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Review

Osteoarthritis in year 2021: biochemical markers

Y. Henrotin † ‡ *

† musculoSKeletal Innovative research Lab (mSKIL), Institute of Pathology, Level 5, CHU Sart-Tilman, Center for Interdisciplinary Research on Medicines (CIRM), Department of Motricity Sciences, University of Liège, Belgium
‡ Department of Physical Therapy and Rehabilitation, Princess Paola Hospital, Vivalia, Marche-en-Famenne, Belgium

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SUMMARY

Objective: To summarize recent scientific advances in protein-derived soluble biomarkers of osteoarthritis.
Design: A systematic search on the PubMed electronic database of clinical studies on protein-derived soluble biochemical markers of osteoarthritis in humans that were published between January 1st 2020 and March 31th 2021. The studies were selected on the basis of objective criteria and summarized in a table. Then they were described in a narrative review.
Results: Out of 1971 publications, 48 fulfilled all selection criteria and 16 were selected by the author for the narrative review. The papers were classified according their clinical significance as defined in the BIPEDS classification. Two papers investigated the "burden of disease", two were dedicated to "investigative biomarkers", four papers question the "prognosis", three the "efficacy of treatment" and five the "diagnosis and phenotyping" value of protein-derived biomarkers.
Conclusions: Currently, biomarkers research is focused on their use as tools to identify molecular endotypes and clinical phenotypes and to facilitate patient screening and monitoring in clinical trials. This approach should allow a more targeted management of patients suffering from osteoarthritis.

Introduction

Although it is the most frequent musculoskeletal diseases, that it is linked to a major handicap, that it contributes to cardiovascular morbidity, and that it is very expensive for society, osteoarthritis (OA) still suffers from a great image deficit and this, due to the notable absence of therapeutic advances[1]. This disappointing result likely has multiple origins. The typically slow and heterogeneous OA course makes trials often fall short in terms of size and length for demonstrating treatment efficacy. This issue is further aggravated by the use of relatively insensitive outcome measures (patient-reported outcome measurements), pain and radiographic joint space changes (X-ray), required by regulatory agencies for a drug to be certified as a Disease Modifying Osteoarthritis Drug (DMOAD)[2]. Finally, an incomplete understanding of the OA pathophysiology obscures identification of proper treatment targets. This is complicated by the increasing knowledge that the physiopathological mechanisms driving the OA process differ between patients, joints localization and disease stage[3].

A challenge for researchers is to identify markers that are linked to OA physiopathology, which allow early diagnosis of the disease and predict its course, which allow among OA patients to select who will be the progressors and predict short-term structural changes or pain/symptoms evolution and finally predict the efficacy and monitor the effects of a treatment at an individual level[4]. Recently, a new challenge is trying to be taken up by scientists and health professionals, which consists in identifying and defining the phenotypes of OA and the endotypes associated with it. Initially, six clinical phenotypes and nine endotypes of knee OA have been described (Fig. 1)[5–7]. A challenge for the future will be to identify the clinical phenotypes and clearly define their constituent molecular endotypes. It is more than likely that the future markers of prognosis or efficacy of a treatment will be part of these molecular pathways.

To help them achieve these objectives, researchers have at their disposal clinical, biological or even medical imaging markers.

* Address correspondence and reprint requests to: Y. Henrotin, musculoSKeletal Innovative research Lab (mSKIL), Institute of Pathology, Level 5, CHU Sart-Tilman, Center for Interdisciplinary Research on Medicines (CIRM), Department of Motricity Sciences, University of Liège, Belgium.
E-mail address: yhenrotin@uliege.be.

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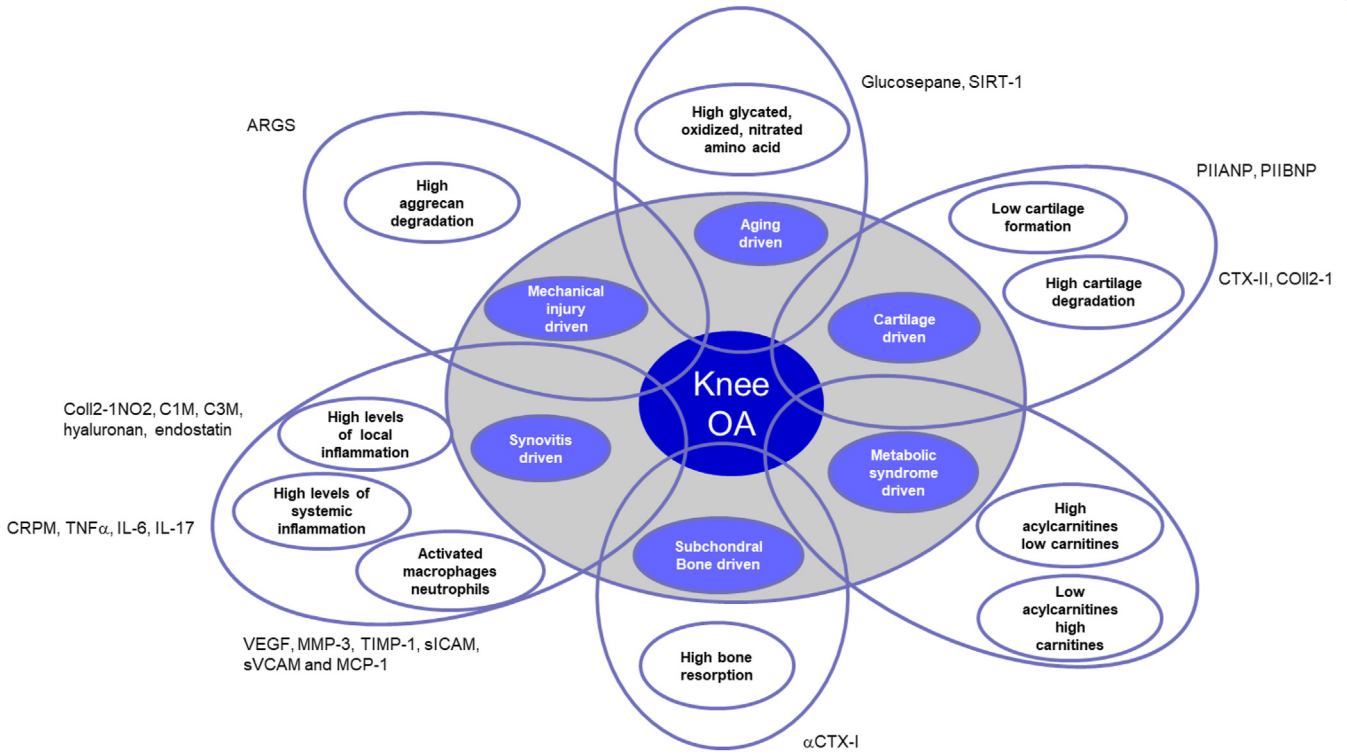

Fig. 1 OA YEAR IN REVIEW 2021 Osteoarthritis and Cartilage

Flower representation of OA phenotypes, endotypes and molecular endotypes (inspired from v6). CRPM: C-Reactive Protein Metabolite; TNF: Tumor Necrosis Factor; IL: InterLeukin; VEGF: Vascular Endothelial Growth Factor; MMP: Matrix MetalloProteinase; TIMP: Tissue Inhibitor of Metalloprotease; sICAM: serum InterCellular Adhesion Molecule; sVCAM: serum Vascular Cell Adhesion Molecule; MCP: Monocyte Chemo-attractant Protein; SIRT: sirtuine.

Among all these markers, soluble biochemical markers have benefited from particular interest because they can be directly detected in biological fluids like urine (u), plasma (p), serum (s) or synovial fluids (sf). In an effort to facilitate research on biomarkers, a working group financed by the NHI proposed a goal-based classification, which was represented by the acronym BIPED to connote the five categories of markers: Burden of disease, Investigative, Prognostic, Efficacy of intervention, and Diagnostic[8]. This classification scheme helps to provide a common language and structure with which to communicate knowledge and advances related to OA biomarkers for both clinical and research applications.

This year 'review aimed to summarize publication with original data on protein-derived soluble biochemical markers and showed them according the BIPEDS classification to allow a continuity with previous review papers.

Method

This narrative review of the literature was performed by searching in titles and abstracts in the electronic PubMed database for publications on clinical studies into protein-derived soluble biochemical markers for OA in humans that were published between 01-01-2020 and 01-04-2021. The following search equation was used for identifying potentially relevant publications in PubMed: ("osteoarthritis"[MeSH Terms] OR "osteoarthritis" [TIAB] OR "arthrosis"[TIAB]) AND ("blood"[TIAB] OR "serum"[TIAB] OR "plasma"[TIAB] OR "urine"[TIAB] OR "urinary"[TIAB] OR "synovial fluid"[TIAB] OR "saliva" [TIAB] OR "SF"[TIAB] OR biomarker*[TIAB] OR marker*[TIAB]). Our search of terms was restricted to title (TI) or abstract (AB) of the articles.

The identified publications were then stepwise selected by the author. First, publications were required to be in English. Second, publications had to present data on humans with OA. Animal or *in vitro* studies were not selected for further review. Third, publication had to present original research work or clinical trials. Fourth, studies should present data on soluble protein-derived biochemical markers in blood, urine, saliva and/or synovial fluid. The markers should relate to matrix metabolism, inflammation and/or other pathobiological processes within joints. Genetic markers were considered outside the scope of this review. Papers discussed in this review were then selected based on the author' evaluation of its content, tabulated and finally discussed following the BIPEDS classification.

Results

PubMed search, paper selection and data extraction

The selection process of potentially relevant publications is shown in Fig. 2. Sixteen publications were selected by the author to be discussed in this review. Selected papers were shown in Table I.

The large majority of studies included patients with knee OA (n = 12), two with both knee and hip OA patients (n = 2), one with lumbar spine OA (n = 1) and finally one with Temporo Mandibular Joint (TMJ, n = 1). Biomarkers were measured in serum (s, n = 11), urine (u, n = 3), synovial fluid (sf, n = 3), plasma (p, n = 1) and saliva (n = 1) using mainly immunoassays. One study investigated the secretome of joint tissues. Two studies investigated the association between biomarker levels and the burden of disease evaluated by X-ray or MRI, four the prognosis value, three the efficacy of treatment, and five diagnosis and molecular endotype.

Burden of disease

By definition, burden of disease markers are biomarkers associated with the severity or extent of disease. Most of the existing biomarkers have been demonstrated to be associated with knee standard X-ray or Magnetic Resonance Imaging (MRI) features of OA severity. Using a subset of 600 patients from Osteoarthritis Initiative (OAI) cohort, a longitudinal cohort study sponsored by the National Institute of Health (NIH), Liem *et al.*[9] demonstrated that of the 19 currently available biomarkers only 4 (s-Coll2-1NO2, s-CS846, s-COMP and u-CTXII) were consistently associated with established radiographic and/or clinical features of knee OA. These biomarkers were independent of one another and provided additional predictive power over, and above established predictors of OA such as age, gender, Body Mass Index (BMI) and race. This suggest that use this cluster of four biomarkers may be considered clinically useful and should be considered first in clinical trials as exploratory endpoint.

In a cross-sectional study of 145 participants with knee pain, biomarkers of innate immunity were associated with MRI features of knee OA[10]. More precisely, serum Lipopolysaccharide Binding Protein (LBP) was associated with meniscal extrusion, and synovial fluid Cluster of Differentiation 14 (CD14) was associated with effusion. Serum Interleukin (IL)-6 was associated with osteophytes, synovitis, effusion, and meniscus extrusion, while synovial fluid IL-6 was only associated with effusion. Similarly, serum Tumor Necrosis Factor alpha (TNFα) was statistically significantly associated with osteophytes, cartilage loss, synovitis, and effusion. None of the biomarkers related to innate immunity was associated with symptoms or radiographic gradings, excepted serum IL-6 which was negatively associated with WOMAC function. This study highlighted the role of Lipopolysaccharide (LPS)/LBP pathways in association with the innate immunity in the pathogenesis of knee OA. LPS, being a Pathogen-Associated Molecular Pattern (PAMP) enhanced in blood of OA patients secondary to increased permeability of the gut, binds to LBP. The CD14 biomarker is predominantly found on activated macrophages and serves as a receptor for

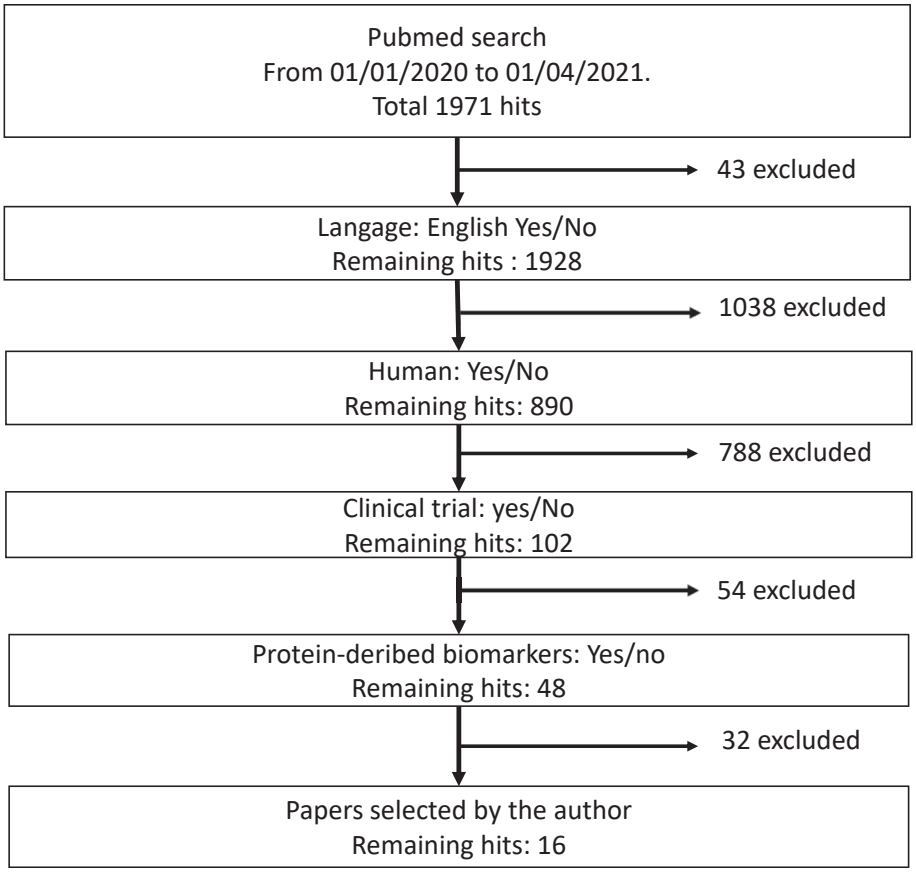

Fig. 2 OA YEAR IN REVIEW 2021 Osteoarthritis and Cartilage

Flow diagram of the stepwise selection process of relevant paper.

Author	Year	Joint	Fluid	Population	Parameters	Results	Conclusion
Burden of disease							
Liem	2020	Knee	Serum Urine	600 OA patients from the OAI data set.	*Biochemical*: sC1, 2C, sC2C, sCPII, sPIIANP, sCol2-1NO2, sCS846, MMP-3, sCTX-I, sCOMP, sHA, sNTX-I, uCTX-II, uC1, 2C, uC2C, uNTX-I, uCTX-1α, uCTX-Iβ, uColl2-1NO2, creatinine *Clinical*: WOMAC and KOOS scores. *Imaging*: JSN, JSW, osteophytes and Kellgren and Lawrence (KL) score on standard X-ray.	Out of the 19 biomarkers only 4 (serum Coll2-1 NO2, CS846, COMP and urinary CTXII) were consistently associated with established radiographic and/or clinical features of OA. Serum Coll2-1 NO2, COMP and urinary CTXII were associated with baseline K&L grade. uCTXII had the strongest and most consistent associations with radiographic and clinical features of OA.	Out of the 19 biomarkers analyzed u-CTXII, s-COMP, CS846 and Coll2-1 NO2 appear to be the most promising biomarkers for OA as they relate to both structural damage as well as symptoms in knee OA.
Rajandran	2020	Knee	Serum Synovial fluid	139 patients with early stage of OA 20 patients with late stage of OA.	*Biochemical*: s and sf LBP, CD14, TLR4, IL-6, IL-8, TNFα *Imaging*: Boston Leeds Osteoarthritis Knee MRI Score	In earlier KOA, sLBP was statistically significantly associated with meniscal extrusion, sfCD14 was associated with effusion and IL-6, IL-8, and TNFα were associated with most MRI features In later stage of KOA, sfLBP was associated with effusion. sfCD14 was associated with cartilage loss and BML. In earlier stage of KOA, the proinflammatory biomarkers IL-6, IL-8, and TNFα were associated with most MRI features	Biomarkers of activated macrophages and synovial inflammation are associated with early MRI features of KOA.
Investigative							
Timur	2020	Knee	Secretomes of cartilage, synovium, Hoffa's fat pad and meniscus. Synovial fluid.	24 patients with end-stage knee OA.	*Biochemical*: Liquid chromatography tandem mass spectrometry, followed by label free quantification. Immunoassays for SLPI, C8, CLU, FN1, RARRES2, MATN3, MMP3 and TNC, IGF2, AHSG, FN1, CFB, KNG and C8.	Proteomic analyses of OA vs non-OA knee synovial fluid revealed 70 proteins with a relatively higher abundance and 264 proteins with a relatively lower abundance in OA synovial fluid. Of the 70 higher abundance proteins, 23 were amongst the most highly expressed in the secretomes of a specific intra-articular tissue measured.	A number tissue-dependent protein release from intra-articular human knee OA tissues had an osteoarthritis-specific abundance in knee synovial fluid. These proteins may serve as novel candidates for OA biomarker development on a tissue specific basis.
Batshon	2020	Knee	Serum	28 subjects including healthy donors, early OA or late OA patients.	*Biochemical*: immunoassays for inactive N-terminal (NT) polypeptide (75SIRT1) and	An increase in sNT/CT SIRT1 ratio in individuals with OA compared with healthy donors, mostly due to	sNT/CT SIRT1 ratio is indicative of early-stage OA.

					a C-terminal (CT) fragment of SIRT-1.	higher NT values. NT values are increased in synovial fluid between healthy donors and late OA.	
Prognosis							
Arnold	2020	Knee Hip	Serum	636 subjects who underwent hip/knee arthroplasty	*Biochemical*: GDF-15 using electrochemiluminescence immunoassay. *All-cause of mortality*: death was ascertained by obtaining the survival status via the respective residents' registration office. Mortality was assessed during follow-up at 6, 12, and 60 months after joint replacement and again, in the years 2014, 2015, and 2019.	GDF-15 was inversely associated with walking distance. The top quartile of GDF-15 demonstrated a 2.69-fold increased risk of dying.	GDF-15 represents a potent predictor of decreased survival over >20 years in OA patients.
Rehm	2020	Knee Hip	Serum	679 OA subjects, undergoing hip or knee replacement followed over 20 years.	*Biochemical*: NT-proBN and high-sensitivity hs-cTnt and hs-cTnI using immunoassays.	Baseline u-CTX-II was associated with elevated risk of radiographic progression in terms of both JSN and KL-grade. The top quartile of NT-proBNP was associated with increased risk of mortality.	Elevated cardiac biomarker concentrations predicted an increased risk of long-term mortality and strongest for NT-proBNP and hs-cTnI.
Bihlet	2020	knee	Urine	1,255 knee OA patients followed for 2 years.	*Biochemical*: uCTX-II using an immunoassay. *Imaging*: JSN and KL score on standard X-ray. *Clinical*: total joint replacement.	A prediction-model incorporating age, sex, BMI, u-CTX-II and KL-grade predicted TJR within the 2-year period. In the absence of baseline radiographic OA severity, u-CTX-II independently contributed to prediction of TJR.	Baseline u-CTX-II was associated with risk of radiographic progression.
Rogers-Soeder	2020	Knee	Blood	987 subjects without radiographic knee OA at baseline and stratified by BMI and DM status.	*Biochemical*: Fasting glucose, and free insulin levels and insulin resistance (HOMA-IR) formula at baseline. *Imaging*: Postero-anterior weight bearing knee radiographs. A knee was defined as having incident tibiofemoral RKOA if there was at least one follow-up radiograph showing RKOA (KL grade 2 or greater) or if there had been a TKR at the time of the visit. The status of every knee was followed from baseline to 84 months.	RKOA were not associated with baseline DM status nor with levels of fasting glucose and HOMA-IR overall and in men. In women, HOMA-IR was inversely associated with odds of incident RKOA.	DM and higher levels of biomarkers of abnormal glucose metabolism were not associated with increased odds of incident RKOA in overall and in men.
Efficacy of treatment							
Conaghan	2021	Knee	Serum Urine	244 participants with primary knee OA which	*Biochemical*: s-CTX-I and u-CTX-II using immunoassay.	Compared with baseline, levels of s-CTX-I and u-CTX-	MIV-711 did not demonstrate any beneficial

(*continued on next page*)

Table I (*continued*)

Author	Year	Joint	Fluid	Population	Parameters	Results	Conclusion
				received either MIV-711 100 ($n = 82$) or 200 mg ($n = 81$) daily or matched placebo ($n = 77$).	*Clinical*: 26-week change in NRS pain score. *Imaging*: change in MRI bone area and cartilage thickness.	II were reduced by MIV-711 and levels of both biomarkers returned to baseline values after MIV-711 treatment stopped.	effects on OA knee pain in this study but reduced bone and cartilage MRI and biochemical markers.
Watt	2020	knee	Synovial fluid	20 individuals undergoing KJD for symptomatic radiographic knee OA.	*Biochemical*: 10 predefined mechanosensitive molecules: Activin A, TGFβ1, MCP-1, IL-6, FGF-2, LTBP2, MMP-3, TSG-6, TIMP-1 and IL-8. *Clinical*: KOOS-4	6/10 sf analytes showed changes between baseline and 6 weeks KJD. Of these, IL-6, MCP-1, FGF-2, LTBP2 and TGFβ-1 showed a predominant increase in levels, while activin A mainly decreased	8 putative mechanosensitive molecules of the inflammatory response (activin A, LTBP2, TGFβ-1, FGF-2, TIMP-1, IL-6, MCP-1, and IL-8) were seen in sf over the period of KJD.
Nambi	2020	Knee	Serum	60 participants with post-traumatic knee OA receiving either virtual reality training (VRT) or sensory-motor training (SMT) or control (supervised conventional exercise programs for the knee muscles).	*Biochemical*: BMP 2, 4, 6, and 7 CRP, TNF-α, IL-2, IL-4, and IL-6 by immunoassays. *Clinical*: VAS and WOMAC index.	BMP 2, 4, 6, and 7 didn't show any significant changes between the groups. Inflammatory biomarkers (CRP, TNF-α, IL-2, IL-4, IL-6) were reduced in VRT group compared to control and sensory-motor training groups.	VRT in PTOA shows beneficial changes in pain, functional disability, and modification of inflammatory biomarkers but no effect on bone morphogenic proteins.
Diagnosis & Phenotyping							
Bianchi	2020	TMJ	Saliva Serum	46 controls. 46 TMJ OA.	*Machine learning*: four machine learning models (Logistic Regression, Random Forest, LightGBM, XGBoost) treating 52 clinical, imaging and biochemical markers. *Biochemical*: serum (angiogenin, BDNF, CXCL-16, ENA-78, MMP-3, MMP-7, OPG, PAI-1, TGF-β1, TIMP-1, TRANCE, VE-Cadherin, VEGF) and saliva (angiogenin, BNDF, CXCL-16, ENA-78, MMP-7, OPG, PAI-1, TGF-β1). *Clinical*: mouth range of motion, age pain, crucial facial pain, worst facial pain, average pain, headache, muscle soreness, vertical range. *Imaging*: radiomics features from the HR-CBCT scans.	For the biomolecular markers, no differences between OA and control subjects were found. Top features to diagnosis TMJ were: mouth range without pain, gender and muscle soreness, VE-cadherin in saliva and headaches, PAI-1 in saliva and headaches, PAI-1 in saliva and range of mouth opening without pain, energy, Haralick correlation, entropy and interactions of TGF-β1 in saliva and headaches, VE-cadherin in serum and angiogenin in saliva.	The final prediction model had an accuracy of 0.823 (SD: 0.029) to predict TMJ OA status using LightGBM + XGBoost with 1,378 features interactions.
Goode	2020	Spine	Serum	74 participants (37 patients with radiographic disc space narrowing (DNS) and low back pain - 37 controls)	*Biochemical*: N-cadherin, Keratin-19, Lumican, CXCL6, RANTES, IL-17, IL-6, BDNF, OPG and NPY. *Clinical*: pressure-pain threshold (PPT) measurements, using a standard mechanical	Significant associations were found between radiographic DSN and OPG, IL-6 and NPY. Relative to a cluster with low levels of biomarkers, a cluster representing elevated levels of OPG,	Individual and combinations of biochemical biomarkers may reflect radiographic DSN.

					pressure-based dolorimeter. *Imaging*: the Burnett atlas on standard X-ray.	RANTES, Lumican, Keratin-19 and NPY and a cluster representing elevated levels of NPY were significantly associated with radiographic DSN.	
Bay-Jensen	2021	Knee	Serum	933 OA patients. 658 RA patients.	*Biochemical*: CRPM by a competitive solid-phase ELISA and CRP by a high sensitivity C-reactive protein assay.	The mean CRPM levels were significantly lower in OA compared to the RA patients; however, a significant subset of OA patients (31%) had CRPM levels (≥9 ng/mL) comparable to RA. OA patients with CRPM levels ≥9 ng/mL were more likely to develop contra-lateral knee OA assessed by X-ray over a 2-year follow-up period.	A subset of OA patients appears to have tissue inflammation comparable to that of RA. High CRPM levels are prognostic of incident knee OA.
Garcia	2021	Knee	Synovial fluid	27 OA patients.	*Biochemical*: OSM, IL-1α, IL-1β, IL-4, IL-6, IL-7, IL-8, IL-10, IL-13, TNF-α, IFN-γ, and IL-1Ra were measured using a multiplex bead assay.	Synovial fluid of OA patients with detectable OSM contained higher levels of other inflammatory cytokines, namely interferon gamma (IFN-γ), IL-1α and TNF-α, likely indicating a more inflammatory state.	OSM might play a prominent role in inflammatory phenotypes of OA.
Luo	2021	Knee	Serum Plasma	253 OA patients.	*Biochemical*: PRO-C2 *Imaging*: risk of radiographic medial joint space narrowing (JSN) over 24 months.	Subjects with low PRO-C2 levels had greater JSN compared with subjects with high PRO-C2.	Serum/plasma level of type II collagen formation, PRO-C2, may be an objective indicator of a low cartilage repair endotype, displaying radiographic progression and superior response to a pro-anabolic drug.

Common abbreviation: AHSG = alpha-2-HS-glycoprotein; BDNF = brain-derived neurotrophic factor; BML = bone marrow lesion; BMP = bone morphogenetic proteins; CD14 = cluster of differentiation 14; C8 = complement 8; CFB = complement factor B; CLU = clusterin; COMP = cartilage oligomeric matrix protein; CRPM = metabolite of C-reactive protein, derived from protease degradation; CS846 = chondroitin sulfate 846 epitope; CTXI, CTXII = C-terminal cross-linked telopeptide of collagen type I and II, respectively; CXC L = chemokine (CXC motif) ligand; DM = diabete mellitus; DSN = disc space narrowing; ENA epithelial neutrophil-activating peptide; FN1 = fibronectin 1; GDF = Growth differentiation factor; HA = hyaluronic acid; hsCRP = high sensitivity C-reactive protein; hs-CTnI = high sensitivity cardiac troponin I; hs-CTnT = high-sensitive cardiac troponin T; IL = Interleukin; IFN = interferon; IGF = insulin growth factor; JSN = joint space narrowing; JSW = joint space width; KOA = knee osteoarthritis; KNG = kinogen; KOOS = knee injury and osteoarthritis outcome score; KL = Kellgren and Lawrence grade; LBP = lipopolysaccharide binding protein; LTBP = latent TGF beta binding proteins; MATN3 = matrilin-3; MCP = Monocyte Chemoattractant Protein; MMP = matrix metal-loproteinase; MRI = magnetic resonance imaging; NPY = neuropeptide Y; NRS = numeric rating scale; OPG = osteoprotegerin; OSM = oncostatin M: PAI = plasminogen activator inhibitor; pro-BNP = N-terminal pro-brain natriuretic peptide type B; PIINP, PIIANP = N-terminal propeptide of type II collagen, and splice variant IIA; OA = osteoarthritis; OAI = osteoarthritis initiative; RA = rheumatoid arthritis; RANTES = regulated on activation, normal T cell expressed and secreted; RARRES2 = retinoic acid receptor responder 2; RKOA = radiological knee osteoarthritis; sf = synovial fluid, s = serum; SIRT = sirtuine; SLPI = Secretory Leukocyte Peptidase Inhibitor; TIMP = tissue inhibitor of metalloproteinase; TNC = Tenascin; TRANCE = TNF-related activation-induced cytokine; TRL = toll like receptor; TGF = transforming growth factor; TNF = tumor necrosis factor; VAS = visual analog scale; VEGF = vascular endothelium growth factor; WOMAC = Western Ontario and Mcmaster Universities osteoarthritis index. Less common abbreviations are explained in the table.

Table I

OA YEAR IN REVIEW 2021

Osteoarthritis and Cartilage

Extracted data from pubmed research of human clinical studies

the LPS/LBP complex. The binding of the LPS/LBP complex to CD14 would then trigger the Toll-like receptor 4 (TLR4) of macrophages, leading to the downstream production of inflammatory mediators and catabolism of chondrocytes[11]. This pathobiological pathways might explain the association between LPS and LBP increase and the severity of MRI changes[12].

Investigative

An Investigative marker is one for which there is insufficient information to allow inclusion into one of the BIPED categories. One way to identify new biomarker candidate is the OMIC analysis of biological fluids or secretome. There is few proteomic analyses of the synovial fluid. Further, for many proteins from OA synovial fluid, their intra-articular tissue of origin remains unknown. This is the reason for which Timur *et al.*[13] have performed comparative proteomic analysis to identify OA-specific and joint tissue-dependent secreted proteins that may serve as candidates for OA biomarker development on a tissue-specific basis. Clearly, the originality of this study was to identify the tissue origin of protein found in synovial fluid. To do that, they performed first, mass spectrometry proteomic analysis of the synovial fluid of non-OA and OA patients and a comparative proteomic analysis of the secretome of Hoffa's fat pad, synovium, meniscus and articular cartilage of OA knees. By this way, they found 62 proteins with a higher abundance in OA than in non-OA knee synovial fluid and 234 with a lower abundance. Interestingly, 39 out of the 62 higher protein were found in the tissue secretome and 56 of the lower detected in the secretome. In total 73 proteins were tissue specific. Among the most increased in OA, they found tenascin, histidine rich glycoprotein, fibronectin and alpha 2 HS glycoprotein and among the most decreased Cartilage Layer Intermediate Protein (CLIP), aggrecan core protein, decorin and fibromodulin. It is interesting to note that this study revealed that in addition to cartilage and synovium, the meniscus and Hoffa's fat pad are also significant contributors to the OA-specific protein composition of the synovial fluid of the knee joint. This study opens new perspectives of diagnostic based on synovial fluid analysis and aiming to classify knee OA according their molecular endotype.

The nicotinamide adenine dinucleotide (NAD)-dependent enzyme Silent Information Regulator 2 Type 1 deacetylase (SIRT1) is a critical intracellular deacetylase in maintaining adult cartilage health by promoting chondrocyte survival and extracellular matrix homeostasis[14]. Mounting data support that SIRT1 is proteolytically inactivated during OA, especially in response to inflammatory stress induced for example by cytokines[15]. It is well known that in chondrocyte SIRT-1 is cleaved by cathepsin B yielding a stable but inactive N-terminal (NT) polypeptide (75SIRT1) and a C-terminal (CT) fragment. Interestingly, Batshon *et al.*[16] found that the NT/CT ratio was significantly increased in OA and this increase was mainly due to NT fragment levels increase. As *in vitro* and animal studies have demonstrated that 1) IL-1β and TNFα increased NT/CT SIRT-1 ratio, 2) that NT/CT SIRT-1 ratio was positively correlated with OA severity in mice, 3) that serum SIRT1 variants were cartilage derived and 4) that senolytic drugs decreased this ratio, NT/CT SIRT-1 ratio can be considered as an investigative marker of OA burden of disease and senolytic drug efficacy.

Prognostic of OA and mortality in OA patients

The key feature of a prognostic marker is the ability to predict the future onset of OA among those without OA at baseline or the progression of OA among those with existing disease. Since it was demonstrated that subjects with OA are at increased risk for cardiovascular and all-cause mortality, some studies attempt to also predict comorbidities or mortality in OA population. Biomarkers of mortality should be considered in existing biomarker classification since it was demonstrated a reciprocal relationship between comorbidities like metabolic syndrome and OA and between OA and cardiovascular diseases[17–19]. Arnold *et al.*[20] investigated the association between growth differentiation factor-15 (GDF-15), a novel stress-responsive cytokine, and long-term all-cause mortality among OA patients. GDF-15 has been measured in the serum of 636 subjects, who underwent hip or knee arthroplasty between 1995 and 1996. During a median follow-up of 19.7 years, a total of 402 deaths occurred. Compared to the bottom quartile (<780 ng/L), subjects within the top quartile of GDF-15 (>1,279 ng/L) demonstrated a 2.69-fold increased risk of dying. They concluded that in subjects with OA, GDF-15 represents a potent predictor of decreased survival over 20 years, independently of conventional cardiovascular risk factors, renal, cardiac, and inflammatory biomarkers as well as walking disability, previously associated with increased mortality and lower extremity OA. This is the first prospective study in patients with OA, demonstrating a strong prognostic value of elevated GDF-15 on decreased survival over 20 years. Further, GDF-15 provided additional prognostic information on all-cause mortality, which was not captured by conventional risk factors, “maximum walking distance” as a measure of gait disability, or other well-established biomarkers and might be a useful biomarker for future risk stratification or targeting preventive measures in such high-risk populations like subjects with OA.

In the same cohort but selecting 679 OA subjects, undergoing hip or knee replacement during 1995 and 1996, Rehm *et al.*[21] measured in serum N-terminal pro-B type natriuretic peptide (NT-proBNP) and high-sensitivity troponins T and I (hs-cTnT and hs-cTnI) which are well-characterized cardiac markers and provide prognostic information. After adjustment for age and sex and several other established covariates (i.e., BMI, smoking status, localization of OA, diabetes mellitus, cholesterol, and cystatin C), the highest quartile of these biomarker concentrations were all associated with increased mortality compared to the lowest quartile. However, after simultaneous adjustment for the cardiac biomarkers, a statistically significant relationship for hs-cTnT was lost. These results suggest that the assessment of cardiac biomarkers may be a useful complement to traditional risk factors for predicting mortality in subjects with OA and may be used for counseling subjects or trigger specific interventions to reduce relevant cardiovascular risk factors.

Previous epidemiological studies have suggested that OA is associated with elevated fasting glucose and is highly prevalent among those with diabetes mellitus[22,23]. Proposed mechanisms include advanced glycation end products, which reduce cartilage mechanical properties[24]. Additionally, systemic inflammation could contribute to changes in cartilage metabolism and integrity, as well as neuromuscular impairment (as a result of symmetric sensory polyneuropathy and autonomic neuropathy from long-standing diabetes mellitus), which could lead to muscle weakness and joint instability. In light of these previous reports, Rogers-Soeder *et al.*[25] evaluated the association of diabetes mellitus and of biomarkers of abnormal glucose metabolism with incident radiographic knee OA of the tibiofemoral joint among participants in the Multicenter Osteoarthritis STudy (MOST)[26,27]. They hypothesized that the presence of diabetes mellitus, as well as hyperglycemia and elevated insulin resistance in participants with and without diabetes, would be associated with increased odds of incident radiographic knee OA, independent of BMI. Baseline fasting glucose and HOmeostasis Model of Assessment – Insulin Resistance (HOMA-IR) formula were associated with increased odds of incident radiographic knee OA in the unadjusted model, as well as in model adjusted for age, race, clinic site and visit. After adjustment for BMI,

these associations were attenuated and lost statistical significance. In women, elevated HOMA-IR was associated with lower odds of incident radiographic knee OA in the model adjusted for sex, age, race, clinic site, visit and BMI. Contrary to their hypothesis, after adjustment for BMI, the authors failed to find increased odds of incident radiographic knee OA in participants with diabetes mellitus nor in participants with higher baseline levels of fasting glucose and HOMA-IR. Surprisingly, after adjustment for BMI, they even observed a protective association of higher levels of HOMA-IR with the odds of incident radiographic OA in women. The author did not explain this result but have excluded a protection by use of diabetic medications. Further investigation of potential protective effects of diabetes mellitus is required to elucidate this unexpected observation.

Using 1,255 patients with painful and radiographic knee OA from two phase III clinical trials designed to study the efficacy and safety of oral salmon calcitonin[28], Bihlet *et al.*[29] have confirmed that baseline u-CTX-II was associated with elevated risk of radiographic progression over 2 years in terms of both joint space narrowing and Kellgren and Lawrence (KL) grade. The statistical significance of the association between u-CTX-II and risk of total joint replacement was lost upon adjustment for the baseline radiographic severity, which suggests that the association between u-CTX-II and total joint replacement may be indirect and driven by the radiographic stage. Then, they studied a model integrating u-CTX-II and demographic and radiological items. They showed that u-CTX-II in association with age, sex, BMI and cumulative knee KL-grade predicted total joint replacement within the 2-year period. The weight of the biochemical marker u-CTX-II was important. Indeed, in the absence of baseline radiographic OA severity, u-CTX-II independently contributed to prediction of total joint. The authors concluded that a composite model combining baseline age, sex, BMI, u-CTX-II and KL-grade predicted total joint replacement during a 2-year period.

Efficacy of intervention

An efficacy of intervention biomarker chiefly provides information about the efficacy of treatment among those with OA or those at high risk of developing OA. Efficacy of intervention markers may be measured prior to therapy to predict treatment efficacy, or may be measured more than once to assess short-term changes that occur as a result of pharmacologic or other interventions.

CMIV-711 is a novel selective cathepsin K inhibitor with beneficial effects on bone and cartilage in preclinical OA models[30,31]. Two-hundred forty-four participants with primary knee OA, were included in a 26-week randomized, double-blind, placebo-controlled phase IIa study and received MIV-711 100 or 200 mg daily or matched placebo. MIV-711 was not more effective than placebo for pain, but it significantly reduced bone and cartilage lesion progression compared to placebo[32]. Significant reductions in bone resorption and cartilage degradation biomarkers were also observed. Compared with baseline, levels of s-CTX-I and u-CTX-II were reduced by 27.8% and 34.4%, respectively in the MIV-711 100 mg group and by 50.3% and 51.6%, respectively in the MIV-711 200 mg group. However, no difference between MIV-711 and placebo group has been reported.

Surgical knee joint distraction (KJD) is a technique where, under anesthesia, an external fixation frame is placed on both sides of the joint, allowing distraction (gradual pulling apart of the joint's bony ends by ~5 mm for 6 weeks). KJD leads to clinical improvement in knee OA and also apparent cartilage regeneration by MRI[33,34]. Watt *et al.*[35] investigated if alteration of the joint's mechanical environment during the 6-week period of KJD was associated with a molecular response in synovial fluid, and if any change was associated with clinical response. They followed 20 patients candidate to KJD during 12 months and collected synovial fluid before, during and after 6 weeks of treatment with KJD. In summary, they showed that IL-6, IL-8, Monocyte Chemoattractant Protein (MCP)-1, Fibroblast Growth Factor (FGF)-2 and Transforming Growth Factor (TGF) β-1 levels increased, while activin A mainly decreased. Those with a relevant increase in IL-8 synovial fluid level during the distraction period had a greater improvement of their algo-functional status over 12 months than those with no change. This unexpected result was not explained by the authors. This study indicates that joint distraction may provide a potential opportunity in the future to validate regenerative biomarker(s) and identify pathways that drive intrinsic cartilage repair.

Non-pharmacological modalities including exercises program have been largely recommended to treat patients with hip and knee OA. However, few studies have investigated the structure-modifying effects of these modalities in human. Recently, Nambi *et al.*[36] have compared the effects of virtual reality training (VRT) and sensory-motor training (SMT) in bone morphogenetic proteins (BMP) and inflammatory biomarkers expression in post-traumatic OA after the anterior cruciate ligament injury. Sixty participants allocated to VRT, SMT or control group underwent training programs for 4 weeks. In the control group, the participants underwent supervised conventional exercise programs for the knee muscles. BMP measures such as BMP 2, 4, 6, and 7 did not show any significant changes with time or between the groups. In both VRT and SMT groups C-reactive Protein (CRP), TNF-α and IL-6 decreased with time while IL-2 and IL-4 increased. In contrast, in the control conventional exercise program, only TNF-α was significantly modified. Further, VRT and SMT groups significantly decreased more pro-inflammatory mediators (CRP, TNF-α, IL-6) and increased more IL-2 and IL-4 than the control group. This indicates that exercises program may modulate inflammation in OA.

Diagnosis and phenotyping

Diagnosis

Diagnostic markers are defined by the ability to classify individuals as either diseased or non-diseased. Most of the existing biomarkers have been demonstrated to statistically distinguish groups of patients with radiological or symptomatic knee OA from those without these characteristics. Rare are those that discriminate groups of patients with spine, hip, ankle or TMJ OA.

TMJ disorders are the second most common musculoskeletal condition affecting 5–12% of the population. Chronic disability in TMJ OA increases with aging. Bianchi *et al.*[37] have analyzed fifty-two clinical, biological and high-resolution Cone-beam computed Tomography (CBCT, radiomics) markers from TMJ OA patients and controls. They screened the diagnostic performance of each feature and built a machine learning models based on the most relevant features. They identified top features to diagnosis TMJ. including mouth range without pain, gender and muscle soreness, VE-cadherin in saliva and headaches, Plasminogen Activator Inhibitor (PAI)-1 in saliva and headaches, PAI-1 in saliva and range of mouth opening without pain, energy, Haralick correlation, entropy and interactions of TGF-β1 in saliva and headaches, VE-cadherin in serum and angiogenin in saliva. Interestingly and even no differences in biochemical markers levels were found between OA and control subjects, prediction models showed that the interaction between VE-cadherin, PAI-1, TGF-β1 in saliva and some clinical features like headaches or range of motion without pain are among the top features with mean contribution to the information gain in the predictive models. As markers of inflammation, VE-cadherin, angiogenin, TGF-β1 and PAI-1 have been previously shown to be

expressed in the TMJ synovial fluid and plasma and to be correlated with the condylar morphology in OA patients.

Recently, osteoprotegerin (OPG) was found to be more elevated in patient with radiographic intervertebral disc narrowing than in those without intervertebral discs narrowing. OPG is a member of the TNF receptor superfamily and has been found to be associated with intervertebral disc degeneration in mice and human tissue samples[38,39]. In propensity score matched regression analyses (matched for age, sex, BMI, knee OA, and hip OA), these authors have also observed significant associations of OPG, IL-6 and neuropeptide-Y with disc space narrowing. Finally, they conducted a cluster analysis to group together participants with similar biomarker profiles, ignoring case/control status. With cluster representing lower levels of biomarkers as the referent, a significant association was found among cases with disc space narrowing compared to controls without disc narrowing for the cluster with higher levels of OPG, Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES), keratin-19 and lumican and neuropeptide-Y. A significant association was also found with cases with disc space narrowing compared to controls without disc space narrowing in the cluster represented with a higher levels of neuropeptide Y[40].

Phenotyping

Another important observation is that during the last year the majority of the papers dedicated to OA biomarkers aimed to better define OA phenotype and clearly identify their constituent molecular endotypes with multiple biomarkers. The two main investigated phenotypes during the last year were the inflammatory/immune/synovitis driven phenotype and the cartilage driven phenotype.

Inflammatory-driven phenotype. CRP is mainly expressed and released from the liver in response to injury and infection, where from it is released in its pentameric form and binds to the site of injury followed by binding to complement and Fc receptors. At this site, CRP may be metabolized by proteases, such as matrix metalloproteinases (MMPs), resulting in the release of the CRP metabolites[41,42]. One such metabolite (M) is the MMP degradation product CRPM. Interestingly, Bay-Jensen *et al.*[43] compared the levels of CRP and CRPM in blood of OA and Rheumatoid Arthritis (RA) patients to investigate if OA patients have inflammation levels comparable to that of RA patients. They used the serum of patients coming from three different cohorts including either early RA, moderate to severe RA or OA subjects. They found that the levels of serum CRP and CRPM were significantly higher in the RA cohort than in the OA cohort but a third of the OA patients had CRPM and half had CRP levels corresponding to the levels observed in RA. This indicated that as significant subset of patients may have an inflammatory signature. Interestingly, only CRPM, and not CRP, was prognostic for OA incidence. Indeed, OA patients with CRPM levels $\geq$9 ng/mL were more likely to develop contra-lateral knee OA assessed by X-ray over a 2-year follow-up period.

Oncostatin M (OSM) is a cytokine from the IL-6 family that has been shown to be detectable in synovial fluid in up to 30% of the OA patients[44]. In line with these findings, Garcia *et al.*[45] demonstrated synovial fluid of knee OA patients with detectable OSM contained higher levels of other inflammatory cytokines, namely interferon gamma (IFN-γ), IL-1α and TNF-α, likely indicating a more inflammatory state.

Taken together these data indicate that OSM and CRPM are candidate biomarker for identification of OA patients with an inflammatory phenotype.

Cartilage-driven phenotype. One study investigated the hypothesis that a low cartilage repair endotype exists and that such endotype is more likely to progress radiographically. In this purpose, Luo *et al.*[46] examined the associations between the level of PRO-C2, the N-terminal propeptide of collagen type IIB reflecting type II collagen formation cartilage formation, in serum of knee OA subjects and radiological OA severity and progression. They found that patients with the lowest PRO-C2 levels at baseline were those with the more important joint space narrowing progression. They had 3.4 times more chance to progress over a 2-year period than the patient with the highest PRO-C2 level. This suggests that low cartilage formation may be associated with an endotype of higher structural loss.

Discussion

The method used to write this review of the papers published between January 2020 and April 2021 was inspired by that used by van Spil and Szilagyi for the year 2019[47]. This approach ensures continuity in the reflection on this theme. However, our work suffers from some limitations. Our search was limited to articles published on PubMed and the paper' selection was made by a single investigator according to arbitrary criteria. This is one reason for which this review can not be considered as a systematic review. This narrative review of the literature shows that interest in biological markers is still very much present in the scientific community. However, despite many efforts made by academic researchers but also by industry, there are still neither marker allowing routine diagnosis, nor biological markers approved by drug agencies such as drug development tools. The main reasons are the absence of an “on-off” biological marker for OA, the lack of sensitivity to changes in existing markers, but also the great phenotype heterogeneity of the subjects included in the cohorts used to validate or qualify biological markers. The absence of surrogate biochemical markers for standard X-ray strongly slows down the development of DMOAD. This is probably one of the reasons that prompted researchers to search biological markers that allow to select rapid OA progressor patients. Identifying these patients would reduce the sample size and duration of clinical trials. During the last 80 months some studies were focused on the use of biochemical markers to better characterized OA patients' phenotypes. Among them, some are actually cellular biomarkers and their clinical relevance in biological fluids needs to be determined. Anyway, this approach by phenotype has been demonstrated in other disease area to be helpful to better select subjects to include in clinical trials according the therapeutic target of the item. This can also lead to significant savings in healthcare management by administrating the right treatment to the right patient.

The second key message coming from this year's review is that there are now models integrating multiple demographic, clinical, imaging and biochemical markers allowing to better predict OA incidence and progression. In the coming months, important data will be disseminated thanks to the European IMI-APPROACH consortium including forty Universities and research organizations and six industries[48,49]. The aim of APPROACH project is creating a platform comprising data on large cohorts of patients and healthy people. The APPROACH cohort is unique in its attempt to select patients primarily from existing cohorts using machine learning models trained using patient data from the CHECK cohort to increase the likelihood of radiographic joint space width (JSW) loss and/or knee pain progression during a limited, 2-year follow-up period. In addition to this unique preselection of patients, the APPROACH cohort combines a very broad spectrum of conventional and novel, explorative, imaging, biochemical, clinical and demographic markers. Using data science techniques suitable to analyze these ‘big data’, algorithms of biomarkers should identify

and predict phenotypes/endotypes of OA that share distinct underlying pathobiological mechanisms with their structural and function consequences, relevant for practical and targeted clinical trials.

In conclusion, this year provides very interesting new data on biochemical markers, mainly on the identification of the phenotypes of patients with OA. The identification and characterization of these phenotypes are important steps towards understanding the disease, its diagnosis and its treatment. This research approach is very promising and will probably make it possible to better specify the indications for current treatments and to accelerate the development of DMOAD by allowing a better selection of patients to be included in clinical trials.

Contributions

YH has performed papers research and written the narrative summary.

Conflict of interest

YH is the founder and the president of Artialis SA. He also received consulting fees from Nestlé, Tilman SA, Naturex, Laboratoire Expanscience, Immunobio and Genequine. The authors also received funding from the Belgian Walloon Region under grant agreement N° 1320131, 7781 and 6905.

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