Randomised, double-blind comparison of a fixed co-formulation of intra-articular polynucleotides and hyaluronic acid versus hyaluronic acid alone in the treatment of knee osteoarthritis: two-year follow-up

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Research Article

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ABSTRACT

Background: A first-year interim analysis of this two-year study suggested that intra-articular injections of highly purified, natural-origin polynucleotides and hyaluronic acid (HA) as a fixed combination (PNHA) might improve knee function and joint pain more effectively than HA alone in patients with knee osteoarthritis (OA). Purpose of the second-year analysis herein described was verifying whether the first-year interim outcomes persist over the whole two-year period.

Methods: Randomised, double-blind, HA-controlled clinical trial in 100 knee OA patients (98 randomised, 69 completing the study) in a high-specialisation tertiary care setting. The hypothesised difference of efficacy between PNHA and HA for the original sample size estimate is 20%. Treatment cycle: 3 weekly intra-articular knee injections of either PNHA or HA. Evaluations: Western Ontario and McMaster Universities (WOMAC) score and Knee Society Score (KSS) as, respectively, primary and secondary endpoints, evaluated at baseline and after 2, 6, 12, and 24 months; synovial fluid levels of proinflammatory mediators (biochemical and immunoenzymatic assays at baseline and the end of the treatment cycle). Adverse

effects investigated at each control visit. Statistical analysis: Kruskal-Wallis test for independent samples (nonparametric one-way analysis of variance) after correction of means for age, Body Mass Index and Kellgren-Lawrence grade. If significant, pairwise post-hoc Sidak multiple comparisons.

Results: KSS total score and KSS pain item: significant improvement in both groups, with significantly more pain improvement in patients treated with PNHA (2-point reduction) than HA (1-point reduction). Both groups experienced significant long-term reductions in WOMAC total scores: significantly stronger in PNHA-treated patients after 24 months with a steady difference of 16% favouring PNHA in WOMAC pain subscore. No clinically significant adverse events in either group.

Conclusions: The outcomes of the 2-year study confirmed that a short cycle of intra-articular treatment (3 weekly double-blind injections) with polynucleotides (long-acting viscosupplementation properties, pro-trophic activity on chondrocytes, pain-relieving properties) in fixed combination with high molecular weight hyaluronic acid is more effective in improving knee function and pain in knee OA patients than HA alone. PNHA may be elective for viscosupplementation in knee OA patients with fastidious and resistant pain, signs of inflammation or worsening disease.

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Registration, 15/04/2015
ClinicalTrials.gov database link:
https://clinicaltrials.gov/ct2/show/NCT02417610?term=NCT02417610&cntry=IT&draw=2&rank=1

KEYWORDS

Knee osteoarthritis; knee function; hyaluronic acid; KSS; knee pain; PN-HPT™; polynucleotides; WOMAC

BACKGROUND

The debate about the real value of hyaluronic acid (HA) as infiltrative therapy of knee osteoarthritis (OA) is far from over in evidence-based guidelines and consensus reports.[1-3] Highly purified polynucleotides from trout gonads, also known with the acronym PN-HPT™ (Polynucleotides Highly Purified Technology), provide persistent viscosupplementation, show trophic properties on chondrocytes and mesenchymal cells, and reduce pain more effectively and more rapidly than HA.[4-6] *In-vitro* and *in-vivo* synergy between PN-HPT™ and HA on chondrocyte trophism and pain control has also been convincingly established—a strong rationale to administer the two viscosupplementation agents together.[7]

The study aimed to verify over two years whether "the association of PN-HPT™ and HA injections would reduce pain in patients affected by knee OA more than HA alone", and whether "it is more effective in improving knee function and pain, in joints affected by OA, compared with HA alone", as suggested by the authors in their first-year interim report.[8] Analysing the final two-year outcomes of the study also aimed to verify whether the clinical synergy between PN-HPT™ and HA, which the

first-year interim analysis suggested, is persistent over a much longer time or it is just a transient medium-term effect.

METHODS

Study Design, Sample Size Estimate and Patient Selection

A hundred knee OA patients, aged between 51and 74 years, were initially screened between mid-September 2014 and mid-July 2015, and 98 randomised, in a double-blind, single-centre, HA-controlled study. Two patients were excluded after failure to meet the inclusion criteria. The authors carried out the study at the Rizzoli Orthopedic Institute, Bologna, Italy, in rigid agreement with the most recent clinical practice guidelines and ethical regulations (for details, see the report that discussed the interim outcomes after the first year of treatment).[8] The final, two-year outcomes are herein illustrated. Demographics and the initially randomised knee OA patients' selection criteria are summarised in Table 1 and Table 2, respectively. The intake of NSAIDs and other drugs was free during the two-year study period; investigators only recorded the NSAIDs consumption since the last visit.

The assumptions initially leading to the sample size calculation and the technicalities adopted for creating the randomisation list and preserving the double-blindness all those involved, patients, investigators, data collectors and outcome assessors, were exhaustively described in the first-year interim report.[8]

The main points about the sample size estimate are herein summarised. With the per cent WOMAC change at 12 months considered as the primary endpoint, the following formula gave an estimate of the needed sample size:[8]

$$\Delta \text{ WOMAC (per cent difference vs baseline)} = \frac{\text{12-month WOMAC} - \text{baseline WOMAC}}{100 - \text{baseline WOMAC}}$$

Based on previous HA literature and exploratory unpublished PNHA little studies, the basic assumption leading to the original sample size estimate was that standard deviations were 26.9% for PNHA-treated patients and 39.1% for HA-treated patients. Further assumptions were that standard deviations would be similar for the two populations to be enrolled. The two intra-articular treatments would differ by at least 20%, in terms of clinical efficacy, under the null hypothesis that the two treatments had similar WOMAC per cent variations. With the assumption of a false-positive (alpha) error of 0.05 and power to avoid false negatives of at least 0.80, a minimum clinically meaningful difference of 20% and a drop-out rate of 10%, the minimum estimated number of patients was 50 per group (100 overall).[8]

The coded packages of PNHA and HA syringes were identical with syringes masked by identical sleeves. The randomisation list reported the numerical code on syringe packages; investigators received the randomisation codes for each patient sealed in an envelope.[8]

Ninety out of initially randomised patients completed the study at [T5] (interim evaluation after the first year of treatment), 46 in the PNPHA study group and 44 in

136 the HA control group; all of them then progressed to [T6] (end of study). Sixty-nine
137 patients completed the 2-year study (final follow-ups: 70%). All the patients who had
138 dropped out at the end of the first year did it for personal reasons.[8]
139

	All patients (n=100)	Study Group (PNHA, n=49	Control Group (HA, n=49)
Age, yrs	50-75 (63.8 ± 5.8)	63.4 ± 6.5	64.2 ± 5.1
Kellgren-Lawrence grade[9]	2 ± 0.7	1.9 ± 0.6	2.1 ± 0.7
Sex, male/female, n	46/54	24/26	22/28
Body Mass Index, kg/m²	28,1 ± 3,5	28,1 ± 3,4	28,1 ± 3,7
Weight, kg	80.0 ± 11.6	80.2 ± 10.2	79.8 ± 13
Height, cm	168,5 ± 9.2	168,9 ± 9.5	168,1 ± 9.0

140 **Table 1** Demographics of the originally screened knee osteoarthritis patients.[8,9]
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Exclusion criteria
Abuse of alcohol or drugs
Pregnancy or breastfeeding
Patients who underwent repeated infiltrative therapies or patients who only underwent a single HA infiltration cycle, although performed less than six months before enrolment
Ongoing treatment with systemic anticoagulants or steroids, or therapy suspended for less than one month
Hypersensitivity to the study products, previous bone fractures, severe knee trauma, joint deformities, rheumatoid arthritis, inflammatory diseases of joints, previous surgical procedures (e.g., meniscectomy, scope debridement)
Haematological diseases or local skin lesions in the site of treatment inoculation

143 **Table 2** The criteria adopted for selecting the 98 enrolled patients.[8]
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Treatments

The regulatory classification of the patented, proprietary fixed PNHA combination investigated in the 2-year study was as a Class-III CE-marked (0373) medical device: pre-filled, single-use, neutral glass 2-mL syringes dosed at 10 mg/mL of natural-origin PN-HPT™ and 10 mg/mL of a biotechnological sodium HA (molecular weight > 1500 kDa) for an overall syringe content of 40 mg in 2 mL of active principles. The European Union's regulatory authorities and several extra-European countries registered the proprietary fixed PNHA combination (brand, POLIART®, Mastelli Srl, San Remo, Italy) for the indication "intra-articular treatment of degenerative chondral disorders". The control HA product (IALART®, Mastelli Srl, San Remo, Italy), is also a Class III CE 0373 commercially available medical device of HA (1200-1500 kDa), industrially obtained from bacterial fermentation and dosed at 40 mg in 2 mL. The formulation of both study products was as absorbable, viscoelastic sterile gels.

Highly skilled specialists performed three weekly intra-articular double-blind infiltrations with 18 to 22 G needles at baseline [T0] and over the following two weeks [T1] and [T2], under aseptic conditions and following standard intra-articular techniques (injected amount at each session, 2 mL). Samples of the synovial fluid (nearly 6 mL of the removed excess synovial fluid) were collected and sent to the laboratory before the first infiltration [T0] and at the end of the treatment cycle [T2] (Figure 1).

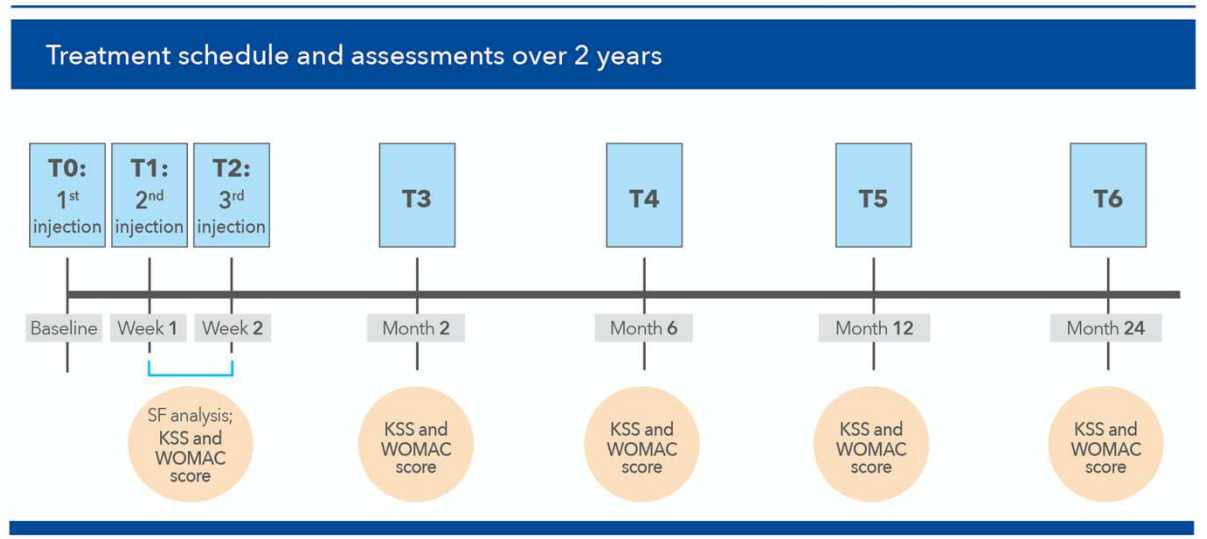

Figure 1 **[T0] to [T2]:** timing of the three double-blind PNHA and HA intra-articular injections and assessments planned over the first two study weeks (synovial fluid analysis and first KSS and WOMAC evaluation); **[T3] to [T6]:** timing of the KSS and WOMAC evaluations planned over the residual 2-year study period.

Follow-Up Assessments

The knee joint function and pain were assessed, with the help of the Knee Society Score (KSS)[10] and the self-administered Western Ontario and McMaster Universities (WOMAC) score [11], at baseline [T0] and after 2 [T3], 6 [T4], 12 [T5] and 24 months [T6] during the 2-year follow-up. A radiographic examination complemented the final clinical evaluation at [T6]. The WOMAC pain subscore was the primary endpoint; KSS, the overall WOMAC score and NSAID consumption were secondary endpoints. Assays of the viscosity of the synovial fluid and the synovial fluid levels of several inflammatory markers — matrix metalloproteinase-1 (MMP1), MM13, tissue inhibitor

of MMP1 (TIMP1), interleukins 1ß (IL-1ß) and IL-6, Tumor Necrosis Factor-α (TNF-α), chemokine IL-8, prostaglandin E_2 (E_2) — were also planned in 40 patients. Assays timing: baseline [T0] and the end of the 2-week treatment cycle [T2] using standard biochemical and immunoenzymatic assays (complete technical details of commercial assays and procedures are available in Ref. 8). As far as possible, all WOMAC and KSS scoring, and indeed all clinical evaluations and biochemical assays on synovial fluid, were performed by the same investigator with only a very few exceptions. Local or systemic side effects were recorded in the electronic clinical report form at each follow-up visit, and the casual relationship immediately assessed and reported for further evaluation.

Statistical Analysis

Descriptive data were tabulated as means ± standard deviations (SD) and graphically as boxplots. The general linear model for repeated measures or Kruskal-Wallis test for independent samples (nonparametric one-way ANOVA test) was applied, after correction of means for age, Body Mass Index (BMI) and Kellgren-Lawrence (KL) grade,[12] to assess for the effect of treatments on the follow-up curves. Using the nonparametric one-way ANOVA test was justified because data (WOMAC, KSS, KSS subscore for pain) were not continuous, although variance was homogeneous (Levene's test). After detecting significant effects of treatments, pairwise post-hoc

Sidak multiple comparisons identified the exact time points of divergence of the curves during the [T3] to [T6] follow-up period.

Regarding the synovial fluid analyses, the Student's t-test for paired samples (one-sample t-test) was used to compare between experimental times within groups and the unpaired t-test (two-sample t-test) for comparisons between groups. The Pearson test for linear relationships between two continuous variables) was used to investigate the correlations between the synovial markers, both among them and between them, and the KSS or WOMAC scores at [T0] and at the end of treatment—[T2] for SF and [T3] for KSS and WOMAC scores. Further statistical details are available in Ref. 8.

Ethical Considerations

The Institutional Review Board of the Rizzoli Orthopedic Institute reviewed all study materials for ethical problems. The principles of the Declaration of Helsinki were always respected. The study was registered in the ClinicalTrials.gov database of privately and publicly funded clinical studies conducted worldwide (ClinicalTrials.gov Identifier: NCT02417610).

RESULTS

Figure 2 illustrates the overall flowchart of the 2-year study. At [T5], the patients of the two groups who progressed towards T6 and the end of the study were still homogeneous for age (p =0.54), Kellgren–Lawrence grade (p = 0.13), gender (p = 0.84), BMI (p = 1), weight (p = 0.86), and height (p = 0.67).

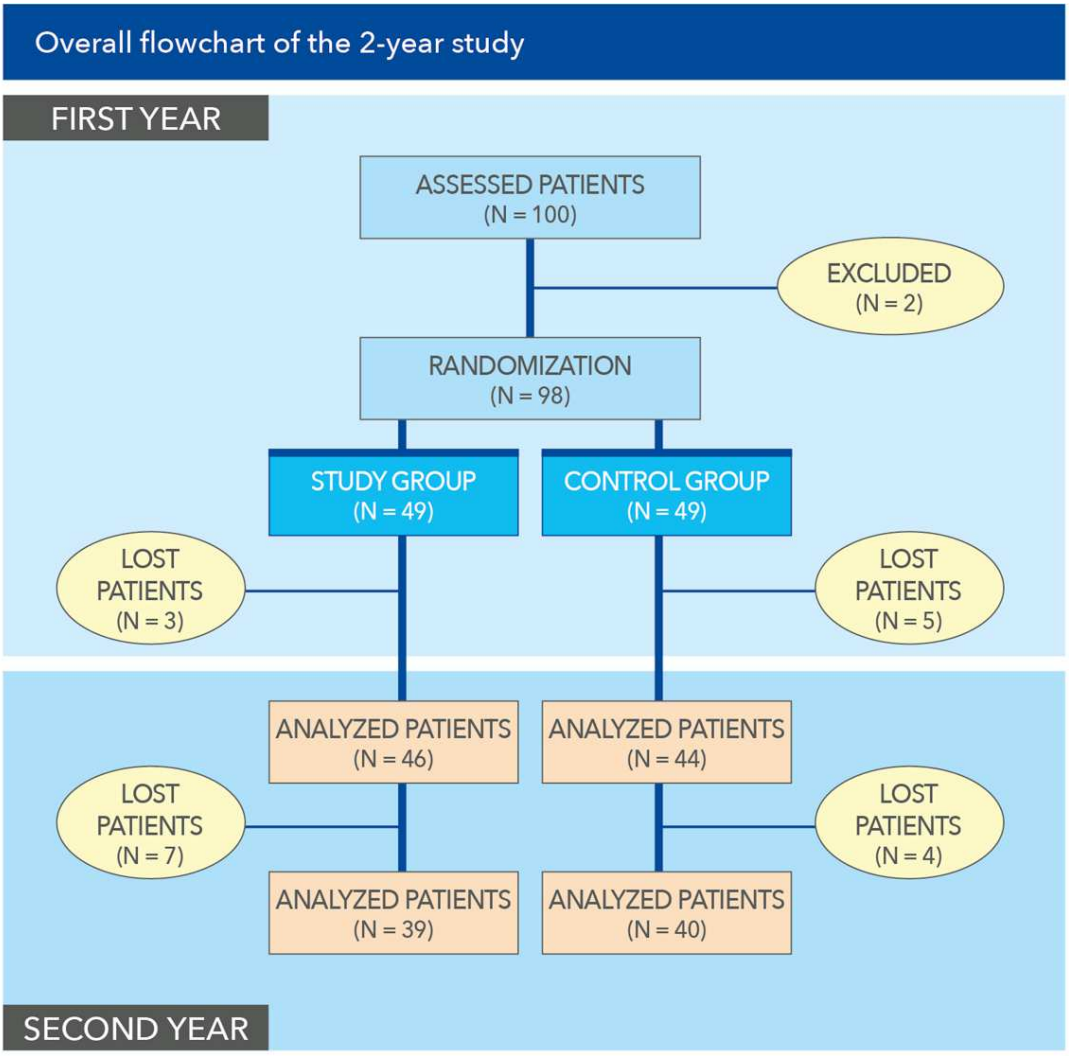

Figure 2 *Upper lighter blue area:* first-year part of the study leading to the interim analysis at the end of the first study year—i.e., outcomes up to [T5] or 12 months discussed in Ref. 8. *Lower darker blue area:* second-year follow-up.

As reported in the interim report, the first year of follow-up saw no infiltration-related complications.[8] Seventy-nine patients completed the study (39 in the PNHA group, 40 in the control HA group), with seven more patients lost in the PNHA group and 4 in the HA group, once again due to personal reasons. As regards the primary endpoint, WOMAC pain score, the pain curves were significantly different at one-way ANOVA (p=0.029; partial eta squared=0.07); divarication of pain curves was both precocious ([T3], p=0.0006 at Sidak test) and steady for two years—[T4] p=0.01, [T5] p=0.001, [T6] p=0.09 (Figure 3).

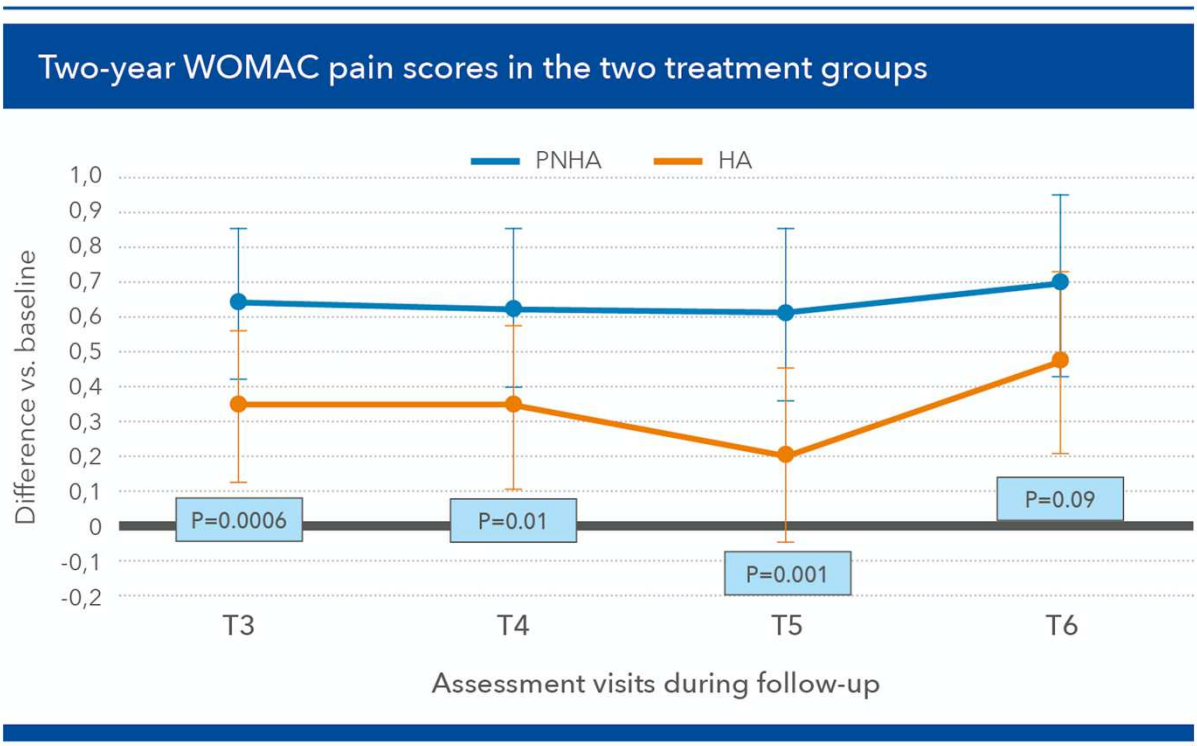

Figure 3 Differences in Western Ontario and McMaster Universities (WOMAC) pain scores (primary endpoint; mean ± SD) vs baseline during the [T3] (2 months) to [T6] (24 months) follow-up period (positive values: improvement vs baseline).

The mean difference in favour of the PNHA group vs the HA control group was about 16%. The improvement of pain showing significant differences at [T4] (p=0.029) and [T5] (p=0.046), and an almost significant difference at [T6] (p=0.059). The other WOMAC items did not show differences between the two groups, with the partial exception of "walking on a flat surface", which was always tendentially easier for patients in the PNHA group and significantly so at [T5] and [T6] (Figure 4). As a result, the mean total WOMAC scores showed a tendency to improve steadily more in the PNHA group than HA controls, over the whole follow-up period (Figure 5), although the difference was statistically significant only at [T6] after corrections for age and other parameters.

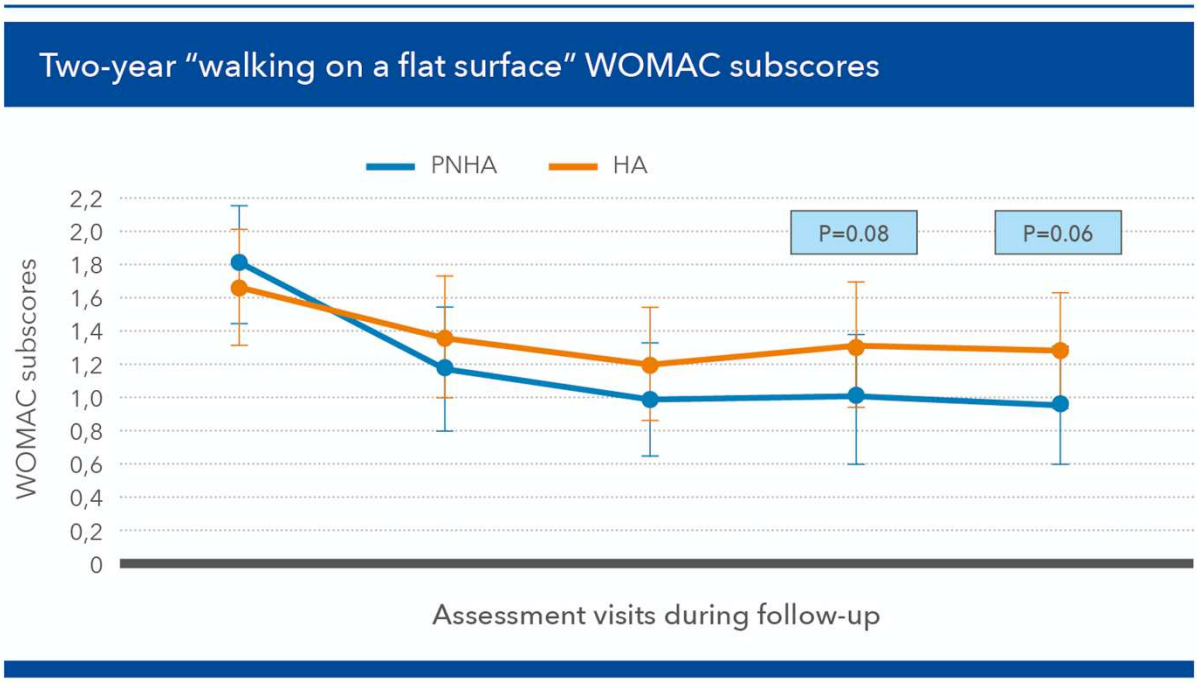

Figure 4 Mean "walking on a flat surface" Western Ontario and McMaster Universities (WOMAC) subscores; mean ± SD) during the [T3] (2 months) to [T6] (24 months) follow-up period (positive values: improvement vs baseline).

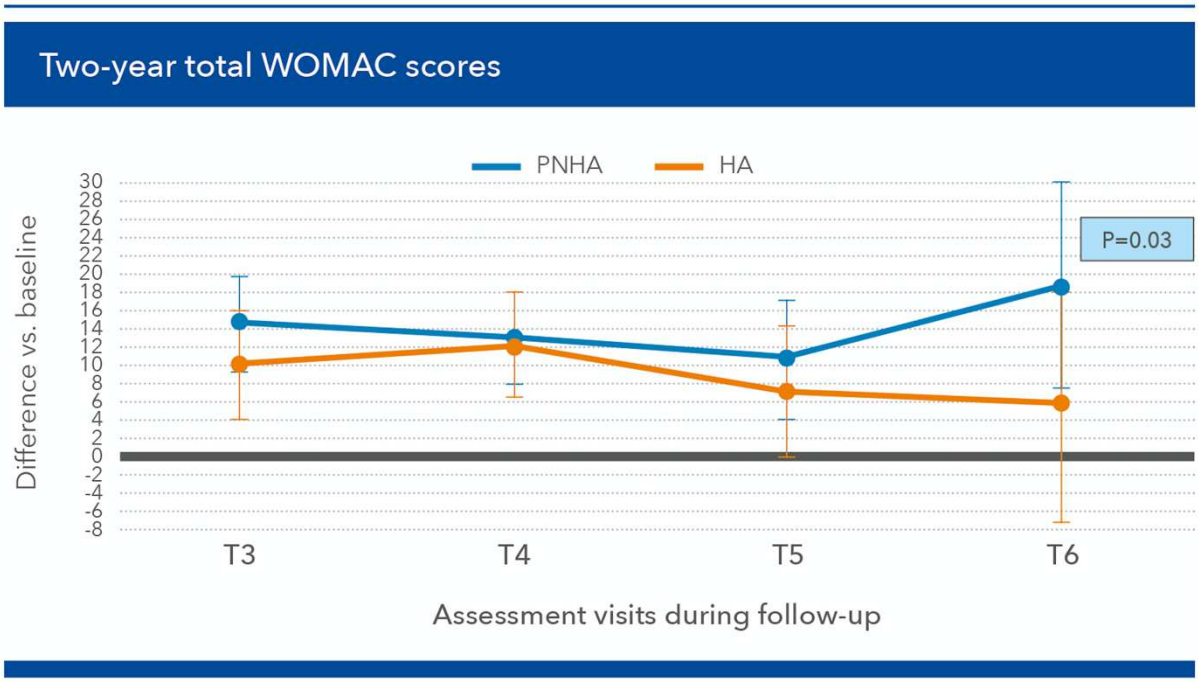

Figure 5 Differences in total Western Ontario and McMaster Universities (WOMAC) scores (mean ± SD) vs baseline during the [T3] (2 months) to [T6] (24 months) follow-up period (positive values: improvement vs baseline).

The KSS total scores over the first year were always significantly higher in the PNHA study group compared with the HA control group at all follow-up assessments ($p=0.02$ at [T3] and $p=0.001$ at both [T4] and [T5]). The 2-year study confirmed the tendency towards a long-term pain benefit for PNHA-treated patients also at the last [T6] assessment (Figure 6).

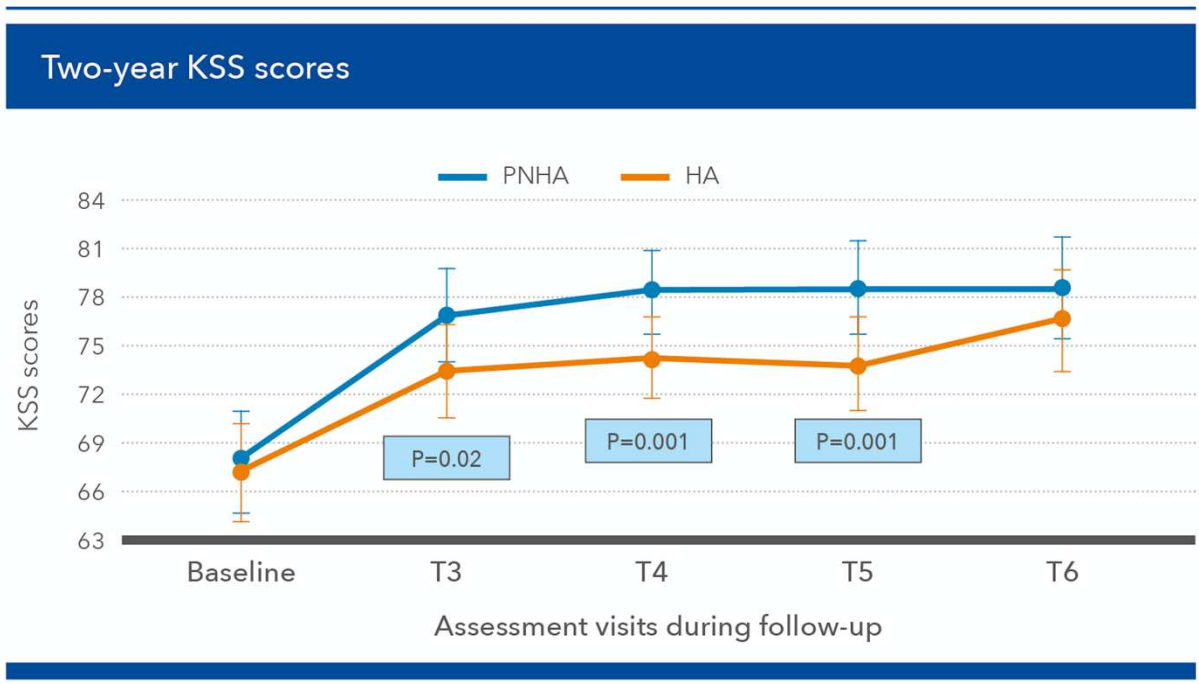

Figure 6 Knee Society Score (KSS) scores (mean ± SD) during the [T3] (2 months) to [T6] (24 months) follow-up period (positive values: improvement vs baseline).

The overall outcomes were similar for the KSS "pain" item subscore ($p < 0.05$ at [T3] and [T5]; [T6] p=0.059 marginally not significant), with 87% of patients of the PNHA treatment group (34 out of 39) and 66% of the HA group reporting an improvement of joint pain (Figure 7).

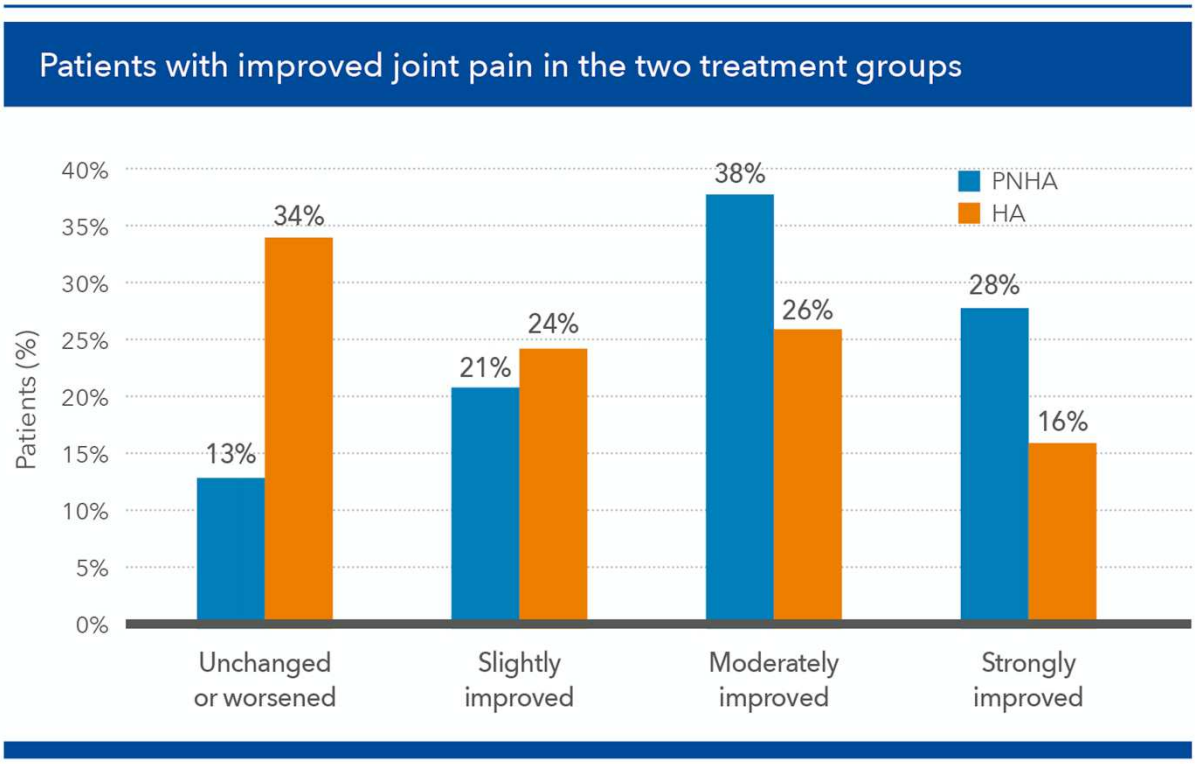

Figure 7 Percent of patients in the fixed combination (PNHA) and hyaluronic acid (HA) treatment groups reporting improvement in Knee Society Score (KSS) pain scores during the [T3] (2 months) to [T6] (24 months) follow-up period.

The degree of improvement in mean KSS pain scores was different in patients of the PNAH treatment group and patients of the HA group as a function of joint damage severity, with a more substantial decrease of pain scores in patients with more severe disease (Figure 8).

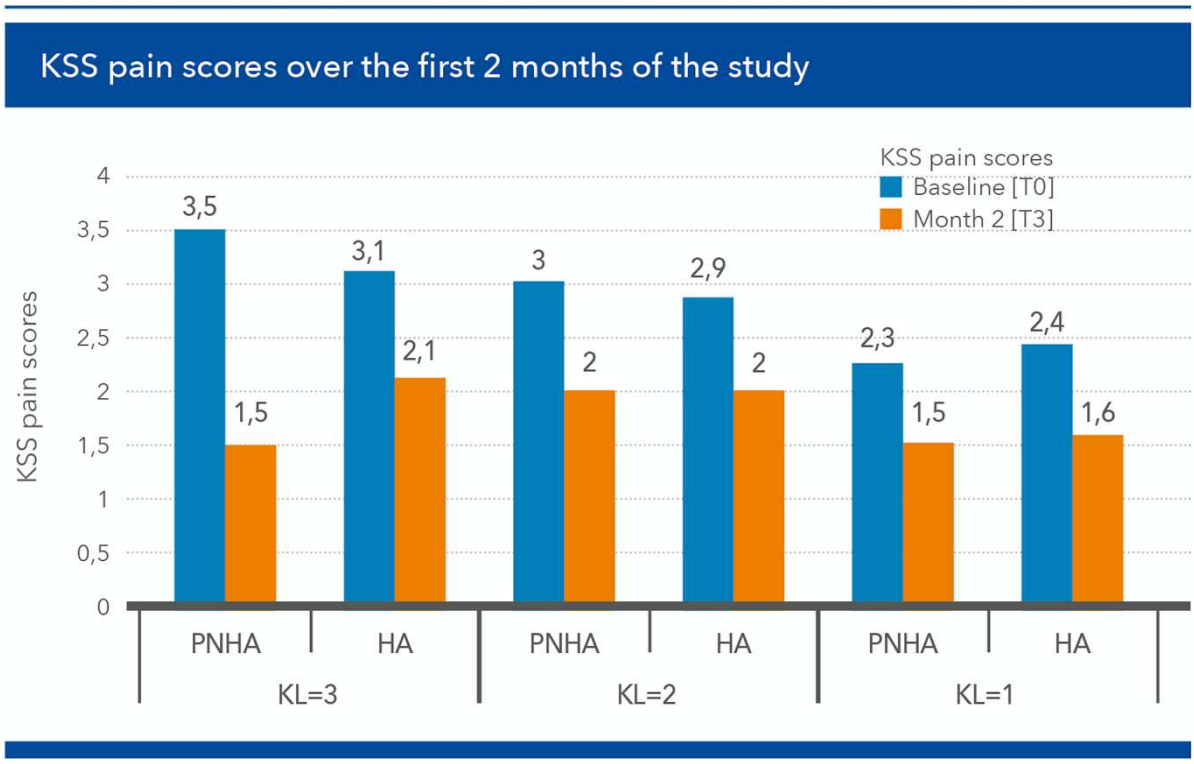

Figure 8 Mean Knee Society Score (KSS) pain scores at baseline and [T3] (2 months) in patients of the fixed combination (PNHA) and hyaluronic acid (HA) treatment groups according to baseline severity (Kellgren–Lawrence grade) of knee joint disease.

Mean KSS pain scores improved by 2 points both early after the end of the treatment cycle [T3] and at the end of the 2-year follow-up [T6] in PNHA-treated patients with more severe knee joint disease; conversely, KSS pain scores improved by 1 point in the HA-treated patients with the same degree of disease severity (Figure 9). Mean improvements were similar in patients with less severe disease; NSAIDs consumption was also similar in the two treatment groups (11 patients in both groups).

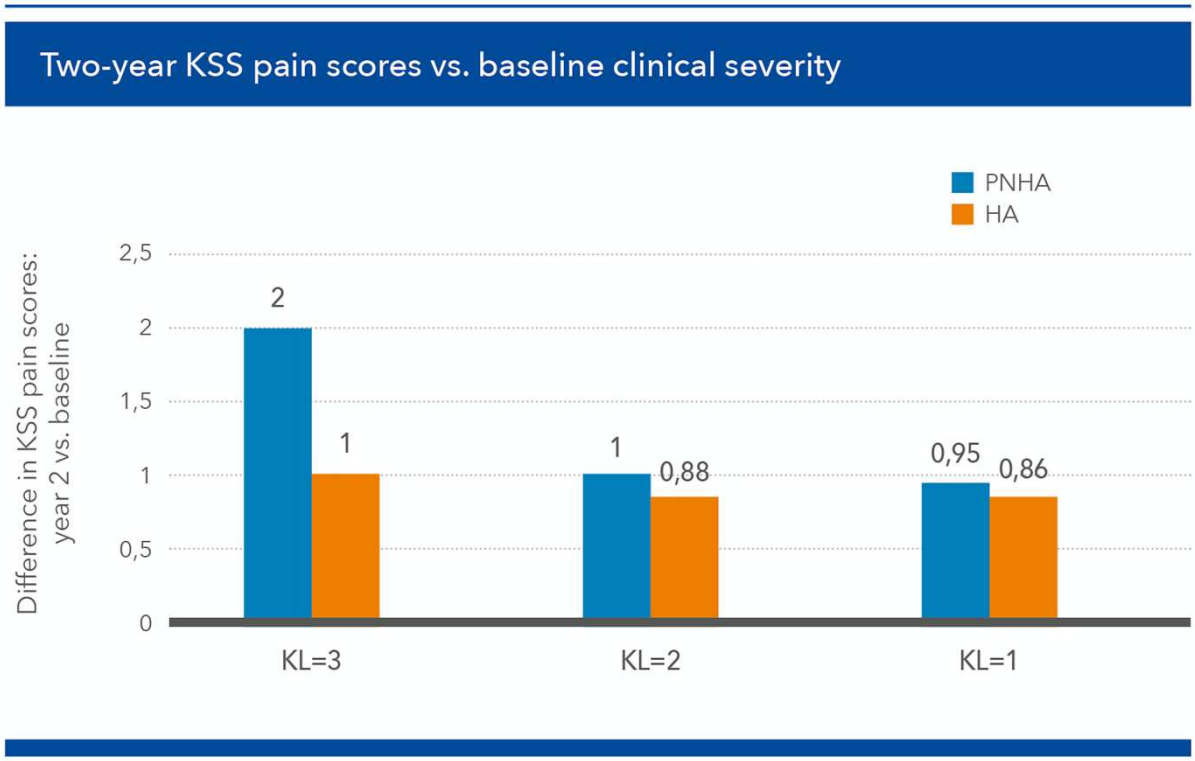

Figure 9 Improvement in mean Knee Society Score (KSS) pain scores, baseline vs [T6] (24 months) in patients of the fixed combination (PNHA) and hyaluronic acid (HA) treatment groups according to baseline severity (Kellgren–Lawrence grade) of knee joint disease.

The synovial fluid samples of all patients were transparent or translucent, showed a well-defined clot, and were of a regular yellow or, more frequently, light yellow colour. The synovial fluid clarity and density (mucin clot test) were also normal in all patients. The total white cell count was always within the non-inflammatory range (< 2000 cells/mm^3). Synovial fluid levels of MMP1, MMP13, IL-6, TNF-α, and PGE_2 showed a tendency to reduction, often quite substantial compared with baseline, after two months of PNHA treatment (e.g., MMP1 –49%, MMP13 –31,2%). Conversely, MMP1, and MMP13 levels increased (+29,5% and +6%, respectively) and only levels of IL-6, IL-8, and PGE_2 appeared reduced after treatment with HA. However, mainly

due to high variability, the low number of patients eligible for synovial fluid sampling and the overall low number of samples, statistical comparison of synovial fluid markers did not yield significant results (data not shown). The main reason was that a set of synovial fluid samples at both [T0] and [T2] was available for only eight patients.

Neither infiltrative treatment was associated with short-term complications or long-term side effects of any clinical significance.

DISCUSSION

The final two-year outcomes of this randomised, double-blind study confirm the preliminary outcomes of the previous 1-year interim report—the intra-articular co-administration of a fixed combination of PN-HPT™ and HA is associated with significant benefits for the knee joint pain, the primary study endpoint, and functional disabilities compared with HA alone.[8] The final two-year outcomes of the study also support the rationale that inspired the development of the fixed PNHA combination—synergy between PN-HPT™ and HA is likely in OA based on the complementary properties of the two viscoelastic agents.[8]

Highly purified, natural-origin PN-HPT™ — linear chains of polynucleotides from trout gonads — release nucleosides, nucleotides, and nitrogen bases by enzymatic cleavage in the synovial space and have shown long-term moisturising, and viscoelastic properties in clinical studies in knee OA.[4-6] PN-HPT™ combine these

properties with a robust trophic activity on mesenchymal tissues and cells and protection of cartilage.[7,12] The biostimulating efficacy of PN-HPT™ appears stronger than HA, which supports the "PN-HPT™ plus HA" synergy concept that inspired the 2-year study herein discussed.[8] PN-HPT™ also seem to have more substantial pain-reduction properties than HA in patients with knee OA.[4]

The more rapid reduction of WOMAC pain scores, the primary endpoint of the study, in PNHA-treated patients compared with the HA group is likely to mirror the synergic short-term viscoelastic contribution of PN-HPT™ to the investigated fixed formulation. Such synergy also likely explains the steady long-term reduction of knee pain, substantial at [T4] and [T5] (months 6 and 12) compared with HA-treated patients, but extending over the whole two-year study period. Without that synergy in the HA treatment group, pain significantly decreased only at the second and fourth months of follow-up ([T3] and [T4]), but not after 12 ([T5]) and 24 months ([T6]). Of course, the use in controls of a low-molecular-weight HA, which may have low elastoviscosity and require frequent infiltrations, might have acted as a confounding factor.[13] The benefits for the WOMAC item "Walking on a flat surface" developed somewhat more slowly in PNHA-treated patient, with still no differences between PNHA and HA at [T3] and [T4], but statistically significant ones at both [T5] and [T6]. PN-HPT™ improved knee OA symptoms more effectively, and possibly earlier than HA in patients with high-grade chondropathy, thus confirming previous observations.[5,6] More specifically, the PNHA treatment group experienced more substantial two-year reductions of both KSS and especially WOMAC mean pain

subscores than the HA treatment group. Pain benefits, already manifest in patients with the least severe disease (KL grade 1), increased progressively with disease severity, from KL grade 1 up to KL grade 3. PN-HPT™ strongly inhibits the migration of inflammatory cells and the local expression of inflammatory markers, and this might be the basis of such reasonable pain control despite advanced joint damage.[14,15] A retrospective stratification of OA severity supports the former observation about the comparative pain benefits progressively increasing in grade-1, grade-2 and grade-3 OA patients. The observation is also limited to pain, meaning caution is warranted. However, compounding this clinical retrospective observation with the PN-HPT™ characteristics described in the literature may help conceive a tentative decisional algorithm to help choose between PNHA or HA in daily clinical practice (Figure 10), with the PNHA doses in the range 2 to 4 mL.

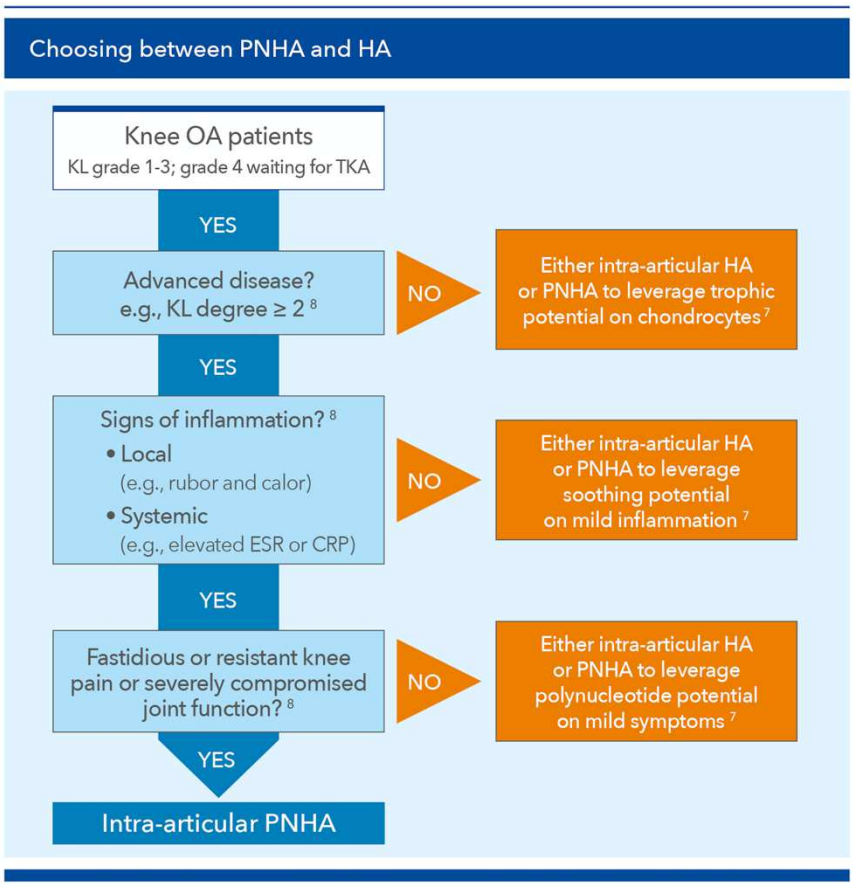

Figure 10 Does an ideal knee OA patient for either PNHA or HA exist? A tentative decisional algorithm.

Regarding the still debated association between pain and synovial fluid inflammation, the one-year interim report discussed assays' rationale.[8] IL1-ß, TNF-α and IL-6 are the proinflammatory cytokines most frequently associated with OA severity, while MMP13 is a primary culprit of the severe damages to joint cartilages.[16-18] The analysis indeed found an inverse correlation between the total KSS score and IL-6 and a trend towards reduced MMP1 and MMP13 synovial levels in the PNHA treatment group. However, no statistical correlation existed with clinical parameters, possibly due to the low number of synovial fluid samples and the short treatment period. As stated in the previous interim report, detecting clinically relevant differences in synovial fluid inflammatory markers might have required more follow-up time after the treatment cycle and more synovial fluid samples.[8]

As a final consideration, the authors acknowledge some weak points of their study: for instance, a three-edged, parallel-group study — placebo, PN-HPT™, PNHA — would have been more discriminating and informative. The study's primary purpose was to identify a role, if any, and possibly a therapeutic niche for PNHA in the current HA-dominated landscape, leading to the two-group design. The authors feel the study fulfilled this limited goal; other considerations, including pharmacoeconomics, will have to wait for future studies. The low mean clinical severity of enrolled patients (2 ± 0.7 for all patients, 1.9 ± 0.6 for the PNHA group) is possibly another weak point. Incorporating more grade-3 patient would have been likely more discriminating in a study of such ambition.

A third point liable to criticism: why falling back to traditional radiology instead of evaluating cartilage trophism with a rapid magnetic resonance imaging technique like 3T MRI? The reason was simple: even in an excellence centre, the risk that MRI resources were overburdened was steadily substantial over the study years.

Summarising, as shown by the two-year evolution of the primary endpoint, the WOMAC pain score, the study demonstrated a steady, long-term improvement of OA-related knee pain in PNHA-treated patients. The pain benefit vs HA was significant at all assessment times and greater in patients with a high KL degree of basal OA severity. Conversely, WOMAC pain control was somewhat unsteady in many patients of the HA treatment group, worsening after six months and one year of follow-up, and, at least tendentially, even after two years. Although some secondary endpoints did not show significant differences, KSS pain control was more rapid, already after two months after the end of the treatment cycle, in PNHA-treated patients.

CONCLUSIONS

The two-year, double-blind study outcomes confirmed natural-origin, highly purified polynucleotides (PN-HPT™) as agents with long-acting viscosupplementation properties and persistent pro-trophic and protective activity on chondrocytes, and a valuable complement to HA for the relief of pain and functional symptoms in knee OA. The suggested PNHA therapeutic range is 2 to 4 mL, but even the lowest dose

used in the trial (2 mL) led to the observed favourable results. The vigorous PN-HPT™ trophic activity on all connective tissues, including joint cartilage, might be especially of value as the basis of the likely *in-vivo* synergy between the two viscoelastic agents.

LIST OF ABBREVIATIONS

3T MRI	3-Tesla Magnetic Resonance Imaging
BMI	Body Mass Index
CRP	C-Reactive Protein
kDa	kilodalton
E_2	Prostaglandin E_2
ESR	Erythrocyte Sedimentation Rate
HA	Hyaluronic Acid
KL	Kellgren-Lawrence grade
KSS	Knee Society Score
IL-1ß	Interleukin 1ß
IL-6	Interleukin 6
IL-8	Interleukin 8 (chemokine)
MMP1	Matrix Metalloproteinase-1
MMP13	Matrix Metalloproteinase-13
NSAID	Non-Steroidal Anti-Inflammatory Drugs
OA	Osteoarthritis
OARSI	Osteoarthritis Guidelines Development Group

PNs	Polynucleotides
PNHA	PNs and HA fixed combination
PN-HPT™	Polynucleotides "Highly Purified Technology"
TIMP	Tissue Inhibitor of MMP1
TKA	Total Knee Arthroplasty
TNF-α	Tumor Necrosis Factor-α
WOMAC	Western Ontario and McMaster Universities

DECLARATIONS

Ethics approval and consent to participate

The Institutional Review Board of the Rizzoli Orthopedic Institute reviewed all study materials such as study protocol, informed consent forms, electronic clinical report form, CVs of authors, Etc. for ethical problems. All relevant documents (IRB approval certificate and approved study materials) are available from Dr Dante Dallari or the corresponding author, Martina Rocchi, MD, on request. The principles of the Declaration of Helsinki were always respected. The study was registered in the ClinicalTrials.gov database of privately and publicly funded clinical studies conducted worldwide (ClinicalTrials.gov Identifier: NCT02417610).

563

564 **Consent for publication**

565 The manuscript contains no individual patient's data in any form—all authors consent
566 to the manuscript's publication.

567

568 **Availability of data and material**

569 The datasets generated and analysed during the current study, not publicly available,
570 are currently archived according to current regulations (with full personal details of
571 all participating subjects) at the Rizzoli Orthopedic Institute, Bologna, Italy. All the
572 datasets are available (after conversion in anonymous form) from the corresponding
573 author on reasonable request.

574

575 **Competing interests**

576 The authors declare that they have no competing or conflicts of interest.

577

578 **Funding**

579 The study was spontaneous and touched off by scientific curiosity arising from
580 available medical literature. The corporate sponsor, Mastelli S.r.l., Sanremo, Italy,
581 provided the unidentifiable code-numbered study samples and financed all the third-
582 part activities associated with the study—design and discussion of the study protocol,
583 electronic clinical report forms and documents provided to the Ethical Committee
584 (available on request), as well as of all other study materials, e.g., for randomisation

procedures, monitoring, and reporting. The only other funding will be the sponsor's financing the article processing charges by *BMC Musculoskeletal Disorders* or the journal that will accept the manuscript (see also "Acknowledgements" subsection).

Authors' contributions

All authors sought and got informed consents by all their knee osteoarthritis patients enrolled in the study. After explaining the benefits and risks they could reasonably expect from intra-articular infiltrations of the two study formulations, they received informed consents from all candidate patients. All authors also personally carried out all double-blind procedures, including baseline and follow-up WOMAC and KSS scoring interviews, at both study sites, always under Dr Dallari's supervision. Paola Torricelli, MSc, also blinded to individual treatments, was responsible for laboratory assays.

Dr Dante Dallari is personally accountable for the clinical and editorial work's accuracy and integrity, leading to the manuscript's submission to *BMC Musculoskeletal Disorders,* including the comments on outcomes expressed in his manuscript.

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Mastelli S.r.l., Sanremo, Italy, is the patent holder and producer of POLIART®, proprietary fixed co-formulation of polynucleotides and hyaluronic acid for intra-articular infiltration, and IALART®, a proprietary formulation of low-molecular-weight hyaluronic acid for intra-articular infiltration. Both formulations were used

and compared in the two-year study. The authors wish to acknowledge the contribution of Mastelli S.r.l. for providing all materials for performing the double-blind study together with some minor financial support to help with the publication costs of the final report of their two-year study (see also "Funding" section).

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Figures

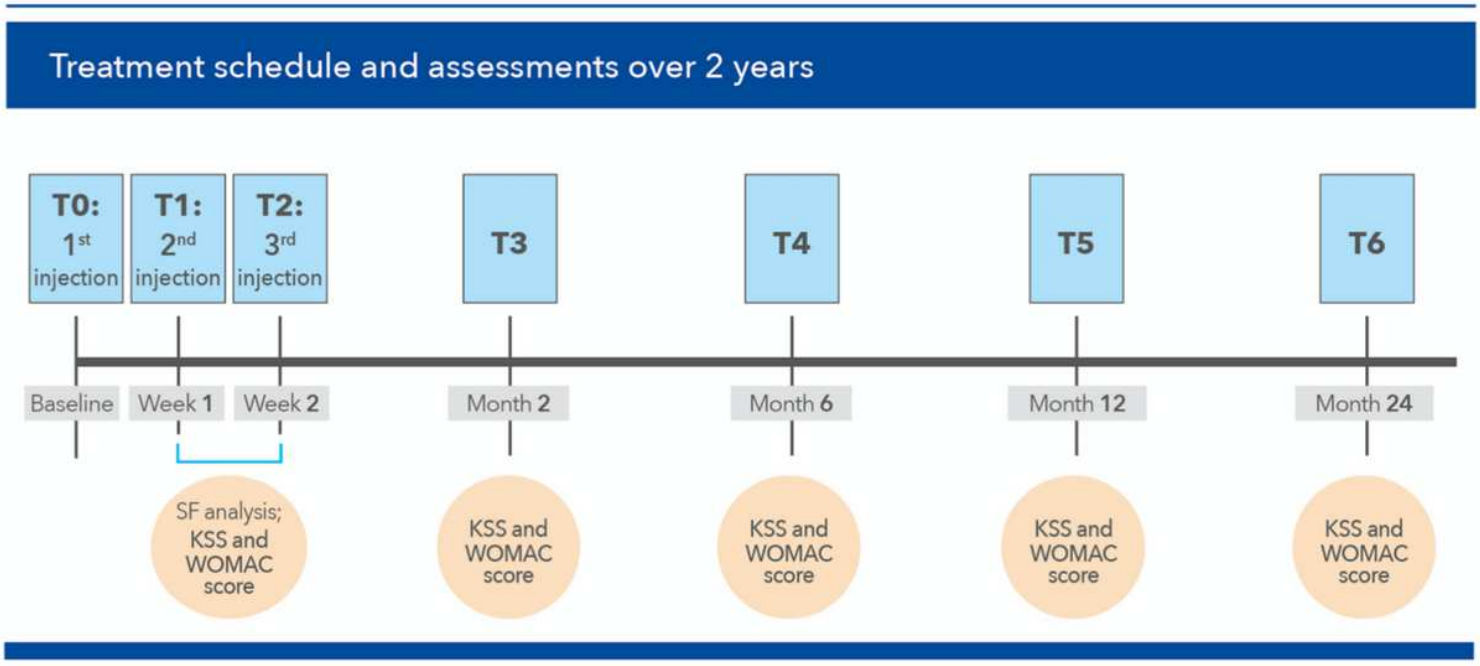

Figure 1

[T0] to [T2]: timing of the three double-blind PNHA and HA intra-articular injections and assessments planned over the first two study weeks (synovial fluid analysis and first KSS and WOMAC evaluation); [T3] to [T6]: timing of the KSS and WOMAC evaluations planned over the residual 2-year study period.

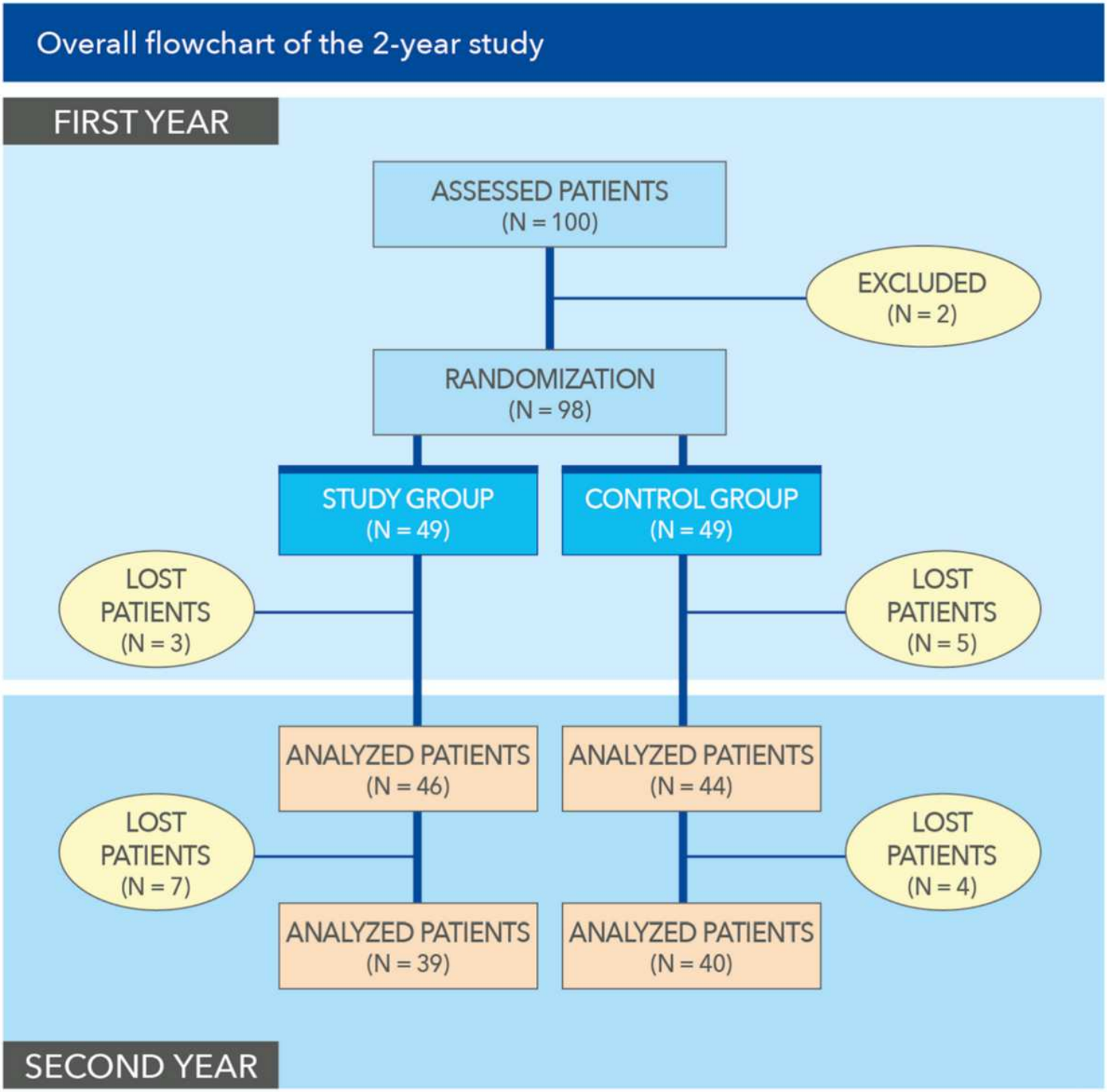

Figure 2

Upper lighter blue area: first-year part of the study leading to the interim analysis at the end of the first study year—i.e., outcomes up to [T5] or 12 months discussed in Ref. 8. Lower darker blue area: second-year follow-up.

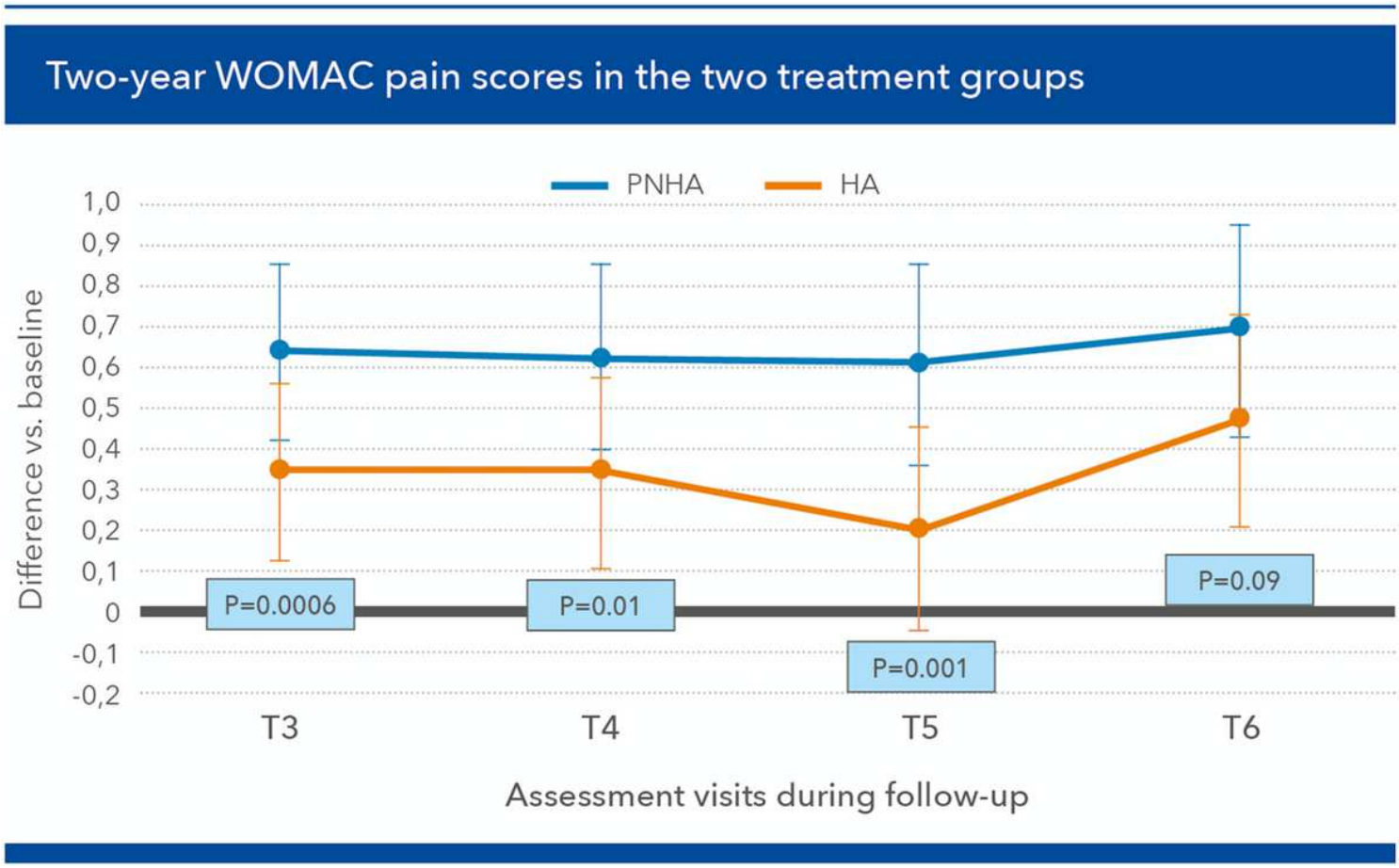

Figure 3

Differences in Western Ontario and McMaster Universities (WOMAC) pain scores (primary endpoint; mean ± SD) vs baseline during the [T3] (2 months) to [T6] (24 months) follow-up period (positive values: improvement vs baseline).

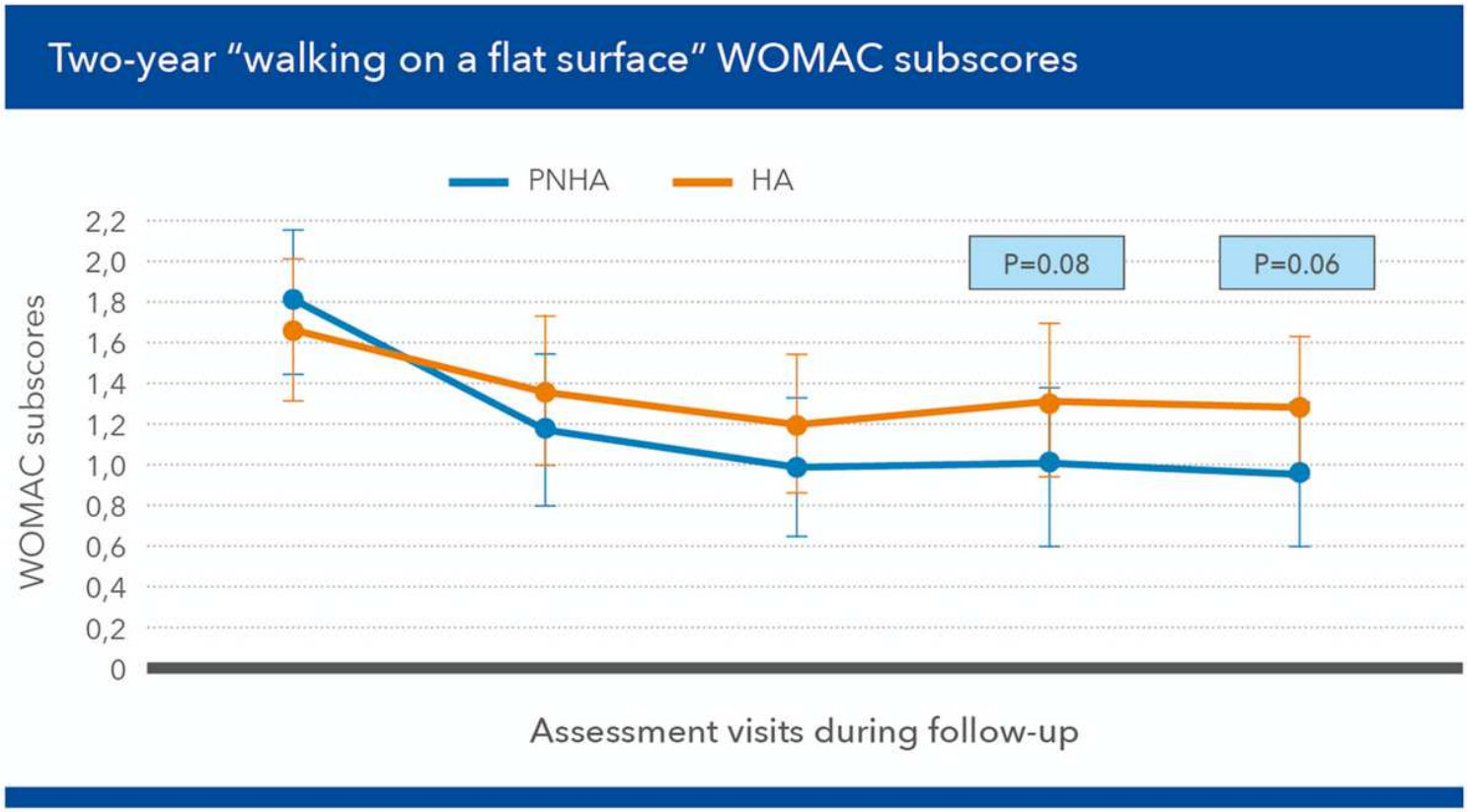

Figure 4

Mean "walking on a flat surface" Western Ontario and McMaster Universities (WOMAC) subscores; mean ± SD) during the [T3] (2 months) to [T6] (24 months) follow-up period (positive values: improvement vs baseline).

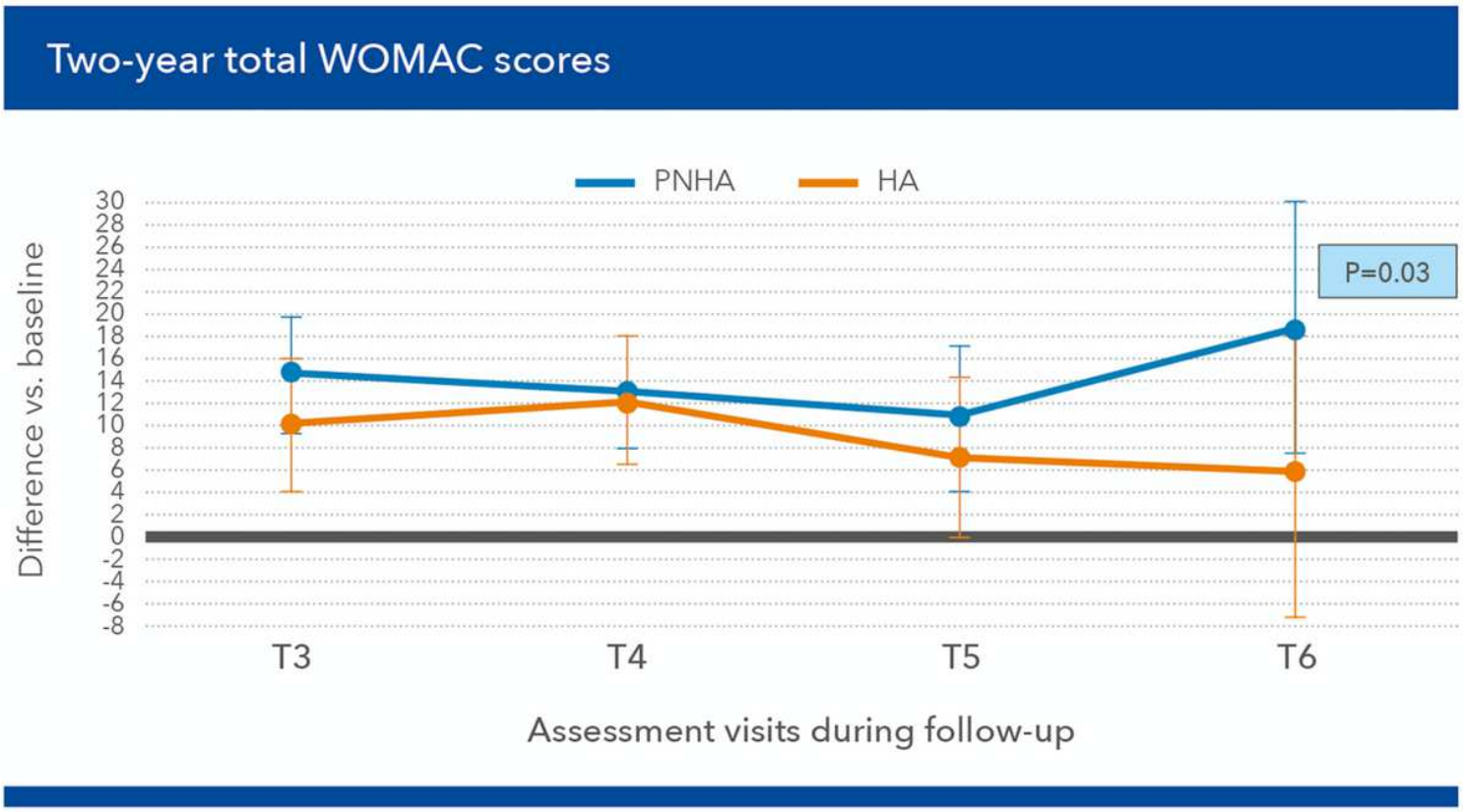

Figure 5

Differences in total Western Ontario and McMaster Universities (WOMAC) scores (mean ± SD) vs baseline during the [T3] (2 months) to [T6] (24 months) follow-up period (positive values: improvement vs baseline).

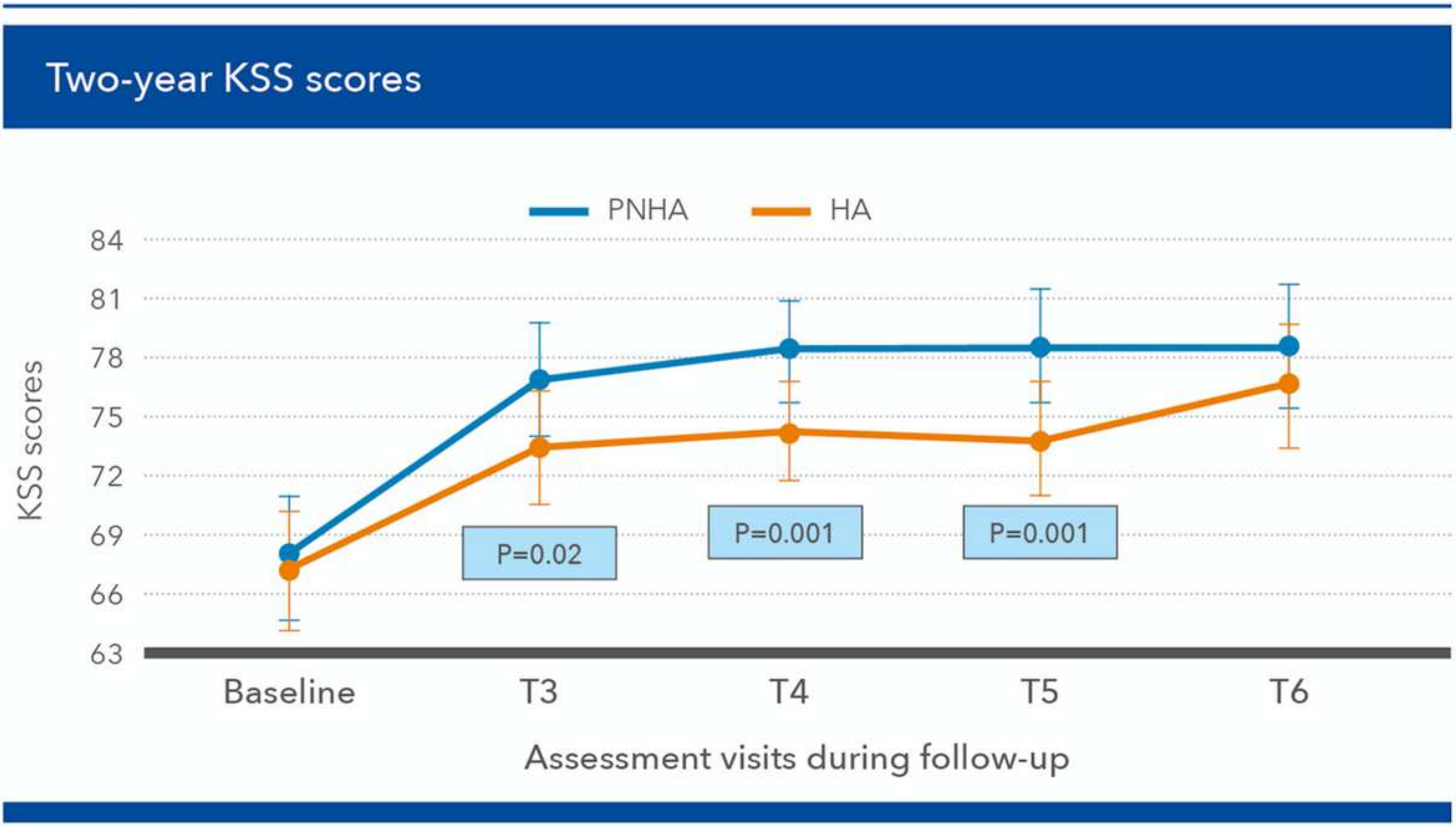

Figure 6

Knee Society Score (KSS) scores (mean ± SD) during the [T3] (2 months) to [T6] (24 months) follow-up period (positive values: improvement vs baseline).

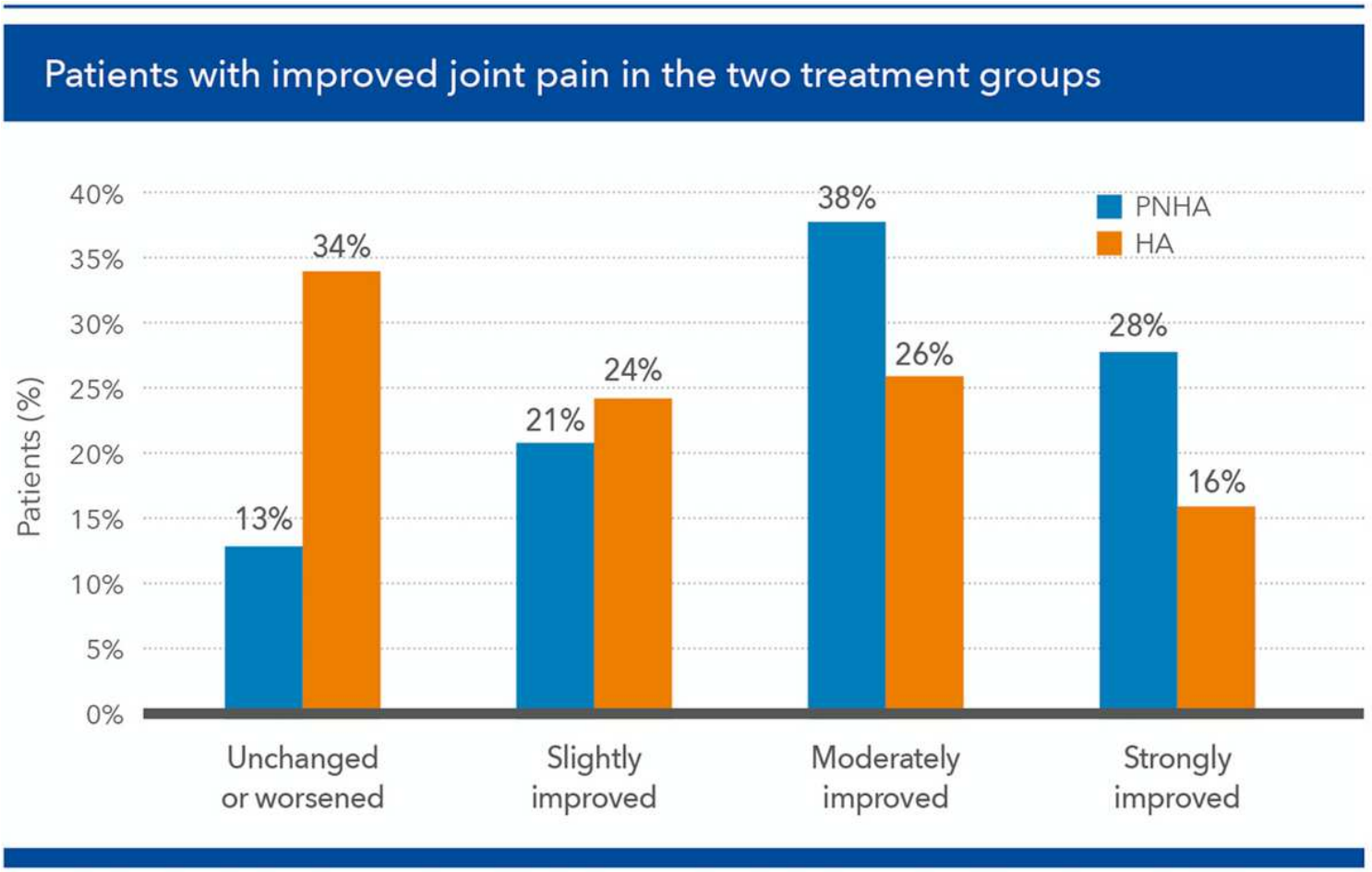

Figure 7

Percent of patients in the fixed combination (PNHA) and hyaluronic acid (HA) treatment groups reporting improvement in Knee Society Score (KSS) pain scores during the [T3] (2 months) to [T6] (24 months) follow-up period.

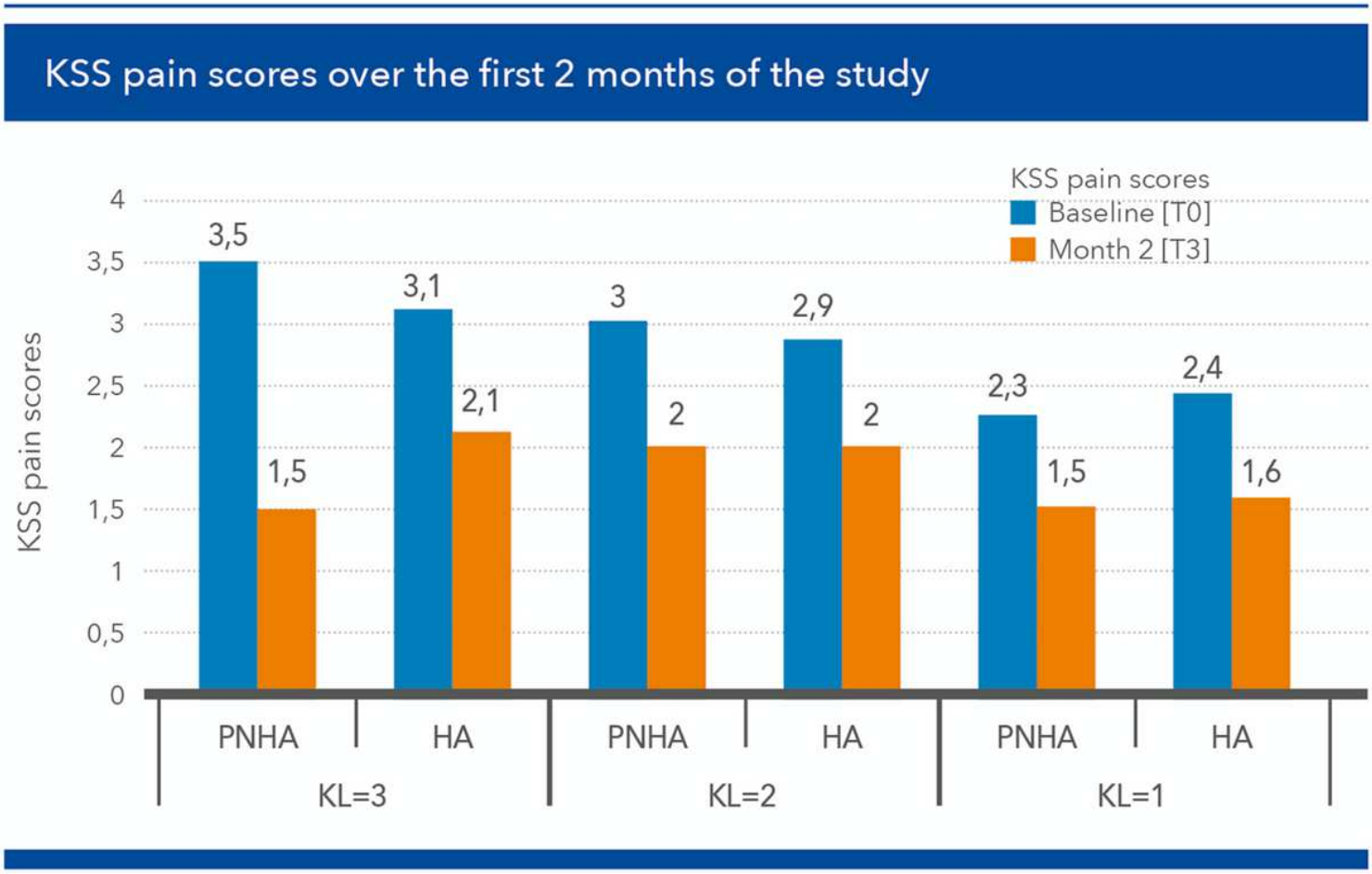

Figure 8

Mean Knee Society Score (KSS) pain scores at baseline and [T3] (2 months) in patients of the fixed combination (PNHA) and hyaluronic acid (HA) treatment groups according to baseline severity (Kellgren–Lawrence grade) of knee joint disease.

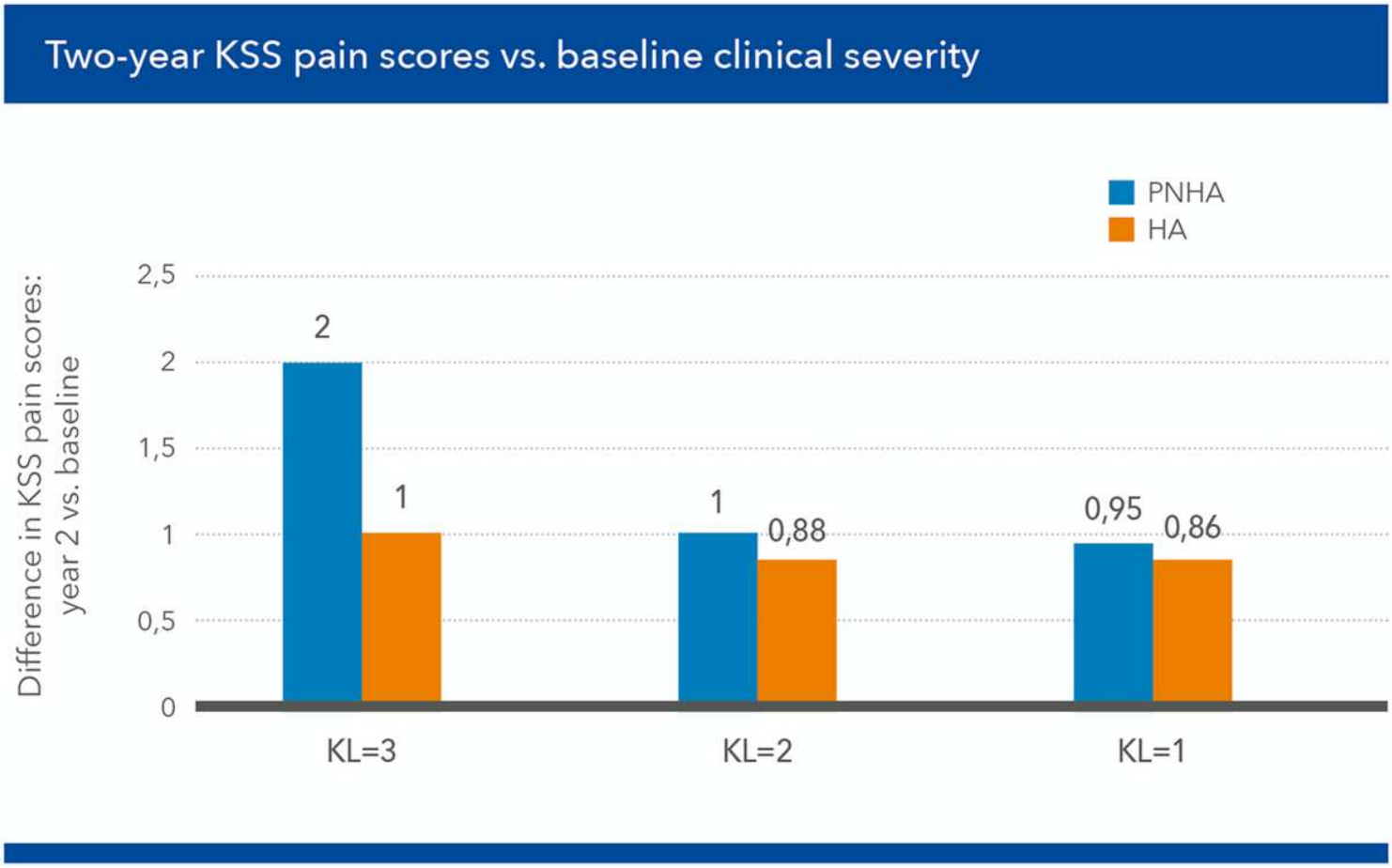

Figure 9

Improvement in mean Knee Society Score (KSS) pain scores, baseline vs [T6] (24 months) in patients of the fixed combination (PNHA) and hyaluronic acid (HA) treatment groups according to baseline severity (Kellgren–Lawrence grade) of knee joint disease.

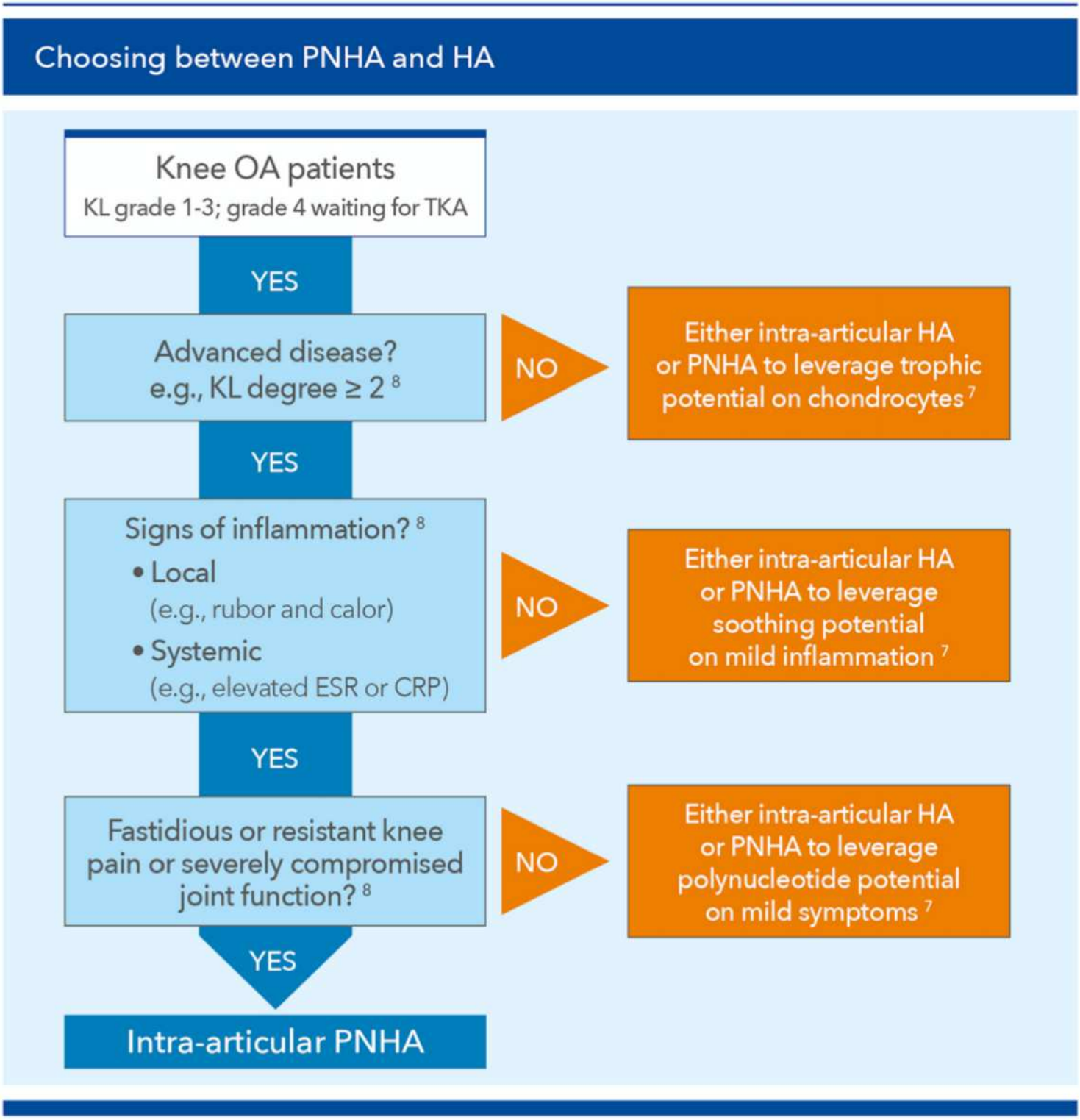

Figure 10

Does an ideal knee OA patient for either PNHA or HA exist? A tentative decisional algorithm.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.png
- Table2.png