Assessment of a New Hyaluronic Acid Filler. Double-blind, Randomized, Comparative Study between Puragen and Captique in the Treatment of Nasolabial Folds

MARIAGIUSEPPINA ONESTI, MARCO TOSCANI, GIUSEPPE CURINGA, STEFANO CHIUMMARIELLO and NICOLÒ SCUDERI

Department of Plastic and Reconstructive Surgery, University of Rome “La Sapienza”, Rome, Italy

Abstract. Fillers represent a field of aesthetic medicine under remarkable expansion. Over the past few years, in the USA, there has been a huge increase in the use of fillers, especially for hyaluronic acid (400% in 2004). The causes of this increase have been the greater tolerability of this reabsorbable filler with respect to the others, and its prolonged efficacy in time due to chemical modifications of its molecular structure. In our study, we report the results of a double-blind comparative study between Puragen (latest-generation hyaluronic acid with double cross-linking) and Captique (second generation hyaluronic acid with single cross-linking), in the treatment of nasolabial folds. Each patient received Puragen in one nasolabial fold and Captique in the contralateral fold, at random. Clinical efficacy was assessed independently by the investigator and the patient 2, 4 and 6 months after baseline or when the optimal cosmetic result was obtained. The tolerability assessment was made by the patient (using a daily diary to record any adverse events) for 2 weeks after each treatment, and by the operator 2, 4, and 6 months after baseline. Sixty-eight patients completed follow up at 6 months. From the results obtained in this study, Puragen remained stably in the treated tissues even after 6 months while less satisfactory results were obtained with Captique.

The term “filler”, in the field of plastic surgery and aesthetic medicine, refers to the vast and heterogeneous group of substances that can be applied by various injection techniques to fill wrinkles and skin sag for aesthetic and curative purposes. Over the past few years, there has been an unstoppable spread of these products in the field of plastic surgery, due to the ever-growing need for techniques that ensure high efficacy but minimum invasiveness. To eliminate blemishes caused by age, fillers currently represent a coded method. They are ever-increasingly refined and designed to meet the many needs in the field of plastic surgery, their use has increased by 25% over the past year and their growth since the year 2000 has been estimated at about 200%. The use of fillers is also indicated for a certain number of diseases in which they play a functional and curative, as well as an aesthetic role. In fact, they are used to correct inherited or traumatic defects of the soft tissues of the face, to cure patients suffering from scleroderma, progressive facial hemiatrophy, facial paralysis and finally, to treat patients suffering from lipodystrophy following treatment with antiretroviral drugs (1). Other indications for their use are represented by unilateral paralysis of the vocal cords, increase in size of the lips and soft palate in patients with labiopalatine cleft, anophthalmia and enophthalmos, and penis augmentation (2-4). The greatest demand for fillers, however, comes from patients with defects or disorders due to skin aging and/or photoaging (5). They are thus used to fill the lacrimal canaliculus/pit, remodel the shape and raise the tip of the nose, fill nasolabial folds and labial commissures, fill the cheeks and raise the zygomas, remodel the mandibular profile, rejuvenate the neck, increase and/or underline the profile and the labial contour. Numerous clinical results and studies conducted on animals have demonstrated a short and long-term efficacy according to the chemical structure and the surface characteristics of the microparticles making up the skin fillers. On the market there are various types of fillers that may be classified as reabsorbable (with temporary effect) and non-reabsorbable (with semi-permanent and permanent effect). Research is permanently oriented towards the discovery of new products that reach the “ideal filler” objective. Today, those that come the closest are those based on hyaluronic acid.

Hyaluronic acid (HA) was isolated for the first time in 1934 by Meyer and Palmer from the vitreous body of the eye of a cow (6). They found a substance containing two saccharidic fractions, one of which was HA.
HA may be defined as a glycosaminoglycan with an unbranched polysaccharide chain produced by the aggregation of thousands of disaccharidic units made up in turn of glucuronic acid residues (a glucose derivative) and N-acetyl glucosamine. In vivo all carboxyl groups of glucoronic acid and N-acetyl-glucosamine are completely ionized giving the hyaluronic acid molecule high polarity and, consequently, high solubility in water. Due to this property, HA can form complexes with numerous molecules of water reaching a high degree of hydration. In the amorphous matrix of a connective tissue, HA therefore maintains the degree of hydration, turgidity, plasticity and viscosity. It can also act as a binding agent, an anti-shock molecule and an efficient lubricant, preventing the damage caused to tissue cells by physical stress. The extremely long structure of the molecule together with its high degree of hydration enables several HA polymers to form a particular structure with the following two main functions: to create a molecular framework that maintains the form and tone of the tissue; and to function as a filter against the dispersion of particular substances, bacteria and infectants in the tissue. Only substances with a sufficiently low molecular weight to pass through the gaps in this mesh can spread freely in the tissue, all substances with a higher molecular weight such as bacteria or viruses will remain trapped in the mesh. It should be noted that many bacteria have hyaluronidase (an enzyme that breaks up HA) and can open a gap for themselves. In its natural form, HA is broken up and reabsorbed quickly due to the action of hyaluronidase and free radicals. In the past, this has considerably limited its use in all medical and specialist applications that require a more lasting effect such as the correction of dermal and subdermal tissue defects, such as wrinkles and scars, and to augment the soft tissues of the face.

The aim of this study was to assess a new-generation HA (Puragen™), in comparison with an old-generation HA (Captique™). Puragen™ is a hydrogel of non-animal origin, with double cross linking. These ultrastructural characteristics increase the resistance of HA to breakdown by enzymes and free radicals (7). Captique™ is a HA of non-animal origin, with single cross-linking.

History and evolution of the concept of filler. In the seventies, when aesthetic plastic surgery was only available to the elite or, in any case, represented a niche, fillers began to constitute an alternative to surgery or an integration of it and the leading pharmaceutical companies began to operate in this sector, which was destined to give huge profits, the current level of which could not even have been imagined. The collagen introduced in the mid seventies represented a turning point in this sector in that it put on the market a potentially safe product that could compete with the only true leader of fillers: silicone (8-10). At the time, this substance represented, for the doctors who used it, the only product that could eliminate blemishes and achieve the desired augmentation in a short time and at limited management costs. The same applies for patients who saw their expectations fulfilled immediately and could be sure that they would obtain a long-lasting result.

Passing through the success of silicone (which represented the only solution) and collagen, in time, we have witnessed the invention of an increasing number of fillers which, in one way or another, contributed to the growth of this market, as they were aimed at an increasingly broad public. In the 80-90s, the use of fillers spread at a breathtaking pace. The attempts to obtain a molecular compound capable of ensuring a long-lasting improvement stable in time led to the spread of fillers of a disputable nature and chemical composition with quite disastrous results. During the same period, silicone was taken off the market (not in all countries). Even today, however, silicone can still be purchased in some European countries.

The attention from the media and doctors and scientists’ awareness of the many cases of “malpractice” attributed to the inappropriate use of the numerous fillers available contributed to the elaboration of a new concept of fillers (10, 11). From a long-lasting result stable in time at all costs, the objective has become a treatment that guarantees safety, efficacy and, where possible, a long-lasting effect (12). Reabsorbable fillers (mainly HA and collagen) have thus grown at a remarkably rapid rate on the international scene (13-16). Therapeutic procedures such as lipofilling may, in some cases, be likened to a genuine “autologous filler”, well tolerated by patients and giving unexpected aesthetic results when compared with traditional fillers (17-21).

Biostimulation and skin revitalization are other concepts that have developed over the past few years, finding their rationale in the injection of substances that nourish and regenerate the layers of the skin (22, 23).

The latest novelty in the vast field of fillers is the use of autologous fibroblasts for eliminating dermal defects (acne scars and wrinkles) (23).

In countertendency, “silicone” returns with a vengeance in the science magazines. The authors sustain with numerous reasons that silicone’s “failure” as an “ideal filler” was due to methodological and production errors (failure to purify the particles). The “love-hate” relationship, as the authors sustain, could bring the historical product back onto the market as a long-lasting “ideal” filler (24-26). According to the experience gained over several decades at the Department of Reconstructive and Plastic Surgery, this hypothesis could take us back in time with very high risks for patients and operators (10).

From the aforesaid considerations, it clearly emerges how the complex world of fillers is a topical subject of debate from many points of view and, above all, at an important crossroads of its growth.
Materials and Methods

Materials. Puragen (Mentor Corporation), is a transparent gel of non-animal origin, based on hyaluronic acid with double cross-linking, made up of particles having a diameter of 240 μm. Captique (Inamed) is a stabilized and cross-linked hyaluronic acid gel (5.5 mg/mL), with particles having a diameter of 500 μm. Both biomaterials are contained in a 1.0 mL syringe with a 30-gauge needle in sterile form.

Patient selection and clinical study. This double-blind patient-investigator randomized study was conducted at the Department of Dermatology and Plastic Surgery of the “La Sapienza” University of Rome. The criteria for inclusion in the study were men and women aged between 25 and 80 years with marked nasolabial folds. All patients recruited on the study were duly informed by the operator, who had them sign the specific informed consent form. The study did not include patients who needed repair of the soft tissues following traumas, facial asymmetries, patients with dermatological problems, systemic diseases (diabetes mellitus, coagulation disorders, connective tissue diseases), patients under immunosuppressive treatment, with reported sensitivity to HA, alcoholics, drug addicts or pregnant women. Patients subjected previously to treatment with fillers were also excluded.

The study was conducted in accordance with the principles of the Helsinki Declaration, and the ICH (International Conference of Harmonization) and GCP (Good Clinical Practice) international guidelines. Each patient received Puragen in one nasolabial fold and Captique in the contralateral fold, in a totally random fashion. To assess clinical efficacy, an investigator worked alongside the operator performing the injections, who did not know the name of the product injected. The treatment was performed with the patient keeping his eyes closed, so that he could not recognize the product.

The patients were assessed 2 weeks after the initial injection implant of Puragen and Captique. If the correction was suboptimal, treatment was repeated with the same product used previously and the patient was reassessed after another 2 weeks, until the “optimal cosmetic result” was obtained; in this way, a baseline of the study was established, following the patients over a period of 6 months.

Injection technique. Use was made of local analgesics (ice, topical anaesthetics for local injection, and/or with 1% lidocaine block) chosen by the operator according to the patients to be treated. The injection implant method used by the operator was linear, serial or in combination. The depth of injection and the volume to be injected were at the operator’s discretion. The skin defects to be treated were filled but not overcorrected. Having completed treatment, a hand massage was performed at the injection site to aid the distribution of the material injected in the adjacent tissues.

Assessment. Clinical efficacy was assessed independently by the investigator and the patient 2, 4 and 6 months after baseline. The tolerability assessment was made by the patient (using a daily diary to record any adverse events) 2 weeks after each treatment, and by the operator 2, 4 and 6 months after baseline.

The efficacy of the treatments was assessed using the Wrinkle Severity Rating Scale (WSRS) and the Global Aesthetic Improvement Scale (GAIS). The WSRS is a scale used to quantify the results obtained in treating nasolabial folds, on the basis of photographic images. The scores for evaluating the nasolabial folds were given according to the length and depth of the folds without reference to pretreatment pictures or baseline (Table I). The GAIS scale assesses the improvement obtained (Global Aesthetic Improvement Scale: Worse, No change, Improved, Much improved and Very much improved) in each fold at baseline and during the follow-up, comparing the results with the pretreatment photographs (Table II).

The main assessment was made by the investigator using the WSRS 6 months after baseline; secondary assessments were made by the investigator and the patient 2 and 4 months later with the WSRS, and 2, 4 and 6 months after baseline the assessment was completed with the GAIS scale.

Statistical analysis. The WSRS (pretreatment) ratings obtained with Puragen and Captique were compared using Mc Nemar’s test. The variable categories (“Puragen is superior to Captique”, “Puragen is equivalent to Captique”, “Captique is superior to Puragen”) were based on the WSRS and GAIS ratings and were expressed as a frequency. A probability of less than 5% was considered statistically significant.
Results

Of the 84 patients initially selected for the study, 74 (70 women and 4 men) continued the comparative study. The average age was 50.2 years. Of this population 68 patients completed 6 months of follow-up; 6 patients withdrew prematurely from the study, 3 because of protocol violation and 3 because of loss to follow-up.

Efficacy. Prior to treatment, the nasolabial folds were assessed (Wrinkle Severity Rating Scale) by the investigator as mild (5%), moderate (50%), severe (35%) or extreme (15%). On achievement of the “optimal cosmetic result” (baseline), no differences were noted between the sides treated with Puragen and those treated with Captique, and most patients improved their result on the WSRS scale by one or two grades, with a larger percentage of patients without folds or with “mild” folds (Figure 1).

Puragen proved significantly superior to Captique in the WSRS rating at all post-baseline check-ups (p<0.05). At six months post-baseline, Puragen was superior to Captique in 60.4% of cases, and Captique proved superior to Puragen in 5.8% of cases (p<0.05) (Table III). According to the GAIS scale ratings, Puragen obtained significantly higher ratings than Captique after baseline (p<0.05). At six months post-baseline Puragen was superior to Captique in 75% of patients, and Captique superior to Puragen in 5.8% (Table IV). The efficacy assessment of the treatments undergone by patients gave equivalent results to those obtained by the investigator (Table V and Table VI).

The number of sessions required to obtain the optimal cosmetic result ranged from 1 to 3 treatments (for an average of 1.5 for both products) and did not reveal any significant differences between the two products.

Pre- and post-treatment photographs with Captique and Puragen are represented in Figures 2 and 3.

Tolerability. After the initial treatment, the adverse reactions at the injection point (recorded by the patients in a diary) were 92% and 90.3% for Puragen and Captique, respectively, mainly of a mild to moderate intensity and short-lived (less than 5 days). The most frequent symptoms for both products were swelling, redness, itching, pain and hardening. The incidence of adverse reactions was less in subsequent sessions (touch-up) with respect to the initial injection implants. During the 6-month follow-up, adverse reactions were observed exclusively at the injection site in 14.7% with Puragen and 11.8% with Captique, in most cases represented by swelling and redness (reactions of mild to moderate intensity).

Complications arising 14 days after the last treatment had a similar incidence between the two products (Puragen 3 cases, Captique 2 cases) (Figure 4); none of these reactions were considered by the investigator as a hypersensitivity response to the implant. All these delayed-onset complications subsided spontaneously within a period of 2 months of treatment.

Discussion

The results of this double-blind, randomized, comparative study have established that both products used, Puragen and Captique are equally effective and safe in eliminating nasolabial folds. The difference between the two products was observed in time, in that the biochemical and structural characteristics of Puragen enabled more long-lasting and stable results to be obtained.

Differences in the behaviour of HAs having different structures have been demonstrated, in vitro, by several authors (7, 27). The first HA based fillers created with a filling function, known as “first generation” fillers, are obtained from two main sources: bacterial fermentation – some species of streptococcus produce HA or extraction from animal tissues, cockerel crests and other animal sources used previously.

The long-lasting effect of the HA depends on 3 factors: Concentration: the higher the concentration of the HA in the filler, the longer-lasting its effect is. Size: the larger the particles of HA, the longer-lasting its effect is. Cross-linking: the greater the cross linking between the HA molecules, the longer-lasting its effect is. The first two factors could also be varied in 2nd generation fillers. The novelty introduced by 2nd generation fillers concerns cross-linking. The functional groups for cross-linking in these fillers are the hydroxyl...
Table III. Investigator’s assessment on the WSRS scale during follow-up: results in three categories (number of patients and frequency).

<table>
<thead>
<tr>
<th></th>
<th>Puragen is superior to Captique</th>
<th>Puragen is equivalent to Captique</th>
<th>Captique is superior to Puragen</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 months</td>
<td>23 (33.8)</td>
<td>40 (58.8)</td>
<td>5 (7.3)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>4 months</td>
<td>35 (51.4)</td>
<td>18 (26.4)</td>
<td>15 (22.1)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>6 months</td>
<td>41 (60.4)</td>
<td>23 (33.8)</td>
<td>4 (5.8)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table IV. Investigator’s assessment on the GAIS scale after baseline (optimal cosmetic result): results in three categories (number of patients and frequency).

<table>
<thead>
<tr>
<th></th>
<th>Puragen is superior to Captique</th>
<th>Puragen is equivalent to Captique</th>
<th>Captique is superior to Puragen</th>
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</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>4 (5.8)</td>
<td>58 (85.3)</td>
<td>6 (8.8)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>2 months</td>
<td>16 (23.5)</td>
<td>47 (69.1)</td>
<td>5 (7.3)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>4 months</td>
<td>45 (66.2)</td>
<td>18 (26.5)</td>
<td>5 (7.3)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>6 months</td>
<td>51 (75)</td>
<td>13 (19.2)</td>
<td>4 (5.8)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
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Table V. Patient’s assessment on the WSRS scale during follow-up: results in three categories (number of patients and frequency).

<table>
<thead>
<tr>
<th></th>
<th>Puragen is superior to Captique</th>
<th>Puragen is equivalent to Captique</th>
<th>Captique is superior to Puragen</th>
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</tr>
</thead>
<tbody>
<tr>
<td>2 months</td>
<td>6 (8.8)</td>
<td>50 (73.5)</td>
<td>10 (14.7)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>4 months</td>
<td>20 (29.4)</td>
<td>40 (58.9)</td>
<td>8 (11.7)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>6 months</td>
<td>43 (63.2)</td>
<td>22 (32.3)</td>
<td>3 (4.4)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table VI. Patient’s assessment on the GAIS scale after baseline (optimal cosmetic result): results in three categories (number of patients and frequency).

<table>
<thead>
<tr>
<th></th>
<th>Puragen is superior to Captique</th>
<th>Puragen is equivalent to Captique</th>
<th>Captique is superior to Puragen</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>6 (8.8)</td>
<td>54 (79.4)</td>
<td>8 (11.8)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>2 months</td>
<td>29 (42.6)</td>
<td>33 (48.5)</td>
<td>6 (8.8)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>4 months</td>
<td>43 (63.2)</td>
<td>19 (28)</td>
<td>6 (8.8)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>6 months</td>
<td>45 (66.1)</td>
<td>19 (28)</td>
<td>4 (5.8)</td>
<td>&lt;0.05</td>
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</tbody>
</table>

In fact, all technologies of this generation of HA (Single-cross-linked) use cross-linked agents to obtain ether bonds (BDDE 1,4- butandiol diglycdyl ether) or ester bonds (DVS divinylsulphone). Breakdown by hyaluronidase is much slower than in 1st generation fillers: in fact, studies conducted on biodegradation times indicate a period ranging from a minimum of 15 to a maximum of 30 days. Viscosity is one of the least analysed rheological properties (7, 27).

Studies show that a 2nd generation HA with a viscosity of 20% has the same hyaluronidase biodegradation time as a natural HA (7). This is very important because if the viscosity of the 2nd generation HA is raised to the level of the natural acid, the half-life obtained quadruples: in other words, if natural HA has a half-life of between 2 and 4 days, this method lengthens the half-life of 2nd generation hyaluronic acid up to 16 days (7, 27). The 2nd generation HAs, defined precursors, have given rise to the 3rd generation HA, which introduces a major chemical and structural innovation represented by the presence of a new and unique feature: a second cross link, in which the particles form an insoluble network of hydrophilic polymers with two binding sites (DXL), one hydroxyl and one carboxyl, leading to the formation of two stable bridges, one ether and one ester. The introduction of the second cross
Figure 2. Clinical case 6 months after treatment (treated with Captique).

Figure 3. Clinical case 6 months after treatment (treated with Puragen).

Figure 4. Complications 1 month after treatment (on left Puragen, on right Captique).
link has led to major differences between the 3rd generation HA (Puragen) and the previous ones. The substantial differences are to be found in the physicochemical properties, biodegradation and stability. The ultrastructural modifications obtained through the presence of an ether (hydroxyl) and ester (carboxyl) double cross link, in place of the single ether link (BDDE or DVS) between two hydroxyl groups present in the 2nd generation HAs, change the rheological properties of the compound. In addition to a higher resistance to biodegradation, this gives the molecule hydrophilic and hydrophobic capabilities very useful in its relations with the other substances present in the extracellular matrix.

The first of the parameters that the operating doctor must take into consideration when choosing a filler is the safety of the product to be used. Reabsorbable fillers guarantee maximum safety. This experimental study is based on the use and composition of HA in various formulations for the aesthetic treatment of a large sample of patients. The differences between 2nd generation HA (Captique), and Puragen, whose particular structural feature is its double cross-linking making the molecule more stable and long-lasting, were assessed.

The results obtained have enabled the establishment of the advantages that Puragen presents with respect to the HA based products used up to now.

From the results obtained in this study, Puragen remained stably in the treated tissues even after 6 months while less satisfactory results were obtained with 2nd generation HA (Captique). The safety of the product is thus ascertained, taking the lack of significant side effects after 6 months into account.

The search for the ideal filler is, in our opinion, at a turning point due to the positive and negative experiences gained over the past thirty years. Irrespective of the filler chosen, the approach to the patient who is to undergo treatment with a filler must be as scrupulous as with a surgical operation.

Detailed and exhaustive information about the product, its limits and possible complications should therefore be given, complete informed consent should be obtained and, above all, it should be remembered that the use of these methods requires an adequate level of experience and specialist professional training.

References


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