The Origin of Soft Tissue Filler Adverse Events

Past, Present and Future

Tom S. Decates

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De oorsprong van filler complicaties

verleden, heden en toekomst

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CHAPTER 1

General introduction

MINIMAL INVASIVE COSMETIC PROCEDURES

Minimally invasive cosmetic procedures have revolutionized the treatment paradigm for both facial and body rejuvenation. With these techniques more invasive surgical procedures such as face-liftings, lip corrections and phenol peels can either be postponed or renounced. Although the efficacy of these methods is usually less significant, advantages are numerous. Down-time and complication rates are favorable compared to surgery. Also improvements in appearance are more subtle and not so ostentatious as surgery frequently makes them. Finally these methods appeal to many because they are less expensive than surgery.

Injection treatment with botulinum toxin and fillers (Figure 1), together referred to as injectables, form the largest part of minimal invasive treatments.[1] Botulinum toxin injections induce a temporary muscle paresis. Injected strategically into specific hyperactive mimical muscles, it diminishes lines and faults of the skin. Hence, botulinum toxin diminishes lines and wrinkles in a n indirect manner. Fillers on the other hand fill the tissue by virtue of their own volume. The concept of fillers injections was initially adopted for the treatment of fine lines and wrinkles by subdermal addition of small amounts of filler. Usually 0,5-1,0 ml is used for this. But in the last decade indications have expanded to include correction of sagginess in the aging face. Sagging of the face results from increased laxity of skin and underlying tissues. This is intensified by to loss of soft tissue volume caused by fat pad atrophy and loss of support as a result from skeletal atrophy. The latter two causes can be counteracted by placing volume in specific areas. For this the amount of filler needed can rise to a total of 15-20 ml delivered during the course various sessions over several months. These fillers are typically injected on periosteum and in deep fat pads.

Psychological well-being

Research has shown that injectable treatments have an overall positive effect on several aspects of clients' mental states. A prospective multicenter trial recorded significant improvements on the FACE-Q scales for psychological well-being, social confidence, and aging appearance (Harmony study 2021).[2] A frequently heard criticism regarding injectable treatment is that the outcome is always somewhat 'fake'. However, these critics fall for a common error in thinking, an example of availability bias. While they recognize only the few excessive examples, they do not recognize the many more cases that go unrecognized because they only display subtle and unnoticeable changes in features. In a prospective study concerning 63 patients, Philipp-Dormston et al. found that hyaluronic acid filler treatment in nasolabial folds and lower face achieved natural-looking results together with long-lasting aesthetic improvement.[3]

The use of soft tissue fillers has increased tremendously over the past ten years.[4] With almost a million procedures in 2019 it has become the second most frequently performed non-surgical

procedure in plastic surgery offices in the United States.[5] There is a notable overall increase of 17.8% in comparison to 2015. The most popular ones, being hyaluronic acid fillers, showed a 26.4% raise (see table 1).[5]

Injectable	2019	2015	Percent Change
Botulinum Toxin (including Botox®, Dysport®, Azzalure®, Bocouture®)	1,712,994	1,454,635	17.8 %
Calcium Hydroxylapatite (Radiesse®)	34,776	64,675	-46.2 %
Hyaluronic Acid (including Juvederm®, Restylane®, Belotero®)	749,409	592,930	26.4 %
Poly-L-Lactic Acid (Sculptra®)	28,100	31,915	-12,0 %

Table 1 Numbers of injectable procedure in 2015 and 2019 in the United States in plastic surgeons clinics

Figure 1. Lip filler treatment

Professional associations and authorities in the Netherlands and other countries have issued several guidelines for cosmetic procedures in general.[6] Many of these consider the nature of cosmetic treatments, being elective and without a medical necessity. Consequently, a strict approach is demanded from doctors applying these techniques. Physicians carry a high burden of providing comprehensive information about the procedure and its expected outcome. Consultation before cosmetic treatment should comprise management of expectations,[7] medical history and examination. The treatment procedure and all potential complications should be addressed, as well as the price. A waiting period after the initial consultation is usually required.

In the Netherlands, filler injection treatment is classified as a procedure with low complexity (grade 1).[6] Not many restrictions are in force. No waiting period after initial consultation is required and no specific requirements are necessary regarding office space and treatment facilities. General rules regarding complication and procedure consent (Figure 2), hygiene, antisepsis, and privacy obviously apply. Every officially registered doctor is allowed to perform injection treatments. There is a legal requirement for physicians to be competent. But this is only assessed after problems.

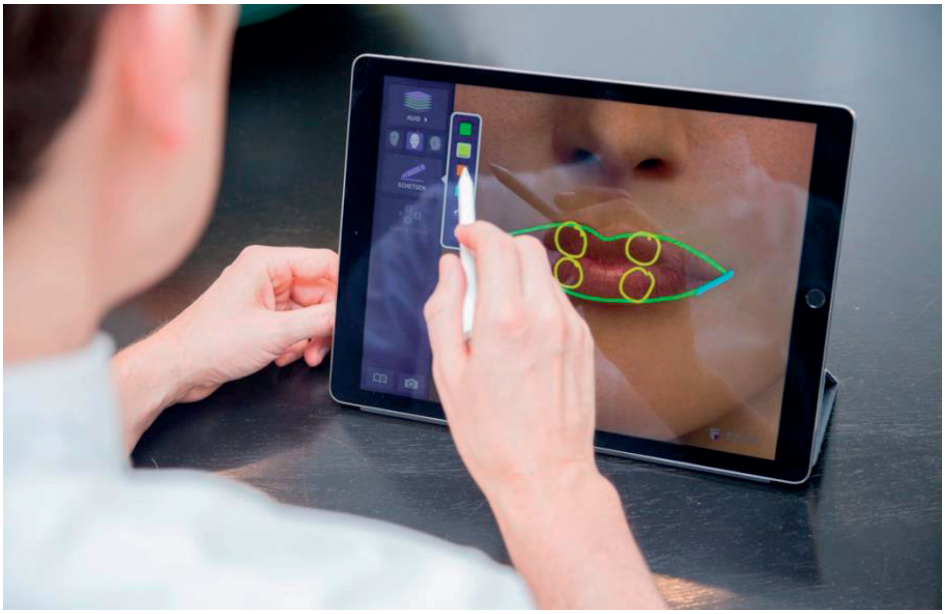

Figure 2. Explanation of the lip filler procedure by the doctor to a patient

Relative contra-indications	Absolute contra-indications
Gravidity or lactation	Patients that do not understand the caveats about outcome
Active auto-immune disorders such as RA, SLE, scleroderma	Body dysmorphic syndrome
Bacterial infection in the treatment area	< 18 years of age

Table 2. Relative and Absolute contra-indications for filler treatments

There is a general notion that patients with permanent fillers anywhere in their face should not be treated with resorbable filler. This is based on cases histories published in which a flare of inflammation around the filler was seen after treatment in other facial regions. Indeed, in a retrospective assessment of the cases in ErasmusMC we noted that in our group 34% of the cases with inflammation after HA injection treatment occurred in patients with permanent fillers.

TYPES OF FILLERS

Although there are many different classifications of soft tissue fillers, for the purpose of adverse event description they are mostly classified by their biodegradability into non-resorbable and resorbable fillers.[8] Non-resorbable fillers remain in the tissue indefinitely. These have been used for many years, however these substances have been banned in many countries (in the Netherlands since 2015).[9] Worldwide several types of non-resorbable fillers are known. Frequently used examples are medical grade silicone, polyalkylimide, polyacrylamide and methacrylate fillers.[10-12] These substances have been tested and initially been found suitable for medical use. But many non-descript, non-medical substances are injected around the world. These come with fancy names as Amazing gel® or biopolymers. Also the term PMMA is used, but the substance referred to under this name is different from the polymethylmethacrylate substance named Artecoll®, that is registered in the USA. These materials are usually not confined to the injection area, but during and after injection spread through the tissues. In particular, allegedly years after injection we have found silicone-like materials injected in buttocks to end up in the lower leg and ankle. Also we have seen cases of polyalkylimide injected in the face giving rise to conspicuous nodules in the neck.

Resorbable fillers consist basically of two variations: hyaluronic acid fillers and bio-stimulatory fillers.

Hyaluronic acid (HA) fillers

HA fillers consist of a naturally occurring linear polysaccharide chains composed of repeating disaccharide units of N-acetylglucosamine and D-glucuronic acid. Hyaluronic acid is a polymer of a disaccharide repeating unit, see Figure 3. The polymer can be broken down in smaller fragments by lyase-type of enzymes (Figure 3).

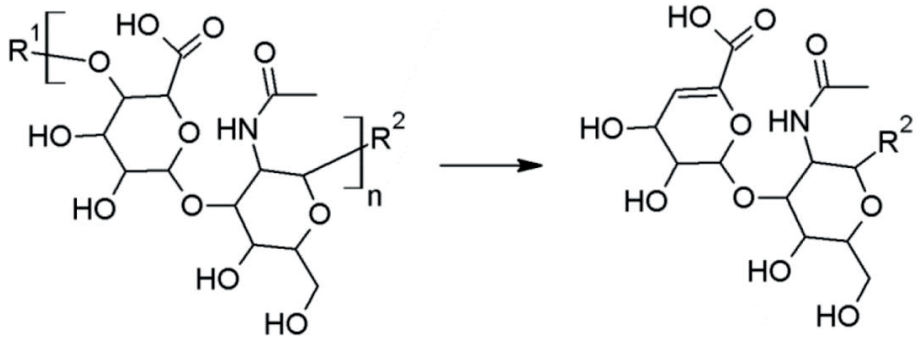

Figure 3. Structure of hyaluronic acid (HA, left) with the repeating unit between brackets. The reaction shown represents the β-(1-4) lyase activity of chondroitinase AC, leading to a fragment containing an unsaturated bond in the glucuronic acid (right). R1 and R2 represent HA repeating units or the terminal alcohols.

HA is of great importance to many living organisms. In the human body, hyaluronic acid is present in almost every organ and synthesized by a great variety of cells, including fibroblast, keratinocytes, endothelial cells, muscle cells.[13] Naturally occurring hyaluronic acid is rapidly degraded with a half-life of only 12 to 24 hours. HA filler material is also broken down and resorbed. But depending on particle size and extent of cross-linking the rate of degradation varies from months up to two years' time or possibly even longer in some cases.[13] The water-binding capacity (hydrophilic character) of the hyaluronic acid makes it an ideal filler.[14] However, to make it a successful product its longevity in the tissue must be expanded. For this the chemico-physical process of cross-linking has been developed. The degree of cross-linking enhances the life span of the filler by increasing the resistance to degradation by native hyaluronidase.[14] Crosslinking of the hyaluronic acid chains also increases its tissue residency and elasticity. However, this may also reduce its biocompatibility, causing foreign body reaction and encapsulation.[15]

Cross-linking of hyaluronic acid-based fillers

The method used most to prevent hyaluronic acid from being metabolized too early after injection is internal cross-linking. Most of the hyaluronic acid-based fillers are cross-linked using butanediol diglycidyl (BDDE).[13] BDDE contains two reactive epoxide groups allowing it to bridge between two strands of hyaluronic acid. Should only one epoxide react with hyaluronic acid, the other epoxide is hydrolyzed, yielding a modified hyaluronic acid. The crosslinking grade can be defined as the percentage of BDDE linked on two hyaluronic acid fragments relative to the total amount of hyaluronic acid fragments. The basic chemical structure of a BDDE-linked hyaluronic acid fragment is shown in Figure 4. In the final stages of the cross-linking procedure residual BDDE should be washes out of the product. One can expect a small amount to remain. What percentage of the total volume this is, remains unclear. It is also unclear which other substance auxiliary substances are used in the production process and how much is contained inside the filler.

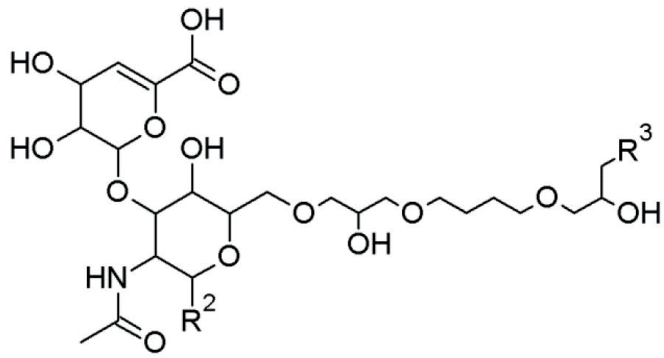

Figure 4. Structure of a BDDE modified HA fragment. R2 and R3 represent an HA fragment or a terminal alcohol.

Every manufacturer presents a range of HA fillers substances with different properties and corresponding applications. Some are intended for very superficial lines, others to render volume in a region such as cheek or temples, and indications in between these two. Basic filler properties are HA concentration, cohesivity, elasticity, viscosity, denoted by mg/ml, G-prime, P-double prime respectively (Table 2).

Variable	Description
Elastic modulus (G′)	Characterizes the ability to rebound to its original shape when acted on by dynamic forces (storage modulus)
	• Quantified in pascals
	• Examples: high elasticity, rubber band; low elasticity, syrup
	• Higher G′ usually correlates with a firmer gel
Viscous modulus (G″)	Characterizes the resistance to dynamic forces (loss modulus)
	• Quantified in pascals
	• Examples: high viscosity, peanut butter; medium viscosity, honey; very low viscosity, water
	• Higher G″ gels are thicker, requiring greater force for extrusion through a needle
	• Lower G″ gels require less force for extrusion through a needle
Tan delta (tan δ)	Characterizes the relative proportions of elastic to viscous moduli (G″/G′)
	• Predominantly elastic gels (e.g., gelatin), have low tan δ (close to 0)
	• Predominantly viscous gels (e.g., honey), have high tan δ (close to 1)
Complex modulus (G*)	Characterizes the overall ability to resist deformation
	• For most HA gels, G′ is much larger than G″ $G^*=\sqrt{(G')^2 + (G'')^2}$
	• For most HA gels, G* is approximately equal to G′
Gel cohesion	Characterizes the capacity to remain intact and not dissociate
	• Attributable to the attraction and affinity between individual molecules
	• Low-cohesive gels dissociate more readily than high-cohesive gels
Gel fluid uptake (SwF)	Characterizes the ability to take up fluid while still in a single phase, referred to as "swelling factor" ratio
	• In vitro measurement only
	• Fully hydrated gel, at equilibrium, will not readily take up more fluid
	• Increasing SwF values indicate a gel is further away from equilibrium
HA concentration (mg/ml)	Total HA in 1 ml of finished product, including both nonextractable (insoluble) and extractable (soluble) HA
	• Nonextractable HA is the bulk of what contributes to a gel's clinical effect
	• Extractable HA is a remnant of the crosslinking process: HA chains, partially crosslinked chains, and fragments that degrade rapidly in vivo

HA, hyaluronic acid; SwF, swelling factor.

Table 3. Rheologic and Physicochemical Properties Relevant to Hyaluronic Acid Gels[16]

Bio-stimulatory fillers

Poly-L-lactic acid (Sculptra®) and calcium hydroxylapatite (CaHa, Radiesse®) are the most used biostimulatory filler.[5,11,12] Their mechanism of action rests on its ability to stimulate fibroblast

proliferation and subsequent neocollagenesis. It is postulated that this is due to a foreign body reaction evoked by the injected material.[17] The resultant formation of a vascularized, connective tissue capsule ensues, which is eventually composed of fibroblasts and new collagen deposits.[18] Radiesse is a biodegradable filler consisting of 30% synthetic CaHA microspheres (diameter of 25–45µm) suspended in a 70% aqueous carboxymethylcellulose gel carrier.[19] The soluble carrier gel evenly distributes the Radiesse CaHA microspheres providing 1:1 correction and gradually dissipates leaving the microspheres at the injection site where they induce neocollagenesis by fibroblast activation.[20-22] In a recent clinical histomorphological study, Radiesse significantly stimulated collagen production over nine months of follow-up.[23] In this manner, Radiesse provides both immediate (replacement volume) and long-lasting (collagen biostimulation) volume enhancement. Fibroblasts are found in all connective tissues and the Radiesse CaHA microspheres are thought to elicit their activation and subsequent collagen production regardless of whether Radiesse is injected intradermally or at the level of the dermal–subdermal junction. The immediate volume correction as well as stimulation of long-term deposition of new collagen surrounding the microspheres contributes to an average duration of effect of 12 to 18 months, though some results have been noted 24 months post-injection.[24]

Sculptra®, Poly-L-latic acid, Polylactic acids were originally synthesized by French chemists in 1954 from the α-hydroxy-acid family and have been used safely as resorbable suture materials, plates and screws in orthopedic, neurologic, and craniofacial surgery.[25-26] Approved by the FDA in 2004 for soft tissue restoration in lipoatrophy in HIV patients, its usage was later expanded to include cosmetic applications in 2009 as Sculptra (Dermik Laboratories, Berwyn, PA). Sculptra functions as more than just a soft tissue filler, but as a biostimulator by stimulating the production and vascularization of collagen. Studies have demonstrated an increase in skin thickness resulting from collagen formation as early as 6 weeks after injection, which remained for up to 96 weeks.[25] Sculptra comes as a powder of poly-L-lactic acid microspheres mixed with sodium carboxymethylcellulose, mannitol. Hours before injections it is dissolved in sterile water for injection. Initial studies showed a high rate of nodule formation, especially in patients with severe atrophy of soft tissue, mainly fat. These nodules resolved spontaneously. This complication was attributed to too superficial product placement together with in the perioral and periocular region.[26] Suspending the material in more water also diminished nodule formation.

The unique advantage of HA fillers over biostimulatory fillers is their reversibility when injected with hyaluronidase, in case of an adverse event.[27]

New soft tissue fillers

New fillers enter the European market regularly. Usually these display new combinations of physical properties as G-prime, concentration, etc. But their intended use is not different from those of other manufacturers. An innovation in fillers application has been the development

Resorbable Fillers	Filler Material	Brandname	Longevity
Hyaluronic Acid (HA)	Non-animal HA	Belotero, Emervel, Juvederm, Restylane, Teosyal, Stylage	6-24 months
Biostimulatory	Calcium Hydroxylapatite (CaHa) 30% in carboxymethyl-cellulose	Radiesse	12-18 months
	Poly-L-latic acid (PLLA)	Sculptra	18-36 months

Table 4. Resorbable fillers, the material they consist of, brandnames and longevity

Nonresorbable (Permanent) Fillers	Filler Material	Brand name
	PAAG 2,5-5% gel	Amazing Gel, Aqualift, Aquamid, Argiform, Formacryl, Interfall
	HEMA/EMA 40% or 60% in HA	Dermalive, DermaDeep
	Polyvinylhydroxyde in PAAG	Evolution, Outline
	PAIG 4% gel	Bio-Alcamid
	PMMA 20% in bovine collagen	ArteColl, ArteFill
	PMMA 30% in carboxynmethyl-cellulose	Metacrill
	Liquid injectable silicone (LIS)	Silikon 1000
	Solid silicone particles in PVP	Bioplastique

Table 5. Nonresorbable (permanent) fillers, the material they consist of and brandnames

of skin boosters. These are HA substances with little or no crosslinking intended to improve physiology and add more resilience to the skin. These are injected superficial in the skin, being deep dermal. Examples are Juvederm Volite®, Restylane Vital®, Belotero Revive®. Introduced between 2010-2016, these are not used much anymore. However, a more popular HA substance with a similar intended use is Profhilo®, available since 2015. The HA molecule is stabilized by an innovative thermal process that rules out the use of any chemical reagents. Profhilo is injected into the subcutaneous fat in only five injection points on each cheek.[28] The HA substance is designed diffuse through the fat. The effects should cover both cheeks completely. A new treatment option consists of a combination of hyaluronic acid and calcium hydroxylapatite.[29] And there is the new filler called Renuva®, an adipose-derived matrix processed from deceased human donors and suitable for allograft transplantation.[30] Such an allograft adipose matrix (AAM) would be an off-the-shelf alternative to either augment or replace autologous fat grafting (AFG) and to act physically and physiologically like autologous fat without requiring a harvest site and the time and morbidity associated with harvesting.

ADVERSE EVENTS

Although manufacturers and various publications claim that the fillers are non-toxic, non-immunogenic and that adverse events are very uncommon, unwanted adverse events occur with all compounds used.[31-35] The terms adverse event and complication are sometimes used in a very loose way and not well defined. In this thesis *adverse event* is defined as any non-intended event related to filler injection treatment, ranging from very mild with no interference in a clients daily activities, to very severe. The latter is regarded as a *complication*, being defined as an injury resulting in prolonged hospital stay, disability at the time of discharge or death.[36] Complications can be viewed as a subset of adverse events.

The severity of an adverse event can be mild (bruising, erythema), intermediate (allergic reaction, inflammation) or severe (blindness, panniculitis necroticans). Table 3 list all adverse events after filler injections reported thus far. Mild adverse events e.g., discomfort, pain, erythema, swelling, bruising, these so-called injection site reactions are common. The happen in the first days after the filler injection. Clients understand that these are normal in any type of injection. [37]

Type of adverse event	**Mild**	**Intermediate**	**Severe**
	Bruising,	Allergic reaction,	Blindness,
	Discomfort,	Inflammation,	Panniculitis necroticans
	Erythema,	Foreign body granuloma,	
	Pain,	Malar edema,	
	Swelling,	Hyperpigmentation,	
	Itching,	Nodule/Adscess	
	Asymmetries		

Table 6. Types of adverse events

Adverse events occur immediately within minutes or days after injection. Delayed occurrence is usually defined to start at least two weeks after treatment but may begin several months or even years later. Adverse events can be of short-term (days-weeks) or of long-term duration (months-indefinite). Some may resolve spontaneously; others require medical interference.

The time until an adverse reaction occurs as well as the type of adverse reaction seems to vary between different soft tissue fillers.[38] In a report from the Injectable Filler Safety Study, a German-based registry for adverse filler reactions, adverse reactions to resorbable fillers were reported to occur after 4.9 ± 5.8 months and reactions to non-resorbable fillers after 18.3 ± 19.0 months. Adverse events to hyaluronic acid-based fillers consisted mainly of swelling, erythema and nodules. Poly-L-lactic acid and polymethylmethacrylate fillers primarily caused development of granulomas.[39] In a European survey, permanent fillers were responsible for severe, persistent, and recurrent adverse effects.[39]

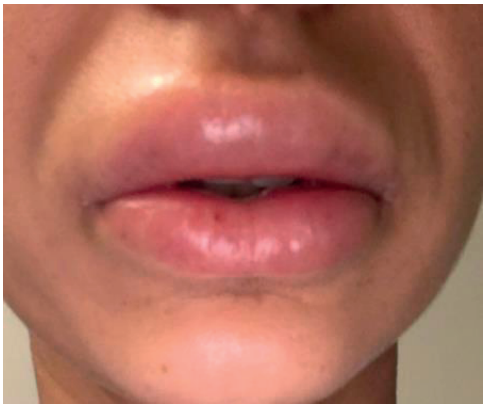

Figure 5. Bruising, redness and swelling one day after a soft tissue filler treatment

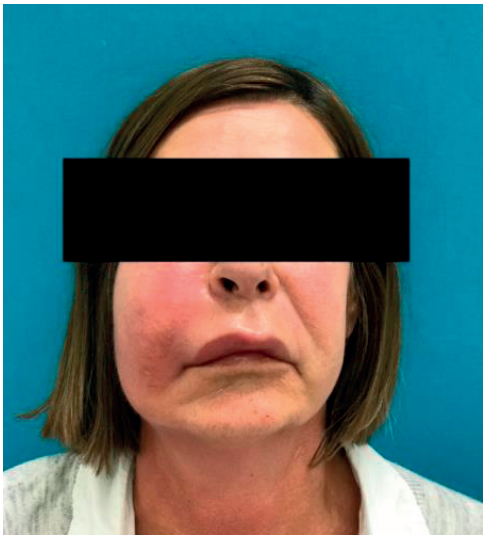

Figure 6. Local infection and abscess of a non-resorbable (permanent) filler

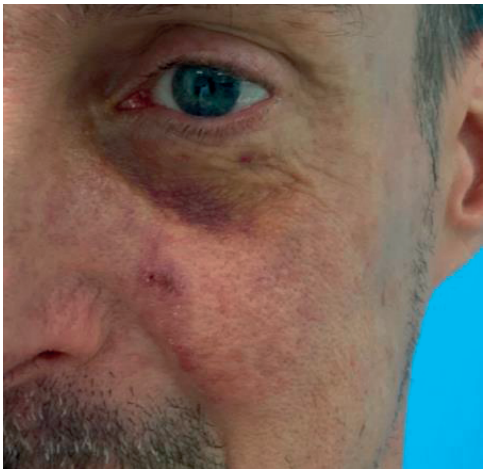

Figure 7. Vascular adverse event (VAE) with redness and skin necrosis after a HA soft tissue filler injection

Aside from the direct harm to the patient, adverse events constitute a considerable financial burden to the healthcare system. In 1999, it was estimated that the total costs of preventable adverse events in the USA lie between $17 billion and $29 billion annually.[36]

Systemic adverse events have also been reported, including flu-like symptoms, lymphadenitis, pneumonitis and ASIA-syndrome.[40]

Implantation of a foreign material always evokes a tissue response. This is a normal immune reaction. Derangement of this response, resulting in excessive local inflammatory, or even systemic adverse reactions, is a multifactorial process which may lead to the adverse events described above.[41] Factors responsible for this may include a direct reaction to the type of filler material, bacterial contamination, and importantly, the genetic immunological profile of the predisposed subject. To date, it is still unknown why some individuals develop adverse events in response to dermal fillers and others have none at all. Several studies have indicated adverse events due to either type of filler material or bacterial contamination.[42,43] Yet, evidence suggests that genetic predisposition of the individual receiving the filler is of major importance and is related to the intensity of their immune and tissue response.[44]

AIMS OF THIS THESIS

Modern resorbable fillers came to the market in the Netherlands around 2002/2003. Before those (and ultimately until 2015) non-resorbable fillers were used. Also, there was no clear training program for doctors applying cosmetic filler injections. These situations have changed in the past five years. Fillers are safe and many doctors are well trained. But new unexplained adverse events such as inflammation, granulomas, vascular blockings, etc. still occur. For this reason, it was felt that more insight in the nature and pathophysiology of adverse events is an urgent necessity.

This led me to investigate:

(1) The number of filler treatments in the Netherlands. This would give some idea about the incidence of filler adverse events and the magnitude of the problem.

(2) Individuals predisposed to developing an adverse event to a soft tissue filler.
In a recent study, Schwarts Navarro and Ali-Jotas Reig[44], showed that individuals with the haplotype HLA-B*08 and HLA-DRB1*03 had a relative risk of 5.9 to develop adverse events, such as granulomas and nodule inflammation, compared to individuals without this particular phenotype. This leads us to believe that a pivotal genetic factor is involved in the development of adverse events to dermal fillers.

(3) The role bacterial contamination during the injection treatment may play in the etiology of adverse events.[42,44,45] In a recent study, biopsies were taken from individuals with adverse events and compared with biopsies from individuals with no adverse events. An increased rate of bacterial contamination was seen in biopsies in the adverse event group. Furthermore, in those biopsies a high rate of immune cells such as macrophages, giant cells, lymphocytes and granulocytes were seen.

(4) The contribution that the filler material itself has in the immune response to the implanted material.[46-47] It is expected that factors indicated in (2)-(4) will enhance the adverse reaction to the soft tissue filler. There might even be a synergy of more than one factor playing together. In this thesis I will address the question which of these factors (singularly and combined) contribute to the occurrence of an adverse tissue response to soft tissue fillers that are currently mostly used in clinical practice. Fir this, a clinical cross-sectional study was set up, comparing subjects treated with soft tissue fillers who show late onset inflammatory adverse reactions (Inflammatory group) and subjects who do not show late onset inflammatory adverse reactions to these fillers (non-Inflammatory group). Inflammatory was defined as the appearance of one or more local clinical signs (edema, angioedema, skin induration, swelling/tender nodules with or without fistulation or discharge of pus or filler material, 3 months or more after initial filler injection) possibly accompanied by systemic complaints including fever, arthralgia, arthritis, skin lesions, eye and mouth dryness, and any other sign or clinical complaint that may be compatible with any autoimmune or immune-mediated systemic disorder.). The non-inflammatory group was formed by people treated with soft tissue fillers at least 3 months before, without any inflammatory complaints. Patients with isolated soft lumps without inflammatory signs were also included in this non-inflammatory group. For the assessment of bacterial contamination, we had the opportunity to use a new and very sensitive technology, the IS-pro method.[48]

(5) Methods for diagnosis and treatment. Although not entirely new ultrasound imaging and intralesional laser therapy showed much promise for diagnosis and treatment of filler complications. Further research will give more insight in the potential of these methods

Chapters 2, 3 and 4 report on the insight gained in the numbers of soft tissue filler treatments preformed in the Netherlands and the number of adverse events after these treatments. The possible involvement of bacterial contamination in the aetiology of soft tissue fillers adverse events is analysed in chapter 5. The role of hypersensitivity and the adaptive immune system is explored in chapters 6 and 7. An exploration on the possible genetic predisposition for the development of adverse events after soft tissue filler injections follows in chapter 8. Diagnostics and treatment options for adverse events after soft tissue filler injections are studied in chapters 9 and 10. Finally chapter 11 considers new hyaluronic acid fillers on the market and new rules and regulations for soft tissue fillers in. Discussion, the last chapter of this thesis, reflects on the main conclusions of this thesis.

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THE PAST
PART II

INCIDENCE ON THE NUMBER OF ADVERSE EVENTS OF SOFT TISSUE FILLER INJECTIONS

CHAPTER 2

Numbers on injectable treatments the Netherlands in 2016

T. Decates
L. de Wijs
T. Nijsten
P. Velthuis

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Objective data on the number of cosmetic injectable treatments (Botulinum Toxin A and fillers) performed annually is lacking. These numbers would be helpfull in establishing the importance of this area in medicine from a medico-social perspective and to determine the incidence of side-effect. Numbers on the number of treatments or global volumes used provided by the American Society of Plastic Surgeons (ASPS) and the International Society of Aesthetic Plastic Surgery are erestricted to their members.[1,2] No organisation has been able to quantify the use of cosmetic injectable treatments nationally across all specialities. The objective of the current postal survey is to identify the physicians administring cosmetic injectable treatments and quantify the number of treatments given.

The sources that we used to identify the doctors performing cosmetic injectable treatments among the 86,588 medical docters in The Netherlands were: Google internet Search, Dutch Archive Data Care Register and memberships lists of professional specialty associations. Doctors were primarily asked for the number of treatments performed in 2016. Should this number be unknown they were requested to give the number of BTX-A vials purchased or the number of zones treated. Depending on the manufactoring company 2.5-5 zones can be treated with one vial. To recalculate other data to numbers of treatments performed, we assumed an average of 3.75 zones per vial. We also assumed an average of 1.5 zone per patient. In case of filler treatments, the numbers of vials used or zones treated were all regarded as one treatment. Doctors were asked to indicate if their numbers were exact or an estimate. In case of an estimate we assumed the uncertainty to be -/+ 20%.

The search yielded a total of 329 eligible doctors (0.0066%) who were send a questionaire by a notary public ensuring the anonimity of participants. A total of 122 responded (response rate of 37%), of whom 60 (49%) provided exact numbers, 62 (51%) gave estimates. The patient male:female ratio was 1:6,4. The results are given in tables 1 and 2.

BOTULINUM TOXIN	RESPONSE (CALCUATING FACTOR)	NUMBER GIVEN	NUMBER CALCULATED	NUMBER EXTRAPOLATED T0 100% (+/- 20%)
Factual	Treatments (x 1)	45.924	45.924	124.119
N=60	Vials (x 3.75/1.5)	6.740	16.850	45.541
	Areas (x 1/1.5)	2.993	1.995	5.392
Estimate	Treatments (x 1)	20.939	20.939	56.592 (45.274 - 67.910)
N=62	Vials (x 3.75/1.5)	2.982	7.455	20.149 (16.119 - 24.179)
	Areas (x 1/1.5)	987	658	1.778 (1.422 - 2.134)
			TOTAL	**253.571 (237.867 - 269.275)**

Table 1. Numbers on Botulinum Toxin A given by respondents (N=122; 37%), recalculated to actual treatments and extrapolated to 100% response.

FILLERS	RESPONSE (CALCULATING FACTOR)	NUMBER GIVEN	NUMBER CALCULATED	NUMBER EXTRAPOLATED TO 100% (+/-20%)
Factual	Treatments (x 1)	30.387	30.387	82.127
N=60	Vials (x 1)	4.020	4.020	10.864
	Areas (x 1)	871	871	2.354
Estimate	Treatments (x 1)	13.276	13.276	35.881 (28.705 - 43.057)
N=62	Vials (x 1)	2.328	2.328	6.292 (5034 - 7550)
	Areas (x 1)	362	362	978 (782 - 1174)
			TOTAL	**138.496 (129.866 - 147.126)**

Table 2. Numbers on Fillers given by respondents (N=122; 37%), recalculated to actual treatments and extrapolated to 100% response.

The estimated number of injections performed in the Netherlands in 2016 with BTX-A was a little more than 250.000 and with filler almost 140.000. Since both the Dutch Society of plastic surgeons and Dutch Society of cosmetic doctors recommends treating only persons between 18-70 years of age, a total of 5.742.227[3] women and 5.781.707[3]. were eligible for treatment. Combined with the male:female ratio and the fact that treatments with BTX-A and fillers are commonly given respectively twice and once yearly (Velthuis et al, unpublished results), this would mean that in the Netherlands in 2016 one out of every 53 women was treated with BTX-A and one out of every 49 with fillers. For men the number are one out of 342 *(5.781.707 / (0.135 x 125.000))* with BTX-A and one out of 305 *(5.781.707 / (0.135 x 140.000))* with fillers. Since many, but not all patients, combine BTX-A and fillers treatments, the number of people undergoing injectable treatment (either BTX-A of fillers) is likely to be higher.

The financial expenditure on cosmetics in the Netherlands in 2016 is approximates the West-European average.[4] Therefore, it can be argued that the attitude of the Dutch people towards medical cosmetic treatments is comparable to that in other Western European countries.

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Upward trend in number of injectable treatments in the Netherlands 2016 – 2019

T. Decates
P. Velthuis
D. Zarringham
L. Bruin
R. Schepers
B. van der Lei

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Little is known about the incidence of injectable cosmetic treatments and adverse events associated with these treatments. The American Society of Aesthetic Plastic Surgery (ASAPS) and the International Society of Aesthetic Plastic Surgery (ISAPS) provide numbers on injectable treatments, but the data are from their members only and the response rates are low (5%).[1,2] Therefore, these results cannot be extrapolated to a general population.

In 2016 we published the results on the number of injectable treatments in the Netherlands based on a postal survey sent to all doctors in the country who performed injectable treatments.[3] To identify these doctors we used Google internet Search, Dutch Archive Data Care Register and memberships lists of all professional specialty associations.

Using this same method, we repeated the survey for the number of treatments performed in 2019. The search yielded a total of 305 eligible doctors. A total of 99 doctors responded (response rate of 32%), of whom 63 (64%) provided exact numbers and 26 (26%) gave estimates. We used the same method to estimate the total numbers of treatments. Total numbers of botulinum toxin (BTX) and soft tissue filler treatments are presented in Table 1. The male/female ratio was 1:8, and the average age was 43.2 years. Since the scientific societies of all professional specialty associations recommend treating only persons between 18 and 70 years of age, a total of 5.907.190[4] women and 5.934.277[4] men were eligible for treatment in the Netherlands. Considering the male/female ratio and the fact that treatments with BTX are usually given twice yearly and soft tissue fillers once yearly (Velthuis et al., unpublished results), this would mean that in 2019 one out of every 53 women (5.907.190 / (0.888 x 124.494) were treated with BTX, and one out of every 41 (5.907.190 / 0.888 x 162.702) with soft tissue fillers.

BOTULINUM TOXIN	RESPONSE (CALCUATING FACTOR)	NUMBER GIVEN	NUMBER EXTRAPOLATED T0 100% (+/- 20%)
2019			
Factual N=63	Treatments (x 1)	48.875	152.734
Estimate N=26	Treatments (x 1)	30.800	96.250 (77.000 - 115.500)
Netherlands		TOTAL 2019	**248.984** (229.734 - 268.234)
Europe		TOTAL 2019	**10.851.806**
2016			
Factual N=60	Treatments (x 1)	64.769	175.052
Estimate N=62	Treatments (x 1)	29.052	78.529 (62.815 – 94.223)
		TOTAL 2016	**253.571** (237.867 - 269.275)
FILLERS	RESPONSE (CALCUATING FACTOR)	NUMBER GIVEN	NUMBER EXTRAPOLATED T0 100% (+/- 20%)
2019			
Factual N=63	Treatments (x 1)	28.365	88.640
Estimate N=26	Treatments (x 1)	23.700	74.062 (59.249 - 88.874)
Netherlands		TOTAL 2019	**162.702** (147.889 - 177.514)
Europe		TOTAL 2019	**7.091.261**
2016			
Factual N=60	Treatments (x 1)	35.278	95.345
Estimate N=62	Treatments (x 1)	15.966	43.151 (34.521 – 51.981)
		TOTAL 2016	**138.496** (129.866 - 147.126)

Table 1. Numbers on botulinum toxin and soft tissue filler treatments given by respondents in 2016 (N=122; 37%) and 2019 (N=99; 32%), recalculated to actual treatments and extrapolated to 100% response. For estimates a 20% margin of uncertainty is given in brackets. European Numbers are extrapolated from the number of inhabitants (18-70 years of age) in Europa in 2019

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CHAPTER 3

Increased usage of botulinum toxin and hyaluronic acid fillers in young adults

D. Zarringam
T. Decates
H.P. Slijper
P. Velthuis

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Over the past decades, dermal fillers and botulinum toxin have become broadly available for the improvement of undesirable skin wrinkles and sagging.[1] Previous studies have mentioned a range of factors resulting in patient motivations for non-surgical aesthetic treatments. Among these were social awareness, acceptance of cosmetic treatments, a growing sociocultural emphasis on beauty and self-image or self-esteem.[2-4] Furthermore, both women and men are increasingly affected by beauty ideals, presented by the mass media, resulting in more dissatisfaction about their self-appearance.[5] A higher engagement of social media usage has been linked to self-image dissatisfaction.[6] Especially since the majority of social media users are young adults, the question arises whether this group is a growing user of aesthetic treatments such as botox and fillers.[7] According to a 2018 survey by the American Academy of Facial Plastic and Reconstructive Surgery, 72 percent of facial plastic surgeons saw an increase in cosmetic surgery or injectables in patients under the age of 30. This would be an increase of 24 percent, compared to 2013.[8] Yet to our knowledge, no study has used a clinical database to assess this trend.

The aim of this study is to quantify the trend of incidence of young adults choosing for non-surgical cosmetic treatments in a multi-center setting. The Electronic Health Reports (EHR) of three medical centers in the Netherlands were examined in this multi-center retrospective observational study: Velthuis Kliniek, Nationaal Huidcentrum and Kliniek Veldhoven. These medical centers give a representative view on a nationwide trend since they contain both high-end locations and accessible ones. Added together, the acquired data originates from ten locations spread across the Netherlands. All-time anonymously data of patients consulting an aesthetic physician for botulinum toxin and hyaluronic acid fillers treatment were considered for inclusion. Only the first consultation of each individual patient was included in this study, excluding checkup appointments and new visits by already registered patients. Young adults are defined as the age group 18-25 years old in this study, based on our clinical categorization of this group.

A total of 12 628 patients were included, spread over the years 2008-2019. Table 1 illustrates the numbers included per year. A number of 8453 patients were excluded due to missing or inaccurate data with respect to age or date of consultation. This missing data was not at random and was more common in the far past due to poorer documentation. No other specific bias was found as a cause. Concerning age, 584 patients (4.6%) fell under the age category of 18-25 years, while 12 044 (95.4%) patients were 26 years or older. Over the eleven years the share of young adults was significantly correlated to the year of initial consultation χ (11) = 62.282, $p < 0.01$. Fig. 1 shows the trend in share of young adults, compared to the total annual percentage of patients. In most years, the share of young adults has increased in the use of botulinum toxin and hyaluronic acid fillers. It is remarkable that the year 2017 forms a significant peak in the share of young adults. Added up, 343 men visited an aesthetic physician compared to 2 662 women, forming a 11.4% minority. A statistically significant correlation was found between the year of the appointment and gender, $\chi(11) = 35.065$, $p < 0.01$. As Table 1 illustrates, the share of men, tends to fluctuate annually while increasing in the more recent years.

		Age group				Gender			
		Age > 25		*Age 18-25*		*Female*		*Male*	
		n	%	n	%	n	%	n	%
year of initial consultation	2008	158	96.9	5	3.1	60	88.2	8	11.8
	2009	326	97.0	10	3.0	129	86.0	21	14.0
	2010	292	97.7	7	2.3	110	88.7	14	11.3
	2011	917	97.9	20	2.1	530	94.3	32	5.7
	2012	583	95.9	25	4.1	273	85.8	45	14.2
	2013	527	97.1	16	2.9	230	92.4	19	7.6
	2014	880	95.8	39	4.2	157	88.7	20	11.3
	2015	1007	95.4	49	4.6	186	89.0	23	11.0
	2016	761	96.9	24	3.1	206	88.0	28	12.0
	2017	940	92.0	82	8.0	222	84.7	40	15.3
	2018	2087	94.2	129	5.8	284	86.9	43	13.1
	2019	3566	95.2	178	4.8	275	84.6	50	15.4

Table 1. Trend in years

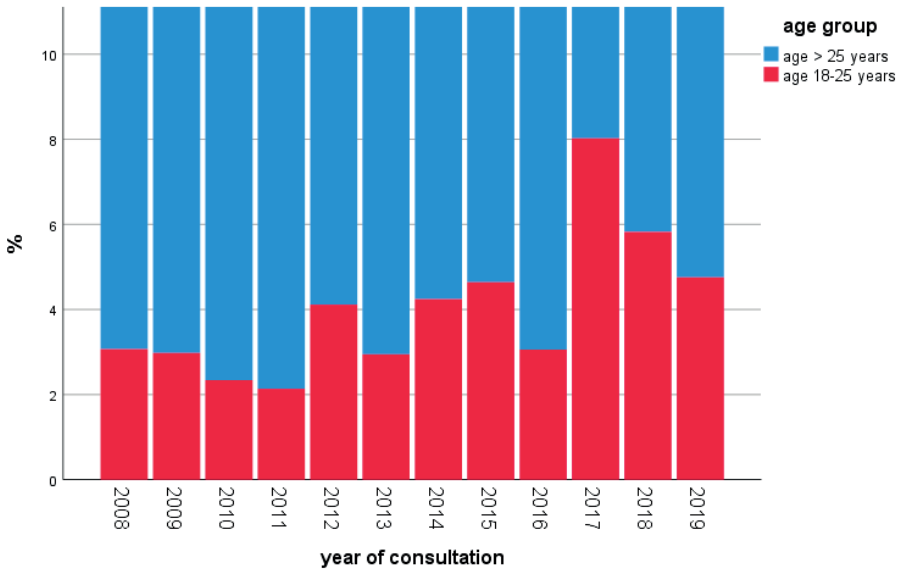

Figure 1. This figure illustrates the percentage of young adults per year. The arrows in between the years represent the statistical significance of the chi-square test between the two adjacent years. A green arrow highlights a statistical significance of $P < 0.05$. The exact numbers are as follows: (i) 2008–2009: $v(1) = 0.003$, $P = 0.955$. (ii) 2009–2010: $v(1) = 0.245$, $P = 0.621$. (iii) 2010–2011: $v(1) = 0.045$, $P = 0.831$. (iv) 2011–2012: $v(1) = 5.099$, $P = 0.024$. (v) 2012–2013: $v(1) = 1.134$, $P = 0.287$. (vi) 2013–2014: $v(1) = 1.586$, $P = 0.208$. (vii) 2014–2015: $v(1) = 0.181$, $P = 0.670$. (viii) 2015–2016: $v(1) = 2.962$, $P = 0.085$. (ix) 2016–2017: $v(1) = 19.829$, $P < 0.000$. (x) 2017–2018: $v(1) = 5.568$, $P = 0.018$. (xi) 2018–2019: $v(1) = 3.244$, $P = 0.072$.

The interpretation of these data remains a point of discussion, since these data are an illustration of the concerning years and the usage of these treatments may be year-dependent. However, the current data does support previous questionnaires among plastic surgeons pointing out the increasing use of botulinum toxin and hyaluronic acid fillers usage in young adults.[9, 10] In conclusion, for the first time the use of these treatments by young adults and men has been expressed in multi-center clinical data.

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ETHICAL APPROVAL

Research concerning anonymously non-traceable data does not require approval by an ethics committee according to Dutch law (WMO).

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CHAPTER 4

Incidence of vascular obstruction after filler injections

L. Schelke
T. Decates
J. Kadouch
P. Velthuis

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An intravascular injection leading to skin necrosis or blindness is one of the most alarming complications in filler treatment.[1-4] A proper calculation on the risk of vascular occlusion has, to our knowledge, never been performed because odds are low and total numbers of injections are generally unknown. In medical literature, frequencies of vascular adverse events (VAEs) are not detailed but estimated to be 1:2000 to 1:10,000 (0.05–0.01%).[3,4]

At the Department of Dermatology at Erasmus University Hospital, we have had a specialized clinic for filler complications since 2011. There are no barriers for patients to visit, because the city of Rotterdam can be reached by train in a maximum of 3.5 hours from every part of the Netherlands. Most physicians in cosmetic medicine in the Netherlands are aware of the problem of vascular occlusion and our competencies, because we have published several papers on filler complications in Dutch as in international journals[5,6] and in the lay press. In the complications debate of the Dutch Society for Cosmetic Medicine, our group has been actively engaged since its foundation. All medical specialties refer patients to our hospital, in particular in acute situations and also after office hours and in weekends.

Recently, we calculated the total number of filler treatments performed in the Netherlands in 2016.[7] For this purpose, we searched Google, the Dutch Archive Data Care Register, and membership lists of professional specialty associations to assess the number of doctors performing such treatments and sent them questionnaires to inquire how many filler injections they had conducted in 2016.

The response rate was 37% (n = 122). The total number of filler treatments was calculated to be 138,496 (min-max. margins: 129,866-147,126).[7] With this information and the knowledge that virtually every patient with an VAE is referred to us, we were able to calculate the incidence of vascular occlusion filler treatments quite accurately.

METHODS

From January 2018 to January 2020 (25 months), we prospectively included patients consecutively referred to our out-patient clinic for filler-induced vascular occlusions. The diagnosis was confirmed by clinical presentation (reticulated bluish pattern with/without pustules and wounds) and doppler-ultrasound images (hypervascular turbulent artery with/without detectable filler blockage).

The reported data consisted of the type of filler product employed, the assessed skin changes and area of the face involved, the artery involved, and whether needle or canula had been utilized. Our treatment for hyaluronic acid filler obstruction is given elsewhere.[5] In calcium

hydroxyapatiterelated vascular blockages, sodium-thiosulphate injections (250 mg/mL-0.2 mL per cm2) were utilized.[8] All patients provided written consent for the treatment procedure. The study was conducted in accordance with guidelines of the Declaration of Helsinki.

RESULTS

A total of 44 patients (3 male, 41 female) with a VAE due to hyaluronic acid or calcium hydroxylapatite fillers were referred to our outpatient clinic (Table 1). The age range of the patients was 18 to 49 years (mean age, 34 years), and the involved areas and arteries of the face are mentioned in Table 2. In some cases, more than one artery was involved. In 3 cases, a canula 25G had been employed. After doppler ultrasound-guided injections of hyaluronidase, all patients fully recovered. The calculation of the risk of vascular occlusion in filler treatments in given in Table 3.

Product used	Artery involved (DUS* identified)	Location(s) of skin changes	Number of treatments with cannula**	Number of patients
HA ***	inferior labial	chin + lower lip		7
	superior labial + columellar	upper lip		5
	angular	nose		4
	superior labial	upper lip		4
	submental	chin		4
	superficial temporal	temple		3
	dorsal nasal	nose tip	1	2
	supratrochlear	forehead		2
	submental	tongue		1
	facial	nasolabial fold		1
	facial + angular	nasolabial fold		1
	angular + superior labial	nose		1
	columellar	nose		1
	columellar	upper lip		1
	transverse facial	cheek	1	1
	infraorbital	midface		1
	zygomaticoorbital	lat corner eye		1
CaHA ****	submental	chin		2
	transverse facial	cheek	1	2
		TOTALS	3	44

Table 1. Patients referred with vascular obstruction. *DUS = doppler ultrasound, **all cases where no cannulas are reported were treated by needle, ***HA = hyaluronic acid filler, ****CaHA = calcium hydroxyapatite filler. Details on a number of these cases were published earlier.[3]

	Location	artery involved (DUS identified)	Delay in treatment time	Nr of treatments	product	Male M Female F
1	nose	angular	1 day	1	HA	F
2	nose	angular	4 hours	1	HA	F
3	nose	angular	1.5 days	2	HA	F
4	nasolabial	facial + superior labial	1 day	2	HA	M
5	lip	superior labial + columellar	3 hours	1	HA	F
6	lip	superior labial	4 hours	1	HA	F
7	lip	superior labial	3 days	1	HA	F
8	lip	superior labial	1 day	2	HA	F
9	lip	superior labial +columella	1 day		HA	F
10	forehead	supratrochlear	8 hours	1	HA	FF
11	forehead	supratrochlear	2.5 days	1	HA	F
12	chin	submental	1 day	1	HA	F
13	chin	inferior labial	1.5 days	1	HA	M
14	chin	inferior labial	8 weeks	1	HA	F
15	parietal area	superficial temporal	3 weeks	2	HA	F
16	lip	superior labial +columellar	3 days	2	HA	F
17	mandibula	transverse facial ,	3 days	2	HA	F
18	lip	superior labial + columellar	3 days	2	HA / C	F
19	chin	submental	1 day	3	HA	F
20	lip	supralabial	3 days	2	HA	F
21	nose tip	columella	4 hours	1	HA	F
22	Infraorbital notch	infraorbital	8 months	1	HA	F
23	nose tip	angularis	8 months	2	HA	F
24	nasolabial	facialis + angularis	3 days	2	HA	F
25	underlip	infralabial	1 day	1	HA	F
26	cheek re	transversal facial	4 hours		CaHA /C	F
27	cheek li		1 day		CaHA	F
28	tongue	submental	1 day		CaHA	F
29	forehead	superficial temporal	3 days	1	HA	F
30	chin	submental	1 day	1	HA	M
31	nose tip	dorsal nasal	4 days	1	HA	F
32	underlip	infralabial	1 day	1	HA	F
33	upperlip	columella	5 hours	1	HA	F
34	nose tip	dorsal nasal	15 days	1	HA /C	F
35	chin	submental	3 days	1	HA	F
36	underlip	infralab art	1 day	1	HA	F
37	temples	supratemp	3 days	1	HA	F
38	underlip	infralabial	1 day	1	HA	F
39	underlip	infralabial	5 hours	1	HA	F
40	nasolabial	facial	14 days	1	HA	F
41	lat corner eye	zygomaticoorbital	1,5 day	1	HA	F
42	nose	dorsal nasal	5 uur	1	HA	F
43	chin				CaHa	F
44	chin				HA	F

Table 2. Consecutive patients referred with vascular obstruction. *DUS = doppler ultrasound, **all cases where no cannulas are reported were treated by needle, ***HA = hyaluronic acid filler, ****CaHA = calcium hydroxyapatite filler, *****/C = 25G canula used. Details on a number of these cases were published earlier [3]

Number of patients referred in 25 months	44
Patients referred per month	1.76
Patients referred per year	21,12
Chance per treatment 21,12 / 138496	1:6558 (0.015%)

Table 3. Calculation on the risk of vascular occlusion in filler treatments.

CONCLUSIONS

We calculated the incidence of VAEs after filler injections to be 1:6558 (or 0.015%). We realize that this calculated measurement of incidence raises some question marks. The number of 41 referrals in 24 months might be an underreport of the real number.

Some physicians may not recognize the problem in their patient, and others may feel reluctant to refer them or prefer to treat the VAEs themselves. However, because of the awareness created in our country by many different channels and the upsetting clinical picture, we are confident the vast majority of cases have been referred to our outpatient clinic. Also, in 2018 to 2019, the total number of filler treatments performed was probably higher than in 2016. Yet underreporting has a larger effect on the outcome than increased treatment numbers. To include under- and overestimation of numbers, we estimated a calculated ±20% as a credible range for a lower and upper estimate of the incidence. We therefore conclude that the chance for VAE is 1:6600 (1:5300-1:8000, rounded to the nearest hundred). Several referrals were from doctors who have practiced cosmetic medicine for more than a decade and are widely recognized as excellent physicians. With a risk of 1:6800 treatments, many physicians will encounter this event more than once during their career.

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THE PRESENT
PART III

BACTERIAL ORIGIN FOR ADVERSE EVENTS AFTER SOFT TISSUE FILLER INJECTIONS

CHAPTER 5

Bacterial contamination is involved in the etiology of dermal filler adverse events

T.S. Decates
A.E. Budding
P.J. Velthuis
Y. Bachour
L.W. Wolters
L.W. Schelke
T. Nijsten
F.B. Niessen

Accepted Plastic and Reconstructive Surgery

ABSTRACT:

Background: The treatment algorithm in late-onset inflammatory adverse events on soft tissue fillers depends primarily on the assumed causative factor, being either immunological or bacterial.

Objective: To assess whether bacteria are associated with late-onset inflammatory adverse events, when using the very sensitive IS-pro method to detect microbiota. Also for the bacteria found, to determine which types are involved.

Methods: Filler biopsies were taken in 29 patients, 13 of whom experienced late-onset inflammatory adverse events (Inflammation group) and 16 who did not (Reference group). Before the biopsy, we acquired skin swabs in 25 of the 29 patients.

Results: A high level of Gram-positive bacteria was found in biopsies of soft tissue fillers, predominantly in patients from the inflammation group. This suggests that these bacteria were introduced during the primary filler injection treatment. The composition of the microbiota on the skin differed markedly from that in the filler indicating that contamination during our sampling process did not influence results.

Limitations: Due to the small sample size, the study needs to be replicated in a larger sample.

Conclusion: Bacteria adherent to soft tissue fillers probably play a causative role in adverse events. Contamination of samples in the biopsies with skin microbiota was excluded.

KEY POINTS

- This article identifies bacterial contamination as a likely cause of late-onset inflammatory adverse events after filler injections.
- This article gives credibility to constant use op topical disinfectants before and during filler treatments. It delivers a strong case for the usage of antibiotics in the treatment of late-onset inflammatory adverse events.

INTRODUCTION

Soft tissue fillers are widely used in dermatology, plastic surgery and aesthetic medicine to reduce the signs of skin aging.[1] The most recent survey of the American Society for Dermatologic Surgery (ASDS) on dermatologic procedures in 2017 reported an increase of 21% in the number of soft tissue filler injections.[2] With more patients undergoing injections with soft tissue fillers, adverse events have also increased.[3]

Several studies have suggested that inflammatory adverse events may be caused by biofilms (Figure 1) resulting from bacterial contamination during the initial treatment.[4-7] This has been reported with other foreign materials[8,9] such as breast implants[10], pacemakers[11] and prosthetic joints.[12]

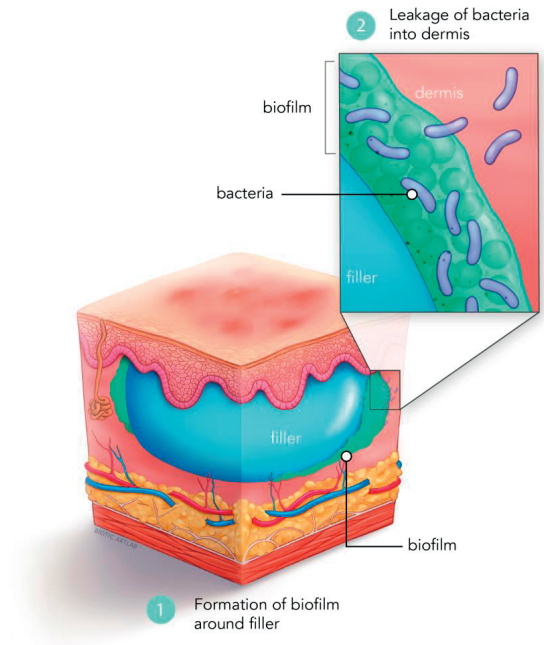

Figure 1. Biofilm surrounding the soft tissue filler; a heterogeneous structure that comprise bacteria embedded within a strong extracellular matrix of secreted polysaccharides

In this paper we addressed the following research questions: is there a correlation between adverse events and the presence of bacteria surrounding the injected filler? And if so, could bacterial contamination during the initial injection be the underlying cause of these adverse events?

To analyze the microbiota, we used a new and very sensitive method, the IS-pro assay. This is a novel, broad-range PCR technique based on sequence variations and length of the 16S-23S ribosomal interspacer (IS) region.[13] IS-pro can also identify bacteria up to species level and detect bacteria at low (<5 colony forming unit cfu) abundance.

MATERIAL AND METHODS

Samples from patients were collected between 2016 and 2018 at the dermatology department, Erasmus University Medical Center, the Netherlands. The local medical ethics committee approved this study.[14] Patient characteristics were collected by completing a questionnaire assessment. All participants provided written informed consent.

We included patients who were willing to undergo a biopsy of the filled tissue at a specialized outpatient clinic for soft tissue filler adverse events. Two groups were defined: an inflammation group with an adverse event and a reference group without such an event. An inflammatory adverse event was defined as the appearance of two or more of the following clinical symptoms/signs of inflammation three months or later after initial filler injection: skin induration, edema, nodules with or without tenderness, with or without fistulation or discharge of pus or filler material.

The reference group consisted of patients treated with soft tissue fillers at least three months before inclusion who did not report any of these inflammatory signs. Cases with isolated soft lumps due to migration of the filler substance, but without any of the above-mentioned inflammatory signs, were also included in the reference group. Both the inflammation group and reference group completed a questionnaire assessment including items on ethnicity, autoimmune diseases, smoking status, allergies and location of the injection.

Of the total sample of 29 patients, 25 had two skin swabs taken on the adverse event side, followed by disinfection with chlorhexidine-alcohol and soft tissue biopsies through fine-needle aspiration. Afterwards, the specimens were collected in sterile specimen containers followed by immediate snap freezing in liquid nitrogen and stored at - 20°C until further analysis.

LABORATORY METHODS

IS-pro assay

Isolated DNA was amplified with the "intergenic spacer profiling" (IS-pro) assay (InBiome, Amsterdam, the Netherlands), according to the manufacturer's protocol. Briefly, IS-pro differen-

tiates bacterial species by the length of the 16S-23S rDNA interspace region, with taxonomic classification by phylum-specific fluorescently labeled PCR primers that have been extensively evaluated for coverage of the phyla included in the assay. Two multiplex PCR reactions are performed. The first contains two fluorescently labeled forward primers and three unlabeled reverse primers. The first forward primer is specific for the phyla Firmicutes, Actinobacteria, Fusobacteria and Verrucomicrobia (FAFV), and the second primer is specific for the phylum Bacteroidetes. The second PCR reaction contains one forward primer specific for the phylum Proteobacteria and seven reverse primers, together covering the phylum Proteobacteria. Amplification was done on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). After PCR, 5 µl of PCR product was mixed with 20 µl of IS-pro™ eMix (IS Diagnostics). DNA fragment analysis was performed on an ABI Prism 3500 Genetic Analyzer (Applied Biosystems).

Data analysis

Data were analyzed with the IS-pro proprietary software suite (InBiome). Automated species calling of IS-pro peaks was done with the dedicated IS-pro software suite (InBiome), in which peaks are linked to a database containing IS-profile information of >500 microbial species. Peaks of <145 relative fluorescence units (RFU) and Proteobacteria peaks <500 were regarded as background noise and were discarded from further analysis. Peaks known to be human contamination and peaks detected in the negative controls that were considered to be contamination were discarded from further analysis.

Statistical Analysis

Bacterial loads were measured by the intensity of their associated fluorescent signal in the CE machine as Relative Fluorescent Units (RFU); summed intensities were used to calculate total bacterial loads. The median load (summed intensity) for each of the three phyla was compared between the inflammation group and reference group. For effect size, the non-parametric Hodges-Lehmann estimate of median difference was used. To assess the statistical significance of differences between the groups, the Mann-Whitney U test was used.

In order to estimate the increased risk of adverse events following soft tissue filler injections in relation to the intensity of the three phyla, a logistic regression analysis was performed, modeling the increased odds of inflammation for each additional 1000 RFU.

For all analyses, the significance level was set to .05. Analyses were conducted with the SPSS software (IBM Corp. Released 2017. IBM SPSS Statistics for Apple, Version 25.0. Armonk, NY: IBM Corp.).

RESULTS

This A total of 29 patients took part in this study. This sample was divided into an inflammation group of 13 patients who experienced late-onset inflammation, and a reference group of 16 patients who did not experience inflammation. The two groups were first compared on several patient characteristics (age, gender, ethnicity, smoking status, autoimmune diseases, allergy, cold sore, filler type, injection location, and filler in situ). Analyses showed that all differences were small, and none was statistically significant.

To determine whether bacterial infection plays a role in adverse events following injection of soft tissue fillers, the presence of bacteria and their loads were compared between the inflammation group and reference group. To further analyze the role of different bacterial phyla, results were stratified into the groups Bacteroidetes (BAC), Firmicutes, Actinobacteria, Fusobacteria, Verrucomicrobia (FAFV) and Proteobacteria (PROTEO).

Figure 2 presents a series of box plots that visualize the distribution of BAC, FAFV, and PROTEO bacterial loads (in 1000 RFU). The median load was markedly higher for the FAFV phylum than for the other two phyla. Bacterial load was lowest for the Proteobacteria. A Friedman test for related samples showed that the differences between the phyla were statistically significant (χ^2 = 27.21, df = 2, p < .001). Figure 2 further shows that the relatively high load of the FAFV phylum occurred mainly in the inflammation group. To compare the inflammation group and reference group on each of the three phyla, Hodges-Lehmann median differences were calculated. The median difference was considerably larger for the FAFV phylum (54.0 95% CI 11.6 to 82.0) than for the BAC (-1.8 95% CI -17.3 to 5.4) and PROTEO (-0.0 95% CI -10.8 to 0.0) phyla. Only for the FAFV phylum the difference was statistically significant (Mann-Whitney U = 50.0, p = .018).

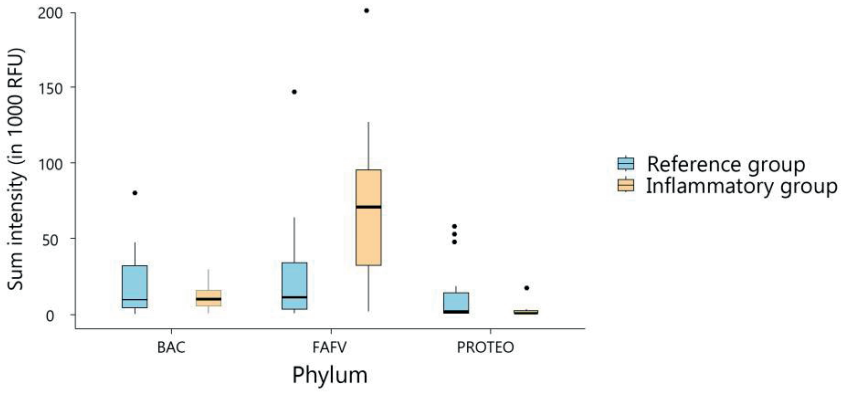

Figure 2. Sum Intensity (in 1000 Relative Fluorescent Units = RFU) for three bacterial phyla in patients with no inflammation (Reference group) and with inflammation (Inflammation group). Only the FAFV phylum showed significantly higher RFU intensity

In summary, a relatively high level of bacterial contamination was found in the biopsies from filled soft tissue, mainly involving the FAFV phylum (which includes most Gram-positive bacteria) of patients experiencing adverse events.

This may indicate that high levels of bacterial contamination are predictive for adverse events. To estimate the increased risk of adverse events in relation to the intensity of the three phyla, a logistic regression analysis was conducted with sum intensity of Bacteroidetes, FAFV, and Proteobacteria (in 1000 RFU) as predictor variables and inflammation status as the binary dependent variable. The analysis showed that, overall, the intensity of the phyla was significantly associated with the odds of adverse events (Nagelkerke's $R^2 = .65$, overall model test $\chi^2 = 19.33$, $p < .001$). However, of all three phyla only FAFV appeared a risk factor (OR = 1.06, 95% CI 1.01 to 1.11, $\chi^2 = 13.66$, $p < .001$).

Bacterial contamination

The relatively high sum intensity for the FAFV phylum in the inflammation group suggested that bacterial contamination during the initial treatment could have caused the high bacterial load that leads to adverse events. To assess this possibility, we collected a total of 40 soft tissue filler biopsies and 26 double skin swabs from the total group of 29 patients. For eight of the patients, 15 additional biopsies were taken from the sites of multiple injected soft tissue fillers. In those cases, the IS profile measurements of multiple biopsies were aggregated on the patient level by taking the arithmetic mean. For three patients it was not possible to obtain skin swabs. These three patients were removed from the analysis. For all 26 patients included in the analyses, two skin swabs were collected. Similarities of these 26 pairs of IS profiles were calculated with the Pearson correlation coefficient. As expected, similarities between the two skin swabs were generally high, with an average correlation coefficient of .85. Therefore, in order to avoid redundancy in the results, analyses of all between-patients similarities involving skin swabs were conducted using only the first skin swab.

Between-patients similarity

The two boxplots of Figure 3 show the distributions of the correlation coefficients for each type of between-patients similarity for soft tissue filler biopsies and skin swabs. As can be seen, the distributions of correlation coefficients for the two types of similarity not only have clearly different locations, with relatively low similarity between skin swab and soft tissue filler biopsy IS profiles, but also different degrees of variation. The largest variation in similarity is found among soft tissue filler biopsy IS profiles, and the lowest among skin swab IS profiles.

The markedly low similarity of skin swab and soft tissue filler biopsy IS profiles (6.5% on average) indicates that soft tissue filler biopsy IS profiles are even more distinctive from skin swab IS profiles than that they are among other soft tissue filler biopsy IS profiles (14.8% similarity on average).

Within-patient similarity

In 26 patients IS profiles were determined for two skin swabs and one soft tissue filler biopsy. This enabled us to determine the within-patient similarity between two skin swabs, as well as between skin swab and soft tissue filler biopsy profiles.

The two boxplots of Figure 4 show the distributions of the correlation coefficients for the two types of within-patient similarities. One regards skin swabs of affected side of the face and the corresponding area on the other side ('Swabs'). The other regards skin swabs compared with soft tissue filler biopsies within the same patient ('Swabs vs Filler'). It is apparent in the figure that the distributions of correlation coefficients for these two types of similarities have different locations. There is high similarity in skin swabs from both sides of the face, but relatively low similarity between skin swab and soft tissue filler biopsy. Both also differ markedly in terms of variation. A Wilcoxon signed-rank test for related samples showed that the difference between the two median correlation coefficients is statistically significant ($Z = 4.37$, $p < .001$). Since the IS profiles representing the type of bacteria in the filler biopsy differ so substantially, this indicates that contamination of biopsies with skin bacteria during the sampling process is very unlikely.

Comparing Figures 3 and 4, it appears that the within-patients and between-patient similarities of skin swab and soft tissue filler biopsy IS profiles are of the same order (9.0% and 6.5%, on average). This suggests that a soft tissue filler biopsy IS profile from a patient is just about as different from other patients' skin swab IS profile as it is from the patient's own skin swab IS profile.

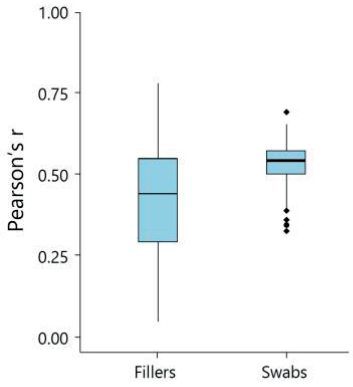

Figure 3. Pearson correlation coefficients between patients. Fillers: boxplot showing all correlation coefficients comparing bacterial loads in the soft tissue filler biopsy from each patient with every other patient; Swabs: boxplot showing all correlation coefficients comparing bacterial loads on the skin swabs from each patient with every other patient.

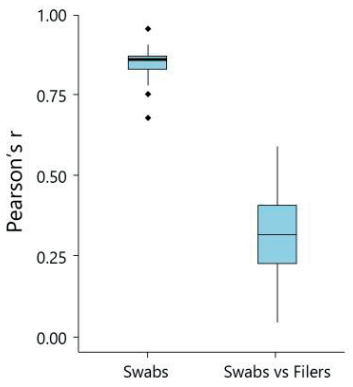

Figure 4. Pearson correlation coefficients within the same patient. Swabs: boxplots showing all correlation coefficients comparing bacterial loads on the skin swabs of the right and left side of the face in every patient; Swabs vs Filler: boxplots showing all correlation coefficients comparing bacterial loads on the skin swab from each patient and bacterial load in the soft tissue filler biopsy from the same patient.

DISCUSSION

This study has shown a strong correlation between bacterial load and late onset inflammatory adverse events in a group of soft tissue filler patients. This correlation applied to the FAFV phyla only and not to the PROTEO or Bacteroides phyla. The FAFV phyla contains the group of gram-positive bacteria including common skin-associated bacteria such as staphylococcus, streptococcus and cutibacterium (Figure 5).

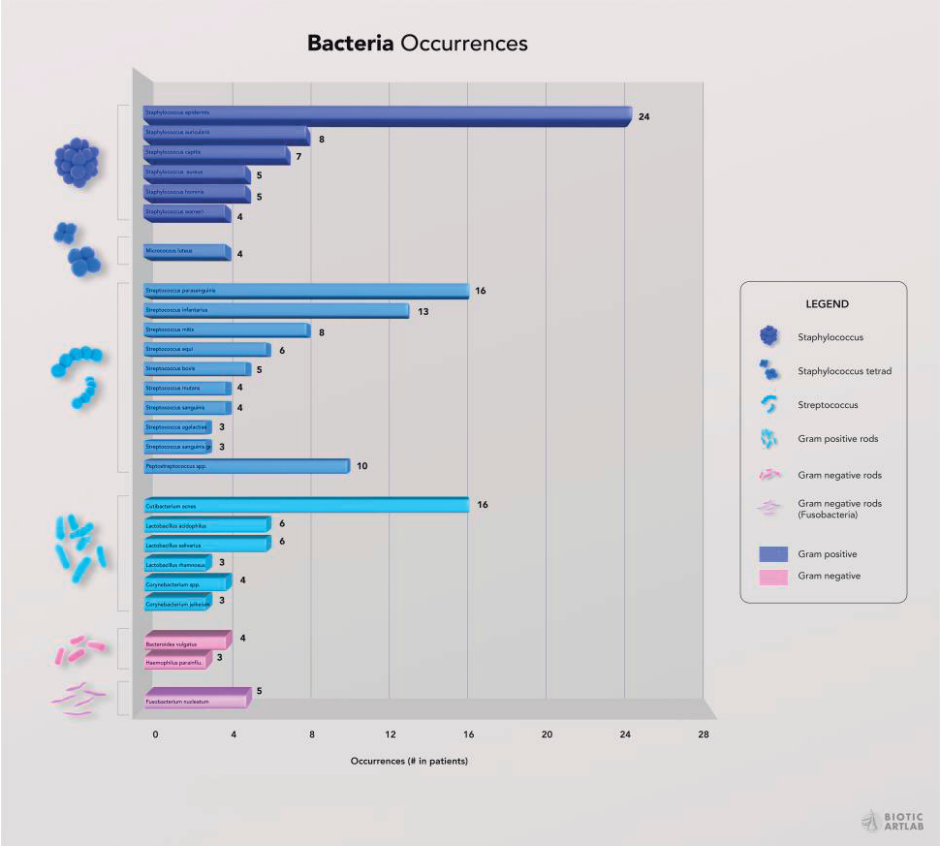

Figure 5. The occurrences of different bacteria. 27 types of skin bacteria were found to occur on 2 or more patients. 42 other types of bacteria occurred in 2 or fewer patients and were not included in this figure.

Although no microscopy was performed, many of the samples taken macroscopically showed filamentous opacities, which is suggestive of biofilm formation. When analyzed on species level, each patient generally showed a dominance of a single species, e.g., streptococcus pyogenes or staphylococcus epidermidis. For the entire patient group, an abundance of different bacteria from the FAFV phyla was found, but with only one dominant species per patient.

This may be indicative of long-term formation of a microbial biofilm, where over time some bacteria benefit more from the environment than others, i.e., circumstances may be more favorable for some bacteria than for others, which can lead to selective outgrowth of a single species (or a very limited number of species). Although the presence of bacteria does not necessarily indicate an infection, we found FAFV bacteria to be significantly more abundant in diseased individuals than in non-diseased ones, concurring with the first two postulates of Koch. This suggests a causative relationship.

It is possible that the bacteria found in the biopsy originated from contamination on the patient's skin during sampling. Other studies investigating the role of bacteria in adverse events of soft tissue fillers have raised this issue,[15] but these studies did not compare skin swabs and tissue samples. In our study this contamination is unlikely; as shown in Figure 2, no correlation between the bacteria phyla surrounding the filler in comparison to the bacteria phyla from the skin swabs was found in individual patients. However, it is quite possible that bacteria from the skin microbiota were co-injected with the filler material, giving rise to a bacterial biofilm with a very distinct signature as compared to the original skin microbiota caused by selectional pressures within the host environment. Therefore, the use of a disinfection agent (e.g. chlorhexidine) for cleaning of the skin before a filler treatment is very important.

In late-onset inflammation after filler injections, the type of antibiotic to be chosen is subject to debate. Many studies have been published on this topic, with many antibiotic treatment options being presented,[16-18] which is due in part to geographical differences in bacterial resistance. However, our data indicate that antibiotics active against gram-positive bacteria, such as vancomycin, may be the best choice. Additional support with an antibiotic that penetrates biofilms, such as rifampicin, can be advantageous. Of course, removal of the filler material with biofilm should still be the cornerstone of every therapeutic regimen.

In conclusion, our findings suggest a causative role for bacteria, probably in biofilm formation, in the development of late-onset inflammatory adverse events to soft tissue fillers.

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PART IV

IMMUNOLOGICAL ORIGIN FOR ADVERSE EVENTS AFTER SOFT TISSUE FILLER INJECTIONS

CHAPTER 6

Immediate nor delayed type hypersensitivity plays a role in late inflammatory reactions after hyaluronic acid filler injections

T.S. Decates*
J.A. Kadouch*
P.J. Velthuis
T. Rustemeyer

*Equally contributed

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ABSTRACT

Purpose: The exact etiology of late inflammatory reactions (LIRs) to hyaluronic acid (HA) fillers is currently unknown. Some argue these result from a hypersensitivity reaction, although evidence to support this is very scarce. Most reports on such reactions are not substantiated by positive skin tests. The purpose of our study was to determine whether immediate or delayed type hypersensitivity reaction follow hyaluronic acid (HA) filler injections.

Patients and methods: Twelve patients were referred for general allergic screening (patch-tests), as well as specific intradermal testing (injection of 0.1cc boluses) on the medial upper arm with a selection of several currently available hyaluronic acid (HA) fillers on the market. A positive allergic reaction was defined as erythema, firmness or swelling.

Results: During the 4 months follow-up no reactions to any of the tested HA fillers was reported. No correlation was found between results from the general allergic screening and a history with LIRs to HA fillers.

Conclusion: The results suggest that neither type I nor type IV hypersensitivity plays a role in late inflammatory reactions (LIRs) to hyaluronic acid (HA) fillers.

INTRODUCTION

Adverse events (AEs) in filler injections treatments are often classified into two types depending on the time of onset (Table 1). Most reports of delayed-onset complications of fillers are based on permanent fillers. However, recently several reports have been published on late inflammatory reactions to hyaluronic acid fillers, with erythema, edema and nodules at and in proximity to the injected sites of the face.[1-3]

For possible hypotheses for such delayed-onset complications exist, namely foreign-body reactions, microbial contamination (in biofilms or otherwise), type-IV hypersensitivity reactions, or adjuvant-based reactions (Figure 1).[4-16] Interestingly, all four etiological factors are believed to be capable of inducing granulomatous immune reactions.[6, 10, 17, 18] Also all filler agents used for soft-tissue augmentation are thought to elicit some degree of granulomatous inflammatory reaction following injection.[4,19] To a certain point this is considered to be part of a normal physiological response to fillers.[20,4]

A genuine granulomatous foreign body reaction (GFBR) is predominantly composed of histiocytes/macrophages and multinucleated giant cells encapsulating filler particles.[4,21] The exact pathophysiology of filler-induced GFBR, or 'filler granulomas', has yet to be elucidated. Current insights have shown that particles larger than 5 μm require the presence of aggregated macrophages, or MNGCs, to be phagocytosed.[22-24] Particles larger than 15 to 20 μm are generally not subject to true phagocytosis. Failure of effective phagocytosis leads to granuloma formation, consisting of macrophages and MNGCs, as well as a contiguous infiltrate of lymphocytes secreting pro-inflammatory cytokines (i.e., tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ) and interleukin 12 (IL-12)).[17]

Some authors have postulated that all granulomatous reactions to fillers are in fact type IV (delayed-type) hypersensitivity reactions.[6] Evidence to support hyaluronic acid as antigen inducing delayed-type hypersensitivity reactions is very scarce, since most cases reported skin-tests were not performed.

We therefore believe it could be of value to further investigate the role of HA-fillers in inducing delayed-type hypersensitivity reactions by using golden-standard intra-dermal skin-testing in patients with late-onset inflammatory AEs to hyaluronic acid fillers.

Early-Onset Adverse Events (days till weeks after injection)	Delayed-Onset Adverse Events (weeks till years after injection)
Injection-site reactions	**Infections (Biofilms)**
Erythema	Erythema
Edema	Edema
Ecchymosis	Pain
Pruritus	Inflammatory nodi
Pain	Ulceration
Technical Errors	**(Granulomatous) Type-IV hypersensitivity reactions**
Non-inflammatory nodi	Erythema
Asymmetry	Edema
Contour irregularities	Pain
	Inflammatory nodi
	Ulceration
Infections	**Foreign Body granuloma**
Erythema	Non-inflammatory nodi
Edema	Erythema
Pain	Edema
Inflammatory nodi	Pain
Abscess	Inflammatory nodi
	Ulceration
Type-I hypersensitivity reactions	**(Pseudo) Abscess**
Erythema	Fluctuating inflammatory swelling, infectious or sterile
Edema	
Urticaria	**Migration (dislocation) of filler material**
Angioedema	Non-inflammatory nodi
(Granulomatous) Type-IV hypersensitivity reactions	**Persistent skincolouring**
Erythema	Hyperpigmentation
Edema	Erythema
Pain	Telangiectasia
Inflammatory nodi	
Ulceration	
Vascular occlusion or emboli	
Tissue necrosis	
Blindness	
Skincolouring	
Tyndall-effect	
Hyperpigmentation	
Erythema	

Table 1. Overview of the different types of filler induced adverse events and clinical symptoms. Injection-site reactions should be interpreted as a brief physiological response to injection-trauma and need no intervention or treatment

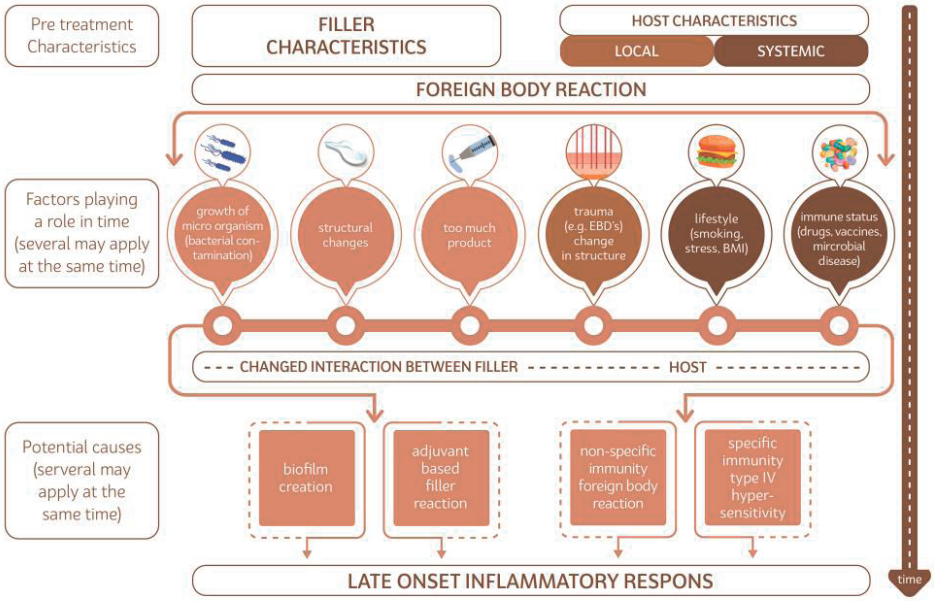

Figure 1. Oversight of the different etiological hypothesis for late inflammatory reactions (LIRs) to fillers.

MATERIAL AND METHODS

From February 2018 to July 2020 a total of 12 patients were enrolled in this prospective study. All were referred to the out-patient clinic of the Amsterdam University Medical Centre (Amsterdam, The Netherlands), for diagnostic evaluation of potential hypersensitivity reactions to hyaluronic acid (HA) fillers.

Only patients >18 years having experienced a late inflammatory response (LIR) in the face following treatment with HA fillers were included. Exclusion criteria were a still active LIR, pregnancy or intent for pregnancy, breastfeeding, any active inflammatory or infectious skin condition at the sites used for testing (arms and back), a known coagulopathy or use of NSIADs, use of steroids or other anti-inflammatory medication, alcohol and/or drug abuse, a severe or unstable (autoimmune) co-morbidity.

All included patients first underwent regular patch testing performed with the European baseline series, the regional series of cosmetic products, fragrance series and own cosmetic products according to recommendations of de Groot.[25] Allergens were tested using Van der Bend test chambers (Brielle, The Netherlands) applied on the back and covered with Fixomull stretch (BSN Medical, Hamburg, Germany). Readings were performed on day (D) 2, D3 or D4, and D7 according to ESCD criteria.[26]

Skin prick tests were performed with 0.05ml of the series of inhalation allergens (ALK, Almere, The Netherlands) and intracutaneous tests with 0.1ml of six fillers which were tested on both inner sides of the upper arms in a randomized manner. The tested fillers were Juvederm® Volbella, Restylane® Kysse, Stylage® M, Belotero® Balance, Etermis® 2 (Figure 2). The skin prick tests and intracutaneous tests were read after 15 minutes. In addition, the intracutaneous tests were read after D2, D3 or D4, and D7. Potential late reactions were monitored after 2 and 4 weeks, and patients were instructed to contact the department in case of any later reaction. A positive allergic reaction was defined as redness, firmness, pain or swelling at the site of injection.

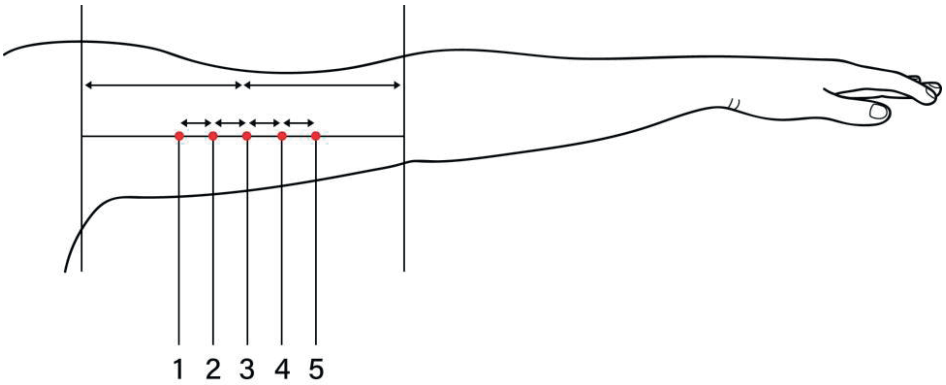

Figure 2. Design of intradermal testing protocol.

All study patients provided written informed consent for the treatment procedure. The study was conducted in accordance with guidelines of the Declaration of Helsinki.

RESULTS

A total of twelve individuals could be included, all female (aged 38-66, mean age 52). Two of which had an atopic history: one patient was known with atopic dermatitis, the other with allergies for cats, avocado and nickel. All patients had been treated with an HA filler before the LIR occurred. In 1 case a permanent filler (liquid injectable silicone) had been injected 20 years ago at the same location (lips).

The affected site concurred with the injected sites in all cases and consisted of the tear trough (n = 2), nasolabial fold (n = 1), marionet lines (n = 1), lips (n = 5), cheeks (n = 2) and full face (n = 1). The reported inflammatory symptoms consisted of erythema (n = 3), swelling/edema (n = 6), nodules (n = 6). The LIRs had been treated by the patient's own physicians with hyaluronidase (n = 7), intralesional corticosteroids (n = 1), intralesional laser therapy (ILT, n = 2) and excision (n = 2).

Patch testing showed positive reactions in three patients. Positive allergens in the first two patients were nickel (n = 2), amerchol-101 (n =1). The third patient was positive for 4-tert-butylphenol butylcarbamate (IPBC), turpentine peroxide, methyl methacrylate and several other reactions on acrylates. No positive reactions were found in the inhalation skin prick tests, or any of the intracutaneous filler tests neither immediately or during the 4 months follow-up.

DISCUSSION

Reviewing the potential sources for the late onset inflammatory reactions, one has to consider every factor that may change in time, both in the filler substance as in the host. These are given in figure 1 and are systematically discussed below.

Filler Characteristics (1): Growth of micro-organisms

Micro-organisms seem a very plausible cause of the delayed inflammatory response in LIR's. In particular, bacteria with low virulence, as e.g., Staphylococcus epidermidis, need time to colonize the filler material. The still controversial biofilm hypothesis states that the mix of bacteria form a slime that protects them from the host immune system.[6,7,10,27-29] Depending on environmental condition one or a small number of the bacterial strains will have a competitive advantage and over time will outgrow the others. At some point these strain(s) can reach numbers that provoke an inflammatory response. Indeed Decates *et al.* found bacteria to be present in a substantial number of samples from inflamed filler sites *(submitted)*. These were almost exclusively gram-positive bacteria with low virulence, i.e., S. epidermidis, Cutibacterium acnes, and others common in skin flora.

Filler Characteristics (2): Structural changes in chemical composition of the filler substance

Degradation of cross-linked HA filler may expose trace substances of BDDE, bacterial proteins, etc. Also, low molecular weight hyaluronic acid (LMW-HA) is believed to exert a direct pro-inflammatory action.[30] Gradual exposure or release of these molecules could trigger the immune the system.

Filler Characteristics (3): Too much product, Faults in choice of filler type or injection technique

Too much filler or filler with incorrect features for a specific area may lead to an immune response which would not have occurred with adequate use of the product. For example, it is known that filler nodules can appear over time by incorrect use or incorrect positioning of filler material, muscle- or gravity-induced displacement or accumulation and capsular contraction.[31] Some filler should not be used in the dynamic areas of the face, others have rheological

properties making them unsuitable for superficial placement. Doing so might prolong or even sustain an inflammatory healing response following implantation.[31, 32]

Host Characteristics: Local, Trauma

Over the years we have observed three cases of patients with local trauma, one after full face fractional CO2 laser treatment, one after 35% TCA peeling of the whole face and one after a scooter accident, that developed palpable and tender nodules on cheeks and/or nasolabial folds. These nodules were hypo-echoic and identified as HA fillers. These resolved after treatment with hyaluronidase.[33]

Host Characteristics: Systemic, Lifestyle change / concomitant systemic disorders

Many colleagues, as we did, have seen cases of LIR in patients after chemotherapy for various cancers. Apparently, the cancers or the effects on the immune system brought about by the chemotherapy changed filler-host interaction sufficiently to provoke a clinically significant reaction. Also, we observed LIR in a patient that lost 60 kilograms of body weight voluntarily (unpublished case dr. Velthuis).

Host Characteristics: Systemic, Altered immune status

Several articles describe the onset of LIRs after alterations in the immune status due to (suspected) infections or flu-like symptoms.[1,6,7,10,27-29] The exact pathophysiological mechanism by which an infectious process induces a LIR is unknown, though probably the clinically non-apparent interaction between filler substance and host immune system is altered and leads to a late-onset inflammatory response. Also, recently COVID-19 vaccination has led to LIR.[34]

We now continue to discuss the potential causes for inflammation in the above-mentioned circumstances.

Specific immunity

The most striking observation of this study is the absence of any response on skin testing of each of the six used HA fillers on the arms in any of the twelve patients. It must be noted that a true allergic reaction is a systemic immune response that should affect all injected sites.[7,35] Since the filler aliquots were injected intradermally, and the degradation of the used HA fillers takes approximately 6-12 months, both type-I and type-IV hypersensitivity reactions are investigated by this approach. Aside from our study limitations, the results of this study suggest that a systemic immune response as etiological basis for LIRs is not probable.

Many authors have postulated that LIRs arise as a consequence of (type IV) hypersensitivity reactions. However, in a literature search on this topic we found only two studies describing true allergic diagnostics on suspected delayed type hypersensitivity reactions.[36,37] Micheels

investigated 8 patients with LIRs to Restylane® (Q.Med, Uppsala, Sweden) and Hylaform® (Biomatrix, Inc., Ridgefield, NJ, USA) using intradermal testing.[37] Of the seven patients with positive reactions, five patients reacted positive to Restylane®, and five to Hylaform®. Histologic samples showed foreign body reactions with giant cells, however no eosinophils. The article was not clear as to how or if the LIRs were treated and if the LIRS reoccurred, something one might expect if untreated and caused by a hypersensitivity reaction. Lowe *et al.* published in 2001 their results of dermal tests performed in five patients with LIRs suspected to be delayed type hypersensitivity reactions.[38] Of the five tested patients, four tested positive. Histologic analysis again showed foreign body reactions with lymphocytes, giant cells and plasma cells, but no eosinophils. All lesions resolved with intralesional triamcinolone 3mg/ml injections, the use of intralesional hyaluronidase injections was not mentioned. In a recent study by Turkmani *et al.* 14 patients suffered LIRs following influenza-like illness.[2] The authors diagnosed the LIRs as delayed hypersensitivity reactions based on clinical presentation, however no epidermal/intradermal allergic testing was performed. Symptoms were treated with oral corticosteroids only (n = 10) of with a combination of oral corticosteroids and intralesional hyaluronidase injections (n = 4). In all cases with complete resolution. Based on these results one would expect that a true delayed type hypersensitivity reaction would persist/reoccur after ending the corticosteroid treatment in patients not treated with hyaluronidase, making this diagnosis less likely in corticosteroid-alone cohort (n = 10). In addition, a bacterial/biofilm etiology would also not resolve after treatment with corticosteroids alone. We must conclude that at least in a number of cases of LIRs, a pathophysiological etiology other than delayed hypersensitivity must be in play.

Non-specific immunity

The generation of a granulomatous foreign body reaction (GFBR) after implantation of a biomaterial, or 'foreign body', is considered a normal physiological response from the host to any foreign body.[4,19] No definition currently exists that differentiates between a physiological and a pathological GFBR.[4,20] Within minutes after implantation, host plasma components are absorbed on the surface of the bio-implant, after which platelets and other components of the coagulation cascade induce clot formation. Platelet adhesion/activation and the release of pro-inflammatory cytokines, chemokines and growth factors sequentially induce the acute and chronic parts of the inflammatory response.[32] Damage-associated molecular patterns (DAMPs), pathogen-associated molecular patterns (PAMPs) and Alarmins present at the implantation site activate the innate immune response through pattern recognition receptors (PRRs), Toll-like receptors (TLRs) and C-type lectin on macrophages, leukocytes and dendritic cells. In the acute phase, neutrophils attempt to degrade the biomaterial through phagocytosis and the release of reactive oxygen species, proteolytic enzymes and neutrophil extracellular traps (NETs, consisting of granular proteins, chromatin DNA, elastase, histones).[32] NETs are also involved in trapping pathogens and prevention of infection spread. This inflammatory phase must slowly

be replaced by an anti-inflammatory phase with secretion of anti-inflammatory cytokines (i.e., IL-10) and recruiting fibroblasts for effective tissue regeneration. The shift towards an anti-inflammatory healing response at the end of a 'normal' foreign body response can be impeded by specific (physicochemical) characteristics of the implanted biomaterial.[31,32] For example, failure of effective phagocytosis (*e.g.* particles larger than 15 to 20 µm are generally too large for ingestion by macrophages or giant cells) leads to a chronic inflammation pathway and granuloma formation, [17,22-24,32] Similarly, excessive production of NETs by neutrophils can impair healing and lead to a chronic inflammation and encapsulation.[32]

Adjuvant based filler reaction

Some authors have postulated that fillers act as adjuvants, rather than as antigens.[8,12,39] Adjuvants are defined as substances that may stimulate immune responses without having specific antigenic properties themselves.[40] Adjuvants are believed to influence both the innate and adaptive immune systems by mimicking evolutionary conserved molecules (*e.g.* PAMPs, DAMPs, Alarmins) capable of binding TLRs, causing the release of T_h1 inflammatory cytokines and increasing the activity of dendritic cells (DCs), lymphocytes and tissue macrophages.[40] Infection, trauma and vaccination may trigger adjuvant activity or act as adjuvants themselves.[8,41,42] The risk of abnormal immune responses is believed to be increased by sequential exposure to (different) adjuvant stimuli.[8] Shoenfeld *et al.* introduced the name 'autoimmune/ inflammatory syndrome induced by adjuvants', in short 'ASIA' or Schoenfeld's syndrome, to describe the spectrum of immune-mediated systemic diseases that may be triggered by previous exposure to an adjuvant stimulus.[43] Whether an adjuvant-induced immune response remains limited or evolves into a full-blown systemic disease (*i.e.* ASIA) is believed to depend on specific adjuvant characteristics and the extent in which innate, adaptive and regulatory immune responses are activated.[8,17,18] A genetic predisposition for the development of ASIA has also been postulated.[8,17]

CONSIDERATIONS

Adjuvants that have been reported to be able to cause ASIA are silicone, aluminum salts, pristane and certain infectious components.[4] Interestingly, also acrylamides and hyaluronic acid compounds have been identified as potential adjuvants.[8,17,18,22] HA-fillers functioning as adjuvants and causing inflammatory TLR-mediated responses through the innate immune system might explain several inconsistencies found in clinical practice and world literature on the topic of LIRs.[2,6,7,30,43] For instance, several authors support a prominent or leading role for bacterial contamination or infection in the origin of LIRs.[2,6] This hypothesis conflicts with the many negative cultures described in the literature, but also with all the positive results reported with anti-inflammatory (*e.g.* corticosteroids) treatment alone. Some have reported complete

resolutions of LIRs using a treatment plan mainly composed of antibiotics and without corticosteroids.[3] However, the antibiotics that were used are known for their immunomodulatory effects and were used simultaneously with intralesional hyaluronidase injections that dissolve the HA filler.

Analogous to these inconsistencies, the diagnosis of delayed hypersensitivity reaction mediated LIRs has its difficulties. Many have described resolution of the LIRs without dissolving or removing the filler.[1,2,44] If all LIRs were caused by delayed hypersensitivity reactions, one would expect inflammatory symptoms to reoccur after ceasing immunosuppressive / immunomodulating therapy, for as long as the filler remains in situ.

A leading role for the innate immune system, possible mediated by adjuvant characteristics of filler materials in genetically predisposed patients, might explain why similar clinical symptoms (*i.e.,* LIRs) have been associated with such diverse therapies, both positively and negatively. Also, bacterial presence would not necessarily mean they act through a biofilm or infection but may execute their (adjuvant) effect by triggering the local innate immune system. It might even be that some patients are genetically predisposed in having a 'sensitive' innate immune system, explaining why some individuals develop LIRs and others not.[17]

Future perspectives

To elucidate the pathophysiology of LIRs more research is necessary. LIRs are known to be invalidating for patients and challenging for physicians. When investigating LIRs authors often look for a common denominator as cause for all LIRs. However, we must keep in mind that different etiologies may give similar clinical phenomena. This is why future research should focus on the different aspects of host immune responses, such as secreted cytokines, upregulated membrane receptors and induced immune pathways. Genetic predisposition for developing LIRs might be elucidated by HLA-typing. But most importantly, authors should refrain from postulating diagnoses and conclusions based on inadequate investigations.

Limitations

This study investigated hypersensitivity reactions to fillers in patients having experienced LIRs. Although we tried to include as many patients as possible, the cohort was limited due to lack of includable individuals. In addition, the diagnostic protocol would not allow all fillers available on the market to be tested, since this number is substantial.

CONCLUSION

In our discussion we evaluated the pathophysiology of LIRs in a broad perspective. Amongst the etiological hypotheses that exist in the scientific literature are biofilms, hypersensitivity reactions, filler characteristics, host genetic predisposition and changes in filler substance of host immune status over time. A concise but complete summery of potential causes is given in figure 2. Although LIRs have often been diagnosed as hypersensitivity reactions, in our study no positive reactions to the six used HA fillers were reported. Both type-I and type-IV hypersensitivity reactions were investigated by our diagnostic protocol. The results suggest that neither type I nor type IV hypersensitivity plays a role in late inflammatory reactions (LIRs) to hyaluronic acid (HA) fillers.

DISCLOSURE

The author reports no conflicts of interest in this work.

Ethical Approval

This study was approved by the local research ethics committee (reference number: MEC-2016-660) and was performed in accordance with the ethical standards set forth in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

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CHAPTER 7

No association found between late-onset inflammatory adverse events after soft tissue filler injections and the adaptive immune system

T.S. Decates
P.J. Velthuis
R. Jhingoerie
S. Gibbs
F.B. Niessen

Submitted

ABSTRACT

Background

To date, it is unknown why some individuals develop late-onset inflammatory adverse events after treatment with fillers. These events may result from various factors, including an immunological response of the adaptive immune system.

Objective

In a pilot study we looked for evidence that is there a relation between late-onset inflammatory adverse events and the presence of immune cells surrounding the injected filler.

Methods & Materials

We included 47 patients, of whom 20 experienced late-onset inflammatory adverse events to different fillers (inflammatory group) and 27 who did not (reference group). A biopsy was taken from the area of the adverse event. Hematoxylin-eosin staining and immunohistochemistry analysis with CD3 (T-cells) and CD68 (macrophages) on paraffin tissue sections was used to assess the biopsies.

Results

Immune cells were found in biopsies obtained from 18 of 47 patients: in 9 biopsies from the inflammation group and 9 from the reference group. All these 18 cases showed CD68-positive immune cells. Virtually no CD3-positive immune cells were found.

Conclusion

Our results indicate that there is no T-cell activity in biopsies from areas with late-onset adverse events after filler injections. The macrophages found in the biopsies are probably not responsible for the inflammatory response.

INTRODUCTION

Since the first soft tissue fillers (STF) were injected for aesthetic purposes, adverse events have occurred.[1-2] The STF market has grown rapidly and has been paralleled by an increased incidence of adverse events, which can lead to lifelong trauma.[3,4]

Four types of resorbable STF are currently available: calcium hydroxylapatite, poly-L-lactic acid, polycaprolactone and hyaluronic acid. Non-resorbable STF consist of materials such as medical grade silicone, polyalkylimide, polyacrylamide and methacrylate.[5-7] Studies on adverse events have suggested incidences of 0.3% to 0.4% for resorbable STF and 5% for non-resorbable STF.[8-11]

Several studies have indicated, both clinically and histologically, that most of the adverse events to STF injections present as an inflammatory response.[5,12] Patients with these inflammatory adverse events present with erythema, edema and nodules at or in proximity to the facial injected sites.[13,14] These adverse events seem to have an immunological basis. Although the pathophysiology is unclear, an exacerbated immune response against foreign body material can play a role, whereby the filler itself, or its degradation products, may act as adjuvants more than as T-cell activators.[15] Adjuvants, as defined by the National Cancer Institute, are agents that stimulate the immune system in a non-specific way.[16] Some authors have postulated that inflammatory adverse events to STF result from type IV (delayed type) hypersensitivity, a reaction type formed by adaptive immunity.[5] These inflammatory adverse events often do not respond well to the regular treatment procedures. Defining the exact etiology could be helpful in treating them.

In this study, we therefore addressed the following research questions: Are late-onset inflammatory adverse events associated with the presence of immune cells surrounding the injected filler? If so, what types of immune cells are involved, i.e., is this a reaction of the innate (non-specific) or the adaptive immune system?

To identify immune cells, we used microscopic assessment of extracted material with hematoxylin-eosin staining (HE) and immunohistochemistry probes (IHC).

MATERIAL AND METHODS

We used a convenience sample of 47 patients of the Dermatology Department, Erasmus University Medical Center (Rotterdam, the Netherlands) in the period between 2016 and 2018. The local medical ethics committee approved this study.[17] All participants provided written informed consent.

An adverse reaction of the body to soft tissue fillers (STF) led to a thick and concentrated 'clump' of cellular debris. We took biopsies of this clump and identified the immune cells as described in the Methods. We included patients who were willing to undergo a biopsy of the STF at our specialized outpatient clinic for adverse events after STF injection. Two groups were defined: an inflammation group and a reference group. The inflammation group consisted of 20 patients who experienced an inflammatory adverse event. The reference group (control group) consisted of 27 patients who did not experience an inflammatory adverse event.

In the inflammation group, an adverse event was defined as the appearance of two or more of the following clinical symptoms/signs three months or longer after initial filler injection: skin induration, edema, nodules with or without tenderness, with or without fistulation or discharge of pus or filler material. The reference group consisted of patients treated with STF at least three months prior to inclusion who did not have any of the above-mentioned adverse events. Cases with isolated soft lumps due to migration of the filler substance, but without any of the above-mentioned adverse events, were also included in the reference group. Both groups completed a questionnaire assessment that included items on ethnicity, autoimmune diseases, smoking status, allergies and location of the injection.

The inflammation group consisted of 20 patients who experienced an inflammatory adverse event. A reference group of 27 patients, who did not experience an inflammatory adverse event, was used as a control group.

Laboratory methods

Biopsies were fixed utilizing buffered 4% formaldehyde for 24 h, subsequently embedded in paraffin and sectioned and routinely stained by hematoxilin-eosin (HE) and immunohistochemistry

staining (IHC) for visual immune cell detection.[18] Immunohistochemical staining on paraffin-embedded 5-µm sections was performed with CD3 and CD68 (all Dako/Agilent, Santa Clara, California, USA, dilution 1:100).[19] Two investigators (RJ and TD), who were blinded to the injected filler type or patient outcome, viewed and assessed the stained slides.

Data analysis

Statistical Analysis

Descriptive statistics of demographic variables were used to characterize the study population and to evaluate similarities between the inflammation group and reference group. Interval estimates of proportions were calculated with the Wilson score method. To assess the association between the presence of immune cells surrounding the injected soft tissue filler and the occurrence of adverse events, contingency table and logistic regression analyses were performed. For all analyses, the significance level was set to .05. Analyses were conducted with the SPSS software (IBM Corp. Released 2017. IBM SPSS Statistics for Apple, Version 25.0. Armonk, NY: IBM Corp.).

RESULTS

Table 1 provides descriptive statistics on several demographic variables for the inflammation group and reference group. As shown in the table, the differences between the two groups were small or non-existent; except for ethnicity, they were statistically non-significant.

		Inflammation	Reference	χ^2	p-value	Fisher's exact test
Gender	Female	17	23	0.00	.986	1.0
	Male	3	4			
Age (in years)	Mean (SD)	62.2 (9.9)	61.9 (7.5)	t = 0.14	.892	
Ethnicity	Non-caucasian	3	0	4.33	.038	.070
	Caucasian	17	27			
Smoking	Yes	4	8	0.56	.454	.517
	No	16	19			
Autoimmune diseases	Yes	7	6	0.94	.333	.511
	No	13	21			
Filler type	Non-Resorbable	19	24	0.55	.458	.626
	Resorbable	1	3			

Table 1. Descriptive statistics for Inflammation and Reference group.

In 18 out of the 47 patients, immune cells were detected in the biopsy (38.3% (95%-CI 25.8% to 52.6%)). As shown in Table 2, immune cells were present more often in the biopsies from the inflammation group (50.0% (95%-CI 29.9% to 70.1%)) compared to the reference group (29.6% (95% CI-15.9% to 48.5%)). However, the absolute risk difference of 20.4% (95%-CI -7.2% to 44.7%) was not statistically significant according to a likelihood ratio test, $\chi^2 = 2.02$, $p = .156$ (Fisher's exact test $p = .226$). A logistic regression analysis showed a fairly wide interval estimation of the odds ratio (OR) (2.38), indicating that the odds of finding immune cells could be up to 7.9-fold higher or 0.7-fold lower for the inflammation group than for the reference group.

Immune cells present	Inflammation	Reference	Total	OR (95% CI)	p-value
No	10	19	29 (62%)	2.38 (0.71, 7.92)	.156
Yes	10	8	18 (38%)		
Total	20 (43%)	27 (57%)	47 (100%)		

Table 2. Presence of immune cells surrounding the injected filler for Inflammation and Reference group.

Subsequent immunohistochemical analysis with CD3 and CD68 staining revealed that in all 18 cases where immune cells were detected in the biopsy, these cells consisted of CD68-positive cells (100% (95%-CI 82.4% to 100%)). No significant presence of CD3-positive cells was detected in any of these samples, thus excluding T-cell involvement at the soft tissue filler treatment site of the patients. A number of cells were CD3 negative and CD68 negative indicating that other cell types were also present in the fillers (Fig 1).

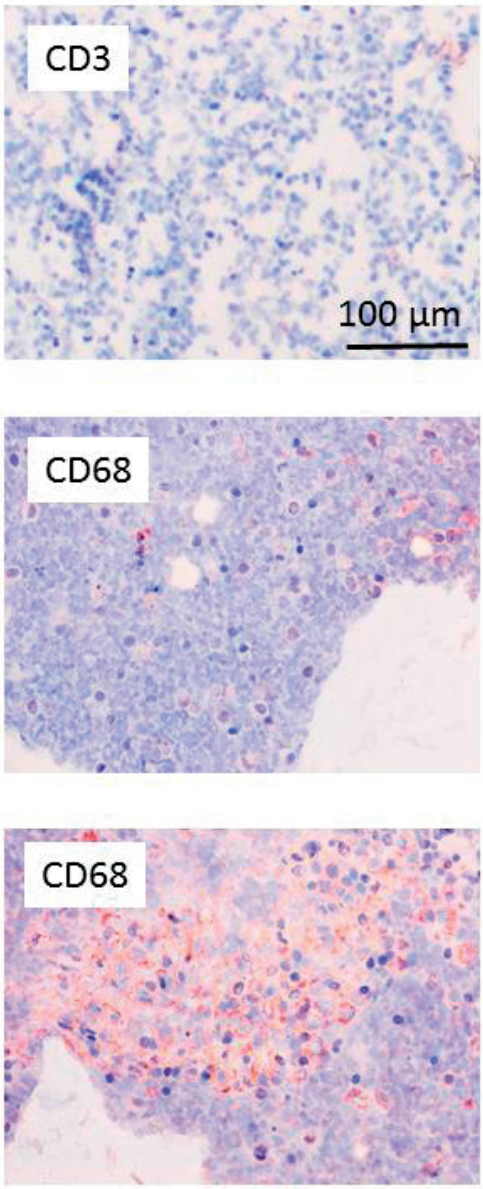

Fig 1. Paraffin-embedded tissue sections were immune stained with either anti-CD3 or anti-CD68. Representative images show absence of CD3-positive cells (upper panel), sporadic (middle panel) and dense clusters (lower panel) of CD68-positive cells in capsules.

DISCUSSION

In this study no statistically significant association between late-onset inflammatory adverse events and the presence of immune cells surrounding the injected filler was found. However, in the samples that tested positive for immune cells, CD68-positive cells were present. This means that in our study macrophages were found in the biopsies along with other yet unidentified CD3 negative, CD68 negative cells.[20] The foreign body reaction is the end-stage response of the inflammatory and wound healing responses following implantation of a medical device, prosthesis, or biomaterial.[21] Macrophages are known to play an important role in the body's defense system after the injection of STF.[21-23] Foreign body reactions can lead to a foreign body granuloma, which are formed by aggregation of macrophages after phagocytoses by macrophages fails.[24-26]

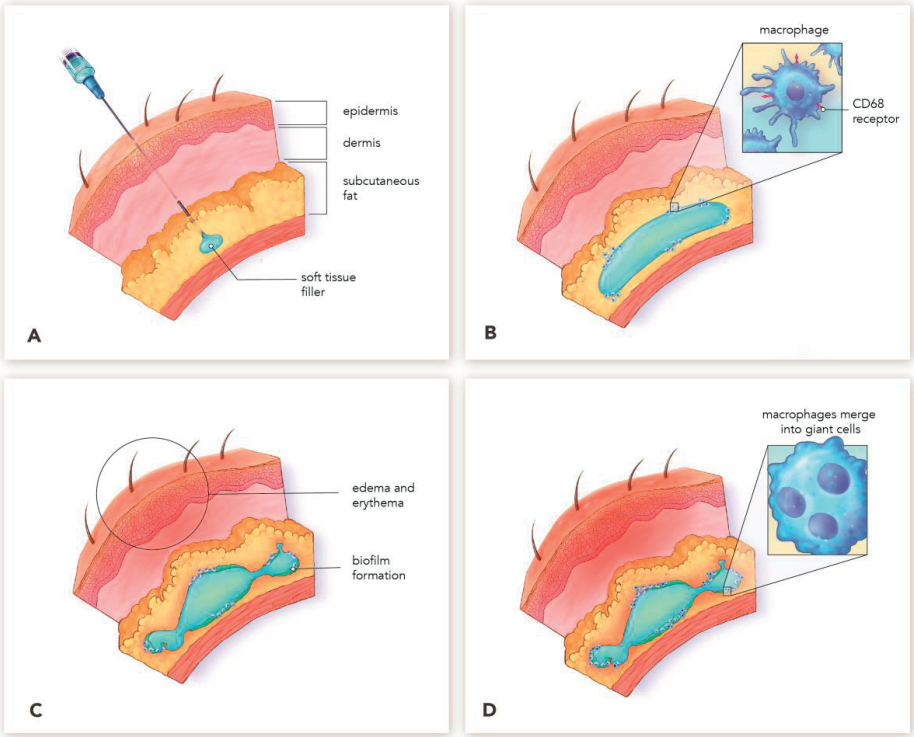

Fig 2. Hypothetical model for late-onset inflammatory adverse event. A: Soft Tissue Filler injection. B: Mobilization of macrophages by the innate immune system. C: Biofilm formation in combination with edema and erythema. D: Late-onset inflammatory adverse event after macrophages have merged into giant cells.

CD3 antibody is a marker for T-cells,[27] which are part of the adaptive immune response.[28] None of our samples had any significant presence of CD3 immune-positive cells. This essentially

excludes T-cell involvement in the adverse reaction at the STF treatment site of the patients in our study. We found no previous studies showing the presence of T-cells being in other implant-related inflammations, but T-cells have been shown to be associated with breast implant-associated anaplastic large cell lymphoma (BIA-ALCL).[29] This is an uncommon T-cell lymphoma that typically presents as a spontaneous periprosthetic fluid collection or a capsular mass on the implant.[30] One hypothesis is that after breast augmentation with implants, a polyclonal reaction consisting of macrophages and T-cells may eventually lead to monoclonal T-cell proliferation.

Some authors have hypothesized that contamination with low virulence bacteria in the form of biofilms can lead to inflammation after filler injection.[7,8] However, in another previous study we analyzed samples from the same patient group for bacteria using a highly sensitive PCR test, which showed high levels of bacterial contamination (Decates et al., submitted). One possibility is that low virulence microorganisms do not provoke neutrophils, but macrophages instead. There is some evidence that macrophages may even promote biofilms. Miranda et al. recently demonstrated the ability of macrophages to influence formation of *C. albicans* biofilm, associated with the prooxidant-antioxidant balance present in biofilm-macrophage co-culture.[31]

Histopathological studies of the excised tissues surrounding the hydrogel implants have indicated that the tissue response progressed from an initial acute inflammation to the chronic inflammatory response characterized by the migration of macrophages.[32] Several other studies have reported that macrophages are the 'first line of defense' against medical device implants.[33-35] An in vivo study by Jeyanthi and Rao (1989) with non-resorbable SFT collagen-p(HEMA) hydrogels showed the presence of macrophages, and other studies have reported that failed total hip replacement is associated with the presence of macrophages.[32-36] If bacteria are brought in with the initial injection of the STF, they are quickly attacked by macrophages. By the time a biofilm is formed, therefore, bacteria and macrophages are constant in a duel for survival.

The fact that we found CD68+ macrophages but no CD3+ T-cells has two potential explanations. First, different kinds of materials may elicit different kinds of foreign body reactions. Initially, all foreign bodies elicit the migration of macrophages, but over time these reactions follow different paths. For example, BIA-ALCL may indeed be provoked by T-cell stimulation, but this has never been reported after STF implants even with tens of millions STF injections each year for decades. Second, the different reactions could be related to the surface area and quantity of foreign body material used. The amount of material used in STF is small, the area of biofilm is small, and bacteria can be attacked by macrophages. Breast prothesis contain more material, have a larger surface areas and a large biofilm; over time, macrophages are insufficient to control bacteria, and T-cells are needed to suppress the late-onset inflammation.

In conclusion, our findings indicate that T-cells do not play a role in late-onset inflammatory adverse events after STF injection thereby suggesting that there is not an association with an adaptive immune response. Although macrophages were prominent, the innate immune system does not appear to be responsible for this inflammatory response since macrophages were also found in the reference group. To predict the change of adverse events after STF injections, one possibility for future research is to look for genetic predisposition for adverse events after STF injections.[36]

The main limitation of this study was due to the small patient group. The findings should therefore be interpreted with caution. To strengthen the evidence, this study should be replicated in larger patient groups.

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PART V

GENETIC ORIGIN FOR ADVERSE EVENTS AFTER SOFT TISSUE FILLER INJECTIONS

CHAPTER 8

Increased risk of late-onset adverse reactions relate to dermal fillers in patients bearing the HLA-B*08-DRB1*03 haplotypes

T.S. Decates
P.J. Velthuis
L.W. Schelke
N. Lardy
E. Palou
S. Schwartz
Y. Bachour
F.B. Niessen
T. Nijsten
J. Alijotas-Reig

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ABSTRACT

Background: Even though manufacturers claim that the dermal fillers are non-toxic and non-immunogenic, adverse events may occur. Clinically and histologically, most of the late onset adverse events present as an inflammatory response.

Objective: To assess whether HLA polymorphisms are associated with late-onset inflammatory adverse events related to dermal fillers.

Methods: A total of 211 patients were included, of whom 129 experienced late-onset inflammatory adverse events to different fillers (Inflammation group) and 82 who did not (Reference group). Patients completed a standardized questionnaire and provided a blood sample or oral swap for HLA testing.

Results: The study population consisted of 188 (89%) women and 23 (11%) men. The two study groups were similar in the distributions of filler type, location of injecting, allergy, autoimmune disease, gender, age, ethnicity, and smoking status. Of the 211 patients in the sample, 25 had the combination of HLA subtype-B*08 and HLA subtype-DRB1*03. This was 16.3% of the inflammatory group and 4.9 % of the reference group. This combination of HLA subtypes was associated with an almost four-fold increase in the odds of developing immune mediated adverse events (odds ratio = 3.79, 95% CI 1.25 to 11.48).

Conclusion: Genetic polymorphisms such as HLA combinations may identify patients at risk of developing late onset immune mediated adverse events to dermal fillers.

INTRODUCTION

An ever-increasing number of people undergo dermal filler injections for aesthetic indications or to reduce the signs of skin aging. Improvements that were previously achieved only by surgery can now be mimicked by dermal fillers treatment at lower cost and with limited-to-none recovery time. For aesthetic reasons alone, almost 2.7 million dermal fillers have been injected in the United States in 2018, a 50% increase since 2010.[1] A recent study in the Netherlands shows that each year, 1 out of 49 women receive at least one injection treatment.[2]

Physicians have different types of dermal fillers at their disposal, consisting either of resorbable or non-resorbable materials. Manufacturers claim that marketed fillers are non-toxic and non-immunogenic, which are requirements for any biomaterial intended for aesthetic uses. However, unwanted adverse events of various clinical relevance may occur in a low percentage of patients implanted with dermal fillers, such as medical grade silicone, polyalkylimide, hyaluronic acid, poly-L-lactic acid, polyacrylamide, or methacrylate fillers.[3-6] Reports suggest incidences of 0.3-0.4% for Hyaluronic Acid (HA)[7] and of 5% for acrylamides. [8-10] Early onset inflammatory adverse events start within two to four weeks after injection.[3] Late onset inflammatory adverse events to dermal fillers appear between 1 and 60 months post-implantation, with 14 months as mean period.[3]

Clinically and histologically, most of the late onset adverse events present as an inflammatory response.[3] In fact, a variety of adverse events has been associated with fillers, including granulomas, inflammatory nodules, angioedema, skin induration, human adjuvant disease, sarcoidosis and sarcoid-like disease, panniculitis, Sjögren syndrome, autoimmune thyroiditis, localized and systemic sclerosis, inflammatory myositis, autoimmune inflammatory syndrome related to adjuvants (ASIA) and cutaneous vasculitis.[14,15] Most of these adverse events seem to have an immunological basis, where the fillers act as adjuvants more than as direct T-cell activators, on a possible background of genetic predisposition.[16] Even though the origin of these complications is not completely clear, they can be due to exacerbated immune responses of the body against foreign body material.[16] Problems with long term safety and reversibility of adverse events due to foreign body material also occur in plastic surgery (breast implants)[11], cardiology (pacemakers)[12] and orthopedics (hip implants).[13]

When looking at the genetic predisposition leading to immune-mediated, adverse reactions, genes within the major histocompatibility complex (MHC) have been shown to be associated with auto-immune diseases as M. Bechterew.[17] And there is also speculation about the HLA subtype-B*08 and HLA subtype-DRB1*03 in immune mediated disorders in women with silicone breast implants.[18-22] Because of the low-prevalence of late-onset immune-mediated complications, we hypothesize about the potential role of genetic polymorphims affecting immune-related gene functions, as a subjacent cause.

Genetic polymorphisms are defined as the occurrence of multiple alleles at a given locus, where at least two alleles occur with a frequency greater than 1%.[23] Accordingly, the human leukocyte antigen (HLA) system is the most polymorphic region within the human genome.[24] The HLA system is a super locus that contains a large number of genes clustered on a 4 Mb-segment of the short arm of chromosome 6 in humans, related to immune system function. Indeed, HLA region comprises six major classical HLA loci that encode structurally homologous proteins which are classified into HLA class I (HLA-A, B, Cw) and class II (HLA-DRB, DQ, DP).[25]

In this candidate gene study, we tested all HLA subtypes A, B and DRB for their association with late-onset immune-mediated adverse reactions related to foreign biomaterials, used as implant fillers.

MATERIAL AND METHODS

Study Population

We used a convenience sample of 211 patients, of which 92 were from of the Systemic Autoimmune Diseases Unit at Vall d'Hebron University Hospital in Barcelona and 119 of the Dermatology Department, Erasmus MC, the Netherlands in the period between 2016 and 2018.

Late onset immune mediated adverse event was defined as the appearance of two or more local clinical signs of inflammation (i.e. edema, skin induration, swollen/tender nodules with or without fistulation or discharge of pus or filler material) 3 or more months after initial filler injection. Patients with these inflammatory complaints formed the Inflammation group (N=129). The Reference group (N=82) was formed by people treated with dermal fillers at least 3 months earlier, without any inflammatory complaints. Patients with isolated soft lumps without inflammatory signs were also included in this Reference group.

Patients with and without late-onset inflammatory complaints were compared through HLA genotyping and a questionnaire regarding age, ethnicity, smoking status, autoimmune diseases, type I or IV allergies, and location of the injection of the dermal filler.

HLA genotyping

DNA was obtained by means of oral swab or blood extraction. Briefly, DNA was isolated from the buccal swabs using QIA-AMP kit according to the manufacturer's instructions (Qiagen, Venlo, the Netherlands). Blood was collected from the antecubital vein of arms through Vacutainer method (Becton Dickinson, Franklin Lakes, NJ, USA). Samples were stored at -80 C until further analyses. Low-intermediate resolution genotyping of HLA-A, HLA-B and DRB alleles of all patients were performed by sequence-specific oligonucleotide probe (SSOP) methods using

the Luminex® microbead technology in Barcelona, Spain (Lifecodes HLA typing kits, Gen-Probe, San Diego, CA, USA) and in Amsterdam, Netherlands. (Sanquin Diagnostic Services, Amsterdam, the Netherlands). This method maps all alleles of the HLA-A, HLA-B and HLA-DRB subtypes.

Statistical Analysis

Descriptive statistics were reported comparing the distribution of patient and filler characteristics possibly associated with adverse reactions to filler injections between the inflammatory group and the reference group. The degree of association between adverse reactions and having a possible specific HLA-A, HLA-B and DRB alleles combination was estimated by the odds ratio (OR) with 95% confidence interval (CI) and Nagelkerke's R^2, along with a likelihood ratio (chi-square) test.

To assess the impact of potential confounders and effect modifiers on the estimated odds ratio, we first conducted contingency table analyses to see if there were any significant associations among the selected characteristics of the patients and both the presence of the HLA combination and the appearance of adverse reactions.

Variables considered as potential confounders or moderators were filler type, location of injecting, allergy, autoimmune disease, gender, ethnicity, and smoking status. A full assessment of possible confounding and effect modification was conducted. For all analyses, the significance level was set to .05. To evaluate the predictive properties of the logistic regression model we also calculated the sensitivity, specificity, and the area under the curve (AUC) parameters. Analyses were conducted with SPSS software (IBM Corp. Released 2017. IBM SPSS Statistics for Apple, Version 25.0. Armonk, NY: IBM Corp.).

The study was approved by the medical ethics committee of both University Hospitals.[26]

RESULTS

A total of 211 patients were eligible for this study, of whom 129 experienced late-onset inflammatory adverse events to different fillers (Inflammation group) and 82 did not (Reference group). Table 1 displays selected characteristics of the two groups. No systemic complaints potentially related to the filler adverse event was seen in the inflammatory group. The distributions of filler type, location of injecting, allergy, history of autoimmune disease, gender, age, ethnicity, and smoking status were comparable between two groups. Both groups were also comparable in types of fillers received, being non-resorbable ones (polyalkylimide, liquid silicone) and resorbable hyaluronic acid fillers (of various brands).

		Inflammation	Reference	χ^2	p-value
Gender	Female	114	74	0.18	.671
	Male	15	8		
Age (in years)	Mean (SD)	53.2 (10.6)	54.3 (10.0)	t = 0.69	.490
Ethnicity	Caucasian	120	77	0.06	.803
	Non-caucasian	9	5		
Smoking	Yes	28	26	2.63	.105
	No	101	56		
Autoimmune diseases	Yes	16	6	1.39	.239
	No	113	76		
Allergy	Drugs	10	5	1.41	.494
	Atopy	25	11		
	No	95	66		
Filler type	Non-resorbable	99	62	0.04	.580
	Resorbable	30	20		
Injection location	Peri-orbital	10	9	5.50	.358
	Lips	27	14		
	Nasolabial folds	35	29		
	Cheeks	26	12		
	Zygoma	27	18		
	Buttocks/legs	4	0		

Table 1. Descriptive statistics of patient characteristics for Inflammation and Reference group.
SD, standard deviation; χ^2, Chi-square value for test for independence.

Of the sample of 211, there were in total 25 patients with the HLA combination of HLA subtype-B*08 and HLA subtype-DRB1*03 (11.8%; 95% CI 8.2% to 16.9%). The proportion of patients with this specific combination was significantly higher in the Inflammation group (n=21, 16.3%) than in the Reference group (n=4, 4.9%), giving $\chi^2 = 6.98$, p = .008.

Table 2 presents the cross tabulation of the presence of HLA subtype-B*08, the presence of HLA subtype-DRB1*03, and the simultaneous combination for both the Inflammatory group and the Reference group. The presence of either the antigen HLA subtype-B*08 or HLA subtype-DRB1*03 was not significantly associated with the risk of developing dermal filler induced adverse reactions (Table 2). However, the combination of HLA subtype-B*08 and HLA subtype -DRB1*03 was associated with an increased risk of late- onset adverse reactions (Nagelkerke's $R^2 = .04$). The odds ratio was 3.79 (95% CI 1.25 to 11.48), indicating that the likelihood of developing adverse reactions may increase by a factor 3.8 in people showing this HLA combination.

The analyses showed that there were no other statistically significant associations among the selected characteristics of the patients and both the presence of the HLA combination and the appearance of adverse reactions (all p-value's > .10). Accordingly, based on the present model,

B*08/DR3	Inflammation	Reference	Total	OR (95% CI)	p-value[b]
No	108	78	186	3.79 (1.25, 11.48)	.008
Yes	21	4	25		
Total	129	82	211		
B*08	Inflammation	Reference	Total	OR (95% CI)	p-value
No	103	71	174	1.63 (0.76, 3.51)	.203
Yes	26	11	37		
Total	129	82	211		
DR3	Inflammation	Reference	Total	OR (95% CI)	p-value
No	98	68	166	1.54 (0.76, 3.10)	.224
Yes	31	14	45		
Total	129	82	211		

Table 2. Risk of adverse events[a] after dermal implantation for Inflammation and Reference group in relation to presence of HLA haplotype (B*08, DR3, or B*08/DR3 combined).
a. Adverse events include granulomas, inflammatory nodules, angioedema, skin induration, human adjuvant disease, sarcoid-like disease, cutaneous vasculitis.
b. Likelihood ratio test. OR, odds ratio; 95% CI, 95% confidence interval.

Table 3. Predictive measures.

Specificity	Sensitivity	AUC
.951	.163	.557

Note. The cut-off value is set to .70. AUC, area under the curve.

the probability of correctly classifying that a patient will experience adverse reactions is about 16.3% (Table 3). The AUC of 55.7% indicates that model-based classification is only marginally better (5.7%) than classification based on random guessing.

DISCUSSION

In this study we found a significant correlation between the combined presence of HLA subtype-B*08 and HLA subtype-DRB1*03 and inflammation in a group of patients with dermal fillers. We used clinical signs to distinguish between inflammatory and non-inflammatory reactions. Also, we had to rely on patient's clinical history regarding the absence of past adverse reactions. An inflammatory response to dermal fillers develops slowly and insidiously and may even spontaneously subside. In fact, it is conceivable that some of the subjects in the Reference group may develop an inflammatory response later in life or may have had sub-clinical symptoms. In this context, the HLA subtype-B*08 and HLA subtype-DRB1*03 combination was found in 5% of the Reference group. The expected percentage within the general population in Europe is 1.6%.[27] Also, in the inflammatory group some patients might have had a non-immunological

condition leading to inflammation, i.e., bacterial infection of the implant. Therefore, it can be argued that both groups are not homogeneous.

Accordingly, in this study positive HLA subtype-B*08 and HLA subtype-DRB1*03 combination has low sensitivity 16,26% and high specificity 95,12%. The HLA combination identifies only one out of six people at risk for an inflammatory adverse event after filler use. Further testing in prospective studies on larger groups and with tighter criteria may reveal higher sensitivity.

For this study HLA class I A, B, and HLA class II DR (and DR subtypes) were selected because of the relationship between HLA-B and HLA-DR and the predisposition to suffer autoimmune and/or granulomatous disorders.[17-22]

HLA-C was left out because of its very high polymorphic complexity and very complicated to relate with human pathology.[28] HLA-D or E appear to play a major role in the viral responses (NK cells and NK-KIR receptors) and in women, with the tolerance degree to embryo-paternal antigens by maternal NK cells and Tregs, but no relation to human diseases have been described as far as we know.[29]

This study suggests a link between specific HLA-subtypes and an immune mediated reaction to a foreign implant, meaning that some persons may be more prone to display a reaction than others based on their immunogenetic profile. We found that people with HLA subtype-B*08 and HLA subtype-DRB1*03 combination have a 3.8-fold increase in risk of developing inflammatory adverse events due to fillers in clinical practice.

Interestingly, certain auto-immune diseases like inflammatory arthritis are also related to the presence of specific HLA subtypes, in particular HLA-B27 and HLA-DR1-11, DR3 and DR4.[30,31] In addition to this, there is increasing evidence that adverse reactions to implanted materials like scleroderma-like syndromes are also related to certain HLA combinations.[24] Bell et al. demonstrated in 1996 that an increased frequency of HLA-A1, -B1 and -B2 in patients with chronic graft-versus-host disease (cGVHD) who developed scleroderma-like complications.[32] This suggests that a genetic predisposition may be linked to the development of more severe scleroderma-like symptoms in cGVHD. Of note, when looking to our patient group, the presence of the combination of HLA subtype-B*08 and HLA subtype-DRB1*03 was also associated higher rates of fibrotic reactions of the skin. None of the patients from the Reference group did show any of those reactions.

This study on late-onset inflammatory adverse events after dermal fillers injections can be a starting point in genetic research for the prediction of adverse events of dermal filler treatments. This study provides for the first time an *in vitro* method for the analysis of the genetic

predisposition of an individual to develop late onset immune mediated adverse events related to dermal fillers.

In conclusion, this is the first study to show that HLAs B*08 and DRB1*03 is associated with late-onset cutaneous inflammatory adverse events to dermal fillers injection, suggesting an immunological pathway for this problem in some patients. Although this HLA combination may be biomarker for these adverse events induced by biomaterials, larger studies including genetic polymorphisms such HLA subtypes are warranted before they can be used to identify people at risk of developing cutaneous inflammatory adverse events due to fillers in clinical practice.

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Conflicts of Interest: JA-R and SS have an issued patent related to the use of HLA haplotypes as risk markers of adverse reactions in dermal filler implantations (P201030604).

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PART VI

DIAGNOSTICAL OPTIONS FOR ADVERSE EVENTS AFTER SOFT TISSUE FILLER INJECTIONS

CHAPTER 9

Ultrasound to improve the safety after hyaluronic acid filler treatments

L.W. Schelke
T.S. Decates
P.J. Velthuis

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INTRODUCTION

Cosmetic medicine is a continuously growing field, including mini- mally invasive treatments with resorbable dermal fillers. Hyaluronic acid fillers are the most commonly used products. According to the American Society of Aesthetic Plastic Surgery, over 2.4 million treatments were performed with hyaluronic acid fillers in 2016.[1] As most patients treated are healthy people looking for a cosmetic improvement, the treatments performed should be as safe as possible. Although these fillers are known for a reliable safety profile, adverse events do occur.[2,3] Complications can be caused by the product itself (too strong cross-linking of the product), the product-host interaction (allergic reactions, inflammatory responses), or the injection technique performed (accumulation or dislocation of the product due to muscle movement, intravascular injection, or vascular compression of filler material).[3,4] In its most serious form, intravascular injection or vascular compression of filler material can lead to skin necrosis or, in rare cases, blindness.[5,6] It has been suggested that the minor signs of vascular compression may be misinterpreted as injection-related bruising, pain, and swelling.[7] Guidelines and other articles focused on hyaluronic acid fillers are published in order to minimize potential damage to skin and underlying tissue.[8-11] Hyaluronic acid fillers come with the advantage of being dissolvable with hyaluronidase in case of complications.[12] If this is necessary, identifying the location of the filler in the skin is important as hyaluronidase should be injected into the filler mass. However, when the filler is placed deep dermally, detection can be very difficult. Doppler ultrasound (duplex) is commonly used in dermatology to evaluate dermatological conditions of the skin and vascular structures,[13,14] specifically in the diagnosis of venous disease of the lower leg. Yet, it can also help to improve the safety of hyaluronic acid filler injections in two distinct ways. First, it is possible to identify the filler in case of a complication.[15,16] Second, prevention of complications will be improved by locating the important vascular structures and earlier filler treatments in the projected area before a new treatment is performed.

ULTRASOUND FOR FILLERS

An ultrasound device consists of a probe and a processor. The probe will generate a sound wave that penetrates body tissue. Sound waves interact with the tissue and become progressively weaker in strength as the waves are absorbed or scattered. Part of the sound waves is being reflected. The reflected sound waves, picked up by the probe and directed to the processor, are transformed into a digital image. Based on echogenicity Table 1, a filler, or its reaction in tissue, will be imaged as hyperechoic (white on the screen), hypoechoic (gray on the screen), and anechoic (black on the screen). Tissues are isoechogenic if they show the same echogenicity as the neighboring tissue, which makes these two tissues indistinguishable.[17]

When a Doppler system is integrated with the ultrasound, the device is named duplex. With a duplex machine, blood flow is made visible on the screen in red and blue colors. Herewith, blood

vessels can be identified in conjunction with other dermal structures. Fillers come in different formulae, but they have hydrophilic or hydrophobic characteristics. All hyaluronic acid fillers are able to bind water and are thus hydrophilic. As water content does not reflect the sound waves, hyaluronic acid appears black (an echogenic) or light gray hypoechoic) on ultrasound Figure 1.[18]

The ubiquitously used hyaluronic acid fillers come in different particle sizes, meant for different applications, and are placed in different layers of the skin and subdermis. Depending on the technique, a treading line of multiple dark deposits can be seen, specifically when a cannula is used Figure 1 or a large dark deposit bolus injections for volume replacement) may be visible Figure 2.

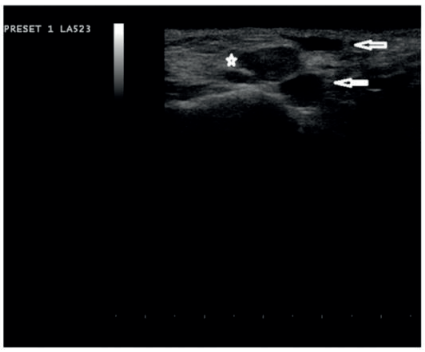

Figure 1. Multiple deposits of hyaluronic acid filler, two anechoic deposits (black) and one hypoechoic deposit*

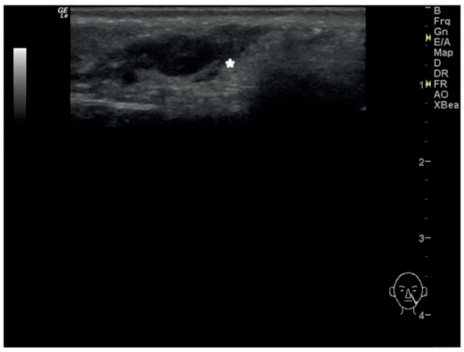

Figure 2. Oval-shaped hypoechoic single deposit of hyaluronic acid

ULTRASOUND TO IMPROVE THE SAFETY OF HYALURONIC ACID FILLER TREATMENTS

At our ambulant cosmetic university hospital clinic, we routinely use ultrasound examination to minimize risks, but also to locate and identify fillers in patients with side effects who are referred to us. With ultrasound/duplex examination, skin, the underlying tissue including muscles, veins, and arteries can be made visible. At the same time, any filler can be brought into sight, measured in pocket size, and the plane of injection can be seen. We experience that the use of duplex provides an important improvement in the safety of dermal filler treatments.

Previous filler treatments

Not only hyaluronic acid but also all fillers are visible with ultrasound.[15,19] Patients who had previous filler treatments may not always remember the type of filler and the place and plane of injection. Yet, different filler substances may give unwanted side effects, when mixed.

Figure 3 shows the lower face of a 61-year-old woman. She was previously injected with poly-methylmethacrylate (PMMA). After a hyaluronic acid filler was injected in the corners of the mouth, she developed an inflammatory response of polymethyl- methacrylate (PMMA). The upper lip and chin were also responding with an inflammatory response, although not treated with hyaluronic acid.

Using ultrasound before a filler treatment can help to distinguish between the different types of fillers used previously and thus to avoid complications Figure 4.

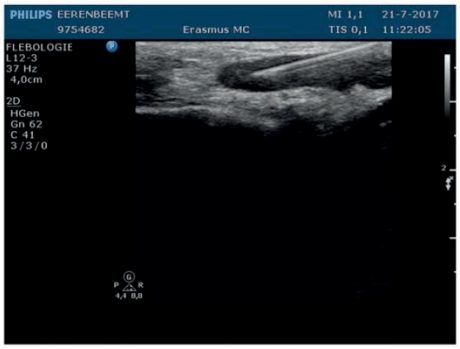

Figure 3. Injection in filler depot under ultrasound guidance

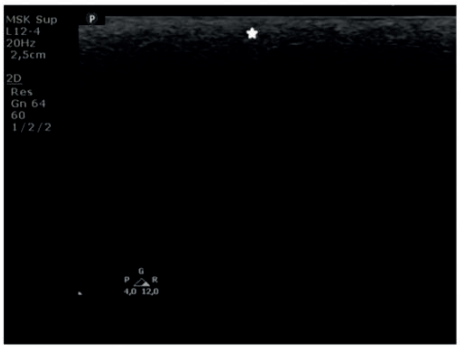

Figure 4. Polymethylmethacrylate visible with ultrasound

Anatomical mapping

Serious complications of filler treatments are intravascular injection or vascular compression of filler material leading to skin necrosis or, in rare cases, blindness. As these vessels are not visible clinically, prevention is extremely important. Guidelines advise to use an adequate injection technique such as cannulas and to inject slowly.[11,20,21] Most of all, anatomical knowledge of the face and the course of veins and arteries is crucial. Unfortunately, individual variations in facial artery anatomy may exist.[22,23] Ultrasound allows visualization of the facial arteries and veins of the proposed treatment area and is a noninvasive imaging tool for vascular mapping before the treatment is started. Vascular structures appear anechoic black, containing liquid) and linear when the transducer is in the same line as the vessel, or circular when the transducer is placed on a section of the vessel. Duplex sonography B-scan ultrasound combined with color Doppler ultrasound) helps to distinguish structures with movement, for example blood moving within vessels. Color Doppler blue vs red) can also be used to determine the direction of the blood flow when needed Figures 5 and 6.

Figure 5. Locating artery with duplex

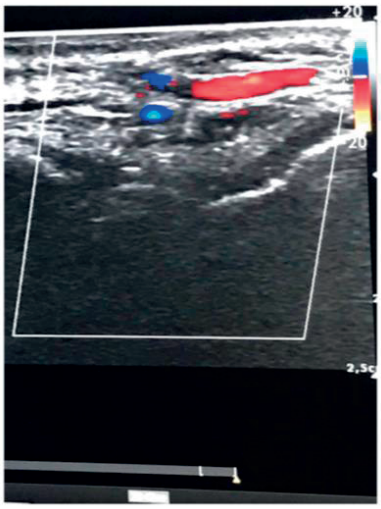

Figure 6. Longitudinal view of corresponding artery

Treatment of adverse events with ultrasound

Recently publicized protocols and guidelines describe how to treat unwanted adverse events.[24] In our experience, dissolving the filler will terminate most of the adverse events. As mentioned above, hyaluronic acid fillers are easily seen with ultrasound. The pocket size and the location of the filler can be brought into sight. Under ultrasound guidance, hyaluronidase can be injected directly into the filler pocket causing the adverse event Figure 7.

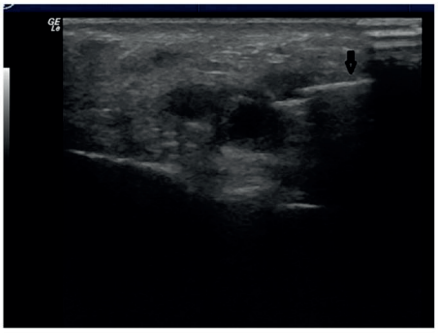

Figure 7. Under ultrasound guidance the needle is inserted in the filler deposit top right

Dislocation, overcorrection of product, and vascular adverse events can be treated in this way to eliminate the cause of the problem. In case of an inflammatory response, temporary medication as antibiotic treatment may be needed as adjuvant treatment. Special attention is given to vascular adverse events as intravascular injection of filler material or vascular compression Figure 8 may lead to severe complications as necrosis.

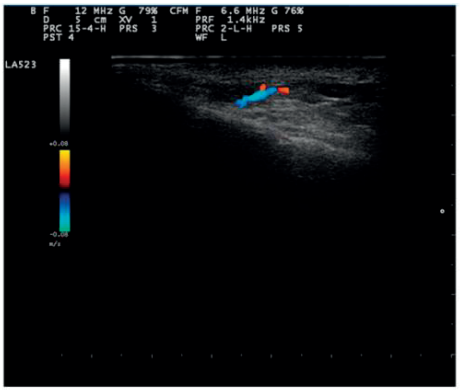

Figure 8. Vascular adverse event, hyaluronic acid deposit compromises vessel

The use of ultrasound is very helpful in the treatment of these complications and in the treatment outcome.

In Figure 9, the beginning of crusting as a result of a vascular adverse event is seen. This was due to a hyaluronic acid filler treatment in the right lower lip to obtain a lip augmentation. The referring physician described pain and blanching during injection. Hyaluronidase (150U) was injected once under ultrasound guidance in the hypoechogenic deposit. Immediate improvement was noted by the patient, continuing throughout the day, with complete recovery of her lip Figure 10.

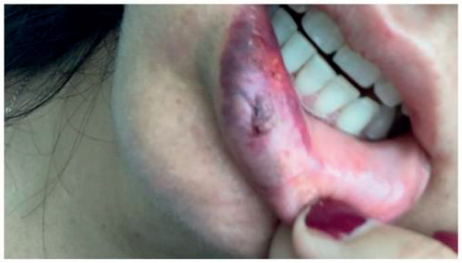

Figure 9. Cursting on under lip

Figure 10. Healing of the under lip, no scarring

CONCLUSION

Ultrasound examination can be an important tool to improve the safety of hyaluronic acid filler treatments. The amount, location, and depth of the injected hyaluronic acid fillers can be identified. With some practice, it makes a precise intralesional delivery of hyaluronidase possible. As prevention, duplex ultrasound can be used to identify vascular structures in the proposed treatment areas Figure 11.

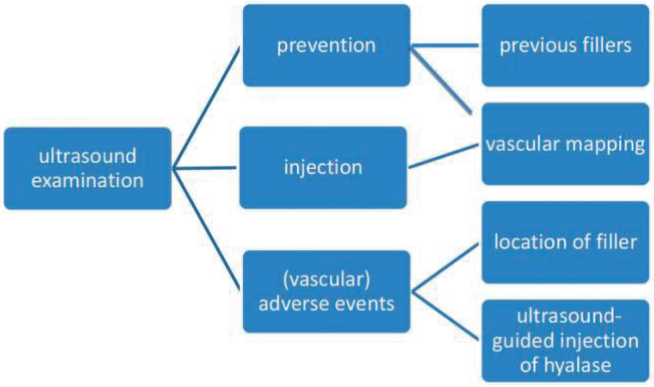

Figure 11. Ultrasound examination for filler treatment

The learning curve to use and interpret duplex ultrasound pictures is, in our experience, not too steep. Small probes with direct connections to tablets are becoming more and more available for reasonable prices. We feel that these devices should be available in any office of a doctor using hyaluronic acid-based fillers.

CONFLICT OF INTEREST

No conflict of interest disclosures.

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Part VII

TREATMENT OPTIONS FOR ADVERSE EVENTS AFTER SOFT TISSUE FILLER INJECTION

CHAPTER 10

Intralesional Laser Treatment for Dermal Filler Complications

L.W. Schelke
T.S. Decates
C.I.M. van der Lugt
L. Pelzer
G. de Mey
P.J. Velthuis

Plast Reconstr Surg. 2018 Jun;141(6):1361-1369

ABSTRACT

Background: For complications caused by filler treatments, in general, two treatment regimens are advised: systemic drugs and surgical removal of the material. Another possible treatment option would be removal of the material by intralesional laser treatment.

Methods: Two hundred forty-two patients with complications caused by fillers were treated with intralesional laser treatment.

Results: In the majority of patients, an improvement was achieved (92 percent), in 9 percent the complication was resolved, and in 3 percent it was not improved (unknown in the rest).

Conclusion: Considering the large number of patients treated until now and the efficacy and good safety profile of this treatment, the authors plead that intralesional laser treatment may be considered as a treatment option before surgery.

INTRODUCTION

Although there is ongoing popularity of dermal filler use and an increasing number of treatments performed, much is unknown about complications with regard to their rate, possible causes, and optimal treatment options. As filler treatments are mainly performed as a cosmetic treatment in healthy clients, not only the treatment itself but also the options if any complications occur should be safe and must avoid severe side effects. Looking at the literature, there is no consensus about the nature of these complications or treatment modalities.[1] One hypothesis of the cause of complications is a chronic foreign body response[2,3]; another theory is biofilm formation around dermal fillers, probably consisting of skin bacteria.[4,5] Both are thought to cause an inflammatory response. In general, two treatment regimens are advised: systemic drugs[1,6] and surgical removal of the material.[2,7] Drugs can be useful to suppress the adverse reactions toward the filler material but they do not remove the filler itself. The drugs used are antibiotics, preferably from the macrolide group 1, as these will treat bacterial inflammatory reactions and suppress foreign body responses by up-regulating the production of anti-inflammatory mediators.[8,9] The latter can also be treated with corticosteroids systemically or injected intralesionally. Surgical excision may remove (parts of) the material but often with tissue damage and scarring as a cosmetically undesirable result. Another possible treatment option for filler complications has been developed and described by Cassuto et al.[10,11] This treatment modality—intralesional laser treatment—is capable of removing the foreign substance in a microinvasive manner. In this article, we describe our treatment outcomes with intralesional laser treatment for dermal fillers.

PATIENTS AND METHODS

In the period between 2011 and 2016, 590 patients consulted our clinics for filler complications. Of these patients, 90 percent (n = 531) were women and 10 percent (n = 59) were men. Each patient's history was taken, especially complaints, onset of adverse event, medication, and earlier treatment regimens. All patients were evaluated with ultrasound examination. With ultrasound, information about the type of filler, the amount, the injection technique, the location and dislocation of the product, and the presence of an acute inflammatory reaction was gathered. Taking into account the degree of cosmetic disfiguration in a patient's face, the patient's complaints, and results from the ultrasound examination, patients were advised either to leave the filler at rest or to have the product evacuated (Fig. 1). All patients gave informed consent for the treatment performed. In 41 percent of the treatments, evacuation of the product was performed with the aid of intralesional laser treatment.

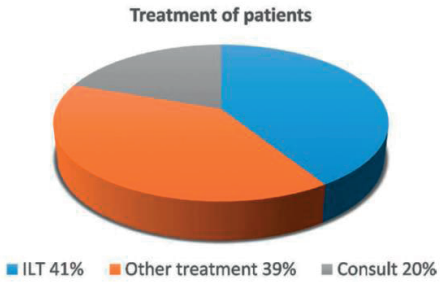

Figure 1. Treatment of patients. *ILT*, intralesional laser treatment

Intralesional Treatment

In our outpatient clinic, two types of lasers are used: a 810-nm-wavelength diode laser and a 1470-nm-wavelength diode laser (continuous wave) (Quanta System, Milan, Italy). Both lasers are developed and used for endovenous laser treatments. The 810-nm laser targets hemoglobin, and the 1470-nm laser heats water in blood and vein wall, secondarily destroying the vein wall. In endovascular laser treatment of varicose veins, different wavelengths are used (810 and 1470 nm). Not the wavelength of the device but the amount of energy and heat delivered to the varicose veins is thought to be most important for achieving success.[12-14] This will probably account for the intralesional treatment, and we found no obvious difference in efficacy for these two different wavelengths. The intralesional laser treatment procedure for dermal fillers consists of inserting a fiberoptic laser into the area of the product. The laser power setting for both lasers depends on the diameter of the fiber used and is on average 3 to 6 W for the 810-nm laser and 0.6 to 0.8 W for the 1470-nm laser, both in continuous-wave mode. Delicate areas such as orbital regions, glabella, and locations that have been treated with corticosteroid injections before were treated with reduced power to avoid skin burns.

The fiber diameter may vary between 200 and 600 μm; the smaller diameter is preferable. If the product is not clinically easily felt or seen, the fiber insertion can be performed under ultrasound guidance. Intradermal anesthesia at the skin entry point is commonly used in all instances. As the pain sensation of the patient is helpful to adjust the delivered energy into the filler, anesthesia is limited to the skin entrance. If too much heat is being delivered to the filler material or if the fiber is not in the right place, heat may be diffused into the surrounding tissues, risking tissue damage or pigmentation.[11]

The patient is instructed to warn the operator if pain or excessive heat is sensed. During the intralesional laser treatment procedure, softening of the product is noticed, which is used as an endpoint. After the laser procedure, the heat-liquefied filler can be (partly) squeezed out by manual compression through an 18-gauge needle entry point or through a small incision made by a no. 11 scalpel (Fig. 2). As we broadly follow the treatment regimen proposed by Cassuto

et al., we have made some additions to their technique based on filler types: hydrophobic and hydrophilic fillers. They behave differently in tissue, but they have also been injected differently into the tissue.

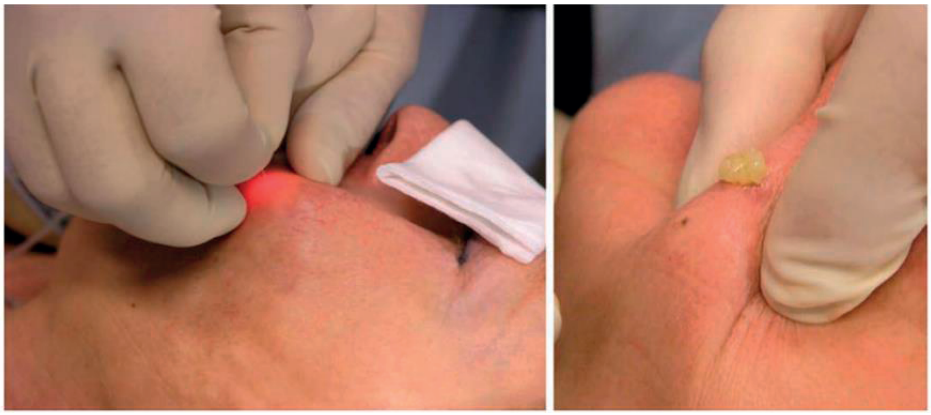

Figure 2. *(Left)* The laser fiver is inserted in the filler. *(Right)* The heat-liquefied filler is squeezed out.

Hydrophilic Fillers

Almost all nonresorbable hydrophilic fillers and resorbable hyaluronic acid fillers with large particles are used as volumizers and are often injected as a bolus. On ultrasound, they appear as hypoechoic pockets or cysts. Known filler types that give rise to complications in this category are polyalkylimide and polyalkylimide fillers and heavily cross-linked hyaluronic acids. Histologic examination indicates that these fillers tend to dehydrate over time. Dehydration may be one of the explanations why these types of fillers are difficult to remove. Before introducing the fiber into Fig. 1. Treatment of patients. ILT, intralesional laser treatment. the filler, 1 to 10 ml (depending on the pocket size) of 0,9% sodium chloride is injected into the filler depot, if needed under ultrasound guidance (Fig. 3).

During heat delivery by the intralesional laser treatment fiber, the injected fluid is bubbling, also visible with ultrasound. [See Video, Supplemental Digital Content 1, which demonstrates the intralesional laser treatment procedure including corresponding ultrasound imaging.]

The intralesional laser treatment of a hydrophilic filler (polyalkylimide) is shown. As it is done under ultrasound guidance, the fiber tip can be inserted accurately in the filler pocket. When the clinical endpoint (softening of the product) is reached, the heat-liquefied filler can be squeezed out by manual compression, in this case through an 18-gauge needle entry point, available in the "Related Videos" section of the full-text article on PRSJournal.com or, for Ovid users, available at http://links.lww.com/PRS/C758.] After the laser procedure, the pocket is irrigated again with saline solution to mechanically flush out as much material as possible.

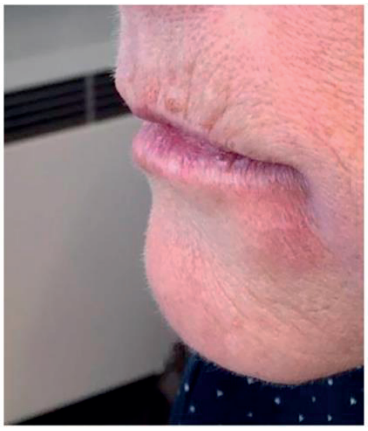

Figure 3. Inflammatory response of polymethylmethacrylate after hyaluronic acid filler is injected in the corners of the mouth. Note: the upper lip and chin are also responding

Hydrophobic Fillers

The most commonly used hydrophobic fillers are polymethylmethacrylate, hydroxyethylmethacrylate, and silicone oil (polydimethylsiloxane). The injection technique used is mainly infiltrating small particles into the tissue, leading to a fibrotic tissue response. The fiber is inserted into the area of the material, by drilling small holes. Little Fig. 2. (Left) The laser fiber is inserted in the filler. (Right) The heat-liquefied filler is squeezed out. Fig. 3. Injection in filler depot under ultrasound guidance. Video. Supplemental Digital Content 1 demonstrates the intralesional laser treatment procedure including corresponding ultrasound imaging. The intralesional laser treatment of a hydrophilic filler (polyalkylimide) is shown. As it is done under ultrasound guidance, the fiber tip can be inserted accurately in the filler pocket. When the clinical endpoint (softening of the product) is reached, the heat-liquefied filler can be squeezed out by manual compression, in this case through an 18-gauge needle entry point, available in the "Related Videos" section of the full-text article on PRSJournal.com or, for Ovid users, available at http://links.lww.com/PRS/C758 droplets dripping out of the insertion openings show filler material (Fig. 4).

Posttreatment Recommendations

For hydrophobic fillers, heat compression followed by gentle massage to push out more product is advised for the first hours after treatment. For hydrophilic fillers, because the skin entrance is much larger (because of a stab incision with a no. 11 blade or an 18-gauge needle), it is advised to leave the skin at rest to prevent inflammatory reactions. For all treatments, our postoperative advice is not to apply any cream or makeup until the entrance skin opening has healed, to prevent any secondary infection. Oral macrolide antibiotic treatment is given to patients at risk such as patients under corticosteroid treatment and immunocompromised patients (e.g., human immunodeficiency virus). Downtime is normally 2 to 4 days after treatment. A sensitive area such as the lips will give rise to a more pronounced swelling and could take 5 days to heal.

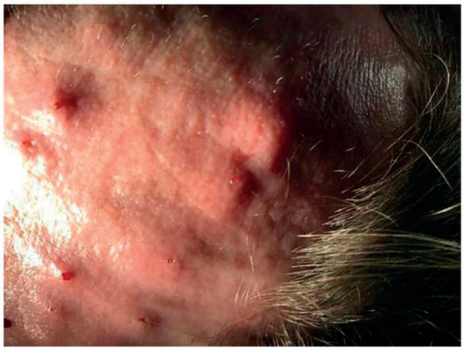

Figure 4. Little droplets of silicone oil after intralesional laser treatment

RESULTS

From January of 2011 to September of 2016, 590 patients visited our clinic. Of these 590 patients, 242 patients (214 women and 28 men) were treated with intralesional laser treatment. The mean age of these patients was 52 years (range, 25 to 78 years). On average, 1.7 treatments were performed per patient (Fig. 5).

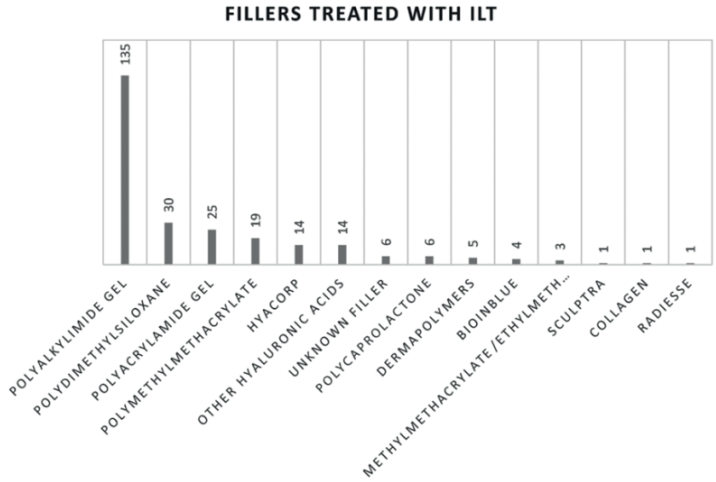

Figure 5. Fillers treated with intralesional laser treatment *(ILT).*

Most complications treated with intralesional laser treatment were caused by polyalkylimide. Clinical symptoms to filler treatment complications were inflammatory reactions, visible lumps and nodules, dislocation and accumulation of the product, and hardening. In Figure 3, the complications treated with intralesional laser treatment are listed per filler for the fillers used most often (Fig. 6).

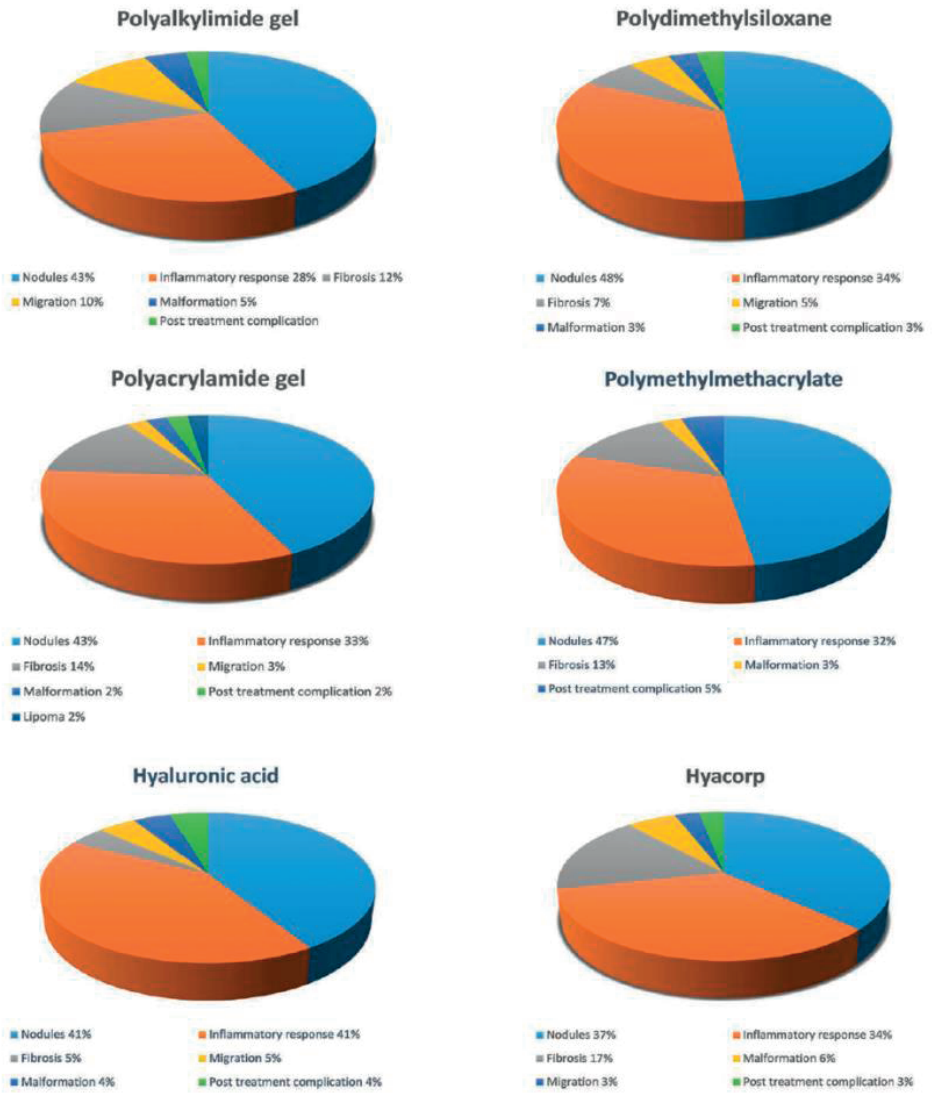

Figure 6. Complications per filler for the six most common fillers.

It is interesting to note that 11 percent of the patients visiting our polyclinic had a complication caused by injections with resorbable hyaluronic acid fillers. Half of these complications are attributable to wrong injection techniques resulting in dislocation of product caused by dynamics of the underlying muscles, excessive edema of the lower eye lid region, or inflammatory responses when volumes that are too large are injected (mostly hyaluronic acid products with a high viscosity and stiffness). The other 50 percent of the complications were attributable to cross-linking (up to 63 percent) of some hyaluronic acid products that were too strong, thus

creating the same complications as seen with permanent fillers. In cases of volumes that are too large and products with excessive cross-linking, hyaluronidase alone was not successful in dissolving the filler, and intralesional laser treatment followed by hyaluronidase injection was necessary to remove it. After treatment, patients were asked whether the treatment improved their cosmetic and physical complaints (Fig. 7).

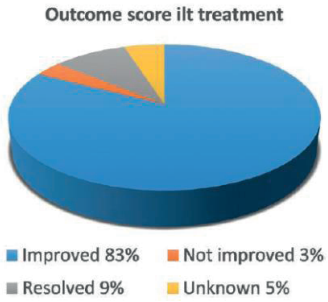

Figure 7. Outcome score of intralesional laser treatment *(ILT).*

To stay in line with the article by Cassuto et al., this was defined as follows: Resolved: All symptoms are completely cured or judged tolerable by the patient. Improved: Cosmetic disturbances and lump visibility are reduced to a degree judged tolerable by the patient. Interrupting the steroid therapy without recurrence is possible. Not improved: no cosmetic and/or physical improvement (Fig. 8).

Figure 8. *(Left)* Before intralesional laser treatment. *(Right)* Improvement after intralesional laser treatment.

The improvement can also be seen with a follow-up ultrasound examination. Hydrophilic fillers decrease in pocket size or disappear, although fibrosis is mostly remaining (Fig. 9).

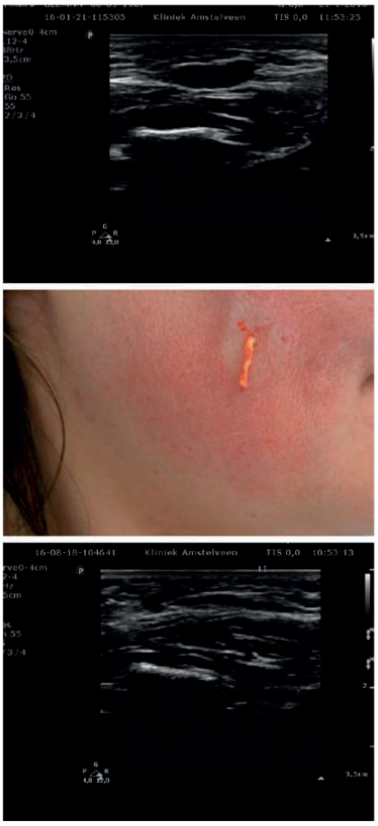

Figure 9. *(Above)* Hydrophilic filler (black pocket on screen) before intralesional laser treatment. *(Center)* Hydrophilic filler is squeezed out after intralesional laser treatment. *(Below)* Decreased pocket size of hydrophilic filler on screen.

The tight fibrotic tissue formed around hydrophobic fillers prevents ultrasound passage (shadowing). After intralesional laser treatment, the visibility of the tissue improves (e.g., teeth become visible again with follow-up ultrasound examination) (Fig. 10).

Patients not improved are mostly patients with an inflammatory reaction after intralesional laser treatment who visited a first aid department of a nearby hospital (not familiar with these types of problems) and, because of the drain placed as a treatment, without any follow-up or wound procedure management, were left with a scar in their face. Furthermore, fillers injected in the orbital region are more difficult to remove, leading to less satisfying results (Fig. 11).

Complications Caused by Intralesional Laser Treatment

Inflammatory reactions as a complication caused by intralesional laser treatment are seen. As there is still no consensus about the cause of this reaction, a macrolide was given as treatment

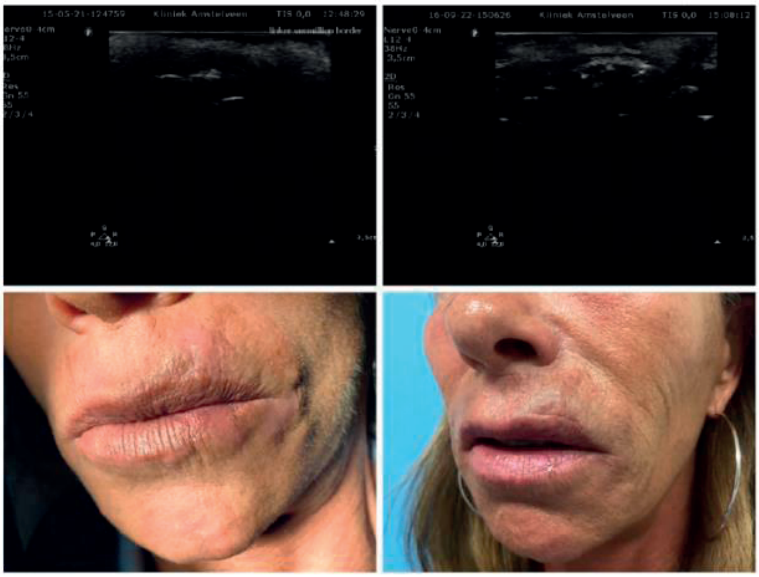

Figure 10. *(Above left)* Fibrotic tissue around silicone oil prevents most ultrasound wave passing through (shadowing). *(Above right)* After intralesional laser treatment, more ultrasound waves can pass through. *(Below left)* Before intralesional laser treatment of silicone oil. *(Below right)* After intralesional laser treatment of silicone oil.

Figure 11. *(Left)* Before intralesional laser treatment. *(Right)* After intralesional laser treatment, showing less improvement in the orbital region.

to cover any bacterial infection and an acute inflammatory response as well. Ibuprofen was given for its pain-reducing and anti-inflammatory characteristics. After a couple of days, the abscess could be evacuated by puncture. Normally, this will leave no visible scarring. Immunocompro-

mised patients are more prone to develop post–intralesional laser treatment inflammatory responses. One patient with large pockets of polyalkylimide injected in the lower orbital region (under the eyelids) was left with an open wound for months. Because of an excess of heat applied into this sensitive area, which had also been treated with cortisone injections previously, damage was done to the overlying skin. In combination with the large amount of filler remaining, there was a very slow healing response. If treating delicate areas such as the periorbital region (lower eyelid region), glabella, or skin treated previously with cortisone injections, the temperature should be adjusted lower. One patient had persistent skin hyperpigmentation after the intralesional laser treatment. This can be prevented by making an insertion hole with a no. 11 scalpel and then inserting the fiber into the pocket of the filler, ensuring that no heat is applied to the skin surface. Placing the tip of the fiber on the skin and heating the fiber thereafter may cause a slight burning reaction that should be prevented in these cases and those with Fitzpatrick skin type III and higher.

DISCUSSION

There is still much to learn about complications caused by filler treatments. The relationship between product and the host response at the time of injection and during the degradation process is still not clear[15,16], thus, treatment options are difficult to standardize. However, in case of complications, it seems logical to at least remove as much filler product as possible. In 2009, a small number of patients (n = 20) treated with intralesional laser treatment were described by Cassuto et al.10 In 2016, the same authors published an article regarding a large number of treated patients (n = 219) who experienced an improvement of their complaints.[11] We underscore these outcomes with our data. Almost all patients noted an improvement after intralesional laser treatment, although not always as much as they hoped for. There may be remainders of the filler or of fibroses. Taking this into account, the minimally invasive manner of the treatment and the limited downtime add to the attractiveness of this method. Considering the large number of patients treated until now, and the efficacy and good safety profile of this treatment, we plead that intralesional laser treatment should be considered as a treatment option before surgery.

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THE FUTURE
PART VIII

THE PROBLEM OF NEW HYALURONIC ACID SOFT TISSUE FILLERS

CHAPTER 11

The Dutch Hyacorp® filler catastrophe. New EU legislation will prevent this from happening again

T.S. Decates
F. Sayghani
J.A.J. van Loghem
P.J. Velthuis

Submitted

ABSTRACT

Background: In 2014, the hyaluronic acid-based fillers Hyacorp-1000 and Hyacorp H-S (H-800) were withdrawn from the Dutch market after concerns about their safety.

Objective: To assess what the most plausible factor of the increased number of adverse events would be, either patient related factors or a factor inherent to the filler itself. We also assessed how new European legislation will affect the approval process for new fillers and prevent safety issues with fillers.

Methods: A total of 42 patients – 37 women (88%) and 5 men (11%) – were included. Consisting of three groups: 13 patients injected with Hyacorp-1000 and Hyacorp H-S (H-800) whom had reported inflammatory adverse events; 12 injected with Hyacorp-1000 and Hyacorp H-S (H-800) who did not; 17 injected with other HA fillers whom had reported inflammatory adverse events.

Results: Patients treated with Hyacorp-1000 and Hyacorp-S (H-800) that reported adverse events were significantly older than those in the Hyacorp-1000 and Hyacorp-S (H-800) group without adverse events. In patients treated with Hyacorp-1000 and Hyacorp H-S (H-800) that reported adverse events, the filler was significantly longer in situ then in patients who had adverse events related to another HA filler.

Conclusion: Hyacorp-1000 and Hyacorp-S (H-800) filler was associated with an increased chance of developing adverse events than other HA fillers probably because it remains in the body for a longer period of time. The upcoming legislative EU update to Medical Device Regulation (MDR) will prevent unsafe filler from entering the EU market or will detect safety problems much earlier.

INTRODUCTION

The modern era of injections with synthetic bioactive materials began several decades ago with the US Food and Drug Administration (FDA) approval of Restylane (Q-Med, Uppsala, Sweden), a hyaluronic acid (HA) filler.[1] Since then, the popularity of soft tissue fillers has continued to increase. Modern filler materials are absorbable and their effect is temporary.[2,3] Next to HA, they comprise a range of substances including collagen, calcium hydroxyapatite, poly-L-lactic acid, and other synthetic polymers.[2,3] Various brands of these materials have been approved for cosmetic medical use by authorities worldwide.[3,4] Soft tissue fillers are classified as medical devices by regulatory agencies based on the intended use and claims made (e.g. the Medical Devices Directives in Europe (EU 93/42/EEC) and the Food and Drug Administration in the United States), as their primary intended action is not pharmacological but mechanical ('the filling effect').[5] The regulation of medical devices involves competing goals of assuring safety and efficacy while providing rapid movement of innovative therapies through the investigative and regulatory processes as quickly as possible. The United States and the European Union have approached these challenges in different ways.[6]

Under the current legislation in Europe, so-called Notified Bodies are responsible for reviewing applications by in depth technical documentation review and on-site CE (Conformité Européenne) audits before new medical devices are allowed to enter the market. When approved, a CE-certificate is issued, indicating conformity with health, safety, and performance requirements of the MDD and applicable standards. After marketing, manufactures are responsible for obtaining data on adverse events through post marketing surveillance.[7] In the United States, according to the Food and Drug Administration (FDA), fillers are also classified as medical devices. To obtain FDA approval, dermal fillers have gone through the controlled clinical testing for safety and effectiveness required for high-risk devices. Pre-market testing is carried out on an average of 120 patients, and many products are also subject to long-term safety studies after marketing. In addition, the FDA maintains a publicly available database of all reported side effects and recalls. In 2020, the minimal requirements in the EU for clinical efficacy and safety has given rise to unforeseen adverse events caused by soft tissue fillers and approximately 160 approved soft tissue fillers on the EU market. Compared to only 10 on the US market.[8]

HA-based fillers are the most popular fillers at this moment.[3] One major disadvantage is their relatively short duration of their effect. Depending on the brand, longevity up to 18 months are reported.[3] To overcome this problem, BioScience GmbH (Bruemmer, Germay) introduced a special line of HA fillers in 2011, named Hyacorp®. This line consisted of five products: Hyacorp H1000, Hyacorp H-S (also known as H-800), Hyacorp L, Hyacorp Face and Hyacorp Lips.[9] Crosslinking in this group was 35% to 60%, much higher than in other regularly used HA fillers such as Restylane, which has crosslinking of 1%.[9,10] A high degree of crosslinking

increases longevity, but usually decreases injectability of the HA filler. Apparently, technicians of the manufacturer had solved this problem and delivered a long-lasting HA-based filler product, which was expected to have a substantial marketing advantage.[9-12] However, in September 2012, almost a year after the introduction of Hyacorp®, the Product and Complication Committee of the Dutch Society of Aesthetic Medicine (NVCG) informed the Dutch Health and Youth Care Inspectorate (IGJ) of a surprisingly high rate of adverse events (AE) after injection of Hyacorp-1000 and Hyacorp H-S (H-800) as a soft tissue filler.[11] These two fillers had the largest particle size and the highest degree of cross-linking in the Hyacorp portfolio. An AE rate of 5% was reported by members of the NVCG, being one-hundred-fold higher than the adverse event rate stated by the manufacturer.[11,12]

The adverse events observed after Hyacorp injections were of late onset (occurring at least 2 weeks after treatment) and included edema, inflammatory nodules, hardening of the product and migration, sometimes with disfiguring outcomes (Fig. 1).[12,13]

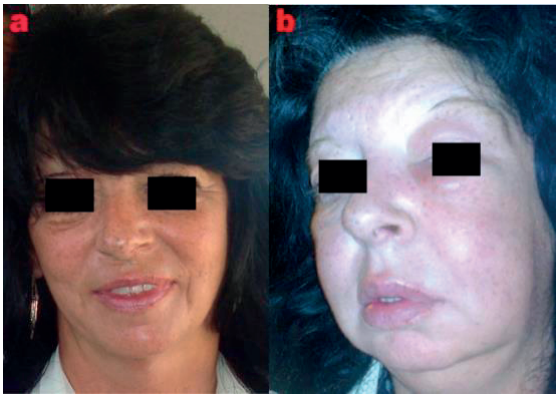

Fig. 1. Same patient, on the left side before (a), on the right side after Hyacorp filler injections (b), showing clinical features of edema, inflammatory nodules and migration of the product.

The distributer was informed about these troubling findings by IGJ. This resulted in withdrawal of the product from the market in 2014.[10-12] In the ensuing investigation by the National Institute for Public Health and the Environment (RIVM) the biodegradability of Hyacorp® fillers was questioned. It was concluded that safety of Hyacorp-1000 and Hyacorp H-S (H-800) was compromised due to its high degree of cross-linking and large particle size. Hyacorp-1000 and Hyacorp H-S (H-800) fillers acted as non-resorbable fillers.[11,12]

In 2020 two new patients presented themselves at the outpatient clinic of our hospital with inflammatory symptoms presumably caused by Hyacorp-1000 and Hyacorp H-S (H-800) filler injections performed between 2011-2014. Given the relatively small market share of Hyacorp compared to the leading companies (Galderma, Merz, Teoxane and Allergan), the number of

Hyacorp® AE cases we have treated, is extraordinarily high. This led us to review clinical data of patients injected with Hyacorp-1000 and Hyacorp H-S (H-800) fillers with and without AE and to compare these with patients with inflammatory AE from other HA fillers to identify triggering factors.

MATERIALS AND METHODS

In this retrospective study we enrolled 25 patients who were treated with a Hyacorp-1000 and Hyacorp H-S (H-800) filler between 2010-2014. Of these, 13 had a delayed inflammatory AE based on the clinical presentation and ultrasound imaging (Hyacorp + AE group) at our outpatient clinic. The remaining 12 persons were responders to a questionnaire sent to a group of 35 who had been treated with Hyacorp-1000 and Hyacorp H-S (H-800) at private practices in the Netherland during the same period, but without history or clinical signs of AE (Hyacorp – AE group). In the third group a total of 17 patients were enrolled, who were treated with another HA filler and had a delayed inflammatory AE (Other HA fillers + AE group) and presented themselves at our outpatient clinic. These other HA fillers were Restylane®, Juvederm®, Belotero® and Teosyal®.

Study Population

Only men and women aged 18 years or older were enrolled. All participants filled out a questionnaire under the guidance of one of the investigators. The questions concerned demographic factors (age, gender, ethnicity), smoking, atopy and use of medication.

Procedure

Data were collected in 2020 at the Dermatology Department of the Erasmus Medical Center (Rotterdam, the Netherlands). The department holds consultation hours specifically intended for patients with adverse events after injection with HA fillers. All persons received their treatments at other aesthetic clinics and persons exhibiting adverse events were referred to this center by dermatologists, plastic surgeons, general practitioners and aesthetic physicians. The study protocol was approved by the Ethics Committee.[11] All patients provided written informed consent.

Statistical Analysis

SPSS was used for data entry and data processing. Frequency analyses were used to describe the data. Testing of differences between the two groups (Hyacorp + AE vs. Hyacorp – AE) and between other two groups (Hyacorp + AE vs. Other HA filler + AE) were performed with a Fisher exact test. The choice for the Fisher exact test was determined by the skewed distribution of the dependent variable.

RESULTS

Table 1 displays selected characteristics of the Hyacorp + AE and Hyacorp – AE group. The distributions of gender, ethnicity, atopy, medication and smoking status were comparable between the two groups. A Fisher's exact test (Table 1) showed that there was a statistically significant difference in age between the two groups (p = .025).

		Hyacorp + AE (N=13)	Hyacorp - AE (N=12)	Fisher's exact test
Gender N (%)	Female	10 (76.9)	11 (91.7)	.593
	Male	3 (23.1)	1 (8.3)	
Age (in years)	Mean (SD)	57.15 (8.93)	49.33 (7.22)	.025
Ethnicity N (%)	Non-caucasian	1 (7.7)	0 (0.0)	1.0
	Caucasian	12 (92.3)	12 (100)	
Atopy N (%)	Yes	2 (15.4)	2 (16.7)	1.0
	No	11 (84.6)	10 (83.3)	
Medication N (%)	Yes	7 (53.8)	3 (25.0)	.226
	No	6 (46.2)	9 (75.0)	
Smoking N (%)	Yes	6 (46.2)	5 (41.7)	1.0
	No	7 (53.8)	7 (58.3)	

Table 1. Descriptive statistics for Hyacorp group with adverse events (Hyacorp + AE) and Hyacorp group without adverse events (Hyacorp - AE)

Table 2 displays selected characteristics of the Hyacorp + AE and Other HA filler + AE group. The distributions of gender, age, ethnicity, atopy, medication and smoking status were comparable between the two groups. Comparison with a Fisher's exact test demonstrated that the filler was significantly longer in the body (filler in situ) in patients in the Hyacorp + AE group.

		Hyacorp + AE (N=13)	Other HA filler + AE (N=17)	Fisher's exact test
Gender N (%)	Female	10 (76.9)	16 (94.1)	.290
	Male	3 (23.1)	1 (5.9)	
Age (in years)	Mean (SD)	57.15 (8.93)	56.00 (8.1)	.715
Ethnicity N (%)	Non-caucasian	1 (7.7)	2 (11.8)	1.0
	Caucasian	12 (92.3)	15 (88.2)	
Atopy N (%)	Yes	2 (15.4)	9 (52.9)	.057
	No	11 (84.6)	8 (47.1)	
Medication N (%)	Yes	7 (53.8)	7 (41.2)	.713
	No	6 (46.2)	10 (58.8)	
Smoking N (%)	Yes	6 (46.2)	4 (23.5)	.255
	No	7 (53.8)	13 (76.5)	
Filler in situ (months)	Mean (SD)	17.8 (18.2)	3.5 (3.6)	.015

Table 2. Descriptive statistics for Hyacorp group with adverse events (Hyacorp + AE) and Other HA filler group with adverse events (Other HA filler + AE)

DISCUSSION

In this study we evaluated whether it is plausible that patient related factors may contribute to the development of inflammatory adverse events after injection with a Hyacorp-1000 and Hyacorp H-S (H-800) filler. The most remarkable outcome was that patients in the Hyacorp + AE group were significantly older than those in the Hyacorp - AE group. With a mean age of 57 at time of referral, it is very likely that a larger proportion of women were at the time of injection in their menopause in the Hyacorp + AE group than in the Hyacorp - AE group. Marusza et al. recognized this as a factor that increases the risk of inflammation.[14]

Comparing both groups with adverse events, we found that fillers in the Hyacorp + AE group were significantly longer in the body compared to the other HA fillers group. Keizers et al. developed an analytical method to validate and identify HA fillers. They reported that several products of the Hyacorp® filler range contained a high modification and crosslinking grade.[9] Hyacorp-1000 and Hyacorp H-S (H-800) fillers remain in the body for a longer period; it actually acts like a permanent filler. Although the groups tested were small in size, we conclude that patients related factors are not the cause, but that it is very probable that Hyacorp-1000 and Hyacorp H-S (H-800) filler themselves were inherently unsafe.

How could this non-safe new type of HA filler appear on the EU market? Compared to their market share, Hyacorp-1000 and Hyacorp H-S (H-800) fillers were overrepresented in the number of serious AEs that were referred to us. Hyacorp-1000 and Hyacorp H-S (H-800) fillers played only a minor role in this market that is dominated by Allergan, Galderma, Merz and Teoxane HA fillers. [15-16] The question is how such a product was granted access to the Dutch market. The current regulations in Europe to approve medical devices are laid down in the Medical Device Directive (MDD 93/42/EEC) which is in force since 1993. Applicants for new devices have to submit a file containing data on intended use, performance and safety of the medical device and production. This system has been criticized due to a lack of uniformity in certification procedures and application of standards. Moreover, the notified bodies have varying levels of expertise, which lead applicants 'shopping' to find those with the most flexible operating standards.

Fortunately, EU-regulations have been subject to change. A new set of rules and regulations for medical devices: MDR (Medical Device Regulation EU 2017/745) has come into force on May 25th, 2017. The 3-year transition period that was envisioned, has been extended by one year due to COVID-19. The new date of application of the MDR is May 26th, 2021. From that moment on, the pathway to CE certification for soft tissue fillers will be much stricter.[17] Next to description of the intended use and specifications on the safety and the performance of the medical device and the production process, as before, the submitted file must provide more clinical

performance test results to establish performance. A very important improvement involves the post-market clinical follow-up. [17] Device manufacturers are required to collect clinical data yearly as part of an ongoing process to assess product safety. Another key aspect in fulfilling the objectives of the MDR is the creation of a European database on medical devices (Eudamed) that should integrate different electronic systems to collate and process information regarding devices on the market. The objectives of the database are to enhance overall transparency, including through better access to information for the public and healthcare professionals.[17]

Finally, the number of notified bodies allowed to approve implant products has been limited to ensure more specialized knowledge. As a result, the new CE certification process will in some aspects, be stricter than the approval process of the FDA in the US (Figure 2). [17] The recent update to MDR can be expected to ensure that the new Medical Device Coordinating Group (MDCG) and the Competent authorities apply very strict requirements before a new soft tissue filler can enter the market as a medical device. [17] Furthermore, some HA fillers now placed on the market as cosmetic product will be controlled by the MDR as well under the Annex XVI products (list of groups of products without an intended purpose). [17] So the soft tissue fillers without any intended medical purpose will have to comply with the MDR requirements as well, will be judged by expert panels and an approval by a notified body is required. [17] It is expected that these changes in procedure may result in some soft tissue fillers, both cosmetic product as well as medical device products, being withdrawn from the market. Increased pricing is anticipated for fillers that remain.

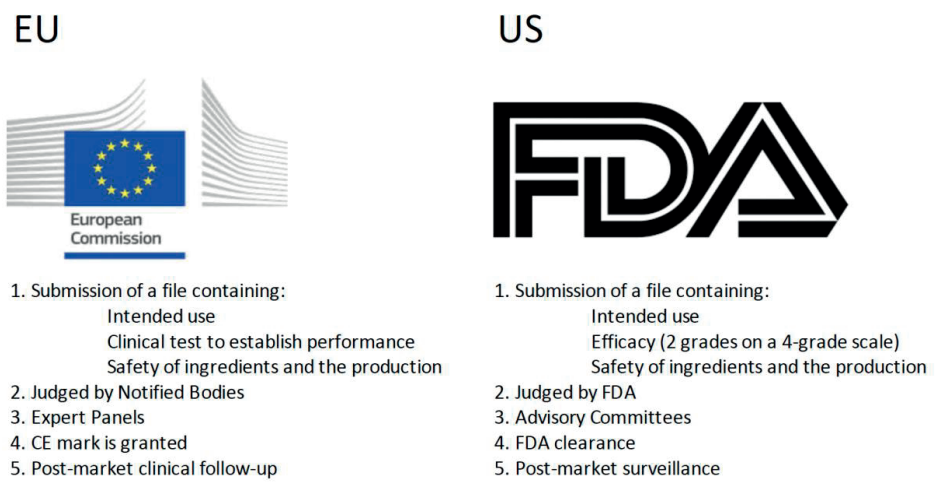

Fig. 2. The differences between the new rules and regulations for soft tissue fillers on the EU market and the US market.

The main limitation of this study was the small sample size. It is therefore possible that the research design had insufficient power to detect effects. However, a larger sample would probably not lead to different results as most p-values were far from reaching significance.

The small sample sizes were caused by several factors. The inclusion criteria were very strict; most patients that presented with Hyacorp-1000 and Hyacorp H-S (H-800) filler adverse events had a history of injection with permanent fillers, and some patients did not know which brand of HA filler was injected.

In conclusion, this study shows that the Hyacorp-1000 and Hyacorp H-S (H-800) fillers, that were used in Netherlands between 2011 and 2014, were associated with a higher chance of developing adverse events than other HA fillers because it stayed in the body for a longer period of time. The recent update of MDR for medical devices should prevent new HA fillers, also the current cosmetic fillers, which did not show safety and (clinical) performance from entering the European market.

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PART IX

DISCUSSION AND APPENDICES

CHAPTER 12

GENERAL DISCUSSION

In general, facial appearance is of tremendous importance for people. For ages individuals have tried to improve appearance by altering their looks through filler injections. Until recently all these attempts dramatically failed. For instance, in the late 1800's, an Austrian plastic surgeon, Robert Gersuny, used mineral oil and paraffin as soft tissue filler.[1] Although the method became popular, in the mid 1920'-s the number of treatments gradually declined because of the associated adverse events, such as granulomatous reactions and infections.[1] Almost 50 years later, bovine collagen was the first agent for cosmetic injection approved by the United States Food and Drugs Administration (FDA). In the 1990's its use slowly diminished because of the short effect (2-3 months) of the collagen filler and concerns about mad-cows' disease. In 1989, Balasz sparked a revolution by discovering the biocompatibility and lack of immunogenicity of hyaluronic acid (HA) as a filler compound.[2] The first actual HA filler, Hylaform® was introduced for cosmetic use in Europe in 1995 as a substance extracted from rooster combs. Only one year later, Restylane®, derived from bacterial fermentation, was the first non-animal stabilized HA filler.[3] In 1998, the first clinical trial with HA was performed.[4] In 2003, almost 10 years after the introduction of HA fillers in Europe, Restylane® was the first FDA approved HA based filler. In 2000, bio-stimulatory filler substances were introduced. With poly-L- lactic acid (PLLA), induction of collagen production was aimed to produce subcutaneous filling in a secondary way. This material entered the US market in 2004 and was followed in 2006 by calcium-hydroxyapatite (Radiesse®) and poly-methylmethacrylate (PMMA).[5]

The primary intended action of fillers is not pharmacological but mechanical (the filling effect). Based on this intended use and other claims made, soft tissue fillers are classified as medical devices by the regulatory agencies both in the United States (FDA) and in Europe (by the European Council based on the Medical Devices Directives (EU 93/42/EEC)). In Europe, new medical devices need to obtain a CE-certificate before being allowed to enter the market.[5] These are issued by Notified Bodies, based on files containing clinical evaluation, review of characteristics and published data of equivalent devices. As part of the CE certification process, the manufacturer must conduct continuous post-market clinical surveillance to provide an ongoing safety assessment. In the United States, the FDA applies more strict requirements for new fillers applications. Most importantly, the FDA demands an efficacy standard for the filler to induce a 2-points decrease in wrinkles score, assessed on a validated 5-point scale. Because of this barrier being much higher in the US than Europe, no permanent filler, except PMMA, was allowed on the US market. In 2010 more than 50 different filler products were available on the European market, compared to only 10 different products in the US.[6] Today, 34 soft tissue fillers are approved in the US[5], and more than 150 across Europe.[7]

A new set of rules and regulations for medical devices, MDR (Medical Device Regulation EU 2017/745) has come into force in Europe on May 25th, 2017. The 3-year transition period that was envisioned, has been extended by one year due to the COVID-19 pandemic. The new date

of application of the MDR is May 26th, 2021. From that moment onwards, the CE-certification process for soft tissue fillers in the EU is much stricter. Requirements for pre-market clinical studies and post-market clinic follow-up have gone up. Regarding post-market follow-up, this is a loosely defined concept[5], that does not require active participation of manufacturers other than when problems are indicated by user-physicians. With the new EU regulations post-market strategy will change from follow-up to surveillance requiring a much more active approach from manufacturers. A reduction in the number of different fillers in EU is expected.

In contrast to the popularity that the use of fillers has gained in the last 25 years, interest in their biology in vivo and over time is virtually absent. Research in cosmetic medicine primarily focuses on outcome and efficacy. The premise that these treatments are safe is regularly not questioned. It is, however, apparent that fillers can lead to complications, as one would expect with any medical treatment. This thesis focuses on three main issues regarding *The Origin of Soft Tissue Filler Adverse Events*. These are categorized by time. *The past* questions what we can learn from the past, most notably in terms of numbers of treatments and side-effects to gain insight in the magnitude of the problem. *The present* uses the material presently hand to establish theories about the pathogenesis, explore new ways for diagnostics and develop a new treatment option. *The future* views beyond the orient into what is in store and comments on governmental strategies and developments in society. Also new possible steps in research are discussed.

THE PAST

In the Netherlands it is not mandatory to report filler complications. However, several organizations register these complications. The official Netherlands Pharmacovigilance Centre LAREB is open for alerts from both lay people and professionals. Because notification of soft tissue filler complications is not compulsory, reports from LAREB are considered warning signals, but do not allow quantification of risk nor identify high risk population.

Dutch cosmetic doctors, being the largest group performing cosmetic filler treatments, report their complications with the Dutch Society of Cosmetic Medicine (NVCG). Twice yearly the 'complication alert group' reports all complications during the general assembly. In cooperating with the Dutch Digital Career Portfolio Company VREST, NVCG is creating an E-portfolio in which Aesthetic Physicians can register their patients' complications much easier.

A very successful example of registering foreign body implants in humans is The Dutch Breast Implant Registry (DBIR),[8] deployed by the Dutch Society of Plastic Surgery (NVPC). The registry has three main goals: 1) to monitor the quality of care (by registration, research, and evaluation using national quality indicators and benchmarking), 2) to monitor the quality of implants (by

registration, research, and evaluation of complications of different types of implants), 3) to trace implants and patients for recall when complications with an implant emerge.[8,9] A unique strength of the DBIR is its opt-out structure.[8,9] This implies that all patients are registered except those that explicitly wish to be excluded. Over 95% of all breast implants and tissue expanders are implanted by plastic surgeons who participate in the registration.[8,9] For the benefit of patient care, it would be of great help should NVCG follow this strategy.

The Inspectorate of Youth and Health (Inspectie Gezondheid en Jeugd, IGJ) has a reporting point for all sorts of problems with medical and cosmetic care (Landelijk Meldpunt Zorg). Also, by law physicians are required to report to IGJ all incidences that have led to death or serious injury.

An intriguing observation about adverse events in fillers is their delayed onset. For example, poly-alkylimide (Bio-Alcamid®) was introduced in 2001.[10] The first indication that this injected filler substance might cause problems became evident when inflammatory reactions were observed in 2005.[10] In 2009 data were published indicating that this substance was associated with a substantial number of adverse events. [11] These data were obtained in a retrospective study with a questionnaire sent to practitioners. Included were 3196 patients, that underwent 4738 treatments. A total of 154 (4.8 %) adverse events were registered. The manufacturer, in contrast, initially had claimed complications in only 0.06%.[10-13] Based on data from the Dutch distributor (AB Medical) it was calculate that 10.000-12.000 patients were treated with this substance in the Netherlands between 2002 and 2007, when distribution of the filler was halted (P. Velthuis, personal communication). In ErasmusMC we have treated at least 500 patients with poly-alkylimide complications over the past 10 years. Apart from inflammation, lumps of this product causing visible nodes/nodules at undesirable places were frequently seen. The prevalence of this latter complication is bound to increased, because with thinning of the subcutis in aging skin, deposits of this material will become apparent in more individuals. Hence, we believe that even the second estimate for complications with Bio-Alcamid is too low. Similar delays in appearance of complications are seen in other permanent fillers, such as liquid silicone and PMMA.[5,14]

Apart from problems adherent to the filler substance itself, other sources of adverse events after soft tissue filler treatment are technical or administration errors during the injections process. For cosmetic medicine, no certified training program existed until 2019. Dutch Authorities have subsidized the development of a training program for doctors in cosmetic medicine. The Ministry of Health has initiated a new independent organization (Stichting Opleiding Cosmetische Geneeskunde, SOCG) that receive a certified status in 2019 for a 2-year educational program. educates and trains physicians in cosmetic treatments. The participants are from different backgrounds. The term, "Cosmetic Physician" (in Dutch: Cosmetisch Arts KNMG), is a protected title that is only awarded to doctors who successfully complete the program. We view this as a major step forward; it allows consumers to recognize and choose

doctors with specific and thorough training in cosmetic medicine. While doctors can refer to themselves by other titles, for example, 'aesthetic physician', all healthcare professionals, by law, must register in the individual Healthcare Professions Act (BIG register), which is available for public consultation. The BIG- register provides clarity about the care provider's qualifications and entitlement to practice in the Netherlands.

Numbers

An intravascular injection leading to skin necrosis or permanent vision loss is the most alarming adverse event in soft-tissue-filler-treatments.[15-18] In order to preserve a baseline measurement, we examined the incidence of vascular adverse events (VAEs) in chapter 4. With this we can hopefully prove in the future, when ultrasound imaging to detect large arteries prior to injection is used universally, that this helps to decrease the number of VAE.

We calculate the incidence to be 1 in every 6,600 treatments.[19] There may be underreporting because many doctors do not recognize the clinical presentation of a VAE, and mistakenly diagnose it as herpes simplex or a bacterial infection. We consider many of these VAEs to be preventable by using ultrasound imaging of the treatment area before injection to identify aberrant vessels. However, with this low incidence, it might be difficult to prove that ultrasound imaging is beneficial for prevention of this complication.

In search for the incidence of soft tissue filler adverse events in general we are looking for a ratio. This is a fraction consisting of the number of complications being the numerator and the total number of soft tissue filler treatments being the denominator. The number of new patients referred to our out-patient clinic for filler complications in the years 2016-2020 were respectively 100, 205, 322, 352 and 341. Increase in volume is most likely due to increased awareness of the existence of filler induced AE among patients and physicians and the introduction of intralesional laser treatment as possible therapy.

These are mostly patients with more serious adverse reactions, that their physicians are unable to treat themselves. These numbers give us an indication about total cases of adverse events every year (the numerator of our fraction). We are, however, well aware of the fact that not every filler serious adverse event is referred to our clinic. Also, not every referral is a complication. We frequently observe mere error in technique leading unwanted nodules. From our studies in chapters 2, we gained an estimate of the total soft-tissue-filler-treatments. For 2016 we calculated 138,496 soft-tissue-filler-treatments performed,[20] rising to 162,702 in 2019.[21] In these studies, we searched for all physicians injecting filler. Yet, we might have overlooked certain groups. One group might be the self-injectors (Figure 1), individuals that buy fillers via internet, a relatively new phenomenon.[22] Or patients who have their treatment abroad because of financial reasons.

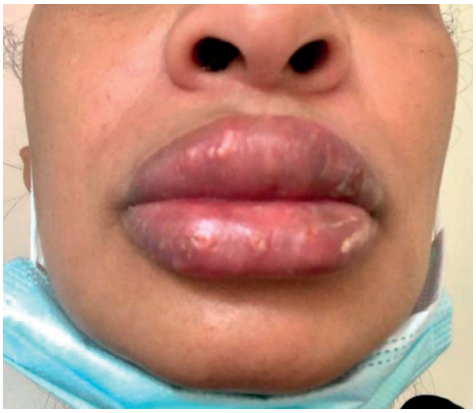

Figure 1 shows the result of self-injecting a 'soft tissue filler', bought on the internet and injected with an air pressure device.

Also, devices that deliver substances subcutaneously without a needle but with compressed air or other methods, have become popular devices for HA filler treatment by non-medicals and self-injectors. From these applications adverse events are being seen.

Another potential source of information about the total numbers of injection treatments, would be soft tissue-filler distributing companies. Unfortunately, these companies declined to disclose numbers, pointing to commercial confidentiality.

International figures are available, but these are not reliable. For instance, the American Society of Aesthetic Plastic Surgery publishes yearly numbers of soft-tissue-fillers-treatments performed by their members. But only 4.7 % of their members shared their numbers of treatments performed.[24] To be able to assess the incidence of filler complications it would be sensible for every cosmetic society in the world to keep track of numbers on soft tissue filler treatments. Once installed as is expected in 2022, the new E-portfolio of the Dutch Society of Cosmetic Medicine can have an exemplary function to other cosmetic societies everywhere in the world. As the new E-portfolio will be part of a quality cycle for accreditation.

In conclusion, it is difficult to obtain indisputable numbers on filler complications. Registrations of both numbers of treatments and numbers of complications are not all-encompassing. Also, filler complication may take years to emerge. But, for now, we assume that the incidence of the serious complications with soft tissue fillers can be regarded as low, but accurate estimates are lacking.

Young adults

A group that warrants special attention are the young adults. There is the common notion that the number of young persons, in particular women doing lip fillers is rising.[24] We are interested in this phenomenon because we expect those individuals to persist in using fillers. Data from geographically distinct Dutch clinics were analyzed in chapter 3.[24] We found indeed an 18% raise in the younger age group during the past 11 years. These individuals are expected to have a longer lifetime exposure to fillers than any generation before. They may even use fillers for the rest of their lives. Unclear is what the effects of a permanent exposure to fillers on the human body will be.

Young adults consist of two age groups: Generation Y, also called 'Millennials' (those born between 1981 – 1996) and Generation Z (those born 1997 – 2012). Being less tied to traditionally demarcated ways, they are now forced to make their own decisions at earlier ages than were former generations.[25] Social media exert high pressure of norms and expectations of the social environment and the social comparisons. This makes some individuals in these generations go astray in cosmetic medicine. The impulse to have filler treatment is combined with a choice based on price, not on quality. Therefore, they are prone to visit low-end clinics with a change of being exposed to not-sufficiently-trained providers (ranging from physicians to hair dressers). So, in the end these impulses can lead to severe adverse events, which can leave scars for the rest of their lives.

Humans do not have a fully grown prefrontal cortex until the age of 24.[26] This area in the brain contains the impulse center for decision-making.[27] Because of this and because of the proved deleterious effect of alcohol on the developing brain, Dutch legislation lifted the age to decide about drinking alcohol in 2014. The age to decide for filler treatments is currently 18. At least one parliamentarian (Joba van den Berg, CDA) has put forward a motion to lift this age-limit to 21. Although science tells us that 25 would be a preferable age for this, any lift increase of age limit would be a very sensible measure.[26]

Some very young patients are excessively influenced by modern celebrity culture. Many of the images that inspire these patients are heavily manipulated, and hence represent an unrealistic perception of what can be achieved with aesthetic intervention. This leads to the phenomenon of 'overfilling'. Overfilling can be a constant trigger for the immune system. Indeed, the ethical principles of non-malfeasance and beneficence require that doctors should decline to treat patients with unrealistic expectations because the risks might outweigh the potential benefits in these individuals.[28]

THE PRESENT

Pathogenesis

Bacteria

Some authors have concluded that bacterial infection is the major cause of inflammatory adverse events (IAE) after soft tissue fillers (SFT) injections.[29-31] However, the mere presence of bacteria in inflamed tissue is not conclusive proof for infection. Our group (and many others), using conventional culturing techniques, are almost invariably confronted with a negative outcome of cultures in IAE. Aggregating low virulent bacteria have received considerable attention recently. Although their existence has never been proved in IAE, biofilm formation has particularly been implied. Biofilms are defined as a structured community of microorganisms encapsulated within a self-developed polymeric matrix and irreversibly adherent to a living or inert surface.[32] Biofilms exert tolerance toward immune cells and antibiotics.[33] When bacteria are brought in with the initial injection of the STF, they are attacked by macrophages.[29-30] Our research established that macrophages are the only immune cells present in the vicinity of fillers. Histopathological studies of the excised tissues surrounding the hydrogel filler indicates that the tissue response progressed from an initial acute inflammation to the chronic inflammatory response characterized by the migration of macrophages.[33] The presence of macrophages is also noted in complications with other foreign body implants such as failed pacemaker, breast implants and total hip replacement, leading to the hypothesis that these immune cell play a role in the inflammation presented clinically. In chapter 5 and 6 we hypothesize that bacteria and macrophages can form an intimate relationship, that perpetuates their mutual presence.

Biofilm formation around the filler substance may be patchy (see figure 1 in chapter 5) and can therefore easily be missed in biopsies and sampling for culturing. In chapter 5 we report on results of biopsies with a PCR technique (IS Pro). Using this we were able to identify bacteria species in the filler material in-situ, that were clearly related to the skin microbiome. Apparently, bacteria were introduced during the filler injection process. It is unclear whether IAE really have an infectious pathogenesis, this finding is an urgent call for all cosmetic injectors to work with outmost hygienic precautions. Therefore, the use of a disinfection agent (e.g. chlorhexidine) for cleaning of the skin before a filler treatment is very important. Clearly the policy that some doctor and clinic deploy to store filler substance after having used only part of a vial, to be utilized later for the same (or sometimes even a different!) patient must be abolished. Alharbi reported in 2019 that vials containing filler stored after opening in low percentages displayed substantial bacterial growth inside the syringe.[34]

The plethora of bacteria found in IAE makes it likely that they play a role in the inflammation reaction. For serious IAE antibacterial treatment should be the first line of treatment. The choice of the most appropriate antibiotic regimen is however difficult, given the world-wide

origin of IAE. If IAE is regarded as an infection this world-wide origin makes the choice of an appropriate antibiotic regimen very difficult. Resistance of bacteria because of excessive usage of antibiotics is a world-wide problem, with great variations in in resistance patterns in different regions.[35] For the Netherlands with its very restrictive antibiotics use, the most reasonable line of attack seems penicillin, macrolides and chinolons. The best advice for antibiotics against infections acquired in other parts of the world seems vancomycin. For severe infections support of a biofilm penetrating antibiotic such as rifampicin can be advantageous.

Immune system

In Chapter 7 we discussed the nature and potential antigens for an immune response after filler injection. We conclude that there are no indications that the adaptive immune system plays a role in the IAE, and that only the innate immune system plays a role in the IAE. However, this gives no indication as to the causative antigen being either bacteria, the filler substance (or its degradation products), or a specific genetic predisposition in the patient. Probably, a combination of these factors is responsible for the pathogenesis of late onset IAE.

An immunogenetic predisposition may play a role in developing IAE. Some individuals may be more susceptible for foreign substances. The human leukocyte antigen (HLA) genes on the MHC region of chromosome 6 are thought to be important in, for example, silicon induced adverse events in breast implant patients. The gene is very polymorphic, multiple single nucleotide polymorphism (e.g., common variants) are associated with different kind of inflammatory diseases. In particular ankylosing spondylitis 90% of Ankylosing Spondylitis patients exhibit HLA-B27. A strong genetic association for systemic sclerosis lies within the MHC region, with loci in HLA-DRB1, HLA-DQB1, HLA-DPB1, and HLA-DOA1 being the most replicated. Important for filler implants, HLA genes have been implicated to play an important role in the development of a variety of autoimmune diseases in women with silicone breast implants.[36-40]

Ideally a Genome-wide association study (GWAS) is performed including large numbers of patients to detect common variants associated with disease or adverse events. In the absence of such large cohorts, a candidate gene approach can be performed. Because of this, our study reported in chapter 8 was designed to perform HLA typing on different groups of subjects. With this we wanted to determine whether patients who experienced late-onset inflammatory adverse events to STF had HLA serologic specificities that differentiate them from patients who did not experienced late-onset inflammatory adverse events after STF injections. HLA subtype-B*08 and HLA subtype-DRB1*03 showed to be a predictor of an adverse reaction to a STF injection. Should this result be confirmed in other studies, this might identify persons with an increased chance for inflammatory reactions after filler injection. Apparently, patients in this group have an immunologic profile that makes them more susceptible to filler complications. It would be interesting to investigate whether these HLA subtypes can also by detected more

frequently in patients reacting to other implants such as breast or hip prostheses, or cardiac implant devices. In the HLA-study only 16% of patients had the specific subtype combination that may render them prone to react on fillers. Thus, in most of the cases another factor or a combination of factors must be implicated. Moreover, the risk estimate of the HLA subtypes was modest making it less good of a predictor in clinical practice.

In 2011, Shoefeld et al. coined the term ASIA (Autoimmune/inflammatory Syndrome Induced by Adjuvants) for an umbrella entity that included several clinical and laboratory features first described by Miyoshi et al. in 1964, together with other new clinical and laboratory parameters related to exposure to diverse external stimuli.[41,42] One of these could be silicone leaking from breast protheses. Typically, patients exert one or several clinical symptoms such as muscle weakness, arthralgia, chronic fatigue, cognitive impairment, dry mouth, and others. Clinical signs may appear days to years after exposure to the external stimulus. Epidemiological data on ASIA in foreign body material are missing.[43] Since a causal relationship between adjuvants and this 'autoimmune-like' syndrome is difficult to establish, for now, ASIA remains a theoretical concept. However, should ASIA eventually be proved to be a genuine disorder, this may impact cosmetic medicine. Given their ability to induce an immune response,[44] soft tissue fillers may also be implied as causative agents in the ASIA syndrome. One can consider it fortunately that all current fillers are coined resorbable and therefore will eventually depart the body. However, with the large amount of HA filler that are used in the so-called liquid facelift technique, decomposition may in some instances take many years. With ultrasound imaging we have detected considerable amount of HA filler material in patients with recurrent unexplained inflammatory reactions several years after filler treatment.[45] The tendency of individuals to start at younger ages with filler injections combined with the anticipation that they will have a decades long exposure to these materials, can also result in more problems in this respect.

New is the observation that COVID-19 vaccines may trigger inflammation around STF.[46,47] In the initial EMA application study from Pfizer company three patients showed inflammatory reaction around previous injected fillers. Surprisingly one control patients had an identical response. Later, other vaccines were also implicated giving the same type of reaction. Related may be the empirical fact of patients exhibit an exacerbation of their adverse event with stress and flu-like symptoms.[48]

So, in conclusion, the answer to the pathogenesis of adverse events after soft tissue filler injections seems not to be uniform. Different pathogeneses may lead to a common clinical outcome

Diagnostics

Imaging Studies

Ultrasound (US) imaging has long been used in dermatology to evaluate vascular structures,[49] in particular for diagnoses in venous disorders of the lower leg. The indications for using US have expanded the past few years and now include hidradenitis suppurativa and detection and characterization of subcutaneous fillers, but also treatments of filler complications. Fillers have characteristic echogenicity's on ultrasound imaging as explained in chapter 9. Hydrophilic (gel-like) fillers such as hyaluronic acid fillers and permanent fillers (Bio-Alcamid of Aquamid) will appear anechoic. An interesting observation that warrants further research is the fact that HA fillers gradually seem to become more hypoechoic or even isoechoic over time. We hypothesize that this change in appearance results from degradation of the filler and concomitant loss of water. Hydrophobic fillers such as silicone oil and PMMA render a hyperechoic image. In these types of fillers helpful artefacts can be found, such as the snowstorm pattern with posterior shadowing (silicone oil) and comet tail artifacts (PMMA) are frequently seen. Bio-stimulatory fillers initially display hypoechoic areas of injection, but after months in situ change their appearance to more hyperechoic.

Another promising area to investigate is the way US imaging can help to control and thus improve the administration of fillers. First, directly after HA filler injection the shape of the filler substance and the anatomical plane it is located, can be traced. In a pilot study, we made surprising observations. For instance, a bolus injection on the edge of the mandible did not display as expected a hypoechogenic pocket on the bone. Instead, it gave rise to masses of material interspersed between fibers of the masseter muscle. Other areas presented unexpected results as well, both regarding the depth and anatomical level of the filler as well as the behavior of the filler during the injection process. Thus, it seems that US can give important feedback for injectors and can help to enhance quality of work.

Treatment

Research presented in this thesis has given some insight regarding pathogenesis of IAE, but it has given us no definitive answer how to treat a patient that walks in with an IAE after filler injections. Since bacteria seem to be almost invariably present, antibiotics remain our first line of treatment, as discussed on page 11.

If not successful within one week, immunosuppressive agents are added. At first this consist of oral corticosteroids. When the prospected treatment period exceeds one month, other immunosuppressants are considered such as ciclosporin, azathioprine and methotrexate (Figure 2). In every case removal of as much as possible of the filler material is advisable, since the foreign material is the essence of the problem, both as substrate for bacterial growth and as trigger for the immune system.

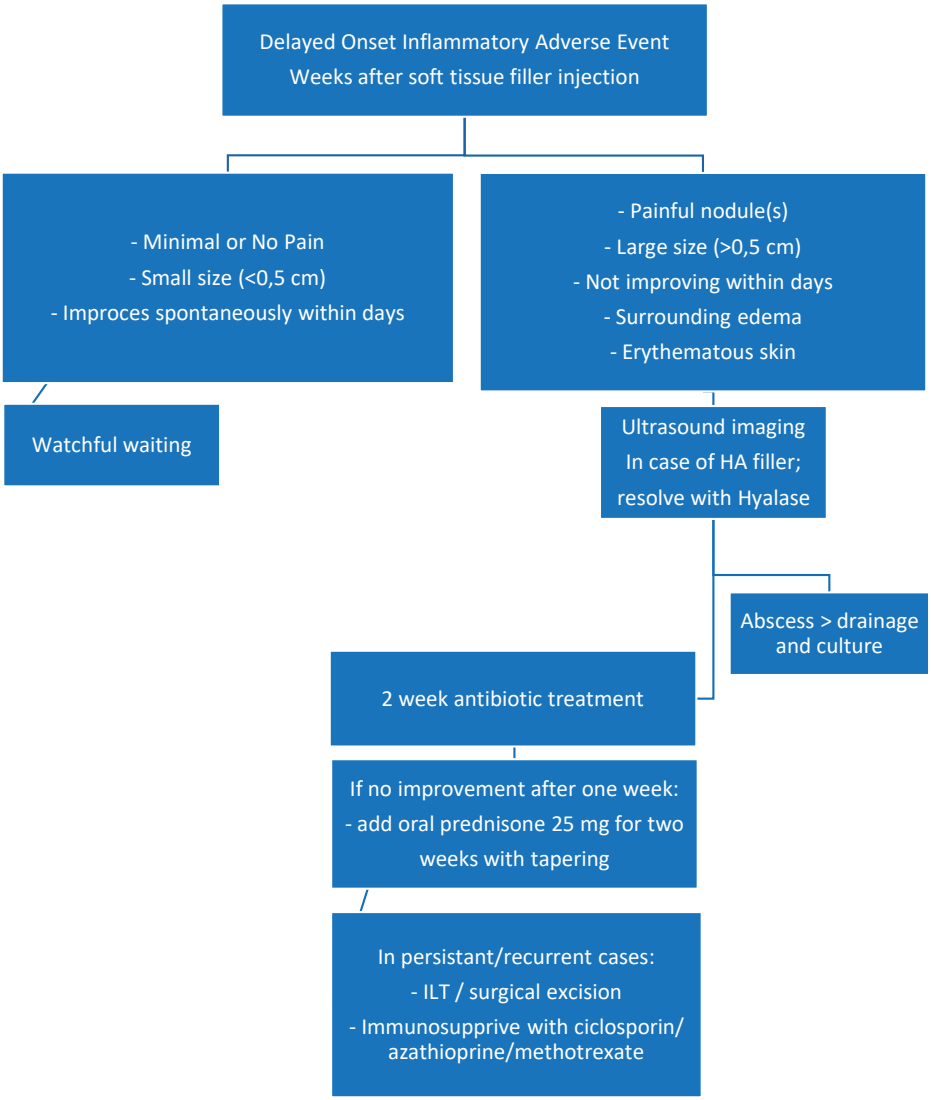

Figure 2 Algorithm for treating delayed onset inflammatory adverse events after soft tissue filler injections.

Although surgical removal seems the most logical approach, in many cases this is impossible. For instance, with injection of liquid silicone in lips the filler material spread through the most parts of the lip. PMMA spreads subcutaneously after injection and its circumference is frequently impossible to delineate. We found intralesional laser therapy (ILT) to be a valuable part of the armamentarium as discussed in chapter 10.[50] With this filler substances can be 'evaporized', and the total load of the offensive agents diminished. In some cases, surgical excision may however be preferable. In a pilot study on silicone filler in lips, we concluded that surgical excision of mucosal surplus rendered a better cosmetic outcome than ILT (unpublished results).

THE FUTURE

As concluded in *The Past* the incidence of serious complications after filler injections is low. However, the impact of a complication or other types of adverse event can be great. Also, given the large and rising scale of injectable treatments, the total number of adverse events is high. Our efforts should continue to be aimed at bringing down the number of adverse events of soft tissue filler injections by increasing the tolerability of the fillers, optimize indications and improve filler administration.

Regulations

In 2013 the Dutch Ministery of Health provided a framework to more stringent regulations in the cosmetic medical sector step by step.[51] This has resulted in some major improvements.

The Cosmetic Care Quality Framework (Kwaliteitskader Cosmetisch Zorg) issued in November 2019 by Zorginstituut Nederland is aimed to ensures safety and quality in cosmetic medical care.[52] This quality framework is based on the patient's perspective. It should always be clear what a patient can expect from the cosmetic healthcare provider and the treatment itself. It also sets rules for clinics and doctors about continuous education, requirements of an in-office quality framework, office environment, equipment, data protection, etc.

New regulations regarding advertising for cosmetic treatments, Code of Medical Cosmetic Treatments Performed by Doctors (CCBA) are in place since October 2017.[53] The concept of 'rational use' is leading. It is not allowed to impose time pressure or to hold promotional campaigns with an obligation to purchase. Advertising should not be misleading, for example not giving guarantees about the result. Claims must be substantiated. Images may be used if they are truthful and should not give the impression that a particular body shape or appearance is preferred. The term 'traceability' applies to any advertisement for medical cosmetic treatments. This means that the title, position, and BIG registration number of the treating physician must be stated. What is special is that every advertisement must be accompanied by the message: "Watch out. Making yourself more beautiful can turn out ugly. A successful procedure starts with a suitable doctor."

In January 2021 the Dutch Health and Youth Care Inspectorate (IGJ) requested the National Institute for Public Health and the Environment (RIVM) to prepare an assessment framework for the risks of new treatment modalities in the cosmetic sector.

European legislations govern the application of medical devices, such as fillers. Regulation of medical devices involves competing goals of assuring safety and efficacy while providing rapid movement of innovative therapies through the investigative and regulatory processes as quickly

as possible. A new set of rules and regulations for medical devices, MDR (Medical Device Regulation EU 2017/745) has recently come into force in May 2021. MDR demands a much more active involvement of medical doctors being the end-users of the materials. They will be part of Expert Panels and post-market surveillance strategies that manufacturers must develop.

In conclusion, in the past five years Dutch and European authorities have done much to improve quality of care in cosmetic medicine. This will help to control excesses such as inapt filler materials and doctors in the future. Yet, we must stay vigilant, as the new devices for self-injecting of fillers prove.

Future research

Although non-resorbable (permanent) fillers are banned in many countries, its worldwide use is still enormous and so. So are its complications. In the Netherlands we are confronted with Dutch inhabitants being injected elsewhere and foreigners with permanent filler problems seeking help with us. Resorbable (non-permanent) filler complications remain an interesting area for further research. However, in this group collecting material is difficult, given the patients reluctance to cooperate with biopsies. To overcome this objective of young cosmetic patients a pilot study is currently employed to detect if needle-biopsy devices will deliver sufficient material for research and will diminish patients' objections. Yet, an integrated approach aligning the different perspectives that follow from immunology, bacteriology and genetic research in both resorbable and non-resorbable fillers would be the next step. Viewing this, a worldwide cooperation of scholars in research, leading to larger sample sizes would obvious be of benefit. In such a cooperation new fillers of poor-quality would become apparent much quicker. With the assistance of industry and key opinion leaders in the field of filling agents new complications can be identified earlier and overall better care can be provided. Combined forces can exert a strong power to change regulations in different parts of the world and ban non-resorbable fillers that devastate peoples lives.

New diagnostic methods can be of help. A innovative in-vitro research method that could be of use is the skin-on-a-chip model.[54] Instead of the traditional Petri dish monocultures, physiologic skin model with different cell types have been engineered. Human skin structures have been integrated onto microfluidic platforms to construct skin-on-a-chip systems that can mimic the complex *in vivo* situation.[55] These models are primarily used to test pharmaceuticals. In this skin-on-a-chip a soft tissue filler can be implanted and the change of biochemical markers in supernatants can be measured. However, there are two challenges to be addressed. One significant challenge is to mimic the structural complexity of living human skin, including vascularization (blood micro vessels), immunity (T-cells, macrophages).[55] Secondly, soft tissue fillers are injected intra- and subdermal, but no skin-on-a-chip models containing subcutis have been created until now.[55]

For a better understanding of the pathogenesis of complications, analysis of local and systemic immunological factors can be of help. In tissue the CD-68 positive cells, that we found in infiltrates, can be further identified more specifically, being either macrophage type 1 or type 2, monocytes, T-cells, or dendritic cells. Also, localization of key cytokines and chemokines (IL-6, IL-8) is of interest as this would lead us to the most reactive areas in the inflammation process. Tissue testing with a more diverse pallet of immunohistochemical markers (IL-2, CD3, CD4, CD8 and HLA-class II: CD20, CD68 and MPO) might be helpful to define the type and extent of the immune response.[56] For systemic aspects blood testing is an option. With a lymphocyte transformation test (LTT) proliferation of circulating T cells to a drug (or in this case a filler) can be tested in vitro. From this one can deduce a previous in vivo sensitization to the substance.[57] Absence of any transformation would strengthen the conclusion that no specific immune response is involved in IAE with fillers.

Another challenge is measurement of the longevity of fillers in situ. The clinical effect may last for 9-18 months. But from the current research with ultrasound, it became clear that hyaluronic acid fillers are present for a much longer time in the tissue. It would be interesting to know what natural course of soft tissue fillers over time in tissues is in terms of dissolvement rate. This is particularly important in HA fillers, because of the natural course of HA filler degradation and the expected exposure of low-molecular weight (LMW)-HA in this process. LMW-HA have a direct proinflammatory effect, whereas high-molecular weight (HMW) HA has primarily an anti-inflammatory effect. LMW-HA can serve as an endogenous danger signal activating the innate immune system. Apart from ultrasound imaging of facial blood vessels a special magnetic resonance angiography (MRA) method has been developed.[58] Assessing the value of this method in comparison to ultrasound or other imaging techniques such as computerized Tomography angiography is worthwhile.[59] Vascular ultrasound imaging may benefit from researching the resistive index (Pourcelot index) in normal and pathological condition in the face. This index is a machine-calculated flow parameter in ultrasound, derived from the maximum, minimum, and mean Doppler frequency shifts during a defined cardiac cycle. Along with the pulsatility index (PI), the resistive index is typically used to assess the resistance in a pulsatile vascular system.[60] Pourcelot indices and PIs have not yet been established for the major facial arteries. Changes in these indexes in arterial obstruction can be helpful in the swift and early detection of obstructed arteries, since with current methods this sometimes proves to be a very cumbersome enterprise. Also measuring these indices may settle the debate whether arterial compression leading to hypoxia is a viable concept.

Given the steady rise in publications on ultrasound imaging in conjunction with filler treatment, and the many requests for doctors in our department for presentation at scientific conferences, we believe that ultrasound will play an important role in the future of cosmetic medicine.

Concluding remarks

Scientific research in cosmetic medicine is still in an early phase. In this research some steps have been taken. Many areas should be explored further. From a clinical perspective diminishing side effects has high priority. Regulations should be implemented to better provide care and track complications with the assistance of industry, authorities, and key opinion leaders in the field of filling agents. With the growing international collaboration that is being set up with other expert groups around the world, a basis has been created to expand our efforts in the future.

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CHAPTER 13

English Summary

Treatment with injectable soft tissue fillers has become an increasingly popular, minimally invasive method for facial rejuvenation and correction of certain medical conditions. Such a treatment requires no anesthesia or extensive preparation and is relatively easily performed in a relatively short period of time. These treatments have limited down time and are relatively inexpensive. In addition, filler injections have an overall low rate of adverse events. However, when adverse events occur, their impact may be considerable.

The aim of the studies described in this thesis, was to investigate the origin of soft tissue filler adverse events. We focused on factors that may provoke the occurrence of an adverse events after soft tissue fillers injections: 1) immunological profile, 2) bacterial contamination, and 3) genetic predisposition. This was done by examining biopsies of patients with adverse events after treatment with soft tissue fillers. Patients were divided in two groups, depending on their clinical status. The Inflammatory group displayed late-onset (2 weeks or later after treatment) symptoms of inflammation and the non-Inflammatory group displayed other non-inflammatory adverse events.

Chapter 1 provides an introduction to soft tissue fillers with an overview of the different types of soft tissue fillers. Soft tissue filler injections, together with botulinum toxin injections are referred to as *injectables*. Research has shown that injectable treatments have an overall positive effect on several aspects of clients mental states. The use of soft tissue fillers has increased tremendously over the past ten years. With almost a million procedures in 2019 it has become the second most frequently performed non-surgical procedure in plastic surgery offices in the United States. Soft tissue fillers are mostly classified by their biodegradability into non-resorbable and resorbable fillers. Resorbable fillers consist basically of two variations: hyaluronic acid fillers and bio-stimulatory fillers.

Also, the definition of the term adverse events is addressed. The terms adverse event and complication are sometimes used in a loose way and are not well defined. In this thesis *adverse event* is defined as any non-intended event related to filler injection treatment, ranging from very mild with no interference in a clients daily activities, to very severe. The latter is regarded as a *complication*, being defined as an injury resulting in prolonged hospital stay, disability at the time of discharge or death. Complications can be viewed as a subset of adverse events. The overall aim of the studies is set out.

Chapter 2 focuses on the number of injectable treatments in the Netherlands in both 2016 and 2019. Objective data on the number of cosmetic injectable treatments (botulinum toxin A and fillers) performed annually are lacking. The objective of the postal survey was to identify the physicians administering cosmetic injectable treatments and quantify the

number of treatments given. A total of 122 physicians responded in 2016 (response rate of 37%), of whom 60 (49%) provided exact numbers, 62 (51%) gave estimates. The calculated number of injections performed in the Netherlands in 2016 with BTX-A was a little more than 250.000 and with filler almost 140.000. A total of 99 doctors responded in 2019 (response rate of 32%), of whom 63 (64%) provided exact numbers and 26 (26%) gave estimates. The calculated number of injections performed in the Netherlands in 2019 with BTX-A was a little less than 250.000 and with filler a little more than 160.000. The numbers in 2016 and 2019 are remarkably consistent, suggesting that they could represent the actual numbers in the general Dutch population. In this 3-year period the number of filler treatments increased by 12%.

Chapter 3 reports about a multi-center retrospective observational study that compares the usage of botulinum toxin and hyaluronic acid fillers in young adults between 2008 and 2019 in the Netherlands. This was undertaken by recording Electronic Health Reports (EHR) of three medical centers in the Netherlands. These medical centers gave a representative view on a nationwide trend since they contain both high-end locations and accessible ones. Added together, the acquired data originates from ten locations spread across the Netherlands. Young adults are defined as the age group 18-25 years old in this study, based on our clinical categorization of this group. A total of 12 628 patients were included, spread over the years 2008-2019. The results show an increasing of botulinum toxin and hyaluronic acid fillers usage in young adults.

Chapter 4 examines the incidence of vascular obstruction after filler injections. In medical literature, frequencies of vascular adverse events (VAEs) are not detailed but estimated to be 1:2000 to 1:10,000 (0.05–0.01%). In this prospective study we included patients referred to our out-patient clinic for filler induced problems. A total of 44 patients (3 male, 41 female) with a VAE due to hyaluronic acid or calcium hydroxylapatite fillers were referred to our outpatient clinic in a period of 24 months. The diagnosis was confirmed by clinical presentation (reticulated bluish pattern with/without pustules and wounds) and doppler-ultrasound images (hypervascular turbulent artery with/without detectable filler occlusion). With the numbers found in chapter 2 and the knowledge that virtually every patient with a vascular adverse event (VAE) is referred to our center, we were able to calculate the incidence of vascular occlusions filler treatments. We calculated the incidence of VAEs after filler injections to be 1:6756 (or 0.015%).

Chapter 5 describes the results of microbiota found in biopsies of 29 patients with late-onset inflammatory adverse events after soft tissue filler injections. We used a new and very sensitive method to detect microbiota, the IS-pro method. This is a novel broad-range PCR technique based on length and sequence variations of the 16S-23S ribosomal interspacer (IS) region. IS-pro can detect bacteria at low abundances and identify them up to species level. To exclude contamination from skin microbiota we compared the microbiota found on skin swabs with that found in biopsies of the same patient. These were found to be non-similar, eliminating

contamination during sampling. However, a high level of Gram-positive bacteria was found in biopsies of soft tissue fillers. This suggests that these bacteria were introduced during the primary filler injection treatment.

Chapter 6 focuses on etiology of late inflammatory reactions (LIRs) after hyaluronic acid (HA) filler treatment. Some argue these result from a hypersensitivity reaction, although evidence to support this is very scarce. Most reports on such reactions are not substantiated by positive skin tests. Twelve patients were referred for diagnostic evaluation of potential hypersensitivity reactions to hyaluronic acid (HA) fillers. The twelve patients were evaluated for general allergic screening (patch-tests), as well as specific intradermal testing (injection of 0.1cc boluses) on the medial upper arm with a selection of several hyaluronic acid (HA) fillers currently available. A positive allergic reaction was defined as erythema, firmness or swelling. At the initial testing and during the 4 months follow-up period no reactions to any of the tested HA fillers were found. Although LIRs have often been diagnosed as hypersensitivity reactions, in our study no positive reactions to the six used HA fillers were reported. The results suggest that neither type I nor type IV hypersensitivity plays a role in late inflammatory reactions (LIRs) to hyaluronic acid (HA) fillers.

Chapter 7 describes a pilot study addressing the question whether there is evidence that the adaptive immune system plays a role in late-onset inflammatory adverse events after soft tissue filler injections. Some authors have postulated that inflammatory adverse events to STF result from type IV (delayed type) hypersensitivity, a reaction type formed by adaptive immunity. These inflammatory adverse events often do not respond well to the regular treatment procedures. Defining the exact etiology could be helpful in treating them. We included 47 patients with adverse events after soft tissue filler injections. Biopsies were taken from the area of the adverse event. In 18 of 47 patients, immune cells were found. All these 18 cases showed CD68 positive immune cells. Virtually no CD3 positive immune cells were found. Our results suggest no role for the adaptive immune system in adverse events after soft tissue filler injections. Mostly macrophages are involved after injections with soft tissue fillers, but their presence is not significantly correlated to the inflammatory response. Macrophages do not seem to play a role in provoking this reaction.

Chapter 8 examines whether HLA polymorphisms are associated with late-onset inflammatory adverse events related to dermal fillers. Most of these adverse events seem to have an immunological basis, where the fillers act as adjuvants more than as direct T-cell activators, on a possible background of genetic predisposition. A total of 211 patients were included. DNA was obtained by means of oral swab or blood extraction. Low-intermediate resolution genotyping of HLA-A, HLA-B and DRB alleles of all patients were performed by sequence-specific oligonucleotide probe (SSOP) methods. Of the 211 patients in the sample, 25 had the combination

of HLA subtype-B*08 and HLA subtype-DRB1*03. This was 16.3% of the inflammatory group and 4.9 % of the reference group. This combination of HLA subtypes was associated with a three-fold increase in the odds of developing immune mediated adverse events (odds ratio = 3.79, 95% CI 1.25 to 11.48). Genetic polymorphisms such as HLA combinations may identify patients at risk of developing late onset immune mediated adverse events to dermal fillers.

Chapter 9 focuses on ultrasound to improve the safety of hyaluronic acid filler treatments. Doppler ultrasound (duplex) is commonly used in dermatology to evaluate dermatological conditions of the skin and vascular structures. Before a filler treatment is performed, with ultrasound previous filler treatments can be brought into sight and avoided. Also, variation in courses of vessels can be mapped. In case of adverse events, the filler itself and the morphology of the surrounding tissues are visible. Dislocation, abscesses, and vascular adverse events can be seen. Under ultrasound guidance, hyaluronidase can be injected precisely into the filler deposit. So, in many ways ultrasound examination can be an important tool to improve the safety of hyaluronic acid filler treatments.

Chapter 10 describes the usage of intralesional laser treatment for dermal filler complications. Although there is ongoing popularity of dermal filler use, much is unknown about complications with regard to optimal treatment options. For complications caused by filler treatments, in general, many doctors advise one of two treatment regimens: either systemic drugs or surgical removal of the material. This chapter gives arguments for a third possible treatment option: removal of the material by intralesional laser treatment. The lasers used for intralesional laser treatment are lasers developed and used for endovenous laser treatments of varicose veins. The intralesional laser treatment procedure for dermal fillers consists of inserting a fiberoptic laser into the area of the product and vaporizing it. 242 patients (214 women and 28 men) were treated with intralesional laser treatment, in the majority of patients, an improvement was achieved (92 percent).

Chapter 11 reviews the story of the hyaluronic acid-based fillers Hyacorp-1000 and Hyacorp H-S (H-800) that in 2014 were withdrawn from the Dutch market after concerns about their safety. We assess what the most plausible factor of the increased number of adverse events with these substances would be, either patient related factors or a factor inherent to the filler itself. We also assess how new European legislation will affect the approval process for new fillers and prevent safety issues with fillers. The results show that patients treated with Hyacorp-1000 and Hyacorp-S (H-800) that reported adverse events were significantly older than those in the Hyacorp-1000 and Hyacorp-S (H-800) group without adverse events. In patients treated with Hyacorp-1000 and Hyacorp H-S (H-800) that reported adverse events, the filler was significantly longer in situ then in patients who had adverse events related to another HA filler. We conclude that increased crosslinking of the filler caused the problems reported. The

upcoming legislative EU update to Medical Device Regulation (MDR) will prevent unsafe fillers from entering the EU market or will detect safety problems much earlier.

Chapter 12 concludes the thesis with an overall discussion of the results and recommendations for future research on the matter of the origin of late inflammatory reaction (LIR) are given. No major cause for these adverse events could be identified. But anomalies were found in bacterial content of the filler in-vivo. And in the immunology of the patient mostly macrophages were found. It seems that either different causes can lead to an identical clinical outcome. Or LIR might be a complex disease, where in the meeting of more than one abnormality in the same timespan leads to inflammation. The most important future research will consist of 1) an innovative in-vitro research method, skin-on-a-chip, in which a soft tissue filler can be implanted and the change of biochemical markers in supernatants can be measured. 2) implementation of ultrasound imaging to follow the natural course of HA filler degradation over time. The biggest development to reduce the number of side effects may lie in new rules and regulations regarding the quality of injecting doctors and fillers. In the past five years Dutch and European authorities have done much to improve quality of care in cosmetic medicine. This will help to control excesses such as inapt filler materials and doctors in the future. Although scientific research in cosmetic medicine is still in an early phase, in this research some steps have been taken.

Together the 12 chapters in this thesis should provide practitioners with a deeper understanding about **the origin of soft tissue filler adverse events**

CHAPTER 14

Nederlandse samenvatting

De behandeling met injecteerbare fillers voor weke delen is een steeds populairdere, minimaal invasieve methode geworden voor de verjonging van het gezicht en de correctie van bepaalde medische aandoeningen. Een dergelijke behandeling vereist geen anesthesie of uitgebreide voorbereiding en kan relatief gemakkelijk in een relatief korte tijd worden uitgevoerd. Deze behandelingen hebben een beperkte hersteltijd en zijn relatief goedkoop. Bovendien hebben fillerinjecties over het algemeen een laag percentage complicaties. Wanneer zich echter bijwerkingen voordoen, kunnen de gevolgen aanzienlijk zijn. Het doel van de in dit proefschrift beschreven studies was om de oorsprong van complicaties van fillers voor weke delen te onderzoeken. We richtten ons op factoren die het optreden van bijwerkingen na injecties met weke delen fillers kunnen uitlokken: 1) immunologisch profiel, 2) bacteriële besmetting, en 3) genetische predispositie. Dit werd gedaan door biopten te onderzoeken van patiënten met complicaties na behandeling met fillers. De patiënten werden in twee groepen verdeeld, afhankelijk van hun klinische status. De inflammatoire groep vertoonde laat ontstane (2 weken of later na behandeling) symptomen van ontsteking en de niet-inflammatoire groep vertoonde andere niet-inflammatoire bijwerkingen.

Hoofdstuk 1 geeft een inleiding tot weke delen fillers met een overzicht van de verschillende soorten weke delen fillers. Injecties met weke delen fillers worden, samen met botuline toxine-injecties, injectables genoemd. Onderzoek heeft aangetoond dat behandelingen met injectables een algemeen positief effect hebben op verschillende aspecten van de mentale toestand van cliënten. Het gebruik van fillers is de afgelopen tien jaar enorm toegenomen. Met bijna een miljoen procedures in 2019 is het de tweede meest uitgevoerde niet-chirurgische procedure in plastische chirurgie klinieken in de Verenigde Staten geworden. Weke delen fillers worden meestal ingedeeld naar hun biologische afbreekbaarheid in niet-resorbeerbare en resorbeerbare fillers. Resorbeerbare fillers bestaan in principe uit twee varianten: hyaluronzuurfillers en bio-stimulatoire fillers. Ook wordt ingegaan op de definitie van de term adverse events. De termen adverse event en complicatie worden soms op een losse manier gebruikt en zijn niet goed gedefinieerd. In dit proefschrift wordt onder adverse events verstaan: elke niet-beoogde gebeurtenis die verband houdt met een injectiebehandeling met fillers, variërend van zeer mild met geen hinder voor de dagelijkse activiteiten van een cliënt, tot zeer ernstig. Het laatste wordt beschouwd als een complicatie, gedefinieerd als een letsel dat leidt tot een langer verblijf in het ziekenhuis, invaliditeit op het moment van ontslag of overlijden. Complicaties kunnen worden beschouwd als een subgroep van adverse events. Het algemene doel van de studies wordt uiteengezet.

Hoofdstuk 2 richt zich op het aantal injectable behandelingen in Nederland in zowel 2016 als 2019. Objectieve gegevens over het aantal cosmetische injectable behandelingen (botuline toxine A en fillers) dat jaarlijks wordt uitgevoerd ontbreken. Het doel van de post-enquête was het identificeren van de artsen die cosmetische injectable behandelingen toedienen en

het kwantificeren van het aantal gegeven behandelingen. In 2016 hebben in totaal 122 artsen gereageerd (respons van 37%), waarvan 60 (49%) exacte aantallen hebben opgegeven, 62 (51%) schattingen. Het berekende aantal injecties dat in 2016 in Nederland is uitgevoerd met BTX-A was iets meer dan 250.000 en met fillers bijna 140.000. In 2019 reageerden in totaal 99 artsen (respons van 32%), van wie 63 (64%) exacte aantallen gaven en 26 (36%) schattingen. Het berekende aantal injecties dat in 2019 in Nederland is uitgevoerd met BTX-A was iets minder dan 250.000 en met filler iets meer dan 160.000. De aantallen in 2016 en 2019 zijn opmerkelijk consistent, wat suggereert dat ze de werkelijke aantallen in de algemene Nederlandse bevolking zouden kunnen vertegenwoordigen. In deze periode van 3 jaar is het aantal fillerbehandelingen met 12% toegenomen.

Hoofdstuk 3 rapporteert over een multi-center retrospectieve observationele studie die het gebruik van botulinetoxine en hyaluronzuur fillers bij jongvolwassenen tussen 2008 en 2019 in Nederland vergelijkt. Dit werd gedaan door het opnemen van Elektronische Gezondheidsrapporten (EHR) van drie medische centra in Nederland. Deze medische centra gaven een representatief beeld van een landelijke trend omdat ze zowel high-end locaties als laagdrempelige locaties bevatten. Bij elkaar opgeteld zijn de verkregen gegevens afkomstig van tien locaties verspreid over heel Nederland. Jongvolwassenen zijn in deze studie gedefinieerd als de leeftijdsgroep 18-25 jaar, op basis van onze klinische categorisering van deze groep. In totaal werden 12628 patiënten geïncludeerd, verspreid over de jaren 2008-2019. De resultaten laten een toename zien van het gebruik van botulinetoxine en hyaluronzuur fillers bij jongvolwassenen.

Hoofdstuk 4 gaat in op de incidentie van vasculaire obstructies na filler injecties. In de medische literatuur zijn de frequenties van vasculaire complicaties (VAE's) niet gedetailleerd, maar ze worden geschat op 1:2.000 tot 1:10.000 (0.05-0.01%). In deze prospectieve studie hebben wij patiënten geïncludeerd die naar onze polikliniek waren verwezen voor door fillers veroorzaakte problemen. In totaal werden 44 patiënten (3 mannen, 41 vrouwen) met een VAE als gevolg van hyaluronzuur- of calciumhydroxylapatiet-fillers verwezen naar onze polikliniek in een periode van 24 maanden. De diagnose werd bevestigd door klinische presentatie (reticulaterend blauwachtig patroon met/zonder pustels en wondjes) en echografische beelden (hypervasculaire turbulente arterie met/zonder aantoonbare filler occlusie). Met de getallen uit hoofdstuk 2 en de wetenschap dat vrijwel elke patiënt met een vasculair adverse event (VAE) naar ons centrum wordt verwezen, konden wij de incidentie van vasculaire occlusies vullerbehandelingen berekenen. Wij berekenden de incidentie van VAEs na filler injecties op 1:6756 (of 0,015%).

Hoofdstuk 5 beschrijft de resultaten van microbiota gevonden in biopten van 29 patiënten met late-onset inflammatoire complicaties na filler injecties. We gebruikten een nieuwe en zeer gevoelige methode om microbiota op te sporen, de IS-pro methode. Dit is een nieuwe PCR-techniek met een breed bereik, gebaseerd op lengte- en sequentievariaties van de 16S-23S

ribosomale interspacer (IS)-regio. IS-pro kan bacteriën met lage abundanties detecteren en ze tot op soortniveau identificeren. Om contaminatie door huidmicrobiota uit te sluiten hebben wij de microbiota die werden aangetroffen op huidswabs vergeleken met die welke werden aangetroffen in biopsies van dezelfde patiënt. Deze bleken niet overeen te komen, waardoor contaminatie tijdens de afname werd uitgesloten. Er werd echter een hoog gehalte aan Gram-positieve bacteriën aangetroffen in biopten van de weke delen fillers. Dit suggereert dat deze bacteriën werden geïntroduceerd tijdens de primaire vulstof injectie behandeling.

Hoofdstuk 6 richt zich op de etiologie van late ontstekingsreacties (LIRs) na een hyaluronzuur (HA) filler behandeling. Sommigen beweren dat deze het gevolg zijn van een overgevoeligheids-reactie, hoewel het bewijs hiervoor zeer schaars is. De meeste meldingen van dergelijke reacties worden niet gestaafd door positieve huidtesten. Twaalf patiënten werden doorverwezen voor diagnostische evaluatie van mogelijke overgevoeligheidsreacties op hyaluronzuur (HA)-fillers. De twaalf patiënten werden geëvalueerd voor een algemene allergische screening (patch-tests), evenals specifieke intradermale tests (injectie van 0,1cc bolussen) op de mediale bovenarm met een selectie van verschillende hyaluronzuur (HA)-vullers die momenteel beschikbaar zijn. Een positieve allergische reactie werd gedefinieerd als erytheem, hardheid of zwelling. Bij de eerste tests en gedurende de follow-up periode van 4 maanden werden geen reacties op een van de geteste HA-vullers gevonden. Hoewel LIR's vaak als overgevoeligheidsreacties worden gediagnosticeerd, werden in onze studie geen positieve reacties op de zes gebruikte HA-vullers gerapporteerd. De resultaten suggereren dat noch type I noch type IV overgevoeligheid een rol speelt bij late ontstekingsreacties (LIRs) op hyaluronzuur (HA) vullers.

Hoofdstuk 7 beschrijft een pilot studie die zich richt op de vraag of er bewijs is dat het adaptieve immuunsysteem een rol speelt bij late ontstekingsreacties na injecties met fillers voor weke delen. Sommige auteurs hebben gepostuleerd dat inflammatoire bijwerkingen van fillers het gevolg zijn van type IV (vertraagd type) overgevoeligheid, een reactietype gevormd door adaptieve immuniteit. Deze inflammatoire bijwerkingen reageren vaak niet goed op de gewone behandelingsprocedures. Het bepalen van de exacte etiologie zou nuttig kunnen zijn bij de behandeling ervan. Wij hebben 47 patiënten geïncludeerd met complicaties na injecties met fillers voor weke delen. Er werden biopten genomen van het gebied waar de complicatie zich voordeed. Bij 18 van de 47 patiënten werden immuuncellen gevonden. Al deze 18 gevallen toonden CD68 positieve immuuncellen. Er werden vrijwel geen CD3 positieve immuuncellen gevonden. Onze resultaten suggereren geen rol voor het adaptieve immuunsysteem bij complicaties na injecties met fillers voor weke delen. Meestal zijn macrofagen betrokken na injecties met weke delen vullers, maar hun aanwezigheid is niet significant gecorreleerd met de ontstekingsreactie. Macrofagen lijken geen rol te spelen in het uitlokken van deze reactie.

In hoofdstuk 8 wordt onderzocht of HLA polymorfismen geassocieerd zijn met late inflammatoire bijwerkingen van dermale fillers. De meeste van deze bijwerkingen lijken een immunologische basis te hebben, waarbij de fillers meer als adjuvans werken dan als directe T-cel activators, tegen een mogelijke achtergrond van genetische predispositie. In totaal werden 211 patiënten geïncludeerd. DNA werd verkregen door orale swab of bloed extractie. Lage-intermediaire resolutie genotypering van HLA-A, HLA-B en DRB allelen van alle patiënten werd uitgevoerd door sequentie-specifieke oligonucleotide probe (SSOP) methoden. Van de 211 patiënten in de steekproef hadden er 25 de combinatie van HLA subtype-B*08 en HLA subtype-DRB1*03. Dit was 16,3% van de ontstekingsgroep en 4,9% van de referentiegroep. Deze combinatie van HLA-subtypes was geassocieerd met een drievoudige verhoging van de kans op het ontwikkelen van immuungemedieerde bijwerkingen (odds ratio = 3,79, 95% CI 1,25 tot 11,48). Genetische polymorfismen zoals HLA combinaties kunnen patiënten identificeren die risico lopen op het ontwikkelen van late onset immuun gemedieerde bijwerkingen van dermale fillers.

Hoofdstuk 9 richt zich op echografie om de veiligheid van hyaluronzuur filler behandelingen te verbeteren. Doppler-echografie (duplex) wordt in de dermatologie vaak gebruikt om dermatologische aandoeningen van de huid en de vaatstructuren te evalueren. Voordat een fillerbehandeling wordt uitgevoerd, kan echografie worden gebruikt om eerdere fillerbehandelingen in beeld te brengen en te vermijden. Ook variaties in het verloop van bloedvaten kunnen in kaart worden gebracht. In geval van bijwerkingen zijn de filler zelf en de morfologie van de omliggende weefsels zichtbaar. Dislocatie, abcessen en vasculaire bijwerkingen kunnen worden gezien. Onder echogeleiding kan hyaluronidase precies in het vulstofdepot worden geïnjecteerd. In veel opzichten kan echografisch onderzoek dus een belangrijk hulpmiddel zijn om de veiligheid van hyaluronzuur filler behandelingen te verbeteren.

Hoofdstuk 10 beschrijft het gebruik van intralesionale laserbehandeling bij complicaties van dermale fillers. Hoewel het gebruik van dermale fillers nog steeds populair is, is er nog veel onbekend over complicaties met betrekking tot optimale behandelopties. Voor complicaties als gevolg van fillerbehandelingen adviseren veel artsen over het algemeen een van de twee behandelingsregimes: ofwel systemische medicatie ofwel chirurgische verwijdering van het materiaal. Dit hoofdstuk geeft argumenten voor een derde mogelijke behandelingsoptie: verwijdering van het materiaal door intralesionale laserbehandeling. De lasers die gebruikt worden voor intralesionale laserbehandeling zijn lasers die ontwikkeld en gebruikt worden voor endoveneuze laserbehandeling van spataderen. De intralesionale laserbehandelingsprocedure voor dermale fillers bestaat uit het inbrengen van een fiber optische laser in het gebied van het product en het verdampen ervan. 242 patiënten (214 vrouwen en 28 mannen) werden behandeld met een intralesionale laserbehandeling; verbetering werd bereikt bij de meerderheid van de patiënten (92 procent).

Hoofdstuk 11 bespreekt het verhaal van de op hyaluronzuur gebaseerde fillers Hyacorp-1000 en Hyacorp H-S (H-800), die in 2014 van de Nederlandse markt werden gehaald na zorgen over hun veiligheid. We beoordelen wat de meest plausibele factor zou zijn van het toegenomen aantal bijwerkingen met deze stoffen, hetzij patiënt gerelateerde factoren, hetzij een factor die inherent is aan de filler zelf. We onderzoeken ook hoe nieuwe Europese wetgeving het goedkeuringsproces voor nieuwe fillers zal beïnvloeden en veiligheidsproblemen met fillers zal voorkomen. Uit de resultaten blijkt dat patiënten die met Hyacorp-1000 en Hyacorp-S (H-800) werden behandeld en bijwerkingen meldden, significant ouder waren dan de patiënten in de Hyacorp-1000 en Hyacorp-S (H-800) groep zonder bijwerkingen. Bij patiënten die werden behandeld met Hyacorp-1000 en Hyacorp H-S (H-800) en bijwerkingen meldden, was de filler significant langer in situ dan bij patiënten die bijwerkingen hadden die in verband werden gebracht met een andere HA-filler. Wij concluderen dat een verhoogde crosslinking van de filler de gemelde problemen heeft veroorzaakt. De aankomende EU-wetgevingsupdate van de Medical Device Regulation (MDR) zal voorkomen dat onveilige fillers op de EU-markt komen of zal veiligheidsproblemen veel eerder aan het licht brengen.

Hoofdstuk 12 sluit het proefschrift af met een algemene discussie van de resultaten en aanbevelingen voor toekomstig onderzoek naar het ontstaan van late ontstekingsreacties (LIR) na filler behandelingen. Er kon geen belangrijke oorzaak voor deze complicaties worden vastgesteld. Maar er werden afwijkingen gevonden in het bacteriële gehalte van de vulstof in-vivo. En in de immunologie van de patiënt werden vooral macrofagen gevonden. Het lijkt erop dat ofwel verschillende oorzaken kunnen leiden tot een identiek klinisch resultaat. Of LIR zou een complexe ziekte kunnen zijn, waarbij het samenkomen van meer dan één afwijking in dezelfde tijdspanne tot ontsteking leidt. Het belangrijkste toekomstige onderzoek zal bestaan uit 1) een innovatieve in-vitro onderzoeksmethode, skin-on-a-chip, waarbij een zachte weefselvuller kan worden geïmplanteerd en de verandering van biochemische markers in supernatanten kan worden gemeten. 2) toepassing van ultrasone beeldvorming om het natuurlijke verloop van de afbraak van HA-vulstof in de tijd te volgen. De grootste ontwikkeling om het aantal bijwerkingen te verminderen ligt wellicht in nieuwe regels en voorschriften betreffende de kwaliteit van injecterende artsen en fillers. In de afgelopen vijf jaar hebben Nederlandse en Europese autoriteiten veel gedaan om de kwaliteit van de zorg in de cosmetische geneeskunde te verbeteren. Dit zal in de toekomst helpen om excessen zoals ongeschikte fillermaterialen en artsen in de hand te houden. Hoewel het wetenschappelijk onderzoek in de cosmetische geneeskunde nog in een pril stadium verkeert, zijn in dit onderzoek al enkele stappen gezet.

Samen moeten de 12 hoofdstukken in dit proefschrift de behandelaars een dieper inzicht geven in het ontstaan van complicaties door filler behandelingen.

CHAPTER 15

List of co-authors
List of publications
Abbreviations
PhD portfolio
Curriculum Vitae
Dankwoord

LIST OF COAUTHORS

Leonie W. Schelke
Department of Dermatology, Erasmus MC, Rotterdam, The Netherlands

Jani A.J. van Loghem
UMA Institute, Aesthetic Medicine Centre, Amsterdam, The Netherlands

Andries E. Budding
InBiome BV, Science Park, Amsterdam, The Netherlands

Junior Lardy
Sanquin, Amsterdam, The Netherlands

Faryha Sayghani
Department of Dermatology, Erasmus MC, Rotterdam, The Netherlands

Yara Bachour
Department of Plastic Surgery, Amsterdam UMC, The Netherlands

Frank B. Niessen
Department of Plastic Surgery, Amsterdam UMC, The Netherlands

Lisanne W. Wolters
InBiome BV, Science Park, Amsterdam, The Netherlands

Danial Zarringam
Department of Dermatology, Erasmus MC, Rotterdam, The Netherlands

Thomas Rustemeyer
Department of Dermatology, Amsterdam UMC, The Netherlands

Hans P. Slijper
Velthuis Kliniek, Arnhem, The Netherlands

Linde de Wijs
Department of Dermatology, Erasmus MC, Rotterdam, The Netherlands

Renushka Jhingoerie
Department of Molecular Cell Biology and Immunology, Amsterdam UMC, The Netherlands

Susan Gibbs
Department of Molecular Cell Biology and Immunology, Amsterdam UMC, The Netherlands

Rutger H. Schepers
Department of Oral and Maxillofacial Surgery, UMC Groningen, The Netherlands

Levi Pelzer
Bijwerkingen centrum Lareb, Den Bosch, The Netherlands

Berend van der Lei
Department of Plastic Surgery, UMC Groningen, The Netherlands

Gabrielle de Mey
Department of Dermatology, Erasmus MC, Rotterdam, The Netherlands

Claudia I.M. van der Lugt
Allizone, Meijel, The Netherlands

Peter J. Velthuis
Department of Dermatology, Erasmus MC, Rotterdam, The Netherlands

Jonathan Kadouch
Department of Dermatology, Erasmus MC, Rotterdam, The Netherlands

Tamar E.C. Nijsten
Department of Dermatology, Erasmus MC, Rotterdam, The Netherlands

Luca Buin
Department of Dermatology, Erasmus MC, Rotterdam, The Netherlands

Chen Hu
Department of Dermatology, Erasmus MC, Rotterdam, The Netherlands

Faryha Sayghani
Department of Dermatology, Erasmus MC, Rotterdam, The Netherlands

Jaume Alijotas-Reig
Systemic Autoimmune Disease Unit, Department of Medicine, Vall d'Hebron University Hospital, Universitat Autonoma de Barcelona, Barcelona, Spain

Eduard Palou
Immunobiology Laboratory for Research and Diagnostic Applications (LIRAD), Barcelona, Spain

Simo Schwartz Jr
Aging Basic Research Unit, Molecular Biology and Biochemistry Research Centerfor Nanomedicine (CIBBIM-Nanomedicine), Vall d'Hebron University Research Institute (VHIR), Barcelona, Spain

Mark Fick
Department of Ophtalmology, Amsterdam UMC, The Netherlands

Laurentius J. van Rijn
Department of Ophtalmology, Amsterdam UMC, The Netherlands

LIST OF PUBLICATIONS

Decates TS, Velthuis PJ, Jhingoerie R, Bachour Y, Schelke LW, Gibbs S, Niessen FB. No association found between late-onset inflammatory adverse events after soft tissue filler injections and the adaptive immune system Submitted

Decates TS, Sayghani F, van Loghem JAJ, Velthuis PJ. The Dutch Hyacorp® filler catastrophe. New EU legislation will prevent this from happening again. Submitted

Decates TS, Kadouch JA, Velthuis PJ, Rustemeyer T. Immediate nor delayed type hypersensitivity plays a role in late inflammatory reactions after hyaluronic acid filler injections. Clinical, Cosmetic and Investigational Dermatology 2021:14 581–589

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ABBREVIATIONS

AE	adverse event
ASIA	autoimmune/inflammatory syndrome induced by adjuvants
BTX	botulinum toxin
CaHA	calcium hydroxylapatite
CT	computed tomography
FDA	food and drug administration
GH	growth hormone
GFBR	granulomatous foreign-body reaction
GHRH	growth hormone-releasing hormone
H&E	hematoxylin-eosin
HA	hyaluronic acid
HEMA/EMA	hydroxyethylmethacrylate/ethylmethacrylate
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
LIS	liquid injectable silicone
MRI	magnetic resonance imaging
PAAG	polyacrylamide gel
PAIG	polyalkylimide gel
PCL	polycaprolactone
PLLA	poly-L-lactic acid
PMMA	polymethylmethacylate
SEM	standard error of the mean
STF	soft tissue filler
TLR	toll-like receptors
TNF	tumor necrosis factor
US	Ultrasound

PHD PORTFOLIO

Name PhD Student:	Tom Servaas Decates
PhD period:	2016-2021
Promotor:	Prof. dr. T.E.C. Nijsten
Co-promotor:	dr. P.J. Velthuis
	dr. F.B. Niessen

Activity	Year	Workload
Courses		
NIHES: Research integrity	2019	0.5 ECTS
Biostatistics	2018-2019	5.0 ECTS
Scientific Writing	2018-2019	5.0 ECTS
Online IATA Infections substance training	2019	0.5 ECTS
BROK good clinical practice	2019	1.5 ECTS
Seminars and Workshops		
Advanced Filler Course, Allergan, Erasmus MC	2017	12 hours
Advanced Life Support, Catharina Ziekenhuis, Eindhoven	2017	24 hours
Belkyra Training, Cappella a/d Ijssel	2018	1.0 ECTS
Werken met meldcode huiselijk geweld Tegen volwassenen W2019, Augeo academy	2019	0.5 ECTS
Conferences attended		
International Congres Nederlandse Vereniging Cosmetische Geneeskunde (NVCG)	2019	1.0 ECTS
International Master Course on Aging Science (IMCAS) Paris, France	2019	1.0 ECTS
International Congres Nederlandse Vereniging Cosmetische Geneeskunde (NVCG)	2018	1.0 ECTS
Aesthetic & Anti-Aging Medicine World Congress (AMWC), Monte-Carlo, Monaco	2018	1.0 ECTS
International Master Course on Aging Science (IMCAS), Paris, France	2017	1.0 ECTS
International Congres Nederlandse Vereniging Cosmetische Geneeskunde (NVCG)	2017	1.0 ECTS
Aesthetic & Anti-Aging Medicine World Congress (AMWC), Monte-Carlo, Monaco	2016	1.0 ECTS
Oral presentations		
Huyd Congres Online, October 10, 2020 Utrecht	2020	1.0 ECTS
ESCAD Teaching Course, February 28-29, 2020 Rotterdam	2020	1.0 ECTS
Echografie bij complicaties van (permanente) fillers Najaarscongres NVMKA, Hoorn	2019	1.0 ECTS
The Nefertiti-Lift F.A.C.E. 2 f@ce Cannes, France	2016	1,0 ECTS
Teaching		
Supervising bachelor thesis of Romain Massot medical student	2020	1.0 ECTS

Supervising master thesis of Faryha Sayghani, medical student	2019	2.0 ECTS
Supervising master thesis of Sepheer Yousufzai, medical student	2018	2.0 ECTS
Supervising master thesis of Linde de Wijs, medical student	2017	2.0 ECTS
Supervising research project PI 'Histological and Microbiological Evaluation of Late Occurring Nodules with Hyaluronic Acid Dermal Fillers' study	2018-2020	2.0 ECTS
Complications of dermal fillers for dermatology interns	2018-2021	1.0 ECTS
The use of Ultrasound in Cosmetic Dermatology for Dermatology interns and cosmetic doctors	2018-2021	1.0 ECTS
Grants		
Contributed together with team members dr. P.J. Velthuis, dr. F.M. Niessen and dr. Y. Bachour to ZonMw application	2016	€230.000
Social Impact		
'Perfect Me' VPRO Tegenlicht (Dutch Television Program)	2021	
Frontpage AD, Dutch National Newspaper	2020	
'Jinek', Dutch talkshow	2020	
'Complicaties van hyaluronzuurpen' Interview by Radar (Dutch Television Program)	2020	
'DNA-test voor filler behandelingen' Interview by RTL Nieuws (Dutch Television Program)	2019	
Frontpage Parool, Dutch National Newspaper	2018	
'Aantallen filler behandelingen in Nederland' RTL Boulevard (Dutch Television Program)	2018	
Frontpage Telegraaf, Dutch National Newspaper	2018	

CURRICULUM VITAE

Tom Decates werd geboren op 23 mei 1980 in Nijmegen en groeide op in Oss. Na het afronden van de havo en meerdere middelbare scholen, behaalde hij zijn atheneum examen op het Canisius College in Nijmegen. Tot zijn geluk werd hij direct ingeloot voor de studie geneeskunde aan de Universiteit van Amsterdam. Na een verdienstelijke periode als voetballer bij Top Oss en hockeyer bij MHC Oss, ging hij in Amsterdam roeien bij Nereus. Tijdens zijn studententijd was hij werkzaam als fietsenmaker, privéchauffeur, dancefeest organisator en als verpleegkundige in de thuiszorg. Na drie jaar als arts-assistent plastisch chirurgie te hebben gewerkt, ging hij verder in de cosmetische geneeskunde. In die periode had hij ook het geluk om zijn vrouw Allison te ontmoeten. Onder de vleugels van dr. Frank Niessen en dr. Peter Velthuis mocht hij in 2016 beginnen aan zijn promotieonderzoek bij de afdeling Plastische Chirurgie van het Amsterdam UMC en bij de afdeling Dermatologie van het Erasmus MC onder begeleiding van zijn promotor prof. dr. Tamar Nijsten. Hij was direct betrokken bij de totstandkoming van het profiel specialisme Cosmetisch Geneeskunde als lid van de onderwijscommissie van de Nederlandse Vereniging Cosmetische Geneeskunde (NVCG). Samen met Allison en hun zoons Raphael en Roman woont hij in Amsterdam.

DANKWOORD

BAM! Mijn proefschrift is af. Ongelooflijk wat vet! Ik had nooit gedacht dat ik het leuk zo vinden om wetenschappelijk onderzoek te doen. Honderden artikelen lezen, in het lab werken en statistiek beoefenen. Het heeft mij volledig gepakt, 'het willen weten', alles in twijfel trekken, waaronder mijn eigen onderzoek. En dit was pas de eerste stap, want ik ben nog lang niet klaar met wetenschappelijk onderzoek doen.

Lieve Allison, bedankt dat je mij de ruimte hebt geboden om dit te doen. Ik houd van je!

Vijf jaar geleden vroegen dr. Peter Velthuis en dr. Leonie Schelke of ik mee wilde helpen met het 'complicatiespreekuur': het oplossen van complicaties na fillerbehandelingen Algauw ontstonden er lange gesprekken over de onduidelijkheid rondom het ontstaan van deze complicaties. Waarom krijgt de ene patiënt een complicatie door een fillerbehandelingen en de andere niet? Toen was het voor mij duidelijk: ik ga dit proberen uit te zoeken!

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Mijn eerste copromotor, Dr. P.J. Velthuis. Beste Peter, het was een eer dat jij mijn copromotor was. 24 uur per dag, 7 dagen in week stond jij voor mij klaar met raad en daad. Of het nou was voor statistiek, grammatica en spelling, politiek of planning, je was er altijd voor mij. Ik heb erg genoten van onze leerzame gesprekken en discussies in Amsterdam, Rotterdam en Zandvoort.

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Geachte leden van de kleine promotiecommissie, prof. dr. Berend van der Lei, dr. Han van Neck en dr. Tjinta Brinkhuizen. Uw bereidheid om dit proefschrift op zijn wetenschappelijke waarde te beoordelen en uw zitting in de promotiecommissie worden ten zeerste gewaardeerd.

De leden van de grote commissie, prof. dr. Thomas Rustemeyer, prof. dr. Benoit Hendrickx, dr. Refaat Karim, dr Armanda Onderdijk en dr. Jonathan Kadouch, hartelijk dank voor uw aanwezigheid op deze bijzondere dag en het wisselen van gedachten over mijn proefschrift.

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Leo, grote vriend en leermeester in de cosmetische geneeskunde. Alweer 12,5 jaar geleden, in het OLVG, liet je mij kennismaken met de technieken en geheimen van fillerbehandelingen. Tijdens iedere behandeling gebruik ik die kennis. Bedankt man!

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Beste Han, in onze gespreken naar nieuwe onderzoeksvragen leerde je mij nieuwe inzichten rondom hyaluronzuur. Ik verwacht dat we in de komende jaren nog nieuw onderzoek naar de biochemische verandering van hyaluronzuur zullen doen.

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Leden van de onderwijscommissie, Maartje, Annemarie, Hayri, Marguerite en natuurlijk Simone, bedankt voor de prettige samenwerking en adviezen over mijn onderzoek.

Oud-bestuursleden van de NVCG, Peter en Paul, wijze cosmetisch artsen KNMG, bedankt voor jullie steun en aanwijzingen in mijn loopbaan als cosmetisch arts en wetenschapper.

Dear Ed, scientific writing is an art which takes years of training to master. With lots of patients you managed to teach me. Later in the process also Charles helped me with finalizing the articles and this thesis. Thank you both!

Sander, grote held, samen met jou rende ik de marathon van Amsterdam, ongelooflijk dat ik dat heb geflikt! Zonder jouw hulp was dat nooit gelukt. De vele trainingskilometers maakten mijn hoofd leeg en zo kon ik steeds opnieuw aan mijn artikelen werken. Emil Zaropek schreef: 'Als je wilt hardlopen, loop dan een mijl. Als je een ander leven wilt ervaren, loop dan een marathon'. Nou dat hebben we geweten. Respect!

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Roel, iedere maandag trainen wij in het Oosterpark, gedachten op nul en knallen. De handstand, pull ups en hardlopen. Gelukkig voor de anderen in het park houden we altijd ons shirt aan.

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