IMPORTANCIA DE LAS ADIPOCITOCINAS, LOS FACTORES CLÁSICOS DE INFLAMACIÓN Y LA ECOGRAFÍA EN LA VALORACIÓN DE LA SEVERIDAD DE LA ARTROSIS DE RODILLA MEDIDA POR CUESTIONARIOS Y RADIOGRAFÍA SIMPLE

María García Manrique de Lara

Universitat Autònoma de Barcelona

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Doctoranda:

María García Manrique de Lara

Tutor:

Dr. Jaume Almirall i Daly

Directores:

Dr. Jordi Gratacós i Masmitjà

Dr. Joan Calvet i Fontova

A mi familia, al Servei de Reumatologia,

a la Unitat Científico Tècnica del Taulí

y a Toni Berenguer, pero, sobre todo,

a Joan

ABREVIATURAS

AP: anteroposterior

AR: artritis reumatoide

AUC: área bajo la curva

CSF-1: factor estimulante de la colonia macrofágica

CTX: telopéptido C terminal del colágeno

DAMP: Damage Activated Molecular Patterns

DL: deep learning

DLP: dislipemia

ELISA: enzimoinmunoanálisis de absorción

EULAR: European Alliance of Associations For Rheumathology

HDL: lipoproteína de alta densidad

HTA: hipertensión arterial

IA: inteligencia artificial

IL: interleucina

IMC: índice de masa corporal

K/L: Kellgren Lawrence

KOIP: Knee Osteoarthritis Inflammatory Phenotypes

KOOS: Knee injury and Osteoarthritis Outcome Score

LCA: ligamento cruzado anterior

LDL: lipoproteína de baja densidad

LS: líquido sinovial

MMP: metaloproteasa

MTF: metatarsofalángica

NGF: factor de crecimiento neuronal

NTX: telopéptido N-terminal del colágeno

OA: artrosis

OARSI: Osteoarthritis Research Society International

OMERACT: Outcome Measures in Rheumatology

PA: posteroanterior

PCR: proteína C reactiva

RMN: resonancia nuclear magnética

SASP: Fenotipo Secretor asociado a la Senescencia

TG: triglicéridos

TLR: Toll Like Receptor

TNF-α: factor de necrosis tumoral alfa

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RESUMEN

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La artrosis ha sido objeto de abundante investigación en los últimos años con una limitada traducción a la práctica clínica, probablemente debido a su gran heterogeneidad. Actualmente, no existe consenso sobre una clasificación integral en fenotipos con relevancia clínica para esta enfermedad ni tampoco medidas terapéuticas específicamente dirigidas a cada paciente.

El objetivo de nuestro estudio es avanzar en el conocimiento de los factores inflamatorios y metabólicos que influyen en la gravedad clínica y radiográfica de las mujeres afectas de artrosis de rodilla con derrame articular. De esta forma evolucionar hacia una medicina personalizada y de precisión.

Para ello se han analizado, tanto en el plasma como en el líquido articular, una serie de factores de inflamación y adipocitocinas; añadiendo la interleucina 8 (IL-8) a este panel previo. Se observó una asociación entre los niveles de IL-8 en líquido sinovial y la gravedad clínica, así como una relación entre diversos factores de inflamación y la IL-8 también en líquido articular. Estos datos no se evidenciaron a nivel plasmático; sugiriendo una mayor implicación de los mecanismos inflamatorios locales que sistémicos y unas posibles vías de inflamación diferenciales en plasma que en líquido articular. Posteriormente, y debido a las asociaciones entre las diversas citocinas y factores metabólicos, se realizó un análisis de clústers, obteniendo cuatro fenotipos distintos dentro del propio fenotipo inflamatorio, KOIPs del inglés (*Knee Osteoarthritis Inflammatory Phenotype*). Estos fenotipos, además de diferenciarse claramente por sus factores inflamatorios y metabólicos definitorios, presentan implicaciones en severidad

clínica y radiográfica. La identificación de estos fenotipos podría tener una aplicabilidad en práctica clínica con implicaciones tanto pronósticas como terapéuticas, sugiriendo nuevas vías para generar futuros tratamientos.

ABSTRACT

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Osteoarthritis has been the subject of abundant research in recent years with limited translation to clinical practice, likely due to its considerable heterogeneity. Currently, there is no consensus on a comprehensive classification into phenotypes with clinical relevance for this disease, nor are there specifically targeted therapeutic measures for each patient.

The aim of our study is to advance the understanding of inflammatory and metabolic factors that influence the clinical and radiographic severity of knee osteoarthritis in women with joint effusion, thus progressing towards personalized and precision medicine.

To achieve this, a series of inflammation factors and adipocytokines were analyzed in both plasma and joint fluid, with interleukin 8 (IL-8) initially added to this panel. An association was observed between IL-8 levels in synovial fluid and clinical severity, as well as a relationship between various inflammation factors and IL-8 also in joint fluid. These associations were not evident at the plasma level, suggesting a greater involvement of local inflammatory mechanisms than systemic ones, and possible differential inflammation pathways in plasma compared to joint fluid.

Subsequently, due to the associations between various cytokines and metabolic factors, a clustering analysis was conducted, resulting in four distinct phenotypes within the inflammatory phenotype, called KOIPs (Knee Osteoarthritis Inflammatory Phenotype). These phenotypes, besides being clearly differentiated by their defining inflammatory and metabolic factors, have implications for clinical and radiographic severity. The

identification of these phenotypes could have applicability in clinical practice with both prognostic and therapeutic implications, suggesting new pathways for future treatments.

1. INTRODUCCIÓN: LA ARTROSIS

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1.1 Definición

La artrosis (OA) es una enfermedad crónica que afecta a la articulación y a sus tejidos circundantes, provocando daño estructural progresivo [1].

Clínicamente se caracteriza por un defecto en el funcionamiento de la articulación, asociado a un grado variable de dolor, discapacidad y disminución de la calidad de vida [2].

Existen diferentes definiciones aceptadas para la OA, pero en general se define por la pérdida focal del cartílago articular por fibrilación, fisuración y ulceración; reacción ósea subcondral y afectación de otras estructuras adyacentes como los ligamentos, meniscos, cápsula, membrana sinovial y músculo periarticular [3]. Según la OARSI (*Osteoarthritis Research Society International*), la sociedad médica dedicada a la investigación en OA, se define como un desorden que afecta a las articulaciones móviles caracterizado por presentar estrés celular y degradación de la matriz extracelular iniciados por micro y macro fracturas que activan respuestas de reparación anómalas [4]. La OA afecta a todos los componentes de la articulación y provoca modificaciones moleculares, bioquímicas, celulares, biomecánicas y morfológicas, que van ocasionando progresivamente cambios estructurales como la degradación del cartílago articular con pérdida progresiva de cartílago, la neoformación de osteofitos, alteraciones en el hueso subcondral y la inflamación de la membrana sinovial, que son rasgos distintivos de la enfermedad [4]. A partir de esta definición, se ha extendido la interpretación de la articulación como un órgano más de nuestro organismo; interpretándose como una entidad compleja y

heterogénea, que no solamente afecta al cartílago articular, sino que implica a todos los componentes de la articulación [5].

Paralelamente, una interpretación que nos permite considerar la OA como una enfermedad de órgano y no solamente del cartílago articular es análisis del dolor, manifestación prioritaria de la artrosis [6]. El cartílago articular es una estructura avascular desprovista de fibras nerviosas, por lo que no se puede considerar el origen del dolor. Las estructuras periarticulares, como la membrana sinovial, están abundantemente inervadas y parecen tener más relevancia en la génesis del dolor; hecho que reafirma la interpretación de la artrosis como una enfermedad de la articulación en global [7].

Las articulaciones más comúnmente afectadas son las rodillas, caderas, columna, manos y pies, aunque la mayor parte de la carga sociosanitaria se relaciona con la OA de rodilla y cadera [8].

En los últimos años se han realizado múltiples trabajos de investigación dirigidos a estudiar la fisiopatogenia de la OA. Fruto de estos estudios, conocemos que factores como la edad, la obesidad, factores genéticos, traumatismos previos, biomecánicos, metabólicos, sustancias que favorecen la degradación del cartílago y la inflamación tanto local como sistémica ejercen un efecto tanto en la aparición como en la gravedad y progresión de la artrosis en distintas localizaciones [9]]. De todos modos, actualmente todavía se considera la fisiopatología de la OA como desconocida o no bien definida [10].

1.2 Prevalencia

La OA es la enfermedad articular más frecuente, y constituye uno de los principales problemas de salud en los países desarrollados [11]. La obesidad, el sedentarismo y la longevidad propias de dichos países se relacionan con un crecimiento continuo de su incidencia [12].

Pero este incremento en su incidencia no se explica sólo por estos factores de riesgo bien conocidos. En un estudio reciente realizado a partir de esqueletos de individuos fallecidos entre los años 1905 y 2015, se objetiva un aumento en la prevalencia de la artrosis de rodilla de más del doble en los individuos de la era postindustrial (2.1, IC 95%: 1.5 a 3.1), independientemente del ajuste por edad e índice de masa corporal [13]. Esto sugeriría la existencia de otros factores no tan bien conocidos, que han aparecido o se han visto incrementados en los últimos tiempos.

La elevada prevalencia de la OA tiene un impacto económico importante en los presupuestos de los países de nuestro entorno, constituyendo una de las principales causas del gasto sanitario en países como Estados Unidos [14]. El consumo de fármacos para el alivio del dolor y el reemplazo articular mediante prótesis en los casos más severos suponen un coste directo muy elevado [15]. Además, habría que considerar los costes indirectos asociados al absentismo laboral, hecho que aumentara sustancialmente el impacto económico de la artrosis sobre el sistema sanitario [16,17].

Para conocer la prevalencia de una enfermedad se utilizan estudios epidemiológicos y encuestas poblacionales de salud. En la artrosis existe una gran variabilidad en cuanto a

los datos epidemiológicos [18]. Esto es así por las diferencias metodológicas y de criterios de inclusión existentes entre los distintos estudios [19].

Dado que en la artrosis existe una disociación clínico-radiológica importante, la prevalencia variará significativamente en función de si los criterios de inclusión utilizados son clínicos o radiológicos [20].

En un análisis epidemiológico realizado en 195 territorios mayoritariamente del continente americano, desde el año 1990 hasta el 2017, se estimó la prevalencia puntual de la OA en 3754.2 (IC 95%: 3389.4 a 4187.6); y una tasa de incidencia anual de 181.2 (IC 95%: 62.6 a 202.4), ambos referenciados por 100.000 habitantes, lo que supuso un incremento con respecto a datos previos [21].

En cuanto a Europa, existen datos que sugieren que existe afectación radiológica severa a nivel de rodilla en el 1% de los individuos entre 25 y 34 años [22], y que este porcentaje aumenta paralelamente a la edad hasta casi el 50% en individuos ≥ a 75 años[23].

Sin embargo, existen diferencias geográficas importantes. Así por ejemplo, en China, la prevalencia parece triplicar a la europea, aumentándose esta diferencia en las comunidades más rurales [24].

Según datos del estudio EPISER2016, que es un estudio transversal basado en la población española, la prevalencia de la OA sintomática en España es de un 29.35% (IC 95%: 27.77 a 30.97), llegando a alcanzar el 52.6% en individuos mayores de 80 años (IC 95%: 46.97 a 58.29), siendo las localizaciones más frecuentemente afectadas las rodillas (13.83%, IC 95%: 12.66 a 15.11); y la columna lumbar (15.52%, IC 95%: 14.30 a 16.83) [25]. Estos porcentajes son superiores a los reflejados en el EPISER2000, y se justifican

no sólo por los cambios sociodemográficos que han tenido lugar en los últimos años, sino por la inclusión de artrosis radiológica [26].

1.3 Etiología

La fisiopatología de la OA es multifactorial y compleja [5]. Actualmente parece claro que se produce por una combinación de factores genéticos, biomecánicos e inflamatorios [27], que actuarían de forma coincidente hasta la aparición del daño articular [28,29]. Estos últimos han adquirido especial importancia en los últimos años, considerándose la OA un estado inflamatorio de bajo grado, tanto local como sistémico [30].

La inflamación sistémica mantenida de los pacientes con artrosis podría explicar su asociación al síndrome metabólico y otros factores de riesgo cardiovascular [31]. La obesidad es un factor de riesgo modificable que se asocia tanto al aumento del riesgo de artrosis de rodilla como a una mayor severidad clínica y radiológica [32].

Referente a los factores genéticos, parece que existe un factor de condicionamiento que explicaría una mayor frecuencia de artrosis en gemelos [33,34]. El factor genético FRZB (*Frizzled Related Protein*), se asocia con un mayor riesgo de artrosis de cadera en mujeres [35]. También se han descrito cambios en la expresión de los genes relacionados con el cartílago (SOX9, ACAN, COL2A1, DKK1, FRZB), a lo largo de la historia natural de la enfermedad, de modo que su expresión disminuye en estadios avanzados [36]. Existe, además, agregación familiar, siguiendo la transmisión con frecuencia las leyes de Mendel [37].

Otros factores de riesgo no modificables ampliamente conocidos son la edad y el sexo (mujer). Aunque se han intentado asociar factores hormonales y relacionados con la

menopausia o los estrógenos con la artrosis [38,39], ninguno de los estudios realizados hasta el momento ha resultado concluyente en este aspecto, por lo que la mayor prevalencia de la OA en mujeres sigue siendo materia de interés. De todos modos, conocemos que tanto la evaluación del dolor, como el grado de marcadores inflamatorios es distinto entre hombres y mujeres, siendo objeto de estudio actual [40,41].

En cuanto a los factores biomecánicos, la actividad física, los traumatismos y determinadas ocupaciones predisponen al desarrollo de la OA [22]. Si bien la actividad física provoca beneficio en las articulaciones al aumentar la masa muscular [42], la actividad deportiva de élite parece relacionarse con un aumento del riesgo de artrosis [43], especialmente en localizaciones dañadas previamente. Los traumatismos articulares aumentan hasta por cuatro veces el riesgo de artrosis [44]. Por otro lado, en un metaanálisis reciente se establece que los trabajos que requieren estar de rodillas o en cuclillas y levantar pesos tienen un riesgo 1.6 veces más elevado de artrosis de rodilla que los trabajos más sedentarios [22]. La debilidad muscular del aparato extensor también se ha presentado como un factor de riesgo para el desarrollo y progresión de la artrosis de rodilla [45,46].

1.4 Medidas de gravedad

Existen dos formas distintas de evaluar la gravedad de la artrosis de rodilla. Una a nivel clínico y otra a nivel de progresión y afectación estructural, para la que actualmente utilizamos los índices radiográficos [47]. De todos modos, las técnicas de imagen como la ecografía y la resonancia nuclear magnética son cada día más utilizadas por su

capacidad para detectar cambios más precoces en la articulación y por su poder de evaluación no solamente de la estructura ósea sino de toda la articulación en su conjunto [48].

No obstante, el curso de la enfermedad es variable y no todos los casos evolucionan al mismo ritmo. Así, según datos obtenidos por modelos de inteligencia artificial (IA) (Deep Learning (DL)), la progresión radiológica ocurre entre el 13% y el 48% de los pacientes en un plazo de 48 meses [49].

Se puede considerar que existen cuatro etapas en la evolución de la OA progresiva: cambios bioquímicos, alteraciones objetivables por resonancia, cambios evidentes en la radiografía simple y fallo articular [50]. Actualmente somos capaces de observar los cambios cuando existe alteración radiográfica, en un momento de la evolución de la enfermedad ya estructurado. La identificación de alteraciones precoces, cuando se inician los cambios bioquímicos o existen alteraciones discretas a nivel de resonancia magnética (RMN), nos permitiría actuar en momentos previos al déficit estructural, y posiblemente hasta prevenir la artrosis, diseñando nuevas estrategias de tratamiento [51].

La investigación reciente en el campo de la artrosis de rodilla se centra en la búsqueda de biomarcadores que permitan identificar a los individuos que progresan frente a los que no, y así predecir la probabilidad individual de evolucionar a una artroplastia total de rodilla [52]. Según EULAR (*European Alliance of Associations For Rheumatology*), la identificación de predictores de progresión de la artrosis es de gran relevancia, pues es lo que permite avanzar en el desarrollo de estrategias de intervención [53]. Se estima

que de los ensayos en fase III, la ausencia de biomarcadores conlleva a un fracaso estimado de 2 de cada 4[54]. La inclusión de biomarcadores clínicos en los modelos de predicción de riesgo ha tenido un éxito moderado [55]. Sin embargo, si se incorporan biomarcadores de laboratorio o de imagen se obtienen mejores resultados [51].

1.4.1. Clínicas

El dolor crónico articular como síntoma principal de la artrosis de rodilla es altamente variable y característicamente no se correlaciona con los cambios estructurales [56]. Generalmente aparece de forma subaguda e insidiosa, aunque también puede desencadenarse súbitamente por un traumatismo [57].

Su origen es multifactorial, y se debe a mecanismos periféricos (nociceptores), de origen sinovial, óseo y de partes blandas; y a mecanismos centrales (de sensibilización y amplificación central), así como a factores psicosociales [57,58].

Se ha descrito un "ritmo artrósico", definido como un dolor que aparece al utilizar la articulación tras un período de reposo, y que mejora tras un tiempo de uso, pero que reaparece si se sobrecarga en exceso. En fases más avanzadas puede aparecer también dolor en reposo [59].

Existen actualmente múltiples herramientas desarrolladas específicamente para medir la repercusión de los síntomas de la OA en la vida diaria, que incluyen cuestionarios y escalas de dolor y de calidad de vida. Estos índices compuestos han demostrado ser válidos para detectar los cambios para los que han sido diseñados, y además son fácilmente reproducibles, por lo que constituyen un elemento útil para monitorizar por ejemplo la respuesta a un fármaco en un ensayo clínico y detectar así a los individuos

respondedores [60]. Nos centraremos en la descripción de las escalas validadas para la artrosis de rodilla, ya que ha sido la estudiada en nuestro trabajo.

El cuestionario posiblemente más utilizado es el WOMAC (*Western Ontario and McMaster Universities Osteoarthritis Index*), que presenta tres dominios que pueden ser evaluados de forma independiente: dolor, de 0 a 20 puntos; rigidez articular, de 0 a 8 puntos; y discapacidad funcional, de 0 a 68 puntos. Esta escala permite una interpretación global, como la suma de los dominios previamente descritos, con una puntuación total que oscila entre 0 y 96, definiendo la enfermedad más grave cuanta más puntuación [61].

Existe otro cuestionario relativamente similar al WOMAC denominado KOOS (*Knee Injury and Osteoarthritis Outcome Score*), que últimamente se usa con mayor frecuencia, posiblemente por el mayor número de dominios que engloba. Concretamente, evalúa de forma independiente los siguientes aspectos: síntomas, con cinco ítems; entumecimiento, con dos ítems; dolor, incorporando nueve ítems; actividades diarias, con 17 ítems; actividades deportivas y recreacionales, en el que se preguntan cinco ítems; y finalmente la calidad de vida, con cuatro ítems. Mediante una fórmula específica se realiza un cálculo de cada uno de los dominios de forma individual, desde 0 a 100, siendo 0 el mayor grado de severidad [62]. Puede realizarse una integración de los dominios preguntados mediante una fórmula específica, pero generalmente se expresa como dominios individuales y rara vez como un global.

Por último, recordar dos cuestionarios más. El primero sería el Lequesne; una de las herramientas más utilizadas en el pasado. Se trata de un cuestionario algofuncional, esto

es, incluye preguntas tanto de dolor como de incapacidad funcional en el mismo formulario, con una suma global de 0-24 puntos, siendo 0 el más leve [63]. El otro cuestionario se ha desarrollado en los últimos años en el seno de OARSI, para evaluar las características heterogéneas del dolor en OA, diferenciando entre el dolor persistente y el intermitente (ICOAP de dolor continuo e intermitente). El dolor persistente incluye 5 preguntas con un resultado de 0-20 y el intermitente 6 preguntas con un resultado de 0-24; presentando mayor gravedad a mayor puntuación [64].

1.4.2. De imagen

Clásicamente para definir la OA según el grado de afectación estructural se han utilizado criterios de severidad radiológicos [65,66]. Sin embargo, estos criterios son de poca utilidad para detectar la enfermedad en fases más precoces, aunque actualmente siguen utilizándose en práctica clínica y para evaluar el grado de afectación por imagen.

Entre las carencias de la radiografía simple cabrían destacar su escasa sensibilidad al cambio, falta de especificidad y falta de reproducibilidad en estudios longitudinales, sobre todo por problemas derivados de la posición [67]. Así, dependiendo de los grados de flexión en los que se realice la prueba se obtienen resultados variables, y una posición incorrecta puede conducir a falsos positivos y negativos [68].

Sobre todo, debido a esta falta de sensibilidad al cambio de la radiografía simple, en los últimos años se han desarrollado trabajos dirigidos a la búsqueda de otros marcadores de gravedad que permitan la detección de la enfermedad en estadios menos avanzados, utilizando pruebas de imagen más complejas, pero de mayor precisión como la ecografía [69] y la resonancia magnética [70,71].

1.4.2.1. Radiografía simple

La radiografía de rodillas constituye un método diagnóstico y de clasificación para pacientes con OA que se encuentra comúnmente disponible y, por lo tanto, es frecuentemente usado para la evaluación de la articulación tibio-femoral; donde los osteofitos marginales, la estrechez del espacio articular, la esclerosis y/o quistes subcondrales reflejan los cambios patológicos [65,72].

El grupo de estudio de OARSI-OMERACT (*Osteoarthritis Research Society International-Outcome Measures in Rheumatology Clinical Trials*), revisó diversas técnicas empleadas en la evaluación radiográfica de la OA de rodillas [73]. La adecuada alineación de los márgenes anterior y posterior del platillo tibial medial, así como el control del grado de rotación, permite una mejor evaluación del espacio articular. Este grupo de estudio determinó que la radiografía anteroposterior (AP) con semiflexión (guiada por fluoroscopia) permite una mejor alineación que las técnicas no guiadas como la posteroanterior (PA) con semiflexión fija (proyección de Schuss-Tunel) o la PA con alineación de la primera articulación metatarsofalángica (PA-MTF) con la placa radiográfica ($p < 0,0001$). En ausencia de fluoroscopia, la proyección de Schuss-Tunel ha demostrado ser superior a la AP en la evaluación de la disminución del espacio articular reduciendo las variaciones en el ángulo femorotibial [74]. Por otra parte, Cline et al [75]no encontraron diferencias entre las proyecciones AP con semiflexión guiada por fluoroscopia, Schuss-Tunel y PA MTP en la evaluación de la progresión radiográfica.

La clasificación de Kellgren-Lawrence (K/L), es la más utilizada y conocida; y todavía hoy en día es la clasificación de referencia para establecer el grado de afectación radiográfica

de las personas con artrosis de rodilla [65]. Evalúa principalmente el grado de formación del osteofito. La clasificación se divide en: Grado 0: ausencia de osteofitos, estrechamiento o quistes; Grado 1: osteofitos dudosos; Grado 2: osteofitos mínimos, posible disminución del espacio articular, quistes y esclerosis; Grado 3: osteofitos moderados o claros con pinzamiento moderado de la interlínea; Grado 4: osteofitos grandes y claro pinzamiento de la interlínea con esclerosis. Su uso es habitual tanto en la práctica clínica como en ensayos clínicos, si bien tiene sus limitaciones, principalmente consecuencia de que sus categorías no son equidistantes, por lo que la proporción de pacientes que progresan de una categoría a otra podría no ser comparable [76].

En al año 2007, el grupo OMERACT publicó un atlas de lectura creando una nueva gradación de la artrosis en diferentes localizaciones, incluyendo la rodilla [77]. Realiza una valoración más precisa del estrechamiento del espacio articular tanto a nivel interno como externo, con una puntuación de 0 a 3, 6 en total. Asimismo, valora la presencia y el grosor de los osteofitos en los compartimentos lateral y medial tanto en tibia como en fémur, puntuándose de 0 a 3 en cada zona, máxima puntuación 12. También se valora de forma dicotómica la presencia de esclerosis medial y lateral y el desgaste articular. Por lo tanto, esta gradación sería más sensible al cambio. Su uso en la práctica clínica no es habitual, ya que es laborioso, por lo que se suele utilizar la clasificación K/L.

Se ha hipotetizado también sobre la utilidad de la determinación de la textura del hueso trabecular como biomarcador de riesgo de progresión de la artrosis de rodilla [78]. El engrosamiento de las trabéculas horizontales precede a los cambios en las trabéculas verticales. En un estudio casos y controles publicado recientemente, se objetivó que los cambios en el grosor del hueso trabecular horizontal obtenidos mediante la aplicación

de un software específico semiautomatizado predicen el riesgo de progresión tanto sintomática como estructural a 24 meses [79].

1.4.2.2. Ecografía

La utilización de transductores de alta frecuencia con mayor resolución para la valoración de las estructuras musculoesqueléticas superficiales ha promovido el uso cada vez mayor de la ecografía en la evaluación del aparato locomotor [80]. A diferencia de la radiografía simple, la ecografía permite visualizar no sólo el hueso cortical, sino también las partes blandas periarticulares. A pesar de ser una técnica dependiente del explorador, su fácil accesibilidad, así como su inocuidad y bajo coste, hacen de ella la prueba de imagen "ideal".

En la OA, además del adelgazamiento progresivo del cartílago, que es una estructura avascular y no inervada, se produce afectación de otros tejidos en los que probablemente radique el origen del dolor, como la membrana sinovial, que frecuentemente está hipertrofiada [81]. Es por esto que la ecografía resulta de gran utilidad en la valoración de la artrosis de rodilla, especialmente en estadios tempranos de la enfermedad en los que la sinovitis es un hecho frecuente, y que se asocia además a una mayor progresión radiológica, y por lo tanto a una mayor severidad [70].

Existen numerosos estudios que tratan de relacionar la presencia de derrame articular valorado por ecografía con el grado de dolor, así como la presencia de señal *Power Doppler*. La mayoría valoran el derrame en el receso suprapatelar, pero este no es el único receso potencialmente afectado, por lo que se deberían explorar todos los recesos articulares (central, posterior y poplíteo) [82]. Hasta un 89.2% de las artrosis de rodilla

presentan derrame grado 2, siendo la prevalencia aproximada de derrame ≥2 de un 50% [83]. La posición del paciente durante la exploración influye notablemente en la valoración del derrame articular [84].

Recientemente, el grupo de trabajo OMERACT ha validado un atlas para la evaluación ultrasonográfica de la artrosis de rodilla, basado en scores semicuantitativos que consideran la sinovitis (0-3), hipertrofia sinovial ≥ 4mm (0-1), efusión ≥ 4mm (0-1), señal *Power Doppler* de los recesos suprapatelar en plano longitudinal, medial y lateral en plano transverso (0-1), osteofitos (0-3), extrusión meniscal medial en plano longitudinal (0-2), y anomalías del cartílago en plano transverso con la rodilla flexionada al máximo (0-3)[85]. Estos scores han demostrado una asociación significativa pero modesta con otras medidas de severidad clínicas y de imagen [86]. Así, tanto la señal *Doppler* como la hipertrofia sinovial y la extrusión meniscal se han relacionado con el dolor.

Se han establecido unos "cut off" a partir de los que la presencia de derrame articular y/o hipertrofia sinovial se consideran patológicos en la valoración ecográfica de la rodilla, y que varían en función del sexo. Así, en los varones, se considera patológico un derrame mayor a 7.4 mm y una membrana sinovial superior a 3.7 mm; mientras que, en las mujeres, estos valores descienden a 5.3 mm y 1.6 mm respectivamente [87].

La detección precoz de la extrusión meniscal medial por ecografía es especialmente interesante, ya que está comprobada su anticipación a la pérdida de cartílago. Un valor límite de 2 mm en sujetos con dolor crónico de rodilla ha demostrado una elevada sensibilidad y especificidad [88,89].

La presencia de quiste de Baker, fácilmente evaluable por ecografía, se ha relacionado con derrame y dolor articular en pacientes con OA de rodilla, aunque el nivel de evidencia es poco consistente [90].

1.4.2.3. Resonancia magnética nuclear (RMN)

El uso de la RMN para la valoración de la artrosis de rodilla es cada vez más común a pesar de su elevado coste [91]. La resonancia, a diferencia de la radiografía, aporta información relevante acerca de los cambios que se producen en diferentes estructuras articulares tanto en el debut como en la progresión de la artrosis de rodilla; como son las partes blandas, el cartílago y el hueso subcondral [2].

La definición clásica de la OA se basa en la presencia de cambios estructurales visualizables en la radiografía simple que no permiten identificar la enfermedad en fases más tempranas. Así, los cambios en el espacio articular tibio-femoral medial se han considerado como la medida primaria de la artrosis de rodilla, y son el método "*Gold Standard*" para valorar la progresión estructural en los estudios de artrosis [74].

Sin embargo, es bien conocido que se producen cambios patológicos articulares previos a la aparición del daño radiológico, que se podrían utilizar como marcadores precoces de la enfermedad. En los últimos años se han desarrollado varios sistemas semicuantitativos de puntuación de la artrosis de rodilla basados en imágenes obtenidas por resonancia que han permitido identificar cambios patológicos a nivel del tejido blando que se asocian con determinados aspectos clínicos y estructurales de la enfermedad.

La proliferación de la membrana sinovial es un hecho característico de la artrosis progresiva, y su engrosamiento medido por resonancia, especialmente utilizando contraste con gadolinio, se ha correlacionado con el grado de afectación radiológica [92].

La patología meniscal (lágrima meniscal, extrusión), se ha presentado como uno de los principales desencadenantes de la aparición y progresión de la artrosis de rodilla [93], aunque su papel real no es del todo bien conocido.

La pérdida de volumen del cartílago femorotibial medial durante 2 años de seguimiento se ha asociado con progresión clínica y sobre todo estructural a 4 años [94]. También se han asociado los cambios en el grosor del cartílago con una mayor probabilidad de artroplastia [95]. Por tanto, este cambio en el grosor del cartílago medido por resonancia podría ser un biomarcador de imagen de progresión de la OA útil y robusto.

El tamaño del edema de la médula ósea ("*bone marrow lesions*"), en imágenes potenciadas en T2, se ha correlacionado con cambios histopatológicos que incluyen microdegradación del hueso trabecular subcondral e intento de reparación de la matriz ósea, y también con deformidad articular progresiva y mayor probabilidad de artroplastia [95]. El edema es un hallazgo frecuente en las rodillas artrósicas, y está presente en el 78% de los pacientes con dolor y hasta en el 30% de los pacientes sin dolor, por lo que su implicación clínica no está aclarada [96].

También se han estudiado biomarcadores de imagen a partir de mediciones del hueso trabecular [97,98]. El hueso trabecular está constituido por laminillas óseas dispuestas en forma de red en cuyo interior se encuentra la médula ósea. Su tamaño oscila entre

los 50 a 200 ⌈m, por lo que su cuantificación es compleja. El aumento del grosor, la pérdida de espacio y el aumento de densidad trabecular (lo que refleja esclerosis del hueso subcondral), ha demostrado asociaciones modestas, pero estadísticamente significativas con la progresión del dolor en 48 meses de seguimiento. Esto se explicaría por razones biomecánicas, ya que en el proceso de remodelado óseo, microtraumatismos repetidos conducirían al daño cartilaginoso; y por razones biomoleculares, ya que el aumento de vascularización conllevaría aumento de secreción de citocinas y factores de crecimiento que desencadenarían defectos en el cartílago y osificación posterior [99].

Utilizando la IA, se han combinado algoritmos de aprendizaje profundo con imágenes de resonancia para obtener modelos predictivos de progresión de la artrosis de rodilla que obtienen mejores resultados que los diseñados a partir de imágenes de radiografía simple [50].

1.5. Marcadores de inflamación

Clásicamente, la OA no se ha considerado una artropatía inflamatoria por la escasez de neutrófilos en el líquido sinovial y la ausencia de manifestaciones sistémicas de inflamación [100]. Así, a menudo se han utilizado los tejidos procedentes de una articulación artrósica como un control no inflamatorio o incluso un sucedáneo del tejido articular normal o sano [101]. En este sentido, las características del cartílago articular (avascular, alinfático y aneural), impiden cumplir los signos clásicos de la inflamación (enrojecimiento, hinchazón, calor y dolor). Sin embargo, gracias a los avances en biología molecular y celular, son múltiples los estudios que demuestran que diversos mediadores

proinflamatorios, como son diversas citocinas, pueden ser importantes en el desarrollo de esta enfermedad [102].

La inflamación crónica de la membrana sinovial produce la liberación de citocinas proinflamatorias que conllevan a la activación de enzimas proteolíticas que son responsables de la afectación de la homeostasis del cartílago articular [103]. Los marcadores séricos reflejan la degradación de las proteínas del cartílago. Por lo tanto, el análisis de citocinas tanto plasmáticas como en líquido articular parece de interés en los pacientes con artrosis de rodilla [104]. De este modo, la identificación de estos marcadores de inflamación a nivel sistémico y local nos permitiría entender mejor la fisiopatogenia de la OA y su implicación en la misma [105]. Existen diversas citocinas que se han asociado previamente con la severidad tanto clínica como radiográfica en la artrosis de rodilla [106,107]. Entre ellas, las más relevantes se comentan a continuación, aunque en ninguna de ellas, los resultados han sido lo suficientemente contundentes como para establecer una translación directa en la práctica clínica, motivo que justifica seguir con su estudio.

1.5.1. TNF-α

El TNF-α es una citocina proinflamatoria clásica sintetizada por una amplia variedad de tipos celulares, incluyendo macrófagos, condrocitos y sinoviocitos. Estudios en humanos *in vitro* han demostrado su relevancia en la patogénesis de la artrosis [30].

Las concentraciones plasmáticas de TNF-α y su receptor soluble predicen la progresión de la artrosis de rodilla, y se asocian con el estrechamiento del espacio articular [108].

Además, algunos estudios previos lo han relacionado con el dolor evaluado por el índice de WOMAC [109].

Sus niveles basales en líquido articular se asocian a mayor progresión a largo plazo en individuos postmeniscectomizados [110].

En un estudio publicado recientemente se objetiva que la concentración del TNF-α dentro de las vesículas plasmáticas extracelulares es mayor en pacientes con artrosis de rodilla, y su concentración basal predice la progresión radiológica [111]. Esto es interesante porque podría explicar en parte la ineficacia de los anti-TNF en el tratamiento de la artrosis [112], aunque pueda ser una citocina relevante asociada a la severidad de la artrosis de rodilla.

1.5.2. IL-6

La interleucina 6 (IL-6), es una proteína pleiotrópica que regula múltiples procesos biológicos, y tiene un papel destacado en la fisiopatología de determinadas enfermedades autoinmunes e inflamatorias [113]. Actúa principalmente mediante tres vías de señalización: JAK/STAT, Ras/MAPK y P13K/Akt.

Participa en el proceso inflamatorio, la respuesta inmune adaptativa e innata, la hematopoyesis, la estimulación del eje hipotálamo-hipofisario-adrenal, el metabolismo óseo y lipídico y la regulación de las respuestas de fase aguda [114].

A nivel local se produce tanto en la membrana sinovial como en la grasa infra-patelar.

Valores elevados de la IL-6 en líquido sinovial favorecen la formación del *pannus* y la resorción ósea como resultado de la osteoclastogénesis en pacientes con artritis reumatoide [101].

Se detectó en líquido sinovial de pacientes con artrosis en el año 1988, siendo las concentraciones más bajas que en la artritis reumatoide [115].

Niveles plasmáticos elevados se han relacionado con progresión radiológica [110]. Su rol en la fisiopatogénesis de la artrosis parece especialmente importante en estadios precoces de la enfermedad, en los que se asocia con mayor severidad clínica [116].

Niveles basales en líquido sinovial se relacionan con mayor progresión radiológica en OA secundarias [110].

También parece clara su relación con la pérdida de capacidad funcional para todos los grados de afectación radiológica [117].

El uso combinado de los niveles séricos de IL-6 asociados al TNF-〈 y a la leptina ha demostrado una elevada sensibilidad para discriminar entre pacientes con OA postraumática y controles sanos (AUC 0.946, sensibilidad 97%, especificidad 61%), resultado similar al obtenido mediante radiografía simple [108].

1.5.3. PCR-hs

La PCR (proteína C reactiva), es una proteína pentamérica de fase aguda utilizada ampliamente como marcador de inflamación, ya que sus niveles aumentan rápidamente en la fase aguda del daño tisular. La forma monomérica de la PCR es más difícil de determinar por ELISA, pero más sensible como indicador de una enfermedad específica,

ya que no se sintetiza en los adipocitos sino en el tejido lesionado [118]. Una publicación reciente demuestra que sus niveles plasmáticos se asocian fuertemente con la artrosis, y aumentan en los estadios más avanzados [119], por lo que podría ser un buen marcador para monitorizar la actividad de la enfermedad y evaluar la eficacia de los tratamientos.

Su importancia no sólo se limita a la reacción inflamatoria, sino que está involucrada en la respuesta inmune innata, activando el complemento y regulando la fagocitosis macrofágica en enfermedades como por ejemplo el infarto agudo de miocardio [120].

En la AR, niveles de PCR persistentemente elevados se asocian con destrucción ósea y progresión estructural.

En la artrosis se han reportado diferencias estadísticamente significativas entre los niveles séricos de los pacientes, que están aumentados, y los controles sanos [121]. Sin embargo, su asociación con la progresión radiológica no está establecida. Diversas publicaciones relacionan la PCR en plasma con la progresión radiológica; mientras que, en otras, esta relación se pierde cuando se controla por el índice de masa corporal (IMC).

En un trabajo reciente no se demostró relación entre los niveles séricos de la PCR-hs, determinados polimorfismos del gen y el riesgo de recambio protésico de rodilla y cadera en una cohorte de mujeres postmenopáusicas con artrosis de rodilla [122].

1.5.4. IL-8

La interleucina 8 es una citocina inflamatoria producida en los condrocitos que provoca la liberación de la MMP-13, y que genera cambios en la membrana sinovial [123].

Sus niveles están aumentados tanto en el suero como en el líquido sinovial de los pacientes con artrosis [124]. También se han hallado niveles elevados en el líquido sinovial de pacientes con artrosis y lesión previa del ligamento cruzado anterior [124].

Se ha evidenciado una asociación débil pero significativa entre sus concentraciones en líquido sinovial y la severidad clínica de la artrosis de rodilla en una cohorte de mujeres sintomáticas con derrame articular [125].

En cuanto al daño estructural, también se ha demostrado su asociación positiva con cambios en la señal de la grasa infrapatelar medida por RMN y con el grado de afectación radiológica según la escala de K/L [92].

1.5.5. Calprotectina

La calprotectina es una proteína de la familia de las alarminas, también conocidas como DAMP (*Damage Activated Molecular Patterns*), que se liberan con la activación de los granulocitos y los macrófagos en múltiples procesos fisiológicos y patológicos [126].

Su presencia fuera de las células actúa como señal de alarma, indicando que algo está provocando la muerte celular.

Ante una situación de daño tisular en el cartílago se produce la liberación de las alarminas, que desencadenan una respuesta inmune innata mediante la activación de distintos tipos de receptores como los TLR (Toll Like Receptor) [127]. La unión de las proteínas S100A8 y S100A9 forma un heterodímero conocido como calprotectina, que se une al receptor Toll-like 4 (TLR-4), favoreciendo la secreción de la IL-1®.

La activación de estos receptores se relaciona con múltiples enfermedades inflamatorias y autoinmunes como la sepsis, la enfermedad inflamatoria intestinal, la esclerosis múltiple y algunas enfermedades articulares. Los niveles séricos y en líquido sinovial del complejo S100A8/A9 están elevados en pacientes con artrosis y artritis reumatoide, correlacionándose con la actividad de la enfermedad [128].

Los niveles elevados de calprotectina se han relacionado con la degradación inicial del cartílago articular y con la presencia de una OA menos evolucionada, sugiriendo su posible utilidad como biomarcador de enfermedad precoz [129]. Lamentablemente, estos hallazgos no se han confirmado en estudios posteriores, por lo que todavía se desconoce con exactitud su relación con la artrosis.

1.5.6. IL-10

La interleucina 10 (IL-10) es la citocina con mayor poder antiinflamatorio, actuando a través de los macrófagos y las células T [130]. Un desequilibrio en la síntesis de IL-10 se ha relacionado con múltiples enfermedades infecciosas, alérgicas y autoinmunes como la hepatitis, la dermatitis atópica, el asma y la colitis ulcerosa [131].

En un trabajo publicado en 2010 en una cohorte de mujeres con artrosis de rodilla [132], se determinó mediante microdiálisis que el ejercicio provocaba un aumento de IL-10 tanto a nivel articular como perisinovial, lo sería congruente con el beneficio demostrado de la actividad física en la artrosis.

Niveles séricos bajos de IL-10 se han asociado con mayor producción local de TNF-〈, degeneración del cartílago y evidencia histológica de enfermedad en conejos con artrosis secundaria a lesión del ligamento cruzado anterior [133].

En un estudio realizado en sujetos con lesión del ligamento cruzado anterior y artrosis de rodilla, se evidenció que las concentraciones séricas de IL-10 eran significativamente menores en el grupo de artrosis severa comparadas con el de artrosis moderada (K/L 4 vs K/L 3, respectivamente), así como en pacientes con lesión del ligamento cruzado anterior [134].

1.5.7. IL-34

La interleucina 34 es una citocina inflamatoria implicada en la inflamación sinovial y en la osteoclastogénesis. Comparte similitudes con el factor estimulante de la colonia de macrófagos CSF-1, por lo que regula la diferenciación, proliferación y supervivencia de las células macrofágicas[135].

Su producción tanto local como sistémica está aumentada en patologías como la artritis reumatoide, por lo que se ha postulado como biomarcador de enfermedades articulares.

Niveles elevados se han asociado con severidad tanto clínica como radiológica en pacientes con artrosis de rodilla [136,137].

1.5.8. IL-17

Citocina inflamatoria producida principalmente por los linfocitos CD4+ (T helper 17).

Se ha evidenciado que sus niveles están aumentados tanto en suero como en líquido sinovial de pacientes con artrosis de rodilla, siendo esta última asociación estadísticamente significativa [138]. Además, sus niveles se han relacionado

positivamente con el grado de afectación radiológica [139]. También hay una correlación positiva con el dolor articular.

1.6. Adipocitocinas

El tejido adiposo expresa y secreta una gran variedad de proteínas que a menudo comparten propiedades estructurales y funcionales con las citocinas, y se clasifican como adipocitocinas [140]. Además de su relación con procesos fisiológicos y metabólicos, son sustancias proinflamatorias que parecen ser el enlace entre la obesidad, síndrome metabólico, inflamación y enfermedades reumáticas. Pasaremos a definir las adipocitocinas más evaluadas en relación con la artrosis de rodilla.

La leptina es una de las más estudiadas. Sus niveles dependen de la cantidad de grasa corporal, y parece tener un efecto importante en la aparición de OA en pacientes obesos, tanto en articulaciones de carga como en las manos [141]. Favorece la secreción de diferentes enzimas responsables de la degradación del cartílago articular; y niveles elevados en líquido sinovial se han relacionado con una mayor severidad de la artrosis [142]. Su producción está elevada tanto en la grasa infrapatelar como en el tejido sinovial de pacientes con artrosis. Sus niveles plasmáticos se han correlacionado con el grado de afectación estructural, tanto evaluado por radiografía simple como por RMN [143,144].

La adiponectina tiene efecto protector metabólico, y sus niveles en plasma se relacionan de manera inversa con el IMC y la resistencia a la insulina [145]. Su producción está aumentada en condrocitos de pacientes con artrosis de rodilla según resultados de estudios *in vitro*, donde induce la liberación de óxido nítrico e interleucinas. Sus niveles

séricos y en líquido sinovial se relacionan con marcadores de inflamación y de destrucción del cartílago articular [145]. Niveles elevados de adiponectina y PCRhs se han correlacionado con la intensidad del dolor articular en la cohorte KHOALA, independientemente de la destrucción articular [146]. Sin embargo, su implicación en la progresión estructural es controvertida con resultados dispares según las distintas publicaciones. Nuestro grupo de investigación ha publicado previamente resultados asociando la adiponectina en líquido sinovial con la gravedad clínica de la artrosis de rodilla [147].

La resistina se relaciona con la insulinorresistencia y con la inflamación sistémica, y sus niveles séricos aumentan de forma proporcional según la actividad de enfermedades reumáticas como la artritis reumatoide y las espondiloartritis [148]. Sus niveles en líquido articular se han relacionado con severidad clínica y radiológica en sujetos con OA de rodilla [149]. Como en el caso de la adiponectina, nuestro grupo de investigación ha relacionado la resistina en el líquido sinovial con la discapacidad funcional en mujeres con artrosis de rodilla con derrame sinovial [150].

La visfatina en modelos de artritis se asocia con inflamación y destrucción ósea. Además, interviene en la vía de activación del factor de crecimiento neuronal (NGF), que está involucrado en la patogenia del dolor en OA [151]. También hemos reportado previamente una asociación negativa de la visfatina con la discapacidad funcional en artrosis de rodilla [152].

La osteopontina podría tener un efecto protector en la fisiopatología de la artrosis, según modelos experimentales, inhibiendo determinadas moléculas catabólicas y de

degradación del cartílago [153]. Sin embargo, diversos estudios relacionan sus niveles plasmáticos y en líquido articular con mayor grado de afectación radiológica, mayor severidad clínica y lesiones histopatológicas [153].

La omentina presenta una elevada correlación con la adiponectina, así como una asociación inversa con los marcadores de síndrome metabólico [154]. Los datos publicados referentes a la asociación de la omentina con la artrosis de rodilla parecen indicar que podría ejercer cierto efecto protector tanto a nivel clínico como radiográfico [155].

La irisina es una miocina emergente secretada por el tejido adiposo y sobre todo por el músculo esquelético en respuesta al ejercicio [156]. Se ha vinculado fuertemente con la salud ósea [157], y niveles séricos bajos pueden incrementar el riesgo de fractura y de padecer enfermedades como la artrosis, la osteoporosis y la artritis reumatoide [158]. Niveles elevados en suero y en líquido sinovial se han correlacionado de forma positiva con la severidad clínica de la artrosis, sobre todo en pacientes con un índice de masa corporal elevado. Sin embargo, su papel en la progresión estructural es controvertido y hay datos que apuntan en ambas direcciones [159]. En los últimos años se ha postulado como potencial diana terapéutica, dado su poder antiinflamatorio y regenerador del cartílago articular [159].

1.7. Factor de crecimiento neuronal (NGF)

El factor de crecimiento neuronal (NGF) es una neurotrofina que regula la estructura y la función neuronal, modulando la sensibilización nociceptiva. En modelos animales y

en humanos, su aporte externo aumenta el dolor tanto a nivel local como sistémico [160], dependiendo de la dosis y la ruta de administración [161].

Actualmente sabemos que en la fisiopatogenia del dolor en la OA intervienen no sólo las terminaciones nerviosas articulares (mecanismos locales), sino que el sistema nervioso central juega un papel importante [162,163].

La producción del NGF y otras citocinas inflamatorias contribuyen a la patogenia de la OA incrementando la degradación del cartílago e induciendo hiperalgesia [164].

Se han detectado niveles elevados en líquido sinovial de pacientes con enfermedades articulares inflamatorias y degenerativas [165].

Además, se ha utilizado como molécula diana para el desarrollo de nuevas terapias [166].

Como podemos ver, se ha estudiado ampliamente la asociación de diferentes marcadores de inflamación, tanto en plasma como en líquido articular, con la artrosis de rodilla. Lamentablemente, ninguno de estos estudios nos ha servido para identificar de forma contundente ningún biomarcador sensible útil en práctica clínica o como diana terapéutica. En este sentido, se considera que la heterogeneidad de la enfermedad dificulta su interpretación a partir de un solo biomarcador. Además, se considera que existen distintos perfiles de pacientes, con lo que un fenotipado de precisión puede ayudar a identificar biomarcadores funcionales particulares de cada fenotipo. Describimos a continuación algunos de los fenotipos previamente reportados.

1.8. Fenotipos en artrosis de rodilla

Actualmente se considera la artrosis como un grupo heterogéneo de enfermedades compuesto por múltiples fenotipos clínicos y endotipos fisiopatológicos que en muchas ocasiones se superponen, en lugar de una entidad única [167].

Un fenotipo clínico se define como las características observables de un organismo que son producidas por la interacción de factores genéticos y ambientales [168]. Estas características similares permiten agrupar a los pacientes de tal forma que se puedan identificar los individuos, por ejemplo, con mayor riesgo de progresión (fenotipo pronóstico), o mayor probabilidad de responder a una intervención específica (fenotipo terapéutico), entre otros [169].

En el caso de la artrosis, la falta de una identificación precisa de subpoblaciones de pacientes con características clínicas, biológicas o estructurales comunes se ha definido como una de las principales causas tanto del fracaso de los ensayos clínicos en fase II/III como de la falta de traslacionalidad de la investigación. Esto se explica fundamentalmente porque los modelos experimentales y animales en los que se desarrollan las primeras fases de investigación de nuevas dianas terapéuticas son puros; es decir, presentan una etiología definida (por inflamación, degradación de cartílago o lesión estructural, entre otras) [170]. En cambio, y como ya se ha comentado, la OA es una enfermedad multifactorial, por lo que la identificación de las principales causas de afectación en cada individuo es primordial para establecer candidatos a recibir tratamientos específicos [60].

A grandes rasgos, los distintos fenotipos de artrosis se podrían clasificar en tres grandes grupos, según estén basados en: pruebas de imagen (RMN, radiografía); marcadores bioquímicos; y en peculiaridades clínicas y variables antropométricas.

En los últimos años se ha utilizado la IA en la búsqueda de distintos fenotipos, especialmente en el área de imagen [171]. Mediante técnicas de *machine learning* se realiza un abordaje de un gran número de datos sin necesidad de una hipótesis previa, lo que permite identificar patrones no previstos que generarían nuevos fenotipos [172].

También se han utilizado técnicas de IA en la búsqueda de modelos predictivos de riesgo de progresión clínica y radiológica. Así, se ha utilizado un modelo de aprendizaje automatizado para predecir la progresión del dolor a partir de radiografías simples, con un AUC de 0.77, que aumentaba hasta 0.81 si se añadían otras variables como la edad, género, raza, IMC, WOMAC y estadio K/L [173].

En otros estudios se utilizaron variables basales y resonancia semicuantitativa para predecir la pérdida de cartílago con un AUC de 0.7 [174].

La combinación de datos de los distintos dominios (forma de presentación clínica, patrones de afectación articular, afectación estructural, biomarcadores), permitiría definir fenotipos más complejos y precisos para clasificar la enfermedad y conocer su pronóstico y respuesta al tratamiento. Se ha evidenciado que para poder discriminar entre distintos grupos se necesita combinar un gran número de variables, incluyendo datos metabólicos, antropométricos e inflamatorios, que permitan definir fenotipos más amplios. Esto, a su vez, permite individualizar la estrategia terapéutica.

El uso de tecnologías "*ómicas*" como la genómica, la proteómica y la metabolómica, que permite manejar un gran número de datos, ofrece un gran potencial para identificar biomarcadores en un futuro próximo [175].

Potencialmente se pueden identificar cientos de fenotipos en función de la definición utilizada y de las variables seleccionadas. En una revisión sistemática de la literatura publicada previamente [176], en la que se analizaron 841 artículos de los que finalmente por cuestiones metodológicas y de calidad se incluyeron 24, se identificaron 37 fenotipos que se unificaban en 6 grandes subgrupos en función de la etiología y progresión: dolor crónico, inflamatorio, metabólico local, síndrome metabólico, sobrecarga mecánica y mínima progresión. Aunque no existen unos fenotipos aceptados a nivel general, esta clasificación ha sido ampliamente utilizada y referenciada. Específicamente, cada fenotipo describe:

- Dolor crónico: se asocia a dolor generalizado y afectación psicológica, orientando a una alteración de los mecanismos neurofisiológicos del dolor y a sensibilización central.

- Inflamatorio: se caracteriza por la implicación de múltiples moléculas mediadoras de la inflamación.

- Metabólico: está definida la asociación entre el síndrome metabólico y la artrosis. La afectación de articulaciones que no están sometidas a carga como por ejemplo es el caso de las manos, indica la existencia de otros mecanismos fisiopatológicos. Se ha evidenciado la relación con la obesidad, la hipertensión, la diabetes y la dislipemia.

- Cambios metabólicos locales: se han relacionado *clusters* de marcadores de remodelado óseo como el CTX, la piridolina y el NTX con la OA.

- Biomecánico: justificaría hasta un 22% de las artrosis de rodilla. Se caracteriza por un exceso de sobrecarga en un área específica de la articulación, y se asocia a mala alineación, debilidad muscular y daño estructural previo. Una mala alineación tipo varo se asocia con afectación del compartimento medial, mientras que el *genu valgo* se asocia a afectación lateral.

- Mínima progresión: se basa en características no etiológicas sino de progresión estructural, constituyendo un subgrupo de baja progresión. Incluye variables séricas (biomarcadores de resorción ósea), variables de imagen (RMN y radiografía simple), y variables clínicas. Su prevalencia oscilaría entre el 17% y el 47%, según los estudios consultados.

Otros fenotipos se han propuesto atendiendo a otras variables clínicas como el estado anímico [177]. Este subgrupo de pacientes con síndrome depresivo asociado se caracterizaría por una mínima afectación radiológica (K/L < a 3), y elevadas puntuaciones en cuestionarios validados para la evaluación de la depresión y ansiedad. Equivaldría en la clasificación de Dell´Isolla al subgrupo de dolor crónico.

Otra clasificación se basa en variables que valoran la discapacidad funcional. Así, en la literatura se describen la dificultad en subir escaleras, la dificultad para levantarse de la silla y la dificultad para caminar como las limitaciones más frecuentes en pacientes con artrosis de rodilla. En base a datos antropométricos y a la dificultad para mantener la bipedestación, la dificultad para deambular de forma prolongada y la dificultad para

subir escaleras, Vongsirinavarat et al. [178] clasificaron a los pacientes en 4 grupos según el grado de limitación funcional: no limitación (31.6%), limitación leve (26.8%), moderada (30.4%), y severa (11.2%).

Recientemente se ha publicado un modelo basado también en 4 subgrupos atendiendo al dolor [179]: debilidad y dolor intenso con múltiples comorbilidades; debilidad y dolor intenso; debilidad y dolor intenso asociado a ansiedad por dolor; y no debilidad y baja sensibilidad al dolor, lo que ocurría en un 17% de los casos, y además se asociaría en mayor medida a daño articular previo y a individuos deportistas.

Esta división indicaría la necesidad de incluir en la estrategia terapéutica un enfoque psicosocial, así como otros factores extraarticulares atendiendo a las comorbilidades de los pacientes.

Previamente [180] otros grupos estudiaron fenotipos de artrosis de rodilla en base al dolor, intentando discriminar factores predisponentes a la cronificación del dolor, independientemente del daño estructural, utilizando datos del estudio MOST, clasificando también a los pacientes en 4 subgrupos, y concluyeron que el grupo de mayor proporción de puntos gatillo asociados a moderada sumación temporal tenían el doble de riesgo de desarrollar dolor articular persistente.

La inclusión de datos de fácil determinación en la identificación de fenotipos favorecería su aplicabilidad en la práctica clínica.

En resumen, actualmente parece clara la necesidad de realizar un fenotipado de precisión para mejorar nuestra comprensión de la OA y clasificar a los pacientes de forma más eficiente. Aunque no existe un consenso universal, la clasificación en estos

seis fenotipos: dolor crónico, inflamatorio, metabólico local, síndrome metabólico, sobrecarga mecánica y mínima progresión, parece la más utilizada actualmente y un buen punto de inicio para seguir estudiando la agrupación de los pacientes con artrosis de rodilla.

2. JUSTIFICACIÓN

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La fisiopatología de la artrosis es multifactorial y compleja, y actualmente todavía no se conoce con exactitud ni en profundidad. El espectro clínico y el pronóstico es muy variable, con diferencias relevantes en cuanto a la exposición a factores de riesgo, por lo que actualmente se considera un síndrome heterogéneo que agrupa distintos subgrupos clínicos (fenotipos), con diferentes rasgos fisiopatológicos (endotipos). La correcta estratificación de los pacientes en subgrupos tendría implicaciones terapéuticas (personalización de tratamientos, nuevas terapias, selección precisa de candidatos para ensayos clínicos…).

Además, el paradigma de la OA ha cambiado en los últimos años, considerándose actualmente una enfermedad inflamatoria de bajo grado. El descubrimiento de la participación de mediadores de la inflamación como las citocinas, las prostaglandinas y las metaloproteasas abre una nueva vía en la búsqueda de biomarcadores que serían de utilidad tanto diagnóstica, como pronóstica y sobre todo como potenciales dianas para futuros tratamientos. De este modo se avanzaría hacia una medicina personalizada y de precisión. De igual modo, durante los últimos años, se ha progresado en el conocimiento y la identificación de distintos fenotipos de artrosis, mayoritariamente de rodilla. Aunque han sido muchos los grupos de investigación dedicados a este fin, como ya se ha descrito en la introducción de esta tesis doctoral, se han definido fenotipos con valor clínico, que ayudan a agrupar mejor a nuestros pacientes, pero con poca implicación en cuanto a modificaciones de la práctica clínica o de los tratamientos prescritos. Por este motivo sigue siendo importante establecer correctos biomarcadores de tales fenotipos para, posteriormente, una vez agrupados los pacientes en ellos, poder estudiar mejor el

comportamiento de los biomarcadores dentro de cada uno de forma individualizada y pormenorizada. En este sentido, consideramos que continúa siendo de interés mayúsculo el estudio de los marcadores de inflamación y su posible relación con la gravedad y severidad de la artrosis de rodilla. Los estudios previos, muestran resultados que en ocasiones parecerían hasta contradictorios o en direcciones opuestas, motivo que justifica seguir estudiando la inflamación.

Creemos que nuestra cohorte, de características muy homogéneas debido a que todos los pacientes incluidos en el estudio son mujeres con artrosis sintomática de rodilla y asociada a derrame articular, considerándose un fenotipo inflamatorio, son un conjunto ideal para evaluar los factores de inflamación.

En segundo término, y como consecuencia de las inconsistencias en los artículos publicados hasta la fecha, nos parece de interés poder evaluar la existencia de distintos fenotipos dentro del propio subgrupo inflamatorio, ya que, como se ha comentado, la artrosis es una enfermedad muy heterogénea.

3. HIPÓTESIS

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Diferentes citocinas y factores metabólicos están relacionados entre ellos y se asocian a la severidad clínica y radiográfica en las mujeres afectas de artrosis de rodilla con componente inflamatorio.

4. OBJETIVOS

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Objetivo principal

- Evaluar de forma integral las distintas citocinas y factores metabólicos tanto en líquido sinovial como en plasma relacionados con la severidad en mujeres con artrosis de rodilla con derrame.

Objetivos secundarios

- Evaluar la relación entre los factores inflamatorios en líquido sinovial con la severidad en artrosis de rodilla con derrame.
- Identificar nuevos fenotipos de pacientes con artrosis primaria con derrame articular en función de su perfil inflamatorio y metabólico.
- Estudiar la asociación de los distintos fenotipos con variables clínicas, radiológicas y ecográficas en el momento del reclutamiento.
- Analizar las posibles asociaciones diferenciales entre las citocinas y la severidad clínica y radiográfica en artrosis de rodilla dentro de cada fenotipo inflamatorio.

5. COMPENDIO DE PUBLICACIONES

5. COMPENDIO DE PUBLICACIONES

5.1. Primer artículo

Synovial fluid but not plasma interleukin-8 is associated with clinical severity and inflammatory markers in knee osteoarthritis women with joint effusion.

María García-Manrique, Joan Calvet, Cristóbal Orellana, Antoni Berenguer-Llergo, Silvia Garcia-Cirera, Maria Llop, Néstor Albiñana-Giménez, Carlos Galisteo-Lencastre, Jordi Gratacós.

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Synovial fluid but not plasma interleukin-8 is associated with clinical severity and inflammatory markers in knee osteoarthritis women with joint effusion

María García-Manrique[1,2], Joan Calvet[1,2✉], Cristóbal Orellana[1,2], Antoni Berenguer-Llergo[3], Silvia Garcia-Cirera[1], Maria Llop[1], Néstor Albiñana-Giménez[4], Carlos Galisteo-Lencastre[1] & Jordi Gratacós[1,2]

Several cytokines and adipokines are related to clinical severity and progression in knee osteoarthritis. The aim of this study was to evaluate the associations of IL-8 with clinical severity and with local and systemic adipokines and cytokines. This is a Cross-sectional study including 115 women with symptomatic primary knee osteoarthritis with ultrasound-confirmed joint effusion. Age, symptoms duration and body mass index were collected. Radiographic severity was evaluated according to Kellgren–Lawrence. Pain and disability were assessed by Lequesne and Knee injury and Osteoarthritis Outcome Score pain, symptoms and function scales. Three inflammatory markers and five adipokines were measured by ELISA in serum and synovial fluid. Partial correlation coefficient (PCC) and corresponding 95% confidence interval were used to evaluate association. Synovial fluid IL-8 was significantly associated with clinical severity scales. After controlling for potential confounders, associations measured by a Partial Correlation Coefficient (PCC) remained essentially unaltered for Lequesne (PCC = 0.237), KOOS pain (PCC = − 0.201) and KOOS symptoms (PCC = − 0.209), KOOS function (PCC = − 0.185), although the later did not reach statistical significance. Also in synovial fluid samples, associations were found between IL-8 and TNF (PCC = 0.334), IL6 (PCC = 0.461), osteopontin (PCC = 0.575), visfatin (PCC = 0.194) and resistin (PCC = 0.182), although significance was not achieved for the later after statistical control for confounders. None of these associations were detected in serum. In conclusion, IL-8 was associated with clinical severity, inflammatory markers and adipokines in synovial fluid, but not in blood. Although the reported associations are weak to moderate in magnitude, these findings reinforce the notion that local and not systemic inflammation is more relevant to clinical severity in knee OA women with joint effusion.

Osteoarthritis (OA) is the most prevalent joint disease and the leading cause of pain and disability in adults[1]. The factors associated with the presence, severity and progression of OA are not well known. Among others, inflammatory factors have been related to OA over the past few decades, but their effects vary widely depending on the different studies[2]. Several previous studies have related some cytokines such as TNF-alpha or IL-6 to OA severity, both in synovial fluid and in serum[3]. However, differences according to disease duration and radiological stage in those studies hinders drawing definite conclusions. Adipokines such as leptin, adiponectin, resistin, visfatin and osteopontin in synovial fluid and plasma have been linked to clinical severity and to knee OA (KOA)

[1]Rheumatology Department, Parc Taulí University Hospital, I3PT Research Institute (UAB), c/Parc Taulí s/n, edifici VII Centenari, 08208 Sabadell, Spain. [2]Departament de Medicina, Universitat Autónoma de Barcelona (UAB), 08003 Barcelona, Spain. [3]Biostatistics and Bioinformatics Unit, Institute for Research in Biomedicine Barcelona (IRB Barcelona), 08028 Barcelona, Spain. [4]I3PT Research Institute (UAB), 08208 Sabadell, Spain. ✉email: joan.calvet.fontova@gmail.com

progression[4–6]. Again, differences occur in the series published concerning disease duration, radiographic stage and the presence of synovial effusion that make hard to extrapolate conclusions.

Interleukin-8 (IL-8), also known as CXCL-8, is an inflammatory chemokine present under pathological conditions. It is produced by human OA chondrocytes and is considered to play an important role in OA pathophysiology as it mediates the release of matrix metalloproteinase-13 and has been related to inflammatory changes in the synovium. In a previous study using cartilage culture, IL-8 has been shown to induce hypertrophy and differentiation of chondrocytes in OA[7,8]. It has also been reported that IL-8 synovial fluid levels are increased in OA patients compared to a control group of younger individuals with anterior cruciate ligament injury, observing that elevated IL-8 plasma is linked to MMP-3 activation[9]. However, to our knowledge no clinical associations between IL-8 and clinical severity has been evaluated in knee OA.

The aim of this study was to evaluate the associations of IL-8 in synovial fluid and plasma with clinical severity in KOA patients with joint effusion. As patients with joint effusion may represent a subset with a higher inflammatory component, we have also studied relevant cytokines and adipokines known to play a role in clinical severity and local inflammation in KOA.

Results

Subject characteristics. Included women with inflammatory KOA had a median age of 68.8 ± 11.1 years old with KOA symptoms duration of approximately 4 years. The median Body Mass Index (BMI) was within the obesity range (30.5 kg/m^2). The Kellgren–Lawrence (KL) grades 2 and 3 were predominant in this cohort (41.7% each) while only 3.5% had KL grade 4. Moderate to high disease activity resulted from the different clinical questionnaires administered to the patients. Strong technical effects associated with round of measurement were identified in the quantification of the inflammatory factors and adipokines, and a statistical correction was carried out to enable comparison between samples (See "Assessments" section). Nevertheless, no cut-off points or comparison between plasma and synovial fluid determinations could be reliably established. The corrected values are shown in Table 1.

Association between IL8 and clinical severity. Synovial fluid IL-8 was mildly but significantly associated with clinical severity independently of the questionnaire used. Partial correlation coefficients (PCC) and their corresponding 95% confidence intervals (CI) for the different severity scales were as follows: 0.291 (0.108 to 0.455) for Lequesne; − 0.216 (− 0.389 to − 0.029) for KOOS pain; − 0.221 (− 0.393 to − 0.033) to KOOS symptoms; and − 0.205 (− 0.380 to − 0.017) for KOOS function . When adjusted for confounders known to influence clinical severity as age, disease duration, KL and BMI, synovial fluid IL-8 remained essentially unaltered and statistically significant for Lequesne (PCC = 0.237; CI 0.045 to 0.411), KOOS pain (PCC = − 0.201; CI − 0.380 to − 0.008) and KOOS symptoms (PCC = − 0.209; CI − 0.387 to − 0.017). KOOS function also preserved the magnitude of it correlation with IL-8, although it did not retained statistical significance (PCC = − 0.185; CI − 0.365 to 0.009). None of these associations were observed for IL-8 in plasma (Table 2).

Associations between IL8 and inflammatory markers. Moderate associations in synovial fluid samples between IL-8 levels and inflammatory markers were also observed, which retained statistical significance after control for potential confounders (age, disease duration, KL and BMI) without relevant changes in the magnitude of the associations. PCC estimations for these associations were 0.334 (CI 0.140 to 0.503) and 0.461 (CI 0.293 to 0.602) for TNF and IL-6, respectively. No association was detected in blood between IL-8 and any of these inflammatory factors (Table 2).

Associations between IL8 and adipokines. When correlation with adipokines was evaluated, mild to moderate associations were observed in synovial fluid between IL-8 and resistin (PCC = 0.201; CI 0.012 to 0.376), visfatin (PCC = 0.256; CI 0.070 to 0.424) and osteopontin (PCC = 0.593; CI 0.455 to 0.703). Again, the magnitude of these correlations did not suffer important changes after controlling by potential confounders (age, disease duration, KL and BMI) for osteopontin (PCC = 0.575; CI 0.430 to 0.692) and visfatin (PCC = 0.194; CI 0.000 to 0.373), although statistical significance was not kept in the case of resistin (PCC = 0.182; CI − 0.012 to 0.362). No additional associations between plasma IL-8 and any of the plasma adipokines were observed (Table 2).

Discussion

This study showed a weak association between synovial fluid IL-8 with clinical severity and local synovial fluid inflammatory markers, both with cytokines and adipokines. On the other hand, plasma IL-8 was not related to clinical severity nor blood cytokines or adipokines.

Previous studies found high levels of IL-8, both in synovial fluid and blood, in KOA patients compared to controls[10]. In contrast to our study, no clinical evaluation or inflammatory markers measurement were conducted in those works, and all KOA patients were analyzed during knee replacement surgery. Furthermore, the control group underwent diagnostic or therapeutic arthroscopy procedures for a knee injury, so, as usually in control groups compared to the OA sample, age-related differences exist. Ruan et al. found an association between plasma IL-8 with clinical (measured by WOMAC) and radiographic severity[11], but as opposed to our study, men and women were included in this work, patients had no joint effusion and, consequently, synovial fluid was not analyzed. A recent study performed in synovial fluid found an association between IL-8 and KL stage, but it involved exclusively end-stage KOA patients who underwent prosthetic surgery with 45% of subjects evaluated in KL stage 4 and 40% in KL 3. In addition, no clinical evaluation was performed in the participants in this study[12]. Thus, to our knowledge, our study is the first to find that synovial fluid IL-8 is associated with clinical severity in knee OA patients.

Variables	Category	Median (IQR)/N (%)
Demographics	Age	68.80 (11.1)
	KOA symptoms duration (months)	50.00 (73.00)
	BMI (kg/m^2)	30.5 (6.35)
Radiographic severity	**KL**	
	1	15 (13.1%)
	2	48 (41.7%)
	3	48 (41.7%)
	4	4 (3.5%)
Clinical questionnaires[a]	Lequesne	14.00 (5.00)
	KOOS pain	42.00 (17.00)
	KOOS symptoms	44.00 (18.50)
	KOOS function	46.00 (19.00)
Inflammatory markers SF[b]	IL-8 SF pg/mL	10.96 (14.00)
	TNF-α SF pg/mL	10.19 (7.97)
	IL-6 SF pg/mL	105.99 (302.64)
Blood inflammatory markers[b]	IL-8 blood pg/mL	3.13 (2.46)
	TNF-α blood pg/mL	5.64 (1.95)
	IL-6 blood pg/mL	2.72 (3.31)
Adipokines SF[b]	Leptina SF pg/mL	42,079.42 (29,565.99)
	Adiponectin SF ng/mL	1734.76 (1352.53)
	Resistin SF pg/mL	2225.65 (2205.79)
	Visfatin SF ng/mL	1.53 (1.18)
	Osteopontin SF ng/mL	57.66 (83.19)
Blood adipokines[b]	Leptin blood pg/mL	37,948.02 (28,243.77)
	Adiponectin blood ng/mL	14,294.05 (9990.22)
	Resistin blood pg/mL	2068.29 (1173.56)
	Visfatin blood ng/mL	3.78 (1.37)
	Osteopontin blood ng/mL	10.97 (7.02)

Table 1. Demographic variables, radiographic severity, clinical questionnaires, inflammatory markers and adipokines in synovial fluid and blood. Medians and interquartilic ranges (IQR) were used to describe continuous variables; categorical data were summarized using absolute frequencies (N) and percentages (%); *KOA* knee osteoarthritis, *KL* Kellgren–Lawrence scale, *BMI* Body Mass Index, *KOOS* Knee injury and Osteoarthritis Outcome Score, *SF* synovial fluid, *IL-8* interleukin-8, *IL-6* interleukin-6, *TNF-α* Tumor Necrosis Factor-alpha. [a]Clinical questionnaires range values and interpretation: Lequesne (range from 0 to 24; where 0 is non and 24 maximum degrees of pain or incapacity) KOOS pain, symptoms, and function (range from 0 to 100; where 0 is maximum and 100 minimum of each condition evaluated). [b]Levels of inflammatory markers and adipokines in synovial fluid and blood were corrected a-priori by measure round.

Previous studies have associated resistin and ostepontin with IL-8 presence in chondrocytes and white adipose tissue in cultures from knee OA patients[13,14]. The impact of IL8 on chondrocytes degradation could be related to OA progression and could point to new therapeutic approaches[15]. The association found in this work between IL-8 and clinical severity in knee OA together with its link with different inflammatory factors and adipokines might suggest that IL-8 could be among the last effectors in the inflammatory cascade of knee OA, as it is associated with a great array of molecules related to knee OA clinical severity.

A high level of IL-8 has been found in the serum and synovial fluid of patients with OA[16]. Different actions of IL8 could explain its involvement in OA, such as neutrophil chemotaxis, activation of leukocytes and migration to the joint or a direct effect on chondrocyte hypertrophy and differentiation or increased matrix metalloproteinase release[17–19], as well as the induction of angiogenic changes related to chronic inflammation[20]. Within the joint, IL-8 is expressed by macrophages[18], OA chondrocytes[19], fibroblast-like synoviocytes[21], and infrapatellar fat tissue[22].

Our study has found an association for IL-8 and clinical severity and inflammatory markers in synovial fluid but not in plasma, which appears to support the hypothesis that changes related to OA physiopathology are more prominent in the joint, and synovial fluid should be the preferred biological fluid to be assessed. These results could indicate that, in this group of women with knee OA with join effusion, local inflammation is more relevant to clinical severity than systemic inflammation.

The main limitation of this study is related to its cross-sectional design, so that conclusions should be interpreted in terms of associations and we should be cautious about extrapolating causal relations. Another limitation is that as all patients were referred to our Rheumatology Unit for specialist care a selection bias towards more severe disease could exist. The lack of a control group did not allow us to confirm that interleukin-8 was

	Synovial fluid IL-8[a]				Serum IL-8[b]			
	Univariate		Adjusted[c]		Univariate		Adjusted[c]	
	PCC [95% CI]	*p* value	PCC [95% CI]	*p* value	PCC [95% CI]	*p* value	PCC [95% CI]	*p* value
Clinical severity								
Lequesne	**0.291 [0.108, 0.455]**	**0.002**	**0.237 [0.045, 0.411]**	**0.016**	0.095 [−0.093, 0.277]	0.320	0.101 [−0.091, 0.287]	0.301
KOOS pain	**−0.216 [−0.389, −0.029]**	**0.024**	**−0.201 [−0.380, −0.008]**	**0.041**	−0.028 [−0.213, 0.160]	0.774	−0.029 [−0.219, 0.162]	0.764
KOOS symptoms	**−0.221 [−0.393, −0.033]**	**0.021**	**−0.209 [−0.387, −0.017]**	**0.034**	−0.076 [−0.258, 0.112]	0.431	−0.071 [−0.258, 0.121]	0.469
KOOS function	**−0.205 [−0.380, −0.017]**	**0.032**	−0.185 [−0.365, 0.009]	0.062	−0.059 [−0.243, 0.128]	0.536	−0.059 [−0.247, 0.133]	0.548
Inflammatory markers								
TNF-α	**0.332 [0.143, 0.498]**	**< 0.001**	**0.334 [0.140, 0.503]**	**0.001**	0.082 [−0.106, 0.265]	0.390	0.094 [−0.099, 0.279]	0.340
IL-6	**0.455 [0.291, 0.594]**	**< 0.001**	**0.461 [0.293, 0.602]**	**< 0.001**	−0.064 [−0.248, 0.123]	0.502	−0.034 [−0.223, 0.158]	0.729
Adipokines								
Leptin	0.076 [−0.119, 0.266]	0.445	0.006 [−0.193, 0.204]	0.955	−0.058 [−0.241, 0.130]	0.547	−0.036 [−0.225, 0.156]	0.718
Adiponectin	0.095 [−0.095, 0.279]	0.326	0.124 [−0.071, 0.310]	0.211	0.068 [−0.120, 0.251]	0.481	0.044 [−0.148, 0.233]	0.654
Resistin	**0.201 [0.012, 0.376]**	**0.037**	0.182 [−0.012, 0.362]	0.066	0.010 [−0.176, 0.196]	0.914	0.024 [−0.168, 0.214]	0.809
Visfatin	**0.256 [0.070, 0.424]**	**0.007**	**0.194 [0.000, 0.373]**	**0.049**	0.037 [−0.151, 0.222]	0.702	0.037 [−0.155, 0.226]	0.708
Osteopontin	**0.593 [0.455, 0.703]**	**< 0.001**	**0.575 [0.430, 0.692]**	**< 0.001**	0.059 [−0.129, 0.242]	0.542	0.066 [−0.126, 0.254]	0.502

Table 2. Synovial fluid and serum IL-8 associations with clinical severity, inflammatory markers and adipokines in synovial fluid and blood. [a]Synovial fluid associations between IL-8 with inflammatory markers and adipokines. [b]Serum associations between IL-8 with inflammatory markers and adipokines. *KOOS* Knee injury and Osteoarthritis Outcome Score, *SF* synovial fluid, *IL-8* interleukin-8, *IL-6* interleukin-6, *TNF-α* Tumor Necrosis Factor-alpha. [c]Effects adjusted by measurement batch and by potential confounders: age, OA evolution time, KL grade and BMI. *KL* Kellgren–Lawrence scale, *BMI* Body Mass Index. Statistical significant associations are in bold type. *PCC* partial correlation coefficient. *95% CI* interval at 95% confidence. Statistically significant results are highlighted in bold.

specifically related to knee osteoarthritis symptoms and would be associated with clinical severity in other joint conditions associated with effusion. Additionally, these observations might be confirmed in a men cohort and were only associated in joint effusion related osteoarthritis women.

A strength of this work is the homogeneity of the patients in this study as only women with significant symptomatic knee OA and joint effusion, with predominantly low-to-moderate radiographic stage and moderate to high clinical severity were included. This homogeneity allows our results to be applied to a well-defined phenotype and increases the statistical power to detect moderate magnitude associations.

In conclusion, synovial fluid IL-8 was related to clinical severity in knee OA patients. The associations between IL-8 and several inflammatory markers and adipokines in synovial fluid, but no in blood, reinforces the hypothesis that local and not systemic inflammation is more relevant for clinical severity in these patients. Replication is warranted, especially in other groups of patients, such as men and different knee OA stages.

Methods

Study design and subjects. Patients systematically included in this cross-sectional study belong to a primary KOA cohort previously reported[23]. We studied 115 women aged 51–83 with symptomatic primary KOA according to ACR criteria and who showed significant joint effusion on physical examination and confirmed by ultrasound (≥ 4 mm on midline supra-patellar line). Included patients had pain intensity ≥ 4 on a 10-cm visual analogical scale despite the use of prescribed analgesic drugs for at least three months and had persisting knee effusion or documented effusion in several consultations. Patients with a history of trauma, meniscal injury, inflammatory rheumatic or septic arthritis, previous knee surgery or patients with any other secondary OA were excluded, as well as those any condition potentially influencing pain perception[4,23,24]. Patients who had received systemic glucocorticoids over the last six months or intra-articular glucocorticoid in the last three months or hyaluronic acid injection in the last 6 months were also excluded. Patients were recruited from October 2013 to June 2016. As there are gender-specific differences related to clinical severity measurement of OA and inflammatory markers or adipokines levels[25–27] only women were included to homogenize the study sample. This study was approved by the Local Ethical Committee at the Hospital Universitari Parc Taulí, Sabadell (2013/591). All

patients included were verbally informed and signed informed consent and all methods were performed in accordance with the relevant guidelines and regulations.

Assessments. The following variables were collected: age, KOA disease duration and body mass index (BMI, kg/m^2). Fasting blood analyses were carried out to assess serum inflammatory markers and adipokines. Joint aspiration was performed during the visit in fasting conditions and at the same time of day for proper evaluation of synovial adipokines. A minimum of 2 mL of aspired synovial fluid was required to include the patient in the study, and the mean and median across the cohort were 13.5 and 9 mL respectively. Non-inflammatory synovial fluid (cell count < 2000 cells) and absence of microcrystals were confirmed. Serum and synovial samples were stored at − 80 °C. Two validated scores (Lequesne index and Knee injury and Osteoarthritis Outcome Score (KOOS) were used to evaluate clinical severity. Subjects participating in this study complete only three out of five KOOS domains, specifically pain, symptoms, and function subscales. Low KOOS scores indicate a worse clinical severity in all domains. Radiographic severity was assessed according to the Kellgren–Lawrence scale (KL) with an antero-posterior knee X-ray examination in standing position performed over the last eighteen months. X-ray were evaluated independently by two rheumatologists (JC, CO).

As previously described, three inflammatory markers (IL-8, TNF-α and IL-6) and five adipokines (leptin, adiponectin, resistin, visfatin and osteopontin) were analyzed by ELISA following manufacturer recommendations for serum and synovial fluid dilutions. IL-6 and TNF-α were analyzed by Luminex HCYTOMAG-60 K-03 (Merck Millipore). Sensibility: IL-6: 0.9 pg/mL, TNF-alpha: 0.7 pg/mL, detection range: 3.2–2000 pg/L, Coef. intra-assay: IL-6: 2%, TNF-alfa: 2.6%, Coef. inter-assay: IL-6: 18.3%, TNF-alpha: 13%. Luminex IL8 Human Procartaplex simplex Kit (ThermoFisher Scientific) was used for analyzing IL8. The sensitivity of the assay tested on plasma is 1.2 pg/mL. Its intra-assay and inter-assay CV are respectively 8.5% and 4.6%. Adipokines were analyzed with Human Leptin ELISA Kit (Biocompare, California, USA). Dilution 1/100. Sensibility: < 8 pg/mL, detection rang: 62.5–10,000 pg/L, Intra-assay: < 6.3%, Inter-assay: < 7.2%. Adiponectin ELISA kit (eBioscience, California, USA). Dilution 1/1000. Sensibility: 0.01 ng/mL, detection rang: 0.78–50 mg/L, Coef. intra-assay: 4.2%, Coef. inter-assay: 3.1%. Human Resistin ELISA Kit (Raybiotech, GA, USA). Dilution 1/100. Sensibility: 1.4 pg/mL, detection rang: 1.4–400 pg/mL, Coef. intra-assay: < 10%, Coef. inter-assay: < 12%. Osteopontin ELISA kit (eBioscience, California, USA). Dilution 1/100. Sensibility: 0.26 ng/mL, detection rang: 0.47–30 mg/L, Coef. intra-assay: 6.7%, Coef. inter-assay: 6.1%. Visfatin ELISA kit (Phoenix Pharmaceuticals, California, USA). Dilution: none. Sensibility: 2.21 ng/mL, detection rang: 0.1–1000 ng/mL, Coef. intra-assay: < 10%, Coef. inter-assay: < 15%. Because of technical reasons inherent to ELISA (configuration of plates used), these markers could not be assessed at the same time for all patients, and non-negligible effects associated to time of measurement were detected[4,24]. For this reason and in order to avoid biases in our estimations due to these effects that are technical in nature, the round of measurement was considered as an adjustment factor in all the statistical analyses performed in this study[4,23,24]. Although this correction allows reliable estimations of association for synovial and plasma measurements, it does not enable inference of either their real range of variability or meaningful cutoff values that can be extrapolated to external data, purposes for which a specific and specialized calibration study would be required[23,24].

Statistical methods. Clinical and laboratory data were described using non-parametric methods. Medians and interquartile ranges were applied to continuous measures, whereas absolute and relative frequencies were used for categorical variables. For laboratory measures, differences in means due to measurement rounds were corrected previously to descriptive calculations. Association analyses were carried out by fitting linear models in which IL-8 was included as outcome, and inflammatory factors and adipokines were transformed suitably to meet the assumptions of the models. As mentioned above, all the models included round of measurement as covariate in order to account for the associated technical variability in the analyses (see "Assessments" section). As in previously published studies, associations were also assessed after statistical control by age, KOA symptom duration, radiographic stage evaluated by KL and BMI, which were included as explanatory variables in the models for this purpose. Partial correlation coefficients (PCC) and adjusted group means derived from the models were used to measure the magnitude of the effects for continuous and categorical variables, respectively[4,23].

A 5% was set as threshold for statistical significance. All statistical analyses were conducted using R.

Ethics approval and consent to participate. Ethical approval was obtained from the Institutional Review-Board of the Parc Taulí University Hospital (Decision Number 2013/591). Participants signed informed consents.

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Author contributions

M.G., J.C., J.G., C.O. contributed to study conception and design, data collection, data interpretation, literature search and writing the report. N.A. contributed to laboratory analysis and experiments, literature search and data interpretation. M.L.L. contributed to study design, data collection, literature search. A.B. contributed to study design, data analysis and interpretation. C.G. and S.G. contributed to patient recruitment, sample process and data collection. All authors reviewed and approved the final version of the manuscript.

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Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to J.C.

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5.2 Segundo artículo

Metabolic and inflammatory profiles define phenotypes with clinical relevance in female knee osteoarthritis patients with joint effusion.

Joan Calvet, María García-Manrique, Antoni Berenguer-Llergo, Cristóbal Orellana, Silvia Garcia Cirera, Maria Llop, Carlos Galisteo Lencastre, Marta Arévalo, Cristina Aymerich, Rafael Gómez, Néstor Albiñana Giménez, Jordi Gratacós.

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British Society for Rheumatology RHEUMATOLOGY OXFORD

Clinical science

Metabolic and inflammatory profiles define phenotypes with clinical relevance in female knee osteoarthritis patients with joint effusion

Joan Calvet [1,2,*], María García-Manrique[1,2], Antoni Berenguer-Llergo [3], Cristóbal Orellana[1], Silvia Garcia Cirera[1], Maria Llop[1], Carlos Galisteo Lencastre[1], Marta Arévalo[1], Cristina Aymerich[1], Rafael Gómez[1], Néstor Albiñana Giménez[4], Jordi Gratacós[1,2]

[1]Rheumatology Department, Parc Taulí Hospital Universitari, Institut d'Investigació i Innovació Parc Taulí (I3PT-CERCA), Sabadell, Spain
[2]Departament de Medicina, Universitat Autónoma de Barcelona (UAB), Barcelona, Spain
[3]Rheumatology Department, Biostatistics and Bioinformatics, Institut d'Investigació i Innovació Parc Taulí (I3PT-CERCA), Sabadell, Spain
[4]Scientific-Technical Unit, Institut d'Investigació i Innovació Parc Taulí (I3PT-CERCA) (UAB), Sabadell, Spain
*Correspondence to: Joan Calvet, Edifici VII Centenari Rheumatology Department, c/Parc Taulí s/n, 08208 Sabadell, Spain. E-mail: jcalvet@tauli.cat

Abstract

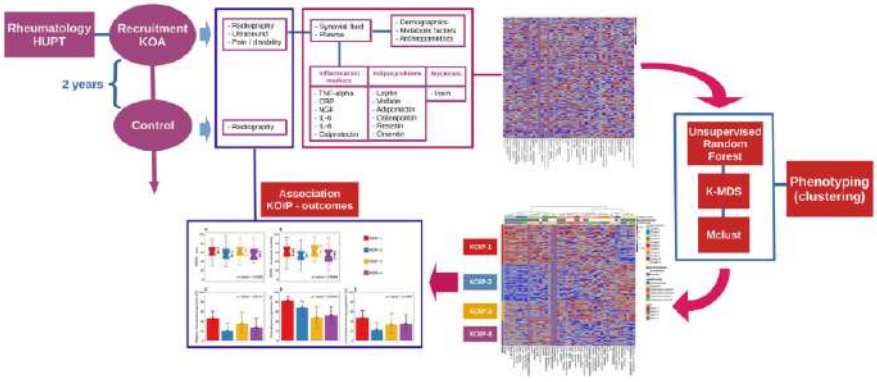
Objectives: Osteoarthritis has been the subject of abundant research in the last years with limited translation to the clinical practice, probably due to the disease's high heterogeneity. In this study, we aimed to identify different phenotypes in knee osteoarthritis (KOA) patients with joint effusion based on their metabolic and inflammatory profiles.

Methods: A non-supervised strategy based on statistical and machine learning methods was applied to 45 parameters measured on 168 female KOA patients with persistent joint effusion, consecutively recruited at our hospital after a monographic OA outpatient visit. Data comprised anthropometric and metabolic factors and a panel of systemic and local inflammatory markers. The resulting clusters were compared regarding their clinical, radiographic and ultrasound severity at baseline and their radiographic progression at two years.

Results: Our analyses identified four KOA inflammatory phenotypes (KOIP): a group characterized by metabolic syndrome, probably driven by body fat and obesity, and by high local and systemic inflammation (KOIP-1); a metabolically healthy phenotype with mild overall inflammation (KOIP-2); a non-metabolic phenotype with high inflammation levels (KOIP-3); and a metabolic phenotype with low inflammation and cardiovascular risk factors not associated with obesity (KOIP-4). Of interest, these groups exhibited differences regarding pain, functional disability and radiographic progression, pointing to a clinical relevance of the uncovered phenotypes.

Conclusion: Our results support the existence of different KOA phenotypes with clinical relevance and differing pathways regarding their pathophysiology and disease evolution, which entails implications in patients' stratification, treatment tailoring and the search of novel and personalized therapies.

Graphical abstract



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Keywords: knee osteoarthritis, phenotype, inflammatory, metabolism, clinical severity, machine learning

Rheumatology key messages

- Despite the abundant research conducted on knee osteoarthritis, there is a lack of translational results.
- Using non-supervised machine learning techniques, we identified four KOA phenotypes.
- The uncovered phenotypes exhibited differences in clinical severity and radiographic progression.

Introduction

OA is the most common form of arthritis worldwide in older adults [1]. Knee OA (KOA) is the most prevalent and the major contributor to OA socioeconomic burden, causing a significant degree of pain and disability [2]. Several studies have evaluated factors associated with clinical severity and radiographic progression in KOA [3–5], including age, obesity, cardiovascular conditions [6], cytokines in plasma and synovial fluid [7, 8], and molecular determinants of cartilage degradation [9]. Numerous sex-related differences have been reported among KOA affected subjects, not only regarding prevalence, but also metabolic conditions, inflammatory factors and levels of pain and function disability [10, 11]. Despite this abundant research, KOA pathophysiology is currently not well understood, the available therapeutic options are limited and, as of the date of writing, there is no specific therapy with a disease-modifying effect [12].

An explanation for this lack of translational results is the great variability across OA patients regarding clinical presentation, exhibit of risk factors and prognosis. Based on these observations, it has been proposed that OA does not correspond to a single entity, but to a multifaceted and heterogeneous syndrome consisting of different subgroups (phenotypes), possibly with specific pathophysiologic traits (endotypes) [13]. The identification of these phenotypes entails clinical implications of high relevance, as they might improve patients' stratification, enable a personalized choice of treatment and a more accurate selection for clinical trials, provide insight into their pathophysiology and generate new hypotheses for future research, especially on targets for novel therapeutic options [14].

Following this hypothesis, various groups aimed their research at the characterization of OA phenotypes, most of them in a hypothesis-driven manner based on one or a few selected features (top-down phenotyping) [15]. In a completely different approach, a few works attempted to identify OA phenotypes by uncovering the patterns and clusters present in patients' data using statistical and machine learning techniques in a totally unsupervised manner (step-up phenotyping) [15]. This approach has been used in a great variety of data, including clinical parameters [16, 17], transcriptomic [18, 19], metabolomics [20, 21] and other biochemical markers [22, 23]. Although all these works suggested the existence of OA phenotypes characterized by features of a different nature, their association with severity and progression and, especially, their clinical applicability was very limited. In agreement with findings previously published [10, 11], some of these studies reported important sex-specificities, suggesting that OA phenotyping should be conducted for women and men separately [23].

Despite these efforts, there is as of now no consensus on a comprehensive classification of OA with clinical relevance. In KOA, the existence of an inflammatory clinical phenotype characterized by the presence of synovitis and higher levels of pain, functional disability and rate of progression is widely acknowledged [24, 25]. In our study, a data-driven approach was used to identify phenotypes in 168 KOA patients with persistent joint effusion from a prospective KOA cohort. To do so, anthropometric parameters, metabolic factors and systemic and local inflammatory markers were analysed using well-established statistical and machine learning methods in a non-supervised manner. To assess their clinical relevance, differences across phenotypes were assessed regarding pain, functional disability, ultrasound and radiographic severity and progression.

Methods

Patients' description

The subjects of this study are part of a prospective cohort of patients that includes men and women with primary knee osteoarthritis (KOA) and persistent joint effusion. The cohort includes 202 KOA patients consecutively recruited after an outpatient visit in our Rheumatology Service from October 2013 to April 2018. At present, the cohort includes 171 women, three of which were excluded from our study due to their high number of missing values in the parameters used for clustering (>10%, five variables), leaving 168 female KOA patients for our analysis (Table 1). We focused the present work on female patients to homogenize the study sample, as previous works have reported numerous sex-related differences in OA regarding metabolic conditions, inflammatory factors and levels of pain and function disability [10, 11]. An exhaustive description of the inclusion and exclusion criteria can be found in the Supplementary Information available at *Rheumatology* online.

All subjects signed an informed consent authorizing the collection of samples and data for their use in the context of KOA studies. The project was evaluated and approved by the ethical committee of our centre (CEIm Parc Taulí) with approval number 2015/539, and was conducted according to the national and international ethical guidelines (Ethical Standards, Declaration of Helsinki).

Samples

Samples from plasma and joint fluid were systematically extracted at recruitment from all patients in the cohort. Synovial fluid was obtained by aspiration (13.5 mean and 9 ml median) and analysed to discard the presence of

Table 1. Main baseline patients' characteristics

			Kellgren–Lawrence			
		All 168 (100%)	Grade 1 19 (11.3%)	Grade 2 65 (38.7%)	Grade 3 78 (46.4%)	Grade 4 6 (3.6%)
Age at recruitment		69.1 [50.9, 83.0]	69.2 [55.7, 80.8]	68.7 [51.7, 83]	69.0 [50.9, 81.4]	74.1 [52.0, 77.4]
Disease evolution time (months)		48 [4, 200]	36 [6, 130]	48 [4, 200]	48 [6, 150]	57 [6, 135]
Obesity		94 (56.0%)	12 (63.2%)	32 (49.2%)	45 (57.7%)	5 (83.3%)
Physical exercise	None	61 (36.3%)	6 (31.6%)	24 (36.9%)	27 (34.6%)	4 (66.7%)
	Sporadic	51 (30.4%)	5 (26.3%)	19 (29.2%)	26 (33.3%)	1 (16.7%)
	Moderate	46 (27.4%)	5 (26.3%)	20 (30.8%)	20 (25.6%)	1 (16.7%)
	Vigorous	10 (6.0%)	3 (15.8%)	2 (3.1%)	5 (6.4%)	0 (0.0%)
Diabetes mellitus		18 (10.7%)	1 (5.3%)	8 (12.3%)	9 (11.5%)	0 (0.0%)
Arterial hypertension		92 (54.8%)	8 (42.1%)	35 (53.8%)	47 (60.3%)	2 (33.3%)
Dyslipidaemia		68 (40.5%)	4 (21.1%)	29 (44.6%)	33 (42.3%)	2 (33.3%)
ATP III metabolic syndrome		61 (36.3%)	8 (42.1%)	24 (36.9%)	28 (35.9%)	1 (16.7%)
KOOS—pain (reversed, 0–100)		58 [28, 97]	58 [29, 87]	58 [33, 89]	58 [28, 97]	58 [42, 92]
KOOS—symptoms (reversed, 0–100)		57 [11, 96]	62 [19, 82]	57 [11, 96]	56 [19, 96]	67 [37, 79]
KOOS—functional disability (reversed, 0–100)		58 [19, 94]	59 [22, 79]	57 [25, 93]	58 [19, 94]	60 [29, 72]
Joint effusion (mm)		9.1 [4.5, 19.1]	8.6 [5.8, 14.1]	9.1 [4.5, 15.2]	9.2 [4.5, 19.1]	9.7 [8.10, 12.30]

Demographic, anthropometric, metabolic, radiographic and clinical factors, for all the KOA patients included in the study and stratified by Kellgren–Lawrence (KL) staging. All subjects are female patients diagnosed with symptomatic primary knee osteoarthritis (KOA) with persistent joint effusion. Continuous parameters are described with their median and ranges (minimum and maximum values), while absolute frequencies and percentages are displayed for categorical variables. No missing values were observed for any of variables displayed in the table ($n = 168$).
ATP III: Adult Treatment Panel III; KOOS: knee injury and osteoarthritis outcome scores (reversed scores).

inflammatory fluid (joint cell count <2500 cells) and microcrystals. Collected samples were appropriately centrifuged and stored at −80°C, until their use for quantifications. Blood extractions and the arthrocentesis were performed at the same day and in fasting conditions.

Data collection

Baseline information regarding demographics, anthropometric and metabolic factors were systematically collected from the patients in the cohort at time of recruitment (Supplementary Table S1, available at *Rheumatology* online). Blood determinations related to metabolic syndrome were assessed as per clinical practice. Synovial and plasma samples were evaluated by ELISA for a set of 13 selected cytokines, in order to assess their local and systemic inflammatory profiles (Supplementary Table S1, available at *Rheumatology* online). In total, a comprehensive panel of 45 parameters clinically relevant for OA were available for the phenotyping analysis, (Supplementary Table S1, available at *Rheumatology* online), which displayed evident structures of correlation among them (Supplementary Fig. S1, available at *Rheumatology* online).

Baseline KOA severity outcomes included: pain, functional disability and symptoms levels as measured by the Knee injury and Osteoarthritis Outcome Scores (KOOS, in reversed order); ultrasound measurements of joint effusion and synovial tissue thickness (mm); Kellgren–Lawrence (KL) radiographic stage; and OARSI atlas lecture, including osteophytes assessment and joint space narrowing (JSN). To assess their radiographic progression at two years, most of the patients ($n = 143$, 85%) also underwent a radiographic evaluation during the follow-up. (Supplementary Table S1, available at *Rheumatology* online). Of the 24 patients without a follow-up radiography, 13 had undergone knee prosthesis surgery (one in baseline KL-1, three in KL-2, seven in KL-3 and two in KL-4). The remaining 11 patients (7%) represent losses in the follow-up.

Clustering analyses for phenotype discovery

A clustering analysis based on well established statistical and machine learning methods was carried out on 45 variables including anthropometric, metabolic and systemic and local inflammatory factors, which were selected from the information available in our KOA cohort for their clinical relevance in OA (see previous sections and Supplementary Table S1, available at *Rheumatology* online). All 45 variables participated in the clustering analyses, and no explicit feature selection was performed prior to the clustering procedure. Of note, this approach was completely unsupervised and no variable expressing KOA severity or progression was involved in the clustering process, which consisted of four steps: (i) missing

values were imputed by Recursively Subtracted Empirical Orthogonal Functions analysis [26]; (ii) pairwise patients' dissimilarities were estimated by unsupervised Random Forest (RF) [27]; (iii) a Kruskal's Non-metric Multidimensional Scaling (K-MDS) was applied to the resulting dissimilarity matrix; and (iv) components derived from K-MDS underwent a model-based clustering analysis based on mixture of Gaussian distributions (Mclust) [28]. Results for the two first K-MDS components showed the most evident data structure as they involved a high number of clusters (four) according to Bayes Information Criterion (BIC) (Supplementary Fig. S2, available at *Rheumatology* online). These clusters showed a strong statistical significance according to a likelihood ratio test (Supplementary Table S2, available at *Rheumatology* online), and remarkable robustness as measured by a number of stability indexes that included Jaccard, adjusted Rand, Fowlkes–Mallows and j-score, and normalized mutual information (Supplementary Table S3, available at *Rheumatology* online). Hence, this configuration was chosen to define the knee osteoarthritis inflammatory phenotypes (KOIP) and used in downstream analyses (Supplementary Fig. S3, available at *Rheumatology* online).

Clustering results were graphically represented using heatmaps at the patient and the cluster level. To facilitate the interpretation of the results, the 45 clustering variables were grouped by their similarity across patients using a hierarchical clustering and a correlation-like distance based on Spearman correlation (SC), Phi coefficients and Glass rank biserial correlations (GRBCorr).

To assist the interpretation of the clusters, a supervised RF classifier was trained to predict patients' KOIP membership. In these analyses, variables contributing the most to KOIP definitions were identified using VSURF [29], a sequential selection procedure based on RF. Finally, and to explore clusters' sex-specificity, male patients with available data ($n = 23$) were assigned to the KOIP groups using a supervised RF model trained with data from the female patients (Supplementary Information available at *Rheumatology* online).

Statistical analysis

Continuous parameters were described by their medians, median absolute deviations and ranges, while categorical variables were summarized using absolute frequencies and percentages. Associations with KOIP groups and outcomes were assessed using non-parametric methods, namely Kruskal–Wallis and Mann–Whitney tests for continuous variables, and Fisher's tests for categorical variables. All data analyses were conducted with R [30] (see Supplementary Information available at *Rheumatology* online).

Results

Phenotype discovery

A clustering analysis was performed on data from 168 women included in a cohort of primary KOA patients with joint effusion (Table 1). Data consisted of a comprehensive panel of 45 variables systematically collected in the cohort and selected for their clinical relevance in OA (Supplementary Table S1,

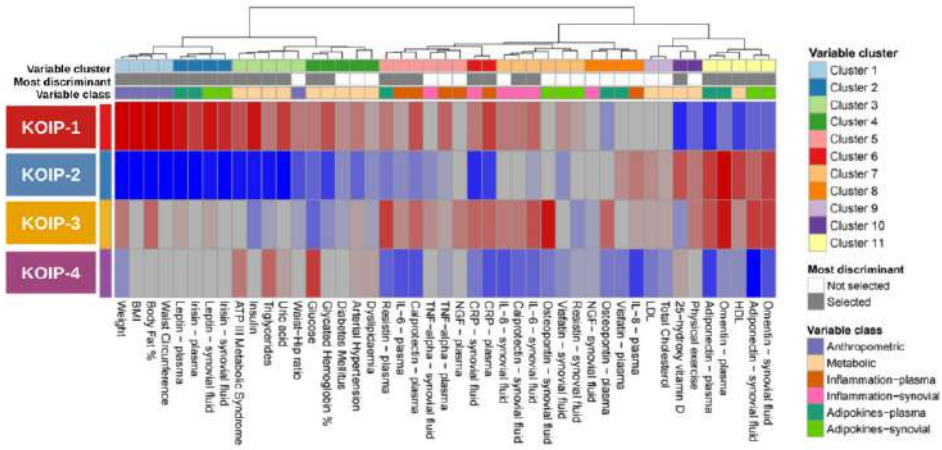

Figure 1. Results at the group level of the phenotype discovery analyses performed on data from 168 female patients of primary knee osteoarthritis (KOA) with persistent joint effusion. Cells show the median (continuous variables) or averages (numerically coded categorical variables) of the standardized (centered and scaled) values of anthropometric, metabolic and systemic and local inflammatory factors involved in the clustering analysis. Red indicates high, blue represents low, and colour intensity expresses more extreme values. Values of binary variables were previously converted to numeric format, where 1 indicated presence and 0 represented absence of the corresponding feature. Physical exercise (four categories) was also converted to numeric format and treated as ordinal. Colour intensities were saturated approximately to percentiles 5% and 95% of the overall values distribution. Patient clusters were derived from a machine learning (ML)-based strategy that used unsupervised random forest (RF), Kruskal's non-metric multidimensional scaling (KMDS) and Gaussian finite mixture models for model-based clustering (Mclust), which selected the optimal number of clusters with objective statistical criteria (Bayes information criterion, BIC). Variables were grouped by a hierarchical clustering using Ward agglomerative method and non-parametric correlation-like measurements, namely: Spearman correlation (continuous *vs* continuous or ordinal variables), Phi coefficient (binary *vs* binary variables) and Glass rank biserial correlation (continuous/ordinal *vs* binary variables). Variables with the highest discriminative power of patients' clusters were identified using a sequential selection procedure based on random forests (VSURF—interpretation mode) and highlighted in the corresponding annotation bar (*Most discriminant*). ATP III: Adult Treatment Panel III; HDL: high-density lipoprotein; KOA: knee osteoarthritis; KOIP: knee osteoarthritis inflammatory phenotype; LDL: low-density lipoprotein; NGF: nerve growth factor

Table 2. Characterization of knee osteoarthritis inflammatory phenotypes (KOIP)

	N (%Miss.)	All 168 (100%)	KOIP-1 55 (32.7%)	KOIP-2 51 (30.4%)	KOIP-3 27 (16.1%)	KOIP-4 35 (20.8%)	*P*-value
Weight (kg)	168 (0.0%)	74.00 (10.90)	84.20 (8.15)	64.40 (7.12)	76.90 (7.56)	71.90 (5.49)	<0.0001
Body mass index	168 (0.0%)	31.04 (4.97)	35.56 (3.49)	26.60 (2.54)	30.78 (3.39)	31.27 (2.86)	<0.0001
Body fat percentage (%)	167 (0.6%)	41.60 (5.49)	46.05 (4.00)	36.40 (3.56)	43.60 (3.26)	42.00 (2.82)	<0.0001
Waist circumference (cm)	167 (0.6%)	101.00 (11.12)	110.75 (7.78)	91.00 (7.41)	100.50 (6.67)	101.00 (5.93)	<0.0001
Leptin—plasma (pg/mL)	167 (0.6%)	36688.11 (19582.18)	54392.71 (25049.17)	23566.59 (10682.27)	39809.19 (18942.95)	35530.72 (13628.15)	<0.0001
Irisin—plasma (ng/mL)	166 (1.2%)	707.78 (256.30)	859.25 (107.21)	437.22 (211.14)	727.20 (174.17)	726.02 (212.84)	<0.0001
Leptin—synovial fluid (pg/mL)	168 (0.0%)	36160.71 (20839.58)	54016.11 (21012.50)	23126.79 (9402.28)	39506.85 (20839.78)	34888.07 (15821.33)	<0.0001
Irisin—synovial fluid (ng/mL)	165 (1.8%)	695.95 (366.25)	938.23 (228.26)	422.07 (289.34)	701.88 (366.00)	686.28 (341.25)	<0.0001
ATP III metabolic syndrome	168 (0.0%)	61 (36.3%)	33 (60.0%)	2 (3.9%)	9 (33.3%)	17 (48.6%)	<0.0001
Insulin (microU/mL)	168 (0.0%)	10.61 (5.95)	15.09 (5.47)	7.20 (3.08)	8.94 (2.49)	11.07 (5.99)	<0.0001
Triglycerides (mg/dL)	168 (0.0%)	114.50 (49.67)	133.00 (56.34)	83.00 (25.20)	110.00 (31.13)	134.00 (48.93)	<0.0001
Uric acid (mg/dL)	168 (0.0%)	4.75 (1.56)	5.60 (1.48)	3.70 (0.74)	4.80 (1.63)	4.90 (1.19)	<0.0001
Waist–hip ratio	167 (0.6%)	0.92 (0.06)	0.94 (0.07)	0.89 (0.06)	0.91 (0.07)	0.92 (0.04)	<0.0001
Glucose (mg/dL)	168 (0.0%)	85.00 (13.34)	90.00 (11.86)	80.00 (7.41)	80.00 (8.90)	93.00 (14.83)	<0.0001
Glycated haemoglobin (%)	168 (0.0%)	5.70 (0.44)	5.90 (0.44)	5.50 (0.30)	5.60 (0.44)	5.70 (0.30)	<0.0001
Diabetes mellitus	168 (0.0%)	18 (10.7%)	10 (18.2%)	2 (3.9%)	2 (7.4%)	4 (11.4%)	0.2069
Arterial hypertension	168 (0.0%)	92 (54.8%)	37 (67.3%)	18 (35.3%)	16 (59.3%)	21 (60.0%)	0.0182
Dyslipidaemia	168 (0.0%)	68 (40.5%)	26 (47.3%)	16 (31.4%)	9 (33.3%)	17 (48.6%)	0.3675
Resistin—plasma (pg/mL)	168 (0.0%)	2115.23 (772.55)	2389.10 (775.51)	1891.16 (542.59)	2657.56 (991.28)	1874.89 (579.99)	0.0001
Interleukin 6—plasma (pg/mL)	167 (0.6%)	2.01 (1.89)	2.28 (2.17)	1.55 (1.48)	2.45 (1.49)	1.27 (1.18)	0.0149
Calprotectin—plasma (ng/mL)	168 (0.0%)	694.27 (257.02)	851.11 (329.44)	613.63 (208.62)	800.12 (272.14)	588.54 (149.27)	<0.0001
Tumor necrosis factor alpha—synovial fluid (pg/mL)	155 (7.7%)	9.02 (4.17)	9.09 (5.62)	8.14 (4.40)	9.90 (3.08)	8.90 (2.56)	0.1239
Tumor necrosis factor alpha—plasma (pg/mL)	167 (0.6%)	6.37 (1.97)	7.18 (2.49)	6.18 (2.47)	6.70 (2.81)	6.08 (1.35)	0.0997
Nerve growth factor—plasma (pg/mL)	167 (0.6%)	1.52 (0.51)	1.52 (0.41)	1.52 (0.38)	1.69 (0.60)	1.52 (0.59)	0.2699
C-reactive protein—synovial fluid (mg/L)	167 (0.6%)	1.23 (0.85)	1.52 (0.86)	0.78 (0.58)	1.61 (1.24)	0.98 (0.67)	<0.0001
C-reactive protein—plasma (mg/L)	168 (0.0%)	4.26 (3.64)	6.87 (3.43)	2.59 (1.83)	6.00 (4.53)	2.90 (2.01)	<0.0001
Interleukin 8 - synovial fluid (pg/mL)	165 (1.8%)	6.21 (4.55)	8.00 (6.32)	6.21 (4.53)	8.21 (9.04)	3.92 (2.69)	0.0012
Calprotectin—synovial fluid (ng/mL)	164 (2.4%)	576.06 (506.81)	763.04 (539.41)	599.99 (640.78)	759.60 (560.50)	420.07 (269.98)	0.0006
Interleukin 6—synovial fluid (pg/mL)	167 (0.6%)	116.33 (142.96)	208.03 (227.13)	91.67 (107.28)	222.45 (208.76)	71.56 (74.70)	0.0012
Osteopontin—synovial fluid (ng/mL)	168 (0.0%)	47.95 (45.78)	48.55 (46.67)	46.75 (44.66)	103.82 (103.73)	36.02 (28.09)	0.0013
Visfatin—synovial fluid (ng/mL)	168 (0.0%)	2.07 (0.96)	2.28 (0.97)	1.88 (0.76)	2.11 (0.89)	1.90 (1.13)	0.5075
Resistin—synovial fluid (pg/mL)	168 (0.0%)	1480.20 (1186.82)	1692.08 (1292.53)	1390.64 (1251.31)	1242.23 (1180.45)	1329.84 (1125.35)	0.4210
Nerve growth factor—synovial fluid (pg/mL)	163 (3.0%)	2.26 (0.65)	2.26 (1.09)	2.26 (0.63)	2.20 (0.48)	2.20 (0.90)	0.0380

(continued)

Table 2. (continued)

		N (%Miss.)	All 168 (100%)	KOIP-1 55 (32.7%)	KOIP-2 51 (30.4%)	KOIP-3 27 (16.1%)	KOIP-4 35 (20.8%)	*P*-value
Osteopontin—plasma (ng/mL)		168 (0.0%)	13.71 (6.07)	12.52 (6.59)	14.12 (6.40)	16.40 (10.18)	12.10 (4.37)	0.1872
Visfatin—plasma (ng/mL)		168 (0.0%)	4.01 (0.99)	4.02 (1.20)	4.23 (1.12)	4.01 (0.54)	3.74 (1.01)	0.2810
Interleukin 8—plasma (pg/mL)		168 (0.0%)	3.19 (1.93)	3.21 (1.78)	3.79 (2.07)	3.52 (2.59)	2.30 (1.28)	0.0152
Low-density lipoprotein (mg/dL)		168 (0.0%)	121.50 (34.84)	123.00 (38.55)	128.00 (34.10)	126.00 (23.72)	113.00 (28.17)	0.2931
Total cholesterol (mg/dL)		168 (0.0%)	208.00 (32.62)	208.00 (32.62)	211.00 (29.65)	213.00 (28.17)	200.00 (32.62)	0.1780
25-hydroxy vitamin D (ng/mL)		167 (0.6%)	19.75 (11.05)	13.80 (7.12)	25.63 (10.50)	17.55 (11.81)	21.30 (8.90)	<0.0001
Physical exercise	None	168 (0.0%)	61 (36.3%)	29 (52.7%)	12 (23.5%)	6 (22.2%)	14 (40.0%)	0.0525
	Sporadic		51 (30.4%)	18 (32.7%)	16 (31.4%)	8 (29.6%)	9 (25.7%)	
	Moderate		46 (27.4%)	6 (10.9%)	18 (35.3%)	11 (40.7%)	11 (31.4%)	
	Vigorous		10 (6.0%)	2 (3.6%)	5 (9.8%)	2 (7.4%)	1 (2.9%)	
Adiponectin—plasma (ng/mL)		168 (0.0%)	16406.09 (9301.69)	11544.55 (6280.30)	22357.78 (10366.17)	21707.37 (7352.75)	11562.39 (5390.11)	<0.0001
Omentin—plasma (pg/mL)		168 (0.0%)	26859.10 (17845.88)	22317.93 (13067.24)	44069.15 (26688.23)	41837.49 (19372.98)	22449.96 (11219.76)	<0.0001
High-density lipoprotein (mg/dL)		168 (0.0%)	60.65 (13.79)	53.30 (11.12)	68.90 (13.49)	63.20 (9.93)	58.00 (17.05)	<0.0001
Adiponectin—synovial fluid (ng/mL)		168 (0.0%)	2420.01 (1470.78)	1937.73 (1263.00)	3047.42 (1271.50)	3372.91 (2111.49)	1501.12 (1186.51)	<0.0001
Omentin—synovial fluid (pg/mL)		165 (1.8%)	4292.68 (3586.44)	3073.42 (2125.52)	6648.21 (4252.38)	6943.02 (4134.11)	2787.84 (2109.62)	<0.0001

Cells show medians and median absolute deviations (continuous) and absolute frequencies and percentages (categorical) for the 45 variables used in the clustering analysis within each KOIP and in the overall series.
Statistical significance was assessed using a Kruskal–Wallis (continuous) or a Fisher's test for contingency tables (categorical variables).
%Miss.: percentage of missing values; KOIP: knee osteoarthritis inflammatory phenotype; N: number of observations.

available at *Rheumatology* online). Based on machine learning techniques and objective statistical criteria for model selection, our methodology identified four robust patient clusters (Supplementary Figs S2 and S3, Supplementary Tables S2 and S3, available at *Rheumatology* online) that displayed clearly differing profiles regarding their anthropometric, metabolic and inflammatory features. Hence, these clusters were used to define four KOA inflammatory phenotypes (KOIP) whose characteristics are detailed in Fig. 1 and Table 2 (see also Supplementary Fig. S4, available at *Rheumatology* online).

- KOIP-1 represented a fat-driven metabolic inflammatory phenotype (55 subjects, 32.7%). Compared with the rest of the patients, this phenotype showed classic hallmarks of metabolic syndrome (MetS) driven by body fat and obesity, such as: higher weight, BMI, body fat, waist circumference and waist–hip ratio; high prevalence of diabetes, arterial hypertension, dyslipidaemia and MetS; increased values of insulin, triglycerides, uric acid, glucose and percentage of glycated haemoglobin; and low high-density lipoprotein (HDL), vitamin D and physical activity. Its inflammatory profile was characterized by the highest levels of leptin and irisin, in contrast to decreased values of adiponectin and omentin, both in plasma and in synovial fluid. Typically, their patients also displayed higher than average values of some inflammatory factors at the local and systemic level, namely IL-6, CRP, calprotectin and resistin, as well as increased values of plasma tumour necrosis factor alpha (TNF-alpha) and synovial interleukin-8 (IL-8) and visfatin.
- KOIP-2 defined a metabolically healthy and mild-inflammatory phenotype (51 subjects, 30.4%). Subjects in this group represented a mirrored picture of the KOIP-1 cluster, as they presented the lowest expressions of the metabolic and obesity features listed above, the lowest levels of leptin and irisin, and increased values of adiponectin and omentin, both in plasma and in synovial fluid. In this phenotype, the expression for the rest of systemic and local inflammatory markers remained around the cohort's average or below, except for plasma IL-8 and visfatin. Patients in the KOIP-2 group were among the most physically active and presented high levels of vitamin D and HDL.
- KOIP-3 depicted a non-metabolic and high-inflammatory phenotype (27 subjects, 16.1%). Although their subjects presented relatively high levels of weight and body fat content, this group was characterized by intermediate values of BMI and average or lower levels and presence of conditions related to MetS and individual cardiovascular risk factors, such as diabetes, glucose, glycated haemoglobin, dyslipidaemia, triglycerides, uric acid and waist–hip ratio. Their patients also displayed intermediate levels of leptin and irisin, high expression of adiponectin and omentin and the highest levels of osteopontin, both systemically

and locally. Regarding the rest of cytokines, and compared with KOIP-1, KOIP-3 subjects showed increased levels of synovial TNF-alpha and plasma nerve grow factor (NGF), lower expression of synovial visfatin and resistin, and a similar profile for the rest of factors. Their vitamin D levels were low, and they were physically active compared to the series average.

- KOIP-4 represented a metabolic low-inflammatory phenotype (35 subjects, 20.8%). This group displayed average values of leptin, irisin and anthropometric factors related with obesity (weight, BMI, body fat, waist perimeter and waist–hip ratio). Their patients, though, showed a high frequency and levels of classic cardiovascular risk factors, especially MetS, triglycerides and glucose, and higher prevalence of arterial hypertension and dyslipidaemia than the cohort's average. Despite that, this phenotype showed low average expression in all the cytokines of our panel, both in plasma and in synovial fluid.

Subjects across KOIP groups did not significantly differ in terms of age or evolution time of their disease although, in median, KOIP-3 subjects were slightly younger (2.2 years) and they had been diagnosed more recently (12 months) compared with the overall series. Subjects in KOIP-4 showed the longest disease evolution (12 months more than the whole patients set, in median). A supervised RF classifier trained to predict KOIP membership achieved a high overall accuracy (89%), with classification errors across KOIP groups ranging from 4% to 17%. A sequential procedure selected up to 27 predictors (out of the 45 analysed) as the most informative for KOIP classification while retaining a similar accuracy in all groups (87%) (Supplementary Table S4, available at *Rheumatology* online). With exploratory purposes, and although the number of male patients in our KOA cohort available for these analyses did not allow for conducting a proper phenotyping analysis ($n = 23$), we observed that the KOIP profiles described for women were only partially reproduced in the male patients of our KOA cohort (Supplementary Fig. S5, available at *Rheumatology* online).

Association of KOIP groups with clinical outcomes

The identified KOIP groups were evaluated for their association with different outcomes, including clinical (KOOS, reversed scale), radiographic and ultrasound severity at baseline and radiographic progression at two years (Fig. 2, Table 3). KOIP-1 and KOIP-3 groups displayed the highest baseline pain across phenotypes, which were statistical significant in the case of KOIP-1 (7 points increase in median compared with KOIP-2 and KOIP-4, *P*-values = 0.0217 and 0.0327) and close to the significance threshold for KOIP-3 (10 points more than KOIP-2 and KOIP-4 in median, *P*-values = 0.0566 and 0.0535), respectively (Fig. 2, Table 3 and Supplementary Table S5, available at *Rheumatology* online). The same pattern was observed for functional disability where, in median,

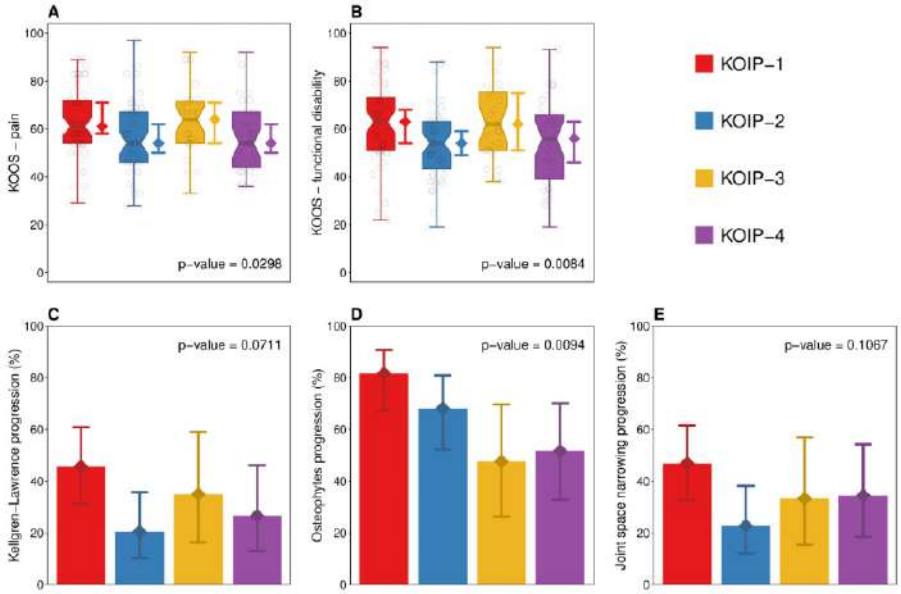

Figure 2. Association of knee osteoarthritis inflammatory phenotypes (KOIP) with clinical severity and radiographic progression. Panels (**A**) and (**B**) represent boxplots showing the distribution of knee injury and osteoarthritis outcome scores (KOOS, reversed scores) across KOIP groups for pain (**A**) and functional disability (**B**). Panels (**C**), (**D**) and (**E**) display the percentage of patients whose disease progressed after a two-year follow-up according to different radiographic criteria, namely Kellgren–Lawrence (KL) stage (**C**), formation of new osteophytes (**D**) and increase of joint space narrowing (JSN) (**E**). Diamond-shaped points and segments show group medians (**A** and **B**), percentages (**C**, **D** and **E**) and their 95% CI. *P*-values are derived from an Kruskal–Wallis test (**A**, **B**) or a Fisher's test for contingency tables (**C**, **D** and **E**). KOIP: knee osteoarthritis inflammatory phenotype; KOOS: knee injury and osteoarthritis outcome scores (reversed scores)

Table 3. Association of knee osteoarthritis inflammatory phenotypes (KOIP) with severity and progression

		N (%Miss)	All 168 (100%)	KOIP-1 55 (32.7%)	KOIP-2 51 (30.4%)	KOIP-3 27 (16.1%)	KOIP-4 35 (20.8%)	*P*-value
KOOS—pain (reversed, 0–100)		168 (0.0%)	58.00 (16.31)	61.00 (14.83)	54.00 (14.83)	64.00 (11.86)	54.00 (17.79)	0.0298
KOOS—symptoms (reversed, 0–100)		168 (0.0%)	57.00 (17.79)	62.00 (17.79)	56.00 (8.90)	57.00 (19.27)	62.00 (19.27)	0.7737
KOOS—functional disability (reversed, 0–100)		168 (0.0%)	58.00 (15.57)	63.00 (17.79)	54.00 (13.34)	62.00 (17.79)	56.00 (19.27)	0.0084
Joint effusion (mm)		168 (0.0%)	9.05 (2.45)	9.30 (2.52)	8.70 (2.37)	9.40 (2.67)	8.80 (1.33)	0.1545
Synovial tissue thickness (mm)		163 (3%)	4.20 (1.78)	4.50 (2.00)	4.20 (1.78)	4.40 (1.78)	4.10 (1.63)	0.6986
Kellgren–Lawrence radiographic grade	1	168 (0.0%)	19 (11.3%)	5 (9.1%)	4 (7.8%)	3 (11.1%)	7 (20.0%)	0.1255
	2		65 (38.7%)	19 (34.5%)	25 (49.0%)	7 (25.9%)	14 (40.0%)	
	3		78 (46.4%)	26 (47.3%)	22 (43.1%)	16 (59.3%)	14 (40.0%)	
	4		6 (3.6%)	5 (9.1%)	0 (0.0%)	1 (3.7%)	0 (0.0%)	
Osteophytes score		167 (0.6%)	3.00 (2.97)	3.00 (2.97)	3.00 (2.97)	4.00 (2.97)	4.00 (2.97)	0.9087
Joint space narrowing	0	168 (0.0%)	63 (37.5%)	15 (27.3%)	22 (43.1%)	9 (33.3%)	17 (48.6%)	0.1391
	1		32 (19.0%)	13 (23.6%)	11 (21.6%)	4 (14.8%)	4 (11.4%)	
	2		42 (25.0%)	10 (18.2%)	12 (23.5%)	9 (33.3%)	11 (31.4%)	
	3		26 (15.5%)	14 (25.5%)	4 (7.8%)	5 (18.5%)	3 (8.6%)	
	4		5 (3.0%)	3 (5.5%)	2 (3.9%)	0 (0.0%)	0 (0.0%)	
Kellgren–Lawrence radiographic progression		140 (16.7%)	45 (32.1%)	21 (45.7%)	9 (20.5%)	7 (35.0%)	8 (26.7%)	0.0709
Osteophytes radiographic progression		143 (14.9%)	95 (66.4%)	40 (81.6%)	30 (68.2%)	10 (47.6%)	15 (51.7%)	0.0093
Joint space narrowing radiographic progression		143 (14.9%)	50 (35.0%)	23 (46.9%)	10 (22.7%)	7 (33.3%)	10 (34.5%)	0.1110

Cells show median and median absolute deviation (continuous) and absolute frequencies and percentages (categorical) for outcomes within each KOIP and in the overall series, including clinical, radiographic an ultrasound severity at baseline and radiographic progression.
Statistical significance was assessed using a Kruskal–Wallis test (continuous) or a Fisher's test for contingency tables (categorical variables).
%Miss.: percentage of missing values; KOIP: knee osteoarthritis inflammatory phenotype; KOOS: knee injury and osteoarthritis outcome scores (reversed scores); N: number of observations.

KOIP-1 and KOIP-3 displayed 8 and 9 KOOS points more than subjects in the KOIP-2 group (*P*-values = 0.0033 and 0.0106) and 7 and 6 more points than KOIP-4 subjects (*P*-values = 0.0595 and 0.0735), respectively (Fig. 2, Table 3 and Supplementary Table S6, available at *Rheumatology* online). Regarding radiographic assessments, KOIP-1 showed the highest rates of progression in all criteria considered, which included Kellgren–Lawrence (KL) (45.7%), formation of new osteophytes (81.6%) and joint space narrowing (JSN) (46.9%) (Fig. 2, Table 3 and Supplementary Tables S7–S9, available at *Rheumatology* online). Although the homogeneity test was non-significant for KL and JSN, these rates were significantly higher compared with the KOIP-2 group for KL (25.2% increase, *P*-value = 0.0143) and JSN (24.2% increase, *P*-value = 0.0178), and for osteophytes progression when compared with KOIP-3 (34.0% increase, *P*-value = 0.0080) and KOIP-4 (29.9%, *P*-value = 0.0093). Importantly, KOIP-1 and KOIP-3 showed both a high progression and a high baseline radiographic severity according to KL and JSN, although baseline differences were not statistically significant across phenotypes for any of the criteria considered (Fig. 2, Table 3). KOIP-3 and KOIP-4 showed similar patterns and rates of radiographic progression (Fig. 2, Table 3). No significant differences were found between phenotypes for KOOS symptoms or ultrasound severity (Table 3).

Discussion

In this study, we aimed to identify different phenotypes of KOA characterized by their anthropometric and metabolic traits and their systemic and local inflammatory profiles. To do so, we applied a non-supervised approach to data from a homogeneous and tightly controlled cohort of female KOA patients with persistent joint effusion, using well-established machine learning techniques and objective statistical criteria for model selection. At present, the existence of a general so-called inflammatory phenotype that includes patients presenting synovitis is widely accepted [31, 32]. Of note, all the subjects studied in our work fall into this category and, hence, they suffered from higher levels of pain, functional disability and probability of progression compared with the non-inflammatory phenotype. Despite this sample homogeneity,

	KOIP-1 Fat-driven MetS High inflammation	KOIP-2 Metabolically Healthy Mild inflammation	KOIP-3 Non-MetS High inflammation	KOIP-4 Non-Fat associated MetS Low inflammation
	55 (32.7%)	**51 (30.4%)**	**27 (16.1%)**	**35 (20.8%)**
Anthropometric / Metabolics	Obesity and MetS	No obesity and metabolically healthy	Overweight and non-abdominal fat	Cardiovascular risk factors
Leptin - Irisin	High	Low	Average	Average
Adiponectin - Omentin	Low	High	High	Low
Rest of cytokines	High	Mild	High	Low
Pain and functional disability	Higher	Lower	Higher	Lower
Radiographic progression	Higher for all criteria	Lower for KL and JSN	Lower for osteophytes	Lower for osteophytes

Figure 3. Knee osteoarthritis inflammatory phenotypes (KOIP). The table reflects the KOIP characteristics regarding anthropometric, metabolic and inflammatory profiles, as well as clinical severity and radiographic progression. JSN: joint space narrowing; KL: Kellgren–Lawrence; KOIP: knee osteoarthritis inflammatory phenotype; MetS: metabolic syndrome

our methodology identified four robust clusters of patients defining four KOA inflammatory phenotypes (KOIP, Fig. 3), which drastically differed in their anthropometric, metabolic and inflammatory profiles, presented substantial differences in clinical severity and suggested different rates of radiographic progression. These results point to differential pathways across these phenotypes regarding pathophysiology and disease evolution (endotypes) and, therefore, implications in treatment tailoring that, in line with previously established hypotheses provide a possible explanation for the current lack of translational results [12, 33–35].

KOIP-1 patients represented the most severe inflammatory phenotype, as shown by their highest levels of pain, functional disability and radiographic stage, and their less favourable evolution according to three different radiographic criteria. We hypothesize that KOIP-1 constitutes what has been previously described as a metabolic osteoarthritis subgroup, whose disease is mediated by a low-grade systemic inflammation promoted by metabolic factors possibly driven by body fat and obesity [36, 37]. Most of the cytokines contributing to the KOIP-1 definition had been previously linked to KOA severity [38]. The identification of this phenotype might be of relevance in clinical practice, to distinguish patients likely to benefit from therapies targeting their metabolic condition [39].

The KOIP-2 group includes patients with a healthy metabolic profile and mild overall inflammation. Their levels of pain and functional disability were the lowest in our series, though were still high compared with patients of a non-inflammatory phenotype [40]. In our data, KOIP-2 showed the lowest proportion of radiographic progression according to KL and JSN criteria. In contrast, their patients frequently suffered from osteophytes formation during their follow-up, a radiographic feature whose prognosis value and relation with cartilage impairment has raised some controversy [41, 42]. Interestingly, the divergence observed in the pattern of radiographic progression suggests the existence of differential mechanisms for the evolution of the disease across these phenotypes. Because the healthy metabolic profile exhibited by KOIP-2 patients probably offers protection against the disease's severity and progression, alternative explanations are needed to clarify the determinants of their KOA onset and inflammatory presentation, which might be related to the mild levels of local inflammation observed in these patients.

KOIP-3 patients are characterized by low or average presence for most metabolic factors and increased values for some plasma and synovial cytokines. Their patients depict a specific inflammatory phenotype that, in contrast to the KOIP-1 group, seems not to be associated to metabolic factors. Similarly to KOIP-1, though, their levels of pain, functional disability and KL radiographic progression were among the highest in our series which, in both groups, was consistent with elevated levels of inflammatory cytokines such as IL-6, IL-8, CRP and calprotectin. These traits correspond to what might be defined as pure inflammatory phenotype, and a selection of KOIP-3 patients might be of interest in clinical trials designed to test a new generation of inflammation-targeting drugs, some of them currently ongoing [43].

KOIP-4 is a metabolic phenotype characterized by cardiovascular risk factors not associated with body fat distribution or obesity and by low inflammation in all markers. Compared with the overall series, their levels of pain and functional disability were relatively low and similar to those in the KOIP-2 group. This phenotype deserves a special consideration as, although all their patients fell into the clinical definition of inflammatory subtype, their cytokines levels were substantially lower than those observed in the rest of KOIP groups. This suggests that KOIP-4 describes a phenotype of patients whose disease is not only mediated by chronic low-grade inflammation linked to MetS as suggested in previous works [44] but also, by different pathways involving the direct action of these cardiovascular conditions [45, 46]. Although different mechanisms related to cardiovascular risk factors have been proposed for KOA onset and severity, none of them provide an explanation for an inflammatory presentation of the disease [47, 48]. Regarding its clinical applicability, patients from this phenotype might benefit from a tight control of their cardiovascular-related comorbidities.

A high number of parameters (27 out of 45) were needed to discriminate the KOIP groups in our data, indicating that these phenotypes were defined by a complex combination of various metabolic, anthropometric and inflammatory factors, rather than fully characterized by a limited number of these features. However, their implementation in clinical practice

would require a panel composed of a small number of biomarkers. In this regard, the use of Omics data derived from high-throughput technologies offers a great potential to identify KOIP-specific biomarkers in the very near future [49], and it is currently an ongoing line of research in our group. Another limitation of our study is that phenotyping was conducted on a homogeneous sample of patients with inflammatory (joint effusion) KOA, and all were recruited from a single hospital, potentially limiting the extrapolation of its results. Although the sample size (168) was large enough to uncover these underlying phenotypes, future studies involving multiple centres and a larger sample size are mandatory to further confirm our findings. Our study was focused on women, as they represented the vast majority of our cohort and several sex-specificities had been reported regarding prevalence, metabolic and inflammatory conditions, and levels of pain and disability [10, 11]. An analysis with exploratory purposes showed that the uncovered KOIP groups were female-specific to a substantial extent, as their features could not be fully reproduced in male patients. Although this result is in agreement with previous works [23], they don't allow for strong conclusions to be derived due to the low number of men in our cohort with available data for these analyses ($n = 23$). An important strength of our study is the exhaustive availability of data, which were systematically collected in the context of a prospective cohort specifically and accurately designed to study the determinants of KOA severity and progression. These data allowed a complete characterization of patients regarding features known for their relevance in KOA, and distinguishes our study from previously published works.

In conclusion, our work identified four groups of KOA female patients with joint effusion that showed differential profiles of anthropometric, metabolic and inflammatory factors, displayed substantial differences in clinical severity and suggested implications in radiographic progression. Our results support the view of KOA as a multifaceted and heterogeneous syndrome consisting of different phenotypes with differing pathways regarding their pathophysiology and disease evolution. If confirmed in larger series of patients, that these findings would entail important implications in research and in clinical practice, as they might boost patients' stratification, the design of personalized therapies and the search for novel treatments.

Supplementary material

Supplementary material is available at *Rheumatology* online.

Data availability

All the code used in the analyses is available upon reasonable request.

Contribution statement

J.C., M.G.-M., A.B.-L. and J.G. contributed to the conception and design of the study. M.G.M., C.O., S.G.C., M.L., M.A. and C.G.L. contributed to the acquisition of data. C.A., R.G. and N.A.G. contributed to the blood sample extraction, processing, storage and analysis performing. J.C., M.G.M., A.B.-L., M.L. and M.A. contributed to the analysis and interpretation of data. J.C. is the paper's guarantor. All authors contributed to drafting the article or revising it critically for relevant intellectual content. All authors gave the final approval of the version to be submitted.

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SUPPLEMENTARY INFORMATION

Inclusion and exclusion criteria

Subjects' inclusion required the presence of symptomatic primary KOA according to the American College of Rheumatology (ACR) criteria [1], with a defined diagnosis provided in the outpatient Rheumatology visits at our hospital, aged between 50 and 85 years old and with joint effusion observed during the physical examination at the recruitment visit and confirmed by ultrasound (≥4 mm on midline suprapatellar line). Symptomatic KOA is defined as the presence of pain greater than or equal to 4 on a 10-cm visual analogue scale, despite the use of prescribed analgesic drugs for at least 3 months. Only patients reporting persisting knee effusion or with documented effusion in several medical visits were included in the study. Exclusion criteria were: secondary osteoarthritis, either due to a history of trauma, menisci injury or previous inflammatory rheumatism; a history of knee surgery; any disease which, in the investigator's opinion, could interfere with the assessment of pain such as, but not limited to, fibromyalgia and polyneuropathies; systemic glucocorticoid intake in the last 6 months; and intra-articular glucocorticoid or hyaluronic acid injection in the last 3 or 6 months before recruitment, respectively. Also, the synovial fluid of patients was analysed to discard the presence of inflammatory fluid (joint cell count < 2500 cells) and microcrystals. The patients were followed up for 2 years after recruitment, and their medical records were regularly checked during this time to discard the onset of conditions for their exclusion.

Samples

Samples from plasma and joint fluid were systematically extracted at recruitment from all patients in the cohort. Synovial fluid was analysed to discard the presence of inflammatory fluid (joint cell count < 2500 cells) and microcrystals. Synovial fluid was obtained by an aspiration procedure and their mean and median were 13.5 and 9 ml, respectively. Collected samples were appropriately centrifuged and stored at -80ºC, until their use for determinations related to metabolic syndrome assessment or the quantifications of cytokines by enzyme-linked immunosorbent assay (ELISA). Blood extractions and the arthrocentesis were performed at the same time and in fasting conditions.

Data collection

Anthopometric and metabolic factors

Baseline information regarding demographics, anthropometric and metabolic factors were collected for all the patients in the cohort at the recruitment visit, including: weight (kg), height

(cm), body mass index (BMI, kg/cm2), waist circumference (cm), waist-hip ratio and percentage of body fat measured by bioelectric impedanciometry (TANITA BC-418MA biologica), following a standard clinical protocol. Obesity was defined BMI ≥ 30. We considered arterial hypertension, dyslipidaemia and diabetes mellitus when the diagnostic was stablished in the medical records, or the patient was taking active medication for this conditions. Metabolic syndrome (MetS) was assessed according to the criteria of the National Cholesterol Education Program Adult Treatment Panel III (ATP III) [2]. Physical exercise was reported by the patients in three categories according to the frequency and intensity of their activity: Never, Sporadic, Regular with Moderate Intensity or Regular-Vigorous.

Quantifications in patients' samples

As per clinical practice, the following determinations were performed in the plasma samples: High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), total cholesterol, triglycerides, glucose, percentage of glycated haemoglobin, insulin, 25-hydroxy vitamin D and uric acid. Patients' synovial and plasma samples were evaluated for the following cytokines: C-reactive protein (CRP, mg/L), Interleukin 6 (IL-6, pg/mL), Interleukin 8 (IL-8, pg/mL), Tumor Necrosis Factor alpha (TNF-alpha, pg/mL), Nerve Growth Factor (NGF, pg/mL), Calprotectin (ng/mL), Leptin (pg/mL), Irisin (ng/mL), Visfatin (ng/mL), Resistin (pg/mL), Osteopontin (ng/mL), Adiponectin (ng/mL) and Omentin(pg/mL) (Figure 1, Supplementary Table S1). Blood extractions and the arthrocentesis for the determinations related to metabolic syndrome and for the quantification of cytokines were performed at the same time (recruitment) and in fasting conditions.

Clinical severity

Baseline clinical severity was assessed at the time of recruitment using the Knee injury and Osteoarthritis Outcome Scores (KOOS; pain, functional disability and symptoms) [3], which were used in reversed order to facilitate the interpretation of the results.

Radiographic severity and progression

Radiographic severity was measured by the Kellgren-Lawrence (KL) [4] scale and following OARSI atlas lecture [5], which includes assessment of osteophytes and joint space narrowing (JSN). It was assessed by examination of an anteroposterior knee x-ray performed with the patient in standing position and, at most, in the last 18 months before recruitment. This evaluation was performed independently by two different clinicians for a subset of patients, and a unweighted Cohen's Kappa was calculated to assess their concordance, which were: 0.884 for KL (135 patients, 95% confidence interval: 0.816 to 0.953); 0.931 for osteophytes evaluation (135 patients, 95% confidence interval: 0.885 to 0.977); 0.782 for JSN (30 patients, 95% confidence

interval: 0.608,to 0.956). To assess their radiographic progression, most of the patients (93%) also underwent radiographic evaluations after two years from their baseline radiography.

Ultrasound severity

Ultrasound measurements of the affected knee were collected regarding joint effusion and synovial tissue thickness (mm). The assessments were performed by a single experienced examiner (JC), using Siemens Acuson Antares with a 5-13 MHz linear array transducer and a standardized protocol based on current guidelines and definitions [6-8]. The knee was scanned in longitudinal and transversal planes with 30° joint flexion. Baseline ultrasound measurements, recorded in millimeters, were: effusion: a ≥4mm hypoechoic or anechoic intraarticular material that was displaceable and compressible in the suprapatellar recess, evaluated using a longitudinal scan; and synovial hypertrophy: a ≥2mm abnormal hypoechoic intraarticular tissue that was non-displaceable and poorly compressible in the suprapatellar recess, measured on a longitudinal scan [9].

ELISA assays

Technical specifications

ELISA assays were conducted according to the manufacturers' recommendations:

- hsCRP: DRG International (USA) ref. EIA-3954, Sensibility: 0.1 mg/L, Detection rang: 0.005 -0.1 mg/L; Intra-assay coefficient: <10%, Inter-assay coefficient: <5%.

- IL-6: Luminex Merck Millipore (Germany) ref HSTCMAG-28SK-02, Sensibility: 0.11 pg/ml; Detection rang: 3.2 - 2000 pg/L, Intra-assay coefficient: <5%, Inter-assay coefficient: <20%.

- IL-8: Luminex ThermoFisher Scientific (USA) ref. EPX01A-10204-901, Sensibility: 1,2 pg, Detection rang: 2,44 - 10 000 pg/ml, Intra-assay coefficient: 4.6 %, Inter-assay coefficient: 8.5 %.

- TNF-alpha: Luminex Merck Millipore (Germany) ref HSTCMAG-28SK-02, Sensibility: 0.16 pg/ml; Detection rang: 3.2 - 2000 pg/L, Intra-assay coefficient: <5%, Inter-assay coefficient: <20%.

-NGF: Luminex ThermoFisher Scientific (USA) ref. EPX01A-12117-901, Sensibility 6,19 pg/mL, Detection rang: 7,32 a 30 000 pg/ml, Intra-assay coefficient: 4.3 %, Inter-assay coefficient: 7.1 %.

- Calprotectin: Calprolab (Sweden) ref. CALP0270, Sensibility: 5ng/ml, Intra-assay coefficient: <5.4%, Inter-assay coefficient: <6.9%.

- Leptin: Biorbyt (UK) ref: orb50067, Sensibility: < 8 pg/ml, Detection rang: 62.5 - 10000 pg/L, Intra-assay coefficient: <6.3%, Inter-assay coefficient: <7.2%.

- Irisin: Cusabio (China) ref. CSB-EQ027943HU, Sensibility: 0.78ng/ml, Detection rang: 3.12 - 200 ng/ml, Intra-assay coefficient: <8%, Inter-assay coefficient: <10%.

- Visfatin: Phoenix Pharmaceuticals Inc. (Germany) ref. EK-003-80; sensibility: 2.21 ng/ml, detection rang: 0.1-1000 ng/ml, Intra-assay coefficient: <10%, Inter-assay coefficient: <15%.

- Resistin: Elabscience (China) ref. ELH-Resistin-002; sensibility: 1.4pg/ml; detection rang: 1.4-400 pg/ml, Intra-assay coefficient: <10%, Inter-assay coefficient: <12%.

- Osteopontin: Bender MedSystems Gmbh (Austria) ref. BMS2066, Sensibility: 0.26 ng/ml, Detection rang: 0.47-30 mg/L, Intra-assay coefficient: <6.7%, Inter-assay coefficient: <6.1%.

- Adiponectin: Bender MedSystems Gmbh (Austria) ref. BMS2032, Sensibility: 0.01 ng/ml, Detection rang: 0.78-50 mg/L, Intra-assay coefficient: <4.2%, Inter-assay coefficient: <3.1%.

- Omentin: Cusabio (China) ref. CSB-E09745h, Sensibility: 0.4 pg/ml, Detection rang: 1.56 - 100 pg/ml, Intra-assay coefficient: <8%, Inter-assay coefficient: <10%.

Batch corrections in ELISA experiments

Due to technical reasons related to the ELISA technology (configuration of plates used), none of these cytokines could be assessed at the same time for all patients. To account for potential effects induced by this technical source, we corrected ELISA values previously to the any formal statistical analysis (two-step correction). For doing so, we independently fitted a linear model to each marker where age, sex and round of measurement (batch) were included as covariates. The coefficient estimations associated with batch were retrieved from the model and subtracted from the original marker values. The resulting quantities were, by definition, an estimation of the marker levels corrected by differences induced by the ELISA batches. Previously to this procedure, the markers were transformed using the Tukey ladder of powers [10], in order to symmetrize their distribution and meet the assumptions of the linear model. The optimal transformations were chosen based on a maximum likelihood criterion [11] and the inspection of the cor-

responding diagnostic plots for these models (Supplementary Table S1). Corrected markers values were then transformed back to their original scale and used in downstream analyses.

Clustering analyses for phenotype discovery

For phenotype discovery, a clustering analysis based on well stablished Statistical and Machine Learning methods was carried out on 45 variables including anthropometric, metabolic and systemic and local inflammatory factors, which were selected from the information available in our KOA cohort for their clinical relevance in OA (see previous sections and Supplementary Table S1). Of note, this approach was completely unsupervised and no variable expressing KOA severity or progression was involved in the clustering process, which consisted in three steps:

1. Data pre-processing and missing imputation.

To avoid the loss of sample size, we first conducted on our data a Recursively Subtracted Empirical Orthogonal Functions (RSEOF) analysis [12], an approach for Principal Component Analysis (PCA) designed to deal with and impute missing data. In these analyses, principal components were derived by RSEOF and used for reconstructing the original data matrix where missing values had been imputed. Previously, numerical variables were transformed using the Tukey ladder of powers [10], in order to symmetrize their distribution and make them more suitable for the PCA (Supplementary Table S1). The optimal transformations had been previously estimated for the whole cohort (202 patients) by fitting to each variable a linear model where age, sex and ELISA kit (when suitable) were included as covariates, and was based on a maximum likelihood criterion [11] and the inspection of the corresponding diagnostic plots for these models. In the RSEOF analysis, binary variables were converted to a numeric format (1 indicating presence and 0 representing absence of the feature), and physical exercise (four categories) was also converted to numeric and treated as ordinal. PCA was performed on centered and scaled variables.

2. Reduction of data dimension

2a) Dissimilarities computation: Data matrix estimated by RSEOF was used as input for an unsupervised Random Forest (RF) analysis [13, 14] from which the matrix containing the pairwise dissimilarities between patients was retrieved. For two given observations, the RF proximity (RFprox) is defined as the fraction of trees in which the two observations fall in the same terminal node. The dissimilarity between subjects was defined as 1 - RFprox. In unsupervised RF, synthetic data are previously generated by randomly sampling from a distribution representing a scenario with no structure in the data. In our case, we used the Addcl1 dissimilarity [14], where these synthetic data was sampled from the product of empirical marginal distributions of the ori-

ginal variables (by independent bootstraping of each variable separately). The algorithm then aims to discriminate synthetic from observed data as in the standard supervised classification setup. Because of the generation scheme for the synthetic data, predictor trees created by RF are enriched with splitted variables with a shared structure of dependence and, hence, the resulting RF dissimilarity measure is built on the basis of these sets of dependent variables. As the RF dissimilarity can vary substantially depending on the synthetic data realization, we averaged the results of 100 different instances, each of them conducted on 10.000 trees (parameter *mtry* = 6).

The RF dissimilarity has proven to be useful for clustering of biomedical and Omic data [15-17], as it is able to deal with a large number of variables and discriminate those who are really relevant to capture complex structures underlying the data. Also, it handles variables of mixed types, it is invariant to monotonic transformations of the input variables and is robust to outliers and to the selection of its parameters values [14].

2b) Dissimilarity representation in a low-dimensional space: the RF dissimilarity matrix was used as input for a Kruskal's Non-metric Multidimensional Scaling (K-MDS) [18], a classic multivariate technique for dimension reduction. In Multidimensional Scaling (MDS), a set of synthetic variables (components) are computed in such a way that euclidean distances between observations are as close as possible to the dissimilarities derived from the original variables. Tipically, this procedure allows to represent the original data in a number of MDS components that is substantially smaller than the number of original variables, while retaining the most of the information contained in it. In K-MDS, this configuration tries to minimize the stress (i.e., the square root of the ratio of the sum of squared differences between the input dissimilarities and those of the configuration to the sum of configuration dissimilarities squared), allowing the original input dissimilarities to differ for a monotonic transformation.

3. Parametric clustering analysis: results derived from different choices of the number of components in the K-MDS analysis (from 1 to 20) were used as input in a model-based clustering analysis based on mixture of Gaussian distributions (Mclust) [19]. Importantly, MDS components have the advantage of being symmetric and, hence, highly suitable for a parametric clustering method as Mclust, where the optimal number of clusters is selected based on Gaussian distributions and objective statistical criteria (Bayes Information Criterion, BIC). Mclust has been widely used in research and applied to broad range of Biomedical data, including epidemiology [20], DNA sequencing [21] and gene expression [22].

Results for the two first K-MDS components showed the most evident data structure as they involved a high number of clusters (four) according to their BIC. Hence, this configuration was

chosen to define the Knee Osteoarthritis Inflammatory Phenotypes (KOIP) and used in downstream analyses (Figure 2 and Supplementary Figures S2, S3 and S4).

Graphical representation of clusters

Clustering results were represented graphically using a heatmap plot at the patient level (Supplementary Figure S4), where cells expressed standardized values (centered and scaled) of original anthropometric, metabolic and inflammatory factors (i.e., with no imputation of missing values). In the heatmap cells, red colour indicated high, blue represented low, and colour intensity expressed more extreme values. Binary variables were previously converted to numeric format, where 1 indicated presence and 0 represented absence of the corresponding feature. Physical exercise (four categories) was also converted to numeric format and treated as ordinal. Continuous variables were standardized (centered and scaled) using their corresponding median and median absolute deviation, while the mean and standard deviation were used for numerically coded categorical variables. Colour intensities were saturated approximately to percentiles 5% and 95% of the overall values distribution, which corresponded to values -1.75 and 1.75.

To facilitate the interpretation of the KOIP groups, a heatmap at the cluster level was also plotted (Figure 2) were cells represented group median values (continuous variables) or averages (numerically coded categorical variables) of the values displayed in the heatmap at the patient level. In this heatmap, colour intensities were also saturated approximately to percentiles 5% and 95% which, in this case, corresponded to values -0.75 and 0.75, respectively.

Finally, a scatter plot was used to represent the clusters in the coordinates of the 2 K-MDS components selected in the phenotyping analysis (Supplementary Figure S3). In this graphic, the size of the points was proportional to the estimated probability of belonging to the assigned cluster (i.e., the certainty of the cluster assignation), so that smaller points were those assigned to their clusters with smaller probability (i.e., larger uncertainty).

Clustering analysis of anthropometric, metabolic and inflammatory variables

To help in the interpretation and in the graphical representation of the KOIP groups, the anthropometric, metabolic and inflammatory variables used in the phenotyping analysis were grouped by their similarity across samples (Supplementary Figure S1). In this case, we used a hierarchical clustering algorithm with Ward agglomerative method and distances based on pairwise correlation-like measurements (1-correlation distance), namely: Spearman correlation (SC) [23] (continuous vs continuous or ordinal variables), Phi coefficient [24] (binary vs binary variables) and Glass rank biserial correlation (GRBCorr) [25] (continuous / ordinal vs binary variables). Physical exercise (four categories) was treated as ordinal in these analyses. These correlation-

like measurements were graphically represented in a heatmap, where red colour indicated positive correlation, blue represented negative correlation, and colour intensity expressed more extreme values of the correlation coefficients. Colour intensities were saturated to 0.5 and -0.5 for positive and negative correlation, respectively. For interpretation purposes, the dendrogram of this clustering was displayed in the heatmaps representing the results of the phenotype discovery analysis at the patient and at the cluster level (Figures 2 and Supplementary Figure S4).

Contributions of variables to clustering

To help in the interpretation of the clusters, a supervised RF classifier was trained to predict patients' KOIP membership (Supplementary Table S2). The RF model was built stratifying by outcome (KOIP), under-sampling without replacement at 80% of the phenotype sizes and using the actual relative frequencies as decision cutoffs for each KOIP (*ntree* = 10.000, *mtry* = 6). To identify the variables contributing the most to the clusters identification, we conducted a sequential selection procedure based on RF models (VSURF) [26, 27] with the same parameters detailed above. Briefly, the procedure consists on three phases: the first step was dedicated to eliminate irrelevant variables from the predictors set (threshold mode); a second step aimed to select all variables related to the response for interpretation purposes (interpretation mode); finally, a third step refined the later selection by eliminating redundancy for prediction purposes (prediction mode). In these analyses, variables importance were computed by averaging the results of 500 RF models.

Expression of KOIP groups in male patients

To assess whether the uncovered phenotypes were also expressed in men, we used the RF model trained in female patients with the 27 most discriminant predictors of the phenotypes (VSURF - interpretation mode, see previous section) to predict the KOIP groups in the male patients available in our cohort. Out of the 31 men in the series, 23 had values for these 27 predictors and, hence, were available for this analysis. Results were represented in heatmaps at the individual and at the phenotype level, analogously to the way described above for female patients (Supplementary Figure S5). As men and women intrinsically display different baseline levels of the parameters used in the clustering process, numeric variables were previously centered (median) and scaled (median absolute deviation) within the female and male patient sets, separately.

Out of the 23 patients with complete information in the selected predictors, 7 were classified a KOIP-1, 6 as KOIP-2, 5 were assigned to KOIP-3 and 5 to KOIP-4. These analyses showed that some important profiles described for female patients were actually reproduced in this small set of men: the antagonism between KOIP-1 and KOIP-2 regarding anthropometric and metabolic factors, the profiles of irisin and leptin in contrast to adiponectin and omentin across pheno-

types, the presence of raised synovial inflammatory markers in KOIP-3 or the similar profiles of HDL, vitamin D and physical exercise across KOIP groups. Nevertheless, other patterns were observed that were not present in female patients, as relative higher levels of some inflammatory markers in the KOIP-2 group compared to KOIP-1, lower levels and prevalences of some metabolic factors in the KOIP-1 group and higher obesity-related factors in KOIP-3 (Supplementary Figure S5). The observation of such differences are in agreement with the sex-specificities reported in previous works regarding the behaviour of anthropometric, metabolic and inflammatory factors [28, 29].

Descriptives and statistical association analysis

For descriptive purposes, continuous parameters were described by their medians, median absolute deviations and ranges, while categorical variables were summarized with absolute frequencies and percentages. Associations with KOIP groups were assessed using non-parametric methods and based on Kruskal-Wallis and Mann-Whitney tests (continuous) or a Fisher's test for contingency tables (categorical variables). Assymptotic intervals at 95% confidence (95%CI) were computed for medians and percentages. Association results between KOIP groups and KOA outcomes were graphically represented in boxplots and stripcharts (continuous variables) or barplots (categorical variables), where the group medians or percentages and their 95%CI were displayed. Threshold for statistical significance was set at 5%. All analyses were conducted with R [30].

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Supplementary Table S1. Cytokines quantifications in the patients series by Knee Osteoarthritis Inflammatory Phenotypes (**KOIP**). Cells show medians and median absolute deviations (continuous) and absolute frequencies and percentages (categorical) for the 13 cytokines evaluated in plasma and synovial fluid samples of the patients' cohort. Statistical significance was assessed using a Kruskal-Wallis (continuous) or a Fisher's test for contingency tables (categorical variables). **KOIP:** Knee Osteoarthritis Inflammatory Phenotype.

		All 168 (100%)	KOIP-1 55 (32.7%)	KOIP-2 51 (30.4%)	KOIP-3 27 (16.1%)	KOIP-4 35 (20.8%)	P-value
SYNOVIAL FLUID	C-reactive protein (mg/L)	1.23 [0.03, 9.64]	1.52 [0.27, 6.02]	0.78 [0.03, 4.10]	1.61 [0.27, 9.64]	0.98 [0.15, 4.76]	<0.0001
	Tumor Necrosis Factor alpha (pg/mL)	9.02 [1.19, 127.49]	9.09 [1.19, 127.49]	8.14 [2.00, 18.17]	9.90 [2.19, 22.16]	8.90 [1.78, 17.98]	0.1239
	Interleukin 6 (pg/mL)	116.33 [0.11, 15683.26]	208.03 [7.55, 15683.26]	91.67 [0.11, 1857.83]	222.45 [22.77, 5820.23]	71.56 [4.01, 652.84]	0.0012
	Interleukin 8 (pg/mL)	6.21 [0.04, 881.40]	8.00 [0.44, 881.40]	6.21 [0.11, 278.44]	8.21 [0.04, 145.52]	3.92 [0.44, 29.02]	0.0012
	Nerve Growth Factor (pg/mL)	2.26 [0.01, 21.63]	2.26 [0.01, 21.63]	2.26 [0.46, 8.25]	2.20 [0.46, 6.90]	2.20 [0.72, 12.17]	0.038
	Calprotectin (ng/mL)	576.06 [36.79, 5957.01]	763.04 [60.31, 5376.84]	599.99 [36.79, 5957.01]	759.60 [197.72, 4129.89]	420.07 [36.79, 2447.75]	0.0006
	Leptin (pg/mL)	36160.71 [1928.12, 522278.22]	54016.11 [14014.43, 522278.22]	23126.79 [5335.19, 58808.60]	39506.85 [1928.12, 79207.03]	34888.07 [1928.12, 101330.36]	<0.0001
	Irisin (ng/mL)	695.95 [61.56, 3099.45]	938.23 [315.26, 3099.45]	422.07 [61.56, 983.08]	701.88 [161.47, 1270.77]	686.28 [242.12, 1527.24]	<0.0001
	Adiponectin (ng/mL)	2420.01 [300.19, 70423.60]	1937.73 [300.19, 6721.39]	3047.42 [689.12, 14901.06]	3372.91 [1194.76, 70423.60]	1501.12 [370.24, 3730.72]	<0.0001
	Omentin (pg/mL)	4292.68 [0.45, 28995.97]	3073.42 [0.45, 9401.45]	6648.21 [496.86, 28995.97]	6943.02 [1081.05, 25688.53]	2787.84 [130.78, 11575.06]	<0.0001
	Osteopontin (ng/mL)	47.95 [0.78, 3253.98]	48.55 [6.88, 3253.98]	46.75 [0.78, 441.11]	103.82 [15.18, 1400.55]	36.02 [6.88, 180.41]	0.0013
	Visfatin (ng/mL)	2.07 [0.13, 553.81]	2.28 [0.49, 70.61]	1.88 [0.13, 553.81]	2.11 [0.42, 7.53]	1.90 [0.53, 12.96]	0.5075
	Resistin (pg/mL)	1480.20 [32.46, 53485.59]	1692.08 [83.70, 53485.59]	1390.64 [52.92, 10386.82]	1242.23 [83.70, 18547.89]	1329.84 [32.46, 14574.82]	0.421
PLASMA	C-reactive protein (mg/L)	4.26 [0.42, 66.38]	6.87 [1.44, 66.38]	2.59 [0.42, 20.38]	6.00 [0.42, 27.13]	2.90 [0.42, 19.50]	<0.0001
	Interleukin 6 (pg/mL)	2.01 [0.08, 51.18]	2.28 [0.08, 42.02]	1.55 [0.12, 51.18]	2.45 [0.16, 8.28]	1.27 [0.08, 11.19]	0.0149
	Interleukin 8 (pg/mL)	3.19 [0.56, 402.21]	3.21 [0.93, 24.91]	3.79 [0.91, 13.13]	3.52 [0.56, 402.21]	2.30 [0.83, 8.22]	0.0152
	Tumor Necrosis Factor alpha (pg/mL)	6.37 [1.77, 22.13]	7.18 [1.77, 22.13]	6.18 [2.94, 14.66]	6.70 [2.59, 10.74]	6.08 [2.63, 8.86]	0.0997
	Nerve Growth Factor (pg/mL)	1.52 [0.22, 14.81]	1.52 [0.40, 14.81]	1.52 [0.22, 5.19]	1.69 [0.40, 8.31]	1.52 [0.44, 6.52]	0.2699
	Calprotectin (ng/mL)	694.27 [205.01, 12307.99]	851.11 [287.17, 6607.95]	613.63 [205.01, 1837.92]	800.12 [365.98, 12307.99]	588.54 [342.92, 1291.50]	<0.0001
	Leptin (pg/mL)	36688.11 [4039.08, 173177.01]	54392.71 [23739.58, 173177.01]	23566.59 [4039.08, 54817.52]	39809.19 [5017.56, 68130.76]	35530.72 [6972.63, 78971.55]	<0.0001
	Irisin (ng/mL)	707.78 [15.67, 1181.91]	859.25 [421.34, 1181.91]	437.22 [15.67, 884.41]	727.20 [234.19, 1059.97]	726.02 [116.43, 1105.78]	<0.0001
	Adiponectin (ng/mL)	16406.09 [1450.48, 59716.25]	11544.55 [1450.48, 52203.90]	22357.78 [10162.63, 59716.25]	21707.37 [7771.73, 40371.01]	11562.39 [4997.58, 42515.20]	<0.0001
	Omentin (pg/mL)	26859.10 [40.42, 114068.21]	22317.93 [40.42, 55037.25]	44069.15 [2444.37, 114068.21]	41837.49 [2444.37, 114068.21]	22449.96 [2444.37, 68850.68]	<0.0001
	Osteopontin (ng/mL)	13.71 [3.40, 64.53]	12.52 [3.40, 64.53]	14.12 [4.78, 55.83]	16.40 [4.91, 43.44]	12.10 [5.55, 29.52]	0.1872
	Visfatin (ng/mL)	4.01 [2.20, 12.58]	4.02 [2.20, 12.58]	4.23 [2.42, 8.37]	4.01 [2.59, 6.04]	3.74 [2.26, 6.93]	0.281
	Resistin (pg/mL)	2115.23 [791.02, 10079.62]	2389.10 [791.02, 8829.96]	1891.16 [958.55, 4435.30]	2657.56 [1045.95, 10079.62]	1874.89 [1194.29, 3812.08]	0.0001

Supplementary Table S2. Cytokines and Knee Osteoarthritis (**KOA**) outcomes evaluated in the patients series. g: parameter value for a Tukey ladder of powers transformation applied to continuous variables (when necessary) to symmetrize their distribution and make them more suitable for graphical representation and linear models for ELISA batch correction.

Set	Variable type	Variable (units / values)	Type (transformation parameter)
Cytokines	Inflammation markers - synovial fluid	C-reactive protein - synovial fluid (mg/L)	Continuous (g = 0.25)
		Interleukin 6 - synovial fluid (pg/mL)	Continuous (g = 0)
		Interleukin 8 - synovial fluid (pg/mL)	Continuous (g = 0)
		Tumor Necrosis Factor alpha - synovial fluid (pg/mL)	Continuous (g = 0)
		Nerve Growth Factor - synovial fluid (pg/mL)	Continuous (g = 0.25)
		Calprotectin - synovial fluid (ng/mL)	Continuous (g = 0.25)
	Adipocytokines / Myokines - synovial fluid	Leptin - synovial fluid (pg/mL)	Continuous (g = 0.25)
		Irisin - synovial fluid (ng/mL)	Continuous (g = 0)
		Adiponectin - synovial fluid (ng/mL)	Continuous (g = -0.25)
		Omentin - synovial fluid (pg/mL)	Continuous (g = 0.25)
		Osteopontin - synovial fluid (ng/mL)	Continuous (g = 0)
		Visfatin - synovial fluid (ng/mL)	Continuous (g = 0)
		Resistin - synovial fluid (pg/mL)	Continuous (g = 0)
	Inflammation markers - plasma	C-reactive protein - plasma (mg/L)	Continuous (g = 0)
		Interleukin 6 - plasma (pg/mL)	Continuous (g = 0)
		Interleukin 8 - plasma (pg/mL)	Continuous (g = -0.25)
		Tumor Necrosis Factor alpha - plasma (pg/mL)	Continuous (g = 0)
		Nerve Growth Factor - plasma (pg/mL)	Continuous (g = 0.25)
		Calprotectin - plasma (ng/mL)	Continuous (g = -0.50)
	Adipocytokines / Myokines - plasma	Leptin - plasma (pg/mL)	Continuous (g = 0.25)
		Irisin - plasma (ng/mL)	Continuous (g = 0.75)
		Adiponectin - plasma (ng/mL)	Continuous (g = 0)
		Omentin - plasma (pg/mL)	Continuous (g = 0.50)
		Osteopontin - plasma (ng/mL)	Continuous (g = -0.25)
		Visfatin - plasma (ng/mL)	Continuous (g = -0.25)
		Resistin - plasma (pg/mL)	Continuous (g = -0.50)
KOA severity and progression	Radiography - baseline	Kellgren-Lawrence radiographic grade (1-4)	Categorical - four categories
		Osteophytes score (0-10)	Continuous
		Joint space narrowing (0-4)	Categorical - five categories
	Clinical severity - baseline	KOOS - pain (reversed, 0-100)	Continuous
		KOOS - symptoms (reversed, 0-100)	Continuous
		KOOS - functional disability (reversed, 0-100)	Continuous
	Ultrasound - baseline	Joint effusion (mm)	Continuous
		Synovial tissue thickness (mm)	Continuous
	Radiographic progression (2 years follow-up)	Kellgren-Lawrence radiographic progression (Yes / No)	Binary
		Osteophytes radiographic progression (Yes / No)	Binary
		Joint space narrowing radiographic progression (Yes / No)	Binary

Supplementary Table S3. Association between cytokines and Knee Osteoarthritis (**KOA**) outcomes in the **complete female KOA patients series**. Table cells show non-parametric correlation-like measures and 95% Confidence Intervals (95%CI) betwen cytokines and clinical, radiographic and ultrasound severity at baseline and radiographic progression. Correlation-like measures are: Spearman correlation (continuous or ordinal outcomes) and Glass rank biserial correlation (binary outcomes). Radiographic progression according to Kellgren-Lawrence staging and joint space narrowing were treated as ordinal in these analyses. Red color indicates positive correlation, blue represents negative correlation, and color intensity expresses more extreme values of the correlation coefficients. Color intensities were saturated to 0.5 and -0.5 for positive and negative correlation, respectively. **IL-6:** Interleukin 6; **IL-8:** Interleukin 8; **TNF-alpha:** Tumor Necrosis Factor alpha; **NGF:** Nerve Growth Factor; **CRP:** C-Reactive Protein; **KOA:** Knee Osteoarthritis. **KOIP:** Knee Osteoarthritis Inflammatory Phenotype.

	KOOS pain	KOOS symptoms	KOOS functional disability	Joint effusion	Synovial tissue thickness	Kellgren-Lawrence Baseline	Osteophytes Baseline	Joint space narrowing Baseline	Kellgren-Lawrence Progression	Osteophytes Progression	Joint space narrowing Progression
CRP - synovial fluid	0.106 [-0.065, 0.273] 0.172647	0.055 [-0.109, 0.218] 0.480493	0.121 [-0.035, 0.272] 0.1183501	0.037 [-0.125, 0.192] 0.631780	0.027 [-0.142, 0.178] 0.737452	0.079 [-0.066, 0.229] 0.308226	-0.015 [-0.157, 0.142] 0.848850	0.069 [-0.093, 0.231] 0.3730562	0.143 [-0.064, 0.343] 0.1718505	0.073 [-0.130, 0.271] 0.476703	0.161 [-0.039, 0.364] 0.115263
CRP - plasma	0.092 [-0.068, 0.250] 0.233347	0.008 [-0.163, 0.171] 0.917722	0.138 [-0.028, 0.297] 0.0746962	-0.033 [-0.187, 0.126] 0.675439	0.003 [-0.159, 0.157] 0.974419	0.068 [-0.076, 0.211] 0.384565	0.003 [-0.151, 0.162] 0.970962	0.019 [-0.131, 0.186] 0.8021990	0.068 [-0.129, 0.283] 0.5162111	0.023 [-0.166, 0.218] 0.824084	0.032 [-0.179, 0.252] 0.755686
IL-6 - synovial fluid	0.097 [-0.055, 0.252] 0.213718	0.133 [-0.030, 0.280] 0.082567	0.125 [-0.022, 0.273] 0.1064135	0.195 [0.036, 0.321] 0.012246	0.051 [-0.102, 0.195] 0.517584	0.052 [-0.093, 0.204] 0.501415	-0.106 [-0.255, 0.051] 0.172672	-0.015 [-0.163, 0.138] 0.8470682	-0.018 [-0.230, 0.198] 0.8606411	0.162 [-0.036, 0.369] 0.117072	0.200 [0.007, 0.410] 0.049946
IL-6 - plasma	0.005 [-0.154, 0.159] 0.943831	-0.003 [-0.149, 0.151] 0.973303	0.036 [-0.111, 0.176] 0.6409276	0.038 [-0.107, 0.200] 0.622594	-0.083 [-0.269, 0.077] 0.290938	0.006 [-0.157, 0.166] 0.942834	-0.114 [-0.267, 0.052] 0.145264	-0.017 [-0.173, 0.137] 0.8264591	0.056 [-0.164, 0.240] 0.5968512	-0.228 [-0.412, -0.032] 0.026496	-0.004 [-0.215, 0.219] 0.965771
IL-8 - synovial fluid	0.130 [-0.036, 0.286] 0.096622	0.196 [0.034, 0.338] 0.011760	0.145 [-0.019, 0.303] 0.0636918	0.009 [-0.139, 0.161] 0.909944	0.054 [-0.094, 0.216] 0.498515	0.176 [0.038, 0.313] 0.023067	0.089 [-0.074, 0.240] 0.258650	0.139 [-0.030, 0.293] 0.0760077	0.117 [-0.083, 0.321] 0.2662429	0.055 [-0.158, 0.259] 0.593650	0.243 [0.053, 0.444] 0.018825
IL-8 - plasma	0.033 [-0.109, 0.192] 0.671038	0.060 [-0.072, 0.210] 0.442820	0.021 [-0.137, 0.156] 0.7876756	0.030 [-0.131, 0.191] 0.699391	0.051 [-0.123, 0.213] 0.518432	0.043 [-0.093, 0.196] 0.578107	0.009 [-0.137, 0.150] 0.906812	0.045 [-0.104, 0.203] 0.5629462	0.004 [-0.186, 0.212] 0.9733046	0.114 [-0.087, 0.307] 0.266380	0.089 [-0.110, 0.258] 0.379714
TNF-alpha - synovial fluid	0.061 [-0.091, 0.233] 0.452481	0.064 [-0.098, 0.221] 0.425905	0.094 [-0.076, 0.263] 0.2471124	0.009 [-0.154, 0.179] 0.907790	-0.031 [-0.192, 0.127] 0.710042	-0.003 [-0.159, 0.162] 0.969022	-0.078 [-0.223, 0.083] 0.337730	-0.084 [-0.265, 0.069] 0.2958517	0.052 [-0.165, 0.257] 0.6384822	-0.002 [-0.209, 0.205] 0.984160	0.148 [-0.078, 0.355] 0.159725
TNF-alpha - plasma	-0.019 [-0.180, 0.133] 0.802994	-0.004 [-0.164, 0.162] 0.962153	-0.026 [-0.183, 0.142] 0.7178056	0.080 [-0.088, 0.233] 0.302833	0.012 [-0.144, 0.165] 0.884367	-0.087 [-0.241, 0.068] 0.281137	-0.154 [-0.306, -0.008] 0.046955	-0.088 [-0.248, 0.065] 0.2563597	0.036 [-0.167, 0.233] 0.7339591	-0.012 [-0.220, 0.183] 0.910722	0.100 [-0.103, 0.307] 0.325756
NGF - synovial fluid	0.048 [-0.106, 0.198] 0.543127	0.064 [-0.094, 0.225] 0.414322	0.106 [-0.051, 0.257] 0.1789914	-0.013 [-0.175, 0.155] 0.868187	-0.050 [-0.216, 0.110] 0.529795	0.047 [-0.116, 0.196] 0.554938	-0.017 [-0.164, 0.140] 0.829499	0.007 [-0.144, 0.155] 0.9299662	-0.046 [-0.246, 0.160] 0.6589828	0.111 [-0.110, 0.303] 0.282672	0.054 [-0.151, 0.280] 0.602053
NGF - plasma	-0.078 [-0.228, 0.071] 0.313855	-0.091 [-0.248, 0.062] 0.241721	-0.099 [-0.260, 0.047] 0.2034301	0.077 [-0.079, 0.211] 0.324298	0.087 [-0.054, 0.224] 0.272677	0.023 [-0.129, 0.174] 0.768014	-0.027 [-0.178, 0.125] 0.727082	0.070 [-0.087, 0.213] 0.3653970	-0.006 [-0.206, 0.194] 0.9567602	-0.123 [-0.321, 0.077] 0.223902	0.062 [-0.127, 0.250] 0.535463
Calprotectin - synovial fluid	0.088 [-0.066, 0.247] 0.262794	0.142 [-0.014, 0.300] 0.070438	0.129 [-0.032, 0.283] 0.0989424	0.110 [-0.038, 0.265] 0.140374	0.135 [-0.031, 0.295] 0.090812	-0.017 [-0.163, 0.135] 0.831931	-0.016 [-0.171, 0.146] 0.833308	-0.053 [-0.214, 0.109] 0.4966793	0.124 [-0.070, 0.328] 0.2420333	0.164 [-0.038, 0.375] 0.112762	0.164 [-0.024, 0.347] 0.113264
Calprotectin - plasma	0.050 [-0.107, 0.211] 0.519412	-0.006 [-0.152, 0.134] 0.938829	0.067 [-0.086, 0.223] 0.3912433	0.023 [-0.135, 0.177] 0.760082	-0.047 [-0.195, 0.102] 0.549679	-0.002 [-0.181, 0.157] 0.984388	-0.013 [-0.169, 0.135] 0.864959	0.001 [-0.158, 0.155] 0.9862902	0.153 [-0.062, 0.361] 0.1439470	-0.007 [-0.185, 0.194] 0.948872	0.063 [-0.164, 0.279] 0.553744
Leptin - synovial fluid	0.135 [-0.013, 0.279] 0.081013	0.088 [-0.060, 0.242] 0.254356	0.225 [0.072, 0.366] 0.0031520	0.090 [-0.056, 0.226] 0.245064	0.043 [-0.116, 0.198] 0.583331	0.105 [-0.040, 0.254] 0.175552	-0.042 [-0.198, 0.096] 0.588616	0.121 [-0.031, 0.275] 0.1179575	0.172 [-0.026, 0.383] 0.1001374	0.067 [-0.120, 0.250] 0.513072	0.157 [-0.030, 0.361] 0.123332
Leptin - plasma	0.106 [-0.040, 0.244] 0.173251	0.029 [-0.113, 0.195] 0.705549	0.176 [0.040, 0.317] 0.0227427	-0.001 [-0.152, 0.159] 0.984908	-0.040 [-0.209, 0.127] 0.611528	0.121 [-0.022, 0.268] 0.119982	-0.019 [-0.178, 0.131] 0.804121	0.117 [-0.022, 0.288] 0.0769468	0.113 [-0.102, 0.327] 0.2809827	0.095 [-0.117, 0.311] 0.357184	0.046 [-0.197, 0.253] 0.649198
Irisin - synovial fluid	0.113 [-0.034, 0.268] 0.141464	0.105 [-0.056, 0.264] 0.177834	0.172 [0.019, 0.317] 0.0271393	0.090 [-0.078, 0.251] 0.251526	0.133 [-0.029, 0.286] 0.093169	0.166 [0.017, 0.306] 0.032891	0.078 [-0.088, 0.213] 0.320132	0.150 [-0.011, 0.297] 0.0552518	0.076 [-0.128, 0.307] 0.4720444	0.001 [-0.214, 0.202] 0.989492	0.111 [-0.103, 0.298] 0.277614
Irisin - plasma	0.020 [-0.139, 0.169] 0.799931	0.008 [-0.151, 0.163] 0.918325	0.144 [-0.018, 0.292] 0.0635070	-0.006 [-0.165, 0.155] 0.943256	-0.026 [-0.183, 0.137] 0.744818	0.134 [-0.024, 0.283] 0.085793	0.001 [-0.160, 0.151] 0.986551	0.159 [0.001, 0.301] 0.0407309	0.072 [-0.144, 0.273] 0.4913428	0.080 [-0.129, 0.273] 0.437514	-0.044 [-0.230, 0.175] 0.668025
Adiponectin - synovial fluid	0.027 [-0.120, 0.169] 0.725210	0.050 [-0.106, 0.211] 0.517209	-0.012 [-0.151, 0.135] 0.8735796	0.162 [0.013, 0.316] 0.033827	0.152 [0.009, 0.298] 0.052435	0.126 [-0.033, 0.287] 0.104630	0.005 [-0.145, 0.161] 0.958345	0.055 [-0.101, 0.207] 0.4777987	-0.128 [-0.330, 0.078] 0.2206469	0.080 [-0.108, 0.282] 0.436538	0.003 [-0.196, 0.202] 0.976359
Adiponectin - plasma	-0.051 [-0.210, 0.109] 0.510409	-0.052 [-0.206, 0.108] 0.505605	-0.079 [-0.238, 0.083] 0.3088593	-0.012 [-0.156, 0.139] 0.882101	-0.055 [-0.217, 0.105] 0.489390	0.084 [-0.080, 0.245] 0.278783	0.082 [-0.082, 0.238] 0.289479	0.072 [-0.088, 0.224] 0.3552915	-0.217 [-0.414, -0.018] 0.0382159	-0.042 [-0.261, 0.166] 0.679950	-0.160 [-0.380, 0.035] 0.114526
Omentin - synovial fluid	0.042 [-0.117, 0.205] 0.587873	0.095 [-0.076, 0.246] 0.225457	-0.003 [-0.167, 0.165] 0.9726616	0.126 [-0.029, 0.274] 0.107853	0.037 [-0.128, 0.195] 0.645883	0.004 [-0.164, 0.160] 0.958346	-0.045 [-0.204, 0.118] 0.586035	-0.042 [-0.194, 0.125] 0.5926123	-0.008 [-0.207, 0.202] 0.9415448	0.017 [-0.210, 0.238] 0.869613	0.056 [-0.148, 0.265] 0.584155
Omentin - plasma	-0.098 [-0.238, 0.055] 0.214799	-0.071 [-0.233, 0.093] 0.360103	-0.105 [-0.250, 0.051] 0.1764601	-0.037 [-0.183, 0.105] 0.634122	0.019 [-0.143, 0.190] 0.805377	0.143 [-0.011, 0.296] 0.064701	0.112 [-0.046, 0.271] 0.151278	0.078 [-0.079, 0.232] 0.3179424	-0.222 [-0.429, -0.009] 0.0318699	-0.006 [-0.232, 0.214] 0.952236	-0.187 [-0.387, 0.027] 0.065946
Osteopontin - synovial fluid	0.193 [0.030, 0.338] 0.012093	0.096 [-0.047, 0.243] 0.217753	0.163 [0.015, 0.304] 0.0349961	0.107 [-0.043, 0.254] 0.160054	-0.087 [-0.231, 0.058] 0.267754	-0.053 [-0.214, 0.086] 0.495447	-0.048 [-0.208, 0.115] 0.513669	-0.073 [-0.221, 0.078] 0.3401345	0.055 [-0.156, 0.263] 0.6000368	-0.100 [-0.303, 0.095] 0.331238	0.174 [-0.031, 0.377] 0.086380
Osteopontin - plasma	0.119 [-0.042, 0.277] 0.125627	0.046 [-0.097, 0.207] 0.555566	0.132 [-0.032, 0.277] 0.0825631	0.003 [-0.143, 0.158] 0.965672	-0.038 [-0.188, 0.110] 0.611939	0.119 [-0.029, 0.278] 0.124017	0.152 [0.006, 0.303] 0.050165	0.147 [-0.007, 0.301] 0.0569982	-0.155 [-0.351, 0.061] 0.1391145	-0.154 [-0.359, 0.051] 0.131487	0.078 [-0.117, 0.294] 0.441023
Visfatin - synovial fluid	0.071 [-0.085, 0.233] 0.361786	0.088 [-0.062, 0.247] 0.258380	0.092 [-0.061, 0.233] 0.2352516	0.036 [-0.119, 0.197] 0.645365	0.089 [-0.061, 0.259] 0.255956	0.151 [0.001, 0.295] 0.050630	0.092 [-0.061, 0.243] 0.259091	0.136 [-0.006, 0.250] 0.0794944	-0.019 [-0.235, 0.185] 0.8581501	0.069 [-0.130, 0.275] 0.499395	0.089 [-0.117, 0.298] 0.382014
Visfatin - plasma	0.025 [-0.123, 0.170] 0.749365	-0.012 [-0.157, 0.130] 0.879680	0.038 [-0.119, 0.194] 0.6260636	0.006 [-0.145, 0.159] 0.954747	0.087 [-0.072, 0.248] 0.271426	-0.003 [-0.152, 0.151] 0.968803	-0.051 [-0.208, 0.099] 0.511867	-0.041 [-0.198, 0.106] 0.5941026	-0.125 [-0.313, 0.072] 0.2326376	-0.106 [-0.310, 0.097] 0.300089	-0.166 [-0.364, 0.019] 0.103136
Resistin - synovial fluid	0.094 [-0.059, 0.252] 0.227636	0.049 [-0.106, 0.207] 0.527293	0.182 [0.036, 0.324] 0.0182283	-0.066 [-0.218, 0.093] 0.391851	-0.106 [-0.255, 0.051] 0.176498	-0.026 [-0.179, 0.120] 0.741997	0.003 [-0.155, 0.163] 0.972354	-0.023 [-0.169, 0.130] 0.7654080	-0.149 [-0.330, 0.053] 0.1564037	0.114 [-0.089, 0.315] 0.268080	0.051 [-0.141, 0.239] 0.617296
Resistin - plasma	0.033 [-0.118, 0.182] 0.670034	-0.076 [-0.222, 0.078] 0.325401	-0.008 [-0.183, 0.150] 0.9175448	0.013 [-0.145, 0.173] 0.864209	-0.077 [-0.235, 0.087] 0.325624	0.001 [-0.143, 0.161] 0.990614	-0.140 [-0.286, 0.024] 0.070645	-0.026 [-0.172, 0.122] 0.7354373	-0.017 [-0.213, 0.197] 0.8706311	-0.071 [-0.276, 0.118] 0.489944	-0.018 [-0.211, 0.189] 0.873064

Supplementary Table S4. Association between cytokines and Knee Osteoarthritis (**KOA**) outcomes in the **KOIP-1 group**. Table cells show non-parametric correlation-like measures and 95% Confidence Intervals (95%CI) betwen cytokines and clinical, radiographic and ultrasound severity at baseline and radiographic progression. Correlation-like measures are: Spearman correlation (continuous or ordinal outcomes) and Glass rank biserial correlation (binary outcomes). Radiographic progression according to Kellgren-Lawrence staging and joint space narrowing were treated as ordinal in these analyses. Red color indicates positive correlation, blue represents negative correlation, and color intensity expresses more extreme values of the correlation coefficients. Color intensities were saturated to 0.5 and -0.5 for positive and negative correlation, respectively. **IL-6:** Interleukin 6; **IL-8:** Interleukin 8; **TNF-alpha:** Tumor Necrosis Factor alpha; **NGF:** Nerve Growth Factor; **CRP:** C-Reactive Protein; **KOA:** Knee Osteoarthritis. **KOIP:** Knee Osteoarthritis Inflammatory Phenotype.

	KOOS pain	KOOS symptoms	KOOS functional disability	Joint effusion	Synovial tissue thickness	Kellgren-Lawrence Baseline	Osteophytes Baseline	Joint space narrowing Baseline	Kellgren-Lawrence Progression	Osteophytes Progression	Joint space narrowing Progression
CRP - synovial fluid	0.057 [-0.212, 0.318] 0.6808475	0.189 [-0.099, 0.453] 0.217488	0.037 [-0.265, 0.324] 0.7880916	0.088 [-0.192, 0.374] 0.524307	0.008 [-0.287, 0.289] 0.953269	0.056 [-0.201, 0.316] 0.685440	0.249 [-0.037, 0.482] 0.071720	-0.057 [-0.312, 0.225] 0.6782933	0.322 [-0.024, 0.620] 0.0625661	-0.033 [-0.519, 0.439] 0.8768724	0.013 [-0.318, 0.364] 0.936124
CRP - plasma	0.018 [-0.254, 0.283] 0.8941563	0.084 [-0.203, 0.368] 0.541826	0.092 [-0.198, 0.374] 0.5044741	-0.152 [-0.440, 0.133] 0.268017	-0.096 [-0.350, 0.169] 0.491954	-0.014 [-0.271, 0.234] 0.910239	0.079 [-0.193, 0.325] 0.565787	-0.169 [-0.399, 0.071] 0.2181278	0.025 [-0.318, 0.360] 0.8860196	0.009 [-0.511, 0.508] 0.6795209	-0.110 [-0.449, 0.226] 0.508546
IL-6 - synovial fluid	-0.122 [-0.364, 0.153] 0.3761797	0.060 [-0.194, 0.328] 0.661141	-0.041 [-0.304, 0.222] 0.7656312	0.031 [-0.232, 0.289] 0.821858	-0.139 [-0.378, 0.135] 0.316026	0.117 [-0.153, 0.368] 0.393270	-0.165 [-0.429, 0.095] 0.228933	0.027 [-0.261, 0.297] 0.8453579	0.029 [-0.319, 0.373] 0.8666328	0.013 [-0.378, 0.467] 0.8760949	[illegible]
IL-6 - plasma	0.007 [-0.293, 0.277] 0.9621484	0.088 [-0.190, 0.339] 0.521256	0.113 [-0.164, 0.376] 0.4132549	0.078 [-0.215, 0.333] 0.573820	0.072 [-0.225, 0.328] 0.604131	0.108 [-0.167, 0.369] 0.434161	-0.083 [-0.340, 0.206] 0.547407	-0.052 [-0.335, 0.225] 0.7068630	0.055 [-0.292, 0.436] 0.7491595	-0.058 [-0.436, 0.333] 0.7861029	0.110 [-0.229, 0.433] 0.434612
IL-8 - synovial fluid	0.030 [-0.266, 0.345] 0.8320551	0.314 [0.059, 0.558] 0.020651	0.199 [-0.110, 0.466] 0.1488220	0.082 [-0.234, 0.372] 0.553326	0.145 [-0.142, 0.404] 0.301267	0.103 [-0.166, 0.345] 0.459411	-0.081 [-0.353, 0.179] 0.558649	0.093 [-0.167, 0.344] 0.5039524	-0.101 [-0.429, 0.255] 0.5616879	-0.006 [-0.442, 0.468] 0.9789212	0.049 [-0.270, 0.389] 0.771999
IL-8 - plasma	0.038 [-0.235, 0.309] 0.7821161	0.192 [-0.064, 0.444] 0.160920	0.164 [-0.102, 0.424] 0.2326517	0.058 [-0.215, 0.346] 0.675558	-0.121 [-0.434, 0.170] 0.383941	0.165 [-0.109, 0.434] 0.228820	0.028 [-0.242, 0.296] 0.840704	0.065 [-0.206, 0.303] 0.6368916	-0.112 [-0.459, 0.268] 0.5153208	0.225 [-0.186, 0.544] 0.2956847	0.164 [-0.167, 0.465] 0.326266
TNF-alpha - synovial fluid	0.028 [-0.272, 0.286] 0.8418532	0.124 [-0.164, 0.388] 0.370218	0.155 [-0.139, 0.421] 0.2648216	0.107 [-0.151, 0.356] 0.439213	-0.067 [-0.339, 0.213] 0.634660	0.103 [-0.173, 0.358] 0.460664	-0.053 [-0.340, 0.229] 0.702761	0.009 [-0.273, 0.303] 0.9470219	-0.236 [-0.560, 0.119] 0.1777694	0.009 [-0.542, 0.400] 0.9683949	0.154 [-0.188, 0.483] 0.362593
TNF-alpha - plasma	0.019 [-0.251, 0.305] 0.8908295	0.070 [-0.216, 0.330] 0.609651	0.028 [-0.285, 0.291] 0.8368486	0.208 [-0.072, 0.480] 0.126096	0.032 [-0.257, 0.309]0.819334	0.038 [-0.226, 0.288] 0.780655	-0.019 [-0.321, 0.269] 0.888887	0.002 [-0.293, 0.279] 0.9864097	0.086 [-0.260, 0.435] 0.6197631	0.179 [-0.398, 0.615] 0.4006709	0.234 [-0.044, 0.573] 0.127874
NGF - synovial fluid	0.026 [-0.274, 0.309] 0.8436837	0.012 [-0.287, 0.304] 0.929738	0.072 [-0.227, 0.369] 0.6103472	0.033 [-0.201, 0.295] 0.814062	0.033 [-0.248, 0.306] 0.816196	-0.001 [-0.303, 0.279] 0.994785	0.060 [-0.194, 0.323] 0.670694	-0.121 [-0.383, 0.170] 0.3861629	-0.139 [-0.471, 0.219] 0.4262136	0.462 [0.036, 0.843] [illegible]	0.060 [-0.264, 0.375] 0.812906
NGF - plasma	-0.211 [-0.445, 0.022] 0.1252997	-0.158 [-0.417, 0.145] 0.253666	-0.181 [-0.460, 0.099] 0.1898113	0.188 [-0.087, 0.414] 0.173928	0.129 [-0.136, 0.405] 0.357333	0.148 [-0.128, 0.413] 0.286865	-0.125 [-0.376, 0.130] 0.366760	0.141 [-0.133, 0.376] 0.3107390	-0.250 [-0.567, 0.064] 0.1492430	-0.262 [-0.683, 0.197] 0.2210059	-0.033 [-0.370, 0.310] 0.843722
Calprotectin - synovial fluid	0.167 [-0.104, 0.427] 0.2374332	0.222 [-0.061, 0.463] 0.113881	0.136 [-0.160, 0.404] 0.3347281	-0.033 [-0.298, 0.222] 0.814926	0.198 [-0.090, 0.462] 0.164977	-0.009 [-0.286, 0.268] 0.951929	-0.047 [-0.310, 0.204] 0.741342	-0.021 [-0.295, 0.242] 0.8831276	-0.039 [-0.430, 0.339] 0.8265385	-0.147 [-0.569, 0.297] 0.4875183	-0.002 [-0.365, 0.325] 0.991202
Calprotectin - plasma	-0.013 [-0.293, 0.283] 0.9252163	0.197 [-0.083, 0.469] 0.148799	0.133 [-0.160, 0.428] 0.3312966	-0.048 [-0.317, 0.215] 0.725346	-0.075 [-0.336, 0.215] 0.588316	0.070 [-0.177, 0.300] 0.609572	0.090 [-0.182, 0.334] 0.515135	0.005 [-0.244, 0.278] 0.9729602	0.181 [-0.204, 0.529] 0.2940672	0.159 [-0.211, 0.512] 0.4857171	0.130 [-0.190, 0.452] 0.434624
Leptin - synovial fluid	-0.076 [-0.386, 0.223] 0.5800336	-0.102 [-0.393, 0.231] 0.457513	-0.024 [-0.325, 0.263] 0.8604284	-0.159 [-0.426, 0.116] 0.245836	-0.152 [-0.422, 0.119] 0.272638	0.154 [-0.120, 0.419] 0.261320	-0.018 [-0.318, 0.236] 0.894325	0.165 [-0.106, 0.426] 0.2280694	0.017 [-0.313, 0.347] 0.9209499	0.463 [0.077, 0.844] [illegible]	-0.043 [-0.396, 0.297] 0.794529
Leptin - plasma	-0.110 [-0.404, 0.180] 0.4294761	-0.077 [-0.374, 0.219] 0.579961	0.015 [-0.252, 0.288] 0.9156144	-0.158 [-0.413, 0.125] 0.253539	-0.238 [-0.462, -0.022] 0.061694	0.072 [-0.186, 0.326] 0.604795	-0.025 [-0.303, 0.244 0.859740	0.100 [-0.166, 0.362] 0.4703454	-0.044 [-0.364, 0.341] 0.8023673	0.403 [-0.031, 0.782] 0.0625738	-0.061 [-0.385, 0.294] 0.717982
Irisin - synovial fluid	-0.086 [-0.335, 0.188] 0.5414093	-0.032 [-0.318, 0.259] 0.822072	-0.011 [-0.263, 0.236] 0.9387161	-0.077 [-0.365, 0.207] 0.582651	0.103 [-0.178, 0.387] 0.466699	0.268 [-0.007, 0.532] 0.052470	0.182 [-0.124, 0.435] 0.192753	0.231 [-0.074, 0.515] 0.0953657	[illegible]	0.158 [-0.328, 0.578] 0.4652117	[illegible]
Irisin - plasma	-0.164 [-0.440, 0.115] 0.2365206	-0.090 [-0.354, 0.182] 0.517365	-0.098 [-0.375, 0.191] 0.4823262	-0.016 [-0.299, 0.275] 0.907568	0.055 [-0.194, 0.307] 0.697348	0.198 [-0.078, 0.462] 0.155702	0.094 [-0.189, 0.355] 0.508657	0.242 [-0.030, 0.515] 0.0777908	-0.038 [-0.424, 0.320] 0.8287400	0.306 [-0.285, 0.743] 0.1345426	-0.238 [-0.546, 0.112] 0.157233
Adiponectin - synovial fluid	-0.115 [-0.390, 0.148] 0.4023430	-0.007 [-0.295, 0.287] 0.961767	-0.055 [-0.320, 0.223] 0.6902647	-0.022 [-0.295, 0.272] 0.871413	0.009 [-0.267, 0.267] 0.947361	0.080 [-0.196, 0.349] 0.560681	-0.024 [-0.331, 0.230] 0.890097	0.059 [-0.217, 0.325] 0.6667851	-0.139 [-0.484, 0.188] 0.4208492	[illegible]	0.070 [-0.268, 0.403] 0.673965
Adiponectin - plasma	-0.251 [-0.482, -0.004] 0.0641238	-0.163 [-0.430, 0.110] 0.234760	-0.197 [-0.439, 0.054] 0.1496734	-0.163 [-0.424, 0.111] 0.234479	-0.173 [-0.430, 0.118] 0.211267	0.022 [-0.249, 0.287] 0.874643	0.021 [-0.243, 0.280] 0.879067	0.140 [-0.132, 0.404] 0.3070675	-0.116 [-0.427, 0.231] 0.5013969	0.311 [-0.180, 0.709] 0.1462014	0.167 [-0.158, 0.498] 0.318803
Omentin - synovial fluid	-0.265 [-0.501, -0.001] 0.0363873	-0.064 [-0.341, 0.218] 0.643342	-0.218 [-0.462, 0.057] 0.1108286	0.143 [-0.145, 0.406] 0.299727	-0.030 [-0.325, 0.242] 0.827211	0.034 [-0.257, 0.296] 0.805906	-0.003 [-0.279, 0.280] 0.984379	0.049 [-0.267, 0.317] 0.7237695	0.208 [-0.149, 0.531] 0.2294128	[illegible]	[illegible]
Omentin - plasma	-0.233 [-0.464, 0.033] 0.0866448	-0.060 [-0.327, 0.224] 0.665817	-0.106 [-0.371, 0.172] 0.4403165	-0.072 [-0.337, 0.169] 0.601943	-0.095 [-0.333, 0.165] 0.492641	0.327 [0.081, 0.555] 0.014888	0.228 [-0.011, 0.455] 0.093663	0.365 [0.097, 0.598] 0.0061557	-0.105 [-0.460, 0.218] 0.5442162	[illegible]	-0.043 [-0.400, 0.310] 0.794529
Osteopontin - synovial fluid	0.097 [-0.183, 0.352] 0.4815616	0.116 [-0.149, 0.364] 0.398833	0.109 [-0.146, 0.359] 0.4303205	-0.013 [-0.309, 0.266] 0.922583	-0.092 [-0.348, 0.186] 0.506222	-0.149 [-0.411, 0.118] 0.278316	-0.120 [-0.375, 0.161] 0.381101	-0.168 [-0.435, 0.133] 0.2193641	0.042 [-0.314, 0.377] 0.8082729	0.211 [-0.208, 0.599] 0.3262690	0.244 [-0.092, 0.582] 0.143416
Osteopontin - plasma	0.134 [-0.176, 0.392] 0.3281941	-0.067 [-0.318, 0.212] 0.628722	0.078 [-0.216, 0.367] 0.5728687	-0.181 [-0.424, 0.089] 0.187217	-0.130 [-0.377, 0.130] 0.348827	-0.047 [-0.341, 0.226] 0.735328	-0.060 [-0.311, 0.174] 0.662341	0.000 [-0.272, 0.264] 0.9978536	-0.322 [-0.637, -0.004] 0.0625984	-0.117 [-0.574, 0.380] 0.5876686	0.047 [-0.289, 0.364] 0.779119
Visfatin - synovial fluid	-0.073 [-0.343, 0.255] 0.5958288	-0.009 [-0.292, 0.271] 0.949144	-0.009 [-0.277, 0.292] 0.9484762	-0.075 [-0.324, 0.214] 0.584413	0.147 [-0.102, 0.420] 0.287049	0.158 [-0.121, 0.431] 0.248905	0.036 [-0.219, 0.294] 0.794322	0.088 [-0.146, 0.322] 0.5216168	-0.149 [-0.489, 0.189] 0.3897290	-0.058 [-0.456, 0.378] 0.7862967	0.065 [-0.303, 0.407] 0.698040
Visfatin - plasma	0.084 [-0.197, 0.379] 0.5431717	0.017 [-0.283, 0.318] 0.900667	-0.038 [-0.312, 0.284] 0.7833750	-0.150 [-0.375, 0.126] 0.274789	0.036 [-0.256, 0.309] 0.797978	0.060 [-0.219, 0.334] 0.662137	0.018 [-0.221, 0.278] 0.894325	-0.020 [-0.299, 0.231] 0.8820434	-0.053 [-0.396, 0.294] 0.7491398	-0.117 [-0.523, 0.274] 0.5876590	-0.013 [-0.333, 0.309] 0.936129
Resistin - synovial fluid	-0.035 [-0.283, 0.225] 0.7977216	-0.069 [-0.326, 0.188] 0.615252	0.066 [-0.220, 0.316] 0.6297848	-0.060 [-0.341, 0.243] 0.662958	0.068 [-0.225, 0.328] 0.617305	-0.036 [-0.323, 0.249] 0.791860	0.023 [-0.243, 0.295] 0.869784	-0.066 [-0.353, 0.229] 0.6337830	[illegible]	0.092 [-0.364, 0.521] 0.6700794	-0.038 [-0.368, 0.308] 0.817789
Resistin - plasma	-0.079 [-0.314, 0.175] 0.5646775	-0.124 [-0.379, 0.131] 0.366273	-0.220 [-0.464, 0.055] 0.1075082	0.012 [-0.273, 0.302] 0.930413	-0.121 [-0.419, 0.191] 0.382289	0.071 [-0.173, 0.317] 0.605001	-0.095 [-0.365, 0.148] 0.488683	-0.015 [-0.277, 0.253] 0.9156636	-0.063 [-0.421, 0.262] 0.7159553	-0.172 [-0.554, 0.258] 0.4234695	-0.013 [-0.337, 0.338] 0.956131

Supplementary Table S5. Association between cytokines and Knee Osteoarthritis (**KOA**) outcomes in the **KOIP-2 group**. Table cells show non-parametric correlation-like measures and 95% Confidence Intervals (95%CI) betwen cytokines and clinical, radiographic and ultrasound severity at baseline and radiographic progression. Correlation-like measures are: Spearman correlation (continuous or ordinal outcomes) and Glass rank biserial correlation (binary outcomes). Radiographic progression according to Kellgren-Lawrence staging and joint space narrowing were treated as ordinal in these analyses. Red color indicates positive correlation, blue represents negative correlation, and color intensity expresses more extreme values of the correlation coefficients. Color intensities were saturated to 0.5 and -0.5 for positive and negative correlation, respectively. **IL-6:** Interleukin 6; **IL-8:** Interleukin 8; **TNF-alpha:** Tumor Necrosis Factor alpha; **NGF:** Nerve Growth Factor; **CRP:** C-Reactive Protein; **KOA:** Knee Osteoarthritis. **KOIP:** Knee Osteoarthritis Inflammatory Phenotype.

	KOOS pain	KOOS symptoms	KOOS functional disability	Joint effusion	Synovial tissue thickness	Kellgren-Lawrence Baseline	Osteophytes Baseline	Joint space narrowing Baseline	Kellgren-Lawrence Progression	Osteophytes Progression	Joint space narrowing Progression
CRP - synovial fluid	-0.192 [-0.472, 0.122] 0.181226	0.006 [-0.320, 0.331] 0.968923	-0.145 [-0.422, 0.148] 0.315037	0.022 [-0.286, 0.329] 0.878007	0.175 [-0.125, 0.454] 0.223185	0.068 [-0.261, 0.370] 0.636965	-0.065 [-0.337, 0.217] 0.655815	0.109 [-0.219, 0.412] 0.449363	0.046 [-0.457, 0.503] 0.834243	0.025 [-0.326, 0.405] 0.8967512	0.268 [-0.242, 0.731] 0.2203176
CRP - plasma	-0.005 [-0.268, 0.258] 0.972142	0.028 [-0.273, 0.308] 0.845896	0.005 [-0.273, 0.274] 0.971821	-0.107 [-0.399, 0.172] 0.456408	0.011 [-0.291, 0.300] 0.941143	-0.083 [-0.347, 0.198] 0.563137	0.020 [-0.304, 0.322] 0.891247	-0.020 [-0.347, 0.283] 0.886972	0.187 [-0.346, 0.641] 0.390714	-0.195 [-0.543, 0.130] 0.3015557	0.062 [-0.404, 0.523] 0.6950003
IL-6 - synovial fluid	0.068 [-0.230, 0.373] 0.637689	0.176 [-0.108, 0.467] 0.222709	0.139 [-0.188, 0.437] 0.335177	0.101 [-0.198, 0.394] 0.484166	0.190 [-0.083, 0.437] 0.194881	-0.080 [-0.367, 0.216] 0.581477	-0.089 [-0.394, 0.218] 0.540704	-0.102 [-0.390, 0.211] 0.480385	-0.248 [-0.683, 0.171] 0.256604	0.138 [-0.333, 0.538] 0.4752297	-0.061 [-0.468, 0.364] 0.7737450
IL-6 - plasma	0.033 [-0.288, 0.330] 0.820779	0.153 [-0.171, 0.426] 0.283983	0.083 [-0.196, 0.360] 0.564184	0.108 [-0.193, 0.377] 0.448654	-0.118 [-0.396, 0.148] 0.418774	-0.103 [-0.398, 0.198] 0.471828	-0.076 [-0.347, 0.197] 0.597763	-0.116 [-0.414, 0.182] 0.417632	0.098 [-0.333, 0.528] 0.651887	[illegible]	-0.138 [-0.590, 0.333] 0.5103045
IL-8 - synovial fluid	0.084 [-0.213, 0.360] 0.564403	0.068 [-0.235, 0.348] 0.640122	0.096 [-0.170, 0.361] 0.512996	-0.292 [-0.549, -0.020] 0.042035	-0.114 [-0.374, 0.142] 0.445487	0.035 [-0.250, 0.339] 0.812890	0.180 [-0.153, 0.454] 0.273189	0.069 [-0.212, 0.338] 0.637051	0.347 [-0.130, 0.745] 0.114339	-0.327 [-0.635, 0.017] 0.0676340	0.306 [-0.126, 0.683] 0.1478229
IL-8 - plasma	0.062 [-0.183, 0.319] 0.667846	0.084 [-0.212, 0.353] 0.558817	0.001 [-0.286, 0.280] 0.993202	0.157 [-0.129, 0.431] 0.270712	0.105 [-0.164, 0.377] 0.473627	-0.109 [-0.336, 0.140] 0.447476	-0.178 [-0.439, 0.117] 0.217639	-0.145 [-0.403, 0.143] 0.310341	-0.308 [-0.619, 0.014] 0.158201	0.362 [-0.019, 0.710] 0.0554000	-0.106 [-0.488, 0.304] 0.6141905
TNF-alpha - synovial fluid	0.186 [-0.110, 0.426] 0.210900	0.149 [-0.140, 0.396] 0.322091	0.234 [-0.037, 0.504] 0.110043	-0.095 [-0.393, 0.243] 0.528795	0.053 [-0.225, 0.316] 0.732315	0.019 [-0.297, 0.312] 0.902523	-0.003 [-0.308, 0.301] 0.983231	-0.186 [-0.429, 0.108] 0.215945	0.153 [NA, NA] 0.508721	-0.159 [-0.548, 0.252] 0.4594471	0.062 [-0.419, 0.518] 0.7722214
TNF-alpha - plasma	-0.207 [-0.480, 0.070] 0.144965	-0.140 [-0.411, 0.131] 0.327216	-0.167 [-0.451, 0.111] 0.241859	-0.141 [-0.373, 0.118] 0.323836	-0.068 [-0.369, 0.234] 0.642354	-0.082 [-0.371, 0.214] 0.567212	-0.108 [-0.388, 0.150] 0.451310	-0.093 [-0.412, 0.229] 0.516493	-0.130 [-0.598, 0.347] 0.550866	-0.190 [-0.560, 0.181] 0.3134999	-0.271 [-0.724, 0.219] 0.1876583
NGF - synovial fluid	0.113 [-0.174, 0.409] 0.446082	0.127 [-0.162, 0.396] 0.390309	0.057 [-0.248, 0.328] 0.698342	-0.029 [-0.377, 0.330] 0.844729	-0.329 [-0.566, -0.033] 0.023810	-0.038 [-0.316, 0.256] 0.798773	-0.167 [-0.433, 0.130] 0.250092	-0.024 [-0.301, 0.231] 0.871536	-0.201 [-0.562, 0.185] 0.359343	-0.201 [-0.613, 0.141] 0.2093873	-0.063 [-0.467, 0.283] 0.7044738
NGF - plasma	0.036 [-0.238, 0.304] 0.800945	0.095 [-0.190, 0.364] 0.507137	-0.043 [-0.322, 0.275] 0.763257	-0.063 [-0.362, 0.240] 0.661225	0.042 [-0.253, 0.327] 0.772301	-0.244 [-0.499, 0.020] 0.084875	0.035 [-0.250, 0.319] 0.808272	-0.197 [-0.439, 0.085] 0.165288	0.102 [-0.305, 0.494] 0.640619	-0.100 [-0.455, 0.282] 0.5956756	0.124 [-0.295, 0.533] 0.5553909
Calprotectin - synovial fluid	0.089 [-0.198, 0.372] 0.537463	0.132 [-0.175, 0.446] 0.360214	0.083 [-0.227, 0.376] 0.568887	0.009 [-0.266, 0.309] 0.952726	0.103 [-0.210, 0.403] 0.486637	-0.206 [-0.472, 0.080] 0.150118	-0.028 [-0.332, 0.282] 0.844953	-0.141 [-0.431, 0.134] 0.329142	0.339 [-0.145, 0.787] 0.180183	0.113 [-0.242, 0.469] 0.5510842	0.321 [-0.118, 0.707] 0.1273816
Calprotectin - plasma	-0.054 [-0.339, 0.232] 0.704307	-0.048 [-0.333, 0.230] 0.736922	-0.134 [-0.376, 0.165] 0.348779	0.101 [-0.164, 0.359] 0.479215	-0.112 [-0.397, 0.182] 0.443180	-0.255 [-0.544, 0.050] 0.070478	-0.092 [-0.357, 0.212] 0.522538	-0.279 [-0.561, 0.001] 0.047831	-0.073 [-0.623, 0.511] 0.737926	-0.314 [-0.638, 0.037] 0.0962037	-0.341 [-0.786, 0.181] 0.1443069
Leptin - synovial fluid	-0.112 [-0.377, 0.161] 0.432544	-0.068 [-0.326, 0.214] 0.636252	-0.074 [-0.350, 0.187] 0.607623	-0.184 [-0.443, 0.084] 0.196678	-0.054 [-0.363, 0.234] 0.711029	0.042 [-0.243, 0.322] 0.768883	-0.129 [-0.379, 0.138] 0.381266	-0.010 [-0.280, 0.239] 0.944671	-0.333 [-0.727, 0.125] 0.128617	-0.181 [-0.534, 0.178] 0.3182829	[illegible]
Leptin - plasma	-0.076 [-0.322, 0.227] 0.595469	0.024 [-0.270, 0.318] 0.868906	0.023 [-0.270, 0.288] 0.874493	-0.201 [-0.472, 0.079] 0.157256	-0.103 [-0.348, 0.181] 0.480889	-0.047 [-0.333, 0.246] 0.743424	-0.088 [-0.380, 0.216] 0.540899	-0.073 [-0.358, 0.199] 0.611302	-0.187 [-0.660, 0.313] 0.390880	-0.145 [-0.536, 0.220] 0.4421415	-0.429 [illegible]
Irisin - synovial fluid	0.105 [-0.158, 0.374] 0.469843	0.196 [-0.095, 0.454] 0.172175	0.142 [-0.146, 0.406] 0.324799	-0.110 [-0.417, 0.200] 0.446436	0.030 [-0.210, 0.267] 0.841649	-0.098 [-0.364, 0.162] 0.496944	-0.130 [-0.432, 0.167] 0.367196	-0.183 [-0.452, 0.129] 0.204229	-0.196 [-0.627, 0.248] 0.370855	-0.177 [-0.519, 0.222] 0.3507954	-0.224 [-0.653, 0.229] 0.2878759
Irisin - plasma	-0.089 [-0.369, 0.196] 0.533790	0.021 [-0.281, 0.290] 0.883898	0.067 [-0.261, 0.369] 0.638313	-0.238 [-0.532, 0.054] 0.098733	-0.091 [-0.369, 0.210] 0.534022	-0.147 [-0.413, 0.144] 0.302119	-0.186 [-0.458, 0.096] 0.190628	-0.109 [-0.378, 0.135] 0.444781	-0.137 [-0.544, 0.300] 0.531689	-0.062 [-0.432, 0.301] 0.7432374	[illegible]
Adiponectin - synovial fluid	0.113 [-0.131, 0.374] 0.429112	0.210 [-0.095, 0.480] 0.138468	0.132 [-0.128, 0.390] 0.355233	0.231 [-0.082, 0.508] 0.102952	0.324 [0.018, 0.575] 0.028268	0.011 [-0.258, 0.289] 0.941083	0.088 [-0.238, 0.369] 0.546396	-0.046 [-0.321, 0.247] 0.749711	-0.003 [-0.494, 0.456] 0.988393	-0.303 [-0.629, 0.028] 0.1088209	0.168 [-0.265, 0.600] 0.3701370
Adiponectin - plasma	0.111 [-0.136, 0.378] 0.439477	0.000 [-0.315, 0.294] 0.998736	0.079 [-0.183, 0.344] 0.582468	0.149 [-0.126, 0.433] 0.296014	-0.026 [-0.319, 0.291] 0.859540	-0.055 [-0.310, 0.240] 0.700631	0.186 [-0.096, 0.453] 0.190198	-0.099 [-0.384, 0.210] 0.488163	0.333 [-0.014, 0.645] 0.126604	-0.093 [-0.506, 0.307] 0.6231517	0.424 [0.124, 0.730] 0.0432420
Omentin - synovial fluid	0.277 [-0.013, 0.550] 0.048755	0.265 [-0.033, 0.515] 0.059832	0.228 [-0.093, 0.527] 0.107389	0.152 [-0.166, 0.461] 0.287251	0.106 [-0.177, 0.414] 0.470266	-0.095 [-0.383, 0.178] 0.506556	-0.022 [-0.325, 0.268] 0.880286	-0.114 [-0.388, 0.195] 0.426190	0.162 [-0.371, 0.682] 0.458123	-0.357 [-0.687, 0.024] 0.0287817	0.147 [-0.340, 0.583] 0.4838389
Omentin - plasma	0.171 [-0.113, 0.462] 0.230413	0.150 [-0.141, 0.410] 0.293784	0.136 [-0.145, 0.436] 0.340476	0.096 [-0.168, 0.363] 0.501140	0.083 [-0.228, 0.398] 0.570988	-0.086 [-0.348, 0.198] 0.546973	0.196 [-0.088, 0.468] 0.168473	-0.134 [-0.412, 0.163] 0.348884	0.133 [-0.260, 0.528] 0.541179	-0.112 [-0.535, 0.319] 0.5537400	0.103 [-0.283, 0.482] 0.6240523
Osteopontin - synovial fluid	0.183 [-0.102, 0.471] 0.198856	0.064 [-0.199, 0.346] 0.654993	0.228 [-0.041, 0.469] 0.107057	0.014 [-0.256, 0.302] 0.921357	-0.236 [-0.476, 0.043] 0.103114	-0.210 [-0.475, 0.076] 0.138804	-0.014 [-0.305, 0.295] 0.922460	-0.104 [-0.417, 0.198] 0.465656	0.121 [-0.344, 0.613] 0.580254	-0.373 [-0.691, -0.021] 0.0402861	0.029 [-0.403, 0.509] 0.8885989
Osteopontin - plasma	0.098 [-0.207, 0.379] 0.494813	0.167 [-0.110, 0.416] 0.240444	0.169 [-0.097, 0.410] 0.237094	0.171 [-0.083, 0.439] 0.229920	-0.085 [-0.382, 0.228] 0.561076	0.176 [-0.114, 0.451] 0.220418	0.248 [-0.048, 0.501] 0.078544	0.197 [-0.091, 0.438] 0.166936	-0.137 [-0.518, 0.289] 0.531689	-0.398 [-0.736, -0.030] 0.0364819	-0.224 [-0.590, 0.145] 0.2872323
Visfatin - synovial fluid	0.089 [-0.179, 0.359] 0.534299	0.133 [-0.138, 0.398] 0.345983	0.124 [-0.152, 0.386] 0.385418	0.197 [-0.104, 0.513] 0.166031	0.137 [-0.143, 0.424] 0.346373	0.126 [-0.131, 0.392] 0.376532	0.172 [-0.128, 0.445] 0.227033	0.196 [-0.077, 0.454] 0.167084	0.162 [-0.245, 0.537] 0.458107	0.021 [-0.338, 0.374] 0.9097187	-0.033 [-0.510, 0.460] 0.8865522
Visfatin - plasma	-0.252 [-0.511, -0.004] 0.074880	-0.253 [-0.492, 0.017] 0.073414	-0.156 [-0.425, 0.121] 0.273054	0.154 [-0.140, 0.405] 0.280186	0.208 [-0.085, 0.468] 0.152450	0.078 [-0.232, 0.347] 0.588722	0.007 [-0.302, 0.289] 0.961830	-0.065 [-0.329, 0.212] 0.651418	[illegible]	-0.143 [-0.561, 0.302] 0.4498818	[illegible]
Resistin - synovial fluid	0.152 [-0.149, 0.425] 0.285488	-0.037 [-0.313, 0.210] 0.795398	0.127 [-0.143, 0.403] 0.375626	-0.077 [-0.371, 0.222] 0.590927	-0.249 [-0.526, 0.037] 0.084067	-0.175 [-0.438, 0.118] 0.218957	-0.034 [-0.299, 0.236] 0.811473	-0.095 [-0.383, 0.174] 0.506133	0.162 [-0.292, 0.579] 0.457790	0.169 [-0.197, 0.522] 0.3706916	0.241 [-0.225, 0.640] 0.2505229
Resistin - plasma	-0.064 [-0.320, 0.222] 0.653606	-0.181 [-0.461, 0.140] 0.202036	-0.100 [-0.355, 0.171] 0.486378	0.113 [-0.160, 0.350] 0.429731	0.099 [-0.174, 0.363] 0.496551	-0.112 [-0.392, 0.191] 0.435977	-0.199 [-0.482, 0.068] 0.161964	-0.194 [-0.455, 0.087] 0.172896	-0.299 [-0.715, 0.123] 0.170036	-0.236 [-0.557, 0.119] 0.2122777	-0.155 [-0.596, 0.342] 0.3776646

Supplementary Table S6. Association between cytokines and Knee Osteoarthritis (**KOA**) outcomes in the **KOIP-3 group**. Table cells show non-parametric correlation-like measures and 95% Confidence Intervals (95%CI) betwen cytokines and clinical, radiographic and ultrasound severity at baseline and radiographic progression. Correlation-like measures are: Spearman correlation (continuous or ordinal outcomes) and Glass rank biserial correlation (binary outcomes). Radiographic progression according to Kellgren-Lawrence staging and joint space narrowing were treated as ordinal in these analyses. Red color indicates positive correlation, blue represents negative correlation, and color intensity expresses more extreme values of the correlation coefficients. Color intensities were saturated to 0.5 and -0.5 for positive and negative correlation, respectively. **IL-6:** Interleukin 6; **IL-8:** Interleukin 8; **TNF-alpha:** Tumor Necrosis Factor alpha; **NGF:** Nerve Growth Factor; **CRP:** C-Reactive Protein; **KOA:** Knee Osteoarthritis. **KOIP:** Knee Osteoarthritis Inflammatory Phenotype.

	KOOS pain	KOOS symptoms	KOOS functional disability	Joint effusion	Synovial tissue thickness	Kellgren-Lawrence Baseline	Osteophytes Baseline	Joint space narrowing Baseline	Kellgren-Lawrence Progression	Osteophytes Progression	Joint space narrowing Progression
CRP - synovial fluid	0.230 [-0.198, 0.591] 0.248541	0.012 [-0.402, 0.396] 0.952873	0.117 [-0.283, 0.504] 0.56014662	0.211 [-0.194, 0.534] 0.285051	0.078 [-0.343, 0.436] 0.698219	0.187 [-0.194, 0.531] 0.324519	0.006 [-0.410, 0.400] 0.9743342	-0.015 [-0.413, 0.392] 0.948885	[illegible]	0.182 [-0.400, 0.722] 0.4813214	0.061 [-0.618, 0.706] 0.8229819
CRP - plasma	0.263 [-0.229, 0.598] 0.188318	0.137 [-0.266, 0.552] 0.435394	0.196 [-0.273, 0.593] 0.22862858	0.221 [-0.071, 0.598] 0.182142	0.334 [illegible]	0.239 [-0.145, 0.565] 0.249881	0.085 [-0.320, 0.511] 0.6378534	-0.058 [-0.487, 0.365] 0.773816	-0.420 [illegible]	-0.189 [-0.656, 0.461] 0.8726548	0.204 [NA, NA] 0.4556380
IL-6 - synovial fluid	0.070 [-0.374, 0.513] 0.730124	0.099 [-0.294, 0.473] 0.621531	-0.138 [-0.529, 0.286] 0.48322588	[illegible]	0.129 [-0.318, 0.522] 0.533671	0.239 [-0.162, 0.562] 0.208806	0.002 [-0.389, 0.375] 0.9938862	0.102 [-0.294, 0.483] 0.612776	-0.231 [-0.758, 0.333] 0.405101	0.273 [-0.245, 0.769] 0.2808457	0.189 [-0.408, 0.778] 0.5059387
IL-6 - plasma	0.098 [-0.367, 0.544] 0.634323	0.108 [-0.330, 0.487] 0.599393	-0.233 [-0.565, 0.202] 0.29583152	0.067 [-0.345, 0.424] 0.746133	-0.237 [-0.615, 0.168] 0.243484	0.070 [-0.380, 0.383] 0.733083	-0.069 [-0.483, 0.376] 0.7374701	0.001 [-0.400, 0.397] 0.995840	-0.024 [-0.514, 0.595] 0.932647	-0.273 [-0.755, 0.189] 0.3020389	0.381 [NA, NA] 0.1869493
IL-8 - synovial fluid	0.222 [-0.229, 0.598] 0.265609	0.329 [illegible] 0.093377	0.062 [-0.429, 0.464] 0.75747769	0.375 [0.039, 0.627] 0.055128	0.273 [-0.143, 0.613] 0.160035	0.215 [-0.203, 0.569] 0.281185	0.288 [illegible] 0.1482444	0.146 [-0.234, 0.518] 0.468515	0.363 [-0.187, 0.820] 0.191848	0.109 [-0.418, 0.655] 0.6726548	[illegible]
IL-8 - plasma	0.104 [-0.329, 0.494] 0.604240	0.045 [-0.314, 0.401] 0.821852	-0.049 [-0.419, 0.353] 0.80987602	-0.287 [illegible] 0.146667	-0.144 [-0.618, 0.308] 0.474399	-0.158 [-0.520, 0.228] 0.432082	0.208 [-0.192, 0.584] 0.3173169	0.084 [-0.283, 0.462] 0.675329	[illegible]	0.055 [-0.469, 0.578] 0.8326894	[illegible]
TNF-alpha - synovial fluid	-0.030 [-0.487, 0.374] 0.881729	-0.018 [-0.415, 0.350] 0.929964	-0.163 [-0.566, 0.286] 0.41308778	-0.051 [-0.459, 0.376] 0.808525	-0.093 [-0.481, 0.368] 0.646096	-0.255 [illegible] 0.206697	0.020 [-0.329, 0.346] 0.9218854	-0.285 [illegible] 0.155553	0.275 [-0.238, 0.750] 0.321912	0.293 [-0.204, 0.763] 0.2598752	[illegible]
TNF-alpha - plasma	-0.123 [-0.481, 0.267] 0.549624	0.095 [-0.291, 0.490] 0.643169	-0.228 [-0.588, 0.211] 0.27036878	0.124 [-0.301, 0.492] 0.545621	-0.019 [-0.564, 0.335] 0.925976	-0.264 [-0.595, 0.189] 0.192837	[illegible]	-0.274 [-0.646, 0.121] 0.175257	-0.189 [-0.762, 0.428] 0.490962	[illegible]	0.071 [-0.524, 0.667] 0.8045709
NGF - synovial fluid	-0.148 [-0.520, 0.257] 0.461117	0.010 [-0.420, 0.461] 0.960321	0.172 [-0.272, 0.576] 0.39219769	-0.014 [-0.434, 0.359] 0.946414	0.033 [-0.317, 0.378] 0.869517	0.144 [-0.295, 0.539] 0.475105	0.196 [-0.175, 0.538] 0.3310559	0.137 [-0.255, 0.486] 0.496782	-0.088 [-0.625, 0.475] 0.747662	-0.227 [-0.716, 0.275] 0.3726152	-0.204 [-0.704, 0.275] 0.4499428
NGF - plasma	-0.339 [illegible]	[illegible]	[illegible]	0.284 [-0.135, 0.678] 0.159438	0.184 [-0.148, 0.513] 0.332852	0.111 [-0.273, 0.504] 0.582444	0.100 [-0.311, 0.465] 0.6207338	0.141 [-0.236, 0.465] 0.482475	-0.132 [-0.677, 0.437] 0.651365	-0.145 [-0.654, 0.364] 0.5701832	-0.274 [-0.751, 0.231] 0.3100580
Calprotectin - synovial fluid	-0.070 [-0.502, 0.371] 0.727934	0.104 [-0.327, 0.509] 0.604177	0.047 [-0.419, 0.479] 0.81577987	0.255 [-0.119, 0.560] 0.199743	0.158 [-0.271, 0.518] 0.436147	0.069 [-0.331, 0.433] 0.733013	0.006 [-0.381, 0.376] 0.9682261	-0.159 [-0.542, 0.261] 0.427295	0.013 [-0.531, 0.548] 0.968395	0.091 [-0.491, 0.618] 0.7247712	0.245 [NA, NA] 0.2786580
Calprotectin - plasma	0.014 [-0.370, 0.406] 0.944137	-0.109 [-0.487, 0.267] 0.588533	-0.143 [-0.533, 0.294] 0.47691883	-0.032 [-0.433, 0.386] 0.875019	0.096 [-0.309, 0.488] 0.634127	0.278 [-0.207, 0.643] 0.159825	[illegible]	0.368 [-0.001, 0.711] 0.065798	0.143 [-0.435, 0.750] 0.606498	0.473 [illegible]	0.265 [-0.291, 0.782] 0.3323127
Leptin - synovial fluid	-0.013 [-0.469, 0.409] 0.946752	0.008 [-0.374, 0.397] 0.969782	0.114 [-0.312, 0.527] 0.57047679	0.057 [-0.288, 0.400] 0.776358	-0.034 [-0.434, 0.354] 0.865467	0.070 [-0.334, 0.449] 0.727011	[illegible]	-0.071 [-0.487, 0.322] 0.723484	-0.209 [-0.714, 0.319] 0.451566	-0.238 [-0.768, 0.219] 0.3599647	0.082 [NA, NA] 0.7653833
Leptin - plasma	-0.007 [-0.387, 0.379] 0.973362	0.068 [-0.302, 0.413] 0.735142	0.062 [-0.358, 0.451] 0.75866749	-0.049 [-0.452, 0.367] 0.808751	0.126 [-0.258, 0.586] 0.532676	0.122 [-0.287, 0.565] 0.543971	[illegible]	-0.103 [-0.487, 0.302] 0.602765	[illegible]	[illegible]	-0.143 [-0.768, 0.509] 0.6815981
Irisin - synovial fluid	-0.013 [-0.410, 0.381] 0.947960	0.079 [-0.322, 0.472] 0.694270	-0.097 [-0.456, 0.294] 0.63091845	0.224 [-0.176, 0.558] 0.262268	0.118 [-0.341, 0.496] 0.563177	0.144 [-0.325, 0.583] 0.473691	-0.335 [illegible]	-0.013 [-0.481, 0.409] 0.948429	-0.319 [NA, NA] 0.250547	[illegible]	0.122 [-0.425, 0.615] 0.6544213
Irisin - plasma	-0.112 [-0.491, 0.331] 0.579430	0.067 [-0.341, 0.463] 0.748877	0.049 [-0.337, 0.432] 0.80990452	0.124 [-0.277, 0.502] 0.537725	-0.028 [-0.445, 0.425] 0.898903	0.208 [-0.212, 0.588] 0.302407	-0.306 [-0.651, 0.070] 0.1209924	0.062 [-0.332, 0.460] 0.758958	-0.429 [illegible]	-0.435 [illegible]	-0.388 [illegible]
Adiponectin - synovial fluid	0.024 [-0.363, 0.378] 0.906550	0.055 [-0.290, 0.424] 0.783724	-0.060 [-0.483, 0.346] 0.76791642	0.283 [-0.145, 0.600] 0.181918	-0.129 [-0.492, 0.302] 0.522620	0.234 [-0.193, 0.641] 0.239710	-0.079 [-0.480, 0.297] 0.6978681	0.287 [-0.143, 0.598] 0.178132	-0.407 [illegible]	0.182 [-0.353, 0.666] 0.4813214	-0.020 [NA, NA] 0.9405282
Adiponectin - plasma	-0.033 [-0.417, 0.362] 0.868757	0.112 [-0.243, 0.437] 0.579828	0.156 [-0.297, 0.559] 0.43782418	-0.098 [-0.481, 0.278] 0.635255	-0.355 [illegible]	0.288 [-0.201, 0.689] 0.157228	-0.161 [-0.527, 0.256] 0.4217865	0.240 [-0.218, 0.600] 0.227335	[illegible]	-0.127 [-0.596, 0.407] 0.6228655	[illegible]
Omentin - synovial fluid	0.165 [-0.198, 0.484] 0.410043	0.206 [-0.315, 0.553] 0.179983	0.237 [-0.172, 0.579] 0.23590323	0.093 [-0.305, 0.462] 0.643962	-0.226 [-0.574, 0.206] 0.256876	0.188 [-0.217, 0.537] 0.426032	-0.219 [-0.578, 0.198] 0.2721665	0.169 [-0.176, 0.520] 0.398809	-0.209 [NA, NA] 0.451566	0.182 [-0.363, 0.694] 0.4813214	-0.020 [-0.667, 0.646] 0.9405282
Omentin - plasma	-0.198 [-0.550, 0.214] 0.341452	0.099 [-0.268, 0.449] 0.622616	0.066 [-0.337, 0.474] 0.74250026	-0.041 [-0.425, 0.342] 0.840559	-0.009 [-0.396, 0.392] 0.962609	0.235 [-0.176, 0.583] 0.238994	-0.285 [-0.618, 0.137] 0.1522435	0.052 [-0.364, 0.439] 0.795726	[illegible]	-0.293 [-0.799, 0.243] 0.2598752	-0.408 [NA, NA] [illegible]
Osteopontin - synovial fluid	-0.076 [-0.478, 0.342] 0.699415	-0.140 [-0.532, 0.332] 0.487456	-0.128 [-0.524, 0.308] 0.52268434	0.184 [-0.228, 0.564] 0.357763	0.168 [-0.264, 0.545] 0.401329	0.143 [-0.342, 0.543] 0.476928	0.035 [-0.387, 0.431] 0.8625164	-0.045 [-0.485, 0.376] 0.822667	0.032 [NA, NA] 0.905583	-0.236 [-0.714, 0.327] 0.3599647	[illegible]
Osteopontin - plasma	0.253 [-0.195, 0.619] 0.282447	0.079 [-0.344, 0.522] 0.694270	0.205 [-0.166, 0.516] 0.30987829	0.012 [-0.368, 0.412] 0.954181	0.119 [-0.291, 0.468] 0.553920	0.466 [illegible]	0.296 [-0.111, 0.609] 0.1366953	0.459 [illegible]	-0.363 [-0.824, 0.187] 0.191048	-0.091 [-0.625, 0.442] 0.7247712	0.386 [-0.308, 0.827] 0.2631189
Visfatin - synovial fluid	-0.112 [-0.467, 0.263] 0.577334	-0.204 [-0.556, 0.251] 0.308513	-0.156 [-0.552, 0.312] 0.43691518	0.010 [-0.355, 0.390] 0.961409	-0.108 [-0.453, 0.267] 0.592386	0.238 [-0.127, 0.568] 0.236855	0.146 [-0.243, 0.540] 0.4748782	0.233 [-0.132, 0.585] 0.242943	-0.231 [NA, NA] 0.405181	0.389 [-0.218, 0.745] 0.2212688	0.204 [-0.373, 0.789] 0.4556380
Visfatin - plasma	0.118 [-0.281, 0.463] 0.584682	0.107 [-0.275, 0.470] 0.594816	0.207 [-0.164, 0.511] 0.30800485	-0.057 [-0.481, 0.398] 0.778259	-0.043 [-0.468, 0.357] 0.831075	-0.024 [-0.395, 0.335] 0.905658	0.043 [-0.374, 0.443] 0.8312879	0.117 [-0.251, 0.489] 0.562260	0.165 [-0.438, 0.736] 0.552287	0.218 [-0.358, 0.709] 0.3861027	-0.082 [NA, NA] 0.7653833
Resistin - synovial fluid	-0.130 [-0.482, 0.261] 0.517824	0.132 [-0.294, 0.513] 0.511300	0.241 [-0.172, 0.593] 0.22615983	-0.146 [-0.549, 0.295] 0.466435	-0.018 [-0.392, 0.429] 0.930092	-0.085 [-0.411, 0.246] 0.672854	0.070 [-0.425, 0.554] 0.7276493	-0.201 [-0.559, 0.246] 0.313698	0.033 [NA, NA] 0.905347	-0.209 [NA, NA] 0.4179997	0.184 [-0.367, 0.725] 0.5017899
Resistin - plasma	0.068 [-0.357, 0.478] 0.734709	0.188 [-0.203, 0.549] 0.347853	0.206 [-0.167, 0.537] 0.30292871	-0.265 [-0.604, 0.125] 0.188875	-0.547 [illegible]	-0.128 [-0.502, 0.297] 0.523673	0.269 [-0.126, 0.596] 0.1749467	-0.033 [-0.399, 0.343] 0.872137	0.319 [NA, NA] 0.250547	0.182 [-0.553, 0.709] 0.4813214	-0.082 [-0.684, 0.537] 0.7653833

Supplementary Table S7. Association between cytokines and Knee Osteoarthritis (**KOA**) outcomes in the **KOIP-4 group**. Table cells show non-parametric correlation-like measures and 95% Confidence Intervals (95%CI) betwen cytokines and clinical, radiographic and ultrasound severity at baseline and radiographic progression. Correlation-like measures are: Spearman correlation (continuous or ordinal outcomes) and Glass rank biserial correlation (binary outcomes). Radiographic progression according to Kellgren-Lawrence staging and joint space narrowing were treated as ordinal in these analyses. Red color indicates positive correlation, blue represents negative correlation, and color intensity expresses more extreme values of the correlation coefficients. Color intensities were saturated to 0.5 and -0.5 for positive and negative correlation, respectively. **IL-6:** Interleukin 6; **IL-8:** Interleukin 8; **TNF-alpha:** Tumor Necrosis Factor alpha; **NGF:** Nerve Growth Factor; **CRP:** C-Reactive Protein; **KOA:** Knee Osteoarthritis. **KOIP:** Knee Osteoarthritis Inflammatory Phenotype.

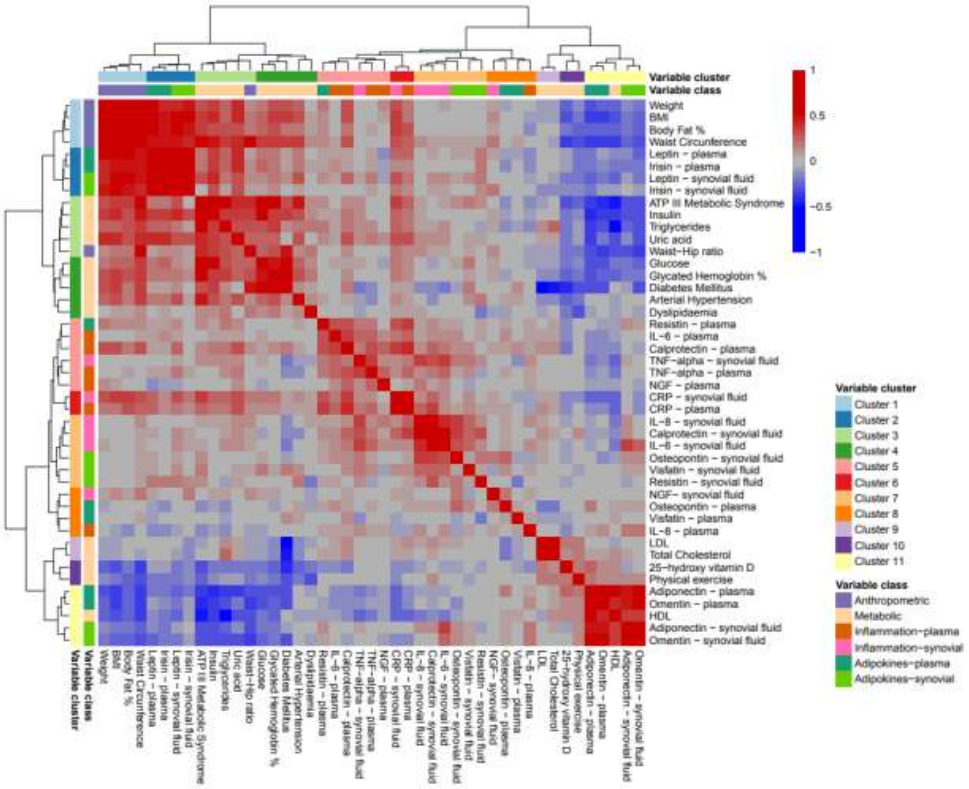
	KOOS pain	KOOS symptoms	KOOS functional disability	Joint effusion	Synovial tissue thickness	Kellgren-Lawrence Baseline	Osteophytes Baseline	Joint space narrowing Baseline	Kellgren-Lawrence Progression	Osteophytes Progression	Joint space narrowing Progression
CRP - synovial fluid	-0.051 [-0.370, 0.265] 0.773244	-0.032 [-0.377, 0.313] [illegible]	0.001 [-0.328, 0.381] 0.994261	[illegible]	-0.219 [illegible]	-0.272 [-0.593, 0.087] [illegible]	-0.224 [illegible]	-0.186 [-0.496, 0.179] [illegible]	-0.006 [-0.523, 0.523] [illegible]	-0.024 [-0.469, 0.412] [illegible]	0.026 [-0.458, 0.565] [illegible]
CRP - plasma	-0.178 [-0.453, 0.152] [illegible]	-0.226 [-0.518, 0.133] [illegible]	-0.074 [-0.391, 0.263] 0.673283	[illegible]	-0.327 [illegible]	-0.140 [-0.464, 0.213] [illegible]	-0.271 [illegible]	-0.072 [-0.384, 0.256] [illegible]	-0.011 [-0.528, 0.456] [illegible]	0.114 [-0.288, 0.547] [illegible]	-0.244 [illegible]
IL-6 - synovial fluid	0.165 [-0.126, 0.427] 0.343170	0.298 [illegible]	0.275 [-0.030, 0.559] [illegible]	0.218 [-0.134, 0.511] [illegible]	0.037 [-0.342, 0.376] [illegible]	-0.272 [illegible]	-0.157 [-0.503, 0.172] [illegible]	[illegible]	0.000 [NA, NA] 1.0000000	0.314 [illegible]	0.232 [illegible]
IL-6 - plasma	[illegible]	[illegible]	-0.203 [-0.417, 0.032] [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	-0.172 [-0.530, 0.203] [illegible]	-0.216 [-0.659, 0.250] [illegible]	-0.186 [-0.635, 0.327] [illegible]	[illegible]
IL-8 - synovial fluid	-0.018 [-0.357, 0.318] [illegible]	0.204 [-0.113, 0.523] [illegible]	-0.025 [-0.362, 0.317] [illegible]	-0.163 [-0.488, 0.188] [illegible]	-0.094 [-0.418, 0.278] [illegible]	0.243 [illegible]	0.137 [-0.183, 0.458] [illegible]	0.089 [-0.270, 0.452] [illegible]	-0.133 [-0.560, 0.260] [illegible]	0.233 [-0.202, 0.625] [illegible]	0.242 [-0.194, 0.667] [illegible]
IL-8 - plasma	-0.092 [-0.416, 0.239] [illegible]	-0.112 [-0.426, 0.218] [illegible]	-0.025 [-0.390, 0.317] [illegible]	0.084 [-0.282, 0.436] [illegible]	[illegible]	0.125 [-0.192, 0.475] [illegible]	0.125 [-0.180, 0.458] [illegible]	0.266 [-0.071, 0.559] [illegible]	0.159 [-0.321, 0.602] [illegible]	[illegible]	0.000 [-0.478, 0.442] 1.000000
TNF-alpha - synovial fluid	-0.012 [-0.400, 0.374] [illegible]	-0.054 [-0.442, 0.360] [illegible]	-0.180 [illegible]	-0.176 [-0.443, 0.245] [illegible]	0.139 [-0.262, 0.542] [illegible]	[illegible]	[illegible]	[illegible]	0.626 [NA, NA] [illegible]	0.091 [-0.392, 0.587] [illegible]	-0.067 [-0.622, 0.447] [illegible]
TNF-alpha - plasma	0.131 [illegible]	0.009 [-0.385, 0.361] [illegible]	0.007 [-0.348, 0.358] [illegible]	-0.076 [-0.437, 0.286] [illegible]	0.090 [-0.294, 0.462] [illegible]	[illegible]	[illegible]	[illegible]	0.114 [-0.325, 0.554] [illegible]	0.152 [-0.300, 0.582] [illegible]	0.064 [-0.369, 0.518] [illegible]
NGF - synovial fluid	-0.052 [-0.343, 0.269] [illegible]	0.111 [-0.235, 0.420] [illegible]	0.047 [-0.257, 0.359] [illegible]	-0.286 [illegible]	0.068 [-0.243, 0.364] [illegible]	-0.031 [-0.303, 0.328] [illegible]	-0.036 [-0.394, 0.311] [illegible]	-0.017 [-0.342, 0.335] [illegible]	0.119 [-0.361, 0.575] [illegible]	0.241 [illegible]	0.274 [illegible]
NGF - plasma	-0.012 [-0.322, 0.308] [illegible]	0.014 [-0.294, 0.346] [illegible]	0.109 [-0.231, 0.433] [illegible]	-0.139 [-0.463, 0.203] [illegible]	-0.064 [-0.456, 0.310] [illegible]	-0.067 [-0.410, 0.253] [illegible]	-0.094 [-0.457, 0.234] [illegible]	0.175 [-0.167, 0.496] [illegible]	0.341 [NA, NA] [illegible]	0.048 [-0.394, 0.490] [illegible]	0.316 [illegible]
Calprotectin - synovial fluid	-0.122 [-0.411, 0.203] [illegible]	0.158 [-0.168, 0.464] [illegible]	0.061 [-0.249, 0.354] [illegible]	-0.195 [-0.518, 0.148] [illegible]	0.006 [-0.350, 0.366] [illegible]	-0.099 [-0.467, 0.220] [illegible]	0.015 [-0.381, 0.391] [illegible]	0.259 [illegible]	-0.220 [-0.655, 0.227] [illegible]	[illegible]	0.000 [-0.478, 0.467] 1.000000
Calprotectin - plasma	-0.141 [-0.472, 0.216] [illegible]	-0.262 [illegible]	-0.216 [-0.534, 0.118] [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	-0.244 [-0.570, 0.080] [illegible]	-0.136 [-0.627, 0.333] [illegible]	-0.258 [illegible]	-0.211 [-0.590, 0.217] [illegible]
Leptin - synovial fluid	0.209 [-0.147, 0.559] [illegible]	0.125 [illegible]	0.285 [illegible]	[illegible]	[illegible]	-0.079 [-0.403, 0.277] [illegible]	0.063 [-0.329, 0.437] [illegible]	-0.055 [-0.401, 0.304] [illegible]	0.284 [-0.250, 0.769] [illegible]	-0.143 [-0.558, 0.276] [illegible]	0.295 [illegible]
Leptin - plasma	0.008 [-0.321, 0.318] [illegible]	-0.030 [-0.369, 0.320] [illegible]	0.031 [-0.268, 0.339] [illegible]	0.344 [illegible]	0.192 [-0.183, 0.520] [illegible]	0.079 [-0.274, 0.430] [illegible]	0.276 [illegible]	0.116 [-0.241, 0.462] [illegible]	0.203 [-0.273, 0.660] [illegible]	0.000 [-0.442, 0.444] 1.000000	-0.005 [-0.529, 0.522] [illegible]
Irisin - synovial fluid	0.048 [-0.310, 0.396] [illegible]	0.181 [-0.177, 0.523] [illegible]	0.178 [-0.156, 0.514] [illegible]	[illegible]	0.263 [illegible]	0.100 [-0.284, 0.517] [illegible]	[illegible]	0.201 [-0.113, 0.513] [illegible]	[illegible]	0.128 [-0.318, 0.571] [illegible]	[illegible]
Irisin - plasma	-0.069 [-0.420, 0.280] [illegible]	-0.099 [-0.427, 0.259] [illegible]	0.022 [-0.298, 0.352] [illegible]	0.119 [-0.242, 0.430] [illegible]	-0.031 [-0.452, 0.390] [illegible]	0.198 [-0.187, 0.532] [illegible]	[illegible]	0.183 [-0.146, 0.508] [illegible]	-0.083 [-0.584, 0.479] [illegible]	0.087 [-0.372, 0.562] [illegible]	-0.244 [illegible]
Adiponectin - synovial fluid	0.014 [-0.399, 0.398] [illegible]	0.150 [-0.222, 0.508] [illegible]	0.143 [illegible]	0.252 [-0.136, 0.598] [illegible]	0.244 [-0.113, 0.569] [illegible]	0.085 [-0.262, 0.400] [illegible]	0.056 [-0.288, 0.391] [illegible]	-0.057 [-0.400, 0.337] [illegible]	0.057 [-0.412, 0.510] [illegible]	0.081 [-0.351, 0.524] [illegible]	0.211 [-0.249, 0.623] [illegible]
Adiponectin - plasma	0.174 [-0.217, 0.512] [illegible]	0.138 [-0.221, 0.495] [illegible]	0.063 [-0.285, 0.397] [illegible]	0.100 [-0.295, 0.451] [illegible]	0.122 [-0.266, 0.480] [illegible]	[illegible]	[illegible]	[illegible]	0.250 [NA, NA] [illegible]	0.000 [-0.457, 0.452] 1.000000	[illegible]
Omentin - synovial fluid	0.131 [-0.240, 0.529] [illegible]	[illegible]	0.099 [-0.341, 0.494] [illegible]	[illegible]	0.232 [illegible]	0.004 [-0.324, 0.344] [illegible]	0.203 [-0.179, 0.526] [illegible]	-0.124 [-0.448, 0.220] [illegible]	0.099 [-0.444, 0.630] [illegible]	0.161 [-0.321, 0.612] [illegible]	0.071 [-0.400, 0.565] [illegible]
Omentin - plasma	0.097 [-0.272, 0.473] [illegible]	[illegible]	-0.054 [-0.378, 0.271] [illegible]	-0.138 [illegible]	0.175 [-0.233, 0.555] [illegible]	0.127 [illegible]	[illegible]	0.242 [illegible]	-0.006 [-0.602, 0.540] [illegible]	0.206 [-0.286, 0.643] [illegible]	-0.068 [-0.534, 0.467] [illegible]
Osteopontin - synovial fluid	[illegible]	[illegible]	0.219 [-0.097, 0.494] [illegible]	0.094 [-0.261, 0.412] [illegible]	-0.118 [-0.417, 0.239] [illegible]	-0.107 [-0.435, 0.244] [illegible]	-0.077 [-0.398, 0.316] [illegible]	-0.181 [-0.473, 0.160] [illegible]	-0.244 [-0.688, 0.244] [illegible]	0.005 [-0.489, 0.441] [illegible]	-0.205 [illegible]
Osteopontin - plasma	0.020 [-0.315, 0.376] [illegible]	0.051 [-0.264, 0.369] [illegible]	0.134 [-0.197, 0.439] [illegible]	-0.044 [-0.372, 0.319] [illegible]	-0.023 [-0.350, 0.306] [illegible]	-0.003 [-0.313, 0.343] [illegible]	0.225 [illegible]	0.052 [-0.285, 0.381] [illegible]	0.081 [-0.363, 0.554] [illegible]	0.124 [-0.395, 0.538] [illegible]	0.295 [NA, NA] [illegible]
Visfatin - synovial fluid	0.230 [illegible]	0.260 [illegible]	0.252 [illegible]	0.017 [-0.320, 0.340] [illegible]	0.061 [-0.335, 0.432] [illegible]	0.046 [-0.288, 0.402] [illegible]	0.034 [-0.375, 0.397] [illegible]	-0.059 [-0.427, 0.294] [illegible]	0.068 [-0.379, 0.524] [illegible]	0.151 [-0.294, 0.558] [illegible]	0.105 [-0.343, 0.522] [illegible]
Visfatin - plasma	0.267 [illegible]	0.102 [-0.222, 0.392] [illegible]	0.288 [illegible]	0.061 [-0.318, 0.421] [illegible]	-0.008 [-0.396, 0.363] [illegible]	-0.247 [-0.525, 0.118] [illegible]	-0.226 [-0.541, 0.099] [illegible]	-0.184 [-0.466, 0.139] [illegible]	-0.057 [-0.534, 0.432] [illegible]	[illegible]	-0.147 [-0.538, 0.304] [illegible]
Resistin - synovial fluid	0.195 [-0.161, 0.509] [illegible]	0.167 [-0.177, 0.496] [illegible]	0.247 [-0.114, 0.557] [illegible]	-0.047 [-0.352, 0.283] [illegible]	-0.247 [illegible]	0.089 [-0.262, 0.413] [illegible]	-0.068 [-0.388, 0.261] [illegible]	0.078 [-0.280, 0.443] [illegible]	[illegible]	0.237 [-0.186, 0.644] [illegible]	-0.211 [-0.643, 0.261] [illegible]
Resistin - plasma	-0.027 [-0.355, 0.328] [illegible]	-0.046 [-0.394, 0.299] [illegible]	-0.022 [-0.421, 0.353] [illegible]	-0.269 [illegible]	-0.064 [-0.456, 0.320] [illegible]	-0.242 [-0.534, 0.079] [illegible]	[illegible]	-0.236 [-0.535, 0.078] [illegible]	[illegible]	-0.248 [-0.676, 0.226] [illegible]	-0.188 [-0.619, 0.317] [illegible]

Supplementary Table S8. Association of synovial and plasma cytokines with clinical severity in Knee Osteoarthritis Inflammatory Phenotypes (KOIP), adjusted for age, time of disease's evolution and Body Mass Index (BMI). The table presents Spearman correlation coefficients, 95% confidence intervals (95% CI) and p-values, illustrating the association between Knee Osteoarthritis (KOA) severity measures (KOOS-pain, KOOS-functional disability, and ultrasound joint effusion) and selected cytokines analyzed in the study. The analyses are adjusted for age, disease duration, and BMI. Results are presented for the entire patient cohort as well as for individual KOIP groups. **KOA:** Knee Osteoarthritis; **KOIP:** Knee Osteoarthritis Inflammatory Phenotypes (KOIP); **KOOS:** Knee injury and Osteoarthritis Outcome Scores (reversed scores); **95%CI:** 95% confidence interval.

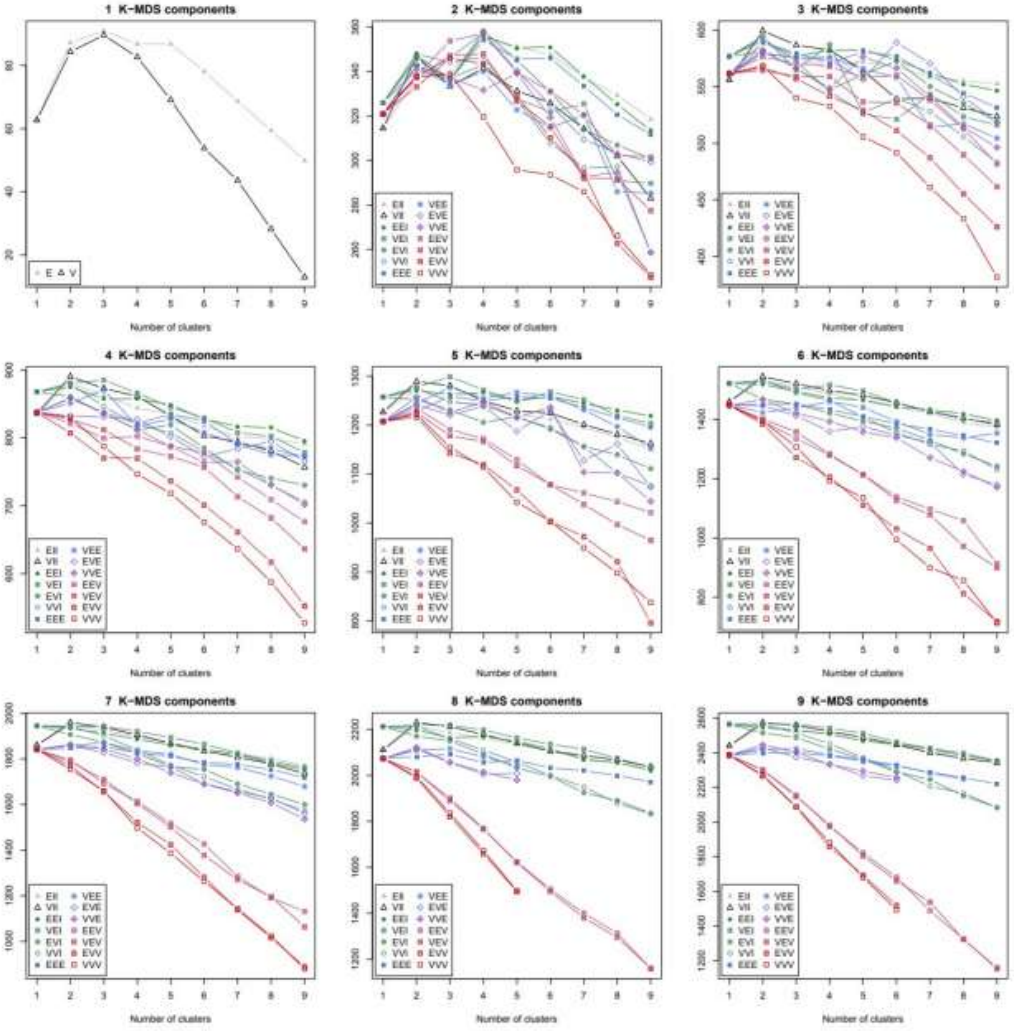
KOA Severity	Cytokine	All		KOIP-1		KOIP-2		KOIP-3		KOIP-4	
		Spearman correlation [95%CI]	P-value	Spearman correlation [95%CI]	P-value	Spearman correlation [95%CI]	P-value	Spearman correlation [95%CI]	P-value	Spearman correlation [95%CI]	P-value
KOOS - Pain (reversed, 0-100)	Omentin - synovial fluid (pg/mL)	0.099 [-0.057, 0.249]	0.21221	-0.241 [-0.487, 0.040]	0.092329	0.300 [-0.025, 0.567]	0.070156	0.146 [-0.209, 0.467]	0.42272	0.231 [-0.199, 0.586]	0.29104
	Osteopontin - synovial fluid (ng/mL)	0.206 [0.055, 0.348]	0.007798	0.118 [-0.176, 0.393]	0.43198	0.114 [-0.207, 0.413]	0.48995	0.098 [-0.349, 0.508]	0.67805	0.461 [0.153, 0.687]	0.0044899
	Interleukin 6 - plasma (pg/mL)	0.007 [-0.147, 0.160]	0.93076	0.047 [-0.240, 0.327]	0.75032	0.027 [-0.261, 0.310]	0.85728	0.130 [-0.366, 0.568]	0.61906	-0.328 [-0.634, 0.067]	0.10145
KOOS - Functional Disability (reversed, 0-100)	Omentin - synovial fluid (pg/mL)	0.060 [-0.095, 0.213]	0.44518	-0.202 [-0.446, 0.069]	0.14207	0.247 [-0.079, 0.524]	0.13584	0.194 [-0.207, 0.539]	0.34369	0.206 [-0.241, 0.581]	0.36769
	Interleukin 6 - plasma (pg/mL)	0.029 [-0.124, 0.182]	0.70824	0.164 [-0.122, 0.425]	0.26152	0.067 [-0.211, 0.335]	0.64094	-0.215 [-0.587, 0.233]	0.34812	-0.299 [-0.565, 0.023]	0.068168
Ultrasound Joint Effusion (mm)	C-reactive protein - synovial fluid (mg/L)	0.007 [-0.146, 0.160]	0.926	0.059 [-0.238, 0.346]	0.70114	0.041 [-0.251, 0.326]	0.78781	0.324 [-0.062, 0.626]	0.097677	-0.570 [-0.763, -0.284]	0.00035628
	C-reactive protein - plasma (mg/L)	-0.029 [-0.181, 0.124]	0.70813	-0.146 [-0.403, 0.133]	0.30445	-0.041 [-0.329, 0.254]	0.78949	0.412 [0.089, 0.657]	0.013912	-0.538 [-0.748, -0.231]	0.0012901
	Leptin - synovial fluid (pg/mL)	0.058 [-0.095, 0.209]	0.45711	-0.123 [-0.410, 0.187]	0.43981	-0.184 [-0.440, 0.099]	0.2007	0.030 [-0.410, 0.458]	0.89965	0.429 [0.091, 0.678]	0.014337
	Leptin - plasma (pg/mL)	-0.062 [-0.213, 0.093]	0.4338	-0.166 [-0.418, 0.110]	0.23865	-0.194 [-0.458, 0.101]	0.19584	-0.034 [-0.420, 0.363]	0.87195	0.253 [-0.099, 0.548]	0.15632
	Irisin - synovial fluid (ng/mL)	0.019 [-0.136, 0.173]	0.81176	-0.183 [-0.434, 0.095]	0.19624	-0.107 [-0.420, 0.228]	0.53419	0.259 [-0.151, 0.593]	0.2124	0.326 [-0.021, 0.603]	0.064766
	Interleukin 6 - synovial fluid (pg/mL)	0.236 [0.086, 0.376]	0.0023444	0.103 [-0.169, 0.361]	0.45915	0.157 [-0.144, 0.430]	0.30618	0.634 [0.390, 0.795]	0.000012813	0.355 [0.035, 0.608]	0.030222
	Interleukin 6 - plasma (pg/mL)	0.055 [-0.099, 0.206]	0.48514	0.118 [-0.169, 0.387]	0.42041	0.142 [-0.149, 0.409]	0.33893	0.051 [-0.351, 0.437]	0.80907	-0.321 [-0.598, 0.025]	0.068546
	Calprotectin - plasma (ng/mL)	-0.003 [-0.156, 0.150]	0.96899	-0.044 [-0.306, 0.224]	0.75206	0.141 [-0.132, 0.394]	0.3115	-0.052 [-0.438, 0.349]	0.8052	-0.308 [-0.554, -0.013]	0.040914
	Omentin - synovial fluid (pg/mL)	0.162 [0.008, 0.308]	0.040004	0.098 [-0.190, 0.370]	0.50697	0.129 [-0.184, 0.418]	0.42145	0.123 [-0.270, 0.480]	0.54479	0.362 [0.021, 0.628]	0.038155

Supplementary Table S9. Association of synovial and plasma cytokines with radiographic progression in Knee Osteoarthritis Inflammatory Phenotypes (KOIP), adjusted for age, time of disease's evolution and Body Mass Index (BMI). The table presents fold-changes, 95% confidence intervals (95% CI) and p-values, illustrating the association between Knee Osteoarthritis (KOA) progression according to different radiographic criteria (Kellgren-Lawrence, Osteophytes and Joint Space Narrowing) and selected cytokines analyzed in the study. Positive fold-changes indicate a higher level of the cytokine in progressors, while negative fold-changes represents a higher level of the cytokine in non-progressors. The analyses are adjusted for age, disease duration, and BMI. Results are presented for the entire patient cohort as well as for individual KOIP groups. **KOA:** Knee Osteoarthritis; **KOIP:** Knee Osteoarthritis Inflammatory Phenotypes (KOIP); **95%CI:** 95% confidence interval.

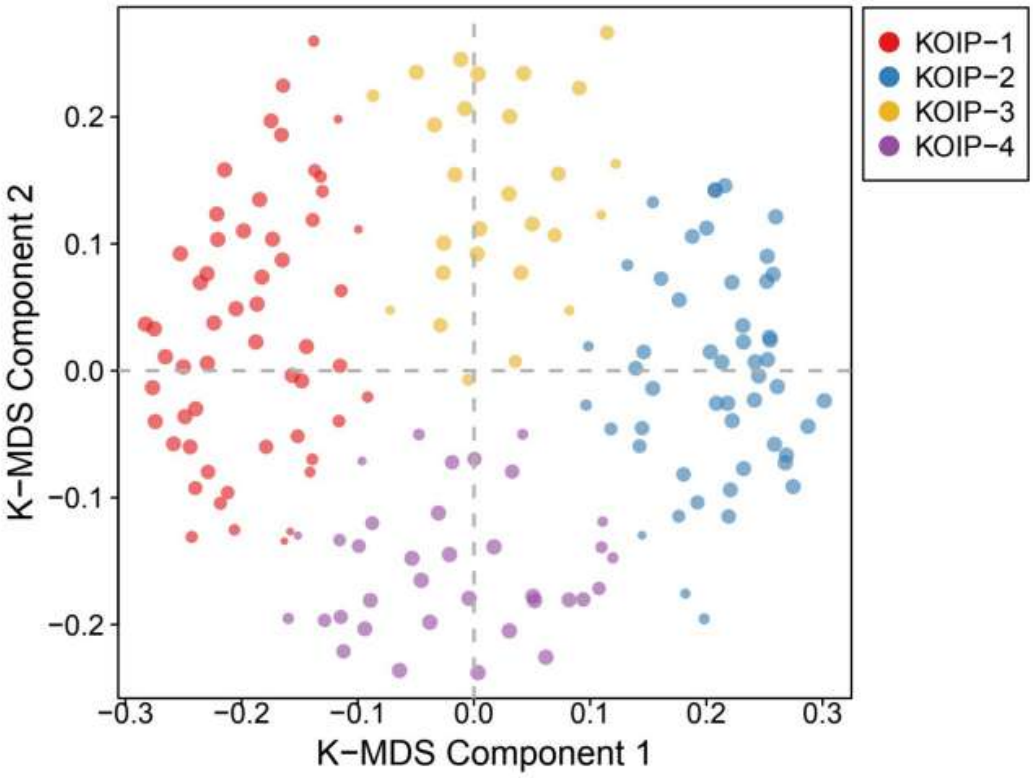
KOA Radiographic Progression	Cytokines	All		KOIP-1		KOIP-2		KOIP-3		KOIP-4	
		Fold-Change [95%CI]	P-value	Fold-Change [95%CI]	P-value	Fold-Change [95%CI]	P-value	Fold-Change [95%CI]	P-value	Fold-Change [95%CI]	P-value
Kellgren-Lawrence Radiographic Progression	Resistin - synovial fluid (pg/mL)	-1.24 [-1.74, 1.18]	0.24462	-2.08 [-3.65, -1.20]	0.012175	1.51 [-1.41, 2.89]	0.27554	1.31 [-2.27, 3.79]	0.50063	-1.24 [-2.91, 1.88]	0.63494
	C-reactive protein - synovial fluid (mg/L)	1.12 [-1.16, 1.49]	0.39035	1.51 [1.05, 2.19]	0.037626	1.06 [-1.70, 1.81]	0.85564	-1.57 [-2.92, 1.13]	0.11665	1.11 [-1.82, 2.86]	0.75714
	Omentin - plasma (pg/mL)	-1.26 [-1.64, 1.04]	0.076489	-1.40 [-2.20, 1.07]	0.12058	1.06 [-1.45, 1.56]	0.81771	-2.70 [-7.00, -1.31]	0.009637	1.05 [-1.66, 1.70]	0.86185
	Irisin - synovial fluid (ng/mL)	-1.04 [-1.18, 1.10]	0.60394	-1.25 [-1.47, -1.06]	0.008212	-1.07 [-1.49, 1.30]	0.68488	-1.16 [-1.74, 1.31]	0.47166	1.39 [1.14, 1.66]	0.0012974
Osteophytes Radiographic Progression	Osteopontin - synovial fluid (ng/mL)	-1.21 [-1.63, 1.10]	0.19176	1.37 [-1.36, 2.56]	0.33122	-1.45 [-2.30, 1.09]	0.12429	-1.10 [-2.64, 2.15]	0.78894	-1.10 [-1.78, 1.41]	0.64559
	Osteopontin - plasma (ng/mL)	-1.14 [-1.37, 1.04]	0.14962	-1.05 [-1.58, 1.40]	0.78026	-1.42 [-1.98, -1.04]	0.031166	-1.38 [-2.66, 1.42]	0.33039	1.11 [-1.27, 1.60]	0.55478
	Omentin - synovial fluid (pg/mL)	1.02 [-1.32, 1.42]	0.88754	1.85 [1.08, 3.37]	0.028051	-1.72 [-2.92, -1.08]	0.03584	1.05 [-3.20, 3.59]	0.90158	1.41 [-1.39, 2.79]	0.28672
	Omentin - plasma (pg/mL)	1.02 [-1.25, 1.31]	0.86418	1.86 [1.10, 3.64]	0.039962	-1.15 [-1.64, 1.26]	0.46302	-1.76 [-5.02, 1.61]	0.24345	1.36 [-1.17, 2.15]	0.19517
	Leptin - synovial fluid (pg/mL)	1.05 [-1.19, 1.30]	0.68843	1.94 [1.03, 2.94]	0.034066	-1.13 [-1.53, 1.19]	0.38546	-1.08 [-2.10, 1.75]	0.80688	-1.41 [-2.35, 1.16]	0.19797
	Leptin - plasma (pg/mL)	1.05 [-1.14, 1.26]	0.61333	1.43 [1.02, 2.01]	0.044663	-1.05 [-1.50, 1.39]	0.84667	-1.68 [-3.47, 1.37]	0.1892	1.02 [-1.37, 1.43]	0.92611
	Irisin - synovial fluid (ng/mL)	-1.08 [-1.22, 1.65]	0.27223	1.09 [-1.13, 1.34]	0.40431	-1.19 [-1.61, 1.12]	0.23591	-1.68 [-2.64, -1.09]	0.031111	1.19 [-1.09, 1.43]	0.066103
	Irisin - plasma (ng/mL)	-1.03 [-1.17, 1.11]	0.66774	1.17 [-1.02, 1.41]	0.087542	-1.15 [-1.59, 1.25]	0.41349	-1.34 [-2.34, 1.24]	0.22328	1.02 [-1.23, 1.28]	0.84938
	Interleukin 8 - plasma (pg/mL)	1.21 [-1.09, 1.55]	0.1495	1.25 [-1.40, 2.05]	0.35869	1.53 [1.06, 2.23]	0.026868	1.49 [-2.98, 6.09]	0.57305	-1.55 [-2.44, 1.01]	0.046448
	Calprotectin - synovial fluid (ng/mL)	1.33 [-1.06, 1.91]	0.12387	1.30 [-2.11, 1.46]	0.41521	1.45 [-1.50, 3.34]	0.3442	2.30 [-1.03, 5.71]	0.066658	2.09 [1.05, 4.29]	0.041223
	Adiponectin - synovial fluid (ng/mL)	1.14 [-1.16, 1.48]	0.36748	1.66 [1.07, 2.51]	0.032595	-1.47 [-2.35, 1.06]	0.099079	-1.05 [-2.86, 2.52]	0.90321	1.16 [-1.47, 2.06]	0.54318
Joint Space Narrowing Radiographic Progression	Osteopontin - synovial fluid (ng/mL)	1.26 [-1.04, 1.68]	0.108	1.31 [-1.28, 2.14]	0.25957	1.02 [-1.62, 1.79]	0.87745	2.59 [1.68, 3.95]	0.00034539	-1.52 [-2.04, 1.17]	0.26025
	JoOmentin - synovial fluid (pg/mL)	1.24 [-1.08, 1.65]	0.16396	1.67 [1.12, 2.50]	0.010901	1.22 [-1.57, 2.07]	0.50537	-1.04 [-2.67, 2.33]	0.84272	1.05 [-1.90, 1.98]	0.89549
	Leptin - synovial fluid (pg/mL)	-1.04 [-1.30, 1.22]	0.74315	-1.06 [-1.56, 1.34]	0.73046	-1.67 [-2.30, -1.20]	0.0013274	1.15 [-1.43, 1.84]	0.59876	1.16 [-1.59, 1.97]	0.54063
	Leptin - plasma (pg/mL)	-1.14 [-1.42, 1.05]	0.15722	-1.07 [-1.44, 1.22]	0.55993	-1.60 [-2.32, -1.09]	0.016944	-1.26 [-2.31, 1.41]	0.46167	-1.07 [-1.54, 1.32]	0.66752
	Irisin - synovial fluid (ng/mL)	1.01 [-1.11, 1.14]	0.84859	-1.12 [-1.30, 1.06]	0.15696	-1.14 [-1.55, 1.21]	0.40734	1.20 [-1.73, 1.76]	0.34398	1.26 [1.05, 1.49]	0.012932
	Irisin - plasma (ng/mL)	-1.16 [-1.33, -1.02]	0.021346	-1.10 [-1.24, 1.03]	0.14487	-1.54 [-2.67, -1.01]	0.045653	-1.22 [-1.92, 1.29]	0.28199	-1.15 [-1.51, 1.11]	0.26456



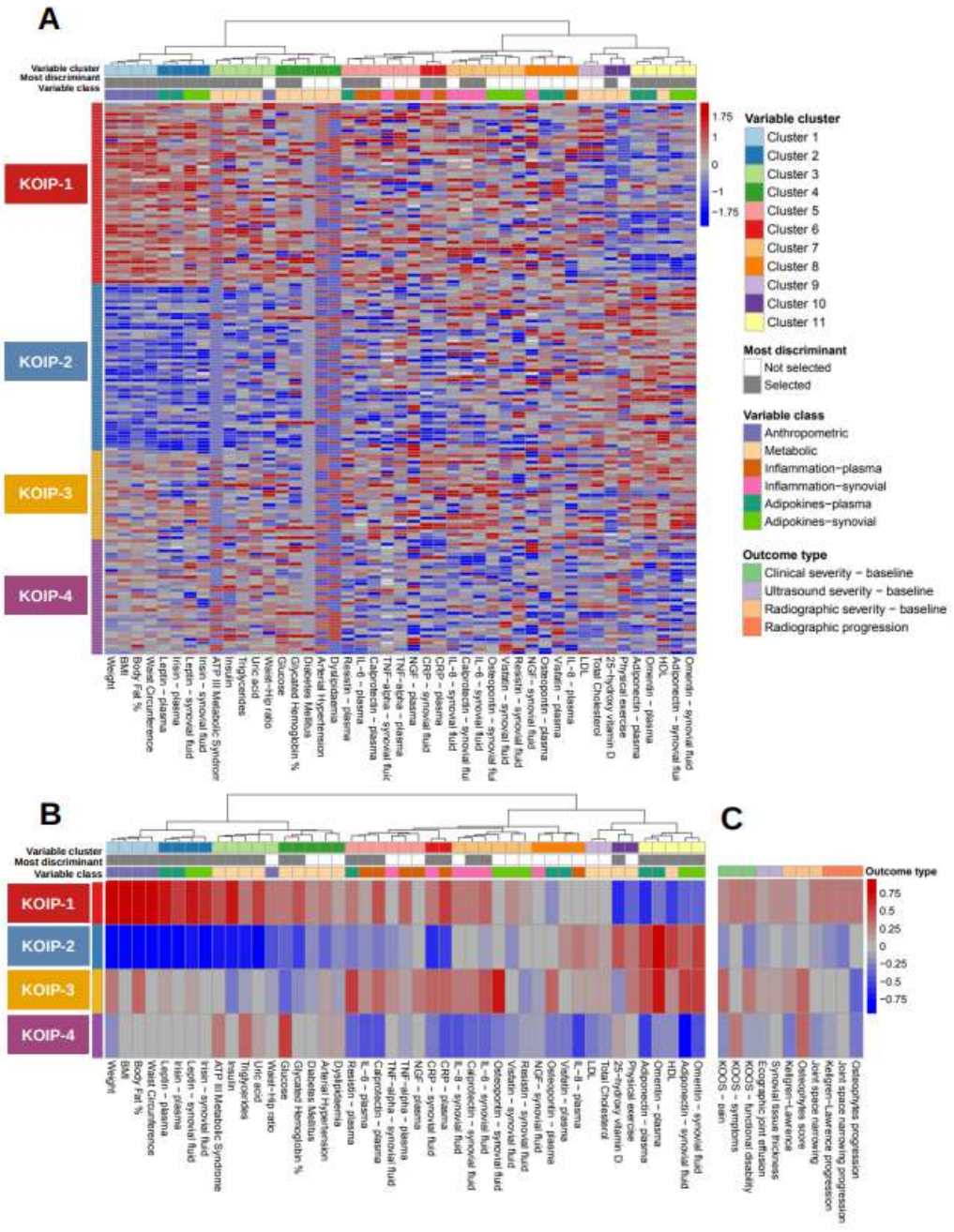
Supplementary Figure S1. Pairwise associations between variables used in the phenotype discovery analysis. The heatmap represents non-parametric correlation-like measurements between anthropometric, metabolic and inflammatory factors from 168 female Knee Osteoarthritis (KOA) patients with joint effusion. Red (blue) color represents positive (negative) association, and the color intensities express the magnitude of the correlations in absolute value. Correlation-like measurements are: Spearman correlation (continuous vs continuous or ordinal variables), Phi coefficient (binary vs binary variables) and Glass rank biserial correlation (continuous / ordinal vs binary variables). Dendrograms in the heatmap represent a hierarchical clustering of variables using these correlations measurements as dissimilarities (ie, 1 - correlation coefficient). Color intensities are saturated to 0.5 and -0.5 values. Physical exercise (four categories) was treated as ordinal in these representations. **BMI:** Body Mass Index; **ATP III:** Adult Treatment Panel III; **IL-6:** Interleukin 6; **IL-8:** Interleukin 8; **TNF-alpha:** Tumor Necrosis Factor alpha; **NGF:** Nerve Growth Factor; **CRP:** C-Reactive Protein; **LDL:** Low-density Lipoprotein; **HDL:** High-Density Lipoprotein.



Supplementary Figure S2. Bayes Informacion Criteria (BIC) for cluster selection in the phenotype discovery analysis. Results are derived from Gaussian finite mixture models fitted by model-based clustering (Mclust), which was applied to the anthropometric, metabolic and inflammatory data from 168 female Knee Osteoarthritis (KOA) patients with persistent joint effusion. Panels show the BIC values for different choices of models (symbol and colors, see legends), the number of clusters (x-axis) and the number of components (each panel, from one to nine) derived from a Kruskal's Non-metric Multidimensional Scaling (K-MDS) analysis. In each case, K-MDS was fitted to 1 minus the proximity measures provided by an unsupervised Random Forest analysis conducted on the patients data. Results from analyses with two K-MDS components showed the most evident data structure involving a high number of clusters (four), and were chosen for downstream analyses. Same analyses were conducted for a selection of 10 to 20 K-MDS components, which produced similar results to the 9 components configuration (data not showed). **E:** equal variance (one-dimensional); **V:** variable/unqual variance (one-dimensional); **EII:** spherical, equal volume; **VII:** spherical, unequal volume; **EEI:** diagonal, equal volume and shape; **VEI:** diagonal, varying volume, equal shape; **EVI:** diagonal, equal volume, varying shape; **VVI:** diagonal, varying volume and shape; **EEE:** ellipsoidal, equal volume, shape, and orientation; **VEE:** ellipsoidal, equal shape and orientation; **EVE:** ellipsoidal, equal volume and orientation; **VVE:** ellipsoidal, equal orientation; **EEV:** ellipsoidal, equal volume and equal shape; **VEV:** ellipsoidal, equal shape; **EVV:** ellipsoidal, equal volume; **VVV:** ellipsoidal, varying volume, shape, and orientation (See Mclust R help for details: https://cran.r-project.org/web/packages/mclust/index.html).

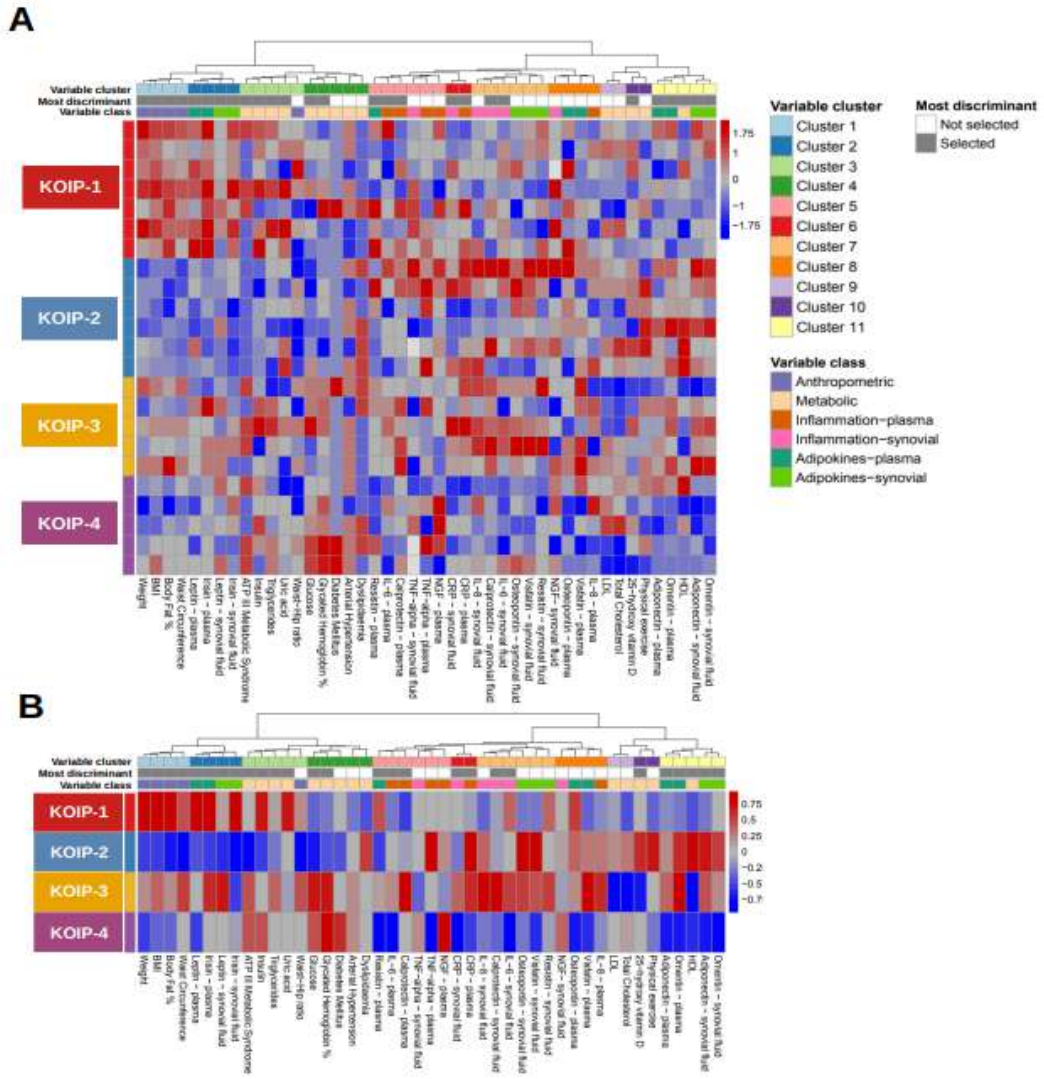


Supplementary Figure S3. Patient clusters derived from the phenotyping analysis. The plot show the patients' scores in the two first components derived from a Kruskal's Non-metric Multidimensional Scaling (K-MDS) for phenotype discovery. The size of the points is proportional to the estimated probability of belonging to the assigned cluster (certainty), so that smaller points are assigned to their clusters with smaller probability (i.e., larger uncertainty). K-MDS was fitted to 1 minus the proximity measures provided by an unsupervised Random Forest analysis applied to the anthropometric, metabolic and inflammatory data from 168 female Knee Osteoarthritis (KOA) patients with persistent joint effusion. Number of components and clusters were chosen according to the Bayes Information Criteria (BIC), whose values were derived from Gaussian finite mixture models fitted by model-based clustering (Mclust). **K-MDS:** Kruskal's Non-metric Multidimensional Scaling; **KOIP:** Knee Osteoarthritis Inflammatory Phenotype.



Supplementary Figure S4. Complete results of the phenotype discovery analyses performed on data from 168 female patients of primary Knee Osteoarthritis (KOA) with persistent joint effusion. **A.** Clustering results at the patient level. Heatmap cells represent standardized (centered and scaled) values of anthropometric, metabolic and systemic and local inflammatory factors, where red indicate high, blue represents low, and color intensity expresses more extreme values. Values of binary variables were previously converted to numeric format, where 1 indicated presence and 0 represented absence of the corresponding feature. Physical exercise (four categories) was also converted to numeric format and treated as ordinal. **B.** Clustering results at the phenotype level. Cells represent phenotype median values (continuous variables) or averages (numerically coded categorical variables) of the values displayed in A. Color intensities were

saturated aproximately to percentiles 5% and 95% of the overall values distribution. Patients clusters were derived from a Machine Learning (ML) -based strategy that used unsupervised Random Forest (RF), Kruskal's Non-metric Multidimensional Scaling (KMDS) and Gaussian finite mixture models for model-based clustering (Mclust), which selected the optimal number of clusters with objective statistical criteria (Bayes Information Criterion, BIC). Variables were grouped by a hierarchical clustering using Ward agglomerative method and non-parametric correlation-like measurements, namely: Spearman correlation (continuous vs continuous or ordinal variables), Phi coefficient (binary vs binary variables) and Glass rank biserial correlation (continuous / ordinal vs binary variables). Variables with the highest discriminative power of patients' clusters were identified using a sequential selection procedure based on Random Forests (VSURF - interpretation mode) and highlighted in the corresponding annotation bar (*Most discriminant*). **C.** Phenotype-averages of standardized (centered and scaled) values of baseline clinical, ecographic and radiographic severity and radiographic progression. Heatmap colors represent median or average standardized values in an analogous way to B. **BMI:** Body Mass Index; **ATP III:** Adult Treatment Panel III; **IL-6:** Interleukin 6; I**L-8:** Interleukin 8; **TNF-alpha:** Tumor Necrosis Factor alpha; **NGF:** Nerve Growth Factor; **CRP:** C-Reactive Protein; **LDL:** Low-density Lipoprotein; **HDL:** High-Density Lipoprotein; **KOA**: Knee Osteoarthritis; **KOOS:** Knee injury and Osteoarthritis Outcome Scores; **KOIP:** Knee Osteoarthritis Inflammatory Phenotype.



Suplementary Figure S5. Results of assignment of Kneee Osteoarthritis Inflammatory Phenotypes (**KOIP**) to 23 male patients from our KOA cohort with available data. **A.** Clustering results at the patient level. Heatmap cells represent standardized (centered and scaled) values of anthropometric, metabolic and systemic and local inflammatory factors, where red indicate high, blue represents low, and color intensity expresses more extreme values. Values of binary variables were previously converted to numeric format, where 1 indicated presence and 0 represented absence of the corresponding feature. Physical exercise (four categories) was also converted to numeric format and treated as ordinal. Continuous variables were standardized (centered and scaled) using their corresponding median and median absolute deviation, while the mean and standard deviation were used for numerically coded categorical variables. Color intensities were saturated aproximately to percentiles 5% and 95% of the overall values distribution, which corresponded to -1.75 and 1.75. **B.** Clustering results at the phenotype level. Cells represent phenotype median values (continuous variables) or averages (numerically coded categorical variables) of the values displayed in A. Color intensities were saturated aproximately to percentiles 5% and 95%, which corresponded to -0.75 and 0.75, respectively. KOIP assignment was made by a Random Forest model trained in the female KOA patients series (where the phenotypes were defined) according to male patient's

6. RESUMEN GLOBAL DE LOS RESULTADOS

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Los resultados muestran una asociación significativa entre la interleucina-8 (IL-8) a nivel de líquido sinovial tanto con la gravedad clínica como con diversas citocinas inflamatorias, sugiriendo la implicación de la inflamación local en la severidad de la artrosis de rodilla en mujeres con derrame articular. Posteriormente se identifican cuatro fenotipos inflamatorios KOIPs (*Knee Osteoarhritis Inflammatory Phenotype*), que se distinguen drásticamente por sus características tanto inflamatorias como metabólicas. De forma relevante estos grupos descritos dentro del propio fenotipo inflamatorio presentan implicaciones tanto en gravedad clínica como en severidad radiográfica.

El KOIP-1 se define por factores metabólicos e inflamatorios asociados a la obesidad como serían la leptina y la irisina. Presenta los mayores niveles tanto de dolor e incapacidad funcional como de progresión radiográfica. En el KOIP-2 predominan las adipocitocinas consideradas “sanas” o con poder antiinflamatorio, como la adiponectina y la omentina. Sería la imagen especular del KOIP-1. A nivel clínico y radiográfico, destaca por la mayor formación de osteofitos; aunque con una progresión radiográfica global menor que los otros grupos, así como menos gravedad clínica. El KOIP-3 destaca por la presencia de citocinas inflamatorias como la IL-8 e IL-6,con niveles próximos al KOIP-1 tanto en dolor como en grados de discapacidad y progresión radiográfica. Finalmente, en el KOIP-4 abundan los factores de riesgo cardiovasculares clásicos y factores metabólicos, con menor afectación tanto clínica como estructural.

Sin embargo, los cuatro fenotipos tienen grados de dolor e incapacidad funcional elevados, así como de progresión radiográfica, si se compararan con grupos de pacientes no inflamatorios. A pesar de ello, existen diferencias claras dentro de cada KOIP.

7. RESUMEN GLOBAL DE LA DISCUSIÓN

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El primer trabajo está centrado en estudiar la asociación entre los niveles plasmáticos y en líquido sinovial de la IL-8 y la severidad clínica de la artrosis de rodilla en una cohorte de mujeres con derrame articular.

Los resultados demuestran una asociación débil pero significativa entre los niveles de IL-8 en líquido sinovial y la severidad clínica, así como con otros mediadores de inflamación, ya sean citocinas o adipocitocinas. Sin embargo, los niveles plasmáticos no se relacionan ni con la severidad clínica ni con dichos mediadores de la inflamación. La originalidad de los resultados observados en este estudio radica en la asociación observada en líquido sinovial pero no en el plasma de la IL-8, tanto con marcadores de la inflamación como con la gravedad clínica. Esto orienta a remarcar la importancia de la inflamación local más que de la posible inflamación sistémica de bajo voltaje en la evaluación de la artrosis de rodilla con componente inflamatorio; e indica la importancia del estudio del líquido sinovial más que del plasma en este grupo de pacientes. Aunque el líquido sinovial es un ultrafiltrado del plasma, es posible que no exista una correlación precisa de las mismas moléculas alteradas en los dos fluidos por lo que el estudio de ambos de forma consonante puede aportar información adicional y complementaria que contribuya al avance en el conocimiento de los mecanismos de severidad en la artrosis de rodilla.

La asociación entre los mediadores de inflamación y la artrosis ha sido objeto de estudio en los últimos años y está ampliamente demostrada, si bien el fallo de las distintas terapias antiinflamatorias en el control de la enfermedad subraya el desconocimiento

parcial de los mecanismos de inflamación y su relación con la degradación del cartílago, posiblemente en muchas ocasiones por el análisis del plasma y mediadores de inflamación a nivel sistémico más que a nivel local.

Clásicamente en la OA se ha dado poca importancia a la membrana sinovial, enfatizando el papel del cartílago y el hueso subcondral. Sin embargo, sabemos que en fases avanzadas de la artrosis la sinovial desarrolla una respuesta inflamatoria que contribuye de manera decisiva en la patogenia y en la expresividad clínica de la enfermedad.

En este sentido, los cambios histopatológicos que se producen en la membrana sinovial de un paciente con OA son similares a los cambios observados en un paciente con artritis reumatoide, con un infiltrado inflamatorio de macrófagos y células T, de ahí la relevancia de identificar factores locales que puedan ser de interés en la evaluación de la artrosis.

Además del componente inflamatorio sistémico de bajo grado de la OA, que ha quedado de manifiesto con la predisposición de diabéticos y obesos a padecer la enfermedad, el eje membrana sinovial-líquido articular tiene un rol fundamental en la patogénesis de la destrucción articular, enfatizando esta posible doble asociación de la inflamación sistémica de bajo voltaje y la local.

La membrana sinovial está formada por sinoviocitos y macrófagos que provocan la liberación de citocinas clave como la IL-1ß, TNF-α, IL-15, IL-6 y la IL-17, que regulan las metaloproteasas encargadas de la degradación del cartílago, pero existe una gran variabilidad en la respuesta inflamatoria de los pacientes con OA de rodilla.

En un abordaje novedoso, que utiliza un análisis multiparamétrico para tratar de identificar fenotipos de respuesta sinovial en la OA de rodilla mediante la evaluación de

la celularidad tisular por citometría de flujo, se vio que la liberación de IL-6 e IL-8 se correlacionaba con dicha celularidad [181], y que ambas moléculas inflamatorias se correlacionaban fuertemente entre sí, lo que reforzaría su rol determinante, aunque no exclusivo, en la patogénesis de la OA.

El líquido sinovial está en contacto directo con el cartílago articular dañado. Su composición en la OA de rodilla está alterada, y en él se pueden identificar moléculas de degradación de la matriz del cartílago antes incluso que en otros fluidos corporales como la sangre o la orina. La concentración de una gran parte de las moléculas de degradación del colágeno tipo II, que es el componente mayoritario de la matriz cartilaginosa, es mayor en el líquido sinovial que en el suero o en la orina, lo que le confiere una mayor sensibilidad y especificidad para detectar estas moléculas. Con respecto a esto último, reflejaría mejor el daño tisular local que la determinación de estos parámetros en otros líquidos biológicos, ya que su composición no se ve tan influenciada por otras condiciones patológicas sistémicas. Esto lo convierte en el medio ideal para estudiar biomarcadores específicos de la artrosis de rodilla, más aún en el caso de la artrosis con derrame articular; y nos permitirá asociarlo con factores sistémicos plasmáticos para intentar extrapolar los resultados.

Numerosos estudios objetivan la elevación de las moléculas de inflamación en el líquido sinovial. Sin embargo, su relación con la severidad clínica es controvertida, pues se obtienen resultados contradictorios. Radojcic et al. encontraron una asociación positiva entre los niveles de IL-6 en el líquido sinovial y el dolor medido por WOMAC (B=0.022, IC95% 0.004 a 0.040) [182], mientras que Brenner et al. [183] reportaron que no existe dicha asociación, al igual que Orita et al [184]. En un trabajo reciente, en el que se busca

la asociación entre diversos mediadores de la inflamación y dolor, se demostró una asociación entre el TNF-α, la IL-1ß y la IL-6 y el dolor en estadios precoces de la enfermedad.

En cuanto a la IL-8, hasta la publicación de nuestro trabajo la evidencia de su asociación con la severidad clínica era escasa. En un estudio que analiza biopsias sinoviales y líquido articular de pacientes con OA de rodilla K/L ≥2 que se someten a una meniscectomía por artroscopia, se evidenció que la expresión de la IL-8 estaba significativamente elevada en los casos más avanzados, pero esta elevación no se correlacionaba con el dolor prequirúrgico valorado por KOOS [185].

También se ha demostrado la asociación entre la IL-8 y la IL-6 en LS obtenido por aspiración directa en una muestra de pacientes con OA, y el dolor articular en movimiento medido por una escala de Likert de 11 puntos, mientras que dicha asociación no se observaba con el dolor en reposo ni con la escala WOMAC [186]. Esto indicaría que los mecanismos responsables de la génesis del dolor en la OA podrían variar en función de las características del dolor.

En cuanto a la severidad radiológica, en nuestro estudio no hemos observado una asociación entre la IL-8 y el estadiaje K/L. No obstante, en un trabajo previo en el que se evalúa la relación entre diferentes citocinas inflamatorias y la afectación estructural de sujetos con OA postraumática, se encontró asociación estadísticamente significativa entre los niveles de IL-8 en LS y la severidad radiológica [106].

Estudios previos demuestran una elevación local de la IL-8. Meehan et al. [187] estudiaron los componentes del líquido sinovial de 51 sujetos con artritis reumatoide,

artrosis de rodilla y controles sanos, y comprobaron que los niveles de IL-8 estaban aumentados en los que padecían alguna enfermedad articular, y que existían diferencias significativas con respecto a su concentración plasmática, lo que apoyaría la teoría de que su determinación en sangre periférica sería poco útil como biomarcador de OA, debido a su inespecificidad en relación a padecer una alteración articular. Resultados similares obtuvieron en un estudio más antiguo Takahashi et al [123].

También se ha demostrado la correlación entre los niveles en líquido articular de IL-8 y otros mediadores de la inflamación como el interferón, IL-6, y metaloproteasas; y alteraciones de la grasa de Hoffa evaluadas por RMN en pacientes con rotura aguda del LCA [188].

Otros trabajos han evaluado la relación de la IL-8 con el género y la edad, siendo la asociación no significativa [189]. No obstante, tanto la IL-8 como la IL-6 forman parte de lo que se conoce como el fenotipo secretor asociado a la senescencia (SASP), que es el resultado del secretoma inflamatorio y proteolítico de muchos tipos de células senescentes, y que provocan aceleración del acortamiento de los telómeros y daño oxidativo del DNA, que se vinculan a trastornos asociados a la edad [190]. Este hecho plantea un campo de investigación de interés en la actualidad, dirigidos a la identificación de factores inflamatorios asociados a la senescencia.

En cuanto a la utilización de la IL-8 como biomarcador, en una revisión sistemática de la literatura publicada recientemente [191], en la que se repasa la utilidad de distintos mediadores de inflamación y moléculas de degradación del cartílago como biomarcadores de la OA (de carga de la enfermedad, de investigación, pronósticos, de

eficacia de intervención y diagnósticos), se concluye que la IL-8 es uno de los más estudiados y más prometedores en el campo de la OA, y tiene un valor tanto diagnóstico, confirmándose niveles más elevados en el LS que en los controles sanos; como de carga de la enfermedad, asociándose a mayor severidad clínica y radiológica; y a nivel de [189] investigación, ya que sus niveles se correlacionan con otros marcadores de inflamación y degradación. Sin embargo, a pesar de la gran cantidad de estudios publicados al respecto, se generan resultados contradictorios, lo que se podría explicar como ya hemos mencionado, la complejidad y heterogeneidad de la OA.

A modo de conclusión, nuestro estudio demuestra una asociación entre los niveles locales de IL-8, medidos en LS, y la severidad clínica de la OA, lo que sugeriría su potencial papel como biomarcador pronóstico y de gravedad. En otro estudio reciente, que investiga los potenciales cambios inflamatorios que ocurren entre la circulación sistémica y local en pacientes con OA, tratando de identificar posibles vías regulatorias, se objetivó que los niveles plasmáticos elevados de IL-8 se asocian a niveles elevados de IL-18 en LS, y podrían estar implicados en la patogénesis de la OA mediante la activación del MMP-3 [192], por lo que ambas interleucinas podrían utilizarse como biomarcadores diagnósticos, en este caso la IL-8 a nivel sistémico.

Dada la multidimensionalidad de la OA, el análisis de una única molécula como biomarcador sin conocer con exactitud sus múltiples interacciones conduciría a conclusiones erróneas. Cada biomarcador jugaría un pequeño papel en el proceso artrósico, por lo que el uso de una combinación de estos permitiría obtener conclusiones más certeras. De ahí la importancia de analizar en global un conjunto de marcadores de

inflamación con medidas antropométricas y factores metabólicos en relación con las medidas de gravedad en artrosis de rodilla; por lo que realizamos el segundo estudio.

En el segundo trabajo, intentamos identificar diferentes fenotipos de OA, dentro de un fenotipo ya definido como es el inflamatorio, basados en características clínicas, antropométricas, inflamatorias y metabólicas.

Como ya se ha comentado en la introducción, la interpretación de la OA como una enfermedad multidimensional implica la aceptación de la existencia de múltiples fenotipos clínicos que reflejan los diferentes mecanismos de acción subyacentes [103]. La existencia de un fenotipo inflamatorio está ampliamente representada en la literatura, e incluiría pacientes con sinovitis asociada a sobreexpresión de diferentes mediadores de la inflamación. Esta inflamación ocurre tanto en fases precoces como en fases avanzadas de la enfermedad [104], aunque las características del infiltrado celular pueden variar a lo largo del proceso, de manera que hay estudios que asocian la proporción de leucocitos y la cantidad de fibrina depositada con la severidad de la OA [193].

En cuanto a la sinovitis, en un estudio longitudinal que valora anomalías macroscópicas de la sinovial valoradas por artroscopia, se demuestra la asociación entre el aspecto inflamatorio (hipervascularización y proliferación), con la progresión estructural [194].

Otro metaanálisis reciente objetiva una correlación positiva entre distintos mediadores de inflamación y anomalías de la membrana sinovial valoradas por RMN [195].

Así pues, queda claro que este fenotipo inflamatorio se asocia con mayor severidad clínica evaluada por dolor y discapacidad funcional, y probablemente también con mayor progresión estructural.

En nuestro estudio, todos los pacientes pertenecen a este fenotipo inflamatorio, por presencia de derrame articular. A pesar de la homogeneidad de la muestra, tras un análisis por clusterización fue posible identificar hasta 4 subgrupos bien diferenciados en cuanto a características antropométricas, metabólicas, inflamatorias con implicaciones clínicas y de progresión estructural.

La clusterización es una técnica que permite, mediante un algoritmo de aprendizaje no supervisado (esto es, sin medida de desenlace predeterminada), asignar observaciones a un grupo en función de la detección de similitudes [171], lo que permite integrar gran cantidad de datos. En el caso de la OA, es útil para identificar fenotipos y trayectorias de progresión de la enfermedad, lo que permitiría, en un futuro, individualizar la estrategia terapéutica y avanzar hacia una medicina personalizada y de precisión.

Nuestro trabajo diferencia drásticamente subgrupos de pacientes con artrosis de rodilla que presentan distintas características inflamatorias y metabólicas con implicaciones en gravedad tanto clínica como radiográfica. El único problema para su implementación es que son necesarias 27 variables analíticas y antropométricas para su definición precisa, aunque son unos resultados relevantes dada su trascendencia clínica.

Tras analizar datos de 168 mujeres con OA con derrame articular, se obtuvieron cuatro clúster (KOIP, *knee osteoarthritis inflammatory phenotypes*), bien diferenciados.

El KOIP-1 clasificaría hasta un 32.7% de los pacientes, y constituiría el fenotipo más inflamatorio y, por tanto, de peor pronóstico en cuanto a severidad clínica y radiológica. Se asocia a señas de identidad propias del síndrome metabólico tales como sobrepeso; mayor proporción de grasa corporal; mayor índice cintura-cadera; alta prevalencia de diabetes, HTA, DLP; valores elevados de insulina, triglicéridos, ácido úrico, hemoglobina glicada; y menor proporción de HDL y actividad física. Además, presentan niveles elevados de mediadores de la inflamación, tales como IL-6, IL-8, PCR y calprotectina; y los niveles más altos de leptina e irisina. A nivel etiopatogénico estaría mediado por un estado inflamatorio de bajo grado promovido por factores metabólicos asociados a la obesidad y a la grasa corporal [169]; por lo que podríamos definirlo como el fenotipo clásicamente asociado a la inflamación sistémica de bajo grado mediada por la obesidad.

La asociación entre obesidad e incidencia de OA en articulaciones de carga, especialmente de rodilla, está bien establecida. Incluso se ha demostrado una asociación significativa entre la cirugía bariátrica y la reducción del dolor e inflamación articular en pacientes con OA [196]. Pero la asociación entre obesidad y OA de articulaciones que no están sometidas a carga, como las manos, parece estar mediada por las adipocitocinas y otros factores inflamatorios posiblemente asociados a la obesidad [197].

Esto es interesante desde el punto de vista terapéutico, ya que el abordaje de estos pacientes sería desde una perspectiva metabólica. La producción excesiva de adipocitocinas por la grasa blanca, responsable del aumento de los procesos inflamatorios, la resistencia insulínica y la diabetes, podría ser el target, por ejemplo, de terapias génicas que mediante la promoción de la termogénesis eviten el desarrollo de

estas enfermedades. También sería un grupo en el que podríamos incidir más directamente en las recomendaciones higiénico-dietéticas realizadas habitualmente en los pacientes con OA, ya que posiblemente sería el grupo en el que se podría observar un mayor beneficio.

El KOIP-2 define un grupo metabólicamente sano, pero con actividad inflamatoria moderada, y clasificaría a un 30.4% de los pacientes. Se traduce en una menor repercusión clínica tanto a nivel de dolor como de capacidad funcional, así como de progresión radiográfica [179]. Esto implicaría un mejor pronóstico y, por consiguiente, menor agresividad a la hora de establecer la estrategia terapéutica. De todos modos, se trata de un fenotipo inflamatorio que, como hemos comentado anteriormente, se asocia a niveles elevados de dolor, incapacidad y progresión estructural. En este caso, existe un claro aumento de las adipocitocinas consideradas sanas, como la adiponectina y la omentina. Sus niveles elevados en líquido articular, reflejan un componente inflamatorio, también asociado a gravedad clínica, aunque en menor medida que en otros fenotipos.

El KOIP-3 clasificaría a un 16.1% de los pacientes, que se caracterizarían por una baja o moderada expresión de los factores metabólicos y niveles aumentados de citocinas pro-inflamatorias tanto a nivel local como sistémico, especialmente del TNF-α, IL-8 y del NGF, formando un fenotipo que podríamos definir como inflamatorio puro, por su ausencia de asociación directa con las medidas de obesidad. El aumento de los mediadores de inflamación conllevaría un peor pronóstico, tanto clínico como estructural. En este caso el abordaje terapéutico debería centrarse en bloquear las moléculas proinflamatorias, lo que actualmente es objeto de investigación [198]. De

esta forma, se abriría la posibilidad de seleccionar pacientes con un fenotipo inflamatorio puro, no mediado por la obesidad, para ensayar nuevas dianas terapéuticas dirigidas, por ejemplo, al inflamosoma o a la inhibición de la IL-1β[199,200].

El KOIP-4 representa a un 20.8% de los sujetos de nuestra muestra, y en este fenotipo los individuos presentan una elevada prevalencia de factores de riesgo cardiovascular clásicos, así como baja expresión de citocinas inflamatorias, lo que describiría un clúster de pacientes en los que la enfermedad no está tan asociada a la inflamación de bajo grado e hiperproducción de adipocitocinas propia del síndrome metabólico y la obesidad, sino a otras causas de índole cardiovascular, y el abordaje terapéutico debería incluir una monitorización estrecha de estas otras causas. A nivel pronóstico presentan valores bajos de dolor y discapacidad funcional, similares a los del KOIP-2; aunque relativamente elevados respecto a otros fenotipos no inflamatorios de artrosis.

La relevancia de los resultados presentados en este trabajo se complementa de forma importante con los datos aportados en la publicación referenciada en los anexos a esta tesis doctoral. En un primer término, la identificación de los cuatro fenotipos inflamatorios con relevancia clínica y radiológica es un hallazgo relevante para seguir progresando en la mejor clasificación y agrupación de nuestros pacientes para poder ofrecerles tratamientos más específicamente dirigidos. Poder definir fenotipos diferenciales dentro de un fenotipo ya establecido previamente, como el inflamatorio, además con implicaciones en severidad tiene interés traslacional. Además, los datos aportados en los anexos ayudan a comprender las múltiples inconsistencias existentes en la literatura referentes a la asociación entre diversos factores de la inflamación y la severidad en artrosis de rodilla, ya sea clínica o radiográfica. En este sentido, podemos

observar como las diferentes citocinas y marcadores de inflamación tienen un comportamiento y asociación diferente con las medidas de desenlace clínicas o radiográficas según el KOIP al que pertenecen. A modo ilustrativo, la omentina en líquido sinovial presenta una asociación negativa en el KOIP-1 y positiva en el KOIP-2 con el dolor. Desde nuestro punto de vista, la relevancia de estos resultados no es tanto la asociación individual de cada marcador de la inflamación con las medidas de desenlace, sino la constatación de la necesidad de un fenotipado de precisión; ya que, debido posiblemente a la heterogeneidad de la artrosis, parece que la implicación en la severidad de la artrosis difícilmente se explicará por una sola molécula sino por la combinación de varias. A su vez, estos resultados pueden contribuir a explicar contradicciones previas en la literatura existente respecto a la asociación de diversas citocinas con medidas de gravedad en artrosis de rodilla, ya que no se han evaluado en función del fenotipo inflamatorio.

7.1. Limitaciones y fortalezas

Nuestro estudio se ha realizado en una cohorte prospectiva de pacientes con osteoartritis de rodilla (KOA), todas ellas mujeres, con derrame articular confirmado por ecografía, lo que constituye un grupo altamente homogéneo de sujetos. Además, todos ellos presentan una artrosis sintomática en el momento de la inclusión con una escala analógica visual del dolor ≥ 4; lo que es una característica diferencial de otros estudios donde se centran en la afectación radiográfica. Referente a este aspecto, cabe destacar que, también como hecho diferencial respecto a otros estudios, hemos reclutado pacientes con grados de afectación radiográfica por K/L de 1 a 4, siendo los grados 2 y 3 los más representados con un 80% de los pacientes. Para la realización del trabajo actual, nos hemos centrado en las mujeres, ya que constituían la mayoría de nuestra cohorte (84%), y existen varias diferencias específicas de género en cuanto a prevalencia, condiciones metabólicas e inflamatorias, así como niveles de dolor y discapacidad previamente definidos en la literatura y ya referenciados anteriormente en este trabajo. Aunque esta homogeneidad puede proporcionar una ventaja para identificar biomarcadores de la enfermedad, reconocemos que también podría limitar la generalización de nuestros resultados. Por lo tanto, se necesitan más estudios en series independientes de pacientes de otros centros, con un tamaño de muestra suficiente y características clínicas y presentaciones diferentes; incluyendo sobre todo hombres y presentaciones no inflamatorias, para evaluar la generalización de estos hallazgos. Lógicamente, la naturaleza del propio estudio, siendo, siendo el primero un corte transversal y el segundo un estudio de cohorte prospectivo permite la identificación de asociaciones, pero no evidencia causalidad, por lo que, en caso de

confirmarse nuestros resultados, referentes a biomarcadores y definición de fenotipos, deberían evaluarse en un estudio prospectivo específicamente diseñado. Otra limitación de nuestro estudio es la ausencia de mediciones cuantitativas puras para la gravedad radiográfica, como las mediciones del ancho mínimo o fijo del espacio articular en milímetros a lo largo del tiempo. Estas mediciones podrían haber proporcionado una mejor resolución, aumentar el poder estadístico y, posiblemente, revelar asociaciones adicionales no identificadas en nuestros análisis actuales. Desafortunadamente, este tipo de cuantificación no está disponible actualmente en nuestra serie de pacientes y constituye un área relevante de investigación para estudios futuros. Por otro lado, nuestro estudio se distingue de los trabajos previamente publicados por su exhaustiva disponibilidad de datos, incluyendo un panel de 13 citocinas cuantificadas en plasma y LS de 168 pacientes. Estas muestras y datos se recopilaron sistemáticamente dentro de los protocolos de una cohorte prospectiva diseñada específicamente para estudiar los factores asociados con la gravedad y progresión de la artrosis de rodilla, lo cual es una fortaleza destacada del estudio. De todos modos, se necesitó un alto número de parámetros (27 de 45) para discriminar los grupos de pacientes con artrosis de fenotipo inflamatorio (KOIP) en nuestros datos, lo que indica que estos fenotipos estaban definidos por una compleja combinación de varios factores metabólicos, antropométricos e inflamatorios, en lugar de estar completamente caracterizados por un número limitado de estas características. Sin embargo, su implementación en la práctica clínica requeriría un panel compuesto por un menor número de biomarcadores. En este sentido, el uso de datos ómicos derivados de tecnologías de alto rendimiento

ofrece un gran potencial para identificar biomarcadores específicos de KOIP en un futuro muy cercano, y actualmente es una línea de investigación en curso en nuestro grupo.

7. CONCLUSIONES

8. CONCLUSIONES

- La interleucina 8 (IL-8) en líquido sinovial de mujeres con artrosis de rodilla y derrame articular se asocia con las medidas de gravedad clínica.
- La IL-8 en líquido sinovial se relaciona con varios factores de inflamación y adipocitocinas en el líquido articular.
- Ninguna de las asociaciones previas se observa con los niveles de IL-8 plasmáticos, evidenciando la importancia del análisis de los factores de inflamación local en este grupo de pacientes.
- Se han identificado cuatro fenotipos de mujeres con osteoartritis de rodilla con derrame articular que mostraron perfiles diferenciales de factores antropométricos, metabólicos e inflamatorios.
- Estos KOIPs discretos presentan implicaciones tanto en la gravedad clínica como radiográfica de la artrosis de rodilla.
- Tanto las citocinas como las adipocitocinas presentan asociaciones diferentes con la severidad clínica y radiográfica en artrosis de rodilla según el KOIP al que pertenecen.

8. INVESTIGACIONES FUTURAS

9. INVESTIGACIONES FUTURAS

En esta tesis doctoral se aprecia la línea de trabajo en pacientes con artrosis y derrame articular. Inicialmente, estudiamos y analizamos un conjunto de factores de la inflamación y adipocitocinas para evaluar su asociación con medidas de desenlace clínicas y radiográficas midiendo la severidad en artrosis de rodilla. Debido a las inconsistencias existentes en la literatura y considerando la artrosis como una entidad heterogénea y multifactorial, el siguiente análisis se centró en la investigación de fenotipos. Hemos sido capaces de identificar cuatro fenotipos dentro de nuestro grupo de pacientes con carácter inflamatorio (KOIPs) que presentan implicaciones en gravedad tanto clínica como radiológica. Su implementación en práctica clínica está limitada por el elevado número de variables necesarias para su definición, por lo que, de forma natural, las investigaciones futuras deben centrarse en:

- Realizar una caracterización más precisa de los distintos KOIPs, mediante técnicas ómicas, inicialmente mediante proteómica. El grupo se ha interesado por la tecnología Olink, basada en ensayos de extensión por proximidad, por su enorme capacidad de discriminar proteínas débilmente expresadas y la necesidad de un bajo volumen de muestra para su realización.
- Una vez caracterizados los KOIPs, se seleccionarán un conjunto de proteínas mínimo y crítico para la definición de los KOIPs, que nos permitan su implementación en práctica clínica.
- Será necesario realizar una validación en una cohorte externa con un grupo independiente de pacientes con artrosis y derrame articular.

- De confirmarse los resultados, se evaluaría su aplicabilidad en una cohorte de hombres con artrosis de rodilla y derrame.
- Igualmente, se valorará la reproducibilidad de estos resultados en una cohorte de pacientes con artrosis de rodilla sin derrame articular, por lo que será necesario estudiar en profundidad el plasma de estos sujetos.
- Se buscarán vías fisiopatogénicas que puedan influir en el desarrollo y la gravedad de la artrosis, intentando priorizar aquellas que se presenten en el plasma, debido a que, en teoría, se podrían generalizar los resultados de forma más directa.
- En caso de confirmarse nuestras hipótesis futuras, podríamos establecer tratamientos específicos en función de cada fenotipo, avanzando hacia una medicina personalizada y de precisión.

9. REFERENCIAS BIBLIOGRÁFICAS

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10. ANEXO

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Specific-cytokine associations with outcomes in knee osteoarthritis subgroups: breaking down disease heterogeneity with phenotyping

Joan Calvet[1,2*], Antoni Berenguer-Llergo[1], Cristóbal Orellana[1], María García-Manrique[1,2], Menna Rusiñol[1], Silvia Garcia-Cirera[1], Maria Llop[1], Marta Arévalo[1], Alba Garcia-Pinilla[1], Carlos Galisteo[1], Cristina Aymerich[1], Rafael Gómez[1], Alejandra Serrano[1], Anna Carreras[1] and Jordi Gratacós[1,2]

Abstract

Background Despite existing extensive literature, a comprehensive and clinically relevant classification system for osteoarthritis (OA) has yet to be established. In this study, we aimed to further characterize four knee OA (KOA) inflammatory phenotypes (KOIP) recently proposed by our group, by identifying the inflammatory factors associated with KOA severity and progression in a phenotype-specific manner.

Methods We performed an analysis within each of the previously defined four KOIP groups, to assess the association between KOA severity and progression and a panel of 13 cytokines evaluated in the plasma and synovial fluid of our cohort's patients. The cohort included 168 symptomatic female KOA patients with persistent joint effusion.

Results Overall, our analyses showed that associations with KOA outcomes were of higher magnitude within the KOIP groups than for the overall patient series (all p-values < 1.30e−16) and that several of the cytokines showed a KOIP-specific behaviour regarding their associations with KOA outcomes.

Conclusion Our study adds further evidence supporting KOA as a multifaceted syndrome composed of multiple phenotypes with differing pathophysiological pathways, providing an explanation for inconsistencies between previous studies focussed on the role of cytokines in OA and the lack of translational results to date. Our findings also highlight the potential clinical benefits of accurately phenotyping KOA patients, including improved patient stratification, tailored therapies, and the discovery of novel treatments.

Keywords Knee osteoarthritis, Cytokines, Phenotype, Inflammation, Clinical severity, Radiographic progression, Machine learning

*Correspondence:
Joan Calvet
jcalvet@tauli.cat
[1] Department of Rheumatology, Parc Taulí Hospital Universitari, Institut d'Investigació i Innovació Parc Taulí (I3PT-CERCA), Universitat Autònoma de Barcelona, c/Parc Taulí s/n, edifici VII Centenari, 08208 Sabadell, Spain
[2] Department of Medicine, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain

Background

Osteoarthritis (OA) is a prevalent musculoskeletal disease that affects millions of people worldwide [1], with knee OA (KOA) being the most affected location and the focus of extensive research in recent years [2, 3]. Patients with OA often experience high levels of pain and disability, which result in seeking healthcare assistance [1–3]. The disease is also associated with numerous

comorbidities, particularly cardiovascular risk factors, contributing to high healthcare costs [3–5].

The pathophysiology of OA is not fully understood, but it is known that age, obesity, genetics, previous trauma, metabolic factors, some molecular determinants of cartilage degradation, and systemic and local inflammation contribute to its onset and progression [5–11]. Sex-related differences have also been identified in OA patients, including prevalence rates, metabolic conditions, inflammatory factors, and levels of pain and functional disability [12, 13]. OA is currently highly prevalent, and its socioeconomic impact is expected to increase in the coming years due to the ageing of the population and increasing rates of obesity in Western societies [5]. Despite extensive research conducted in the past decades, the development of targeted drugs capable of effectively alleviating pain or halting the structural deterioration in OA remains an unmet need [14].

OA is now understood to be a complex, multi-tissue disease that affects various joint structures including the articular cartilage, bone, subchondral bone, synovial membrane, capsule, ligaments, menisci, and periarticular muscles [1, 5]. Inflammation and metabolic factors are recognized as crucial factors in the development and progression of OA [9–11]. Previous studies have focused on identifying specific inflammatory markers, such as adipocytokines and cytokines found in the blood, synovial fluid, and synovial membrane of OA patients, which have been linked to pain, disability, and radiographic changes [15, 16]. However, much of this research has provided inconclusive or inconsistent results regarding the strength and nature of the association between these cytokines and the severity and progression of OA [11, 17–19].

One possible explanation for these inconsistencies and the lack of major translational research may be the heterogeneity of OA patients regarding clinical presentation, exhibit of risk factors and prognosis. In this regard, it has been suggested that OA may not be a single entity, but rather a complex and heterogeneous condition made up of different subgroups (phenotypes) with specific pathophysiological traits (endotypes) [20, 21]. The identification of these phenotypes could lead to better assessment of severity and prognosis biomarkers, resulting in significant clinical implications for patients' stratification, therapy tailoring, and exploration of novel treatments [22]. In this regard, several groups have described distinct OA phenotypes characterized by the presentation of diverse features, such as clinical parameters [17, 23], transcriptomics [24, 25], metabolomic data [26, 27], and other biochemical markers [18, 28]. Despite these efforts, a comprehensive and clinically relevant classification system for OA has yet to be established.

In KOA, though, it is generally accepted the existence of an inflammatory clinical phenotype characterized by synovitis, increased levels of pain and disability, and a faster rate of disease progression [29, 30]. In the last years, our group has focussed on the metabolic and inflammatory profiles of KOA patients in this inflammatory phenotype [10, 19, 31–34]. In doing so, our objectives were to better understand the inflammatory mechanisms underlying KOA and to identify specific risk and prognostic factors associated with this inflammatory phenotype. As a result, we have recently identified four knee osteoarthritis inflammatory phenotypes (KOIP) using well-established statistical and machine learning methods applied to a cohort of 168 female patients with primary KOA and joint effusion [35]. The analysis included a comprehensive panel of 45 variables describing the patients' anthropometric and metabolic status, as well as their inflammatory profile measured by a set of 13 cytokines in plasma and synovial fluid. These phenotypes showed marked differences in their anthropometric, metabolic, and inflammatory profiles and demonstrated significant differences in clinical severity and radiographic progression [35].

In this study, we aimed to further characterize the four KOA inflammatory phenotypes (KOIP) recently proposed by our group, by evaluating inflammatory factors linked with the severity and progression of KOA in a KOIP-specific manner. The identification of such factors is of relevance as they might point to different underlying inflammatory mechanisms for the onset and evolution of the disease across these phenotypes. To do so, we assessed the association between the panel of 13 cytokines available in our KOA cohort and the disease's severity and radiographic progression within each KOIP separately, both in plasma and in synovial fluid.

Methods

Patients' description

The study was carried out on a prospective cohort of 168 female patients with symptomatic primary knee osteoarthritis (KOA) and persistent joint effusion [35]. Plasma and joint fluid samples were available for all patients. We focussed the analysis on female patients to homogenize the study sample, as numerous sex-related differences have been previously reported in KOA regarding metabolic conditions, inflammatory factors, and levels of pain and function disability [12, 13]. Subjects' inclusion required the presence of symptomatic primary KOA according to the American College of Rheumatology (ACR) criteria, with a defined diagnosis in the outpatient rheumatology visits, aged between 50 and 85 years old, and with joint effusion observed during the physical examination at the recruitment visit and confirmed

by ultrasound (≥ 4 mm on midline suprapatellar line). Symptomatic KOA was defined as the presence of pain greater than or equal to 4 on a 10-cm visual analogue scale, despite the use of prescribed analgesic drugs for at least 3 months. The exclusion criteria comprised secondary osteoarthritis, either due to a history of trauma, menisci injury, or previous inflammatory rheumatism; a history of knee surgery; any disease which, in the investigator's opinion, could interfere with the assessment of pain such as, but not limited to, fibromyalgia or polyneuropathies; systemic glucocorticoid intake in the last 6 months; and intra-articular glucocorticoid or hyaluronic acid injection in the last 3 or 6 months before recruitment, respectively. The recruitment period was from October 2013 to April 2018.

Samples

Samples from the plasma and joint fluid were extracted at the patient's recruitment. Collected samples were appropriately processed and stored at −80 °C, until their use for quantification of cytokines by enzyme-linked immunosorbent assay (ELISA). ELISA assays were conducted according to the manufacturer's recommendations. Synovial and plasma samples were evaluated for the following cytokines: C-reactive protein (CRP, mg/L), interleukin 6 (IL-6, pg/mL), interleukin 8 (IL-8, pg/mL), tumour necrosis factor alpha (TNF-alpha, pg/mL), nerve growth factor (NGF, pg/mL), calprotectin (ng/mL), leptin (pg/mL), irisin (ng/mL), visfatin (ng/mL), resistin (pg/mL), osteopontin (ng/mL), adiponectin (ng/mL), and omentin (pg/mL). Due to technical reasons related to the ELISA technology (configuration of plates used), these markers could not be assessed at the same time for all patients. To account for potential effects induced by this technical source, and as described previously [35], we corrected the ELISA values previously to any formal statistical analysis (two-step correction), after applying a transformation using the Tukey ladder of powers to symmetrize their distribution and meet the assumptions of the linear model (Additional file 2: Table S2).

Data collection

Baseline information regarding demographics and anthropometric and metabolic factors was collected for these patients as described previously [35]. Baseline clinical severity is available for these patients as measured by the Knee injury and Osteoarthritis Outcome Scores (KOOS; pain, functional disability, and symptoms) [36], which were used in reversed order to facilitate the interpretation of the results. Ultrasound measurements were collected regarding joint effusion and synovial tissue thickness (mm). The assessments were performed by a single experienced examiner (JC), using Siemens Acuson Antares with a 5–13-MHz linear array transducer and a standardized protocol based on current guidelines and definitions [37–39]. Radiographic severity was measured by the Kellgren-Lawrence (KL) scale [40] and following the OARSI atlas lecture [41], which includes an assessment of osteophytes and joint space narrowing (JSN). This evaluation involved an anteroposterior knee X-ray conducted with the patient in a standing position, performed within the last 18 months before recruitment. The follow-up radiographic evaluation was blind to the results at baseline. Two different clinicians independently conducted readings for a subset of patients. Concordance between the readers was assessed using unweighted Cohen's kappa, yielding values of 0.884 for KL (135 patients, 95% confidence interval 0.816 to 0.953), 0.931 for osteophytes evaluation (135 patients, 95% confidence interval 0.885 to 0.977), and 0.782 for JSN (30 patients, 95% confidence interval 0.608 to 0.956). Most of the patients (n = 143, 85%) underwent a radiographic evaluation during the follow-up to assess their radiographic progression at 2 years. To assess the radiographic progression at 2 years, the majority of patients (85%) underwent a radiographic evaluation after their initial radiography with a median interval of 26 months (over 18 months for 90% and over 24 months for 69% of the patients in the study). Radiographic progression was defined by comparing the follow-up and baseline radiographs, using each of the three different measures available: an increase in the radiographic Kellgren-Lawrence (KL) stage in the follow-up evaluation (KL progression), the appearance of new osteophytes (osteophyte progression), and a reduction in the space between joint bones (JSN progression). More details about patients, samples, and data collection are available in our previous work [35].

Statistical analysis

Continuous parameters were described by their medians, median absolute deviations, and ranges, while categorical variables were summarized using absolute frequencies and percentages. Associations with KOIP groups and outcomes were assessed using non-parametric methods, namely the Kruskal-Wallis and Mann-Whitney tests for continuous variables and Fisher's tests for categorical variables.

Univariate associations between cytokines and KOA outcomes were assessed for the overall series and within each KOIP independently. Given the low sample size available, we deliberately opted for non-parametric methods for their robustness against bias due to the extremely high influential values used. These methods included Spearman correlation (SC) [42] (continuous or ordinal outcomes) and Glass rank biserial correlation (GRBCorr)

[43] (binary outcomes). Baseline Kellgren-Lawrence (KL) staging and joint space narrowing were treated as ordinal in these analyses. Asymptotic 95% confidence intervals (CI) were computed for SC, while bootstrap intervals were computed for GRBCorr coefficients (1.000 resamples). For binary outcomes, fold changes (FC) of the median groups were also calculated to quantify the magnitude of the cytokines differences between the patient groups, along with their bootstrap 95% confidence intervals. To aid interpretation, FCs below one were inverted and prefixed with a minus sign, so that a negative FC indicates a higher level of the cytokine in the reference group. In each case, statistical significance was assessed with non-parametric asymptotic methods (SC test for continuous or ordinal outcomes; Mann-Whitney test for binary outcomes). No adjustment by multiple contrasts was performed for these analyses, since they are considered as exploratory when examined individually.

These results were graphically represented in a heatmap, where red colour indicated positive correlation, blue represented negative correlation, and colour intensity expressed more extreme values of the correlation coefficients. Colour intensities were saturated to 0.5 and −0.5 for positive and negative correlation, respectively. For graphical representation, we also used scatter plots (KOOS scores and ultrasound joint effusion) and boxplots and stripcharts (radiographic progression) where the cytokines were displayed in their transformed scale (Additional file 2: Table S2, see the "Samples" section).

To further examine the associations between cytokines and KOA outcomes, we conducted statistical analyses while controlling for age, disease evolution time, and body mass index (BMI). For continuous KOA outcomes (KOOS-pain, KOOS-functional disability, ultrasound joint effusion, and synovial tissue thickness), we computed adjusted Spearman correlations using probability-scale residuals and cumulative probability models as previously described and implemented [44], along with their corresponding 95% confidence intervals and p-values. For binary KOA outcomes (radiographic progression based on KL, osteophytes, and joint space narrowing), each cytokine was individually fitted to a linear model. In these models, patients' status (progressors or not-progressors) and the confounding variables were included as explanatory factors. To ensure the assumptions of the linear models, we incorporated the cytokines values in their transformed scale (Additional file 2: Table S2) and, when necessary, applied transformations to the confounding variables (Tukey ladder of powers: $g = 0.25$ for disease evolution time; $g = -0.75$ for BMI; no transformation for age). These methodologies were selected for their robustness to avoid or, at the very least, attenuate biases induced by extreme values. p-values were calculated using the Wald test to assess the significance of differences in cytokine levels between the two patient groups. To quantify the association, we extracted the means from the model for each patient group, transformed them back to the original scale of the cytokine, and used these values to estimate a FC between patients with and without radiographic progression. This FC can be interpreted as the ratio of cytokine medians across the patient groups in the original scale of the cytokine [45], assuming that the transformation applied to the response variable allows it to meet the linear model's assumptions. Confidence intervals for these FCs were computed through simulation from the linear model, following a previously described approach [46].

The magnitudes of the associations observed within each KOIP were compared with those obtained from the whole female patients' series. To do so, absolute values of the correlation coefficients were computed and compared pair-wise using a Wilcoxon test. The results of these analyses were graphically displayed in a boxplot and a stripchart. The threshold for statistical significance was set at 5%. All analyses were conducted with R [47].

Results

Recently, our group identified four distinct inflammatory KOA phenotypes (KOIP) using data from 168 female subjects included in a cohort of primary KOA patients with joint effusion [35]. These phenotypes drastically differed in their anthropometric, metabolic, and inflammatory profiles and exhibited substantial differences in clinical severity and radiographic progression [35]. To gain further insight into these phenotypes and their underlying inflammatory mechanisms, we used the same series (Table 1) to assess, in each of these phenotypes, the association between KOA severity and progression and the panel of 13 cytokines evaluated in the plasma and synovial fluid of our cohort's patients (Additional file 2: Tables S1 and S2). A global view of these results showed that associations with KOA outcomes were of higher magnitude within the KOIP groups than for the overall patients' series (all p-values < 1.30e−16) and that some of the cytokines showed a KOIP-specific behaviour regarding these associations (Fig. 1, Additional file 1: Fig. S1 and Additional file 2: Tables S3–S7).

To illustrate that, we point out some results observed for the clinical severity parameters. In KOIP-1 subjects, a negative correlation was observed for synovial omentin with baseline KOOS pain (Spearman correlation, SC = −0.265, p-value, pv = 0.050) and functional disability (SC = −0.218; pv = 0.110). In contrast, roughly the same magnitude of positive correlation was found in the KOIP-2 group for both pain (SC = 0.277, pv = 0.049) and functional disability (SC = 0.228, pv = 0.1074) (Fig. 2,

Table 1 The main baseline patients' characteristics. Demographic, anthropometric, metabolic, and radiographic factors for all the KOA patients included in the study and stratified by knee osteoarthritis inflammatory phenotypes (KOIP). All subjects are female patients diagnosed with symptomatic primary knee osteoarthritis (KOA) with persistent joint effusion. Continuous parameters are described with their median and ranges (minimum and maximum values), while absolute frequencies and percentages are displayed for categorical variables

		All, 168 (100%)	KOIP-1, 55 (32.7%)	KOIP-2, 51 (30.4%)	KOIP-3, 27 (16.1%)	KOIP-4, 35 (20.8%)	*p*-value
Age at recruitment		69.1 [50.9, 83.0]	70.4 [50.9, 81.4]	68.2 [51.4, 83.0]	66.9 [54.4, 80.8]	70.4 [51.1, 80.5]	0.5195
Kellgren-Lawrence radiographic grade	Grade 1	19 (11.3%)	5 (9.1%)	4 (7.8%)	3 (11.1%)	7 (20.0%)	0.1261
	Grade 2	65 (38.7%)	19 (34.5%)	25 (49.0%)	7 (25.9%)	14 (40.0%)	
	Grade 3	78 (46.4%)	26 (47.3%)	22 (43.1%)	16 (59.3%)	14 (40.0%)	
	Grade 4	6 (3.6%)	5 (9.1%)	0 (0.0%)	1 (3.7%)	0 (0.0%)	
Disease evolution time (months)		48 [4, 200]	45 [4, 150]	48 [4, 200]	36 [6, 125]	60 [6, 172]	0.7291
Obesity		94 (56.0%)	51 (92.7%)	5 (9.8%)	16 (59.3%)	22 (62.9%)	< 0.0001
Physical exercise	None	61 (36.3%)	29 (52.7%)	12 (23.5%)	6 (22.2%)	14 (40.0%)	0.0123
	Sporadic	51 (30.4%)	18 (32.7%)	16 (31.4%)	8 (29.6%)	9 (25.7%)	
	Moderate	46 (27.4%)	6 (10.9%)	18 (35.3%)	11 (40.7%)	11 (31.4%)	
	Vigorous	10 (6.0%)	2 (3.6%)	5 (9.8%)	2 (7.4%)	1 (2.9%)	
Diabetes mellitus		18 (10.7%)	10 (18.2%)	2 (3.9%)	2 (7.4%)	4 (11.4%)	0.1101
Arterial hypertension		92 (54.8%)	37 (67.3%)	18 (35.3%)	16 (59.3%)	21 (60.0%)	0.008
Dyslipidaemia		68 (40.5%)	26 (47.3%)	16 (31.4%)	9 (33.3%)	17 (48.6%)	0.2321
ATP III metabolic syndrome		61 (36.3%)	33 (60.0%)	2 (3.9%)	9 (33.3%)	17 (48.6%)	< 0.0001

ATP III Adult Treatment Panel III

Additional file 1: Fig. S2). Other notable findings were the negative association between plasma IL-6 and both pain (SC = −0.405, pv = 0.016) and function disability (SC = −0.305, pv = 0.075) in the KOIP-4 group, where synovial osteopontin was also positively correlated with KOOS pain (SC = 0.411, pv = 0.014) (Additional file 1: Figs. S3–S5).

The results stratified by KOIP also showed phenotype-specific associations with radiographic evolution. According to the KL and the JSN criteria, progression was associated with high synovial irisin in KOIP-4 (FC = 1.61, pv = 0.002; FC = 1.53, pv = 0.016, respectively), but also with low levels of this cytokine in KOIP-1 (FC = −1.27, pv = 0.014; FC = −1.27, pv = 0.033) (Fig. 3 and Additional file 1: Fig. S20). Based on the KL criteria only, progression was associated with low levels of synovial resistin in KOIP-1 (fold change (FC) = −1.70, pv = 0.008) and low values of synovial CRP (FC = −2.32, pv = 0.036) and plasma omentin (FC = −2.59, pv = 0.029) in KOIP-3 (Additional file 1: Figs. S6–S8).

Synovial omentin and adiponectin showed statistically significant associations with osteophytes progression in KOIP-1 (FC = 1.73, *p*-value = 0.004; FC = 1.83, *p*-value = 0.005, respectively), which were of opposite direction in the KOIP-2 group (FC = −1.26, *p*-value = 0.059; FC = −1.27, *p*-value = 0.107, respectively). Osteophyte progression also showed associations of opposite directions for leptin in KOIP-1 (FC = 1.35, pv = 0.025 in the synovial fluid; FC = 1.43, pv = 0.063 in the plasma) and KOIP-3 (FC = −1.62, pv = 0.360 in the synovial fluid; FC = −1.67, pv = 0.057 in the plasma), and for plasma IL-8 in KOIP-2 (FC = 1.48, pv = 0.055) and KOIP-4 (FC = −1.45, pv = 0.022). Other phenotype-specific associations with osteophytes progression were found for osteopontin in KOIP-2 (FC = −1.22, pv = 0.049 in the synovial fluid; FC = −1.42, pv = 0.036 in plasma), irisin in KOIP-3 (FC = −1.95, pv = 0.024 in the synovial fluid; FC = −1.46, pv = 0.091 in the plasma), and synovial calprotectin in KOIP-4 (FC = 1.80, pv = 0.015) phenotypes (Additional file 1: Figs. S9–S19).

According to the JSN criteria, radiographic progression was also associated with high synovial osteopontin in KOIP-3 (FC = 5.72, pv = 0.009), high synovial omentin in KOIP-1 (FC = 1.81, pv = 0.009), low levels of leptin (FC = −1.52, pv = 0.007 in synovial fluid; FC = −1.61, pv = 0.041 in plasma), and low plasma irisin in KOIP-2 patients (FC = −1.83, pv = 0.038) (Additional file 1: Figs. S20–S25).

As other interesting examples, and despite no association was found between the KOIP groups and ultrasound severity in our previous work, some cytokines showed a phenotype-specific correlation with joint effusion, such as synovial IL-6 in KOIP-3 (SC = 0.541, *p*-value = 0.004; Fig. 4). Interestingly, several cytokines were associated with joint effusion in KOIP-4, including positive correlations with leptin (SC = 0.405, pv = 0.016 in the synovial

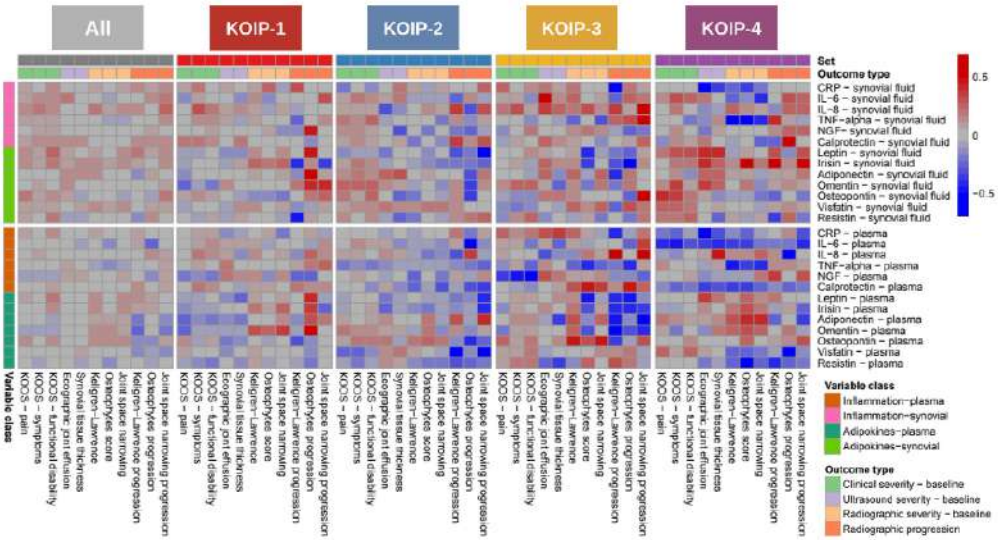

Fig. 1 Association of cytokines with knee osteoarthritis (KOA) severity and progression stratified by KOA inflammatory phenotypes (KOIP). The heatmap colours represent non-parametric correlation-like measurements to assess the association of the cytokines evaluated in our study with KOA outcomes, including clinical, radiographic and ultrasound severity at baseline, and radiographic progression at 2 years. Associations were assessed for the overall series and within each KOIP independently using Spearman correlations (continuous or ordinal outcomes) and Glass rank biserial correlations (binary outcomes). Baseline Kellgren-Lawrence staging and joint space narrowing were treated as ordinal in these analyses. Red indicates positive, blue represents negative, and colour intensity expresses more extreme values of the correlation coefficients. Colour intensities were saturated to 0.5 and −0.5 for positive and negative correlation, respectively. IL-6, interleukin 6; IL-8, interleukin 8; TNF-alpha, tumour necrosis factor alpha; NGF, nerve growth factor; CRP, C-reactive protein; KOOS, Knee injury and Osteoarthritis Outcome Scores (reversed scores); KOA, knee osteoarthritis; KOIP, knee osteoarthritis inflammatory phenotype

fluid; SC = 0.344, pv = 0.043 in the plasma), synovial irisin (SC = 0.405, pv = 0.016), and omentin (SC = 0.363, pv = 0.041) and negative correlations with CPR (SC = −0.590, pv = 0.0002 in the synovial fluid: SC = −0.512, pv = 0.002 in the plasma) and plasma IL-6 (SC = −0.368, pv = 0.030) and calprotectin (SC = −0.336, pv = 0.049) (Additional file 1: Figs. S26–S33).

Finally, it is noteworthy that, despite the relatively small sample sizes within each KOIP group (ranging from 27 to 55 patients), the strength and statistical significance of the associations described above (summarized in Additional file 1: Figs. S2–S33) remained largely unchanged in most of the cases after adjusting for age, disease evolution time, and BMI (Additional file 2: Tables S8 and S9).

Discussion

In this study, we identified a set of cytokines that are differentially associated with severity and radiographic progression across a recently proposed classification of inflammatory phenotypes in KOA (KOIP). In the last years, extensive research has been conducted on the role of several markers on OA severity and progression, including some of the ones evaluated in the present work [10, 11, 15, 17–19, 48]. Although these studies have provided valuable information about the pathophysiology of the disease, none of their results has been transferred to the clinical practice, either to improve the diagnosis or prognosis of their patients or to develop new therapeutic targets with a disease-modifying effect [14]. On the contrary, many of them have provided inconclusive or inconsistent results which, together with the heterogeneity of the disease, has given rise to the hypothesis of the existence of multiple phenotypes in OA [20]. However, despite great efforts have been invested in this line of research, there is still no consensus on a comprehensive classification of OA with clinical relevance.

In our previous work, we identified four different phenotypes of inflammatory KOA that exhibited differential profiles of anthropometric, metabolic, and inflammatory factors and displayed substantial differences in clinical severity and radiographic progression [35]. In the present study, we used this classification as a framework to shed light on the inconsistencies and lack of translational results of previous research. To accomplish this,

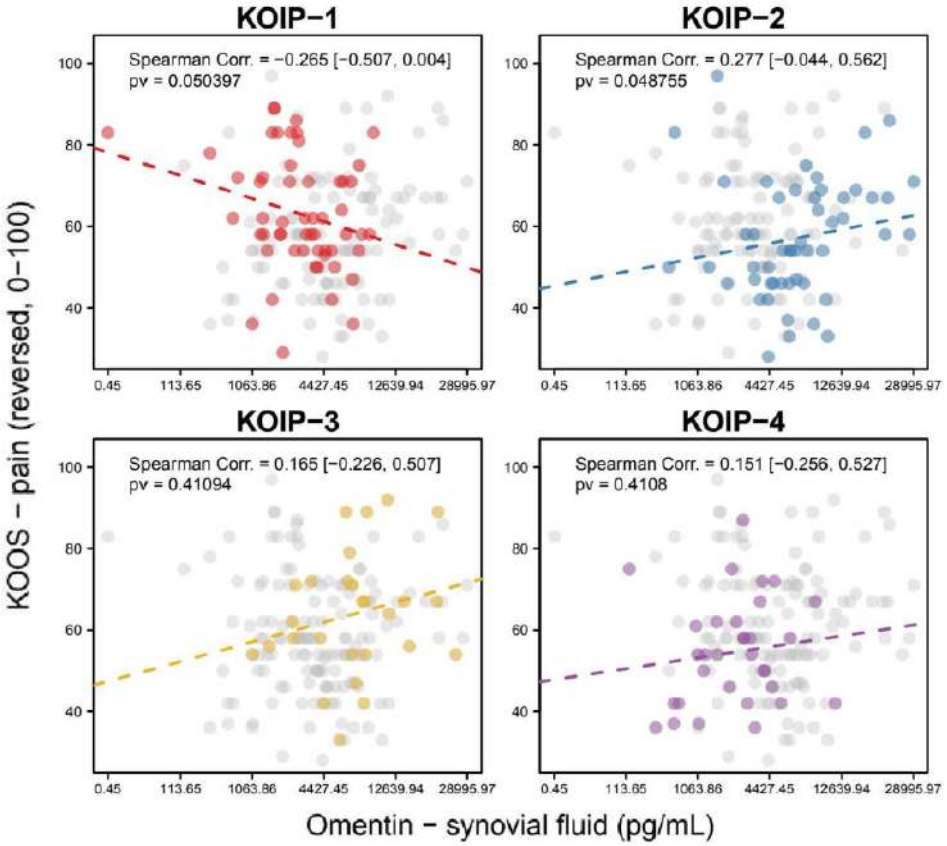

Fig. 2 Association of synovial omentin with baseline pain measured by Knee injury and Osteoarthritis Outcome Score (KOOS, reversed score) within each Knee Osteoarthritis Inflammatory Phenotype (KOIP). The panels show the scatter plots for omentin and the KOOS scores in each KOIP group separately, the Spearman correlation coefficient, and its corresponding asymptotic 95% confidence interval (between brackets) and p-value. Omentin values are represented in a transformed scale according to Tukey's ladder of powers, to symmetrize their distribution and make them more suitable for graphical representation (transformation parameter, $g = 0.25$); x-axis labels are shown in the original scale. Values from patients not belonging to the indicated KOIP group are represented in grey. Corr., correlation; pv, *p*-value; KOOS, Knee injury and Osteoarthritis Outcome Scores (reversed scores); KOIP, knee osteoarthritis inflammatory phenotype

we assessed the association with severity and progression of a panel of 13 cytokines quantified in the plasma and the synovial fluid of patients with inflammatory KOA, separately for each KOIP group in our cohort. When comparing these results globally, associations with KOA outcomes were significantly of higher magnitude within the KOIP groups than for the overall patients' series, and a KOIP-specific behaviour was observed for some of the analysed cytokines. In our opinion, the primary significance of these results does not lay in the results of these specific cytokines themselves, whose interpretation is limited by the sample size, but rather in underscoring the crucial role of phenotyping in advancing our comprehension of the disease.

For purely illustrative purposes, we point to the case of omentin, which has been studied by us and others with mixed results [10, 35, 49, 50]. In our previous study, we showed that extreme values of these cytokines contributed to characterize phenotypes in agreement with their metabolic profile (high for KOIP-2 and KOIP-3 and low for KOIP-1 and KOIP-4), but with different levels of clinical severity (more severe in KOIP-1 and KOIP-3 than in

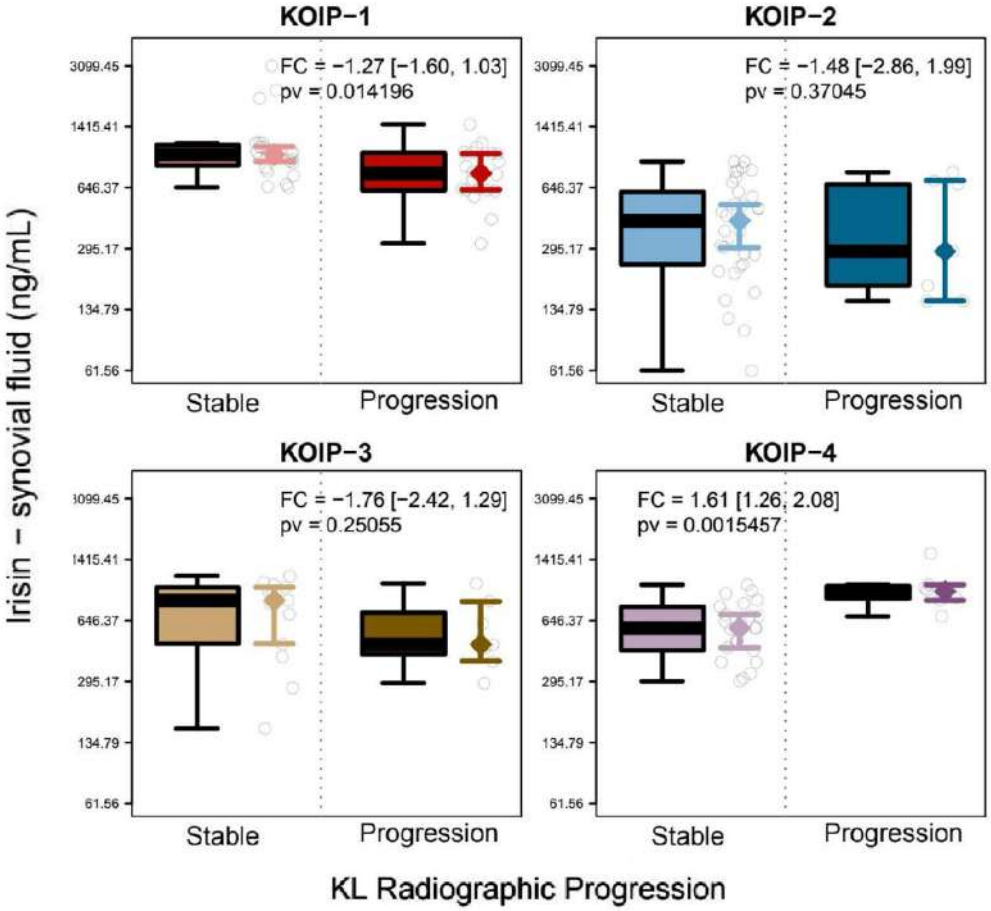

Fig. 3 Association of synovial irisin with radiographic progression according to the Kellgren-Lawrence (KL) criteria within each knee osteoarthritis inflammatory phenotype (KOIP). The panels show the boxplots and stripcharts for irisin by patient groups in each KOIP group separately according to the patients' progression status, as well as the group medians and their corresponding 95% confidence intervals. The legends display the fold changes (FC) between the groups, their 95% confidence intervals (between brackets), and the *p*-value for group comparisons derived from a Mann-Whitney test. A positive FC indicates higher average levels of irisin in progressors while negative FCs represent higher irisin levels in stable patients. Irisin values are represented in a transformed scale according to Tukey's ladder of powers to symmetrize their distribution and make them more suitable for graphical representation (transformation parameter, $g = 0$); *y*-axis labels are shown in the original scale. FC, fold change; pv, *p*-value; KL, Kellgren-Lawrence; KOIP, knee osteoarthritis inflammatory phenotype

KOIP-2 and KOIP-4) [35]. In this work, we also showed that this cytokine displays an association with severity and radiographic progression of opposite sign depending on the KOIP considered (positive in KOIP-2 but negative in KOIP-1). These stratified analyses revealed several other examples of such KOIP-specific associations with KOA outcomes for the evaluated cytokines, both in the plasma and the synovial fluid, which are provided in the "Results" section and the supplementary material of this work. Importantly, most of these associations retained their magnitude and statistical significance after adjustment by age, disease evolution time, and BMI. While the analyses in this study were based on a small number of patients (ranging from 27 to 55) and

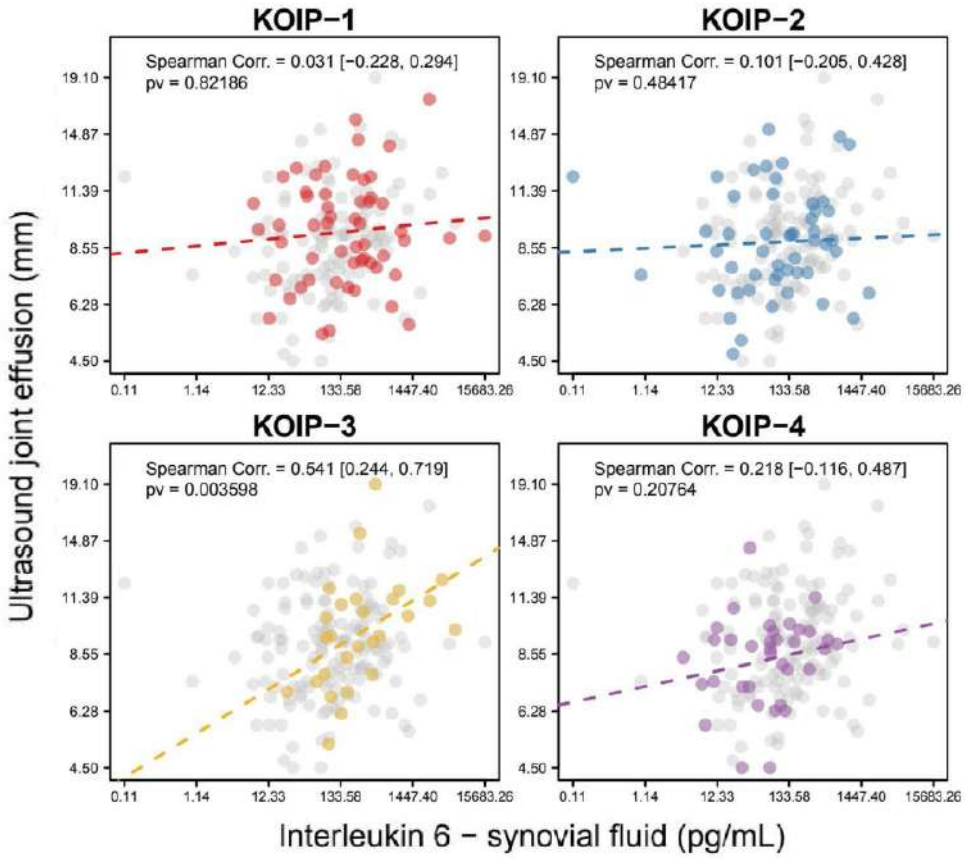

Fig. 4 Association of synovial interleukin 6 with joint effusion within each knee osteoarthritis inflammatory phenotype (KOIP). The panels show the scatter plots for interleukin 6 protein and joint effusion (mm) measured by ultrasound in each KOIP group separately, the Spearman correlation coefficient, and its corresponding asymptotic 95% confidence interval (between brackets) and *p*-value. Interleukin 6 protein values are represented in a transformed scale according to Tukey's ladder of powers, to symmetrize their distribution and make them more suitable for graphical representation (transformation parameter, $g = 0.25$); *x*-axis labels are shown in the original scale. Values from patients not belonging to the indicated KOIP group are represented in grey. Corr., correlation; pv, *p*-value; KOOS, Knee injury and Osteoarthritis Outcome Scores (reversed scores); KOIP, knee osteoarthritis inflammatory phenotype

should be interpreted with caution, their results provide further evidence of specific risk and prognosis factors across these KOIP phenotypes and divergent pathophysiological pathways and disease evolution (endotypes). These results also suggest that differential inflammatory mechanisms may be responsible for the inconsistencies observed in previous research on OA biomarkers and, therefore, the current lack of translational results, likely due to variations in the distribution of KOA phenotypes among the subjects selected for these studies [21, 51].

Importantly, OA pathophysiology is probably too complex to be attributed to a few numbers of cytokines [20, 52–54]. Hence, the purpose of our study was not to point the relevance of a specific set of cytokines or inflammatory factors regarding the clinical or radiographic severity in KOA, an objective for which a larger sample size would be required. Rather, we aimed to illustrate the potential of a precise phenotyping in identifying the inflammatory and metabolic pathways of the disease (endotypes). In contrast to other rheumatic conditions,

the inflammatory profile of OA patients is characterized by a relatively lower number of markers that are highly altered in its clinical presentation, and the interaction and modulatory effects may play a significant role in this context of a low-grade, persistent inflammation state [16, 55, 56]. It is noteworthy that correlations exceeding 0.25 (in absolute value) between cytokines and outcomes were observed in varying proportions among KOIP groups, ranging from 12% (KOIP-1) to 24% (KOIP-4). When we raised this threshold to 0.40, the percentages ranged from 3% (KOIP-1 and KOIP-2) to 9% (KOIP-3). While these proportions are considerably higher compared to those observed in the entire patient series (1% for > 0.25; 0% for > 0.40), we acknowledge that the effect sizes are not exceptionally large, even within the identified phenotypes. For this reason, the identification of specific biomarkers remains a critical challenge for improving patient classification and elucidating the molecular mechanisms underlying each phenotype. The identification of such biomarkers would have significant implications for research and clinical practice, as they may facilitate tailored treatments and the discovery of new therapeutic targets [22]. In this regard, the use of Omics technologies holds great potential for making advances in this objective, and our group is currently pursuing this line of research.

Our study was conducted on a prospective cohort of female KOA patients with joint effusion, which constitutes a highly homogeneous group of subjects. Our study was focused on female patients, as they were the majority in our cohort (84%), and several sex-specific differences exist in terms of prevalence, metabolic and inflammatory conditions, and pain and disability levels [12, 13]. Although this homogeneity can provide an advantage for identifying disease biomarkers, we acknowledge that it might also limit the generalization of our results. Hence, further studies are needed in independent series of patients from other centres, with a sufficient sample size and different characteristics and clinical presentations, including males and non-inflammatory presentations, in order to evaluate the generalization of these findings. In addition, and as highlighted earlier in this section, our study's primary objective was not to emphasize the relevance of specific cytokines in relation to clinical or radiographic severity in KOA, an objective that would require a larger sample size. Beyond the general picture represented in Fig. 1 and Additional file 1: Fig. S1, the results for each cytokine are considered exploratory when considered individually and that was the reason for not adjusting them for the large number of comparisons performed. Hence, the interpretation of these results at the cytokine level should be approached with caution, as they also require further validation in future studies specifically designed for this purpose. Together with the moderate effect sizes found in these analyses and the scarce knowledge in the literature on KOA phenotypes and their specific pathophysiology, our study does not allow for strong interpretations at this level, as they would be too speculative. Another limitation in our study is the absence of pure quantitative measurements for radiographic severity, such as the minimum or fixed joint-space width measurements in millimetres over time. These measurements could have provided better resolution, enhance statistical power, and, possibly, reveal additional associations not identified in our current analyses. Unfortunately, this kind of quantification is not currently available in our patients' series and constitutes a relevant area of research for future studies. On the other hand, our study is distinguished from previously published works by its exhaustive availability of data, including a panel of 13 cytokines quantified in the plasma and synovial fluid of 168 patients. These samples and data were systematically collected within the protocols of a prospective cohort specifically designed to study the factors associated with KOA severity and progression, which is a remarkable strength of the study.

Conclusion

Overall, our study provides further evidence to support the notion that KOA is a multifaceted syndrome composed of multiple phenotypes with differing pathophysiological pathways, which provide a possible explanation for the inconsistencies observed across previous studies on the role of cytokines in OA and the lack of translational results to the date. Our findings also highlight the potential benefits of accurate phenotyping of KOA patients for both research and clinical practice, including patient stratification, personalized therapy design, patient selection for clinical trials, and the discovery of novel treatments. Moving forward, large-scale studies using Omics-based biomarker technologies are needed to confirm and allow the reproducibility of the KOIP classification, evaluate its generalizability to other patient populations, and precisely determine its clinical relevance.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13075-023-03244-y.

Additional file 1.

Additional file 2.

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Authors' contributions
JC, AB, CO, and JG contributed to the conception and design of the study. MR, MG, CO, SG, MLL, MA, and CG contributed to the acquisition of the data. CA, RG, AS, and AC contributed to the blood sample extraction, processing, storage, and analysis performing. JC, AB, CO, MG, MLL, MA, MR, SG, and CG contributed to the analysis and interpretation of the data. JC is the papers' guarantor. All authors contributed to the drafting of the article or to the critical revision for relevant intellectual content. All authors gave the final approval of the version to be submitted.

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Availability of data and materials
All data and code used in this study are available upon reasonable request.

Declarations

Ethics approval and consent to participate
The project was evaluated and approved by the ethical committee of our centre (CEIm Parc Taulí) with approval number 2015/539. This study involves human participants. The study is being conducted in compliance with the protocol, Good Clinical Practice (GCP), the Declaration of Helsinki, and applicable ethical and legal regulatory requirements. All participants have received oral and written information and provided written informed consent authorizing the collection of samples and data for their use in the context of knee osteoarthritis studies.

Consent for publication
The data used in the current study are available from the corresponding author upon reasonable request.

Competing interests
The authors declare that they have no competing interests.

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Publisher's Note

Supplementary Table S1. List of variables used in our study. The list Includes the anthropometric, metabolic and inflammatory factors used in the discovery analysis of Knee Osteoarthritis (KOA) Inflammatory Phenotypes (KOIP) and measures of KOA severity and progression. **g:** parameter value for a Tukey ladder power transformation applied to continuous variables (when necessary) to symmetrize their distribution and make them more suitable for Principal Component Analysis (PCA) and linear models for ELISA batch correction. **KOA:** Knee Osteoarthritis; **KOIP:** Knee Osteoarthritis Inflammatory Phenotype; **ATP III:** Adult Treatment Panel III; **KOOS:** Knee injury and Osteoarthritis Outcome Scores.

Set	Variable type	Variable (units / values)	Type (transformation parameter)
Phenotype Discovery (KOIP)	Anthropometrics	Weight (Kg)	Continuous (g = -0.50)
		Body Mass Index	Continuous (g = -0.75)
		Waist Circunference (cm)	Continuous
		Waist-Hip ratio	Continuous (g = -2)
		Body Fat percentage (%)	Continuous (g = 2)
	Metabolic factors	Arterial Hypertension (Yes / No)	Binary
		Dyslipidaemia (Yes / No)	Binary
		Diabetes Mellitus (Yes / No)	Binary
		ATP III Metabolic Syndrome (Yes / No)	Binary
		High-Density Lipoprotein (mg/dL)	Continuous (g = 0)
		Low-Density Lipoprotein (mg/dL)	Continuous (g = 0.75)
		Total Cholesterol (mg/dL)	Continuous
		Triglycerides (mg/dL)	Continuous (g = 0)
		Glucose (mg/dL)	Continuous (g = -1.50)
		Glycated Hemoglobin (%)	Continuous (g = -2)
		Insulin (microU/mL)	Continuous (g = 0.25)
		25-hydroxy vitamin D (ng/mL)	Continuous (g = -0.25)
		Uric acid (mg/dL)	Continuous (g = 0.50)
		Physical exercise (None / Sporadic / Regular / Vigorous)	Ordinal
	Inflammation markers - plasma	C-reactive protein - plasma (mg/L)	Continuous (g = 0)
		Interleukin 6 - plasma (pg/mL)	Continuous (g = 0)
		Interleukin 8 - plasma (pg/mL)	Continuous (g = -0.25)
		Tumor Necrosis Factor alpha - plasma (pg/mL)	Continuous (g = 0)
		Nerve Growth Factor - plasma (pg/mL)	Continuous (g = 0.25)
		Calprotectin - plasma (ng/mL)	Continuous (g = -0.50)
	Inflammation markers - synovial fluid	C-reactive protein - synovial fluid (mg/L)	Continuous (g = 0.25)
		Interleukin 6 - synovial fluid (pg/mL)	Continuous (g = 0)
		Interleukin 8 - synovial fluid (pg/mL)	Continuous (g = 0)
		Tumor Necrosis Factor alpha - synovial fluid (pg/mL)	Continuous (g = 0)
		Nerve Growth Factor - synovial fluid (pg/mL)	Continuous (g = 0.25)
		Calprotectin - synovial fluid (ng/mL)	Continuous (g = 0.25)
	Adipocytokines / Myokines - plasma	Leptin - plasma (pg/mL)	Continuous (g = 0.25)
		Irisin - plasma (ng/mL)	Continuous (g = 0.75)
		Visfatin - plasma (ng/mL)	Continuous (g = -0.25)
		Resistin - plasma (pg/mL)	Continuous (g = -0.50)
		Osteopontin - plasma (ng/mL)	Continuous (g = -0.25)
		Adiponectin - plasma (ng/mL)	Continuous (g = 0)
		Omentin - plasma (pg/mL)	Continuous (g = 0.50)
	Adipocytokines / Myokines - synovial fluid	Leptin - synovial fluid (pg/mL)	Continuous (g = 0.25)
		Irisin - synovial fluid (ng/mL)	Continuous (g = 0)
		Visfatin - synovial fluid (ng/mL)	Continuous (g = 0)
		Resistin - synovial fluid (pg/mL)	Continuous (g = 0)
		Osteopontin - synovial fluid (ng/mL)	Continuous (g = 0)
		Adiponectin - synovial fluid (ng/mL)	Continuous (g = -0.25)
		Omentin - synovial fluid (pg/mL)	Continuous (g = 0.25)
KOA severity and progression	Radiography - baseline	Kellgren-Lawrence radiographic grade (1-4)	Categorical - four categories
		Osteophytes score (0-10)	Continuous
		Joint space narrowing (0-4)	Categorical - five categories
	Clinical severity - baseline	KOOS - pain (reversed, 0-100)	Continuous
		KOOS - symptoms (reversed, 0-100)	Continuous
		KOOS - functional disability (reversed, 0-100)	Continuous
	Ultrasound - baseline	Joint effusion (mm)	Continuous
		Synovial tissue thickness (mm)	Continuous
	Radiographic progression (2 years follow-up)	Kellgren-Lawrence radiographic progression (Yes / No)	Binary
		Osteophytes radiographic progression (Yes / No)	Binary
		Joint space narrowing radiographic progression (Yes / No)	Binary

Supplementary Table S2. KOIP membership prediction for the KOA female patients included in the study using supervised Random Forest (RF). Columns show the prediction accuracy for all variables participating in the clustering analysis (All variables, 45 in total) and the most informative predictors according to VSURF, a sequential selection procedure based on RF models,which selected those variables related to the response for interpretation purposes (interpretation mode, 27 variables) and those remaining after removing redundancy for prediction purposes (prediction mode, 15 variables). **KOIP:** Knee Osteoarthritis Inflammatory Phenotype.

	All (45 predictors)	VSURF Interpretation mode (27 predictors)	VSURF Prediction mode (15 predictors)
KOIP-1	83.3%	83.3%	81.2%
KOIP-2	95.3%	93.0%	90.7%
KOIP-3	96.0%	88.0%	84.0%
KOIP-4	84-6%	80.8%	73.1%
Overall	89.4%	86.6%	83.1%

Supplementary Table S3. Association of Knee Osteoarthritis Inflammatory Phenotypes (KOIP) and **pain** measured by Knee injury and Osteoarthritis Outcome Score (KOOS, reversed score). Table columns display: group medians and their 95% confidence intervals (95%CI); p-values for pairwise comparisons between groups (Mann-Whitney test); and the global p-value for assessing differences across phenotypes (Kruskal-Wallis test). **KOIP:** Knee Osteoarthritis Inflammatory Phenotype; **95%CI:** 95% confidence interval.

	KOOS - pain (reversed, 0-100)	Pairwise p-values				Global p-value
	Median [95%CI]	KOIP-1	KOIP-2	KOIP-3	KOIP-4	
KOIP-1	61.0 [58.0, 71.0]		0.0217	0.8313	0.0327	0.0298
KOIP-2	54.0 [50.0, 62.0]	0.0217		0.0566	0.7711	
KOIP-3	64.0 [54.0, 71.0]	0.8313	0.0566		0.0535	
KOIP-4	54.0 [50.0, 62.0]	0.0327	0.7711	0.0535		

Supplementary Table S4. Association of Knee Osteoarthritis Inflammatory Phenotypes (KOIP) and **functional disability** measured by Knee injury and Osteoarthritis Outcome Score (KOOS, reversed score). Table columns display: group medians and their 95% confidence intervals (95%CI); p-values for pairwise comparisons between groups (Mann-Whitney test); and the global p-value for assessing differences across phenotypes (Kruskal-Wallis test). **KOIP:** Knee Osteoarthritis Inflammatory Phenotype; **95%CI:** 95% confidence interval.

	KOOS functional disability (reversed, 0-100)	**Pairwise p-values**				**Global p-value**
	Median [95%CI]	**KOIP-1**	**KOIP-2**	**KOIP-3**	**KOIP-4**	
KOIP-1	63.0 [54.0, 68.0]		0.0033	0.8667	0.0595	0.0084
KOIP-2	54.0 [49.0, 59.0]	0.0033		0.0106	0.7052	
KOIP-3	62.0 [51.0, 75.0]	0.8667	0.0106		0.0735	
KOIP-4	56.0 [46.0, 63.0]	0.0595	0.7052	0.0735		

Supplementary Table S5. Association of Knee Osteoarthritis Inflammatory Phenotypes (KOIP) and **radiographic progression** at two-years of follow-up according to **Kellgren-Lawrence** scale. Table columns display: the number of progressors over the total of subjects; the group progression percentages and their 95% confidence intervals (95%CI); the p-values for pairwise comparisons between groups; and the global p-value for assessing differences across phenotypes (Fisher's test for contingency tables). **KOIP:** Knee Osteoarthritis Inflammatory Phenotype; **95%CI:** 95% confidence interval.

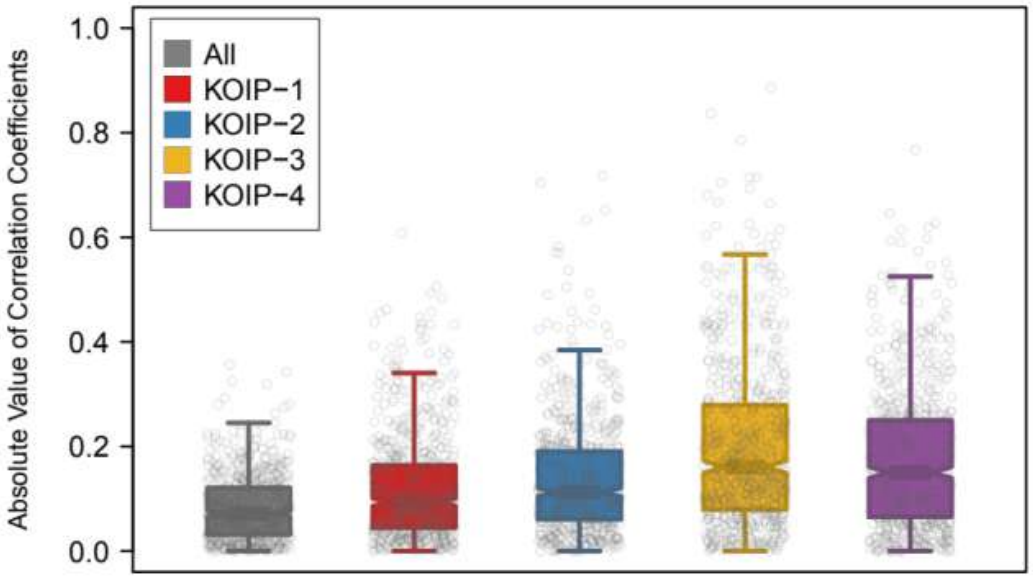
	Kellgren-Lawrence radiographic progression		Pairwise p-values				Global p-value
	Nb progressed / Total	% [95%CI]	KOIP-1	KOIP-2	KOIP-3	KOIP-4	
KOIP-1	21/46	45.7% [31.2, 60.8]		0.0143	0.5886	0.1468	0.0709
KOIP-2	9/44	20.5% [10.3, 35.8]	0.0143		0.2294	0.5814	
KOIP-3	7/20	35.0% [16.3, 59.1]	0.5886	0.2294		0.5467	
KOIP-4	8/30	26.7% [13.0, 46.2]	0.1468	0.5814	0.5467		

Supplementary Table S6. Association of Knee Osteoarthritis Inflammatory Phenotypes (KOIP) and **radiographic progression** at two-years of follow-up according to formation of **osteophytes**. Table columns display: the number of progressors over the total of subjects; the group progression percentages and their 95% confidence intervals (95%CI); the p-values for pairwise comparisons between groups; and the global p-value for assessing differences across phenotypes (Fisher's test for contingency tables). **KOIP:** Knee Osteoarthritis Inflammatory Phenotype; **95%CI:** 95% confidence interval.

	Osteophytes radiographic progression		Pairwise p-values				Global p-value
	Nb progressed / Total	Median [95%CI]	KOIP-1	KOIP-2	KOIP-3	KOIP-4	
KOIP-1	40/49	81.6% [67.5, 90.8]		0.1545	0.008	0.0093	0.0093
KOIP-2	30/44	68.2% [52.3, 80.9]	0.1545		0.1723	0.2191	
KOIP-3	10/21	47.6% [26.4, 69.7]	0.0080	0.1723		0.9999	
KOIP-4	15/29	51.7% [32.9, 70.1]	0.0093	0.2191	0.9999		

Supplementary Table S7. Association of Knee Osteoarthritis Inflammatory Phenotypes (KOIP) and **radiographic progression** at two-years of follow-up according to the increase of **joint spacing narrowing**. Table columns display: the number of progressors over the total of subjects; the group progression percentages and their 95% confidence intervals (95%CI); the p-values for pairwise comparisons between groups; and the global p-value for assessing differences across phenotypes (Fisher's test for contingency tables). **KOIP:** Knee Osteoarthritis Inflammatory Phenotype; **95%CI:** 95% confidence interval.

	Joint space narrowing radiographic progression		Pairwise p-values				Global p-value
	Nb progressed / Total	Median [95%CI]	KOIP-1	KOIP-2	KOIP-3	KOIP-4	
KOIP-1	23/49	46.9% [32.8, 61.6]		0.0178	0.4297	0.3463	0.1110
KOIP-2	10/44	22.7% [12.0, 38.2]	0.0178		0.3811	0.2951	
KOIP-3	7/21	33.3% [15.5, 56.9]	0.4297	0.3811		0.9999	
KOIP-4	10/29	34.5% [18.6, 54.3]	0.3463	0.2951	0.9999		



Suplementary Figure S1. Magnitudes of the associations between cytokines and KOA outcomes stratified by Knee Osteoarthritis Inflammatory Phenotypes (**KOIP**). The boxplot shows the absolute value of the correlation-like measurements used for assess the associations between the cytokines evaluated in the study and baseline clinical (KOOS pain, functional disability and symptoms), ultrasound (effusion and synovial tissue thickness), radiographic severity (Kellgren-Lawrence, osteophytes and joint space narrowing), and progression at two years of follow-up according to the same radiographic criteria. Correlation-like measurements were: Spearman correlations (continuous or ordinal outcomes) and Glass rank biserial correlations (binary outcomes). Kellgren-Lawrence staging and joint space marrowing at baseline were treated as ordinal in these analyses. Differences between each KOIP and the whole dataset (*All*) were statistically significant according to a Wilcoxon test (all p-values < 1.30e-16). **KOIP:** Knee Osteoarthritis Inflammatory Phenotype.

Suplementary Figures S2-S33. Association of synovial and plasma **cytokines** with **clinical severity and radiographic progression** within each Knee Osteoarthritis Inflammatory Phenotype (**KOIP**). En each graphic, the panels show scatter plots, the Spearman correlation coefficient and its corresponding asymptotic 95% confidence interval (between brackets) and p-value (baseline pain, functional disability and joint effusion); or boxplots, stripcharts, group medians, Fold-Change (**FC**) between groups, their corresponding 95% confidence interval and the p-value derived from a Mann-Whitney test (radiographic progression). A positive FC indicates higher average levels of Irisin in progressors while negative FCs represents higher Irisin levels in stable patients. Cytokine's values are represented in a transformed scale according to a Tukey's ladder of powers, to symmetrize their distribution and make them more suitable for graphical representation (see Supplementary Table S2); axis value labels are showed in the original scale. **Corr.:** Correlation; **FC:** Fold-Change; **pv:** p-value; **KOOS:** Knee injury and Osteoarthritis Outcome Scores (reversed scores); **KL:** Kellgren-Lawrence; **JSN:** Joint Space Narrowing; **KOIP:** Knee Osteoarthritis Inflammatory Phenotype.

KOOS functional disability

- S2: synovial Omentin
- S3: plasma Interleukin-6

KOOS pain

- S4: synovial Osteopontin
- S5: plasma Interleukin-6

KL radiographic progression

- S6: synovial Resistin
- S7: synovial C-reactive protein
- S8: plasma Omentin

Osteophytes radiographic progression

- S9: synovial Irisin
- S10: synovial Leptin
- S11: synovial Adiponectin
- S12: synovial Omentin
- S13: synovial Osteopontin
- S14: synovial Calprotectin
- S15: plasma Irisin
- S16: plasma Leptin
- S17: plasma Omentin
- S18: plasma Osteopontin
- S19: plasma Interleukin-8

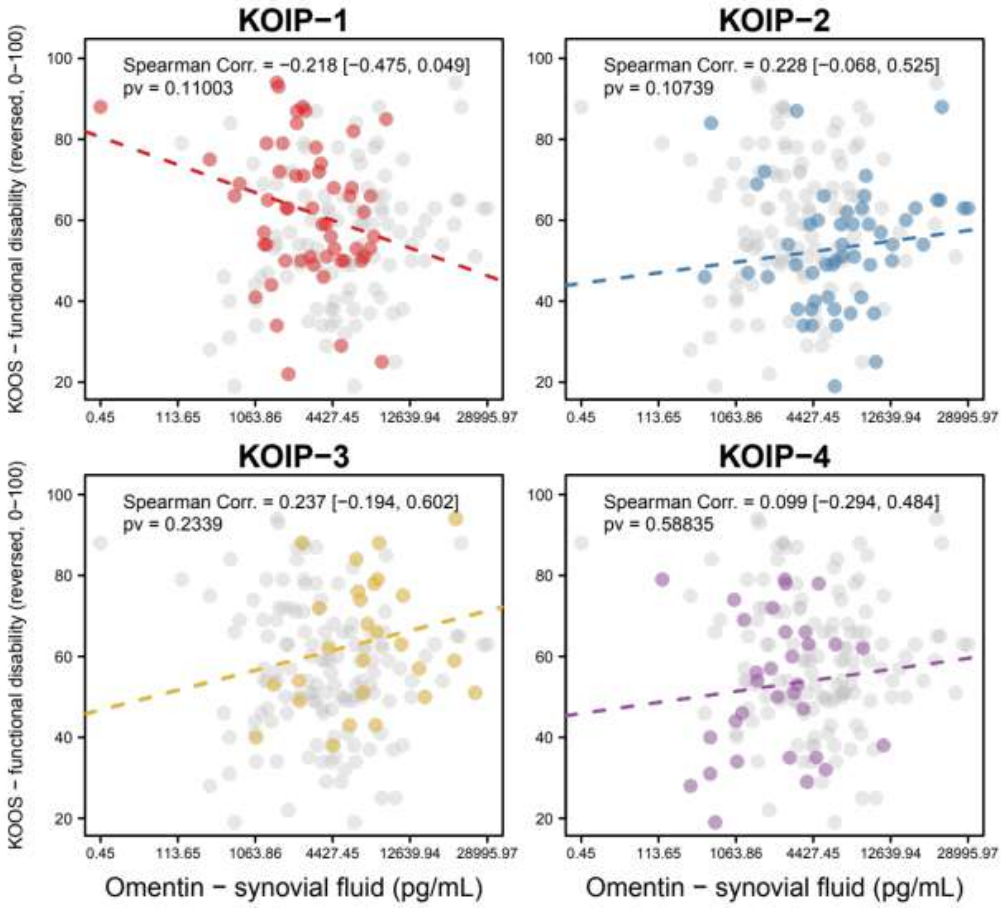
JSN radiographic progression

- S20: synovial Irisin
- S21: synovial Leptin
- S22: synovial Omentin
- S23: synovial Osteopontin
- S24: plasma Irisin
- S25: plasma Leptin

Ultrasound Joint Effusion

- S26: synovial Irisin
- S27: synovial Leptin
- S28: synovial Omentin
- S29: synovial C-readtive protein
- S30: plasma Leptin
- S31: plasma Calprotectin
- S32: plasma Interleukin-6
- S33: plasma C-reactive protein

S2: KOOS functional disability - synovial Omentin



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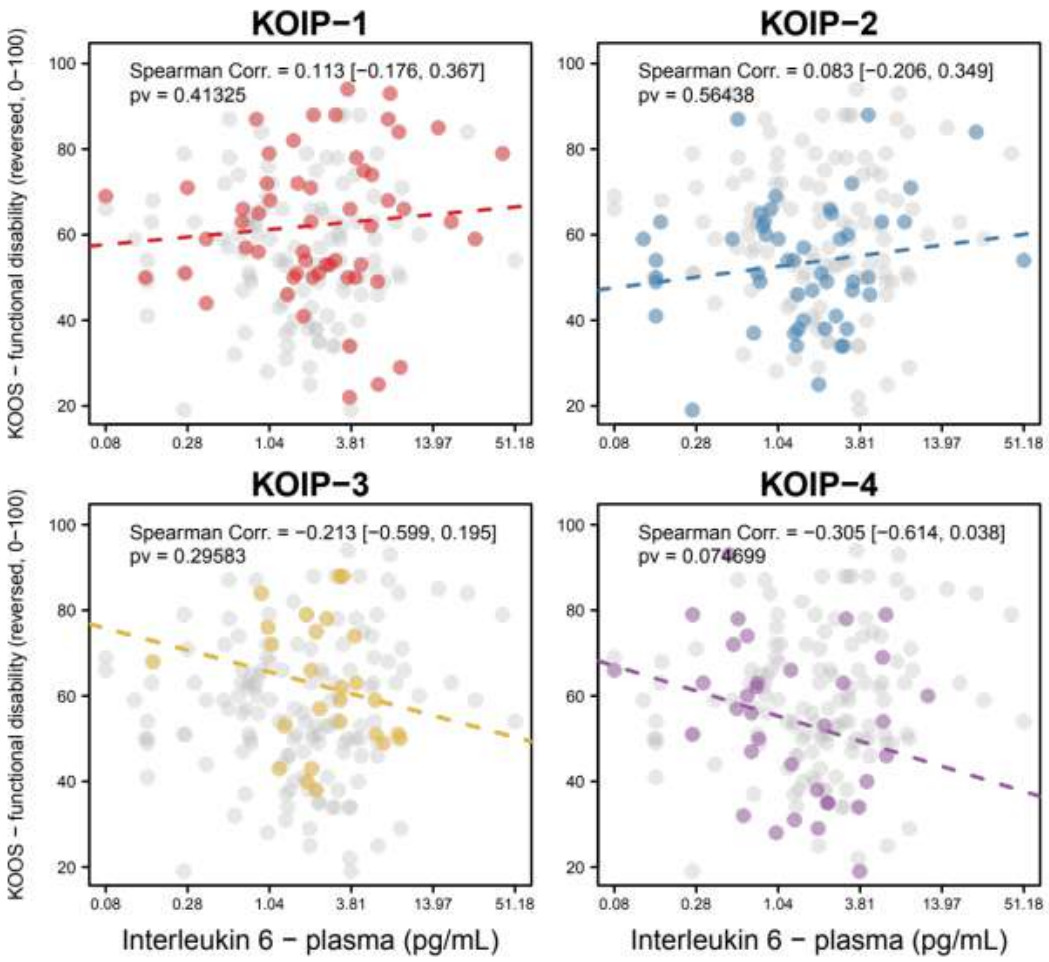
S3: KOOS functional disability - plasma Interleukin-6



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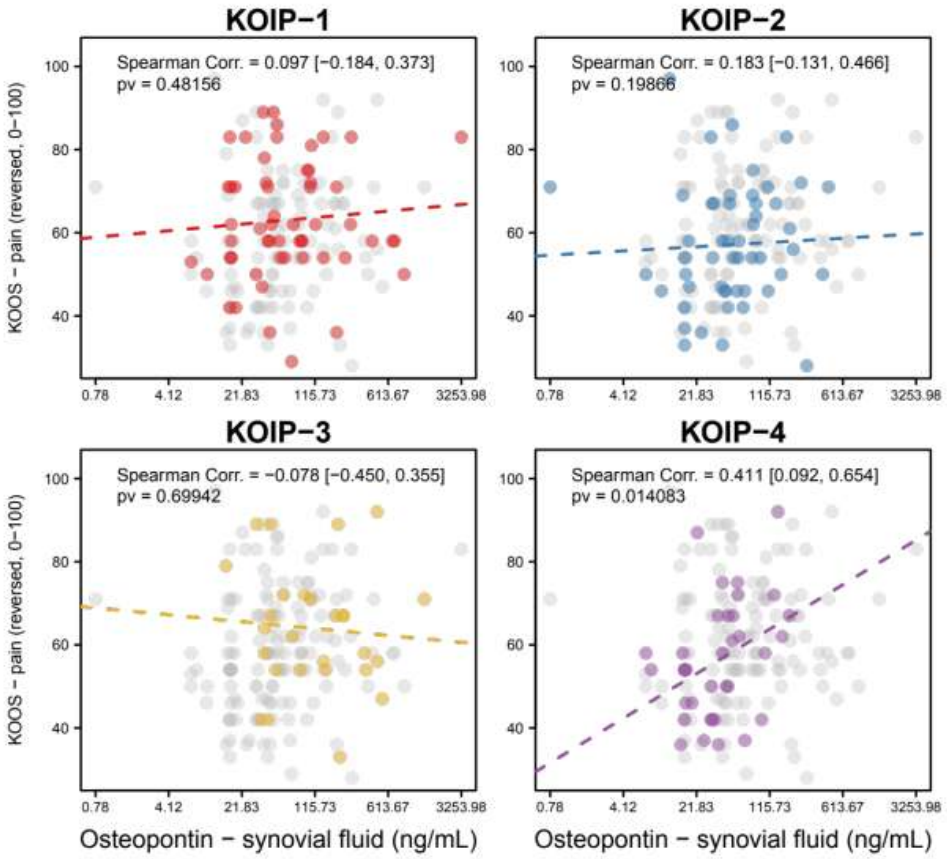
S4: KOOS pain - synovial Osteopontin



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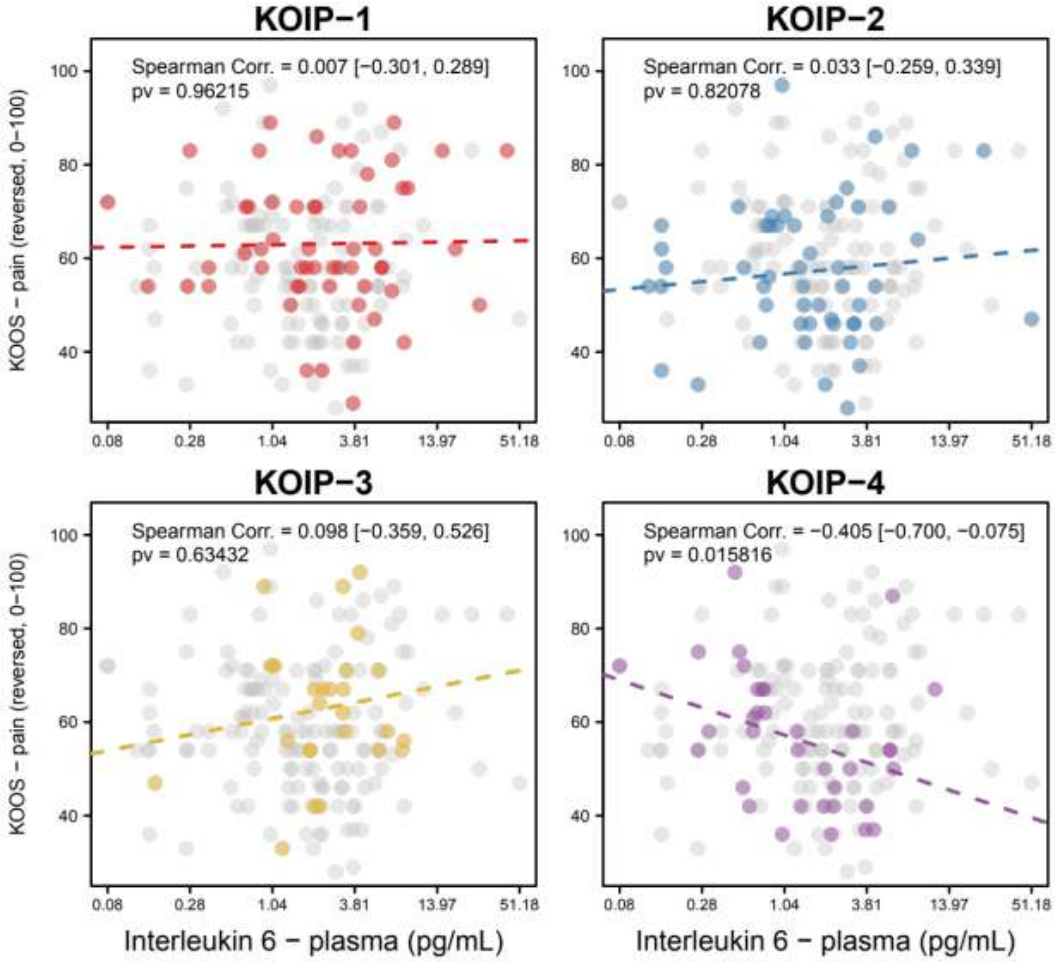
S5: KOOS pain - plasma Interleukin-6



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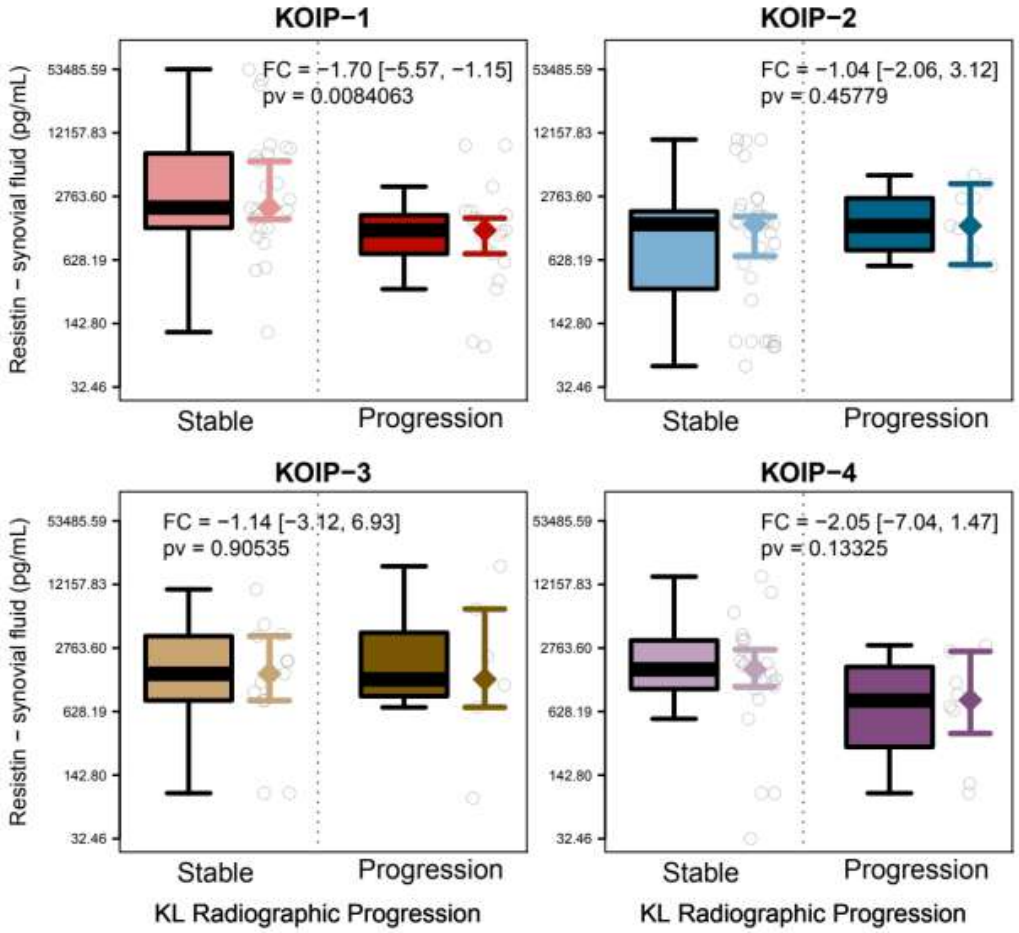
S6: KL radiographic progression - synovial Resistin



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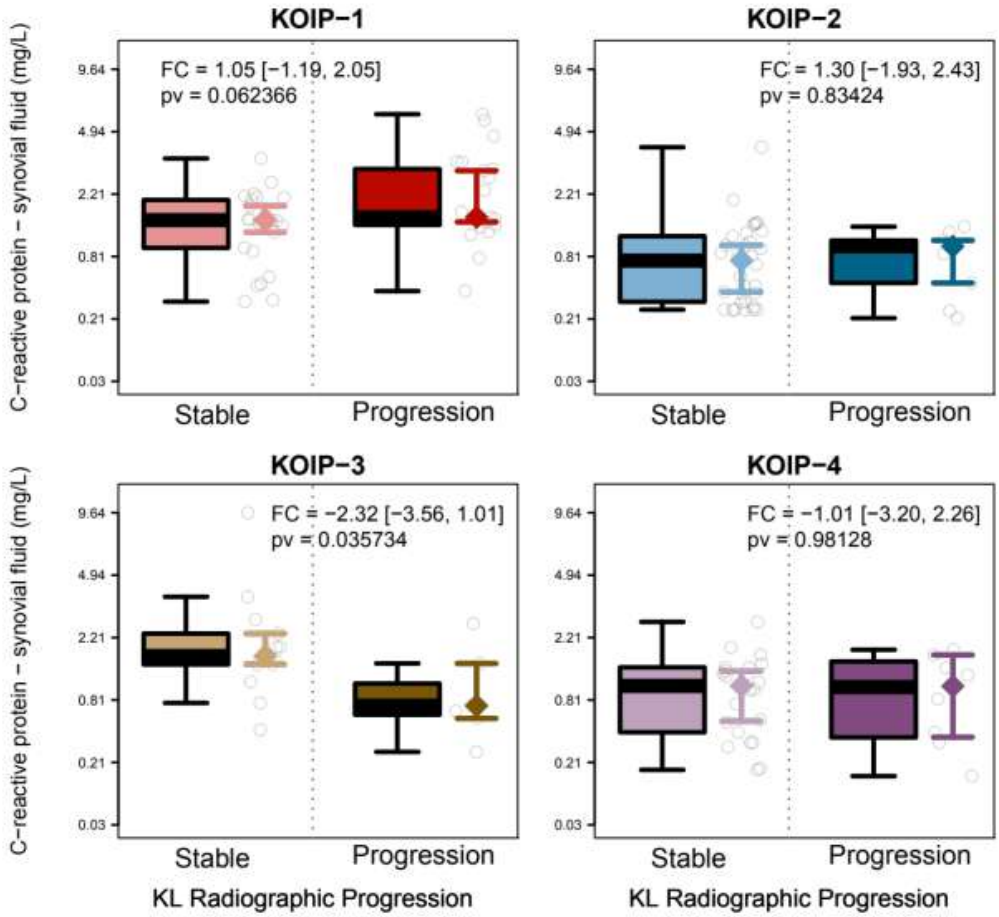
S7: KL radiographic progression - synovial C-reactive protein



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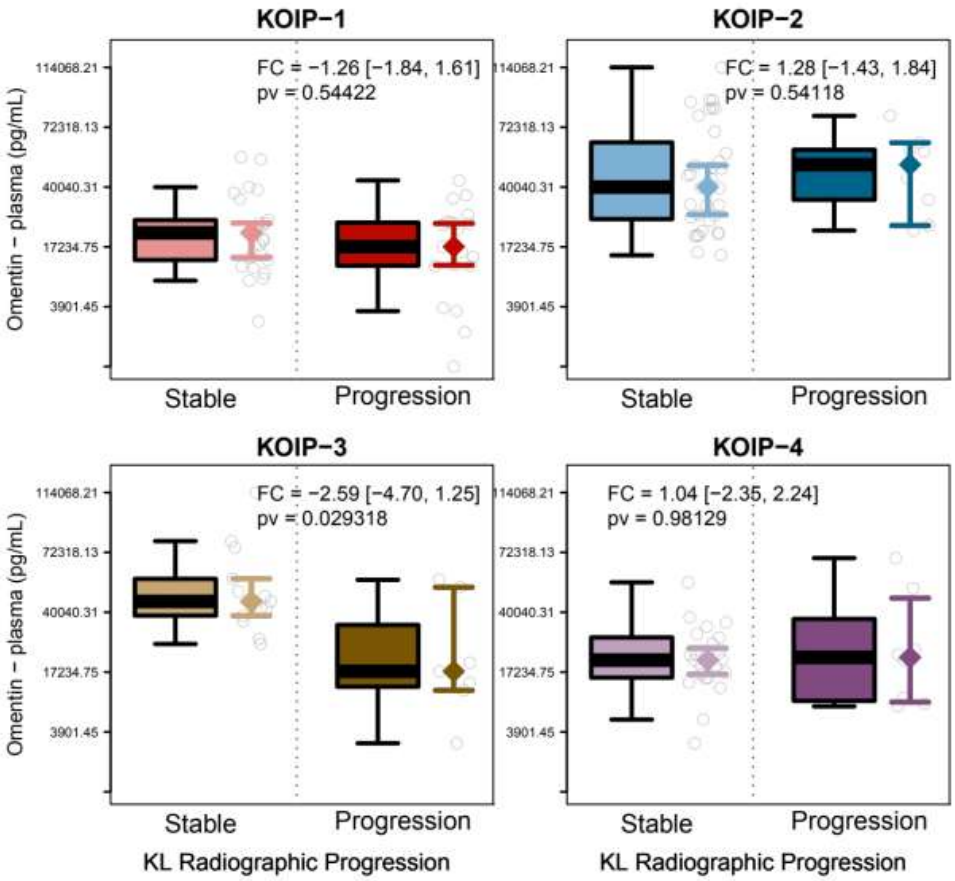
S8: KL radiographic progression - plasma Omentin



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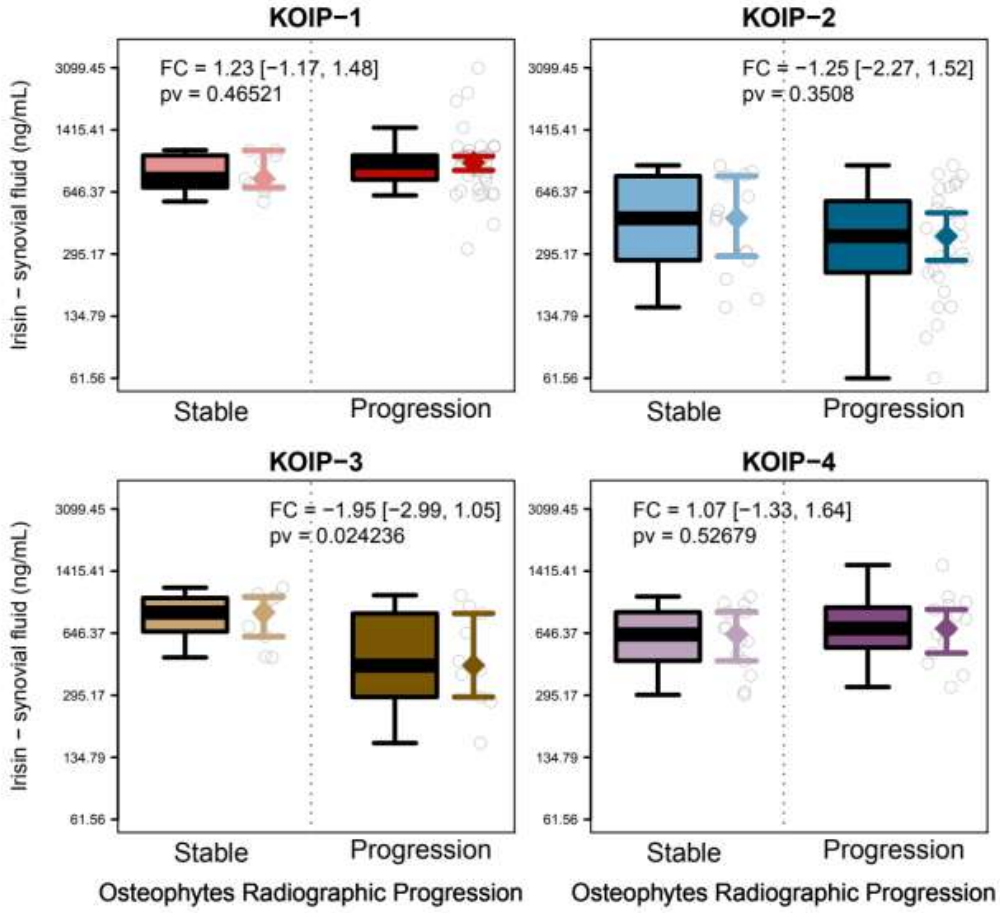
S9: Osteophytes radiographic progression - synovial Irisin



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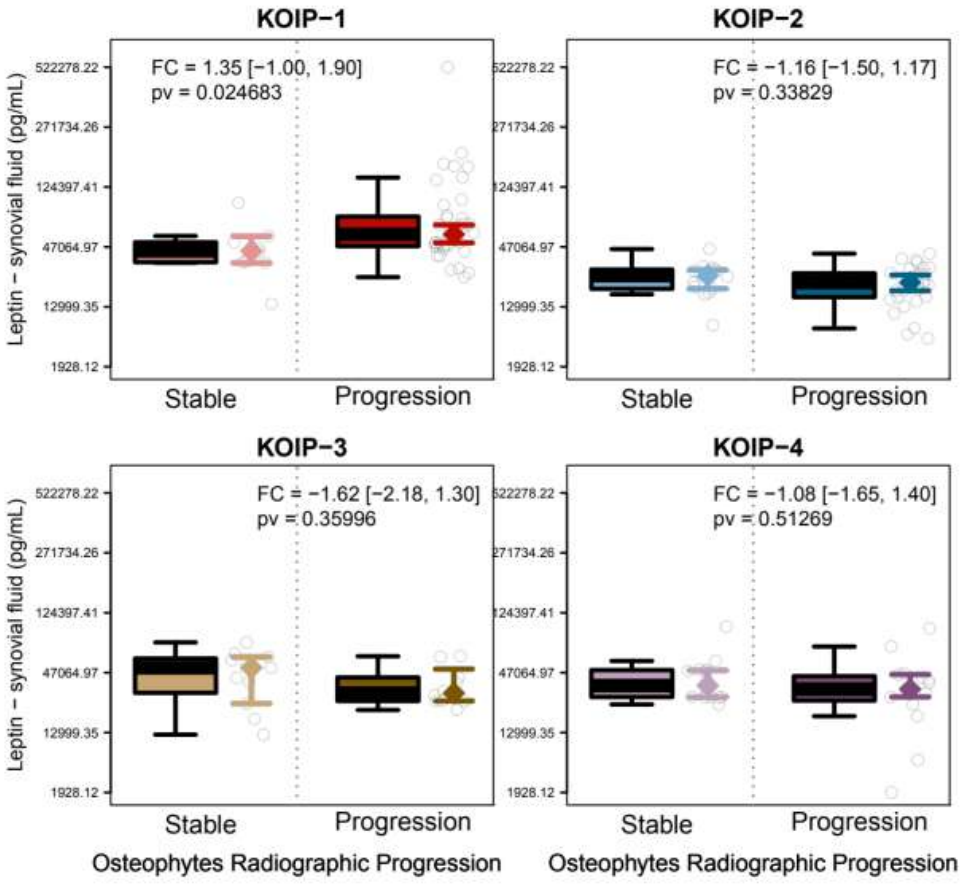
S10: Osteophytes radiographic progression - synovial Leptin



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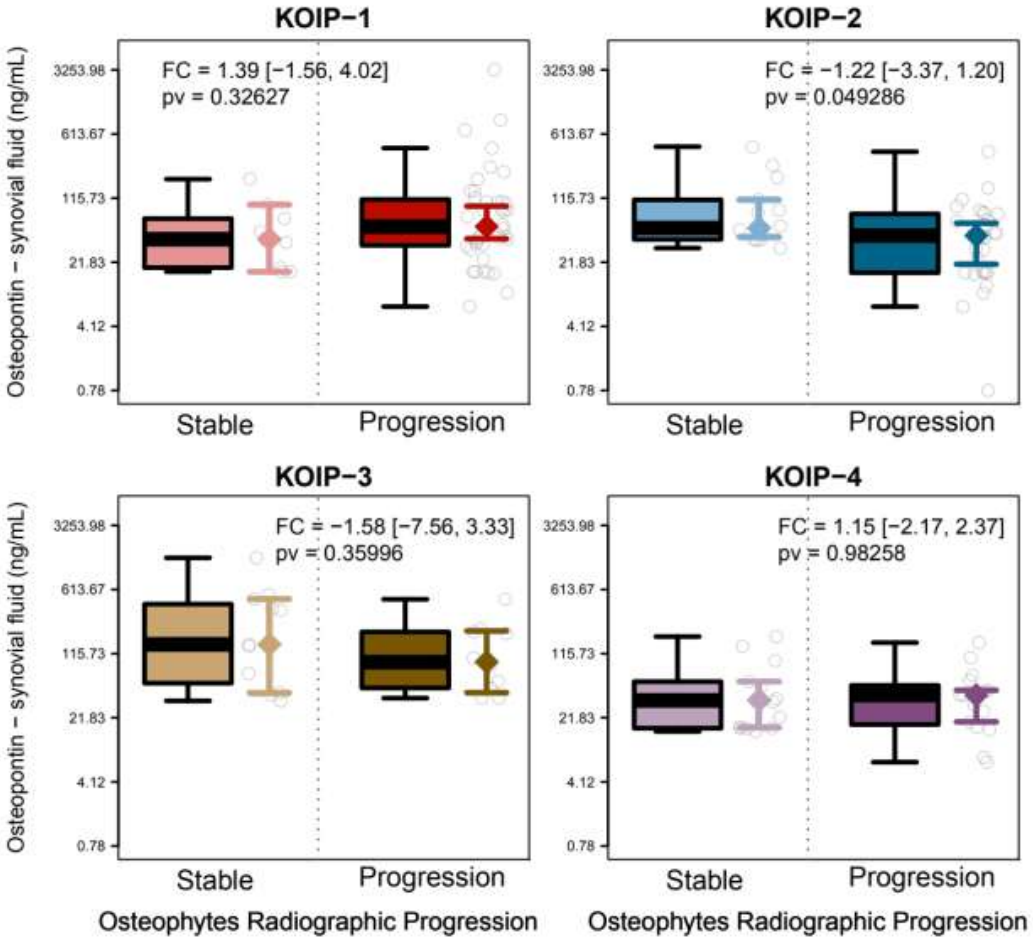
S13: Osteophytes radiographic progression - synovial Osteopontin



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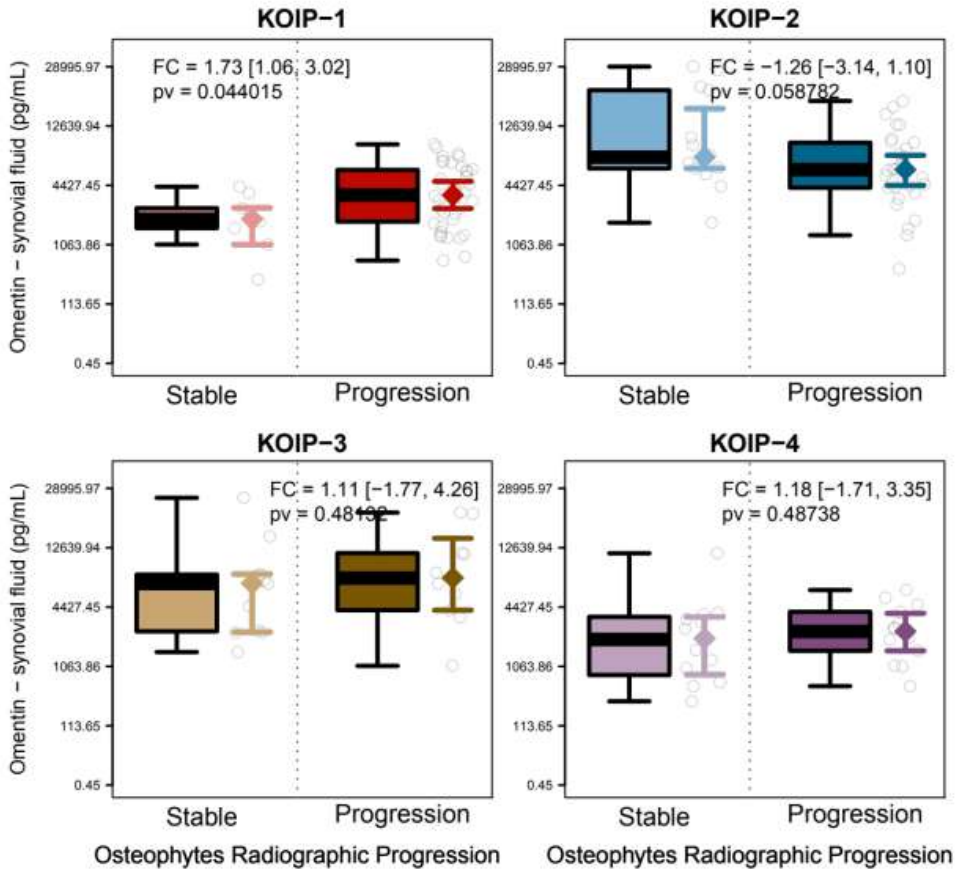
S12: Osteophytes radiographic progression - synovial Omentin



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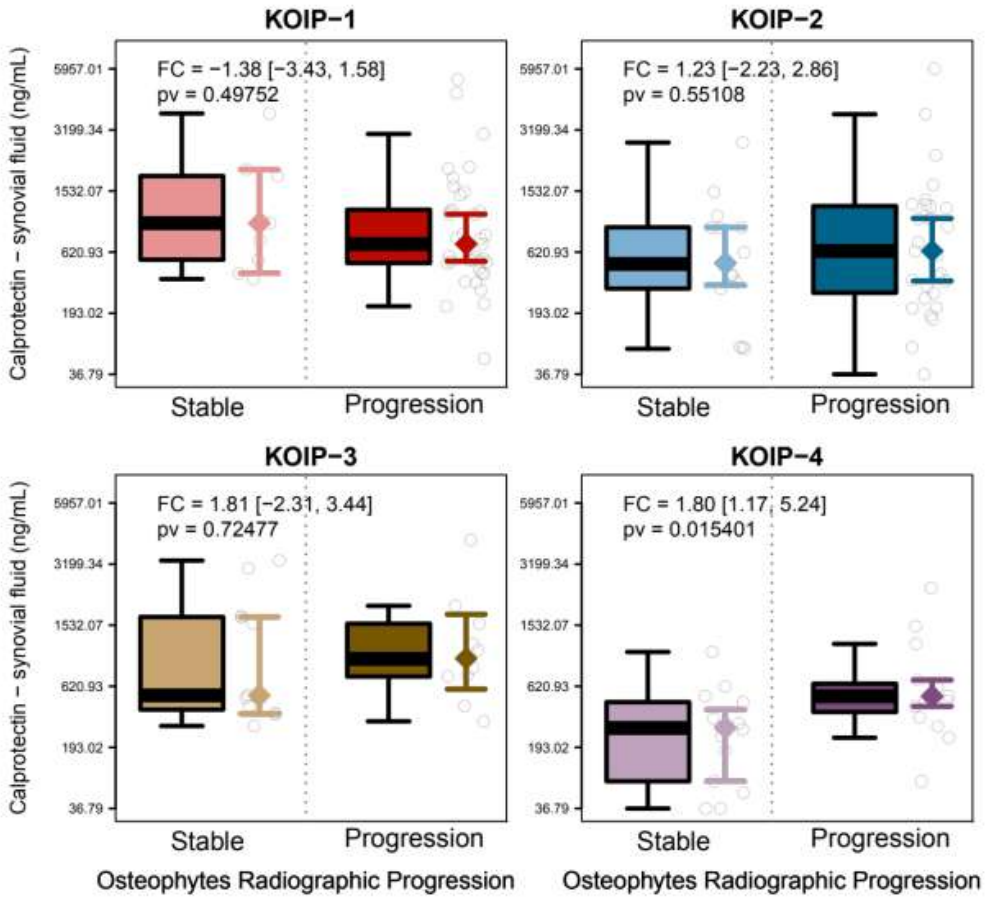
S14: Osteophytes radiographic progression - synovial Calprotectin



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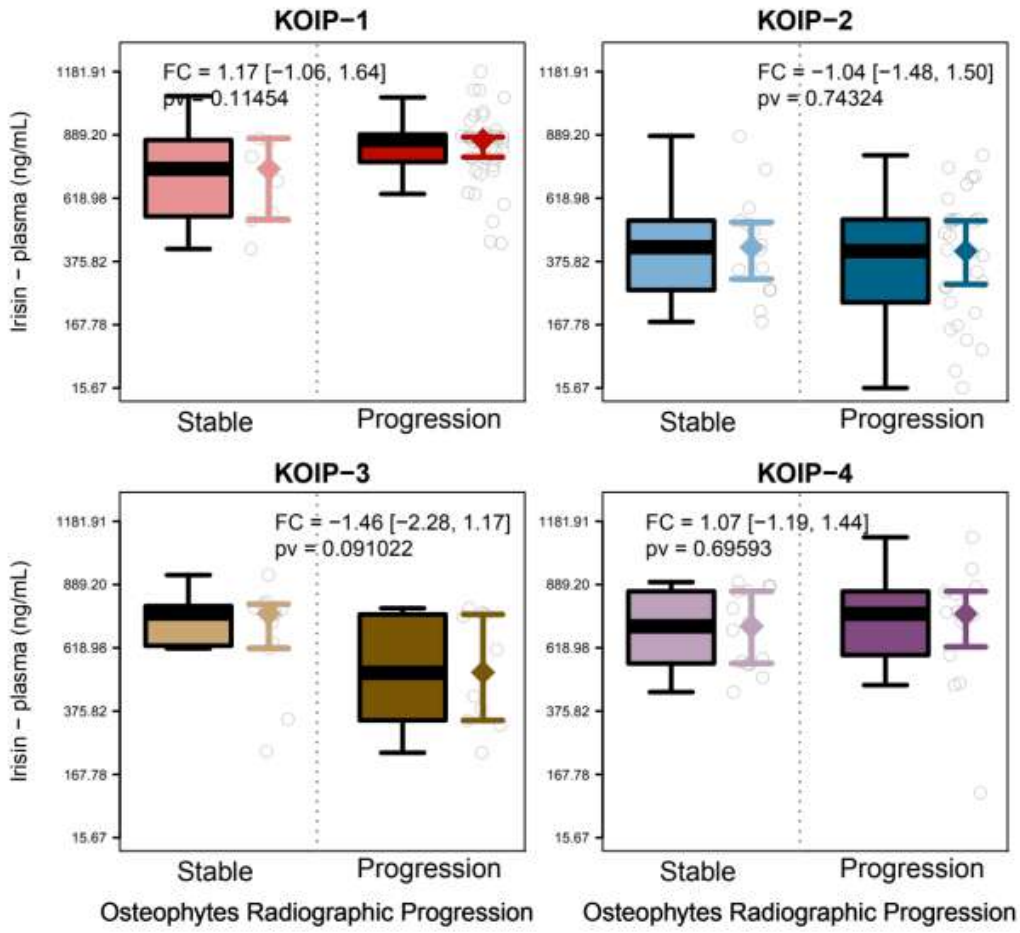
S15: Osteophytes radiographic progression - plasma Irisin



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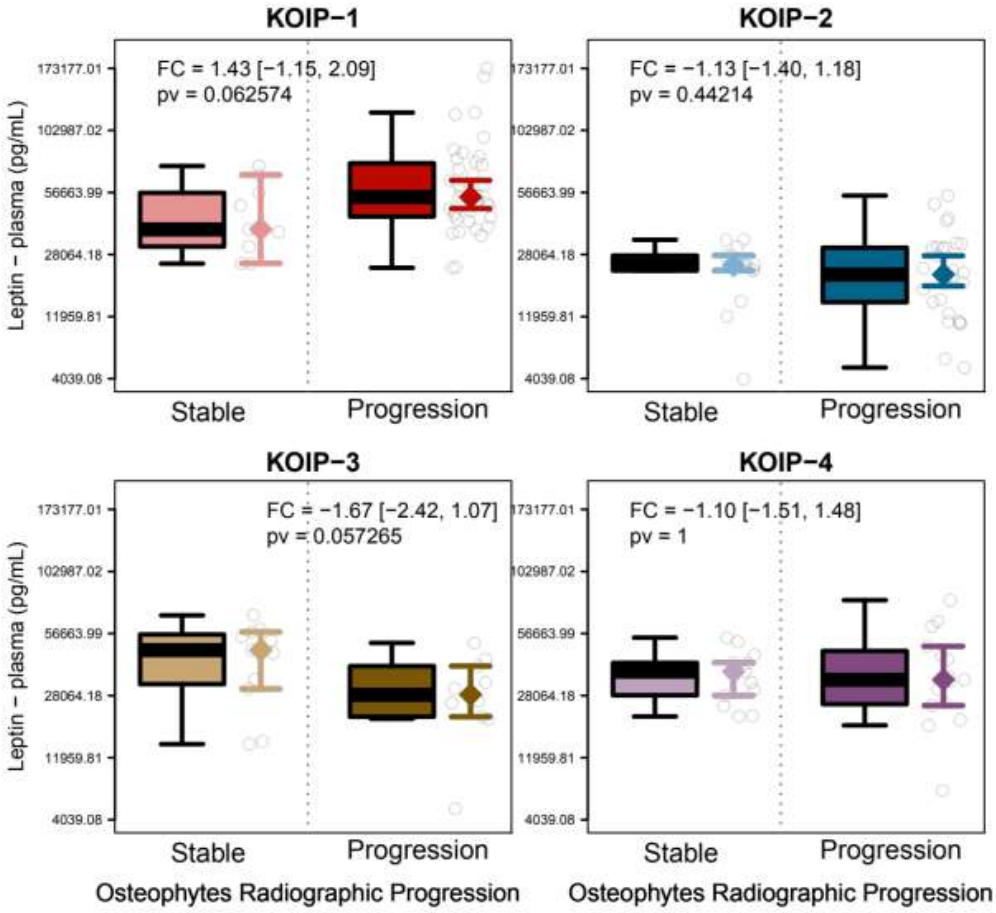
S16: Osteophytes radiographic progression - plasma Leptin



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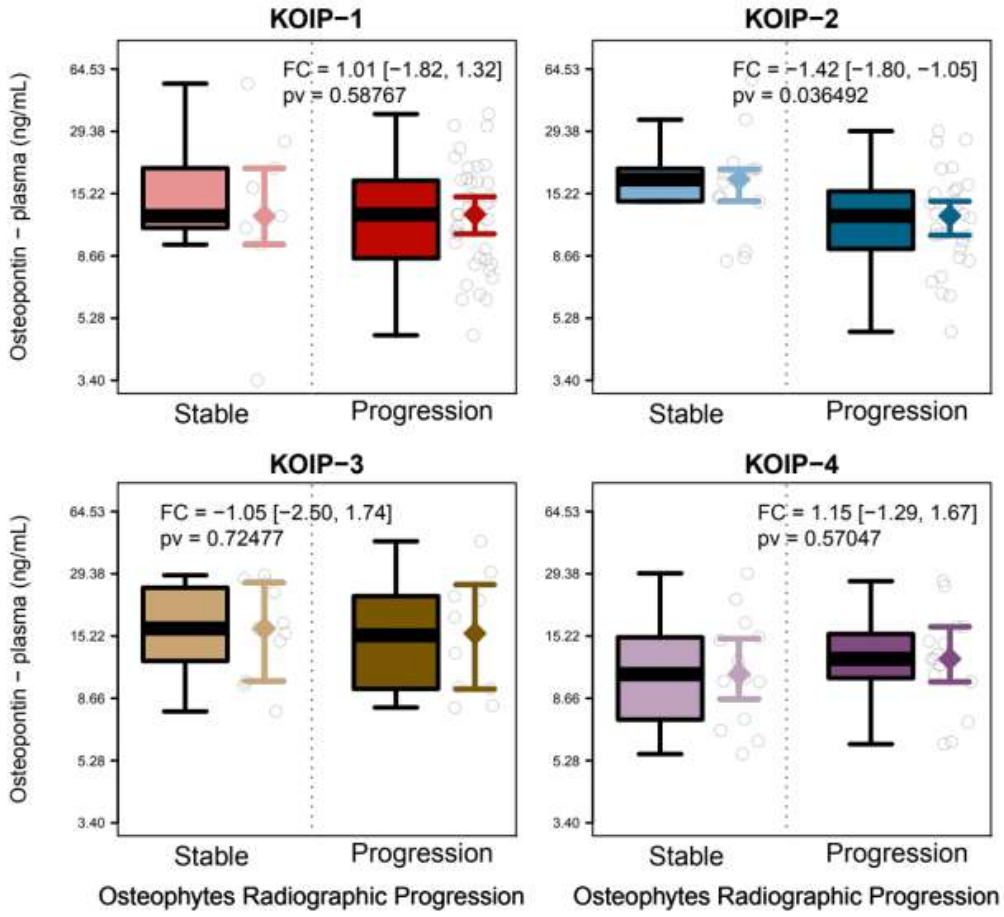
S18: Osteophytes radiographic progression - plasma Osteopontin



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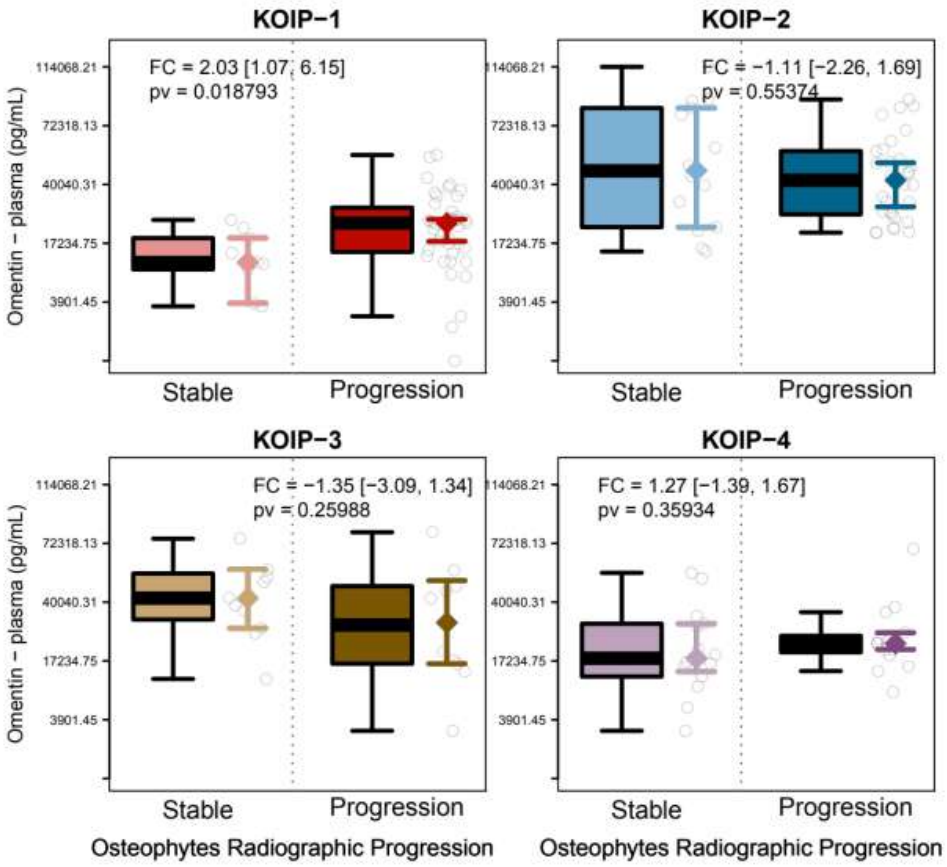
S17: Osteophytes radiographic progression - plasma Omentin



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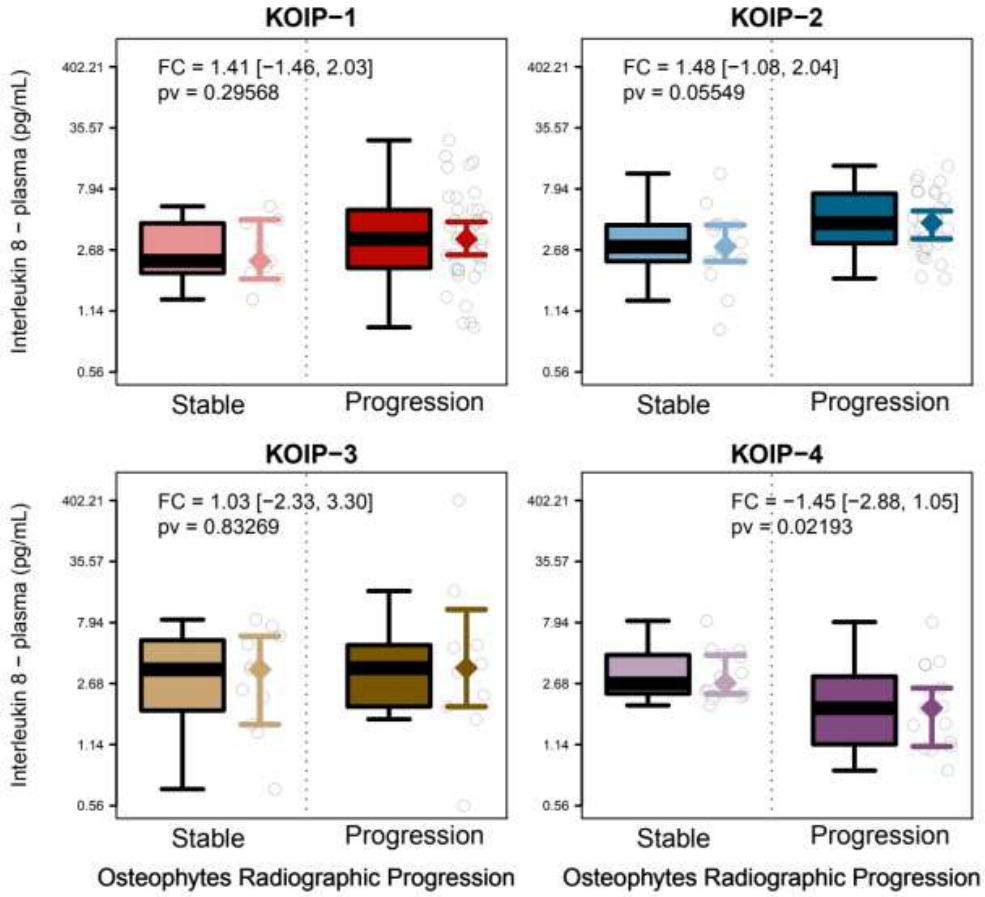
S19: Osteophytes radiographic progression - plasma Interleukin-8



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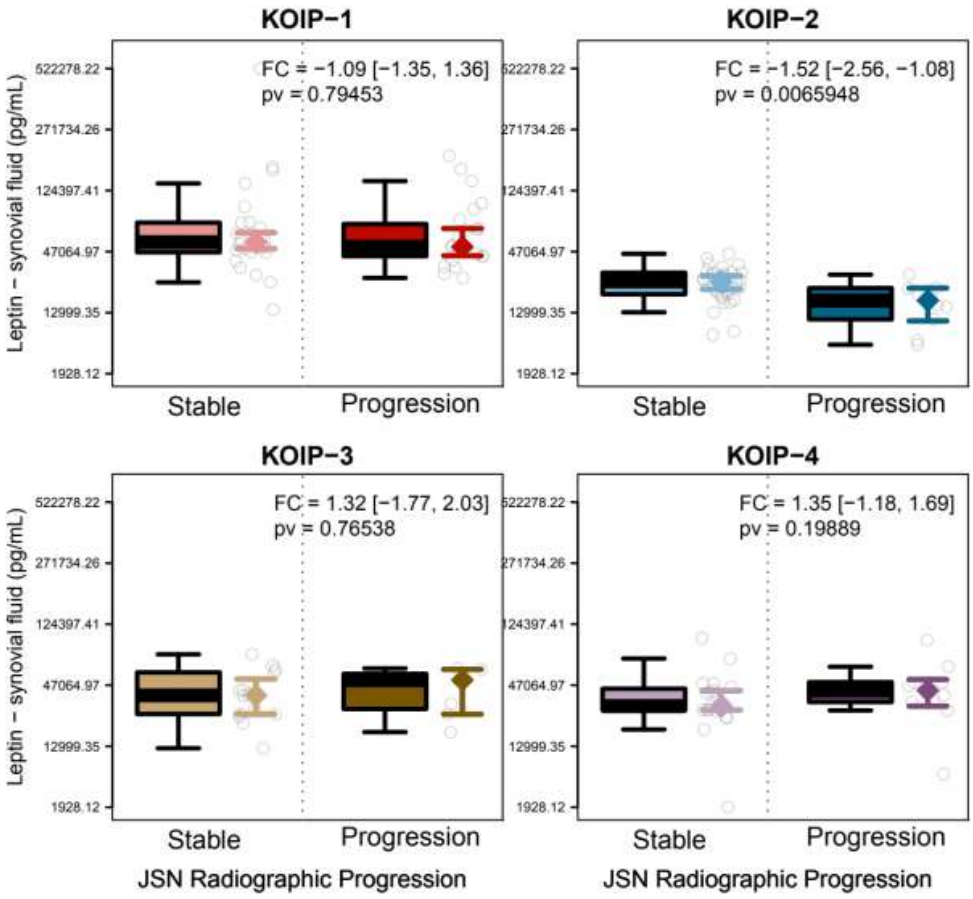
S21: JSN radiographic progression - synovial Leptin



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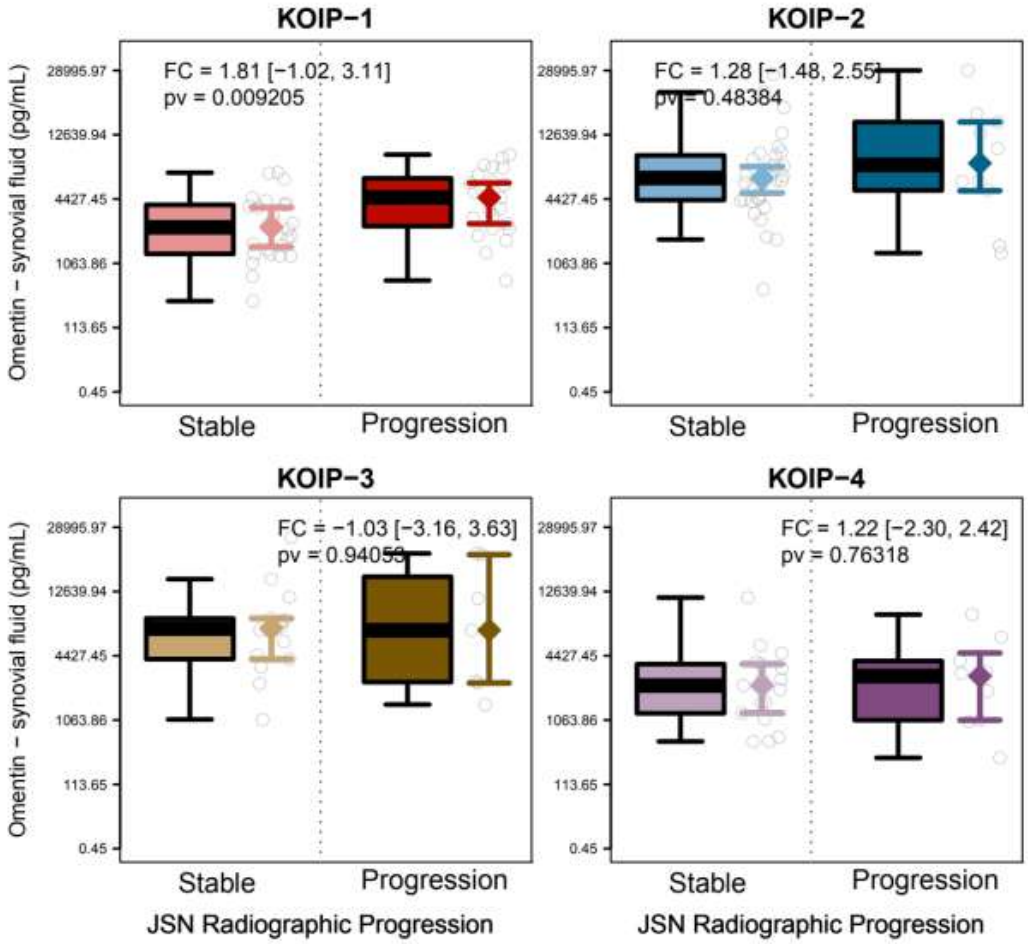
S22: JSN radiographic progression - synovial Omentin



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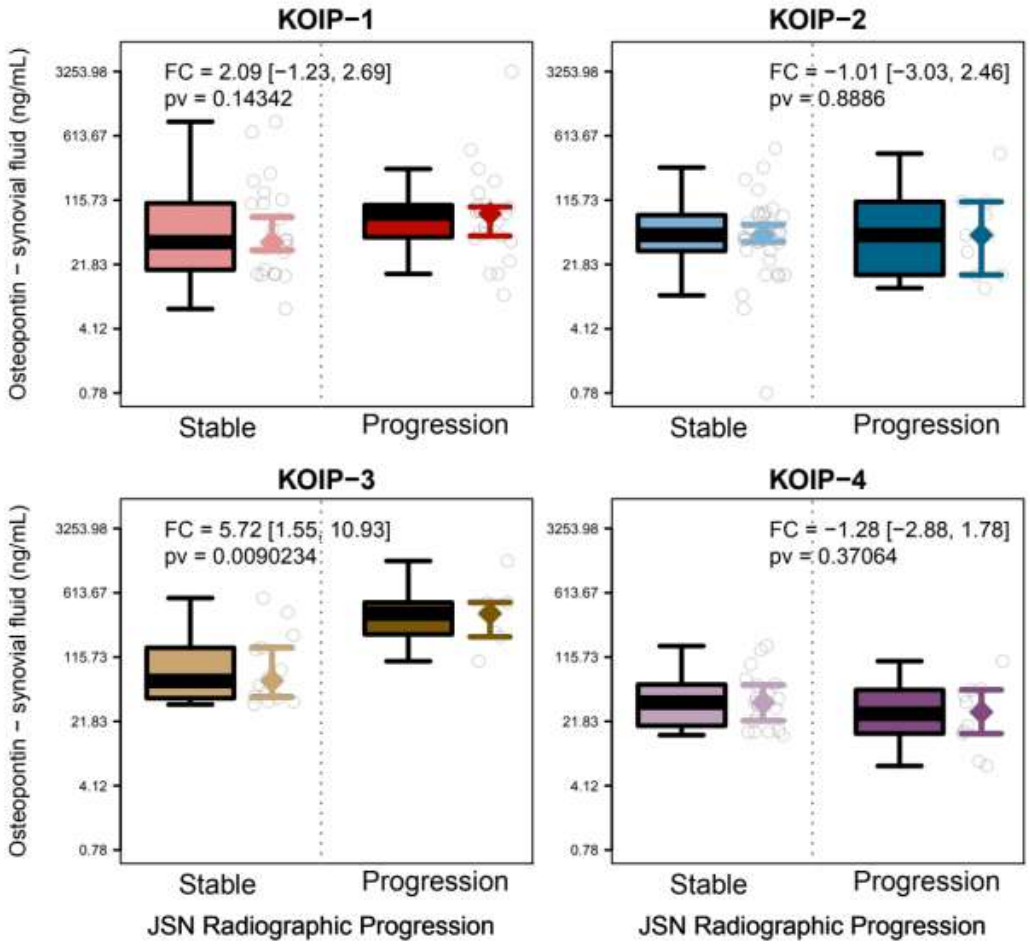
S23: JSN radiographic progression - synovial Osteopontin



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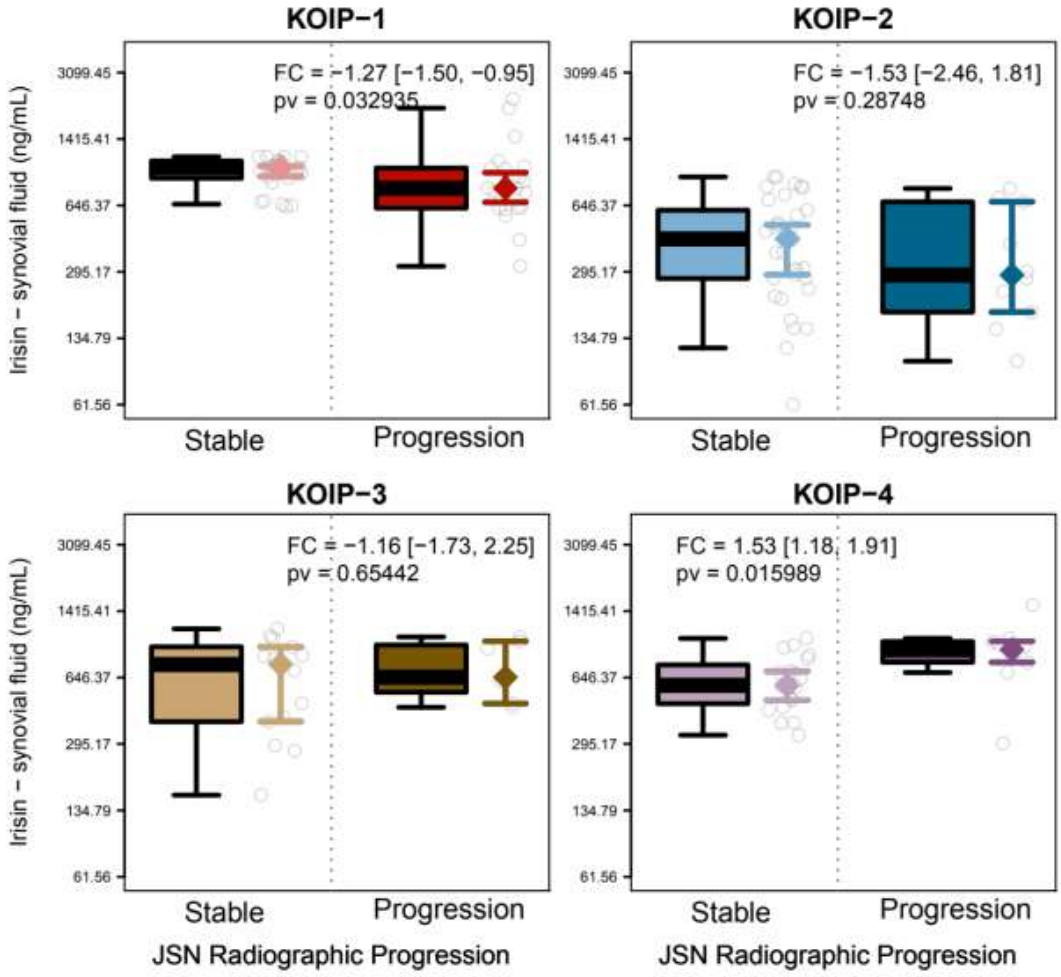
S20: JSN radiographic progression - synovial Irisin



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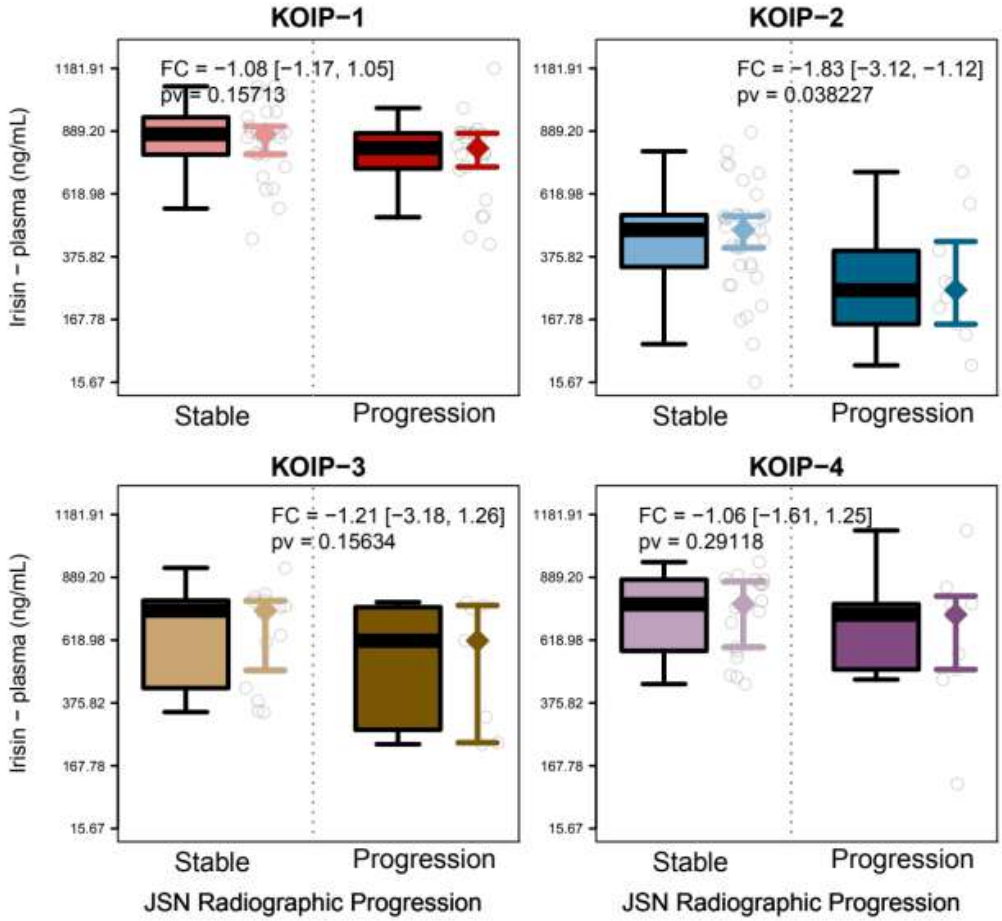
S24: JSN radiographic progression - plasma Irisin



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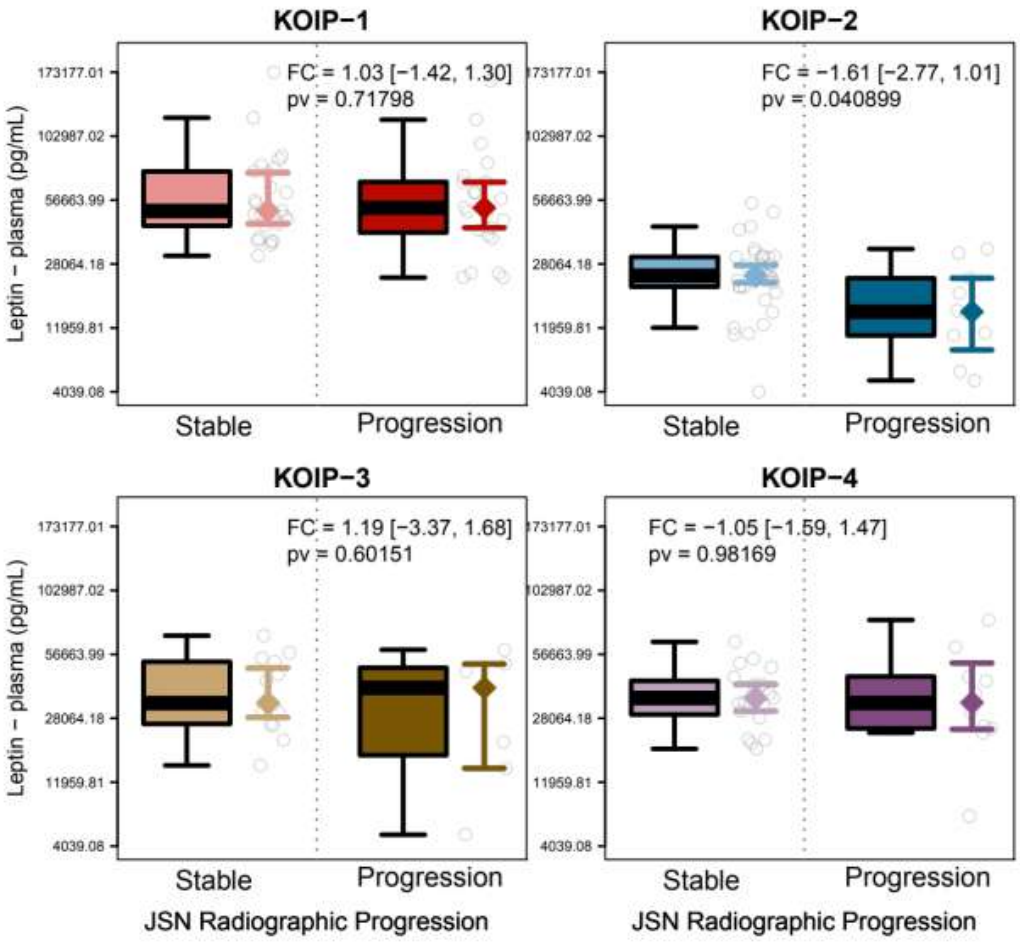
S25: JSN radiographic progression - plasma Leptin



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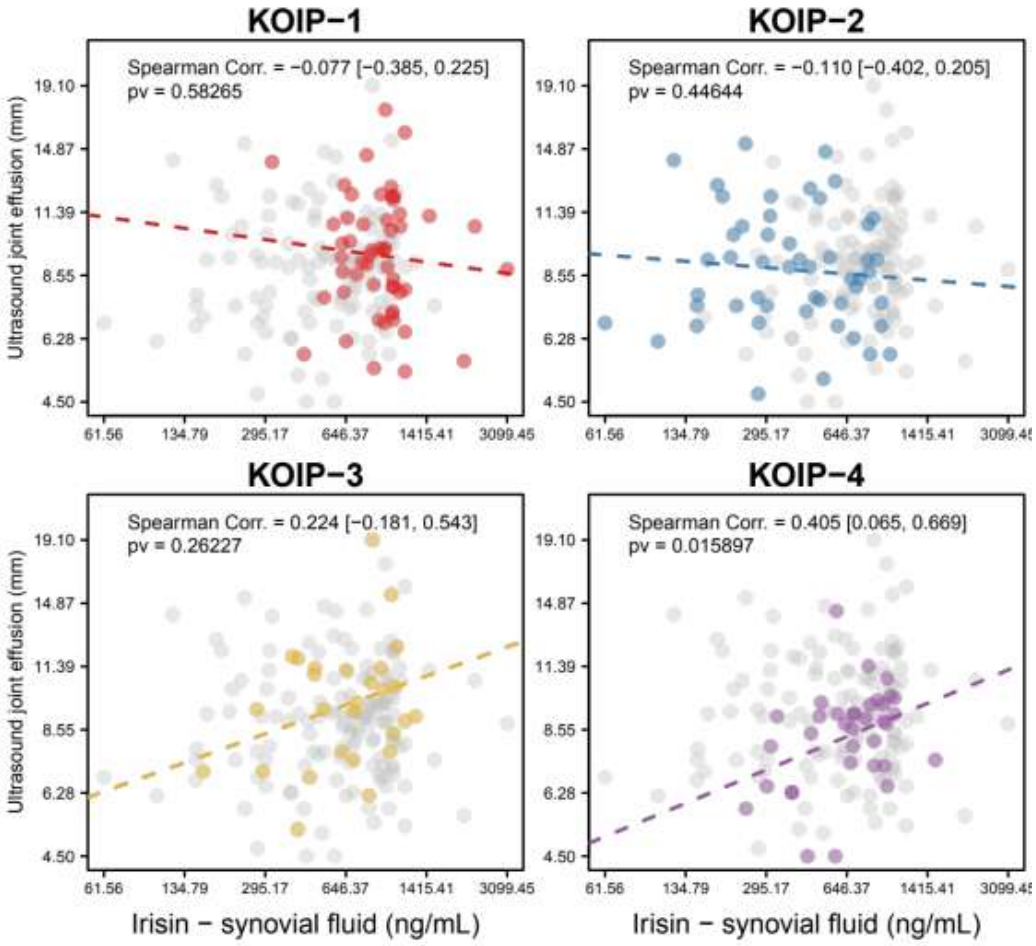
S26: Ultrasound Joint Effusion - synovial Irisin



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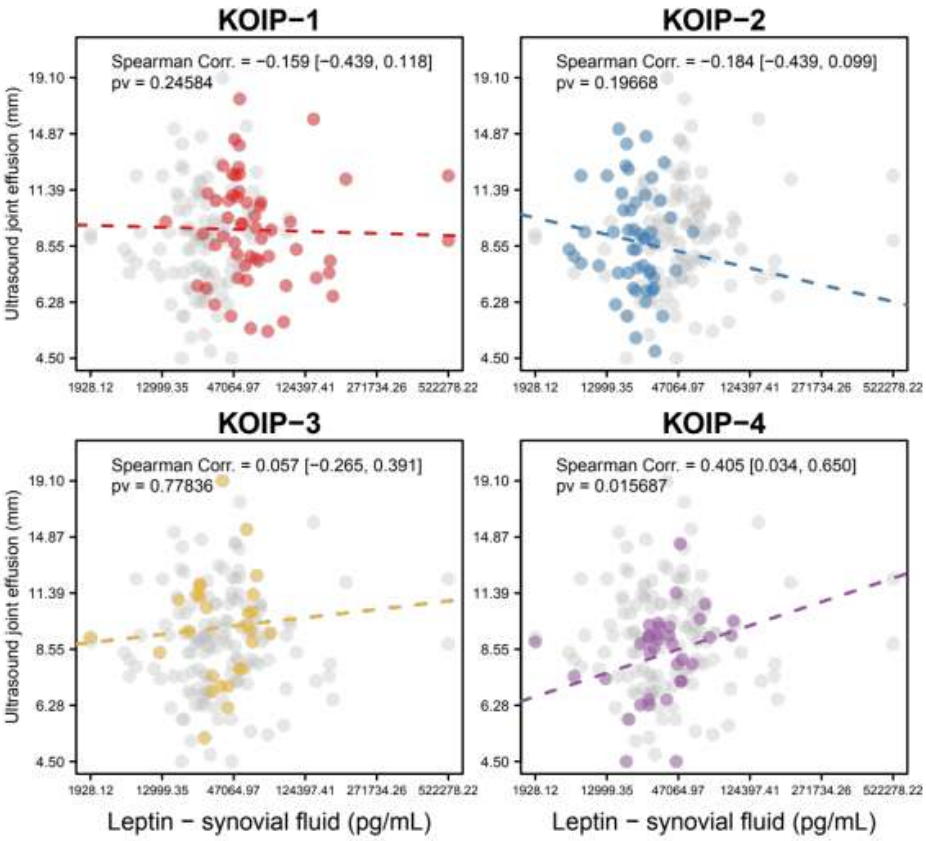
S27: Ultrasound Joint Effusion - synovial Leptin



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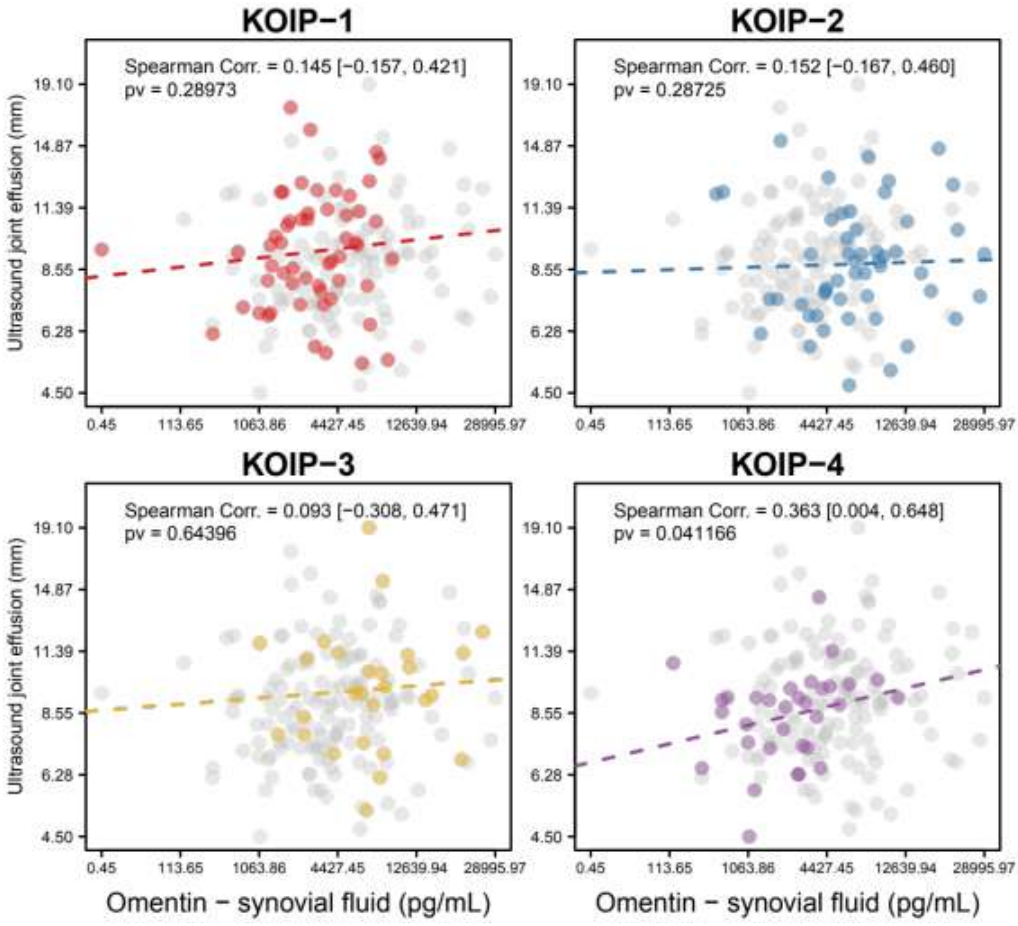
S28: Ultrasound Joint Effusion - synovial Omentin



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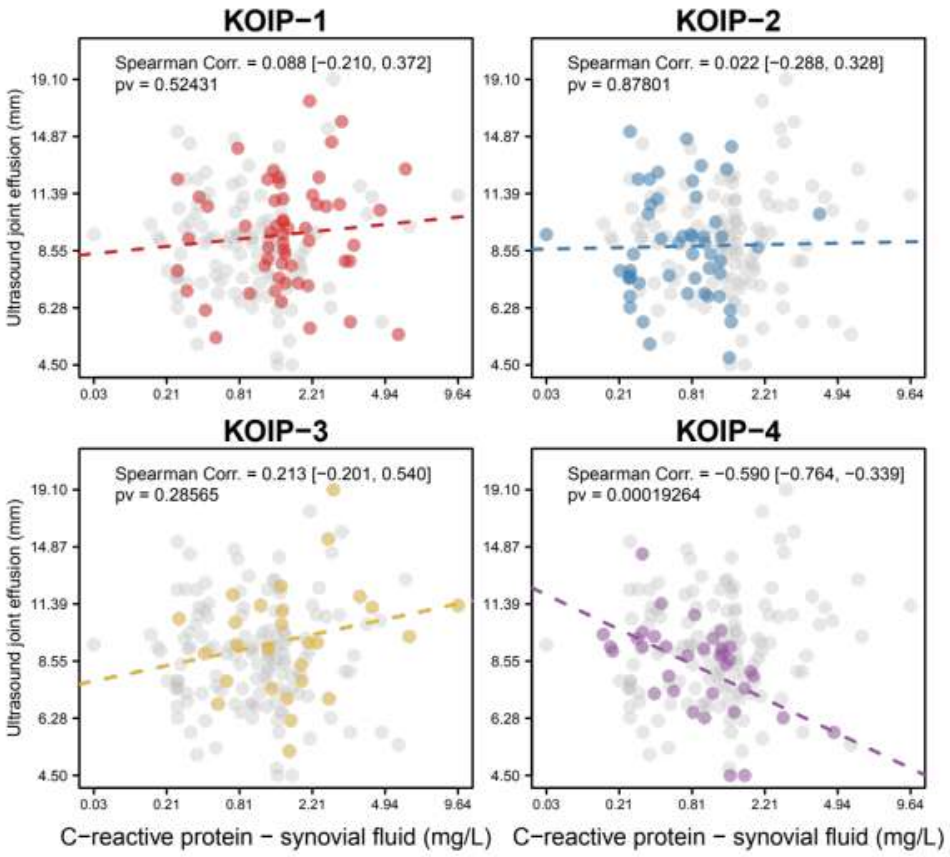
S29: Ultrasound Joint Effusion - synovial C-Reactive Protein



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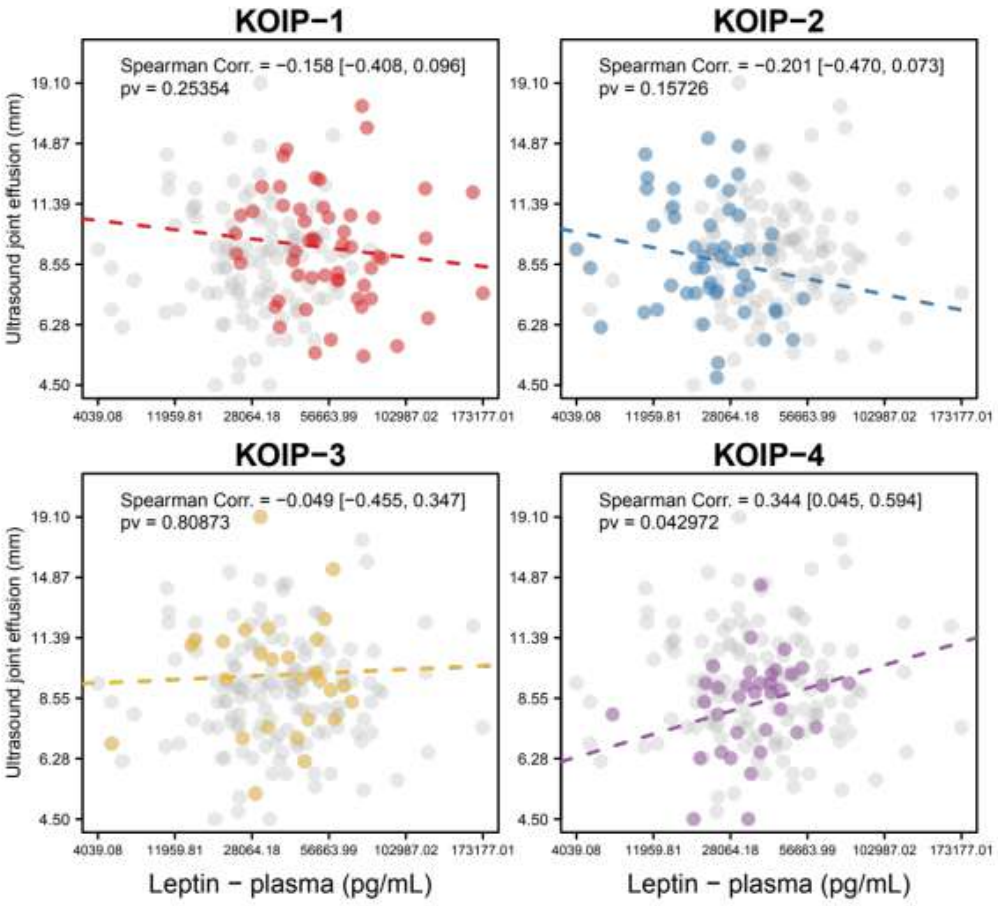
S30: Ultrasound Joint Effusion - plasma Leptin



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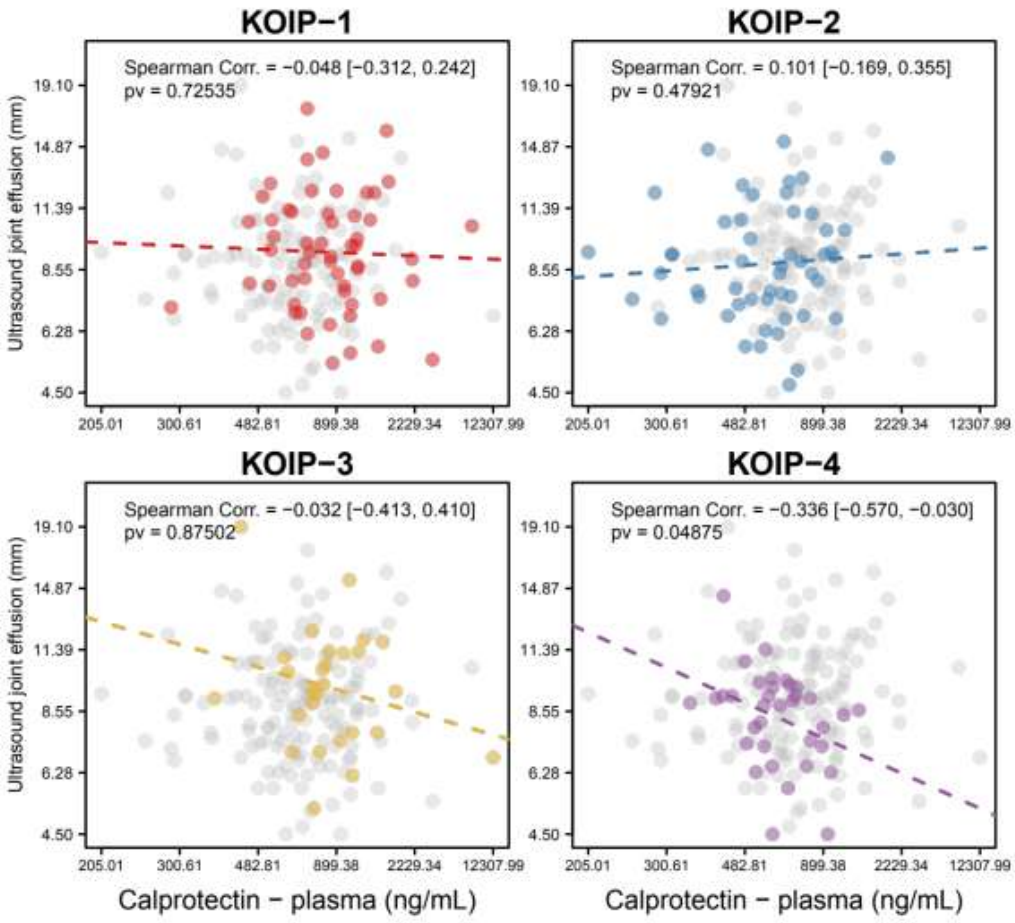
S31: Ultrasound Joint Effusion - plasma Calprotectin



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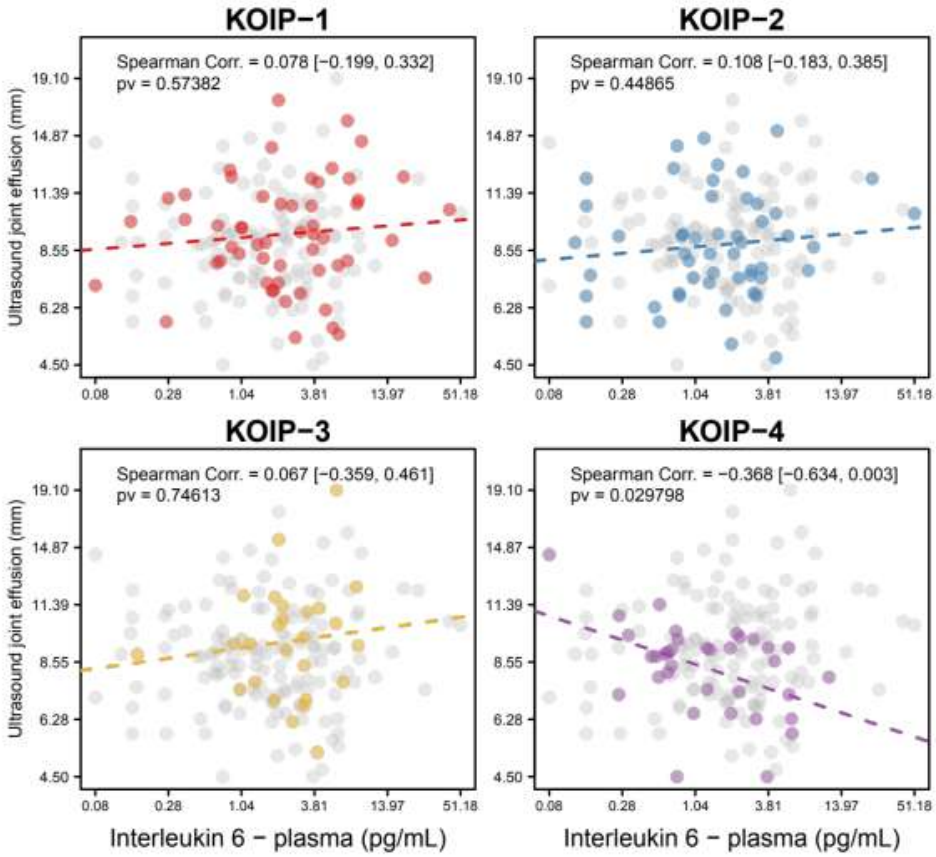
S32: Ultrasound Joint Effusion - plasma Interleukin-6



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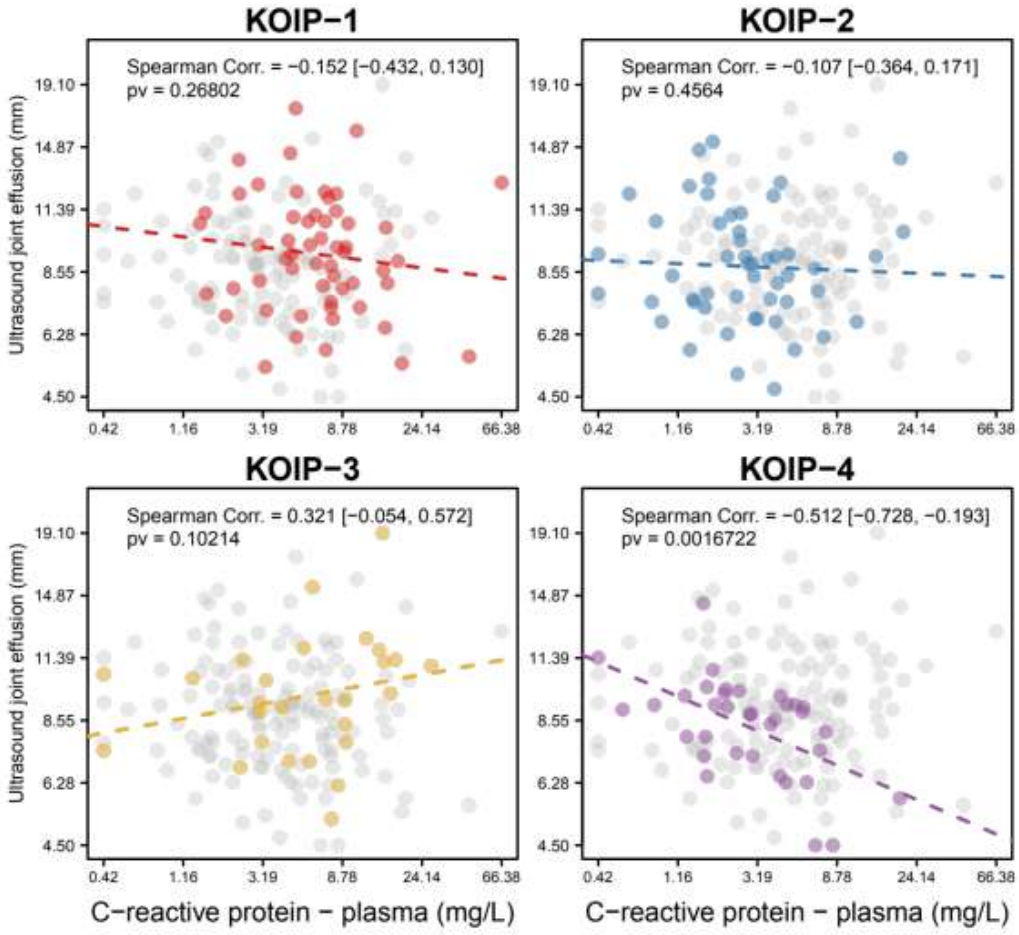
S33: Ultrasound Joint Effusion - plasma C-reactive protein



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