Differences among Branded Hyaluronic Acids in Italy, Part 1: Data from *In Vitro* and Animal Studies and Instructions for Use

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ABSTRACT

BACKGROUND: The use of hyaluronic acid (HA) for intra-articular (IA) injection is widespread around the world for patients affected by osteoarthritis.

AIM: The aim of this study is to identify scientific evidence from *in vitro* and *in vivo* studies supporting the use of IA HAs marketed in Italy. We also evaluated the accuracy of indications and contraindications reported in the leaflets of such HAs compared with the available scientific evidence.

MATERIALS AND METHODS: An extensive literature search was performed to identify all *in vitro* and *in vivo* model studies reporting on the effects of various HAs marketed in Italy for IA use. Data reported in the leaflets of different HA-based products for IA use were extracted and analyzed alongside evidence from *in vitro* and *in vivo* model studies.

RESULTS: Nine *in vitro* studies and 11 studies on animal models were examined. Comparing results with what is reported in the leaflets of HAs marketed in Italy, it was observed that many branded formulations are introduced in the market without any reporting of basic scientific evidence. Only 12.82% and 17.95% of branded products had been shown to be effective with scientific evidence from *in vitro* and *in vivo* studies, respectively. The rationale of use of these products is based on their nature, as if a class effect existed such that all HAs would yield similar effects.

CONCLUSIONS: Data on HAs deriving from *in vitro* and *in vivo* studies are scarce and relate to only a small percentage of products marketed in Italy. Many indications and contraindications are arbitrarily reported in Italian HA leaflets without the support of scientific evidence. Larger and brand-specific studies are necessary and should be reported in the leaflets to guide clinicians in making an appropriate choice regarding HA-based IA therapy.

KEYWORDS: osteoarthritis, hyaluronic acid, hylan G-F 20, *in vitro*, animal

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Introduction

Hyaluronic acid (HA) for intra-articular (IA) injection is widely used for patients affected by osteoarthritis (OA) all around the world. Guidelines regarding the use of HA for IA injection have been contradictory in the past few years, and no definitive criteria yet exist regarding the use of HA.[1–7] Several HA-based products for IA use are available in the health market, and the large majority is classified as medical devices. Little is known about the differences among all the HA-based products merchandised in Italy and in other countries, and it is, therefore, impossible to accurately choose the right product for different stages and patterns of OA. Although all HA products marketed in Italy have been approved for IA intra-articular use, only a small number of them have been shown, with scientific evidence, to be suitable for this use. The aim of this study is to highlight which HA products marketed in Italy are supported, in their indications and contraindications, by scientific literature. We have utilized an accurate systematic revision of all HA-based products available in the Italian health market, performed by the Technical Expert Panel of the ANTIAGE (Italian National Association for Intra-Articular Therapy of the Hip by Ultrasound Guidance) (www.antiagefbf.it (http://www.terapiainfiltrativa.it/category/prodotti/acido-ialuronico). From this starting point, we show the differences in scientific evidence by analyzing three different items as follows: *in vitro* evidence, *in vivo* evidence, and differences in product leaflets.

This is the first of two studies regarding the evidence gathered about HA. The second study will focus on evidences regarding the use of various HAs for IA injection in knee OA and in other joints affected by OA.

Usually, HAs are considered as a class of compounds sharing common properties, but the aim of the present study is to point out the differences existing between merchandised HAs. HA products differ in molecular weight, concentration, and molecular structure, and it is thus not correct to assume that study results for a certain HA may be extended to other HAs that differ in composition. With this rationale, we aimed to identify the evidence for HAs marketed in the Italian market.

The first part of this study focuses on the evidences regarding how different HA products commercialized in Italy may provide benefits in OA treatment as demonstrated by *in vitro* bioevaluation studies.

The second part of this study focuses on the reported effects of different HA brands as evidenced in animal models.

The third part of this study aims to analyze what is reported in the leaflets of different HA products commercialized in Italy and compare such claims and characteristics with the evidence reported in the scientific literature.

Materials and Methods

For the first part of this study, focused on *in vitro* evidence, a literature search was conducted for *in vitro* studies published in PubMed, restricted to English language. We searched with three different parameter sets, as follows. In the first search, without time restriction, search terms were *HA* and *in vitro* studies. The second search was restricted from 2002 to 2014 to isolate more recent studies, using the following MeSH terms separately or in combination: *osteoarthritis, viscosupplementation, hyaluronic acid, hylans, sodium hyaluronate, intra-articular injection/infiltration*, and *in vitro*. The third search was conducted using the trade names of the 57 Italian HA products (Fig. 1).

Experimental and human studies were excluded. Any sources of literature encompassing broad terms denoting HA efficacy in OA without clarifying the type of the study were included for a preliminary full manuscript review, and then excluded if lacking an explicit reference to evidence *in vitro*, or if the product was not in the Italian market or not specified. The search was widened using the references reported within the included articles. After the study selection, extracted data were transcribed onto standardized data collection sheets.

Regarding the second part of the study, a literature search was conducted for animal model studies published in PubMed, restricted to English language, using alternatively the terms *hyaluronan, hyaluronic acid,* or *hylan*, all associated with *OA* and *animal model*.

All articles regarding the topic of *in vivo* effects of HA on OA animal models were gathered and analyzed for data extraction. Only articles focusing on the effects of branded HA available in Italy were taken into account for examination.

In the final part of the study, we extracted data as reported in the leaflets of different HA-based products for IA use.

We compared HA-based branded products for the following characteristics: concentration (mg/mL), source of HA, joint for which usage is indicated, suggested dosage, expected duration of effect, classification as drug or medical device, the presence of references regarding studies on the same branded HA or on other kinds of HA product, indications, and contraindications. Classification for molecular weight was performed, and products were defined as low molecular weight products (800–1200 kD), medium molecular weight products (>1200 kD but <2400 kD), and high molecular weight products (>2400 kD). Regarding indications, we categorized the products for joint pain, articular mobility reduction, OA, and synovial fluid (SF) substitution. Regarding contraindications, they were categorized by six different items as follows: hypersensitivity (HS) to HA, cutaneous infections, joint infections, joint inflammation, pregnancy/breastfeeding, and venous or lymphatic stasis.

Results

Evidence from *in vitro* studies. The selection process for the studies included in our analysis is presented in Figure 1. Evidence from nine *in vitro* studies concerning experiments with HA-based products commercialized in the Italian health market was evaluated (Table 1). Products are listed by trade name in alphabetical order: Artz®, Durolane®, Hyalgan®, Hymovis®, Ostenil®, Synvisc®, and Synvisc-One® (Table 1).[8–16] In 1997, Homandberg et al.[8] tested the ability of Artz® 0.1 mg/mL of HA to suppress fibronectin fragment-mediated cartilage chondrolysis *in vitro*, and a significant downregulation of inflammatory mediators was found, suggesting that IA injection of HA in combination with statins might feasibly slow the progress of OA. Durolane® was tested by Henriksson in 2012 and demonstrated a better result than hydrogel (PuraMatrix®) and adhesive tissue glue gel (TISSEEL®), but was not a suitable cell carrier for cell therapy.[9] Lisignoli et al demonstrated that 500–730 kD HA (Hyalgan) exerts an antiapoptotic effect on anti-FAS-induced chondrocyte apoptosis by binding its specific receptors (CD44 and ICAM-1), but did not affect spontaneous chondrocyte apoptosis.[10] It was also reported that this HA fraction may be able to slow down chondrocyte apoptosis in OA by regulating the processes of cartilage matrix degradation.

Brun et al demonstrated that Hyalgan® was able to enhance human chondrocyte proliferation and survival under conditions of oxidative injury,[11] which may be one possible therapeutic mechanism of HA in OA. This effect showed dose-dependent response. HYADD®4-G/Hymovis® is a chemically modified amphiphilic HA. Its lubricating effect on bovine articular cartilage *in vitro* was tested in a musculoskeletal biomechanics laboratory by Schiavinato and Whiteside.[12] This

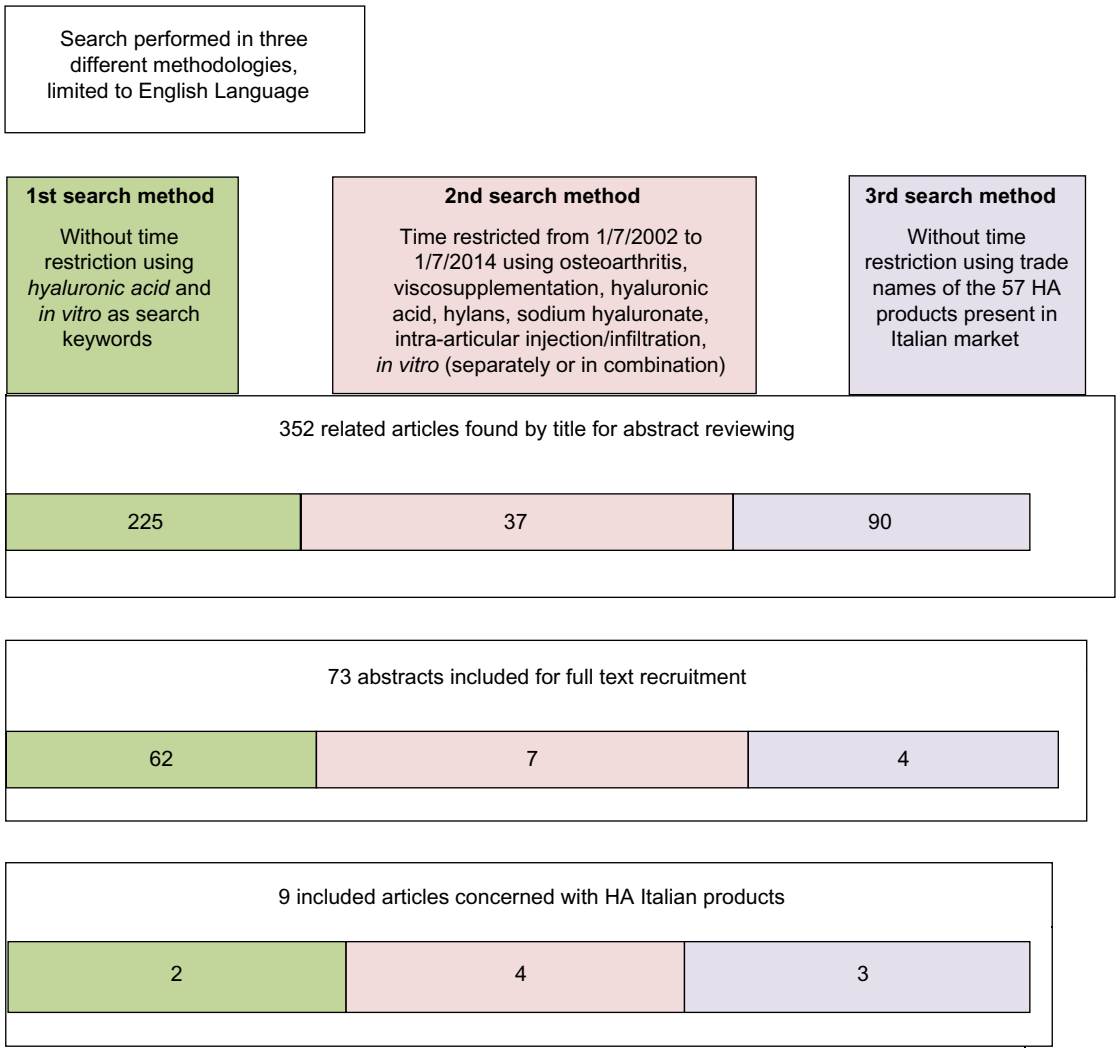

Figure 1. Flow chart reporting the search process of articles regarding effects of Italian branded hyaluronic acids *in vitro*.

study provided evidence that HYADD®4-G may serve as an effective lubricant and protect the articular surfaces against mechanical wear. One year later, an *in vitro* study by Smith et al concluded that Hymovis® has beneficial effects on human osteoarthritic chondrocytes and synoviocytes superior to those of unmodified hyaluronans.[13]

Waller et al found that the use of hylan G-F 20 provides joint lubrication that may prevent chondrocyte apoptosis by lowering the coefficient of friction.[14] Peña Ede examined the analgesic effects of Synvisc® on joint pain *in vitro*, compared with nonelastoviscous solutions, evaluating the opening probability of stretch-activated channels.[15] Synvisc® was more potent in reducing the opening probability, which suggests that the analgesic effects of IA injections may be elastoviscous dependent, possibly due to the reduction of sensitivity to mechanical forces on stretch-activated channels present in the membrane of joint mechanonociceptors. In 2009, Mathieu et al compared Synvisc-One® to a linear HA of bacterial origin (ARD) of 1.14 × 106 Da, exploring the changes in rheologic behavior of OA SF after adding Synvisc-One® or the linear HA.[16] Both linear and cross-linked HAs induced different changes in the OA SF rheologic properties when added *in vitro*, suggesting interactions between SF proteins and exogenous HA. The non-Newtonian behavior in SF shown in this study was dependent on both the level of viscosity and HA concentration.[16]

In the second part of this study, evidence from animal model studies showed that 144 studies were found on HA in OA animal models. We excluded 73 studies in which HA was cited but did not appear in the aim of the study. Of the remaining 71studies, 54 reported directly on the effects of different HAs in animal models and the other 17 reported on combinations of HA plus other substances, always in animal models. The 73 excluded studies reported on platelet-rich plasma, stem cell or chondrocyte transplants, and their effect on the production of HA. Most of the studies about the association of hyaluronan and other substances were published in the last four years. Of the 54 studies that dealt only with hyaluronan, we found 11studies comparing different HA products (Fig. 2, Table 2).[17–27]

Data reported in leaflets. Regarding the third part of the present study, we observed that branded products fundamentally

Table 1. Studies reporting on the *in vitro* effects of hyaluronic acids products merchandised in Italy for intra-articular use.

AUTHOR (YEAR)	TRADENAME OF HA TESTED	COMPOSITION	MW (KDA)	COMPARATOR	EXPERIM. MODEL	OBJECT OF ANALYSIS	FINDINGS
Homandberg GA (1997)	Artz/Supartz®	HA	600–1200	60 and 250 Kda HA	Cartilage condrocyte	Proteoglican production and inhibition of metallo-proteinases	Artz/Supartz slows down Chondrolysis and has some reparative potential
Henriksson H (2011)	Durolane®	NonAnimal Stabilized HA	NA	Hydrogel (Puramatrix®), adhesive tissue (TISSEEL®).	Human mesenchymal cells, IVD cells, chondrocytes	Ttest as gel carrier for cell-based intervertebral disc therapy	Durolane® showed best result of the tested gels; BUT it was not a suitable cell carrier for cell therapy.
Lisignoli G (2001)	Hyalgan®	Sodium hyaluronate	500–730	2 HA with molecular weights 40–65 and 1,000;	Human OA knee chondrocytes	Reduction of Anti-fas-induced apoptosis in chondrocytes	Antiapoptotic effect on anti-FAS–induced chondrocyte apoptosis by binding its specific receptors (CD44 and ICAM-1).
Brun P (2003)	Hyalgan®	Sodium hyaluronate	$5–7 \times 10^5$	Non	Normal and damaged human chondrocyte	Effect on survival of normal and damaged chondrocytes	Increase of the viability of chondrocytes and restored cell viability after oxidative cell injury.
Schiavinato A (2012)	Hymovis®	HA (idrogel)	500–730	Synovial fluid and phosphate buffered saline	Bovine articular cartilage	Efficacy on lubrication of articular cartilage	Hymovis® may serve as an effective lubricant and protect the articular surfaces against mechanical wear.
Smith MM (2013)	Hymovis®	HA (idrogel)	500–730	0, 0.5, 1.0 or 1.5 mg/mL of HA (500–730)	Human Chondrocytes and fibroblasts	Effects on human OA chondrocytes and synoviocytes	Superior beneficial effects of Hymovis® on cell expression of catabolic enzymes and inflammatory cytokines.
Waller KA (2012)	Synvisc®	A cross linked HA	6000	Synovial Fluid and saline	Bovine cartilage bearing system	Preventing friction induced chondrocyte apoptosis,	Prevention of chondrocyte apoptosis by lowering the coefficient of friction
Peña Ede (2002)	Synvisc®	A cross linked HA	6000	(1) Hylan A, 0.8% MW, 6 2) Hylan A 0.8% t96,000 MW); (3) Barth's solution	Xenopus oocytes	Analgesic effect of Synvisc through stretch-activated ion channels	The analgesic effects of hylans are due to a reduction of the sensitivity to mechanical forces of stretch-activated channels present in the membrane of joint mechanonociceptors.
Mathieu P (2009)	Synvisc One®	A cross linked HA	8×10^6	A linear HA of bacterial origin 1.14 x 106 Da	Synovial Fluid of OA human knee and shoulder	Changes in the OA synovial fluid rheologic properties	Linear and cross-linked HA induce different changes in the OA SF rheologic properties *in vitro*

Abbreviation: HA, Hyaluronic Acid.

differ in both molecular weight and concentration (Table 3). Following the classification for molecular weight described earlier, we found 22 low molecular weight products, 23 medium molecular weight products, and 3 high molecular weight products, as well as 9 products for which the molecular weight was not reported. It was not possible to perform a classification by molecular weight for three products, Jonexa, Durolane, and Hymovis, due to cross-linking molecular processes. Jonexa is the result of the combination of a medium molecular weight HA and Hylastan, obtained by the cross-linking of HA with divinyl sulfone. Durolane is composed of a non-animal stabilized HA in gel formulation (NASHA), while Hymovis is composed of HYADD4 (sodium hyaluronate hexadecylamide). Regarding the origin of HA, from biofermentation (BioF) or direct extraction by rooster combs, 5 products were extracted from rooster combs, 50 products were formulated by BioF, and for 2 products, Artrosulfur HA and Structovial, it was impossible to gather data regarding their origin.

Data on the joint indicated for IA use of the products also showed that products with similar composition, origin, and molecular weight may still have different indications reported in the leaflet. An indication for knee joint was only reported on 15 products; 12 products reported an indication for use in big joints, such as shoulder, hip, ankle, elbow, and knee; 9 products reported an indication for small joints, such as trapeziometacarpal joint, carpometacarpal joints, metacarpophalangeal joints, temporomandibular joint, and other small joints; and for 15 products, it was impossible to gather data regarding which joint they are indicated for.

Regarding dosage and effect duration, data are even more scarce. Of the 57 products, 18 reported that a single injection per cycle is sufficient to obtain the desired effect. Of those 18 products, only 10 reported the expected duration of effect, while for 8 products there were no data on this topic in the leaflet. In the leaflet of 15 products, it was reported that a cycle of 2–3 injections was necessary to obtain the desired effect, and out of those 15 products, only 1 reported the expected duration of effect. A further 14 products reported that 4–5 injections were needed to obtain the desired effect, and out of those 14 products, only 4 reported the duration of effect. Five brands did not report the number of injections needed or the expected duration. Similar products report an expected duration of effect that may vary from 3 to 12 months. Brands reporting an expected duration of more than six months were combinations of HA with other substances. Only Durolane, Ostenil, Synvisc, Orthovisc, Synolis VA, and Artz (Supartz) reported studies on their own products in the references of the leaflet, while Arthrum, Fermathron, and Go-on reported studies on generic HA. Data on indications and contraindications of the analyzed products are reported in Tables 4 and 5.

Categorization for clinical indications showed that 45 of 57 products were indicated for the relief of joint pain, 33 of 57 for improving joint mobility, 48 of 57 for OA, and only 6 of 57 for substitution of SF.

About contraindications, 32 of 57 products reported that their use is contraindicated in case of HS to HA, and 36 reported contraindication in case of cutaneous infection in the zone to inject. Joint inflammation was reported as a contraindication to IA injection for only 27 products, and joint infection for only 37 products. Interestingly, pregnancy and breast feeding were reported as contraindications for IA use in only 6 products, despite the lack of data on the use of HA products in such conditions. Similarly, only 6 products reported that the use of HA is contraindicated in cases of lymph stasis, although it is clearly reported in the literature that metabolism of HA is exerted through the lymphatic system.[28]

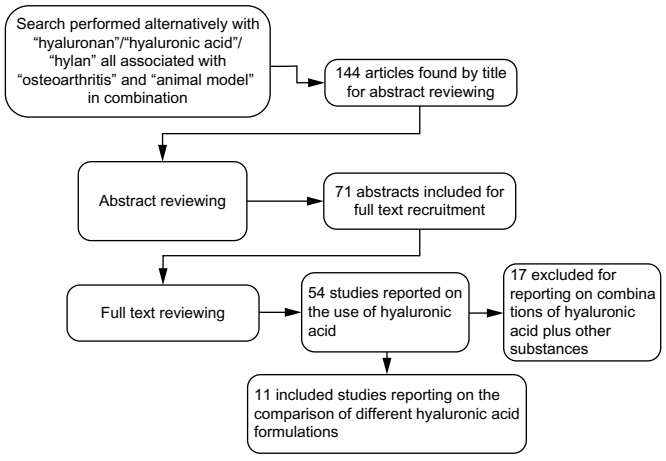

Figure 2. Search methodology for studies on animal models and results obtained.

Discussion

The first aim of this study was to investigate, through a systematic review, the scientific evidence from both *in vitro* and *in vivo* studies for each HA formulation commercially available in the Italian market. Unfortunately, only 12.82% and 17.95% of branded products were described in the scientific evidence of *in vitro* and *in vivo* studies, respectively. In addition, the diversity of aims, methodology, and cellular systems used between the *in vitro* studies rendered a comparison of formulations impossible.

Many branded formulations are introduced in the market without any kind of basic scientific evidence. The rationale of use of these products is based on the product's nature, as a compound of HA, reporting the characteristics of generic HA as a class effect. This occurs because, in Italy, regulatory laws for the marketing of medical devices do not require high levels of evidence. HA formulations are not all the same, not only in their characteristics of extraction, molecular weight, and concentration, but also in the scientific evidence relating to each formulation. Some properties shown by a particular formulation in an *in vitro* or animal study must not be extended generically to all products of the same class. This produces a confusing framework. Clinicians should be in a position to consider evidence for each branded product and use branded

Table 2. Studies reporting on the effects on animal models of hyaluronic acids brands merchandised in Italy for intra-articular use.

AUTHOR, YEAR	AIM OF THE STUDY	EXPERIMENTAL MODEL	PRODUCT UTILIZED	IS THE EFFECT MW-DEPENDENT?	EVIDENCE
Schiavinato et al, 2002	Tolerability	Rabbit knee joints	Hyalgan®, Artz/ Supartz®, Synvisc®	Yes in favour of Hyalgan® and Artz® (LMW > HMW)	Synvisc® causes mild inflammation compared to native hyaluronic acid;
Gomis et al, 2004	Sensitivity of nociceptors	Normal and inflamed rat knee joint	Synvisc®, Orthovisc®, Hyalgan®	Yes in favour of Synvisc® (HMW > LMW)	Elastoviscous properties are determining factors in reducing pain-eliciting nerve activity
Goomer RS et al, 2005	Immunologic reactions	guinea pigs	Hyalgan®, Synvisc®	Yes in favour of Hyalgan® (LMW > HMW)	Native hyaluronan produces less hypersensitivity than cross-linked HA
Greenberg DD et al, 2006	Matrix turnover and inflammation	canine synovial and cartilage explants	Hyalgan®, Synvisc®	Yes, slightly in favour of Synvisc® (HMW > LMW)	A possible delay in onset with Hyalgan®; anti-inflammatory mechanism similar in Hyalgan® and Synvisc®
Ottaviani et al, 2007	Inflammatory and immunological effects	Air pouches established in BALB/c mice	Hyalgan®, Supartz®, Synvisc®, saline	Yes in favour of Hyalgan®/Supartz® (LMW > HMW)	All 3 preparations cause an inflammatory reaction, but only the non-hyaluronan portion of the Synvisc® created an immunological response
Cake et al, 2008	Ground reaction forces (GRFs) and joint health	Bilateral meniscectomia induced OA in sheep	Hymovis®, Hyalgan®	Yes; slightly in favour of Hymovis®	Both preparations showed gait improvement, no modulation of OA severity or sinovial fluid parameters. Significant functional improvement after Hymovis®
Smith et al, 2008	Pathological changes in synovial membrane	Bilateral meniscectomia in sheep	Hymovis®, Hyalgan®, saline solution	No	Hyalgan® decreased aggregate score, vascularity and fibrosis. Hymovis® decreased vascularity, intimal hyperplasia and increased high-MW HA synthesis.
Gomis et al, 2009	Movement-evoked nociceptor impulse activity	Guinea pig nociceptor impulse activity	Hymovis®, Hyalgan®	Yes; slightly in favour of Hyalgan®	HAs reduce nociceptor activity and attenuates the enhanced impulse response to joint movements
Boettger MK et al, 2011	Antinociceptive effect	Rat knee joints	Durolane®, Synvisc®, Hyalgan®, saline soliution	Yes in favour of Durolane® and Synvisc® (HMW > LMW)	All HAs provided significant antinociceptive effects. Its duration is more prolonged after NASHA and Hylan GF20.
Galois L et al, 2012	Influence on chondral and synovial lesions	Rat knees with ACLT	Hyalgan®, Synvisc®, sham injection, saline solution,	No	HAs exert simultaneously exerting chondroprotective properties and long-term subacute synovitis.
Elmorsy S et al, 2014	Joint lubrication and chondroprotective effect	Bilateral ACLT in rabbits	Artz®, Synvisc®, saline solution	Yes, in favour of Synvisc® (HMW > LMW)	Synvisc® exerts chondroprotective effects and produces superior friction coefficients. Synvisc® improves joint lubrication significantly

Abbreviations: ACLT, anterior cruciate ligament transection; G, group; LMW, Low Molecular Weight; HMW, High Molecular Weight.

Table 3. Main characteristics of hyaluronic acid based products for intra-articular use merchandised in Italy.

TN	CONC	V	COMP	Src HA	MW	JOINT	DOS	DUR	DR/MD	REF	CL	CONS
Arthrum®	40 mg (2%)	2	HA	Bio	2400	K	1X3	NR	MD	NR	N	2 to 30
Arthrum 2,5%®	75 mg (2,5%)	3	HA	Bio	2400	K	1	NR	MD	GEN	N	2 to 30
Artrosulfur HA®	32 mg (1,6%)	2	HA	NR	1000	Nr	1X3	NR	MD	NR	N	2 to 30
Artz®/Supartz®	25 mg (1%)	2,5	HA	RC	600–1200	K	1X5	NR	DR/MD	PROP	N	<25
Condrovisc®	20 mg	2	HA + Carnosine	Bio	600–1000	Nr	1X4	3–6	MD	NR	N	<25
Coxarthrum®	75 mg (2.5%)	3	HA	Bio	2400	H	1	NR	MD	NR	N	2 to 30
Durolane®	60 mg	3	NASHA	Bio	NA	K, H	1	NR	MD	PROP		2 to 25
Durolane SJ®	20 mg	1	NASHA	Bio	NA	A, E, W, HN, FT	1	NR	MD	PROP		2 to 25
Euflexxa®	20 mg (1%)	2	HA	Bio	2400–3600	K	1X3	6	MD	NR	N	2 to 25
Fermathron S®	69 mg	3	HA CL 1,4 butandiol-diglicidil ether)	Bio	NR	K, H, S	1	NR	MD	GRN	Y	<25
Fermathron plus®	30 mg (1,5%)	2	HA	Bio	2000	All	1X3	NR	MD	GEN	N	<25
Go-On®	25 mg (1%)	2,5	HA	Bio	800–1500	K, S	1X5	>6	MD	NR	N	<25
Go-On Mini®	10 mg (1%)	1	HA	Bio	800–1500	HN, TMJ, HN, FT, spine	1X3	>6	MD	NR	N	<25
Go-On matrix®	40 mg (2%) 80 mg sorbitole	2	HA + sorbitol	Bio	800–1500	K	1X3	>6	MD	NR	N	<25
Hyalart®	20 mg/2 mL (1%)	1	HA	RC	500–730	K	1X5	NR	MD	NR	N	<25
Hyalgan®	20 mg (1%)	2	HA	RC	500–730	K	1X5	NR	DR	NR	N	0 to 25
Hyalubrix®	30 mg (1.5%)	2	HA	Bio	>1500	NR	1X3	NR	MD	NR	N	<25
Hyalubrix® 60/ Hyalone®	60 mg (1.5%)	4	HA	Bio	1500–2000	K, H	NR	NR	MD	NR	N	<25
Hymovis®	24 mg	3	HYADD® 4	Bio	–	K	1X2	NR	MD	NR	Y	0 to 25
Inartral®	30 mg (1.5%)	2	HA	Bio	>1500	NR	1X3	NR	MD	NR	N	<25
Intragel® 0.8%	16 mg (0.8%)	2	HA	Bio	800–1200	NR	1X3–1X5	NR	MD	NR	N	<25
Intragel® 1.6%	32 mg (1.6%)	2	HA	Bio	800–1200	NR	1X3	NR	MD	NR	N	<25
Intragel® Mini	8 mg (0.8%)	1	HA	Bio	800–1200	HN, W, FT, TMJ	1	4–6	MD	NR	N	<25
Jointex®	16 m (0.8%)	2	HA	Bio	800–1200	NR	1X3–1X5	NR	MD	NR	N	<25
Jointex® Starter	32 mg (1.6%)	2	HA	Bio	800–1200	NR	1X3	NR	MD	NR	N	<25
Jointex® Mini	8 mg (0.8%)	1	HA	Bio	800–1200	HN, FT, W, TMJ	1	4–6	MD	NR	N	<25
Jonexa®	42 mg (80% gel CL; 20% HA)	4	Hylastan SGL-80 (gel HA CL)	Bio	900–1300	K	1	NR	MD	NR	Y	2 to 30
Kartilage®	15,5 mg	2	HA + mannitole	Bio	1500	S, K, H, A, IP	1X3	NR	MD	NR	N	2 to 25
Kartilage cross®	16 mg	2,2	HA + mannitole	Bio	NR	S, H, K, A, IP	1	NR	MD	NR	N	2 to 25

(*Continued*)

Table 3. (*Continued*)

TN	CONC	V	COMP	Src HA	MW	JOINT	DOS	DUR	DR/MD	REF	CL	CONS
MonoVisc®	80 mg	4	HA	Bio	NA	K	1	NR	MD	NR	Y	2 to 25
Orthovisc®	30 mg (1.5%)	2	HA	Bio	1100–2900	NR	1X3	NR	MD	PROP, GEN	N	2 to 25
Orthovisc® mini	15 mg (1.5%)	1	HA	Bio	1450	HN, W, FT, A, S, TMJ	NR	NR	MD	PROP, GEN	N	2 to 25
Ostenil®	20 mg (1%)	2	HA	Bio	1000–2000	K, H, S, A	K 1X3, H 1	12	MD	PROP	N	2 to 25
Ostenil® Plus	40 mg (2%) Mannitol: 10 mg (0.5%)	2	HA + mannitole	Bio	1000–2000	K	1	6	MD	PROP	N	2 to 25
Ostenil® mini	10 mg (1%)	1	HA	Bio	1000–2000	HN, FT, TMJ, facets	1X3–1X5	NR	MD	PROP	N	2 to 25
Proial®	20 mg	2	HA	NR	NR	K, other	1X3–1X5	NR	MD	NR	N	0 to 25
Promovia®	24 mg 40 mg 82,8 mg	2, 3, 6	HA	Bio	1200–1800	NR	1X3–1X5	6	MD	NR	N	<25
RenehaVis®	LMW 15.4 mg (2.2%) + HMW 7 mg (1%)	1,5	HA	Bio	1000 and 2000	K	1	6	MD	NR	N	Nr
Rhizarthrum®	20 mg (2%)	1	HA	Bio	2400	HN, FT, TMJ	1	6	MD	NR	N	2 to 30
Sinovial®	16 mg (0.8%)	2	HA	Bio	800–1200	NR	1X3–1X5	6	MD	NR	N	<25
Sinovial® Forte	32 mg (1.6%)	2	HA	Bio	800–1200	NR	1	6	MD	NR	N	<25
Sinovial® Mini	8 mg (0.8%)	1	HA	Bio	800–1200	HN, W, FT, TMJ	1	4–6	MD	NR	N	<25
Sinovial® One	50 mg (2%)	2,5	HA	Bio	800–1200	NR	1	NR	MD	NR	N	<25
SportVis®	12 mg (1%)	1,2	HA	Bio	NR	PERI	1X2	NR	MD	NR	N	Nr
Structovial®	20 mg (1%)	2	HA	NR	NR	NR	1X3–1X5	NR	MD	NR	N	2 to 25
Synocrom®	20 mg (1%)	2	HA	Bio	1600	All	1X3–1X5	NR	MD	NR	N	0 to 25
Synocrom® mini	10 mg (1%)	1	HA	Bio	1600	All	NR	NR	MD	NR	N	0 to 25
Synocrom® Forte	40 mg (2%)	2	HA	Bio	2100	All	NR	NR	MD	NR	Y	0 to 25
Synocrom® Forte One	80 mg (2%)	4	HA	Bio	2100	All	NR	NR	MD	NR	Y	0 to 25
Synolis® V-A	HA: 20 mg (2%) Sorbitol: 40 mg (4%)	2	HA + sorbitole	Bio	2200	All	1X3	NR	MD	PROP	N	2 to 25
Synvisc®	16 mg (80% HA HMW CL; 20% gel CL)	2	Hylan G-F 20	RC	6000	K, H, S, A	K 1X3, H, S, A 1	6–12	MD	PROP	Y	2 to 30
Synvisc® One	48 mg (80% HA high MW cross-linked; 20% gel CL)	6	Hylan G-F 20	RC	6000	K	1X6M	12	MD	PROP	Y	2 to 30
Yaral®	16 mg (0.8%)	2	HA	Bio	800–1200	NR	1X3–1X5	NR	MD	Nr	N	<25
Yaral® Forte	32 mg (1.6%)	2	HA	Bio	800–1200	NR	1X3	NR	MD	NR	N	<25

Yaral® Mini	8 mg (0.8%)	1	HA	Bio	800–1200	HN, FT, W, TMJ	1	4–6	MD	NR	N	<25
Viscoplus®	20 mg	2 ml	HA	Bio	2000	K, H, S, FT, TMJ, spine	1X3–1X5	6	MD	NR	N	2 to 25
Viscoplus gel®	75 mg (2.5%)	2 ml	HA	Bio	NR	K, H, S, FT, TMJ, spine	1	6	MD	NR	N	2 to 25

Abbreviations: TN, Tradename; Conc, Conentration in mg/ml (%); V, Volume in ml; Comp, Composition; Src HA, Source of Hyaluronic Acid; MW, Molecular Weight in KDa; Joint, Joint the HA is indicated for; Dos, Dosage; 1x2 1, 1 injection per week for 2 weeks; 1x3, 1 injection per week for 3 weeks; 1X4, 1 injection per week for 4 weeks; 1X5, 1 injection per week for 5 weeks; 1x6M, 1 injection every 6 months; Dur, Expected duration of effect in months; Dr/MD, Drug or medical device; DR, Drug; MD, Medical Device; Ref, Presence of references in the leaflet; CL, Crosslinked or not (Y, Yes, N, No), Cons, Conservation in°C; HA, Hyaluronic Acid; Bio, biofermentation; RC, Rooster Combs; K, Knee; H, Hip; S, Shoulder; A, Ankle; E, Elbow; TMJ, Tempuromandibular joint; IP, Interphalangeal; W, Wrist; HN, Hand; FT, Foot; PERI, Periarticular; MD, Medical Device; Prop, References on the HA to which the leaflet belongs; NR, Not reported; NA, Not Applicable; GEN, References on generic HA; LMW, Low Molecular Weight; HMW, High Molecular Weight.

products with a documented rationale; to that end, regulators should require a sufficient amount of studies before introducing a new brand in the market. Manufacturers should introduce in the leaflet all studies regarding their own product, with the related references, and should avoid using generic evidence or class properties only.

In vivo evidence is far more developed for the low molecular weight products such as Hyalgan, Supartz, and Hylan G-F 20 (Synvisc). Five studies were also conducted with HYADD4-G (Hymovis), a low molecular weight formulation with a modified molecule. One study each was conducted on Orthovisc and NASHA (Durolane).

Studies performed with the addition of other substances were conducted with self-made HA derived from bacterial fermentation.

The studies conducted in animals deepen the understanding of aspects such as the effects of HA use after traumas such as anterior cruciate ligament tear, meniscus injury, articular cartilage injury, histologic changes in cartilage and synovial tissue after use of HA, and adverse reactions, all of which are relevant to establish the rationale for the use of HA in human beings. Unfortunately, some brands are launched in the market without animal or clinical evidence. This is due to the products' registration as medical device, which in Italy may be performed without the production of RCTs reporting on efficacy and safety profiles of the given medical device.

Analyzing all data of *in vitro* and animal model studies and then comparing the findings with what is reported in the leaflets of branded compounds, it is clear that leaflets are incomplete and often lack the basic data necessary for an aware administration of such compounds. First, leaflets for the large majority of HA-branded products for IA use do not include references supporting indications and contraindications reported. This is a major flaw for products whose use should be carefully evaluated. References supporting the use in terms of dosage, expected duration of effect, target joint, and, above all, clinical conditions representing indications or contraindications, should be clearly reported, so that physicians dealing with IA therapies could be supported by scientific evidence in their therapeutic approach. Also, in terms of health insurance, the presence of solid references reporting on scientific evidence would lead to a more secure approach to the therapeutic use of IA injection of HA.

Curiously, some HA-based products that have been studied reported indication for OA, but not all of them. Some reported indication for joint pain relief, although they did not specify any joint alteration that might be inducing pain for which they are indicated and did not provide the related evidence. Again, some branded products reported to be indicated for SF temporary substitution or for the improvement of joint mobility, but no data regarding the cause of SF alterations or reduced joint mobility, such as OA or other conditions, were reported in the leaflet. If the leaflet was to be strictly followed,

Table 4. Indications as reported on the leaflet of the hyaluronic acid brands merchandised in Italy for intra-articular use.

TRADENAME	PAIN	REDUCED MOBILITY	OA	SYNOVIAL LIQUID SUBSTITUTION	NO TKR	POST TRAUMATIC	AFTER ARTHROSCOPY OR SURGERY
Arthrum®	0	0	1	0	1	1	0
Arthrum 2,5%®	1	0	1	0	1	1	0
Artrosulfur HA®	1	1	1	0	0	1	0
Artz®/Supartz®	0	0	1	0	0	0	0
Condrovisc®	0	0	1	0	0	1	0
Coxarthrum®	0	0	1	0	0	0	0
Durolane® (AF)	1	0	1	0	0	0	1
Euflexxa®	1	0	0	0	0	0	0
Fermathron S (AF)®	1	1	1	0	0	1	0
Go-On® (AF)	1	0	1	0	0	0	0
Hyalart®	0	0	1	0	0	1	0
Hyalgan®	0	0	1	0	0	0	0
Hyalubrix®	0	0	1	1	0	0	0
Hyalubrix® 60*	0	0	1	1	0	0	0
Hymovis®	1	0	1	0	0	0	0
Inartral®	0	0	1	1	0	0	0
Intragel® (AF)	1	1	1	0	0	1	0
Jointex® (AF)	1	1	1	0	0	1	0
Jonexa®	1	1	1	0	0	0	0
Kartilage (AF)	1	1	1	0	0	1	0
MonoVisc®	0	0	1	1	0	0	0
Orthovisc® (AF)	1	0	1	0	0	0	0
Ostenil®	1	1	1	0	0	0	0
Ostenil® Plus	1	1	1	0	0	0	0
Ostenil® mini	1	1	1	0	0	1	0
Proial®	1	0	0	0	0	0	0
Promovia® (AF)	1	1	0	0	0	0	0
RenehaVis®	1	1	0	0	0	0	0
Rhizarthrum®	1	1	0	0	0	0	0
Sinovial® (AF)	1	1	1	0	0	1	0
SportVis®	1	1	0	0	0	0	0
Structovial®	1	1	1	0	0	1	0
Synocrom® (AF)	1	1	1	0	0	1	0
Synolis® V-A	1	0	1	0	0	0	0
Synvisc®	0	0	0	1	0	0	0
Synvisc® One	0	0	0	1	0	0	0
Yaral® (AF)	1	1	1	0	0	1	0
Viscoplus®	1	1	0	0	0	0	0
Viscoplus gel®	1	1	1	0	0	0	0

Note: *Also Hyalone.
Abbreviation: AF, all formulations of the same brand.

we would have products indicated for OA, products indicated for symptomatic OA, products indicated for reduced joint mobility, and products indicated for SF substitution due, or not, to OA. This is all confusing and, at least, imprecise and arbitrary, especially when such indications are not supported by adequate scientific references. Similarly, it is of extreme relevance to underline another aspect of the management of HA: conservation of HA-based products. The majority of leaflets reported the optimal temperature for the conservation of HA for IA use, but this aspect is often considered as secondary.

Table 5. Contraindications as reported on the leaflet of the hyaluronic acid brands merchandised in Italy for intra-articular use.

TRADENAME	HYPERSENSITIVITY TO HA	SKIN INFECTION	JOINT INFECTION	JOINT INFLAMMATION	PREGNANCY/ BREAST FEEDING	VENOUS OR LYMPHATIC STASIS	HEPATHOPATHIES	CHILDREN	BLEEDING DISORDERS
Arthrum®	0	0	0	0	0	0	0	0	0
Arthrum 2,5%®	0	0	0	0	0	0	0	0	0
Artrosulfur HA®	0	1	1	1	0	0	0	0	0
Artz®/Supartz®	1	0	0	0	1	0	1	1	0
Condrovisc®	0	1	1	1	1	0	0	0	0
Coxarthrum®	1	0	1	1	0	0	0	0	0
Durolane® (AF)	0	1	0	0	1	0	0	1	0
Euflexxa®	1	1	1	0	1	0	0	0	0
Fermathron® (AF)	1	1	1	0	0	0	0	0	0
Go-On®	1	0	0	1	0	0	0	0	0
Go-On® Mini	1	0	0	1	0	0	0	0	0
Go-on® matrix	1	1	1	1	0	1	0	0	0
Hyalart®	1	0	0	0	0	0	1	0	0
Hyalgan®	1	0	0	0	0	0	1	0	0
Hyalubrix®	1	1	0	0	0	0	0	0	0
Hyalubrix® 60*	1	1	0	0	0	0	0	0	0
Hymovis®	1	1	0	0	0	0	0	0	0
Inartral®	1	1	0	0	0	0	0	0	0
Intragel® (AF)	0	1	1	1	0	0	0	0	0
Jointex® (AF)	0	1	1	1	0	0	0	0	0
Jonexa®	1	1	1	0	0	1	0	0	0
Kartilage (AF)	1	0	0	1	0	0	0	0	0
MonoVisc®	1	1	0	0	0	0	0	0	1
Orthovisc® (AF)	1	0	0	0	1	0	0	0	0
Ostenil® (AF)	1	0	0	0	0	0	0	0	0
Proial®	1	1	1	0	0	0	0	0	0
Promovia®	0	1	0	0	0	1	0	0	0
RenehaVis®	1	0	0	0	0	0	0	0	0
Rhizarthrum®	1	0	1	1	0	0	0	0	0
Sinovial® (AF)	0	1	1	1	0	0	0	0	0
SportVis®	1	1	0	0	0	0	0	0	0
Structovial®	1	0	1	0	0	0	0	0	0
Synocrom® (AF)	1	0	1	0	0	0	0	0	0
Synolis® V-A	1	1	1	0	1	0	0	1	0
Synvisc® (AF)	0	1	1	1	0	1	0	0	0
Yaral® (AF)	0	1	1	1	0	0	0	0	0
Viscoplus® (AF)	0	0	0	0	0	0	0	0	0

Note: *Also Hyalone.

Abbreviation: AF, all formulations of the same brand.

Both physicians and patients, who often buy such products, should be carefully informed regarding the conservation of HA, to avoid alterations of the products that may lead to inefficacy of the therapy. Regarding contraindications to the use of HA, it is evident that certain clinical conditions represent absolute contraindications to the use of IA HA, such as skin or joint infection. It is important to report that not all branded products stated such clinical conditions as contraindications to the IA administration of HA, and this again represents a major flaw in the leaflets of many products. The presence of infections in the site of injection or the presence of infectious arthritis should represent absolute contraindications to the use of IA HA, as already reported in literature.

Similarly, bleeding disorders should be carefully considered, as IA injections represent an invasive procedure. Although there are several recent reports on the use of viscosupplementation in patients affected by hemophilic arthropathies,[29,30] the IA injection of compounds in patients affected by bleeding disorders should be reported as potentially dangerous. Severe hepatopathies are reported by some HA-based products as contraindications to their use for IA injection, but again no references to support such data are reported. Furthermore, we were not able to find any reliable data in scientific literature regarding the relevance of hepatopathies in the IA administration of such compounds, although the elimination of HA administered by IA injection relies upon hepatic metabolization.[31]

Regarding venous and lymphatic stasis, the role of the lymphatic system in the metabolism of HA has already been reported.[28] It is logical to suppose that impairments of the lymphatic system such as lymphatic or venous stasis may interfere with the normal metabolism of HA administered by IA injection, and some of the products report such clinical conditions as contraindications to the use of HA. Again, no scientific evidence regarding the role of venous or lymphatic stasis is present in literature to support this contraindication.

Another condition to consider for IA injection of HA is pregnancy. At present, there are no reports regarding the safety and efficacy of the use of HA in pregnant patients, and this lack of data represents the cause for a relevant warning regarding its use. Probably, the prevalence of OA in pregnant patients is low, due to the usual young age of pregnant patients, but in cases of symptomatic OA during pregnancy, the use of HA should be avoided because of the complete absence of scientific evidence regarding its safety. Similarly, a complete lack of data exists regarding the use of IA HA during breast feeding. For safety reasons, this lack of data should be clearly reported in all leaflets.

Three of the examined products report that the use of HA should be avoided in children. It is unclear what the role of viscosupplementation in children could be, and again, no references supporting this contraindication are reported.

Conclusions

Evidence from *in vitro* and *in vivo* studies regarding HA products marketed in Italy is scarce and relates to only a small proportion of the available branded products. Our analysis of the content of leaflets for various HAs marketed in Italy suggests that many reported indications and contraindications are arbitrary and not supported by scientific evidence, thus confounding the decision to prescribe the products. Larger and brand-specific studies are necessary in order to understand and support the correct use of HA for IA injection and to guide clinicians in making a correctly targeted choice when prescribing an HA-based IA therapy.

Author Contributions

Conceived and designed the experiments: AM. Analyzed the data: AM, EB, CF, ODL, ADS, Asmaa M, MB. Wrote the first draft of the manuscript: EB, ODL, ADS, Asmaa M. Contributed to the writing of the manuscript: AM, CF, MB. Agree with manuscript results and conclusions: AM, CF, EB, ODL, ADS, Asmaa M, MB. Jointly developed the structure and arguments for the paper: AM, CF, ODL, EB, ADS, Asmaa M, MB. Made critical revisions and approved final version: AM, EB, CF, ODL, ADS, Asmaa M, MB. All authors reviewed and approved of the final manuscript.

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