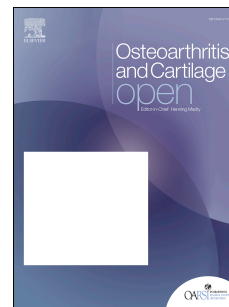


Journal Pre-proof

The Use of Technology in the Subcategorisation of Osteoarthritis: a Delphi Study Approach

Claire Mennan, Timothy Hopkins, Alastair Channon, Mark Elliott, Brian Johnstone, Timor Kadir, John Loughlin, Mandy Peffers, Andrew Pitsillides, Nidhi Sofat, Caroline Stewart, Fiona E. Watt, Eleftheria Zeggini, Cathy Holt, Sally Roberts, & The OATech Network+ Consortium



PII: S2665-9131(20)30072-8

DOI: <https://doi.org/10.1016/j.ocarto.2020.100081>

Reference: OCARTO 100081

To appear in: *Osteoarthritis and Cartilage Open*

Received Date: 30 January 2020

Revised Date: 22 May 2020

Accepted Date: 28 May 2020

Please cite this article as: C. Mennan, T. Hopkins, A. Channon, M. Elliott, B. Johnstone, T. Kadir, J. Loughlin, M. Peffers, A. Pitsillides, N. Sofat, C. Stewart, F.E. Watt, E. Zeggini, C. Holt, S. Roberts, & The OATech Network+ Consortium, The Use of Technology in the Subcategorisation of Osteoarthritis: a Delphi Study Approach, *Osteoarthritis and Cartilage Open*, <https://doi.org/10.1016/j.ocarto.2020.100081>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Published by Elsevier Ltd on behalf of Osteoarthritis Research Society International (OARSI).

The Use of Technology in the Subcategorisation of Osteoarthritis: a Delphi Study Approach

Claire Mennan¹, Timothy Hopkins¹, Alastair Channon², Mark Elliott³, Brian Johnstone⁴, Timor Kadir⁵, John Loughlin⁶, Mandy Peffers⁷, Andrew Pitsillides⁸, Nidhi Sofat⁹, Caroline Stewart¹, Fiona E. Watt¹⁰, Eleftheria Zeggini¹¹, Cathy Holt¹², Sally Roberts¹ & The OATech Network+ Consortium¹².

Affiliations

1. The Robert Jones & Agnes Hunt Orthopaedic Hospital NHS Foundation Trust & School of Pharmacy & Bioengineering , Keele University, Oswestry, Shropshire, SY10 7AG, UK.
2. School of Computing & Mathematics, Keele University, Staffordshire, ST5 5BG, UK.
3. Institute of Digital Healthcare, WMG, University of Warwick, Coventry, CV4 7AL, UK.
4. Department of Orthopaedics and Rehabilitation, Oregon Health and Science University, Portland, Oregon, 97239, USA.
5. Optellum Ltd, Oxford Centre for Innovation, Oxford, OX1 1BY, UK.
6. Biosciences Institute, International Centre for Life, Newcastle University, Newcastle upon Tyne, NE1 3BX, UK.
7. Institute of Ageing and Chronic Disease, The University of Liverpool, L69 7ZX, UK.
8. Comparative Biomedical Sciences, The Royal Veterinary College, London, NW1 0TU, UK.
9. Institute of Infection and Immunity, St Georges University of London, SW17 0RE, UK.
10. Centre for Osteoarthritis Pathogenesis Versus Arthritis, Kennedy Institute of Rheumatology, NDORMS, University of Oxford, OX3 7FY, UK.
11. Helmholtz Zentrum München - German Research Center for Environmental Health, Institute for Translational Genomics, Ingolstädter Landstr. 185764 Neuherberg, Germany.
12. Professor Cathy Holt, Cardiff University, Queen's Buildings, The Parade, Cardiff, CF24 3AA, UK.

Claire Mennan Claire.Mennan@nhs.net

Timothy Hopkins Timothy.Hopkins@nhs.net

Alastair Channon A.D.Channon@keele.ac.uk

Mark Elliott	M.T.Elliott@warwick.ac.uk
Brian Johnstone	johnstob@ohsu.edu
Andrew Pitsillides	apitsillides@rvc.ac.uk
Nidhi Sofat	nsofat@sgul.ac.uk
Timor Kadir	Timor.Kadir@optellum.com
John Loughlin	John.Loughlin@newcastle.ac.uk
Mandy Peffers	M.J.Peffers@liverpool.ac.uk
Caroline Stewart	Caroline.Stewart9@nhs.uk
Fiona Watt	Fiona.Watt@kennedy.ox.ac.uk
Eleftheria Zeggini	Eleftheria.Zeggini@helmholtz-muenchen.de
Sally Roberts	Sally.Roberts4@nhs.net
OATech Network+	Holt@cardiff.ac.uk

Corresponding Author

Sally Roberts

Spinal Studies & Cartilage Research Group

PhaB (Keele University)

Robert Jones & Agnes Hunt Orthopaedic Hospital NHS Foundation Trust

Oswestry

Shropshire

SY10 7AG, UK

Running Headline

Subcategorising Osteoarthritis

ABSTRACT**Objective**

This UK-wide OATech+ Network consensus study utilised a Delphi approach to discern levels of awareness across an expert panel regarding the role of existing and novel technologies in osteoarthritis research. To direct future cross-disciplinary research it aimed to identify which could be adopted to subcategorise patients with osteoarthritis (OA). **Design**

An online questionnaire was formulated based on technologies which might aid OA research and subcategorisation. During a two-day face-to-face meeting concordance of expert opinion was established with surveys (23 questions) before, during and at the end of the meeting (Rounds 1, 2 and 3, respectively). Experts spoke on current evidence for imaging, genomics, epigenomics, proteomics, metabolomics, biomarkers, activity monitoring, clinical engineering and machine learning relating to subcategorisation. For each round of voting, $\geq 80\%$ votes led to consensus and $\leq 20\%$ to exclusion of a statement.

Results

Panel members were unanimous that a combination of novel technological advances have potential to improve OA diagnostics and treatment through subcategorisation, agreeing in Rounds 1 and 2 that epigenetics, genetics, MRI, proteomics, wet biomarkers and machine learning could aid subcategorisation. Expert presentations changed participants' opinions on the value of metabolomics, activity monitoring and clinical engineering, all reaching consensus in Round 2. X-rays lost consensus between Rounds 1 and 2; clinical X-rays reached consensus in Round 3.

Conclusion

Consensus identified that 9 of the 11 technologies should be targeted towards OA subcategorisation to address existing OA research technology and knowledge gaps. These novel, rapidly evolving technologies are recommended as a focus for emergent, cross-disciplinary osteoarthritis research programmes.

Keywords (4-6 words).

Stratification; Osteoarthritis; Technology; Phenotype; Omics; Biomarkers.

Journal Pre-proof

2
3 It is predicted that there will be a 4- to 6-fold increase in the number of total joint replacements
4 for osteoarthritis (OA) in the coming decades^[1]. Despite the increase in prevalence and the large
5 body of literature existing on the subject, definitions of OA subcategories, whether in clinical or
6 research environments, are often disparate. The OATech Network+, a multidisciplinary
7 consortium, had identified this as a potential limitation to furthering OA research. Whilst X-rays
8 are one of the most commonly used technologies for studying OA for decades, there have been
9 many recent technological developments applied to the field, for example, in genomics and other
10 'omics', different forms of imaging, and computational analysis of big data.

11
12 The OATech Network+ organised a consensus meeting combining experts in a broad range of
13 existing and novel technologies (with basic scientists and clinicians) to appraise the potential of
14 existing and new technologies and improve OA subcategorisation. A Delphi approach was
15 adopted, aiming to recommend improved targeting of technology for OA subcategorisation so
16 that existing and emerging treatments could be applied more effectively to selected patients or
17 subgroups.

18
19 The meeting commenced with experts in the fields of engineering, rheumatology, orthopaedic
20 surgery, radiology, physiotherapy, biology and OA pain perception sharing their experience of
21 OA research. Experts in more recently developed technologies lectured on their OA research
22 application, summarised below.

23 24 **Genetics and genomics**

25
26 The field of complex trait genetics has witnessed a revolution in technological advances over the
27 last decade, enabling the genome-wide interrogation of sequence variation, leading to the

28 discovery of thousands of genetic risk loci. Recent methodological advances have also enabled
29 deep molecular characterisation of disease-relevant tissues collected from human patients or
30 studied in cellular and organismal models of disease. Together, these can help enhance our
31 understanding of the mechanisms underlying disease development and progression^[2]. Large-
32 scale genetics can help improve our understanding of the genetic aetiology of OA and related
33 sub-groups by interrogating big data in genetics, genomics and medically-relevant phenotypes
34 from rich epidemiological resources, patient collections and disease registries. Functional
35 genomic approaches for integrated molecular phenotyping of relevant cell types can help
36 translate insights from genomics into mechanisms of disease in order to overcome the critical
37 barrier of there being currently no disease-modifying treatment for OA. The relevant diseased OA
38 tissues are readily available from joint replacement surgery, enabling the study of molecular
39 processes in the appropriate tissues, both to fill a gap in our fundamental understanding of
40 biology and to identify novel therapeutic avenues.

41

42 **Epigenetics and Functional Analysis**

43

44 Epigenetics is a mechanism used by the cell, tissue and organ to regulate gene expression in a
45 dynamic manner by reversible chemical changes to the genome. There are three epigenetic
46 markers: DNA methylation, histone modification and the activity of regulatory RNAs^[3]. Epigenetic
47 changes are context specific and show temporal and spatial effects. They act during
48 skeletogenesis and joint formation, and have a role in OA^[3-5]. As for genomic studies, the
49 diseased joint tissues such as articular cartilage, synovium or bone, are used in relatively large
50 quantities to extract DNA, chromatin and RNA for epigenetic analysis. Such studies have led to
51 subcategorisation of OA by, for example, identifying individuals who appear to have an
52 inflammatory component to their disease^[4].

53

54 **Proteomics and Metabolomics**

56 Proteomics and metabolomics can be used to identify molecules as possible predictors of early
57 disease, disease progression and response to treatment. Synovial fluid contains systemic
58 proteins and metabolite markers of disease and holds significant potential for the discovery of
59 proteins and metabolites to aid subcategorisation of the disease.

60

61 Whilst transcriptomics can indicate the proteome, the relationship between mRNA and proteins
62 is complex and thus identifying proteins in a sample and how they vary is paramount.
63 Quantitative proteomic differences between sample groups can be identified using either
64 absolute or relative quantification, with or without labelling (reviewed^[6]). Absolute quantification
65 has been used to measure up to 20 targeted proteins in a single experiment^[7]. Label-free relative
66 quantification using synovial fluid has been used and predictors of treatment outcome with
67 autologous chondrocyte implantation (ACI) have been investigated for a number of biomarkers^[8].
68 Nuclear magnetic resonance (NMR) and MS have been used in assessing metabolomics, being
69 non-destructive, quantitative, reproducible and cost effective. Both techniques have identified
70 up to 32 differentially expressed metabolites in synovial fluid from OA and rheumatoid arthritis^[9].

71

72 Degradomics is another proteomic method that may be useful in OA subcategorisation, assessing
73 cleavage products at different stages in OA^[8]. A further development, Matrix Assisted Laser
74 Desorption Ionization Mass Spectrometry Imaging (MALDI-IMS), has been used to identify
75 proteins and neopeptides altered in cartilage ageing and OA^[8].

76

77 **Molecular signatures and biomarkers**

78

79 All the above techniques (genomics, epigenomics, proteomics) can assist in the search for OA
80 biomarkers, in terms of the “Burden of disease, Investigative, Prognostic, Efficacy of intervention
81 and Diagnostic (BIPED)” classification scheme^[10]. To date, many candidate proteins,

82 carbohydrates and lipids^[11] have been investigated^[12]. Several are associated with disease
83 progression in OA cohorts, but are not able to stratify individuals^[13]. A 'molecular signature'
84 representing multiple protein or non-protein markers may be more realistic for OA than finding a
85 single biomarker, perhaps better indicating relevant shared mechanisms within the disease.

86

87 Although singleplex antibody-based assays remain the mainstay for investigation of candidate
88 protein biomarkers, multiplexing with higher sensitivity and specificity for complex biological
89 fluids is now possible by proprietary adaptive immunoassay approaches, such as
90 electrochemiluminescence or proximity extension assays (combining antibody and PCR
91 technology)^[14]. Whether using immunoassay or mass cytometry (e.g. CyTOF), antibodies limit the
92 absolute number and combinations possible, whereas non-antibody approaches circumvent
93 these issues. Modified aptameric assays (aptamers being short sequences of nucleotides which
94 are selected for their specificity to bind proteins in much the same way as an antibody) can be
95 multiplexed to quantify thousands of proteins simultaneously in a single sample. These
96 approaches have the ability to identify molecular endotypes (molecular subgroups in disease) or
97 to predict drug toxicity and transform the way we are able to dissect molecular pathways or
98 identify molecular signatures as biomarkers in biological fluids.

99

100 **Clinical Engineering**

101

102 The International Classification of Functioning, Disability and Health (ICF) provides a framework
103 for understanding disability which links the body functions and structures to activity and
104 participation. Clinical movement analysis, in particular 3D gait analysis, allows clinicians to
105 measure the impact of OA on walking. This is important as patients often perceive their walking
106 pattern as a cause as well as a consequence of the disease. Patients with unilateral disease often
107 develop bilateral symptoms^[15].

108

109 Previous work^[16] has described gait in patients with single joint disease, who do not have a
110 typically antalgic gait pattern, but have knee loading which is high throughout the stance phase,
111 giving them a high moment impulse, combined with muscular co-contraction. This co-
112 contraction, measured using electromyography (EMG) further increases contact forces in the
113 joint. 3D gait analysis can detect bilateral overloading in both hip and knee joints in patients with
114 unilateral, single joint disease. The adopted tentative gait pattern seems to predispose other
115 joints to OA .

116
117 Whilst knee pain and loading measures improve after knee arthroplasty, some patients improve
118 more than others and abnormal loading patterns often persist^[16]. 3D gait analysis is useful in
119 understanding the control and loading of the joints during movement and interpreting how these
120 change in OA gait is important in providing appropriate therapies, such as bracing or biofeedback.

121
122 In knee OA populations biomechanical measures at baseline have also been used to predict
123 radiographic disease progression^[17], future total knee arthroplasty (TKA)^[18] and stratify response
124 to interventions such as and lateral wedge insoles and TKA^[16].

125

126 **Activity monitoring**

127

128 Recent OARSI guidelines have advocated the use of activity monitoring devices to collect
129 objective measures of physical activity^[19]. It is important for individuals with OA to remain
130 physically active. Evidence indicates that it can reduce OA related pain, in addition to increasing
131 muscle strength, joint range of motion and cardiovascular fitness^[20]. Physical activity levels
132 measured in OA populations over the longer term (3-12 months post-surgery) show no
133 substantial increases in activity after 12 months^[21]. Therefore more behavioural interventions are
134 required to promote physical activity in the recovery period; a conclusion that could be missed
135 when using more subjective self-reported measures.

137 Activity monitoring technology is rapidly advancing but for subgrouping of OA requires large
138 amounts of data. Smart phones and wearable technology now offer the potential to collect this
139 data outside of the laboratory and unobtrusively.

140

141 **Machine Learning and ‘Big Data’**

142

143 Much of the technology described with potential to improve OA stratification creates very large
144 data sets which require computational analysis; as the quantity of data increases, meaningful
145 analysis becomes more challenging. The use of complex artificial neural network architectures
146 or machine learning (ML) have been shown to be capable of representing and learning
147 predictable relationships in many diverse types of data. These computational tools hold promise
148 for transforming the future of ‘omics’ and other technologies which acquire huge data sets or
149 Big Data^[22].

150

151 Imaging modalities such as MRI are used as clinical diagnostic tools and contain vast amounts of
152 information which lend themselves well to analysis via ML. In the following example, ML is
153 applied to image analysis of OA in the spine, thus demonstrating the potential value of this
154 technology in identifying subgroups of OA. ML has been used to develop an automated method
155 for grading degeneration in the spine and intervertebral disc on MRIs^[23, 24], as used in the
156 Pfirrmann Score^[25] for degenerative disc disease or OA of the spine (developed as ‘SpineNet’).
157 The system can robustly extract measurements for this, in addition to having the potential to
158 identify other phenotypes such as spinal stenosis or ‘Modic’ changes in the vertebral endplates.
159 This approach requires well defined cohorts of patients with appropriate levels of consent for
160 this type of data storage and analysis, both for developing the program and then subsequently
161 independent cohort(s) for validation. SpineNet also has the capability of producing so-called
162 ‘Hotspots’ or saliency images that can be used to visualize the parts of the MRI that are the likely

163 source of the output^[23], so possibly defining completely new phenotypes from this unbiased
164 approach.

165

166 A prerequisite for imaging biomarker discovery is the extraction of robust and discriminative
167 radiological measurements from joint MRIs; however, the lack of imaging biomarker
168 standardisation within the research community, the inherent intra- and inter-reader variability
169 and time and cost has hampered research to date. Clearly ML is providing a powerful tool to aid
170 in the analysis of 'big data' and medical images with diverse applications too numerous to
171 discuss here. Future ML, computational analysis and the development of automated programs,
172 can offer robust, repeatable and rapid analysis of large datasets (MRI images or any other
173 potential 'biomarker', provide important tools for subcategorization and identification of OA
174 biomarkers . As novel markers of OA emerge across the biological, biomechanical, clinical and
175 imaging interfaces, their combination will provide increasingly powerful datasets and
176 opportunities for ML applications across OA diagnostics and classification domains.

177

178 In summary, the technologies mentioned above have developed rapidly in the last decade. For
179 example, a literature search for 'genomics' or 'epigenomics' (using Medline and Embase) over
180 the last 30 years highlights the increased awareness and use of such technology. From 1990-
181 1999 genomics or epigenomics shows a total of 10 publications, 2000-2009 shows 7,322 and
182 2010-2019 shows 23,426. With the continuous evolution of these technologies, it seemed
183 appropriate that the OATech Network+ should address the topic of the potential of technologies
184 for subcategorising OA and it was felt that a Delphi meeting would be an appropriate approach.

185

186 **METHODS**

187

188 This Delphi study consisted of a two-day focus group meeting (see programme in Supplementary
189 Table 1), together with online surveys using 'Google Forms', to assess the level of agreement on

190 a number of statements relating to OA and the use of different technologies (see Supplementary
191 Table 2). The group consisted of a number of different specialists (listed in Table 2), all with
192 expertise and significant interest in OA (Supplementary Table 3). A questionnaire was
193 formulated based on the most widely used technologies and research tools which may aid
194 subcategorisation of OA. The technologies were chosen by the organisers from their knowledge
195 of the field and review of the literature, including a search performed for this study. Selected
196 examples of OA categorisations were taken from the recent literature through primary searches
197 (using Medline, EMBASE and PubMed with 'definition of osteoarthritis' as a search term) and
198 articles known to the authors. Questions requiring free-text opinions of panel members were
199 included in the questionnaire, for example, 'were any questions missing' and 'what was their
200 personal definition of OA?'. Answers to the latter were used to start discussions at the meeting
201 and to assess the similarity of expert definition and understanding of OA. Expert consensus was
202 reached for each statement when $\geq 80\%$ participants agreed with the statement and rejected if
203 $\leq 20\%$ of participants agreed, as commonly used in previous Delphi studies^[26].

204
205 The questionnaire was tested on 3 world leading experts in the field of OA (Professors Richard
206 Loeser, Mary Goldring and Virginia Kraus) and modified slightly on their advice, before being
207 sent to the Delphi panel electronically. Panel members were asked if they agreed/disagreed
208 with each of the statements. Round 1 was completed before the two day meeting. Talks were
209 given at the start of the meeting by experts in the technologies presented in the Introduction. All
210 statements in Round 1 were retained for Round 2, viewed 'live' on the Delphi on Google Form;
211 any questions/statements which did not reach consensus in Round 2 were discussed in fine
212 detail with participants suggesting potential improvements to statements. Once unanimous
213 agreement on the wording was achieved, the wording was altered in the survey for voting on in
214 Round 3 at the end of day 2. These changes to wording are shown in Table 1.

215 Please insert Table 1 here

216

218 The aims of the Delphi study were to determine, using a panel of experts, 1. whether novel and
219 existing technologies could aid in the subcategorisation of patients with osteoarthritis (OA) and
220 2. whether there is good knowledge and awareness of these technologies. This could then help
221 define what technology gaps exist to allow recommendations on the focus of future
222 collaborative and cross disciplinary research.

223

224 **Participant identification and inclusion**

225

226 Experts were selected from a wide range of disciplines relevant to the field of OA. All 130
227 members of the OATech Network+ were invited to take part. The Delphi questionnaire was
228 emailed to 36 potential Delphi panel experts, who were all active in the OA field and expressed
229 an interest in attending the meeting. The minimum requirement for all invited experts was to
230 complete all three rounds of the Delphi and attend the meeting.

231

232 **RESULTS**

233

234 Thirty three experts responded and completed the Round 1 questionnaires and attended the
235 meeting, so becoming the Delphi panel (Supplementary Table 3). This consisted of basic science
236 researchers, orthopaedic surgeons, physiotherapists, rheumatologists, engineers, radiologists,
237 veterinary researcher and a clinical efficacy researcher from the UK (n=31), America (n=1) and
238 the Netherlands (n=1). However, several members were multi-faceted, e.g. being clinically
239 active and performing basic research and running clinical trials. The questionnaire showed 37%
240 of the panel members were actively treating patients whilst 63% were not, but might have
241 patient contact. Twenty seven percent of panel members had been working in the field of OA
242 for 0-5 years, with 24% being involved for >20 years (Supplementary Figure 1). Although the
243 Delphi panel was made up of a diverse group of experts, none were experts in Delphi

244 methodology. However, several panel members had significant, relevant experience of the
245 process to mitigate this limitation.

246

247 The wording in the statements and the results of the Delphi questionnaire over 3 rounds are
248 shown in Table 1 and summaries of the definitions of OA provided by participants from different
249 disciplines in Table 2. Not all panellists answered the question on defining OA as all questions
250 were optional for panel members, so results are shown from those available, with only small
251 variations between and within professions.

252

253 None of the six categorisations of OA taken from recent literature reached consensus in any
254 round (Table 1). Furthermore, 4 of the 6 literature-derived definitions demonstrated a decrease
255 in agreement between Rounds 2 and 3 (following the face-to-face meeting).

256

Insert Table 2 here.

257 In contrast, there was unanimous agreement in Rounds 1 & 2 that the latest technological
258 advances could be used to improve OA subcategorisation (Table 1 & Figure 1). Of the
259 technologies identified, only the statement 'X-rays alone can be used to categorise OA
260 phenotype' failed to reach consensus in rounds 1 and 2, whilst there was no consensus in Round
261 2 for either X-rays or ultrasound as technologies which would to improve clinical OA
262 subcategorisation (Table 1).

263

Insert Figure 1 here.

264 The technologies which gained greatest consensus in Round 2 for being of use in improving
265 subcategorisation of OA were: ML (100%), genetic analysis and MRI (both 97%), proteomics and
266 wet biomarker analysis (both 93.8%), activity monitoring (90.9%), metabolomics (both 90.6%),
267 epigenomics and clinical engineering (both 88%). Eighty three percent of participants thought X-
268 rays could aid subcategorisation of OA in Round 1, but this reduced to 49% in Round 2, whilst for
269 ultrasound this changed from 59% in Round 1 to 67% in Round 2. Ultrasound was described as
270 useful for identifying inflammation in the knee and could therefore be valuable in

271 subcategorising OA, although some members did not feel that there was sufficient evidence
272 presented to make an informed decision as this technology was not presented at the meeting.

273

274 There was much discussion on the usefulness of X-rays and the commonly used Kellgren-
275 Lawrence (KL) score for staging disease. Discussions highlighted that radiography is considered
276 outdated and flawed, but that X-rays are still the gold standard (alongside clinical criteria) for
277 diagnosis and assessing OA in the clinic, e.g. for suitability for arthroplasty.

278

279 **DISCUSSION**

280

281 Whilst OA has long been recognised as a heterogeneous multi-faceted disorder, progress into
282 defining subgroups or categories has been poor; this is a likely reason why several clinical trials
283 of novel pharmaceuticals or Disease Modifying Osteoarthritis Drugs (DMOADs) have failed^[27-29].

284 In other areas of medicine such as asthma, subcategorisation has been achieved according to
285 the pathological mechanisms (i.e. molecular endotyping) and clinical phenotyping^[30]. It is to be
286 hoped that this can be achieved for OA, resulting in improved diagnosis, understanding of
287 disease mechanisms, identification of novel therapeutic targets, the development of new
288 therapies and, subsequently better stratification and improved treatment of patients. Indeed,
289 this was a conclusion of the inaugural meeting of an EPSRC-funded UK initiative for the OATech
290 Network+, with the subsequent decision to utilise a Delphi-style process to address this topic.

291

292 As technology becomes more sophisticated and specialised there is a danger of working
293 increasingly in silos. This process, including expert participants (>20% having >20 years' OA
294 research experience), from several disciplines, facilitated an appraisal across key areas where
295 technology has made great advances. The benefits associated with this were indicated in
296 participant feedback, for example, the change in consensus on technologies such as clinical
297 engineering. The process highlighted a consensus belief that adopting key existing and emerging

298 technologies (ML, genetic analysis, MRI, proteomics, wet biomarker analysis, activity monitoring,
299 metabolomics, epigenomics and clinical engineering), would increase successful delivery of
300 improved OA subcategorisation and discussions raised many suggestions as detailed below. In
301 contrast, existing literature provided little agreement on the approach to OA categorisations and
302 indeed, other studies that have highlighted the urgent need for updated definitions and
303 categories^[31,32].

304 X-rays, discussed at length, are well known to have limitations, especially with regard to the KL
305 scoring system for radiographic diagnosis of OA^[33,34]. The inclusion of clinical and non-clinical
306 participants was particularly beneficial with orthopaedic surgeons highlighting that X-rays
307 remain a valued clinical technology, being relatively simple, cheap, readily available and
308 routinely and useful for diagnosis and treatment decisions. The KL radiographic classification
309 scheme for OA, first described in 1957^[33], remains the most widely used clinical tool for the
310 radiographic diagnosis of OA^[34], despite its known limitations. Hence X-rays should be retained in
311 OA studies and based on previous improvements^[35], the optimistic aim is to enhance their use
312 through further application of ML and AI.

313
314 Epigenetic changes can modulate the impact of risk-conferring alleles of DNA polymorphisms
315 that are associated with OA. For example, if a polymorphism is in a gene-regulatory element and
316 the risk allele reduces gene expression, its effect can be attenuated or aggravated by DNA
317 methylation of that element in an allele-specific manner^[4]. As such, subgrouping OA patients by
318 their genetic and epigenetic profile might reduce the heterogeneity seen across patients and
319 enhance the interpretability of functional studies of genetic risk.

320
321 Large datasets generated from activity tracking through the increased adoption of smartphones
322 and wearables, are likely to provide further opportunities to aid the stratification of OA
323 populations. Activity monitoring research in OA populations has, in the past, been limited to
324 measurements over short durations (i.e. up to 7-days), hence providing limited insight. Fitness

325 trackers and smart phones have revolutionised the opportunities to collect continuous activity
326 data more reliably and over longer time periods. Objective measures of physical activity can be
327 used for monitoring recovery e.g. following joint arthroplasty, to measure short term recovery in
328 terms of daily step count change over the first four weeks post-surgery^[36]. Extending this
329 approach over a large sample population would allow an expected trajectory of recovery to be
330 developed such that patients deviating from it could, for example, be flagged for follow-up
331 consultation. Deeper analysis and modelling of the inertial sensor data collected by wearables
332 will be important for categorising OA populations. For example, multi-dimensional analyses of
333 activity data have been found to be more accurately associated with functional test outcomes
334 than step-count and sedentary time measures alone^[37]. Similarly, studies have investigated
335 longer term monitoring with follow-up measures at 3-12 months post-surgery^[21]. Interestingly,
336 there was no substantial increases in activity after 12-months, concluding that more behavioural
337 interventions are required to promote physical activity in the recovery period.

338
339 ML was the only technology reaching 100% consensus in its ability to improve OA
340 subcategorisation in Round 2 of the Delphi, highlighting recognition of its potential use . During
341 discussions, the importance of integrating data, especially 'big' data, across disciplines and the
342 application of ML approaches was highlighted as being of great importance. In big data science,
343 ML is based on computer algorithms that can learn to identify complex patterns based on real
344 data^[38, 39]. The goal of ML is to enable an algorithm to learn from past and/or present data and
345 then to make predictions or decisions for unknown future events^[40].

346
347 ML/AI is of paramount importance to all technologies generating 'big data', such as genomics,
348 all omics and imaging modalities now used in biomarker and molecular signature discovery in
349 OA. The use of ML/AI in integrating these advanced analytical techniques, , provides the
350 opportunity to build and test complex models incorporating important non-biomarker
351 covariates. Multi-omics data has enabled biomarkers generations for the stratification of

352 patients into subgroups e.g. in oncology and other chronic diseases such as asthma^[41,42]. This
353 allows subcategorisation into groups based on genetic variability and other biomarkers so that
354 medications may be tailored to individuals^[43, 44]. Big data systems using multi-omics (genomics,
355 proteomics, metabolomics and epigenomics), enables, understanding of interactions and
356 functions of the genome, often identifying unexpected functions or possibly illustrating the
357 interplay between the genome, the cellular environment and the progression of disease.

358

359 In summary, a Delphi-type exercise was undertaken as a route to obtaining expert consensus
360 from a range of disciplines, regarding the role of novel experimental technology in OA research.
361 It provided a valid route to recommendations for the focus and direction that should be adopted
362 by the cross-disciplinary OA research community. Rather than employing individual
363 technologies, it is likely that combining several identified technologies (eg proteomics, imaging
364 and clinical engineering, together with machine learning), across sites, focussing on one or more
365 OA subgroups will reap real benefits and provide important advances in the field of
366 osteoarthritis research.

367

368 **Acknowledgments**

369

370 The authors wish to thank all members of the Delphi panel, the EPSRC, Tissue Engineering and
371 Regenerative Centre Versus Arthritis and MRC for financial support (grant numbers:
372 EP/N027264/1, Versus Arthritis (VA) 21156, VA 18450 and MR/L0104531/1). Professor Roberts,
373 Dr Mennan and Mr Hopkins acknowledge support from VA 21156. The authors also wish to
374 acknowledge Professors Mary Goldring, Virginia Kraus and Richard Loeser for help and advice.
375 Professor Loughlin acknowledges the support of VA (grant 20771), and the Medical Research
376 Council and Versus Research via the Centre for Integrated Research into Musculoskeletal Ageing
377 (CIMA, grant references JXR 10641, MR/P020941/1 and MR/R502182/1). Dr Watt is a UKRI
378 Future Leaders Fellow and acknowledges the support of the Centre for Osteoarthritis

379 Pathogenesis Versus Arthritis (grants 20205 and 21621) and NIHR Oxford Biomedical Research
380 Centre. The views expressed are those of the authors and not necessarily those of the NHS, the
381 NIHR or the Department of Health. The authors also wish to thank the Francis Costello Library,
382 RJAH, for assistance with literature searches.

383

384 **Author contributions**

385

386 All authors contributed to the ideas, questionnaire and writing the manuscript. All authors gave
387 final approval of the version submitted.

388

389 **Role of the funding source**

390

391 This meeting was funded by OATech Network+ (EP/N027264/1) and CM by Versus Arthritis
392 Tissue Engineering and Regenerative Therapies Centre (Grant number 21156).

393

394 **Conflict of interest**

395

396 The authors report that there is no conflict of interest.

397

399

- 400 1. Ryd L, Brittberg M, Eriksson K, Jurvelin JS, Lindahl A, Marlovits S. et al. Pre-
401 Osteoarthritis: Definition and diagnosis of an elusive clinical entity. *Cartilage*. 2015; 6(3):
402 156-165.
- 403 2. Zengini E, Hatzikotoulas K, Tachmazidou I, Steinberg J, Hartwig FP, Southam L. *et al.*
404 Genome-wide analyses using UK Biobank data provide insights into the genetic
405 architecture of osteoarthritis. *Nature Genetics*. 2018; 50: 549–558.
- 406 3. Barter, MJ, Bui C, Young DA. Epigenetic mechanisms in cartilage and osteoarthritis: DNA
407 methylation, histone modifications and microRNAs. *Osteoarthritis Cartilage*. 2012; 20,
408 339-349.
- 409
- 410 4. Reynard LN. (2017) Analysis of genetics and DNA methylation in osteoarthritis: What
411 have we learnt about the disease? *Semin. Cell Dev. Biol.* 2017; 62, 57-66.
412
- 413 5. van Meurs JB. Osteoarthritis year in review 2016: genetics, genomics and epigenetics.
414 *Osteoarthritis Cartilage*. 2017; 25, 181-189.
415
- 416 6. Wong JW & Cagney G. An overview of label-free quantitation methods in proteomics by
417 mass spectrometry. *Methods Mol Biol*. 2010. 604: 273-83.
418
- 419 7. Cox DM, Zhong F, Du M, Duchoslav E, Sakuma T, McDermott JC. Multiple reaction
420 monitoring as a method for identifying protein posttranslational modifications. *J Biomol*
421 *Tech*. 2005; 16(2): 83-90.
422
- 423 8. Peffers MJ, Smagul A, Anderson JR. Proteomic analysis of synovial fluid: current and
424 potential uses to improve clinical outcomes. *Expert Rev Proteomics*. 2019. 4:287-302.
425 doi: 10.1080/14789450.2019.1578214.
426
- 427 9. Anderson JR, Chokesuwattanasakul S, Phelan MM, Welting TJM, Lian LY, Peffers MJ et al.
428 ¹H NMR Metabolomics Identifies Underlying Inflammatory Pathology in Osteoarthritis
429 and Rheumatoid Arthritis Synovial Joints. *J Proteome Res*. 2018; 11:3780-3790. doi:
430 10.1021/acs.jproteome.8b00455.
431
- 432 10. Kraus VB, Blanco FJ, Englund M, Henrotin Y, Lohmander LS, Losina E, et al. OARSI Clinical
433 Trials Recommendations: Soluble biomarker assessments in clinical trials in
434 osteoarthritis. *Osteoarthritis Cartilage*. 2015;23(5):686-97.
435
- 436 11. Zhang W, Sun G, Likhodii S, Liu M, Aref-Eshghi E, Harper PE, et al. Metabolomic analysis
437 of human plasma reveals that arginine is depleted in knee osteoarthritis patients.
438 *Osteoarthritis Cartilage*. 2016;24(5):827-34.
439
- 440 12. Watt FE, Corp N, Kingsbury SR, Frobell R, Englund M, Felson DT et al. Towards
441 prevention of post-traumatic osteoarthritis: report from an international expert working
442 group on considerations for the design and conduct of interventional studies following
443 acute knee injury. *Osteoarthritis Cartilage*. 2018;26(3):312-8
444
- 445 13. Kraus VB, Collins JE, Hargrove D, Losina E, Nevitt M, Katz JN, et al. Predictive validity of
446 biochemical biomarkers in knee osteoarthritis: data from the FNIH OA Biomarkers
447 Consortium. *Annals of the rheumatic diseases*. 2017;76(1):186-95.
448

- 449 14. Watt FE, Paterson E, Freidin A, Kenny M, Judge A, Saklatvala J, et al. Acute Molecular
450 Changes in Synovial Fluid Following Human Knee Injury: Association With Early Clinical
451 Outcomes. *Arthritis & rheumatology*. 2016;68(9):2129-40.
452
- 453 15. Metcalfe AJ, Andersson ML, Goodfellow R, Thorstensson CA. Is knee osteoarthritis a
454 symmetrical disease? Analysis of a 12 year prospective cohort study. *BMC*
455 *musculoskeletal disorders*. 2012;13(1):153.
456
- 457 16. Metcalfe AJ, Stewart CJ, Postans NJ, Biggs PR, Whatling GM, Holt CA et al. Abnormal
458 loading and functional deficits are present in both limbs before and after unilateral knee
459 arthroplasty. *Gait & posture*. 2017;55:109-15.
460
- 461 17. Miyazaki T, Wada M, Kawahara H, Sato M, Baba H, Shimada S. Dynamic load at baseline
462 can predict radiographic disease progression in medial compartment knee osteoarthritis,
463 *Ann. Rheum. Dis*. 2002. 61: 617–622.
464
- 465 18. Hatfield GL, Hubley-Kozey CL, Astephen Wilson JL, Dunbar MJ. The Effect of Total Knee
466 Arthroplasty on Knee Joint Kinematics and Kinetics During Gait, *J. Arthroplasty*. 26 (2011)
467 309–318. doi:10.1016/j.arth.2010.03.021.
468
- 469 19. Dobson F, Hinman RS, Roos EM, Abbott JH, Stratford P, Davis AM et al. OARSI
470 recommended performance-based tests to assess physical function in people diagnosed
471 with hip or knee osteoarthritis. *Osteoarthritis and Cartilage*. 2013; 21. 1042e1052.
472
- 473 20. Fransen M, McConnell S, Harmer AR, Van der Esch M, Simic M, Benne KL et al. Exercise
474 for osteoarthritis of the knee. *Cochrane Database Syst. Rev*. 2015; 1, CD004376.
475
- 476 21. Jeldi A J, Deakin AH, Allen DJ, Granat MH, Grant M, Stansfield BW. Total Hip
477 Arthroplasty Improves Pain and Function but Not Physical Activity. *J. Arthroplasty*. 2017;
478 32, 2191–2198.
479
- 480 22. Jamshidi A, Pelletier J-P & Martel-Pelletier J. Machine-learning-based patient-specific
481 prediction models for knee osteoarthritis. *Nature Reviews Rheumatology*. 15 (2019) 49-
482 60.
483
- 484 23. Jamaludin A, Lootus M, Kadir T, Zisserman A, Urban J, Battié MC et al. The Genodisc
485 Consortium. Automation of reading of radiological features from magnetic resonance
486 images (MRIs) of the lumbar spine without human intervention is comparable with an
487 expert radiologist. *European Spine Journal*. 2017; DOI 10.1007/s00586-017-4956-3.
488
- 489 24. Jamaludin A, Kadir T, Zisserman A. SpineNet: Automated Classification and Evidence
490 Visualization in Spinal MRIs. *Medical Image Analysis*. 2017; 41: 63-73.
491
- 492 25. Pfirrmann CWA, Metzdorf A, Zanetti M, Hodler J & Boos N. Magnetic Resonance
493 Classification of Lumbar Intervertebral Disc Degeneration. 2001. *SPINE*. 26(17):1873–
494 1878.
495
- 496 26. Zhang W, Moskowitz RW, Nuki G, et al. OARSI recommendations for the management of
497 hip and knee osteoarthritis, Part II: OARSI evidence-based, expert consensus guidelines.
498 *Osteoarthritis. Cartilage*.2008. 16: 137-62.
499
- 500 27. Hellio le Graver MP, Clemmer RS, Redifer P, Brunell RM, Hayes CW, Brandt KD, et al. A 2-
501 year randomised, doubleblind, placebo-controlled, multicentre study of oral selective
502 iNOS inhibitor, cindunistat (SD-6010), in patients with symptomatic osteoarthritis of the

- 503
504
505 28. Karsdal MA, Byrjalsen I, Alexandersen P, Bihlet A, Andersen JR, Riis BJ, et al. Treatment
506 of symptomatic knee osteoarthritis with oral salmon calcitonin: results from two phase 3
507 trials. *Osteoarthritis Cartilage*. 2015; 23.
508
- 509 29. Watt FE, Corp N, Kingsbury SR, Frobell R, Englund M, Felson DT et al. Towards
510 prevention of post-traumatic osteoarthritis: report from an international expert working
511 group on considerations for the design and conduct of interventional studies following
512 acute knee injury. *Osteoarthritis Cartilage*. 2019. 1:23-33. doi:
513 10.1016/j.joca.2018.08.001.
514
- 515 30. Svenningsen S & Nair P. Asthma Endotypes and an Overview of Targeted Therapy for
516 Asthma. *Frontiers in Medicine*. 2017; 4: 158.
517
- 518 31. Kraus VB, Blanco FJ, Englund M, Karsdal MA, and Lohmander LS. Call for Standardized
519 Definitions of Osteoarthritis and Risk Stratification for Clinical Trials and Clinical Use.
520 *Osteoarthritis & Cartilage*. 2015; 8: 1233–1241.
521
- 522 32. Kingsbury SR, Corp N, Watt FE, Felson DT, O’Neill TW, Holt CA et al. Harmonising data
523 collection from osteoarthritis studies to enable stratification: recommendations on core
524 data collection from an Arthritis Research UK clinical studies group. *Rheumatology*.
525 2016; 55.
526
- 527 33. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. *Ann Rheum*
528 *Dis*. 1957;16:494–502. doi: 10.1136/ard.16.4.494.
529
- 530 34. Braun HJ, Gold GE. Diagnosis of osteoarthritis: imaging. *Bone*. 2012;51:278–288. doi:
531 10.1016/j.bone.2011.11.019.
532
- 533 35. Tiulpin A, Thevenot J, Rahtu E, Lehenkari P & Saarakkala S. Automatic Knee
534 Osteoarthritis Diagnosis from Plain Radiographs: A Deep Learning-Based Approach. *Sci*
535 *Rep*. 2018; 8: 1727.
536
- 537 36. Toogood PA, Abdel MP, Spear JA, Cook SM, Cook DJ, Taunton MJ. The monitoring of
538 activity at home after total hip arthroplasty. *Bone Jt. J*. 2016; 98–B, 1450–1454.
539
- 540 37. Sliepen, M, Mauricio E, Lipperts M, Grimm B, & Rosenbaum D. (2018). Objective
541 assessment of physical activity and sedentary behaviour in knee osteoarthritis patients–
542 beyond daily steps and total sedentary time. *BMC musculoskeletal disorders*, 19(1), 64.
543
- 544 38. Kononenko I. Machine learning for medical diagnosis: history, state of the art and
545 perspective. *Artif Intell Med*. 2001;23(1):89–109.
546
- 547 39. Lane HY, Tsai GE, Lin E. Assessing gene-gene interactions in pharmacogenomics. *Mol*
548 *Diagn Ther*. 2012;16(1):15–27.
549
- 550 40. Landset S, Khoshgoftaar TM, Richter AN, Hasanin T. A survey of open source tools for
551 machine learning with big data in the hadoop ecosystem. *J Big Data*. 2015;2:24.
552
- 553 41. Lin E, Lin CG, Wang JY, Wu LS. Gene-gene interactions among genetic variants from
554 seven candidate genes with pediatric asthma in a Taiwanese population. *Curr Topics*
555 *Genet*. 2009;3:83–8.
556

557 42. Bailey MH, Tokheim C, Porta-Pardo E, Sengupta S, Bertrand D, Weerasinghe A et al.
558 Comprehensive Characterization of Cancer Driver Genes and Mutations. *Cell*. 2018,
559 173:371-385.
560

561 43. Lin E, Tsai SJ. Novel diagnostics R&D for public health and personalized medicine in
562 Taiwan: current state, challenges and opportunities. *Curr Pharmacogenomics Person*
563 *Med*. 2012;10:239–46.
564

565 44. Lin E, Hwang Y, Tzeng CM. A case study of the utility of the HapMap database for
566 pharmacogenomic haplotype analysis in the Taiwanese population. *Mol Diagn Ther*.
567 2006;10:367–70.
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609

Journal Pre-proof

611 **Figure 1.A.** Frequency histogram indicating change of panel members' response as to whether
612 different technologies were able to improve OA stratification in Round 1 (before the focus meeting)
613 and Round 2 (after the instructive lectures at the start of the meeting). Nine of the 11 technologies
614 reached consensus after the 2nd round. B. The modified question related to X-ray and ultrasound
615 technologies for the 3rd round for the clinic and research and the % agreement.

616

617

618

619

620

621

622

623

624

625

626

627

628

629

630

631

632

633

634

635

636

637

638

639

640

641

642

643

644

645

646

647

648

649

650

651

652

653

654 **Table 1.** Statements used in the DELPHI and the percentage of participants who agreed with the
655 statements at each Round.

DELPHI statement/Question		Round 1	Round 2	Modified question for round 3	Round 3	
		Percentage agreement with statement				
1	OA is a disease of i. Bone ii. Cartilage iii. Bone and cartilage	1. <u>2.9</u> 2. <u>5.7</u> 3. 91.4	1. <u>3.1</u> 2. <u>0</u> 3. 96.9			
2	OA always involves other tissues in the joint in addition to bone and or cartilage	63.9	87.9	OA involves other tissues in the joint in addition to bone and cartilage	100	
3	Early OA needs categorising differently to 'established OA	86.1	87.9	Panel decided not to take this question forward		
4	Osteoarthritis needs re-defining	65.7	69.7			
5	OA is a continuum	88.6	97			
6	Subcategorising OA is useful	94.3	100			
7	The definition of OA needs to be joint specific	55.6	69.7	The definition of OA needs to encompass joint specific differences	66.7	
8	OA phenotypes should rely on underlying mechanisms	73.5	84.8			
9	X-rays alone can be used to categorise OA phenotype	<u>5.6</u>	<u>6.1</u>			
10	The Kellgren-Lawrence (KL) is the most appropriate for categorising OA on X-ray	50	74.2	There is a need for an improved scoring system than the Kellgren-Lawrence for X-rays	93.9	
11	MRI has no role to play in categorising OA	<u>2.8</u>	<u>9.1</u>			
12	A universal OA categorisation system can be used for all clinical cases of OA	44.4	56.3	Panel decided not to take this question forward		
13	The same categorisation system for OA can be used in the clinic and or research studies	57.1	59.4	The same categorisation system for OA should be used in the clinic and or research studies	78.8	
14	The latest technological advances can be used to improve OA subcategorisation	100	100			
15	Please say if you agree or disagree that the application of the following technologies can improve clinical OA subcategorisation Epigenomics Genetic analysis MRI <i>X-ray</i> <i>Ultrasound</i> Metabolomics Proteomics Wet biomarker analysis Machine learning (AI) Activity monitoring Clinical engineering	84.8 91.4 100 82.9 58.8 78.8 87.9 97.1 88.9 68.6 72.2	87.5 97 97 48.5 66.7 90.6 93.8 93.8 100 90.9 87.5		Clinical 87.9 75.8	Research 75.8 69.7

16	Different OA subcategorisation	Journal	Pre-proof		
	systems have been suggested in the literature recently. Please say if you agree or disagree with the following statements taken from the literature.				
	A. Examples of OA can be: Hip/knee/hip and or knee ^[45]	58.3	72.7		51.5
	B. Pain, symptoms, clinical examination and X-rays are the most useful factors in diagnosing early OA ^[46]	45.7	36.4		42.4
	C. Pain, psychological distress, radiographic severity, BMI, muscle strength, inflammation and comorbidities are all associated with clinically distinct OA phenotypes ^[47]	60	69.7		51.5
	D. Minimal joint disease, malaligned, biochemical, chronic pain, inflammatory metabolic syndrome and bone and cartilage metabolism are all main phenotypes of OA ^[48]	61.8	72.7		48.5
	E. Knee OA phenotype is defined by patient reported frequent knee pain, cartilage damage and the presence of degenerative meniscal tissue ^[49]	58.8	48.5		39.4
	F. OA can be classified by symptomatic radiographic OA (primary criteria) and pain alone (secondary criterion).	31.4	24.2		36.4

656
657
658
659
660
661
662
663
664
665
666
667
668
669
670

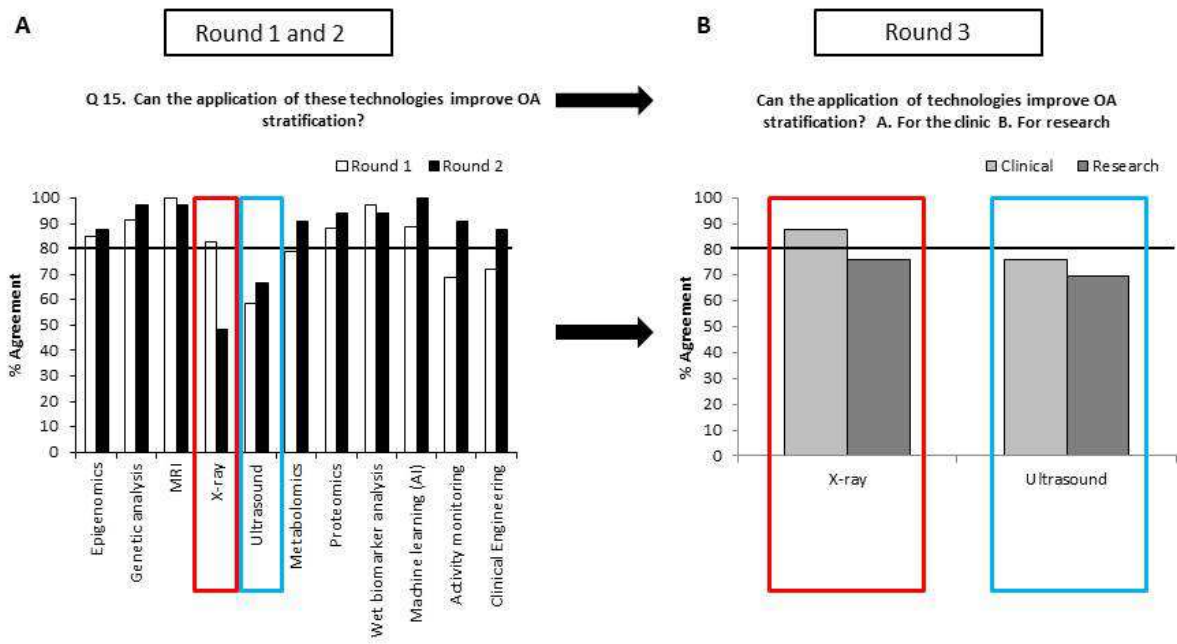
671 Table 2. Definitions of OA from different professions on the Delphi panel.

Profession	OA definition
Physiotherapists	A syndrome affecting the joints of the body
	Joint pathology leading to pain and functional limitation that involves genetics and epigenetic factors
Rheumatologists	Structural alteration of cartilage and bone in a joint which results in pain and loss of function
	A disease of the whole joint with distinct clinical and structural phenotypes
	A disease of many tissues of the joint including cartilage and bone, associated with pain or stiffness
	Osteoarthritis is a whole-joint disease, affecting articular and periarticular tissues. It has components of degeneration, regeneration and low-grade inflammation that differ in extent and clinical consequences between joints, disease stages and patients
Orthopaedic Surgeons	Structural and biological derangement of joint (that isn't rheumatoid/ankylosing spondylosis/psoriatic)
	A painful condition involving changes in multiple tissues of the joint
Engineers	A disease of the joint, characterised by pain, loss of function and degeneration/progressive damage of structures in/around the joint
	Musculoskeletal disease possibly triggered by altered joint biomechanics and biological signalling leading to joint tissue degeneration, inflammation and pain
Radiologist	Degenerative joint change currently based on exclusion of other causes
Vet	Degenerative whole joint disease with an inflammatory component
Scientists (researcher)	Joint disease that results in cartilage degeneration, bone changes and pain
	Degenerative disorder of the joint
	A degenerative disease of the bone and cartilage. Can lead to cartilage loss, joint inflammation, changes in the bone and pain

672 * The number of comments shown indicates the number of people who provided definitions in each
 673 profession.

674
 675
 676
 677
 678
 679
 680
 681
 682
 683
 684
 685
 686
 687
 688
 689
 690
 691
 692

694 Figure 1.



695

Journal Pre