Hyaluronic Acid Fillers, Needle Contamination by Fastidious Microorganisms, and Risk of Complications

Maurizio Cavallini, MD,* Andrea Casasco, MD, PhD,†‡ Fulvio Ferrara, MD,§ Mauro Raichi, MD,‖ and Giuseppina Spinelli, MSc¶

BACKGROUND Complications are becoming ever more common with the increased use of hyaluronic acid (HA) fillers in aesthetic medicine. Complications due to needle contamination with fastidious microorganisms are no exception.

OBJECTIVE To perform, in a top Italian aesthetic medicine facility, what the authors think is the first monitoring program of microbial needle contamination of cross-linked HA gel fillers after the prefilled syringes with gel residues were stored for retouches after the first aesthetic procedure.

METHODS Needle contamination monitoring study, performed between January and November 2019, on 35 needles (caliber, 30 and 27 G) stored at 4°C in their resealed filler packages for possible retouch after a first aesthetic treatment involving the middle and lower facial thirds. Women's age: 35 to 70 years old.

RESULTS The search for contaminating agents of the 3 monitored bacterial contaminants (*Staphylococcus aureus*, *Streptococcus pyogenes*, and anaerobes) as well as yeasts and molds always tested negative. In the days and months after treatment, no patients in post-treatment controls showed evidence of infection in the treated areas.

CONCLUSION The observational retrospective study confirms that good storage conditions, including monitored refrigeration, avoid the risk of contamination of partially used HA gel fillers by fastidious microorganisms.

For years, fillers based on cross-linked hyaluronic acid (HA) have been the most frequently used worldwide aesthetic medicine to correct wrinkles, facial profiles, and volumes. Persistent biophysical stability in tissues, low immunogenicity, and prompt reversion of effects with hyaluronidase are advantages over other filling materials that contributed to the success of HA gel fillers.[1,2] According to the authoritative Fortune Business Insights, "*the global HA-based dermal fillers market size was valued at USD 2,680.9 Million in 2018 and is projected to reach USD 4,884.6 Million by 2026, exhibiting a compound annual growth rate of 7.8%*".[3]

With the increase in HA procedures, complications have increased—vascular problems in most cases. Examples are the compression or embolization of the supratrochlear and supraorbital terminal branches of the ophthalmic artery, possibly leading to blindness and emergency salvage treatment; more occasionally, the facial artery, near the distal margin of the zygomatic major muscle, is involved.[4,5] Regarding herpes simplex and other viral and bacterial complications, poor preinjection skin cleansing and the failure to use gloves and needle tip contamination, possibly after repeated facial injections, qualify as risk factors. Early or late reactive nodules are also possible.[6–8]

This study, performed in a top-tier Italian aesthetic medicine clinic, is presumably the first one reporting on a microbiological monitoring program of needle contamination from cross-linked HA gel syringes with gel residues, stored for retouches after the first use for aesthetic corrections of the face.

Methods and Materials

The needle contamination monitoring study, performed between January and November 2019 at the Dermatosurgery and Aesthetic Medicine Service (surgical outpatient clinic) at Centro Diagnostico Italiano in Milan (Italy), involved 35 thirteen-mm needles (caliber, 30 and 27 G) stored in their resealed filler packages with partially used Vycross technology 17.5 to 25-mg cross-linked HA gel fillers (Allergan Aesthetics, Rome, Italy). Storage of the resealed filler packages was in refrigerated vaults at 4°C.

Description of the Original and Touch-Up Procedures

The preinjection preparatory protocol was always the same: undressing; wearing of protective gown, shoes, and cap; and anamnesis. All women who ended up in the observational cohort, aged between 35 and 70 years, self-certified as nonpregnant, without ongoing anticoagulant therapy, local or systemic infections, or a recent history of dental

*From the *Dermatology and Dermatosurgery Department, CDI Centro Diagnostico Italiano, Milan, Italy; † CDI Centro Diagnostico Italiano, Milan, Italy; ‡ Department of Public Health and Experimental and Forensic Medicine, University of Pavia, Pavia, Italy; § Laboratory Medicine Department, CDI Centro Diagnostico Italiano, Milan, Italy; ‖ Clinical Pharmacology Consultant, Milan, Italy; ¶ Laboratory Medicine, Medical Genetics and Pathology Department, Clinical Microbiology Section, CDI Centro Diagnostico Italiano, Milan, Italy*

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Address correspondence and reprint requests to: Maurizio Cavallini, MD, Servizio di Dermatochirurgia e Medicina Estetica, Centro Diagnostico Italiano, Via Saint Bon 20, 20147 Milan, Italy, or e-mail: maurizio.cavallini@libero.it

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TABLE 1. Looking for Microbiological Contamination of Needles of Partially Used Cross-Linked HA Fillers Stored for Follow-Up Maintenance Treatments at the Dermatosurgery and Aesthetic Medicine Service, Centro Diagnostico Italiano, Milan, Italy: Methodological Details

Medium	Incubation	Incubation Period
Blood agar	Anaerobiosis	24–48 h
MacConkey agar	5% CO_2, 37°C	24–48 h
Sabouraud agar	5% CO_2, 37°C	24–48 h

HA, hyaluronic acid.

treatments or viral infections. No woman had a history of autoimmune skin diseases.

The indications for the initial treatments involving the middle and lower facial thirds were:

- Deep volumetric filling above the cheek's periosteum and medial fat compartments (treatment: 20-mg/mL cross-linked HA; mean injected total volume, 3 mL).
- Subcutaneous filling of wrinkles in the nasogenic folds and labial and perioral areas (treatment: 17.5-mg/mL cross-linked HA; mean injected total volume, 2 mL).
- Deep subcutaneous or supraperiosteal filling to correct mandibular profiles and hypoplasia (treatment: 25-mg/mL cross-linked HA; mean injected total volume, 2 mL).

After make-up removal, the patients underwent 30-second skin cleansing with an antiseptic solution (0.5% chlorhexidine and 96% ethyl alcohol in purified water solution). The operator performed all manoeuvers using nonsterile latex gloves during all preinjection and post-injection procedures. The number of injections per face was between 3 and 5, with a needle for each 1-mL vial.

After the initial treatment, a new sterile needle replaced the used needle; an identification tag newly applied to the resealed filler packages stated the patient's name and date of birth. If some HA remained after the touch-up session, the syringe was again stored with a new sterile needle. A periodic review of the log of stored HA syringes helped to discard syringes older than 1 year or that had reached the expiration date.

Before each touch-up session, a swab of the needles (eSwab collection and transport technology, Copan Italia SpA, Brescia, Italy) was sent to the internal laboratory for automated seeding and broad-range search for contaminating microorganisms (WASP automated microbiology specimen processing technology, Biomérieux, Grassina, Italy) with utmost care for secondary contaminations. Special care was devoted to looking for the following typical skin flora and environmental contaminants:

- *Staphylococcus aureus*
- *Streptococcus pyogenes*
- *Anaerobic bacteria*
- Yeasts (e.g., *Candida* spp.) and molds (e.g., dermatophytes and *Aspergillus* spp.)

Seeding media: blood agar (general purpose medium and discrimination of hemolytic properties), MacConkey agar (selective medium for Gram-negative, lactose-positive and lactose-negative *Enterobacteriaceae*), Schaedler blood Agar (selective medium used for isolating and cultivating anaerobic species), and Sabouraud agar (yeasts and molds).

Results

The search for contaminating agents of the 3 fastidious targets always tested negative (Table 1). In the days and months after treatment, no patients in post-treatment controls showed evidence of infection in the treated areas.

Discussion

Stored cross-linked HA fillers are frequently used for small-volume follow-up maintenance treatments after a few weeks or even re-treatment when the HA augmentation effect subsides after many months. Alternatively, it may happen to open a HA gel filler to perform a minor "touch-up" and to store the residual content of syringes for further touches-up.

The rationale for follow-up maintenance HA injection is solid. Recently, Smith and colleagues reiterated the formerly stated rationale for HA "retouches" 4.5 to 9 months after the first treatment to maintain the long-term tissue augmentation.[9,10]

Although of apparent economic merit, the reuse of stored HA gel fillers exposes to risks of aerobic or anaerobic bacterial or fungal contamination and infectious complications. Several recent studies discussed this concern over the last few years.

Most studies and reviews found no evidence of contamination associated with reused HA gel fillers regardless of concentration and original injection site and no correlation between length of storage time and contamination risk.[1,2,11–13] Storage in original syringes at room temperature in nonaseptic conditions for up to 12 months likely increases the risk.[11] Regarding direct evidence of contamination-related adverse effects in injected individuals, a somewhat dated retrospective review reported no infection in the only subject-based study that looked for adverse events among individuals injected with HA from stored filler syringes.[2] Conversely, although sparse, evidence of contamination does seem in the literature. A Saudi in vitro study reported no fungal and acid-fast bacilli–positive cultures and 5 positive bacterial cultures with normal skin surface flora after a mean storage period of 57.8 months. Storage at room temperature was a possible risk factor and bias of that study.[12]

The observational retrospective study confirms that good storage conditions, including monitored refrigeration, avoid the risk of contamination of partially used cross-linked HA fillers by fastidious microorganisms. However, all operators should not overlook the wise suggestions by a Saudi author for touch-up visits with reinjection of HA from partially used syringes: "*We suggest squeezing out tiny drops of the HA (less than 0.05 mL) and changing to a new needle before commending the augmentation*" and "*The syringe should be discarded if the contents are cloudy*."[12]

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