas terapéuticas disponibles en el mercado. Así, se contribuiría a determinar que opciones terapéuticas se deberían emplear de forma rutinaria.

Conclusiones

Las conclusiones obtenidas en los trabajos de investigación que conforman la presente tesis doctoral son las siguientes:

1. Los condiloma/s en el canal anal en pacientes infectadas por el VIH, especialmente entre los hombres que practican sexo con hombres, es una patología prevalente respecto a la población general. La exploración sistemática para la identificación de esta patología debería ser instaurada en la práctica clínica en pacientes infectados por el VIH.
2. Las personas infectadas por el VIH con un diagnóstico de condiloma/s en el canal anal, presentan una infección por el VPH en el canal anal por más de un genotipo. Es decir, este grupo de pacientes se caracteriza por estar infectados por genotipos de VPH de bajo riesgo oncogénico propios de los condilomas y por genotipos de alto riesgo oncogénico. Esto hace que sean un grupo de pacientes con un alto riesgo de desarrollar un CEA.
3. Como relación y consecuencia de la conclusión 2, la presencia de condiloma/s en el canal anal se asocia a una alta prevalencia de lesiones escamosas intraepiteliales del canal anal en personas infectadas por el VIH. Por tanto, la coexistencia de dos patologías relacionadas con el VPH el canal anal: condiloma/s y lesiones escamosas intraepiteliales, es prevalente en personas infectadas por el VIH. Esto corrobora que sean un grupo de pacientes con un alto riesgo de desarrollar un CEA.
4. Los diferentes tratamientos usados en el manejo de los condiloma/s del canal anal en la práctica clínica presentan una alta tasa de recidiva entre las personas infectadas por el VIH. Es necesario realizar ensayos clínicos que aporten evidencias sobre la eficacia y seguridad de los tratamientos, y permitan personalizar cuál es el mejor tratamiento a ofrecer a nuestros pacientes.

Bibliografía

1) Del Amo J, Campbell C, Navarro G, Segura F, Suárez I, Teira R, Brañas F, Serrano-Villar S, Moreno S, Morillo R, Román I, Marrugat J, Fernández E, Marco MP, Blanch J, Castaño M, Pujol F, Fuster MJ, Hernández JS, García-Goñi M, Nuño-Solinís R, Elizondo N, Nuño-Solinís JEDL, Gol-Montserrat J. **HIV in Spain 2017: policies for a new management of chronicity beyond virological control.** Rev Esp Salud Publica. 2018; 92. pii: e201809062.

2) Park IU, Introcaso C, Dunne EF. **Human Papillomavirus and Genital Warts: A Review of the Evidence for the 2015 Centers for Disease Control and Prevention Sexually Transmitted Diseases Treatment Guidelines.** Clin Infect Dis. 2015; 61 Suppl 8:S849-55. doi: 10.1093/cid/civ813. Review.

3) Dietrich A, Hermans C, Heppt MV, Ruzicka T, Schauber J, Reinholz M. **Human papillomavirus status, anal cytology and histopathological outcome in HIV-positive patients.** J Eur Acad Dermatol Venereol. 2015; 29(10):2011-8. doi: 10.1111/jdv.13205.

4) Oette M, Mosthaf FA, Sautter-Bihl ML, Esser S. **HIV-Associated Anal Dysplasia and Anal Carcinoma.** Oncol Res Treat. 2017; 40(3):100-105. doi: 10.1159/000456715. Review.

5) Iribarren-Díaz M, Ocampo Hermida A, González-Carreró Fojón J, Alonso-Parada M, Rodríguez-Girondo M. **Practical considerations for high resolution anoscopy in patients infected with human immunodeficiency virus].** Enferm Infecc Microbiol Clin. 2014; 32(10):676-80. doi: 10.1016/j.eimc.2013.07.017. Review.

6) Berry JM, Palefsky JM, Jay N, Cheng SC, Darragh TM, Chin-Hong PV. **Performance characteristics of anal cytology and human papillomavirus testing in patients with high-resolution anoscopy-guided biopsy of high-grade anal intraepithelial neoplasia.** Dis Colon Rectum. 2009; 52(2):239-47. doi: 10.1007/DCR.0b013e31819793d9.

7) Tamalet C, Ravaux I, Dhiver C, Menard A, Colson P, Stein A. **Feasibility and Acceptability of** Anal **Self-Sampling for Human Papillomavirus Screening in HIV-Infected Patients.** Intervirology. 2016; 59(2):118-122. doi: 10.1159/000452710.

8) Nahas CS, da Silva Filho EV, Segurado AA, Genevcius RF, Gerhard R, Gutierrez EB, Marques CF, Cecconello I, Nahas SC. **Screening anal dysplasia in HIV-infected patients: is there an agreement between anal pap smear and high-resolution anoscopy-guided biopsy?** Dis Colon Rectum. 2009; 52(11):1854-60. doi: 10.1007/DCR.0b013e3181b98f36.

9) Duvoisin Cordoba C, Clerc D, Vanoni A, Pache B, Hübner M, Demartines N, Hahnloser D. **Screening for anal cancer: is it the same as for cervical cancer?** Rev Med Suisse. 2018; 14(611):1230-1236.

10) Poynten IM, Waterboer T, Jin F, Templeton DJ, Prestage G, Donovan B, Pawlita M, Fairley CK, Garland SM, Grulich AE. **Human papillomavirus types 6 and 11 seropositivity: risk factors and association with ano-genital warts among homosexual men.** J Infect. 2013; 66(6):503-11. doi: 10.1016/j.jinf.2013.03.005.

11) Darwich L, Cañadas MP, Videla S, Coll J, Piñol M, Cobarsi P, Molina-López RA, Vela S, García-Cuyás F, Llatjos M, Sirera G, Clotet B; HIV-HPV Can Ruti Team. **Association of Anal Condylomata with Human Papillomavirus Infection and Cytological Abnormalities in the anal canal in HIV-infected Men.** HIV Med 2012; 13(9):549-57. DOI: 10.1111/J.1468-1293.2012.01013.X.

12) Johnstone AA, Silvera R, Goldstone SE. **Targeted ablation of perianal high-grade dysplasia in men who have sex with men: an alternative to mapping and wide local excision.** Dis Colon Rectum. 2015; 58(1):45-52. doi: 10.1097/DCR.0000000000000241.

13) Goldstone RN, Goldstone AB, Russ J, Goldstone SE. **Long-term follow-up of infrared coagulator ablation of anal high-grade dysplasia in men who have sex with men.** Dis Colon Rectum. 2011; 54(10):1284-92. doi: 10.1097/DCR.0b013e318227833e.

14) Goldstone RN, Hasan SR, Drury S, Darragh TM, van Zante A, Goldstone SE. **A trial of radiofrequency ablation for** anal **intraepithelial neoplasia.** Int J Colorectal Dis. 2017; 32(3):357-365. doi: 10.1007/s00384-016-2679-2.

15) Anderson CA, Boller AM, Richardson CJ, Balcos EG, Zera RT. **Anal condyloma: a comparison between HIV positive and negative patients.** Am Surg. 2004; 70(11):1014-8.

16) D'Ambrogio A, Yerly S, Sahli R, Bouzourene H, Demartines N, Cotton M, Givel JC. **Human papilloma virus type and recurrence rate after surgical clearance of anal condylomata acuminata.** Sex Transm Dis. 2009; 36(9):536-40. doi: 10.1097/OLQ.0b013e3181a866a3.

17) Hillman RJ, Cuming T, Darragh T, Nathan M, Berry-Lawthorn M, Goldstone S, Law C, Palefsky J, Barroso LF, Stier EA, Bouchard C, Almada J, Jay N. **2016 IANS International Guidelines for Practice Standards in the Detection of Anal Cancer Precursors**. J Low Genit Tract Dis. 2016; 20(4):283-91. doi: 10.1097/LGT.0000000000000256.

18) Medina-Laabes DT, Suarez-Perez EL, Guiot HM, Muñoz C, Colón-López V, Tirado-Gómez M, Ortiz AP. **Human Papillomavirus Correlates With Histologic Anal High-Grade Squamous Intraepithelial Lesions in Hispanics With** HIV**.** J Low Genit Tract Dis. 2018; 22(4):320-325. doi: 10.1097/LGT.0000000000000416.

19) Chuang E, Lim E, Milne C, Zhu X, Agsalda M, Killeen J, Miller FD, Hernandez BY, Shiramizu B. **Human Papillomavirus at Multiple Sites Associated with Anal Squamous Intraepithelial Lesions in HIV-Seropositive Individuals.** Ann Clin Cytol Pathol. 2016; 2(4). pii: 1029.

20) Bennetts LE, Wagner M, Giuliano AR, Palefsky JM, Steben M, Weiss TW. **Associations of Anogenital Low-Risk Human Papillomavirus Infection With Cancer and Acquisition of HIV.** Sex Transm Dis. 2015; 42(10):541-4. doi: 10.1097/OLQ.0000000000000319. Review.

21) Apaydin KZ, Nguyen A, Borba CPC, Shtasel DL, Ulery S, Mayer KH, Keuroghlian AS. **Factors associated with anal cancer screening follow-up by high-resolution anoscopy.** Sex Transm Infect. 2018 Jun 22. pii: sextrans-2017-053515. doi: 10.1136/sextrans-2017-053515.

22) Vyas NS, Pierce Campbell CM, Mathew R, Abrahamsen M, Van der Kooi K, Jukic DM, Stoler MH, Villa LL, da Silva RC, Lazcano-Ponce E, Quiterio M, Salmeron J, Sirak BA, Ingles DJ, Giuliano AR, Messina JL. **Role of histological findings and pathologic diagnosis for detection of human papillomavirus infection in men.** J Med Virol. 2015; 87(10):1777-87. doi: 10.1002/jmv.24238.

23) Buzard CL, Rizzolo D. **An overview of anal intraepithelial neoplasia.** JAAPA. 2018; 31(7):1-5. doi: 10.1097/01.JAA.0000534979.69236.e7. Review.

24) Elorza G, Saralegui Y, Enríquez-Navascués JM, Placer C, Velaz L. **Anal intraepitelial neoplasia: A narrative review.** Rev Esp Enferm Dig. 2016; 108(1):31-9. Review.

25) Wasserman P, Rubin DS, Turett G. **Review: Anal Intraepithelial Neoplasia in HIV-Infected Men Who Have Sex with Men: Is Screening and Treatment Justified?** AIDS Patient Care STDS. 2017; 31(6):245-253. doi: 10.1089/apc.2017.0063.

26) Schlecht HP, Fugelso DK, Murphy RK, Wagner KT, Doweiko JP, Proper J, Dezube BJ, Panther LA. **Frequency of occult high-grade squamous intraepithelial neoplasia and invasive cancer within anal condylomata in men who have sex with men.** Clin Infect Dis. 2010; 51(1):107-10. doi: 10.1086/653426.

27) Abramowitz L, Benabderrahmane D, Ravaud P, Walker F, Rioux C, Jestin C, Bouvet E, Soulé JC, Leport C, Duval X. **Anal squamous intraepithelial lesions and condyloma in HIV-infected heterosexual men, homosexual men and women: prevalence and associated factors.** AIDS. 2007; 21(11):1457-65.

28) Durot C, Dohan A, Boudiaf M, Servois V, Soyer P, Hoeffel C. **Cancer of the Anal Canal: Diagnosis, Staging and Follow-Up with MRI.** Korean J Radiol. 2017; 18(6):946-956. doi: 10.3348/kjr.2017.18.6.946.

30) Goldstone S. **Effectiveness of infrared coagulation ablation of high-grade anal dysplasia: are the results too good to be true?** AIDS. 2013; 27(7):1194-5. doi: 10.1097/QAD.0b013e32835f1fec.

31) Willems N, Libois A, Nkuize M, Feoli F, Delforge M, DeWit S. **Treatment of anal dysplasia in HIV-positive men who have sex with men in a large AIDS reference centre.** Acta Clin Belg. 2017; 72(1):29-35. doi: 10.1080/17843286.2015.1116725. Epub 2016 Jun 16.

32) Goldstone SE, Kawalek AZ, Huyett JW. **Infrared coagulator: a useful tool for treating anal squamous intraepithelial lesions.** Dis Colon Rectum. 2005; 48(5):1042-54.

33) Cranston RD, Hirschowitz **SL**, Cortina G, Moe AA. **A retrospective clinical study of the treatment of high-grade anal dysplasia by infrared coagulation in a population of HIV-positive men who have sex with men.** Int J STD AIDS. 2008; 19(2):118-20. doi: 10.1258/ijsa.2007.005665.

34) Silvera RJ, Smith CK, Swedish KA, Goldstone SE. **Anal condyloma treatment and recurrence in HIV-negative men who have sex with men.** Dis Colon Rectum. 2014; 57(6):752-61. doi: 10.1097/DCR.0000000000000080.

35) Fazendin EA, Crean AJ, Fazendin JM, Kucejko RJ, Gill HS, Poggio JL, Stein DE. **Condyloma Acuminatum, Anal Intraepithelial Neoplasia, and Anal Cancer in the Setting of HIV: Do We Really Understand the Risk?** Dis Colon Rectum. 2017; 60(10):1078-1082. doi: 10.1097/DCR.0000000000000890.

36) Manzione Tda S, Nadal SR, Calore EE, Nadal LR, Manzione CR. **Local control of human papillomavirus infection after anal condylomata acuminata eradication.** Rev Col Bras Cir. 2014; 41(2):87-91.

37) Silverberg MJ, Lau B, Justice AC, Engels E, Gill MJ, Goedert JJ, Kirk GD, D'Souza G, Bosch RJ, Brooks JT, Napravnik S, Hessol NA, Jacobson LP, Kitahata MM, Klein MB, Moore RD, Rodriguez B, Rourke SB, Saag MS, Sterling TR, Gebo KA, Press N, Martin JN, Dubrow R; North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) of IeDEA. **Risk of anal cancer in HIV-infected and HIV-uninfected individuals in North America**. Clin Infect Dis. 2012; 54(7):1026-34. doi: 10.1093/cid/cir1012.

38) Videla S, Darwich L, Cañadas MP, Coll J, Piñol M, García-Cuyás F, Molina-Lopez RA, Cobarsi P, Clotet B, Sirera G; HIV-HPV Study Group. **Natural history of human papillomavirus infections involving anal, penile, and oral sites among HIV-positive men**. Sex Transm Dis. 2013; 40:3-10. doi: 10.1097/OLQ.0b013e31827e87bd.

39) Mistrangelo M, Dal Conte I, Volpatto S, DI Benedetto G, Testa V, Currado F, Morino M. **Current treatments for anal condylomata acuminata.** Minerva Chir. 2018; 73(1):100-106. doi: 10.23736/S0026-4733.17.07554-X. Review.

40) Websky MWV, Wesselmann PC, Schwarze-Zander C, Vilz TO, Stoffels B, Boesecke C, Rockstroh J, Kalff JC, Pantelis D. **Treatment of HPV-associated** Anal **Lesions in HIV-positive Patients - Comparison of Surgical Treatment and Topical Therapy with Imiquimod].** Zentralbl Chir. 2018 Jul 3. doi: 10.1055/a-0632-2017. [Epub ahead of print] German.

41) Braga EA, Lopes GJ Filho, Saad SS. **Argon plasma versus electrofulguration in the treatment of anal and perianal condylomata acuminata in patients with acquired immunodeficiency virus.** Acta Cir Bras. 2017; 32(6):482-490. doi: 10.1590/s0102-86502017006000009. Erratum in: Acta Cir Bras. 2017 Sep 28;:0.

42) Leszczyszyn J, Łebski I, Łysenko L, Hirnle L, Gerber H. **Anal warts (condylomata acuminata) - current issues and treatment modalities.** Adv Clin Exp Med. 2014; 23(2):307-11. Review.

43) Mistrangelo M. **Surgical treatment of anal condylomata acuminata.** Dis Colon Rectum. 2009; 52(10):1803; author reply 1803. doi: 10.1007/DCR.0b013e3181ab3185.