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ORIGINAL ARTICLE

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

Tom S. Decates MD, PhD[1] | Peter J. Velthuis MD, PhD[1] | Renushka Jhingoerie MSc[2] | Susan Gibbs MD, PhD[2,3] | Yara Bachour MD, PhD[4] | Frank B. Niessen MD, PhD[4]

[1]Department of Dermatology, Erasmus Medical Center, Rotterdam, The Netherlands

[2]Department of Molecular Cell Biology and Immunology, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

[3]Department of Oral Cell Biology, Academic Centre for Dentistry (ACTA), University of Amsterdam and Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

[4]Department of Plastic Surgery, Amsterdam University Medical Centre (location VUmc), Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

Correspondence
Tom S. Decates, Department of Dermatology, Erasmus Medical Center, Postbus 2040, 3000 CA Rotterdam, The Netherlands.
Email: t.decates@erasmusmc.nl

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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 and 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: Nine biopsies from the inflammation group and nine 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.

KEYWORDS
adaptive immune system, adverse events, cosmetic dermatology, filler, soft tissue fillers

1 | 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]

The following 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%–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] Patients with these inflammatory adverse events present with erythema, edema, and nodules at or

in proximity to the facial injected sites.[12,13] 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.[14] Adjuvants, as defined by the National Cancer Institute, are agents that stimulate the immune system in a non-specific way.[15] 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, that is, 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).

2 | METHODS AND MATERIALS

We used a convenience sample of 47 patients of the Dermatology Department, Erasmus MC, the Netherlands, in the period between 2016 and 2018. The local medical ethics committee approved this study.[16] 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. The biopsies were taken by incisional approach from the site of the inflammation (inflammation group) or where the lesion was shown on ultrasound (reference group).

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 as follows: 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 3 months or longer after initial filler injection: skin induration, erythema, 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 3 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.

TABLE 1 Descriptive statistics for Inflammation and Reference group

		Inflammation	Reference	χ^2	*p*-Value	Fisher's exact test
Gender	Female	17	23	0.00	0.986	1.0
	Male	3	4			
Age (in years)	Mean (SD)	62.2 (9.9)	61.9 (7.5)	$t = 0.14$	0.892	
Ethnicity	Non-Caucasian	3	0	4.33	0.038	0.070
	Caucasian	17	27			
Smoking	Yes	4	8	0.56	0.454	0.517
	No	16	19			
Autoimmune diseases	Yes	7	6	0.94	0.333	0.511
	No	13	21			
Filler type	Non-Resorbable	19	24	0.55	0.458	0.626
	Resorbable	1	3			
Inflammation episodes	Mean (SD)	2.2 (0.9)	1.9 (0.9)	$t = 0.97$	0.336	
First episode to visit (years)	Mean (SD)	12.5 (3.7)	14.1 (2.9)	$t = 1.69$	0.098	

TABLE 2 Presence of immune cells surrounding the injected filler for inflammation and reference group

Immune cells present	Inflammation	Reference	Total	OR (95% CI)	*p*-Value
No	10	19	29 (62%)	2.38 (0.71, 7.92)	0.156
Yes	10	8	18 (38%)		
Total	20 (43%)	27 (57%)	47 (100%)		

The inflammation group consisted of 20 patients who experienced an inflammatory adverse event. Within the inflammation group 9 had an inflammatory reaction with concomitant edema, erythema, and induration, whereas 11 presented with a nodule only. A reference group of 27 patients, who did not experience an inflammatory adverse event, was used as a control group.

2.1 | Laboratory methods

Biopsies were fixed utilizing buffered 4% formaldehyde for 24 h, subsequently embedded in paraffin and sectioned and routinely stained by hematoxylin–eosin (HE) and immunohistochemistry staining (IHC) for visual immune cell detection.[17] Immunohistochemical staining on paraffin-embedded 5-μm sections was performed with CD3 and CD68 (all Dako/Agilent, dilution 1:100).[18] Two investigators (RJ and TD), who were blinded to the injected filler type or patient outcome, viewed and assessed the stained slides.

2.2 | Data analysis

2.2.1 | 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 0.05. Analyses were conducted with the SPSS software (IBM Corp. Released 2017. IBM SPSS Statistics for Apple, Version 25.0: IBM Corp.). The Medical Ethical Committee Erasmus Medical Center MEC-2016-660 and Medical Ethical Committee Vall d'Hebron University Hospital PR(AG)-19/2008) approved this study.

3 | 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.

Table 1.

In 18 out of the 47 patients, immune cells were detected in the biopsy (38.3% [95%-CI 25.8%–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%–70.1%]) compared with the reference group (29.6% [95%-CI 15.9%–48.5%]). However, the absolute risk difference of 20.4% (95%-CI 7.2%–44.7%) was not statistically significant according to a likelihood ratio test, $\chi^2 = 2.02$, $p = 0.156$ (Fisher's exact test $p = 0.226$). 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.

Table 2.

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

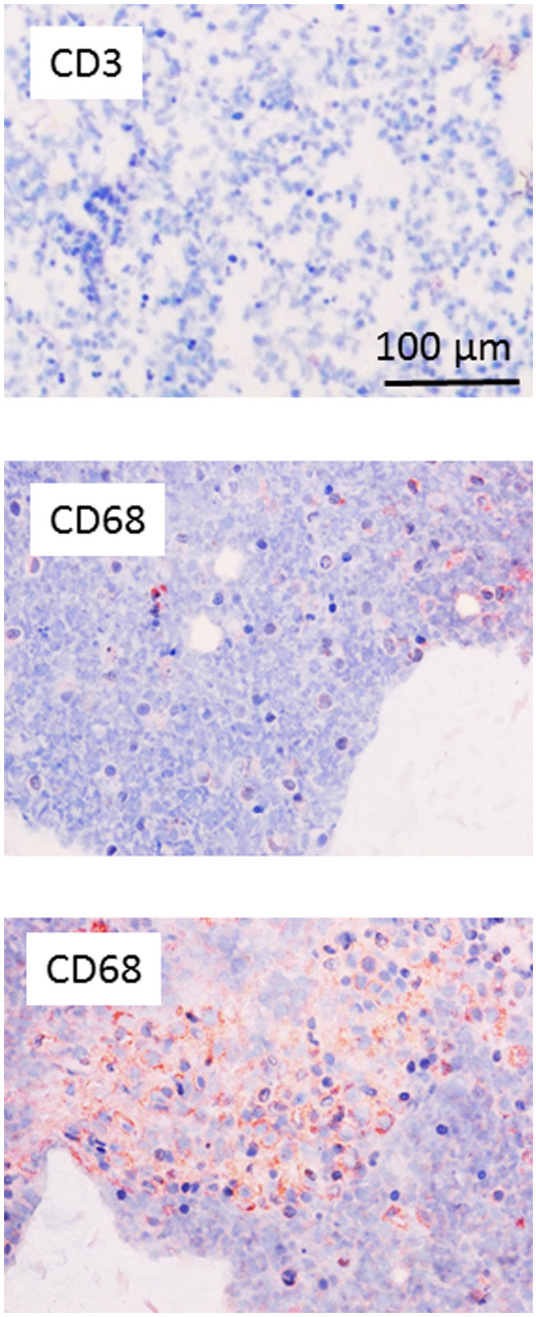

FIGURE 1 Paraffin-embedded tissue sections were immune stained with either anti-CD3 or anti-CD68. Representative images show absence of CD3-positive cells (upper panel), sporadic (middle panel) and dense clusters (lower panel) of CD68-positive cells in capsules

(100% [95%-CI 82.4%–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 (Figure 1).

4 | 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.[19] 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.[20] Macrophages are known to play an important role in the body's defense system after the injection of STF.[20–22] Foreign body reactions can lead to a foreign body granuloma, which are formed by aggregation of macrophages after phagocytoses by macrophages fails.[23–25] Figure 2.

CD3 antibody is a marker for T-cells,[26] which are part of the adaptive immune response.[27] 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.

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

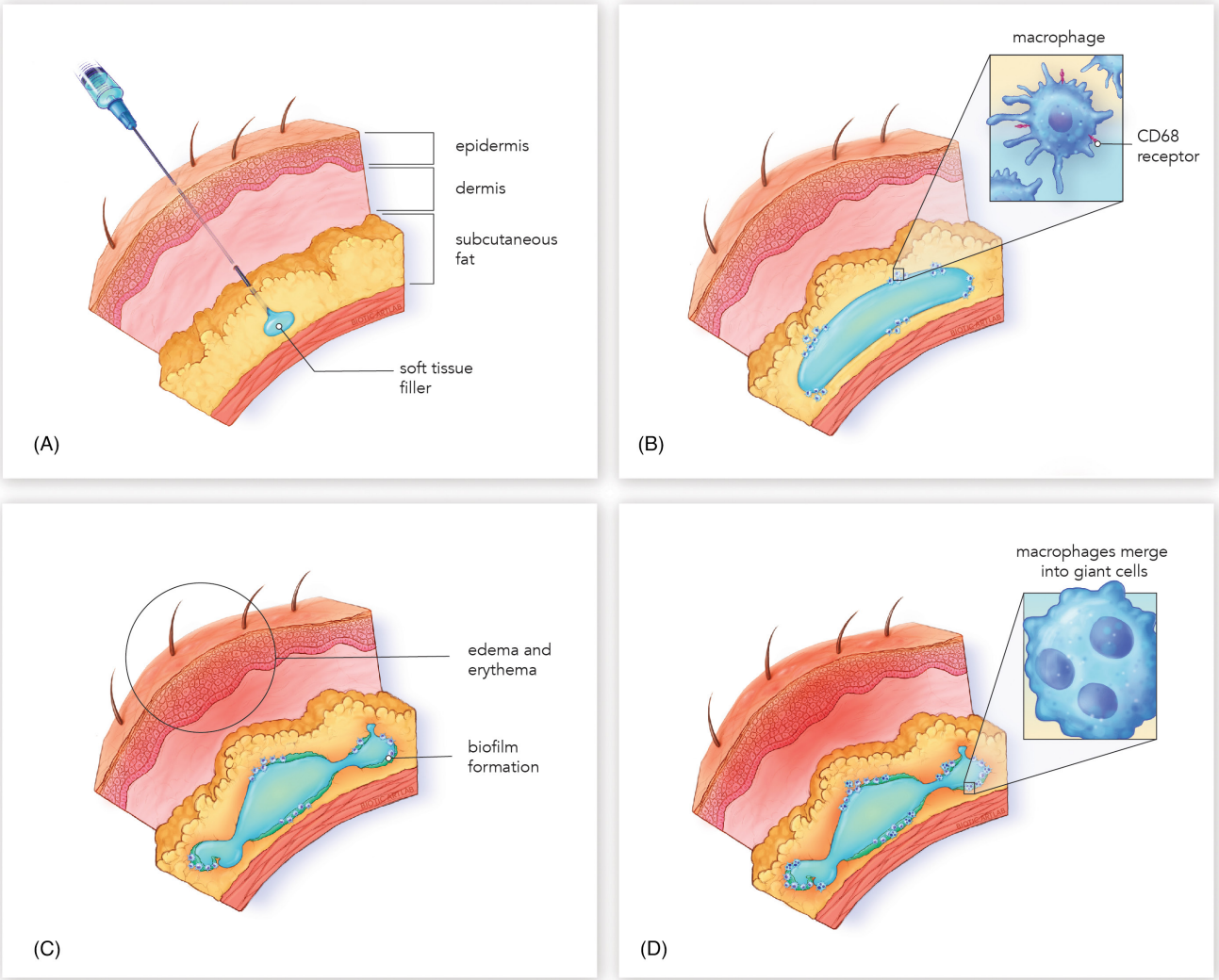

FIGURE 2 Hypothetical model for late-onset inflammatory adverse event. (A) Soft Tissue Filler injection. (B) Mobilization of macrophages by the innate immune system. (C) Biofilm formation in combination with edema and erythema. (D) Late-onset inflammatory adverse event after macrophages have merged into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

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.[28]

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.[29] Several other studies have reported that macrophages are the "first line of defense" against medical device implants.[20,30,31] 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.[20,29–32] This is in line with our study where, out of the 47 patients in this study, four (9%) were treated with resorbable fillers and the other 43 (91%) with non-resorbable STF. 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 constantly 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, Breast Implant-Associated Anaplastic Large Cell Lymphoma (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, with our methodology, we could not find an association between late-onset inflammatory adverse events after STF injections and an adaptive immune response since we did not find a marker for T-cells. One has to bear in mind that this T-cells absence could be time and area dependent. 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.[32]

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.

AUTHOR CONTRIBUTIONS

T.D. and P.V. performed the research and analyzed the data. T.D., P.V., and F.N. designed the research study. R.J. and S.G. analyzed the biopsies in the lab. T.D., P.V., F.N., and S.G. wrote the paper.

CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICAL APPROVAL

Ethics committee approval was obtained from the Medical Ethical Committee Erasmus Medical Center MEC-2016-660 and the Medical Ethical Committee Vall d'Hebron University Hospital PR(AG)-19/2008).

ORCID

Tom S. Decates https://orcid.org/0000-0002-1929-2010

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