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TITOLO TESI

Hyaluronidase for muscle stiffness in spasticity

Ialuronidasi per la rigidità muscolare nella spasticità

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Introduction

Spasticity is defined as a motor disorder characterized by a velocity-dependent increase in muscle tone with exaggerated tendon jerks, resulting from hyper excitability of the stretch reflex, as one component of the upper motor neuron syndrome [1]. The incidence of spasticity in patients with spinal cord injury is 65–78% [2]. In contrast, only around 35% of the patients with persistent hemiplegia after stroke manifest spasticity [3]. More specifically, the prevalence of spasticity is about 19% within three months after stroke, but rises to 39% after 12 months [4]. Spasticity commonly presents as muscle over-activity, reduction in the ability to relax, hypertonia, paresis, muscle spasms, and loss of the fine motor control, which are attributed to neural mechanisms [5]. However, less understood symptoms include increased stiffness in the soft tissue, muscle fatigue, and postural changes in the limbs, which may be explained by non-neural contributions having secondary effects on skeletal muscles [6, 7]. Lundstrom et al. postulate that spasticity cannot be considered an immediate consequence of CNS injury because it progresses during the weeks and months after the injury [8].

Several studies have demonstrated that muscular changes in spasticity cannot be explained by classic interpretations of the effects of neural changes alone [9, 10]. Crone et al. found that there is no correlation between the degree of spasticity (assessed by the Ashworth scale) and the degree of reduced "reciprocal inhibition" [11]. Gracies et al. proposed that muscular changes in spasticity cannot be fully explained by hyperreflexia or alterations in the central processing of sensory input in the spinal cord alone [12, 13]. Studies show that individuals presenting with spasticity after spinal cord injury on clinical examination turn out not to have any signs of hyperreflexia [14, 15]. In addition, it was shown that reflex stiffness did not differ significantly between the impaired spastic and the less impaired contralateral limb in individuals with spastic hemiparesis [16]. It has therefore been argued that structural and functional changes in and around skeletal muscles may account for the late presentation of disabling spasticity after CNS injury. These changes range from rounding of

the muscle fibers, increase in the inter-fiber space in hypertrophic fibers, and fiber atrophy which are all associated with deposition of excessive connective tissue in the muscle [17-20].

Peripheral alterations in spasticity

Several studies support the involvement of peripheral tissues, as muscle fibers and connective tissue, in spasticity. Mirbagheri et al. found that intrinsic muscle stiffness was increased in patients with spasticity [21]. Wood et al. describe the complex interactions between muscle cells and the extracellular matrix that affect the contractile properties of skeletal muscle fibers relevant to changes that occur with spasticity [22]. Lieber et al. showed that spastic muscle bundles are stiffer than isolated single spastic fibers; only about 40% of spastic muscle bundles were occupied by muscle fibers in contrast to 95% in normal muscle bundle, suggesting increased intramuscular connective tissue deposition in spastic muscle bundles [23]. Three different mechanisms have been proposed to explain the 'increased resistance to stretch' or intrinsic muscle stiffness [9]: (1) active muscle stiffness where increased stiffness is caused by an increase in the number of cross-bridges attached during muscle contraction; (2) neurally-mediated reflex stiffness caused by descending influences on the monosynaptic reflex between muscle spindle afferents and the alpha motor neurons; and (3) passive muscle stiffness caused by fibrosis within the muscle tissue or a change in the properties of the muscle fibers.

Active muscle stiffness: alterations in the extracellular matrix

The extracellular matrix is composed by adipose cells, glycosaminoglycans and hyaluronic acid. Hyaluronic acid is however the chief component [24]. Immobilization or paresis decreases the normal turnover of the extracellular matrix, increasing its concentration within and between the muscular compartments. It is known that hyaluronic acid behaves like a non-Newtonian fluid at high

concentrations and becomes more viscous [25]. The increased viscosity of the loose connective tissue may cause decreased gliding between the layers of collagen fibers, which may be perceived by patients as stiffness [26]. In a longitudinal study examining the effects of immobilization in rat soleus muscle, hyaluronic acid concentrations were increased and sarcomere length was shortened by one week [27]. These changes can lead to an increase in the number of cross-bridges attached during contraction [28, 29], producing active muscle stiffness. Note that at this stage the arrangement of collagen fibrils in the endomysium is still longitudinal, but becomes more circumferential by 4 weeks [27]. It is known that under homeostatic conditions hyaluronic acid has important roles in tissue structural integrity. However, a series of events that leads to muscle contracture may begin with subtle changes in the turnover of hyaluronic acid [30, 31] and the properties of the extracellular matrix very early on after CNS injury (Fig. 1).

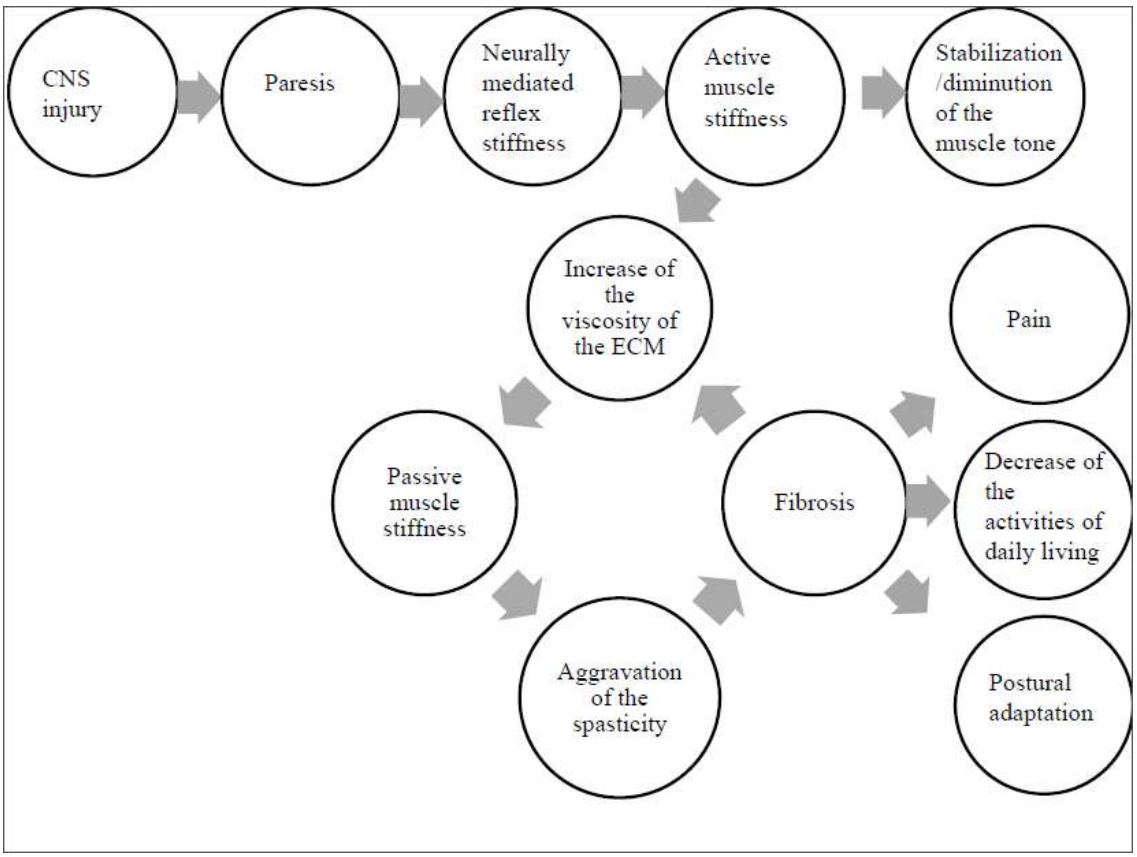

FIG. 1. Proposed model of the evolution of the spastic process.

We hypothesize that the non-Newtonian behavior of hyaluronic acid may be especially responsive to injection of hyaluronidase enzyme that depolymerized it and lower molecular mass polymers are generated [30]. Stern et al. found that small polymers of hyaluronic acid, smaller than 1000 Dalton, may be able to catalyze specific inflammatory reactions [31]. Thus biochemical control of the properties of hyaluronic acid early on may help restore the quality of hyaluronic acid and re-establish normal tissue sliding mechanisms involving the endomysium, perimysium, epimysium and deep fascia.

Neurally-mediated muscle stiffness: the role of muscle spindles

The muscle spindles are enclosed in a capsule and have both sensory and motor components. The motor component consists of several small specialized muscle fibers known as intrafusal fibers, localized at either end of the muscle spindle. The sensory component is localized in the central area of the spindles, where specialized nerve endings termed annulospiral and flower-spray endings are present. The annulospiral endings (Ia afferents) provide information about the length and velocity of muscle contraction, whereas the flower spray endings (group II afferents) only provide information on muscle length but these sensory fibers respond even when the muscle is at rest. When the muscle lengthens and the muscle spindle is stretched, mechanically-gated ion channels in the sensory dendrites are opened. This leads to a receptor potential that triggers action potentials in the muscle spindle afferents; the firing of the spindle Ia afferents stimulates the alpha motor neurons in the spinal cord causing reflex contraction of the extrafusal muscle fibers. At the same time Ia spindle afferents synapse in the posterior horn of the cord and stimulate inhibitory interneurons which then depress alpha motor activity to the antagonistic muscles. Thus the simple myotatic/stretch reflex acts as a servo-mechanism to maintain correct muscle tone. Muscle tone (residual muscle tension or tonus) is the continuous and partial contraction of the muscles, or the muscle's resistance to passive stretch

during rest. If the muscle spindles cannot be activated or are activated too easily, the regulation of muscle tone will be compromised [5, 32, 33].

When the relationship between muscle spindles and the intramuscular connective tissue is considered, the role of the muscle spindles in peripheral motor coordination becomes evident. The muscle spindles are localized in the perimysium and their capsule is connected to the epimysium and fascial septae [34, 35]. Strasmann et al. analyzed the septum of the supinator muscle and found that many muscle spindles are inserted directly into the connective tissue of the septum [36]. Studies have demonstrated that the extra- and intra-fusal muscle fibers possess complex biophysical properties that contribute to muscle stiffness and/ or laxity [37, 38]. Increased stiffness of connective tissue in an immobilized muscle reduces its compliance. This in turn increases spindle stimulation to a given stretch, as the pull is transmitted more efficiently to the spindles in a less extensible muscle [39]. Such increase in spindle responses has been demonstrated after immobilization in a shortened position, which augments stretch reflexes and eventually contributes to the stretch-sensitive forms of muscle overactivity [40-42]. Thus Gracies proposed that the etiology of spasticity involves increased stretch-induced stimulation of spindles in muscles with stiffer connective tissue (endomysium, perimysium and epimysium), which contributes to abnormal feed-back and feed-forward control of the extrafusal muscle fibers [43]. However, the relative contribution of intrinsic muscle stiffness versus neurally-mediated spindle responses may vary by body region affected [44].

It is commonly noted that voluntary movements that stretch a spastic muscle produce a reflex in the antagonistic muscle opposing the initiating movement [45]. Constant overuse of spastic antagonist muscles can augment the production [46], and retention [47] of hyaluronic acid in the connective tissue. The increased concentration of hyaluronic acid on the spread surface can lead to its further self-association, leading to dramatically increased viscoelasticity [48]. The higher viscosity extracellular matrix can alter the perimysium as well the muscle spindle capsule leading to hyper stretching of the annulospiral ring, and further decrease the threshold of activation of the spindle

afferents and the alpha motoneuron with increasing spasticity in the corresponding motor unit. In fact Beres-Jones [49] has demonstrated that repetitive clonic muscle contractions are more likely to be associated with impaired interaction of central and peripheral mechanisms than with recurrent stretch reflex activity. Elderly individuals however often show a decrease in basal tone and in reflex activity, perhaps due to sarcopenia and consequent alterations in muscle tone and muscle spindle activation [50]. Disabling spasticity occurs more commonly in young survivors (less than 65 years) or after a first stroke [51], further necessitating that we understand the mechanisms and initiate appropriate treatment early.

Passive muscle stiffness: deposition of collagen and fibrosis

In the chronic phase, histolopathological studies show a generalized increase in extracellular connective tissue in spastic muscles; specifically an increase in the concentration of collagen [18, 19]. Stecco C. at al. reported similar findings in patients after 10 months of immobilization that could simulate the period of flaccid paralysis after CNS injury [52]. The authors dissected several recently amputated legs of patients that had been immobilized with a tibio-femoral external fixator due to severe trauma to the knee region. They showed the existence of fibrotic zones in the subcutaneous tissue that created adherences between the skin and deep fascia in the ankle region, which had not been injured. The entire ankle region demonstrated alterations in the subcutaneous tissue, and the deep fascia showed disorganized arrangement of the fibrous bundles. Histological study showed that the loose connective tissue separating the subcutaneous planes was absent, and that all the structures were replaced by homogeneous fibrous tissue. Studies by Järvinen et al. [53] reveal that immobilization results in a marked increase in the endo- and perimysial connective tissue of the muscle, the majority of the increased endomysial collagen being deposited directly on the sarcolemma of the muscle cells. The connective tissue becomes very dense and the number of irregularly oriented collagen fibers is markedly increased. Further, immobilization clearly disturbs the normal structure

of the endomysium making it impossible to distinguish the various networks of fibers from one another. One might hypothesize that similar findings would be seen in individuals with chronic stroke. In fact the presence of dense connective tissue (fibrosis) has been recently confirmed through ultrasonography by Picelli et al. [54]. The authors quantified the disruption of normal muscle architecture in spastic muscles by evaluating and grading muscle echo intensity. The hyper echogenicity in the muscle in the chronic stage is due to increase in collagen fiber both within and between the muscle bundles (Fig. 2). Excessive collagen deposition is thought to lead to the development of abnormal postures, and greater amounts of collagen predict a lower response to conventional treatments for spasticity such as Botulinum toxin injections.

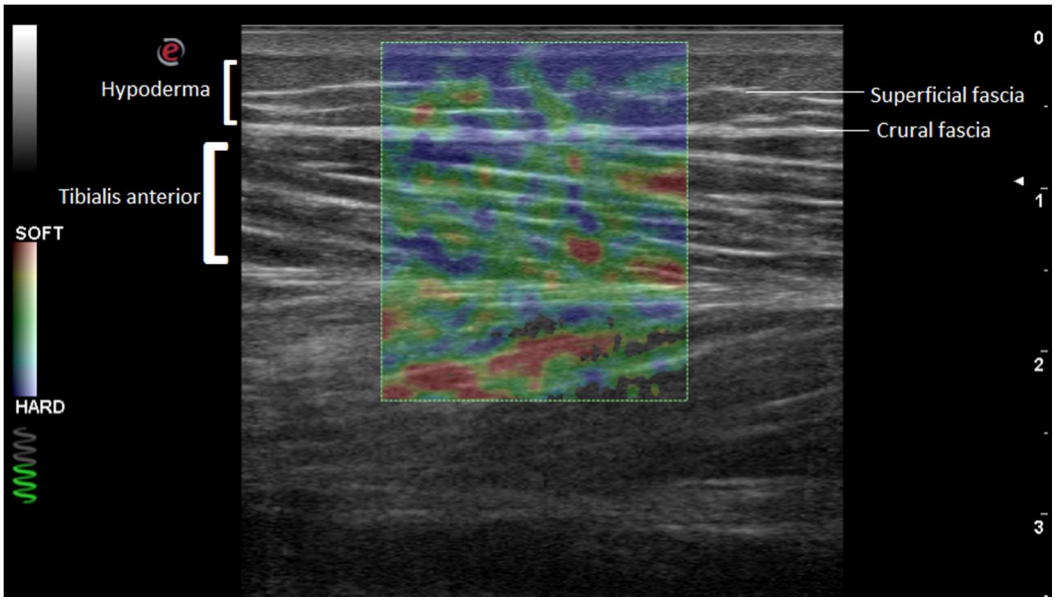

FIG. 2. Ultrasonography and elastosonography (to assess tissue stiffness) imaging in a spastic tibialis anterior muscle.

Although muscle fibers are the main tissues studied in spasticity, there is no agreement on the role of muscle fiber pathology and abnormal muscle activity in spasticity [9, 23]. Muscle biopsies from patients with spasticity show abnormalities such as increased variability in fiber size, increased numbers of 'rounded' fibers, and 'moth-eaten' fibers [17, 20, 55, 56]; these are however non-specific changes also noted in other disease states, and may be directly attributed to the effect of

immobilization. Morphometric and histochemical investigations show changes in mechanical muscle fiber properties [17, 28, 57] that might contribute to spastic muscle tone, indicating an increase in passive stiffness of a muscle to stretch in patients with stroke and spasticity due to subclinical contractures. Indeed studies have found that functional alterations in patients with muscle hypertonia are associated with subclinical muscle contracture rather than with reflex hyper-excitability [6, 13, 16, 58-60]. Thus the data are congruent with the idea that instead of spasticity causing contracture, contracture may actually potentiate spasticity in some patients [59]. Consequently, the Ashworth scale and modified Ashworth scale remain low-sensitivity instruments for distinguishing between soft tissue and neural contributions to hypertonia [61]. Electromyographic measurements can quantify the resistance to passive movement, but are still unable to determine how much resistance is produced by the tonic stretch reflex and how much is produced by soft tissue stiffness [5].

Role of peripheral mechanisms in generating pain

Post-stroke spasticity is often associated with secondary complications such as pain and limitation in mobility. Motor and sensory impairments are associated with an increased risk for stroke-related pain [8]. Patients with spasticity produce compensatory movements and postures due to changes in their muscles and soft tissues. Such compensations are also noted with common musculoskeletal dysfunction. Indeed Stecco at al. have documented alterations in the soft tissue and muscle in patients with chronic myofascial pain [26], and have correlated the alteration in the loose connective tissue of the deep fascia with patients' stiffness and pain. The connective tissue is well innervated with free nerve endings and proprioceptors as Pacini and Rufini corpuscles [62-64]. It has been shown that change in the visco-elasticity of the connective tissue shapes the dynamic response of the mechanoreceptors in the tissue, which may become the source of pain [65, 66]. Thus there may be greater similarities between non-specific musculoskeletal pain and spastic muscle pain.

Implications for treatment

Surgical, pharmacological, and physiotherapy techniques are among the most common interventions offered to mediate spasticity [67,68]. Pharmacological agents are the most commonly used intervention to treat poststroke spasticity. The options for reducing spasticity are oral medication, such as benzodiazepines, baclofen, tizanidine hydrochloride, and dantrolene. Diazepam is one of the oldest and most commonly used benzodiazepines for treating spasticity. Botulinum toxin type A is used for focal treatment of overly spastic muscles, while Intrathecal baclofen is commonly used to reduce spasticity in individuals with spinal cord injury. However, despite significantly reducing spasticity, the use of pharmacological agents does not always translate into an increase in functionality [69], but also make the muscles weak [12]. As a result, restoration of active movement and function may be compromised.

While the changes in muscle structure may be partly attributed to immobility ensuing from the initial neurologic injury, the changes in function cannot be explained by classic interpretations of the effects of neural changes alone. Here we propose a novel hypothesis to explain the role of non-neural factors in the development and exacerbation of spasticity, and describe a new treatment approach based on this hypothesis in the case series below.

The hyaluronan hypothesis

We hypothesize that immobility, after central nervous system injury, increases the viscosity of hylauronan in the extracellular matrix due to its non-newtonian properties. The increased viscosity leads to stiffness of the loose connective tissue in and around the muscles and increases tension in the force transmitted by the dense connective tissue (including the endomysium, perimysium and epimysium) reducing its compliance. The capsule of the muscle spindle is a specialized portion of the epimysium and is continuous with the perimysium [70]. Thus reduced perimysial compliance will increase spindle stimulation and spindle response to a given stretch-force, as the stretch will be more efficiently transmitted to the spindles in a less extensible muscle producing hyperreflexia (Fig. 2a).

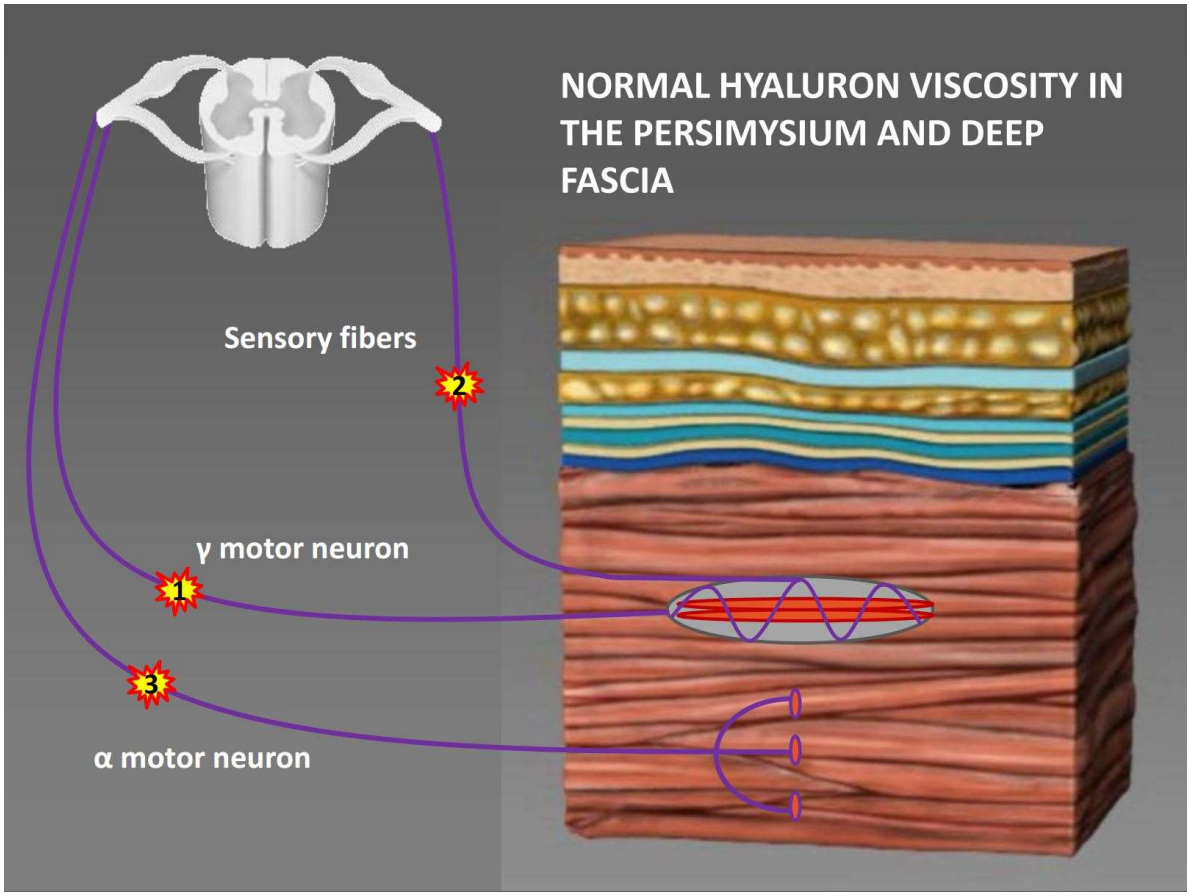

Fig 2a: muscle spindle mechanism

If immobility continues, self-association of the chains of hyaluronan will dramatically increase the viscoelasticity of the extracellular matrix in and around the muscles producing generalized muscle stiffness. The stiffness and spasticity may predominate in some muscles such as the pectoralis major, the biceps brachii, pronator teres and wrist and finger flexors of the upper limb because these muscles have large myofascial expansions that connect them together leading to posturing of the joints in flexion (Fig. 2b).

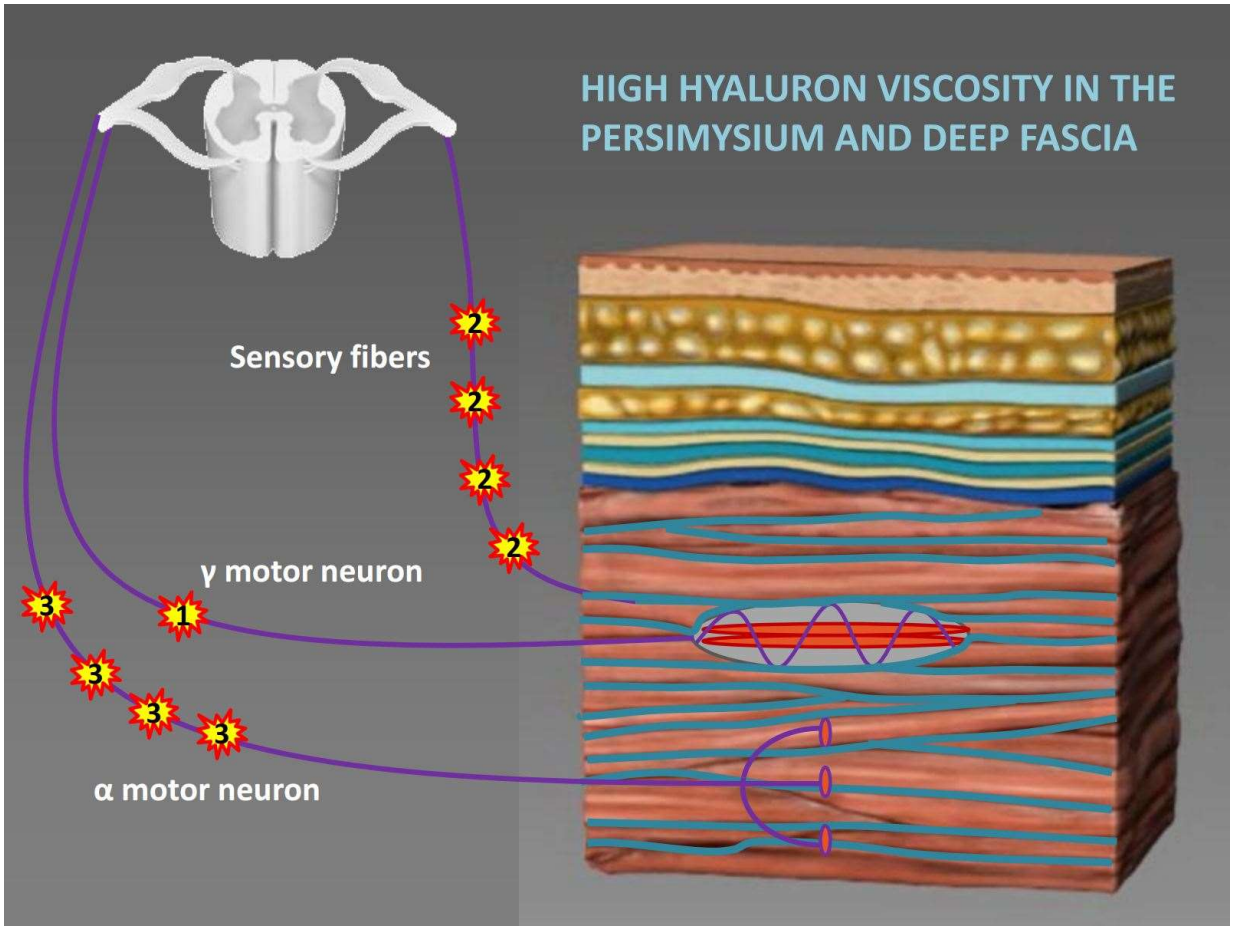

Fig 2b: dysfunction of the muscle spindle

Untreated and unchecked the abnormal chronic posturing can lead to fibrosis and contracture. On the other hand, voluntary movement that stretches a spastic muscle will produce a reflex in the antagonistic muscle to oppose the voluntary movement [71], further stimulating specific cells in the loose connective tissue called fasciacytes to produce more hylauronan [46]. The aggregation of the hyaluronan will disturbed balance of forces between the agonist and antagonist muscles leading to co-contraction, excessive torques during movement and muscle fatigue. The hyaluronan hypothesis can thus mechanistically partially explain the clinical presentations of individuals with spasticity.

The purpose of this case series is to describe the safety, tolerability, and preliminary efficacy of human recombinant hyaluronidase-saline injections given intramuscularly to restore the viscosity of the extracellular matrix and reduce muscle stiffness in individuals with unilateral spastic hemiparesis from a brain injury. Human recombinant hyaluronidase is FDA approved since 2005 as a tissue

permeability modifier. While it is recommended that hyaluronidase be used for subcutaneous injections only, Moore DC [73] reports as many authors have performed nerve blocks entailed injections into muscle layers and deep fascial compartments. Moore DC concluded, in his review, that there is no contraindication to infiltrating solutions containing hyaluronidase into deep tissues if the normal precautions for intravascular injections are observed. Hyaluronidase is supplied as a sterile, clear, colorless, non-preserved, ready-for-use solution. Each mL contains 150 USP units of recombinant human hyaluronidase with 8.5 mg sodium chloride, 1.4 mg dibasic sodium phosphate, 1 mg albumin human, 1.5 mg L-methionine, 0.2 mg polysorbate 80, and hydrochloric acid and sodium hydroxide added for pH adjustment; it has a pH of ~7.0 and an osmolality of 280 to 340 mOsm/kg. Hyaluronidase modifies the permeability of connective tissue through the hydrolysis of hyaluronan by splitting the glucosaminidic bond between C1 of an N-acetylglucosamine moiety and C4 of a glucuronic acid moiety. This temporarily decreases the viscosity of the extra cellular matrix.

Injection with recombinant hyaluronidase

From May 2014 to September 2015, twenty-one individuals (14 male and 7 female) between the ages of 10 and 77 years with moderate-to-severe upper limb spasticity in more than one joint consented to received off-label injections of recombinant hyaluronidase in combination with preservative-free normal saline, in the outpatient hand clinic at the Hospital for Joint Diseases, New York University Langone Medical Center. The individuals were existing patients that had exhausted all available options with limited benefit, or were referred to the clinic by providers seeking this specific treatment. The institutional review board approved the case series, and the study was registered in Clinicaltrials.org. All procedures were performed in accordance with the Guidelines for Good Clinical Practice as issued by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. The investigation and the use of patient data for research purposes were in accordance with the Declaration of the World Medical

Association, and the study was performed in accordance with ethical standards on human experimentation as per the Helsinki Declaration.

The inclusion criteria for the present case series were: informed consent for the procedure and for documentation of video recordings of range-of-motion (ROM) for clinical and academic purposes, unilateral brain injury producing the upper motor neuron syndrome, moderate-to-severe spasticity with a Modified Ashworth Scale (MAS) score of ≥2 in more than one joint. Exclusion criteria were severe sensory aphasia, bilateral spasticity making it difficult to comply with a home exercise program involving assistance with the unaffected limb, concurrent treatment of spasticity with other injectable agents such as Botulinum toxin injections, recent changes in the treatment of spasticity or in underlying medical problems. The 21 cases are described in Table 1.

Case #	Age	Sex	Side	Etiology	Time since injury (months)	Prior treatments	No. of units of Hyaluronidase used*	Volume injected (ml)	No. of areas injected	Location of injections
1	67	M	R	1	29	BT	450	6	0	Pectoralis minor, triceps, pronator theris, extensor digitorum communis, extensor carpi ulnaris, flexor carpi radialis.
2	48	F	R	2	64	BT	450	6	0	Pectoralis minor, pronator theris, extensor digitorum communis, flexor digitorum, extensor carpi ulnaris, flexor carpi radialis.
3	59	M	L	1	61	BT	450	6	0	Pectoralis minor, pronator theris, extensor digitorum communis, flexor digitorum, extensor carpi ulnaris, flexor carpi radialis.
4	53	F	L	2	54	BT	450	6	7	Pectoralis minor,pronator theris, bicept brachi, extensor digitorum communis, extensor carpi ulnaris, flexor carpi radialis, abductor pollicis
5	66	M	R	2	41	BT	450	6	0	Pectoralis minor, pronator theris, bicept brachi, extensor digitorum communis, extensor carpi ulnaris, flexor carpi radialis.
6	43	F	L	1	45	none	450	6	0	Pectoralis minor, pronator theris, extensor digitorum communis, brachialis, flexor digitorum, palmaris aponevrosis
7	43	M	R	1	10	BT	600	8	0	Pectoralis minor, pronator theris, teris major, extensor digitorum communis, flexor digitorum, palmaris aponevrosis, extensor carpi ulnaris, flexor carpi radialis,
8	59	M	R	1	24	BT	600	8	0	Pectoralis minor, pronator theris, teris major, extensor digitorum communis, flexor digitorum, palmaris aponevrosis, extensor carpi ulnaris, flexor carpi radialis,
9	64	M	R	2	85	none	600	8	0	Pectoralis minor, pronator theris, bicep brachi, extensor digitorum communis, flexor digitorum, palmaris aponevrosis, extensor carpi ulnaris, flexor carpi radialis,
10	15	M	R	1	5	none	600	8	0	Pectoralis minor, biceps brachi, brachialis, pronetor theris, flexor digitorum, extensor digitorum communis, palmaris aponevrosis, flexor carpi ulnaris,

11	11	F	L	6	114	BT	450	6	0	Pectoralis major, biceps brachi, pronetor theris, flexor digitorum, palmaris aponevrosis, flexor carpi ulnaris, estensor carpi radialis
12	38	F	R	1	18	none	600	8	0	Pectoralis minor, biceps brachi, brachialis, pronetor theris, flexor digitorum, extensor digitorum communis, palmaris aponevrosis, flexor carpi ulnaris,
13	10	M	R	1	152	BT	300	4	0	Pectoralis minor, biceps brachi, flexor digitorum, flexor carpi radialis, extensor digitorum communis, flexor carpi ulnaris.
14	39	M	L	1	35	BT	450	6	0	Pectoralis minor, biceps brachi, brachialis, pronetor theris, extensor digitorum communis, palmaris aponevrosis, flexor carpi ulnaris, interosseus III-IV and IV-V
15	11	M	R	4	8	none	450	6	0	Pectoralis minor, biceps brachi, extensor digitorum communis, palmaris aponevrosis, flexor digitorum, flexor carpi ulnaris, flexor carpis radialis, interosseus III-IV and IV-V
16	10	F	R	2	17	none	300	4	0	Pronator theris, brachialis, extensor ulnaris carpi, flexor digitorum, hypothenar eminance
17	15	F	R	4	13	BT	600	8	0	Elevetor scapule, Pectoralis minor, biceps brachi, brachioradialis, pronetor theris, flexor digitorum, extensor digitorum communis, extensor carpi radialis, flexor carpi ulnaris, palmaris aponevrosis,
18	58	M	R	2	12	none	600	8	0	Romboid minor, pectoralis minor, biceps brachi, brachialis, pronetor theris, flexor digitorum, extensor digitorum communis, flexor carpi ulnaris,
19	77	M	L	1	19	none	450	6	0	poster part of the deltoid, pectoralis minor, pronetor theris, extensor digitorum communis, flexor carpi radialis,
20	59	M	R	2	5	BT	600	8	0	pectoralis minor, biceps brachi, brachialis, pronetor theris, flexor digitorum, extensor digitorum communis, flexor carpis radialis, palmaris aponevrosis
21	37	M	R	4	7	BT	600	8	0	Elevetor scapule, pectoralis minor, brachialis, pronetor theris, flexor digitorum, extensor digitorum communis, flexor carpi ulnaris, abductor pollicis, palmaris aponevrosis
m.v.	**42**	**15 M**	**15 R**		**38.95**		**500**	**6.67**	**0.33**	
S.D.	**21.98**	**6 F**	**6 L**		**38.79**		**98.74**	**1.32**	**1.53**	

Table 1: location and amount of medicine used in each single patient. BT= botulinum toxin injection. 1= ischemic stroke; 2=hemorrhagic stroke; 3= cancer; 4=Sturg Weber (hemicraniectomy for hemispherectomy)

The dose of hyaluronidase was determined according to the each patient's individual pattern and extent of spasticity, with the total dose not exceeding 600 IU and not more than 75 IU per single injection site in this cohort. The dosage of hyaluronidase selected for the patients were between four and eight times more than normal therapeutic dose, but still nontoxic based on the ratio of toxicity that is 200,000 to 1 [74]. The dilution was standardized: one vial (150 IU) was diluted with 1 ml

normal saline, thus each ml of injected fluid contained ~ 75 IU of hyaluronidase. This dilution was selected based on the rate of diffusion, that is proportionate to the amount of enzyme, and the rate of extent, that is proportionate to the volume of solution [75]. Prior to the procedure a preliminary skin test for hypersensitivity to recombinant Hyaluronidase was performed. An intradermal injection of approximately 0.02 mL (3 Units) of a 150 Unit/mL solution was injected. No erythema, itching or wheal were noted in and around the injection site at 5 or 20 minutes in any of the patient's injected. All injected areas were cleaned with chloroprep swabs prior to injecting. The injections were administered using sterile aseptic technique by a single operator with a 0.7 mm 30G needle. Surface anatomical landmarks [76] were used to guide the location of the muscles injected and precautions were taken to avoid intravascular injection. Multiple synergic muscles, in one or more spatial planes, were injected in the upper limb to address stiffness along the myofascial chain as shown in Fig. 3.

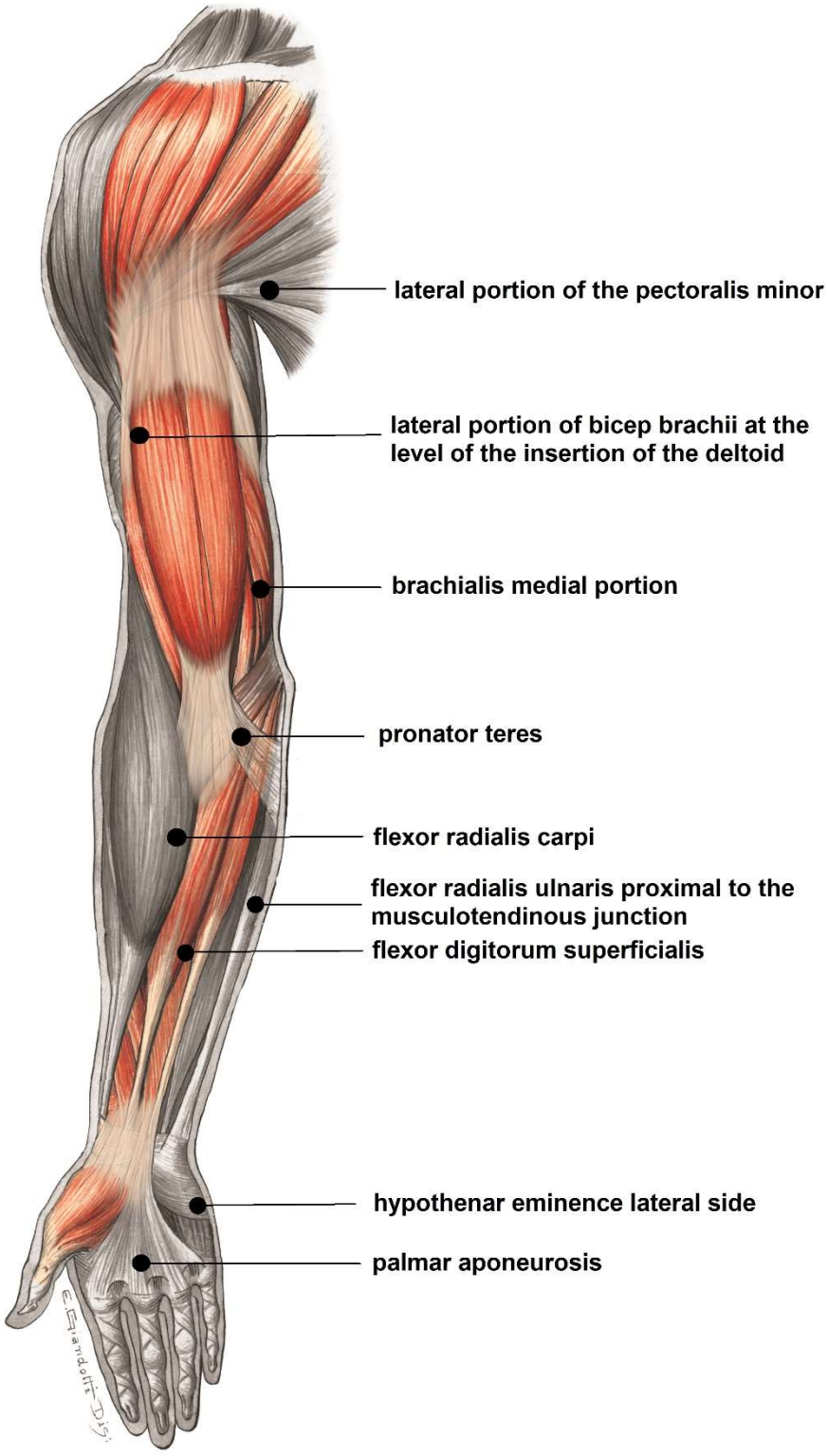

Fig 3: location of the injections

All the patients were evaluated pre-injection, and at three time points post-injection: between 3-10 days, 4-6 weeks and 12-15 weeks. Safety was assessed by examination for adverse events and clinically significant changes in vital signs (body temperature, diastolic and systolic pressure, heart rate) post-injection and over the period of follow-up for at least up to 15 weeks for this case series, and for longer in most cases. Adverse events were sought through interviews, during which patients were encouraged to report any problems. Each patient was assessed by the same treating physician at study entry and throughout the course of the study. All complaints were also assessed by an unbiased clinic nurse and documented in the medical record. Patients were allowed to continue with current therapy and encouraged to follow a home exercise program of stretching and passive movements of the upper limb joints to prevent further muscle stiffness. Compliance with the home exercise program was documented in the medical record.

Efficacy of treatment was assessed by change in spasticity on the MAS, and in passive and active ROM measured from video recordings. ROM of shoulder abduction and forearm pronation and supination (in the frontal plane) and shoulder forward flexion, elbow flexion and extension, and wrist flexion and extension (in the sagittal plane) were recorded with the camera placed perpendicular to the plane of the movement. Each movement was repeated three times passively, and when possible, actively. Angular excursions of the joints were extracted using commercially available video analysis software (Dartfish 7.0), which has been shown to be a viable and reliable method for quantitative clinical movement analysis with a reported interclass correlation coefficient (ICC) of 0.45 to 0.94 [77].

The regions of the injections were focused in relation at the limitation presented by the patients. For this reason, it was decided to focus the injection for restore ROM that was less than 85% of the normal range reported by American Association of Orthopedic Surgeon (AAOS). For the same reason the dates of the active and passive ROM that reach the 85% of the normal range at baseline were excluded from our statistical evaluation. For the passive ROM we increment the limit to 95% for elbow extension; 90% elbow flexion; 100% forearm pronation and supination and wrist flexion due

to the good ROM presented by most of the patients at baseline. The evaluation of the supination and pronation were performed from a frontal position with the camera at the level of the forearm, 20 cm from the hand. The landmark for evaluate the pronation and supination was the line between the head of the II and V metacarpal bone. The force of the operator was applied at the level of the wrist. This procedure has generated a bias increasing the axial rotation of the hand of the patients. For this reason it was decided to decrease of 15% all the values of the pronation and supination in all the evaluations.

Role of the funding source

There was no funding for this case series

Statistical analysis

Data analysis was performed by using the SPSS statistical package (version 20.0) for Windows. Motor tests were analyzed as change from baseline at 1, 4 and 12-15 weeks with Wilcoxon signed-rank test.

The changes at post-baseline visits were calculated for each assessment of motor function. Statistical testing was done to assess whether the absolute change and, where applicable, percentage change from baseline and each postoperative visits was significantly different from zero. The Wilcoxon signed-rank test was used to test whether changes in the clinical global impression of severity score and clinical global impression of improvement score at each postoperative visits were significantly different from baseline.

Efficacy was defined as a significant ($p<0.05$) reduction in spasticity from baseline.

Results:

All patients tolerated the injections without immediate adverse effects. None of the patients demonstrated a hypersensitivity reaction to the skin test. Patients 1-5 were followed daily for the first 3 days after the injections for close monitoring and to examine onset of therapeutic effect, if any. The

most common adverse reaction was soreness at the injection sites with onset 24 hours after the injection, lasting 24-48 hours. Sun exposure caused a mild rash in the injected arm on days 1-3 which disappeared spontaneously. Thereafter patients were advised to avoid direct sun-exposure for the first week after the injections.

Patients reported reduction in muscle stiffness as early as 2-3 days post-injection, once the post-injection soreness wore off. The stiffness reduced further over the subsequent weeks. Compliance with the home exercise and stretching program facilitated reduction in stiffness. All 21 patients showed clinically significant reduction in spasticity on the Modified Ashworth Scale in all the upper limb joints 1 week post-injection (T1) which persisted at 1 month (T2) and 3-month (T3) follow up (Table 2).

	SHOULDER							
	FLEXION				**ABDUCTION**			
	PRE (T0)	**POST (T1)**	**1st FU (T2)**	**2nd FU (T3)**	**PRE**	**POST**	**1 FOLL**	**2 FOLL**
mean value	2.41	0.82	0.65	0.56	2.47	0.79	0.63	0.56
SD (+/-)	0.80	0.73	0.70	0.73	0.77	0.79	0.76	0.78
P value		0.00	0.00	0.00		0.00	0.00	0.00
		T0-T1	T0-T2	T0-T3		T0-T1	T0-T2	T0-T3
	ELBOW							
	FLEXION				**EXTENSION**			
	PRE	**POST**	**1 FOLL**	**2 FOLL**	**PRE**	**POST**	**1 FOLL**	**2 FOLL**
mean value	2.29	0.64	0.67	0.60	2.31	1.05	1.02	0.98
SD (+/-)	0.56	0.76	0.84	0.80	0.51	0.65	0.78	0.77

P value		0.00	0.00	0.00		0.00	0.00	0.00
		T0-T1	T0-T2	T0-T3		T0-T1	T0-T2	T0-T3
	FORARM							
	PRONATION				**SUPINATION**			
	PRE	**POST**	**1 FOLL**	**2 FOLL**	**PRE**	**POST**	**1 FOLL**	**2 FOLL**
mean value	2.28	0.85	0.65	0.53	2.28	0.80	1.00	0.89
SD (+/-)	0.60	1.04	0.88	0.70	0.60	1.06	1.08	0.99
P value		0.00	0.00	0.00		0.00	0.00	0.00
		T0-T1	T0-T2	T0-T3		T0-T1	T0-T2	T0-T3
	WRIST							
	FLEXION				**EXTENSION**			
	PRE	**POST**	**1 FOLL**	**2 FOLL**	**PRE**	**POST**	**1 FOLL**	**2 FOLL**
mean value	2.35	0.47	0.47	0.38	2.41	0.65	0.91	0.97
SD (+/-)	0.49	0.62	0.72	0.62	0.51	0.79	0.87	0.94
P value		0.00	0.00	0.00		0.00	0.00	0.00
		T0-T1	T0-T2	T0-T3		T0-T1	T0-T2	T0-T3

Table 2: Modify Ashword Scale. (SD= standard deviation)

The mean passive range of motion increased for shoulder flexion, abduction and wrist extension at 1 week post-injection and persisted at approximately the same level at 1 and 3 month follow up. The increase in elbow flexion, forearm pronation and wrist flexion was more modest at 1 week post injection and regressed slightly over subsequent follow up. Elbow extension and forearm supination also increased, but much more variably across patients at 1 week post-injection but became statistically significant over subsequent follow up, especially at 3 months (Table 3).

	SHOULDER							
	FLEXION				**ABDUCTION**			
	PRE	**POST**	**1 FOLL**	**2 FOLL**	**PRE**	**POST**	**1 FOLL**	**2 FOLL**
mean value	114.44	129.50	131.00	126.72	114.61	134.50	141.22	135.78
SD (+/-)	25.34	28.36	26.67	26.96	28.80	33.75	34.50	29.46
p value		0.00	0.00	0.01		0.00	0.00	0.00
		T0-T1	**T0-T2**	**T0-T3**		**T0-T1**	**T0-T2**	**T0-T3**
	ELBOW							
	FLEXION				**EXTENSION**			
	PRE	**POST**	**1 FOLL**	**2 FOLL**	**PRE**	**POST**	**1 FOLL**	**2 FOLL**
mean value	128.1	133.5	132.3	130.6	161.89	168.33	167.11	168.56
SD (+/-)	6.45	7.88	10.34	10.82	12.98	15.63	14.61	14.78
p value		0.05	0.19	0.23		0.13	0.14	0.05
		T0-T1	**T0-T2**	**T0-T3**		**T0-T1**	**T0-T2**	**T0-T3**
	FORARM							
	PRONATION				**SUPINATION**			
	PRE	**POST**	**1 FOLL**	**2 FOLL**	**PRE**	**POST**	**1 FOLL**	**2 FOLL**
mean value	78.07	85.71	85.64	81.21	71.44	83.89	80.67	81.11
SD (+/-)	13.10	14.25	13.30	12.90	23.42	14.64	22.30	20.64
p value		0.01	0.03	0.46		0.10	0.05	0.02

		T0-T1	T0-T2	T0-T3		T0-T1	T0-T2	T0-T3
	WRIST							
	FLEXION				**EXTENSION**			
	PRE	**POST**	**1 FOLL**	**2 FOLL**	**PRE**	**POST**	**1 FOLL**	**2 FOLL**
mean value	67.86	76.57	75.36	74.43	39.12	55.35	53.71	55.71
SD (+/-)	12.85	14.63	17.31	16.36	12.37	20.07	16.55	17.12
p value		0.01	0.09	0.10		0.00	0.00	0.00
		T0-T1	**T0-T2**	**T0-T3**		**T0-T1**	**T0-T2**	**T0-T3**

Table 3: Passive Range of motion of the patients in degree. (SD= standard deviation)

The mean active range of motion showed more variability across patients, but increased significantly for shoulder flexion, shoulder abduction, elbow extension, forearm supination and wrist extension at 1 week post injection and the improvement was generally maintained at 3 months. The increase in wrist extension became statistically significant at 1 month rather than at 1 week and persisted at 3 months. Interestingly, active elbow flexion and forearm pronation showed the greatest amount of variability across patients which decreased over time, but did not show statistically significant change (Table 4).

	SHOULDER							
	FLEXION				**ABDUCTION**			
	PRE	**POST**	**1 FOLL**	**2 FOLL**	**PRE**	**POST**	**1 FOLL**	**2 FOLL**
mean value	75.21	99.21	94.71	101.71	88.80	102.53	101.67	105.73

SD (+/-)	51.04	39.66	54.32	48.01	30.11	33.05	38.15	37.12
p value		0.006	0.005	0.004		0.032	0.070	0.062
		T0-T1	**T0-T2**	**T0-T3**		**T0-T1**	**T0-T2**	**T0-T3**
	ELBOW							
	FLEXION				**EXTENSION**			
	PRE	**POST**	**1 FOLL**	**2 FOLL**	**PRE**	**POST**	**1 FOLL**	**2 FOLL**
mean value	125.00	129.90	131.00	129.60	124.43	141.57	144.86	146.57
SD (+/-)	9.39	13.49	12.87	8.47	30.07	26.71	29.27	28.33
p value		0.230	0.259	0.273		0.047	0.086	0.094
		T0-T1	**T0-T2**	**T0-T3**		**T0-T1**	**T0-T2**	**T0-T3**
	FORARM							
	PRONATION				**SUPINATION**			
	PRE	**POST**	**1 FOLL**	**2 FOLL**	**PRE**	**POST**	**1 FOLL**	**2 FOLL**
mean value	43.27	46.33	46.47	53.27	22.93	35.00	41.36	35.43
SD (+/-)	31.05	41.98	36.05	18.61	45.98	45.20	43.54	46.66
p value		0.660	0.610	0.254		0.022	0.011	0.005
		T0-T1	**T0-T2**	**T0-T3**		**T0-T1**	**T0-T2**	**T0-T3**
	WRIST							
	FLEXION				**EXTENSION**			
	PRE	**POST**	**1 FOLL**	**2 FOLL**	**PRE**	**POST**	**1 FOLL**	**2 FOLL**
mean value	32.71	41.79	47.21	48.64	-0.07	13.50	16.36	14.50
SD (+/-)	25.09	23.81	18.72	21.95	29.42	24.73	25.52	25.71
p value		0.036	0.004	0.046		0.076	0.038	0.056
		T0-T1	**T0-T2**	**T0-T3**		**T0-T1**	**T0-T2**	**T0-T3**

Table 4: Active Range of motion of the patients in degree. (SD= standard deviation)

Discussion:

In this case series, 21 patients with moderate-to-severe chronic upper limb spasticity received multiple intramuscular human recombinant hyaluronidase-saline injections and were followed-up for 12-15 weeks. The injections were found to be safe and well tolerated. Spasticity was significantly reduced across all the joints in the entire upper limb and the improvement was maintained throughout the follow up period. Although there were significant inter-individual differences, significant improvements in passive range of motion, and remarkably also in active range of motion, were noted at several joints which were maintained over the follow up period. These results provide preliminary evidence in support of the hyaluronan hypothesis of spasticity.

The long lasting decrease of the HA viscosity cannot be related at the single effect of the drug due to it short effect. Indeed Hechter [78] found that the natural HA barrier was partly restored in twenty-four hours and completely restored in forty-eight [79]. At the opposite our results show an improvement of the ROM at 48 hours and later. We suppose that a normalization of the viscosity of the deep fascia and perimysium, that surround the muscle spindle, should restore the normal activation of the primary and secondary sensory fibers, decreasing the activation of the alfa motor neuron. We also believe that this decrease of the muscle tone, following the normalization of the activity of the muscle spindle, should not last so long without the contemporary treatment of the synergic and antagonist muscle belly. It is postulated that the tension generated by the hyper tonicity of the primary spastic muscle can affect, thought the "myofascial expansion" [80], synergic mono or biarticular muscle in the same segment, or in the proximal / distal segments. In our previous publication [81] it was proposed that, from an active muscle stiffness, generated by the CNS lesion, we can have a new neurally mediated reflex stiffness that can involve muscles that perceive the tension generated thought the "myofascial expansion". Overall Kaper DG [45] showed that a voluntary movement stretching a spastic muscle may produce a reflex in the antagonistic muscle that

would oppose the voluntary movement. This constant overuse activity of the antagonist muscles could perpetuate the activation of specific cell "fasciacytis", localized in the deep fascia as well in all the connective tissue [46], that produce HA. This excessive production can generate retention of the hyaluronic acid [47] in the connective tissue. Matteini P [48] has demonstrated that, by increasing the concentration of HA in a spread surface, HA can self-associate generating a dramatically increased of the viscoelasticity. The presence of higher viscosity extracellular matrix in a distal or proximal area, respect the primary spastic muscle, can altering the perimysium as well as the muscle spindles capsule that modulate the anulospiral ring. This impairment of the anulospiral ring could higher activate the afferent “Ia” fibers and then the alfa motoneurons with consequent spasticity in the corresponding motor unit. This cascade can “spread” the muscle stiffness or exacerbate the spasticity in patients also after months or years from the central lesion.

We want to underline the importance of the location of the injections that should select muscles localized in different body segments that work in synergy or antagonism. The successful results of this study support our methodology that targets the restoration of the biomechanical of the full upper limb. The locations of the injections are also important due to the limit capacity of penetration of the Hyaluronidase. Indeed it can penetrate a fibrin barrier only if injected into it; otherwise, the drug diffuses around it and follows the path of least resistance [78]. It is important to know that the fascial planes and periostium are barrier to solutions containing hyaluronidase [73]. For this reason it is required a specific knowledge of the fascial planes to perform properly the injections.

Study limitations:

The limitations of this study are the possible effects of confounding variables. Another limitation is the small number of patients and the lack of a control group. For this reason we cannot be certain that the differences observed can be attributed solely to the effects of the hyaluronidase. Any suggestion of possible efficacy on some of the motor measures, that were previously outlined, could be spurious and indicate a placebo response, investigator bias, or other factors.

The evaluation of the active movements have showed a moderate intra-operator rehabilitee due to the vicious patter of movements that the patients made during the evaluation. Since the movements were performed according to the participant preferences, we did not control how tasks were performed; thus, some variation in motion requirements were present.

Improvements were also seen in other secondary outcome measures, such as activities of daily living. Most of these changes were clinically significant, and they might have important day-to-day benefits for patients. Nevertheless, caution must be exercised when interpreting the preliminary findings from this unblinded and uncontrolled study. Several treatments for spasticity that have appeared promising in open-label studies have failed to show efficacy under more rigorous controlled and blinded conditions. For this reason further testing in greater numbers of patients and for longer is required.

Conclusion:

This open-label study provides preliminary evidence for the safety and potential symptomatic efficacy of hyaluronidase as a treatment for spasticity. If the purpose in relieving spasticity is to improve physical functioning and ultimately quality of life [83], hyaluronidase treatment will allow the decreasing of the spasticity without affect the functionality of the muscles. These results are very encouraging for develop a new treatment that allow the patients to improve the active movements. Unfortunately the medication by itself it is not efficacy if not injected in the correct points. The selection has to be performed through an accurate evaluation of the body following specific biomechanical criteria.

Despite the limitations of our study, the data provide some insights for discussion and provide a justification for future research. A larger, prospective, controlled, double-blind, clinical trial is needed to assess further the findings from this preliminary study. Although we need to confirm these associations in a larger trial, disease severity and, specifically, the presence botulinum toxin

unresponsive features might need to be taken into account when the entry criteria for patients are defined in future studies.

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Declaration of Interest

All the authors disclose all forms of financial support, both monetary and equipment. The author has developed a patent in conjunction with the New York University.