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A Roadmap to Safer Hyaluronic Acid Injections

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A Roadmap to Safer Infections

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Abstract

Non surgical aesthetic treatments have become increasingly more popular in the United States (*2020 Plastic Surgery Statistics Report* 2021). Hyaluronic acid (HA) fillers are the second most popular non-surgical aesthetic treatment behind Botulinum toxin injections (The Aesthetic Society, 2021). The increased use of these fillers has led to increased rates of complications With the release of new long lasting fillers in 2014, providers began to see an increase in delayed onset nodules (DON). A DON is considered in the aesthetic field to be a nodule that occurs 30 days to years after filler injection (Convery et al., 2021). A systematic review of the available literature looking at DONs and their potential causes was completed. While the review of the literature was not conclusive, three trends were identified as potential causes: product choice, injection technique, and timing. Allergan's low molecular weight Vycross fillers were identified as higher risk for DONs. Biofilms caused by poor aseptic technique and bacterial infections were also identified. Timing related to vaccination, viral infections, and dental procedures may also pose a risk of DONs. This data can be used to help providers better understand HA fillers and provide a roadmap to safer injections.

*Keywords/phrases:*delayed onset nodule filler, delayed inflammatory reactions filler, foreign body granulomas filler, hyaluronic acid filler complications, dermal filler adverse effects, Vycross filler complications, crosslinked filler complications.

A Roadmap to Safer Hyaluronic Acid Injections

Background and Significance

Precedence Research (2022) estimates that by 2030 the non-surgical aesthetic industry will be a $150.6 billion dollar industry. Hyaluronic Acid (HA) filler injections are the second most popular non-surgical treatment in the industry behind Botulinum toxin injections (The Aesthetic Society, 2021). In 2020 there were 3.4 million filler procedures done in the United States (*2020 Plastic Surgery Statistics Report* 2021). These fillers are injected into different layers of the skin for cosmetic purposes to replace lost volume, correct fine lines and wrinkles, and give an overall more youthful appearance to the face (Daoud & Weiss, 2021).

HA naturally exists in dermal tissue and is made up of polysaccharide molecules that bind with water (Coleman 2006). It acts as structural support as well as attracts water giving the skin a youthful and volumized look (Daoud & Weiss 2021). Natural HA diminishes with age. The first HA filler, Restylane™, was approved by the FDA in 2003 for cosmetic purposes (Daoud & Weiss, 2021). HA fillers typically last 6-18 months and are broken down by the body using a combination of phagocytosis and enzymatic breakdown (Daoud & Weiss 2021).

Over the last several years, pharmaceutical companies have gained FDA approval for a wide range of HA fillers to treat signs of aging. HA fillers are categorized in two ways, by the gel particle size and by the degree of crosslinking in the gel (Daoud & Weiss 2021). Gel particle size allows different molecular weights of HA to be injected depending on the amount of volume needed and the depth of the injection. Crosslinking means that intermolecular bonds have been applied to the HA chains allowing them to last longer in the dermis and attract more water (Daoud & Weiss 2021).

The injection of HA fillers for cosmetic purposes is generally considered a safe procedure. However, there are rare but significant complications that can arise (Convery et al., 2021). In 2014, aesthetic companies began to release a new generation of HA fillers that would last longer after being injected, these are considered crosslinked fillers. Allergan released HA fillers named Volbella™, Vollure™, and Voluma™. Galderma released Refyne™, Defyne™ and Kysse™. Pharmaceutical companies attempted to improve the longevity of the fillers by developing lower molecular weight HA's and crosslinking the HA chains with butanediol diglycidyl ether (BDDE). This makes them more resistant to the body's natural mechanism to break them down (Cohen et al., 2022) With the release of these new crosslinked fillers, providers in the aesthetic field began to see increased rates of late onset complications in practice (Wu et al., 2021).

A delayed onset nodule (DON) is a rare but significant complication that can occur as a result of HA filler injections. Rates of DONs have increased since the cross linked fillers have been released (Wu et al., 2021). A DON is a nodule that appears under the skin after the initial swelling from the injection itself has subsided (Convery et al., 2021). DONs can happen weeks to years after filler injections. DONs present themselves as firm lumps and can present with or without pain. While treatable, they can cause significant distress to patients. Researchers have debated on the cause of these nodules (Artize et al. (2016), Beleznay et al. (2015), Humphrey et al. (2020), Convery et al. (2021). Potential causes of DONs are biofilms due to poor aseptic technique, foreign body granulomas. delayed hypersensitivity reaction, autoimmune disorders, infections related to dental procedures, vaccinations, and viral triggers like COVID-19. (Convery et al., 2021).

The purpose of this systematic review was to examine the causes of delayed onset nodules. After a clear understanding of the literature was obtained the information was used to create a screening tool to flag potentially high risk patients. This tool was then incorporated into a roadmap for safer injections for providers to easily use in practice. The goal of this tool is to better educate patients and decrease the rates of these rare, but significant complications.

Methods

Study Purpose and Design

A systematic review examining current literature related to Delayed Onset Nodules (DONs) after HA filler injections was completed. It was conducted using the Preferred Reporting Items of Systematic Reviews and Meta-Analyses (PRISMA) statement and checklist. Relevant prospective studies, retrospective studies, and case reports on delayed onset nodules after HA filler injection were reviewed. The data was used to create a screening tool to flag potentially high risk patients. This screening tool was incorporated into a roadmap for safer injections for providers to use in order to decrease the risk of these rare, but significant complications. The following questions were considered for this review:

Does current evidence support a) that a subgroup* of patients are at an increased risk for delayed onset nodules following hyaluronic acid filler injections? and b) Does current evidence support the use of a screening tool at the initial visit to decrease the rate of delayed onset nodule occurrence?

**Subgroup of patients are those with autoimmune diseases, recent infections, or recent immune stimulating procedures like viral infections or vaccinations.*

Search Strategy

A thorough search of PubMed, Science Direct, Google Scholar, and San Jose State University Library resources was used to search for articles. Key terms/phrases included in the search were: delayed onset nodule filler, delayed inflammatory reactions filler, foreign body granulomas filler, hyaluronic acid filler complications, dermal filler adverse effects, Vycross filler complications, crosslinked filler complications. Articles selected reference pages were analyzed to search for other potential articles relevant to the review.

Inclusion and Exclusion Criteria:

Articles in the last ten years were included in the search. Only articles examining HA fillers were included. Articles that compare HA filler to non HA filler were examined for relevance to the topic. Articles that compare crosslinked fillers to non-crosslinked fillers were included. Case studies that aim to understand potential causes of delayed onset reactions were included. Articles looking at fillers that are not FDA approved in the United States were excluded. Articles looking at other HA filler complications, vascular occlusion, blindness, infection were also excluded.

Data Extraction

The articles were organized into an evaluation table (see appendix A). This table allows for organization of article type, author, year, sample size, setting, variables examined. The data was extracted and organized into a spreadsheet in order to see trends in patients who developed DONs. Data points of interest are type of filler, amount of filler injected, age of patient, area injected, timing of reaction, type of reaction, triggering events identified i.e. cold/flu or dental procedures, histologic studies, medical history, aseptic techniques used. If the article provided treatment of the DON used this data was documented as well.

Quality Appraisal

Once the literature search was complete each selected article was individually analyzed for quality. The tool used was Hierarchy of Evidence for Intervention Studies adapted from Evidence Based Practice in Nursing and Healthcare: a guide to best practice. (see appendix B)

Results

The initial literature search of the databases found 107 articles using the keywords. Eighty three articles were eliminated due to duplication or being irrelevant to the topic. After examination of the remaining 24 articles and implantation of the inclusion and exclusion criteria there were eleven articles to include in the systematic review (see Figure 1).

Four of the articles included are from the United States, four from Canada, one from Israel, one from Saudi Arabia, and one from Spain. All of the articles examined HA fillers with FDA approval in the United States. Five of the articles analyzed were retrospective chart reviews of individual clinics. One article was a Manufacturer and User Facility Device Experience (MAUDE) review analyzing mandatory reports made to the FDA from manufacturers as well as voluntary reports from healthcare providers and patients. Four articles were case studies that present relevant information to the topic including histology results.. One article was a literature review analyzing biofilms and their potential role in DON's after HA filler injections. Three distinct themes were found in the reviewed literature: Higher rates with certain products, biofilms from poor aseptic technique or infection, and timing of viral illness, vaccination, and dental procedures may increase the risk of DONs.

Product

Five of the articles state that there are certain fillers that are higher risk for DON's than other fillers on the market. This hypothesis comes from retrospective chart review, relevant case studies and histologic analysis of nodules. It is unclear in the research why some patients develop

this reaction to the filler and some do not. Artize et al. (2016), Sadeghpour et al. (2019). Cohen et al. (2022), and We et al. (2021), state that the Allergan family of crosslinked fillers Volbella™, Vollure™, and Voluma™ cause a heightened immune response during the bodies natural breakdown of the filler. Rates of DON's in these studies ranged from 1%-4.25%, which is much higher than industry standard of 0.2% (Artizi et al. 2016).

Artizi et al. (2016) completed a retrospective chart review of patients treated with Allergan's cross linked filler Volbella™ in the lips or tear trough. Of the 400 patients injected over a two year span, 17 developed DONs (4.25%). One patient had a biopsy revealing florid granulomatous dermatitis, composed of epithelioid histiocytic granulomas. All fungal, bacterial, and mycobacterial cultures of the lesion were negative (Artizi et al., 2016). None of the 17 patients had pre-existing skin conditions or recalled any immune triggering episodes.

In a similarly designed study, Sadeghpour et al. (2019) completed a retrospective chart review that looked at patients in their clinics that received crosslinked filler over a 12 month period (Sadeghpour et al., 2019). They specifically looked at the Allergan HA crosslinked fillers with trade names, Volbella™, Vollure™,and Volluma™. For reference they also examined a non-crosslinked filler made by Galderma (Restylane Silk™), commonly used in the lips. They found that 1029 patients were injected with Allergan crosslinked filler treatments over a twelve month period. Five of these patients developed DONs (Sadeghpour et al., 2019) These patients had all received Volbella™ with a DON incidence of 1.0%. No DONs were found in Vollure™ or Voluma™ (Sadeghpour et al., 2019). Rates of DON with Restylane silk™ was 0.25%. One patient reported a potential immune triggering event, dental cleaning 3 weeks prior to treatment. Sadeghpour et al (2019) agree with Artizi et al (2016) and state that Volbella™ should be associated with higher risk of DONs than other FDA approved filler (Sadeghpour et al., 2019).

Cohen et al. (2022) completed a retrospective review of delayed events reported to the Manufacturer and User Facility Device Experience (MAUDE) FDA database (Cohen et al., 2022). The database consists of safety issues that are derived from mandatory reports from manufacturers and voluntary reports from health care professionals and consumers. Cohen et al. (2022) looked at crosslinked fillers from Galderma, Allergan, and Revance companies. They found 585 total reports related to crosslinked fillers. Of these reports 195 were confirmed delayed reactions to crosslinked fillers (Cohen et al., 2022). The authors report very few reports of delayed reaction with non-crosslinked fillers during this time frame. The authors' research support claims that crosslinked fillers have higher rates of delayed events. The Allergan brand of crosslinked fillers had the highest rates of nodules reported, 86.8% (Cohen et al., 2022).

Wu et al. (2021) used the database at one institution in New York to search for histopathologic reactions to HA filler from 2104-2019. Fifteen cases of nodules were found. In 11 of the 15 cases where the filler was known, all nodules were formed after the use of Allergan's Vycross fillers (Volbella™, Voluma™, Vollure™). The authors examined biopsy specimens of these granulomas. Results were consistent with an inflammatory response; histopathologic pattern of discrete foci of tightly cuffed palisaded granulomas with eosinophils (Wu et al., 2021). There was no evidence of bacteria, fungi, or microorganisms found in the samples thus the authors state that with the lack of neutrophils these delayed reactions are unlikely biofilms. Wu et al. (2021) state that the increase in DONs is likely related to the crosslinking molecules used by Allergan (which are proprietary), the low molecular weight of the filler, or a combination of both, to be proinflammatory.

A case study to further support this stance is presented by Perz-Perez et al, (2017). A healthy 49 year old patient who received 2mls of Vycross fillers in her lower face (Volluma™

and Vollure™) developed hard painless nodules in the area of treatment four months after treatment (Perez-Perez et al., 2017). Ultrasound was used to locate the nodules and found edema and vascularity in the area. A skin biopsy showed lymphocytic inflammatory infiltrates in the adipose tissue (Perez-Perez et al, 2017). A patch test of Voluma™, Vollure™, BDDE, pet, and lidocaine revealed negative results. According to the American Academy of Dermatology (2021) a patch test involves placing small amounts of allergens on the skin and covering it with a patch. The area is assessed for reaction after 48 hours and again in 4-7 days. Intradermal injection into the right forearm showed no results at 20 minutes and 96 hours, but both turned positive two months after placement (Perez-Perez et al. 2017). Perz-Perz et al. (2017) agree with Artizi et al.(2016) that there may be a late immune response to these Vycross fillers, but found the reaction unpredictable as this patient had no triggering event or any medical history.

Timing

Five of the articles state that immune triggering events like viral illness, vaccinations, or dental exams trigger an inflammatory response that can increase the risk for DON development. Beleznay et al. (2015), Humphrey et al. (2020) and Rivers et al.(2021) and Turkman et al. (2019) agree that DONs are immune mediated, but state it is not the breakdown of the filler alone that causes the DON. They hypothesize that when low molecular weight crosslinked fillers are injected into certain high risk patients and there is an immune trigger like a viral illness, dental work, or trauma a DON is more likely to develop (Beleznay et al., 2015, Humphrey et al. 2020, and Rivers et al. 2021). All of the retrospective studies found seasonal trends with higher rates of DON's in fall and winter months where viral illnesses are more common (Beleznay et al., 2015, Humphrey et al 2020, and Rivers et al. 2021).

Beleznay et. al. (2015) started to see more incidences of DON in their practice with the release of crosslinked fillers. A retrospective chart review identified 4,702 patients that received the crosslinked filler Juvederm Voluma™ over a 68 month span. Twenty-three of these patients developed DONs or 0.5% of patients, three to five months after treatment (Beleznay et al., 2015). In this study nine of the 23 cases (39%) recalled experiencing an immunologic trigger like a viral illness, dental work or trauma prior to nodule onset (Beleznay et al., 2015). The identified immunologic trigger and seasonal trend lead Beleznay et. al (2015) to hypothesize that a triggering event is a prerequisite for development of a DON (Beleznay et al., 2015).

Rivers et al. (2021) and Humphrey et al. (2020) both had similar results from retrospective chart reviews. Humphry et al. (2020) had 4200 patients who received Allergan's HA filler Voluma™. Forty four patients experienced delayed onset reactions (0.98%) (Humphrey et al., 2020). Again, the median onset of the delayed nodule was 4 months. Fifteen of these patients (34%) identified an immune trigger prior to the nodule event; viral infection,vaccination, bacterial infection, or dental exam. More than half of these events occurred between the months of October and January (Humphrey et al., 2020). In Rivers et al.(2021) retrospective review, 2139 patients received Vycross fillers, seven of these patients (0.33%) developed DON's. Analysis found that six of the seven patients (86%) had undergone dental procedures prior to the event and again a seasonal trend was identified. 71% of DONs developed between September and December. One patient had histologic evaluation showing a foreign body granuloma.

To further support this stance, Turkman et al. (2019) presented 14 case studies. Each patient had a delayed reaction to HA filler after a flu-like illness (Turkmani et al., 2019). The fillers the patients received were crosslinked fillers or a combination of crosslinked and non-crosslinked fillers. In each case the patient started to have a reaction to the filler 3-5 days

after a flu-like illness (Turkmani et al., 2019). The immunologic reaction between the viral infection and the fillers is poorly understood, but the authors believe this as a type 4 hypersensitivity reaction (Turkmani et al., 2019).

Since the onset of mass vaccination against COVID-19 many case studies have been reported of patients experiencing DON after vaccination. Reig et al. (2022) presents 20 cases of inflammatory immune mediated adverse reactions induced by COVID-19 vaccines. While the fillers used are not specifically identified this reaction is consistent with other reports of DONs after immune triggering events and providers should be aware of the risk. Michlon (2021) presented two more specific case studies, one had Volbella™ injected to the tear trough 6 months prior to getting vaccinated against COVID-19. One day post vaccination pain, swelling, and erythema was noted in the area of injection. The second case was a patient previously injected with Vycross filler to multiple areas on her face. Nine months after injection she received COVID 19 vaccine and developed facial swelling, erythema, and pain. After reviewing the articles by Belnezay et al. (2015) and Turkman et al. (2019), Reig et al. (2022) concluded that a systemic inflammatory response and immune reaction likely caused the filler reaction. Reig et al. (2022) also notes the importance of not delaying vaccination, but that providers should be aware of the potential response.

Technique

Beer and Avelar (2014) recognize that DONs have been attributed to immune reactions, but state that biofilms play an important role. Beer and Avelar (2014) stress the importance of aseptic technique during skin preparation and injection and suggest that small amounts of bacteria can adhere to the gels. These bacteria can be slow growing and missed in culture due to the short incubation times. Beer & Avelar (2014) state that different techniques to look for

biofilms should be used to better identify pathogens, specifically polymerase chain reaction (PCR) and fluorescent in situ hybridization (FISH) . HA fillers can last in the skin for years and infection may result from later exposure to bacteria through the skin. Beer & Avelar (2014) state the prevention of biofilms should be an important part of injection education.

It was also noted that Artize et al. (2016) and Humphrey et al. (2020) both concluded that an immune response was likely the cause of DONs, but both listed oral antibiotics as an important part of treatment of DONs. This further supports that biofilms can not fully be ruled out. Although there were limited amounts of cultures done for DON in the articles reviewed, none that were reported had any positive results.

Discussion

The exact cause of DONs is debatable. A particular subgroup of patients who are at higher risk for DONs was unable to be identified. Trends throughout the research show that the Allergan crosslinked fillers have higher rates of DONs than any other crosslinked or non crosslinked fillers on the US market. Artize et al. (2016), Sadeghpour et al. (2019). Cohen et al. (2022), and We et al. (2021) point to something in the proprietary ingredients in the Allergan hyaluronic acid fillers. The low molecular weight HA with BDDE crosslinking ingredient causes some patients to develop a late immune reaction to the filler. Histology reports in several case studies prove an immune response, but the number of biopsies is limited (Artizi et al. 2021, and Wu et al. 2016). Belnezay et al. (2015), Turkman et al.(2019), Reig et al. (2022) concluded that a systemic inflammatory response after an immune triggering event, like dental work, viral infection, or vaccination likely contributes to the formation of DONs. The case of biofilms can not be completely ruled out and must be considered (Beer and Avelar 2014).

With an understanding of the literature, a roadmap to safer injections was created (see figure 3). At the initial consultation patients should be screened for autoimmune disease, recent viral illness, vaccinations, dental work, and bacterial infections on the skin (see figure 4). Patients with any identifiable red flags must lead the provider to STOP. These findings trigger further investigation and consideration of delaying treatment. If there are no red flags, the provider provides proper consent and education to the patient on the risk and benefit of HA filler with the risk of DON being clearly stated. Once a patient is properly consented the patient can decide whether to accept or not accept the risk. Once consent is obtained, the provider decides which HA filler to use. If a crosslinked filler is chosen then further discussion of DON should be considered by the provider. The provider must then ensure proper aseptic technique to prevent formation of biofilms. All makeup is removed from the patient's skin and skin is prepped with antibiotic solution. Needles should be changed frequently if multipoint injections are used and cannulas are not allowed to drag across the skin surface. Once the procedure is complete the provider must provide proper after care education to the patient. Patients should avoid touching the face and wearing makeup until injection points have healed. Patients should be instructed to follow up with questions or concerns keeping in mind that the average onset of DONs in the research was 4 months after injection. (See figure 3 for Roadmap)

Limitations and Gaps

Several limitations were found in the articles. The follow up time with patients varied from four months to two years. This may affect the results as some patients can develop delayed reactions years after filler placement. Each practice has unique injection techniques. There are many variables to consider including how patients skin is prepped prior to injection, was a needle or cannula used, how often was the needle changed, what was the depth of the product being

placed, and how much product was injected; in some studies these details were not included. Additionally, the majority of nodules are treated without biopsy, so understanding the cell type and culture results make it difficult to fully understand what causes delayed onset nodules and how to prevent them. Finally, the reporting of filler reactions is voluntary thus many providers do not report complications. The aesthetic industry is changing rapidly with new fillers to gain FDA approval in the next few months; during the time of this project new research will have to be taken into consideration. Much of the data gathered in these articles is subjective. Patients need to self report DONs and need to be able to recall any triggering events.

Conclusion and Practice Implications

While the rates of DONs remain low in the literature, experiencing them in practice can be time consuming and cause distress to the patients. Having an in-depth understanding of the literature regarding DONs will prepare providers to provide safer injections. With the rapid growth of aesthetic medicine it is more important than ever to understand that filler injections while elective are medical procedures. They should be done by medical providers who are educated in risks and dedicated to providing safe injections. The screening tool and roadmap created using the data in this systematic review can easily be incorporated into daily practice and shared with colleagues to create safer injections for patients everywhere.

Figure 1

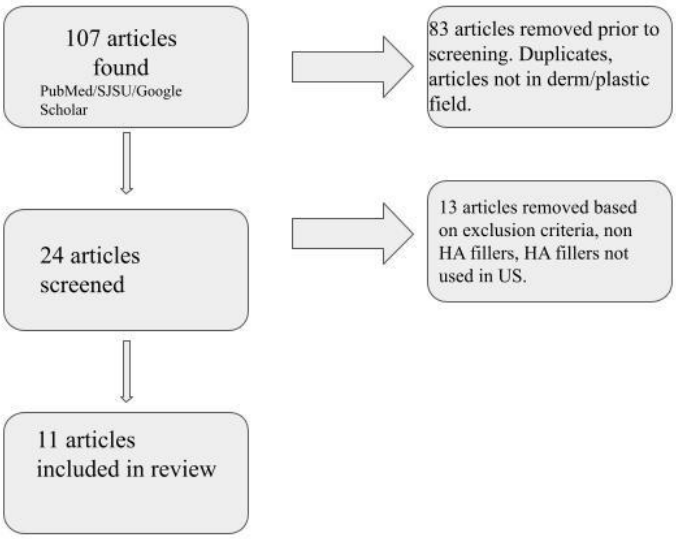
107 articles found
PubMed/SJSU/Google Scholar
83 articles removed prior to screening. Duplicates, articles not in derm/plastic field.
24 articles screened
13 articles removed based on exclusion criteria, non HA fillers, HA fillers not used in US.
11 articles included in review

Figure 2

Hierarchy of Evidence for Intervention Studies

Type of evidence	Level of evidence	Description
Systematic review or meta-analysis	I	A synthesis of evidence from all relevant randomized controlled trials.
Randomized controlled trial	II	An experiment in which subjects are randomized to a treatment group or control group.
Controlled trial without randomization	III	An experiment in which subjects are nonrandomly assigned to a treatment group or control group.
Case-control or cohort study	IV	Case-control study: a comparison of subjects with a condition (case) with those who don't have the condition (control) to determine characteristics that might predict the condition. Cohort study: an observation of a group(s) (cohort[s]) to determine the development of an outcome(s) such as a disease.
Systematic review of qualitative or descriptive studies	V	A synthesis of evidence from qualitative or descriptive studies to answer a clinical question.
Qualitative or descriptive study	VI	Qualitative study: gathers data on human behavior to understand *why* and *how* decisions are made. Descriptive study: provides background information on the *what*, *where*, and *when* of a topic of interest.
Expert opinion or consensus	VII	Authoritative opinion of expert committee.

Adapted with permission from Melnyk BM, Fineout-Overholt E, editors. Evidence-based practice in nursing and healthcare: a guide to best practice [forthcoming]. 2nd ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams and Wilkins.

Figure 3

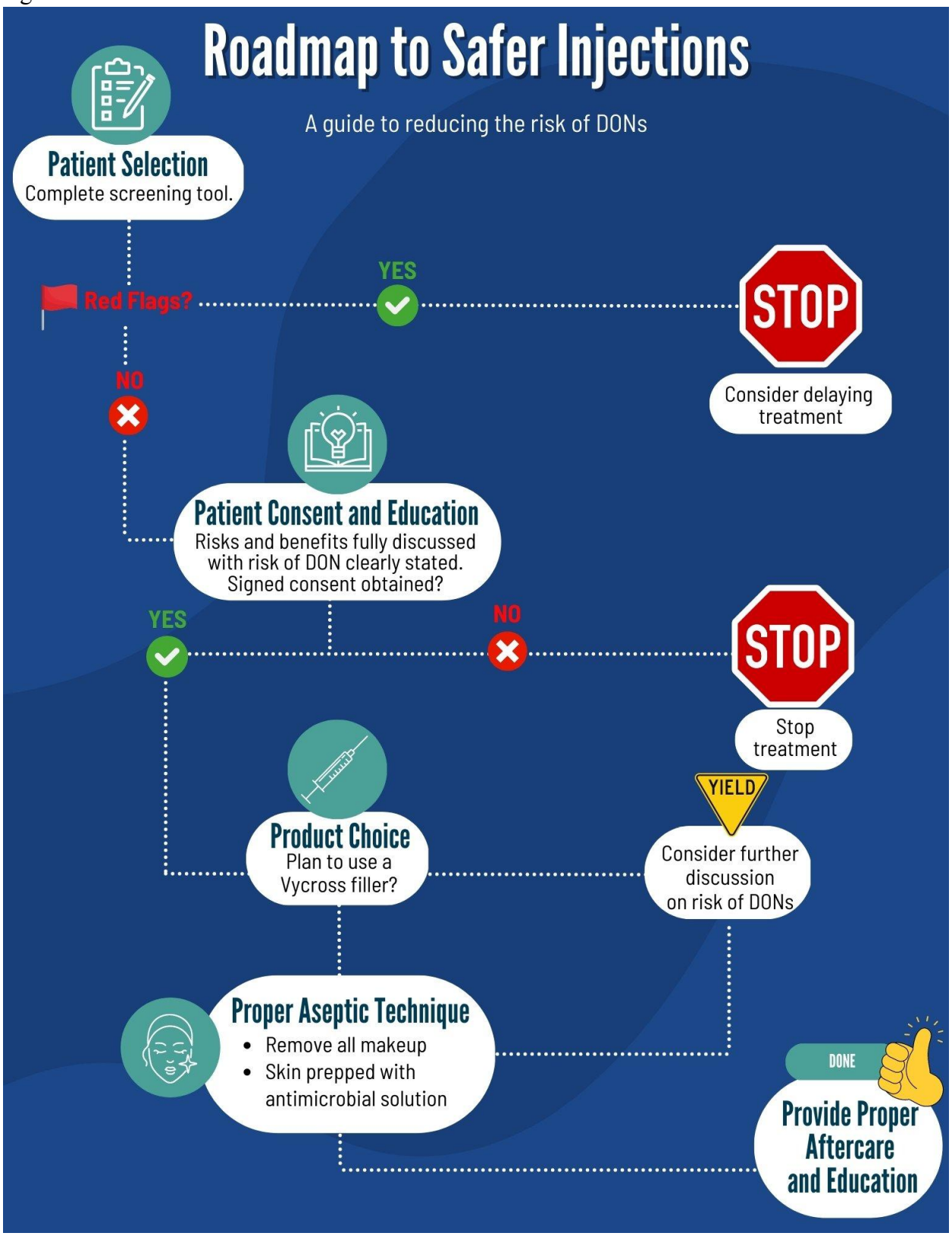
Roadmap to Safer Injections
A guide to reducing the risk of DONs
Patient Selection
Complete screening tool.
Red Flags?
YES
STOP
Consider delaying treatment
NO
Patient Consent and Education
Risks and benefits fully discussed with risk of DON clearly stated. Signed consent obtained?
YES
NO
STOP
Stop treatment
YIELD
Product Choice
Plan to use a Vycross filler?
Consider further discussion on risk of DONs
Proper Aseptic Technique
Remove all makeup
Skin prepped with antimicrobial solution
DONE
Provide Proper Aftercare and Education

Figure 4

Roadmap to Safer Injections Screening Tool

Patient Name_____

Do you take any medications?

- ☐ Yes
- ☐ No

Do you have any history of autoimmune diseases?

- ☐ Yes
- ☐ No

Have you had any dental work in the last month?

- ☐ Yes
- ☐ No

Do you have any plans for dental work in the next few months?

- ☐ Yes
- ☐ No

Have you had a recent cold or flu?

- ☐ Yes
- ☐ No

Do you have active Acne or bacterial infection on your face?

- ☐ Yes
- ☐ No

Have you had any recent vaccinations?

- ☐ Yes
- ☐ No

Do you have plans to receive any vaccinations in the next few months?

- ☐ Yes
- ☐ No

Further explanation of any of the above:

_____

_____

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_____

Table 1

Author and Title	Year	Design method	Sample/Setting	Major Varriables	Measurment	Results	Data Analysis	Relative to practice	Authors Stance on cause of DON	Level of Evidence
Artizi et al. Resistant and Recurrent Late Reactio to HA ACid -Based Gel	2015	Retrospective chart review	400 pts were examined. study of Medical Records in Isreal at 4 medical centers; 2 physicians.	Juvederm Vobella injected in lips or tear trough only. All other HA's all over face.	Number of DON associated with each filler	17-4.25% developed DON with Vobella	17/400 developed a reaction to the filler vobella. 8/17 had 1 additional product, 4/17 had 2 products, 5 had 3 aditional products. Average reation was 8.4 weeks after. The authors compared these results to a seperate chart review looking at tear trouch with othe HA fillers over a 6 moth period. only 1/302 patients (0.3%) Deveoped DON. Only difference was the Vobella vs other HA	Yes- Vobella is associated with higher rates of DON's than other FDA approved fillers in this study.	Biofilm not ruled out but authors believe Vobella to be more imunoggenic thatn any other filler.	VI
Beer, K., & Avelar, R. Relationship Between Delayed REactions to Dermal Fillers and Biofilms: Facts and Considerations.	2014	Literature review	N/A	Incubation time of cultures, skin prep during fillers, lack of different lab tests to show bacteria	n/a	Case of isolating bacteria typically found in the mouth. Case of linking the bacteria to the ice used after treatment.	Difficulty in culturing the bacteria due to traditional culturing techniques does not allow suffient time for incubtion. The biofilm may exist in a dormant stte and become activated by external factors. ie trauma, manipulation and injections.	Education on proper skin prep prior to injection. Dental work may play a role in biofilms as well as repeat injections. This is an important step in my **ROAD MAP TO SAFER INJECTION**	Biofilm	VII
Beleznay, et al. Delayed-Onset Nodules Secondary to a Smooth Cohesive 20 mg/mL Hyaluronic Acid Filler: Cause and Management.	2015	Retrospective chart review	HA-Voluma between February 1, 2009, and September 30, 2014, to evaluate for delayed-onset nodules.	Filler looked at was Juvederm Voluma. 4,702 treatments using 11,460 mL of Voluma	Number of DON associated with Voluma	Twenty-three patients (0.5%) experienced DONs.	The median time from injection to reaction was 4 months, and median time to resolution was 6 weeks. Nine of the 23 (39%) had an identifiable immunologic trigger such as flu-like illness before the nodule onset.	Better consents and education to patients as far as risk. Delay dental treatments after injections.	Immune mediated	VI
Cohen et. al Post market safety surveillance of delayed complications for recent fda approved HA dermal fillers	2022	Retrospective study Reports from MAUDE were extraced for multiple HA fillers from 2016-2020	585 reports were examined. MAUDE is Manufacturer and User Facilty Device Experience Database	Fillers looked at were Restylane Refyne, Defyne, Kysse. Juvederm Vobella, Vollure, Voluma, RHA 2,3,and 4	type of adverse event	585 MAUDE reports- 195 delaeyd AE's 71.8% were nodules, 21.5 hypersensitivity, 6.7 granuloma.Of these events 74.4 were Vollure, 12.3 Vobella, 5.1 Defyne 3.6 refyne, 2.1 RH2	Very few reports of nodules in non crosslinked fillers. Fewer reports prior to 2016 when crosslinked fillers were released.	These were only looking at events actually reported. No mandate to report these. Important for providers to be aware of these.	n/a	VI
Humphrey, et al. Retrospective review of delayed adverse events secondary to treatment with a smooth, cohesive 20-mg/mL hyaluronic acid filler in 4500 patients	2020	retrrospective Chart review	Charts from patients who received HA- Voluma between February 1, 2009, and February 28, 2018 from 2 clinics were analyzed.	Voluma was only filler authors looked at. They analyzed time of year, amount of filler, and immunologic triggers	DON associated with Voluma	In 4500 patients who received 9324 treatments with HA-V, 44 DON were identified, for a combined incidence of 0.98% per patient, 0.47% per treatment, and 0.23% per syringe.	Pts with DON eceived a slightly larger cumulative amount of HA-V than those who did not. Reactions and occurred a median of 4 months after treatment, with an increase in frequency between October and January. About a third were preceded by an identifiable immunologic stimulus.	Better consents and education to patients as far as risk. Delay dental treatments after injections.	Authors hypothesize that an increase in fragmentation during the HA-V degradation process may trigger an inflammatory response **AFTER** an immunologic trigger.	VI

Michon, Alain. Title: Hyaluronic Acid Soft Tissue Filler Delayed Inflammatory Reaction Following COVID-19 Vaccination – A Case Report.	2021	Case Report	Case reports were from Authors Clinic in Canada in 2021	Pt one was injected with Juvederm Vollite in tear trough 10/2020 Covid vaccine 4/2021 experienced swelling 2 days after along with flu like symptoms. Pt 2 was injected with multiple syringes of vycross filler in July of 2020. in April 2021 had Covid vaccine and within a few days devoloped swelling and tenderness in to sites of filler.	Delayed reaction to filler after Covid vaccine	2 case studies with Delayed inflamation following COVID vaccine	Both patients had vycross fillers. Both resolved quickly. Not true DON.	providers should be conscious of the risks posed by the interaction of such vaccines in patients who previously had or seeking hyaluronic acid soft tissue filler injections. Authors suggest at least a 3 week pause after vaccine for filler	These reactions are mediated by T-lymphocytes and can be triggered by flu-like illnesses, including SARS-CoV-2 infection. Vaccination may also induce hypersensitivity. THIS IS SOMETHING TO EXPLORE MORE!	VI
Perez, et al. Delayed Adverse Subcutaneous Reaction to a New Family of Hyaluronic Acid Dermal Fillers With Clinical, Ultrasound, and Histologic Correlation	2017	Case study	Ultrasound and histologic results of a nodule-biopsy, skin patch test	Vyross fillers from Allergan	histology exams	US showed edema, skin biopsy thickening of fibrous subcutaneos septa and lymphocytic inflammatory infiltrate in adipose lobules. Skin patch test to Voluma, Vollure, BDDE pet and lidocaine were negative. Intradermal injection of Voluma and Vollure negative at 20min, 96hours, but possitive 2 months later	Late inflammatory reaction, pt improved with treatment but had relapse in nodules 4 months after treatment.	Authors requested full ingrediants from Allergan but were denied	Agree that activation of nodules is immune response. Suspect that lowerer HA molecular wt plays a role	VI
Rivers, Jason K. Incidence and Treatment of Delayed Onset Nodules after VYC filler injections to 2139 patients at a single Canadian clinic	2019	Retrospective chart review	retrospective chart review of patients in a single clinic in Canada that were injected with Vycross HA Fillers. N=2139.	Vycross fillers from Allergan	Number of DON	7 female patients (mean age, 62 years) developed DON- 0.33%.	A potential inflammatory trigger (reported by 6 patients) occurred 1–168 days prior to nodule development. Nodule biopsy in 1 patient confirmed a foreign-body granuloma. 6 out of 7 devolped september-decmeber. dosing was similar in all patients	DON incedence was lower than earlier reports from previous studies. Closer to industry standards. Author states that standardized injection protocol will decrease DON!	Multifactorial	VI
Sadeghpour et. al Delayed onset Nodules to differentially crossliked HA acits: comparative incidence and risk assessment	2019	Retrospective Chart Review	1029 patients who recieved 1250 Vycross fillers in One Dermatology practice	Fillers looked at were Juvederm Vycross Family: Vobella, Vollure, and Voluma. Compared to Restylane silk for comparison	Number of DON associated with each filler	5 pts developed DON. All of these had Volbella. Vobella had a 1% risk of nodules. No DON with Vollure of Voluma. Rest Silk 0.2% Onset of nodule was 35.8 wks from 1st treatment.	Patients who developed nodules were asked for relevant history. Dental procedures, URI, trauma near site, vaccine administration or Auntoimune disease. 1 patient had a dental cleaning 3 weeks post filler	Yes- Vobella is associated with higher rates of DON's than other FDA approved fillers	Delayed immune respone	VI
Turkmani et. Al Delayed hypersensitivity reaction to hyaluronic acid dermal filler following injluenza like illness	2019	Case studies	14 cases in 2 dermatology centers in Saudi Arabia and Belgium between 2016 and 2018	Patients ranced from 22-65 all were female. In all cases reaaction started 3-5 days after flu like illness. Multiple injections over 4 years with variety of filler.	Reaction post flu like illness	Case study of 14 patients	Author has chart of fillers, number of treatments location, time to onset of symptoms. All patients treated with oral steroids		type 4 hypersensitivity following HA implantation and injluenza ifection.	VII
Wu et al. Histopahologic reaction patterns to differentially cross-linked hyaluronic acid fillers: A retrospective case series.	2021	Retrospective search for histopathologic reations to HA from 1/14-12/19 in NYC	Biopsy analysis of 15 granulomas after HA filler were retrieved and analyzed	Not all fillers identified; but of the 11 known were Vycross: 5 vobella, 5 voluma, 1 vollure. Area of injection: lip/cheek/chin/temple	Granuloma biopsy results	10 cases hare a distinct hisopoathologi pattern; multiple discrete foci of tightly cuffed palisades of multinucleated histocytes surrounding HA deposits with varying number of	10/15 descrete foci of tightly cuffed palisades of multinucleated histocytes surrounding HA deposits with varying number of eosinophils . The remaining 5 were nondecript inJlammatory patterns.	sample size is small but important to look at histolgy results. Helps to identify causes.	Authors state distinct inImmatatory patterns suggest immune response. May be LMW-HA with BDDE crosslinking.	VI

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Appendix- Annotated Bibliography

Artizi, O., Loizides, C., Verner, I., & Landau, M. (2016, January). Resistant and Recurrent Late Reaction to Hyaluronic Acid-Based Gel. *Dermatologic Surgery*, *42*(1), 31-37.
Artizi et al. (2016) completed a retrospective chart review, N=400. Patients were treated with cross linked filler Volbella. These patients were treated by 2 different physicians in a nine month span in 2013-2014. The patients had Volbella placed in lips, tear trough or both. Of the 400 patients, 17 developed a late reaction to the filler. The reaction occurred an average of 8.41 weeks after filler. The patients were all treated with a combination of antibiotics, corticosteroids, and hyaluronidase injections. One patient had a biopsy that revealed florid granulomatous dermatitis composed of epithelioid histiocytic granulomas, with numerous multinucleated foreign body-type giant cells surrounding amorphous material. All cultures of the lesion were negative (Artizi et al., 2016). Each of the 17 patients had no pre-existing skin conditions or any immune triggering episodes. The authors rate of DONs with Vobella were 4.25%, much higher than the industry standard of 0.02%. Artizi et al (2016) state the cause of the reactions remains unclear, but Volbella has properties that make it more likely to trigger an immune response. The authors also state that it is appropriate to place patients on antibiotics since biofilm reaction can not be completely ruled out (Artizi et al., 2016). This is an interesting article and one that is often referenced in other studies. The 4.25% risk of DON is far above industry standards. This will be interesting to compare to other reports.

Beer, K., & Avelar, R. (2014, November). Relationship Between Delayed REactions to Dermal Fillers and Biofilms: Facts and Considerations. *Dermatologic Surgery*, *40*(11).
Beer and Avelar (2014) recognize that DONs have been attributed to hypersensitivity reactions but state that biofilms play an important role. The authors (Beer and Avelar 20014) stress the importance of aseptic technique during skin preparation and injection and argue that small amounts of bacteria can adhere to the gels. These bacteria can be slow growing and missed in culture due to the short incubation times. The authors (Beer & Avelar, 2014) state that different techniques to look for biofilms should be used to better identify pathogens . HA fillers can last in the skin for years and infection may result from later exposure to bacteria through the skin. The authors feel that the prevention of biofilms should be an important part of injection education (Beer & Avelar, 2014).
This is a useful source as it will help me to create my roadmap to safer injections. Since research can not fully eliminate biofilm as a cause of DON, it will be important to educate on proper skin prep prior to injections.

Beleznay, K., Carruthers, J., Carruthers, A., Mummert, M., & Humphrey, S. (2015, August). Delayed-Onset Nodules Secondary to a Smooth Cohesive 20 mg/mL Hyaluronic Acid Filler: Cause and Management. *Dermatologic Surgery*, *41*(8).
Authors of this article started to see more incidences of DON in their practice (Beleznay et al., 2015). A retrospective chart review found N=4,702 patients that received the crosslinked filler Juvederm Voluma over a 68 month span. Twenty-three of these patients developed DONs or 0.5% of patients, three to five months after treatment. In this study nine of the 23 cases had an immunologic trigger like a viral illness, or dental work prior to nodule onset (Beleznay et al., 2015). The most common and most successful treatment

of DONs in this practice was a course of oral prednisone, the authors also used Hyaluronidase into the nodules in many cases (Beleznay et al., 2015). The authors of this article state that delayed reactions are immune mediated (Beleznay et al., 2015). This source will help to guide me to better develop consents for patients as viral illnesses can not be predicted. In my roadmap it will be recommended to delay dental treatments as this research supports it may be a triggering event for DONs.

Cohen, J. L., Hicks, J., Nogueira, A., Lane, V., & Andriopoulos, B. (2022, February). Postmarket Safety Surveillance of Delayed Complications for Recent FDA-Approved Hyaluronic Acid Dermal Fillers. *Dermatologic Surgery*, *48*(2).
The purpose of this retrospective study is to review delayed events reported to the Manufacturer and User Facility Device Experience (MAUDE) FDA database (Cohen et al., 2022). The database consists of safety issues that are derived from mandatory reports from manufacturers and voluntary reports from health care professionals and consumers. Cohen et al. (2022 looked at crosslinked fillers from Galderma, Allergan, and Revance companies. They found 585 total reports of delayed reaction. Of these reports 195 were confirmed delayed reactions to vycross filler (Cohen et al., 2022). The authors report very few reports of delayed reaction with non crosslinked fillers during this time frame. The authors support research that claims crosslinked fillers have higher rates of delayed events. The Allergan family of crosslinked fillers had the highest rates of nodules reported. Due to the fact that there is no required reporting it is difficult to fully understand the rates of these complications, but health care providers need to be aware and prepared to treat them when they arise (Cohen et al., 2022) This is an important look as it looks at cases as a whole not in just one practice. This may represent less bias.

Humphrey, S., Jones, D., Carruthers, J., Carruthers, A., Beleznay, K., Wesley, N., Black, J., Vanderveen, S., & Minokadeh, A. (2020, July). Retrospective review of delayed adverse events secondary to treatment with a smooth, cohesive 20-mg/ml hyaluronic acid filler in 4500 patients. *Academy of Dermatology.*
Humphrey et al. (2020) completed a retrospective chart review from 2009-2018 of patients in multiple clinics who received the cross linked filler Juvederm Voluma, N=4500. Forty four patients experienced delayed onset reactions (0.98%) (Humphrey et al., 2020). The median onset of the delayed nodule was 4 months. More than half of these patients injected with Voluma, the product was diluted down with Saline or Lidocaine. Fourteen of these patients identified an immune trigger prior to the nodule event; viral infection, bacterial infection, or dental exam. Patients with larger volumes of the fillers were also more likely to develop nodules (Humphrey et al., 2020). The patients in this study were treated with oral steroids, hyaluronidase (filler dissolving), and antibiotics. The authors state that during the breakdown of the Voluma the inflammatory response may be triggered causing these nodules to appear (Humphrey et al., 2020). This a large study, in over half of the cases the filler was altered with saline or lidocaine which may have played a role in the DOlN formation. It will be difficult to compare these results, but I do think this is a good look at Voluma and relevant to the topic.

Michon, Alain. Title: Hyaluronic Acid Soft Tissue Filler Delayed Inflammatory Reaction Following COVID-19 Vaccination – A Case Report. (June 26, 2021). Journal of Cosmetic Dermatology, 20 (9), 2684-2690.
The author presents two case studies of patients who developed delayed inflammatory reactions to HA filler after COVID-19 vaccinations. In Case 1 the patient had Vycross

filler Vobella injected to her tear trough in October of 2020. Two days after Covid-19 vaccination in April 2021 the patient developed erythema and edema to the left tear trough accompanied by flu-like symptoms. This patient's symptoms resolved spontaneously with watchful waiting. Case number two had multiple syringes of Vycross filler to her face in June of 2020. The patient had Covid 19 Vaccine in April of 2021 and developed flu like symptoms and non erythematous edema to areas of filler injection several days after vaccination. The edema fluctuated for 72 hours and was treated with hyaluronidase (filler dissolving enzyme). The author goes into discussion on recent research for delayed reaction after HA filler and points out the significance of immune triggering events. While these cases are not true DON's as there was edema but no nodule, I do think these cases are relevant to this review. This is an important education point for patients and providers.

Perez, L., & Garcia-Gavin, J. (2017, April). Delayed Adverse Subcutaneous Reaction to a New Family of Hyaluronic Acid Dermal Fillers With Clinical, Ultrasound, and Histologic Correlation. *Dermatologic Surgery*, *43*(4), 605-608.

This article is a case study of a healthy 49 year old patient who received 2mls of Vycross fillers in her lower face (Volluma and Vollure) (Perez & Garcia-Gavin, 2017). Four months after treatment this patient developed hard painless nodules in the area of treatment. The authors used ultrasound to locate the nodules and found edema and vascularity to the area. A skin biopsy showed lymphocytic inflammatory infiltrates in the adipose tissue (Perez & Garcia-Gavin, 2017). A patch test of Voluma, Vollure, BDDE, pet, and lidocaine revealed negative results. Intradermal injection into the right forearm showed no results at 20 minutes and 96 hours, but both turned positive two months after placement (Perez & Garcia-Gavin, 2017). The authors agree with the studies done by the authors above, that there can be a late immune response to these Vycross fillers. They showed that injecting 0.05mL of filler into the forearm can produce a reaction.

This case study provides facts rather than speculation. Although only one case study is presented, the delayed reaction was repeated on the arm. This result further supports the delayed immune response to certain fillers.

Rivers, Jason K. (2022, April). Incidence and Treatment of Delayed Onset Nodules after VYC filler injections to 2139 Patients at a Single Canadian Clinic. *Journal of Cosmetic Dermatology, 21; 2379-2386.*

This article is a retrospective chart review of patients in a single clinic in Canada that were injected with Vycross HA Fillers. N=2139. A total of seven patients (0.33%) developed DON's between 2010 and 2019. The seven patients were analyzed for amounts injected, areas injected, type injected and immune triggering events. Analysis found that six of the seven patients had undergone dental procedures prior to the event. However the timing was variable ranging from 1 day to 168 days after the event. One patient underwent histologic evaluation showing a foreign body granuloma. The author also analyzed current research on DONs after HA filler injection and compared it to his findings. The discussion analyzes several studies that are chosen for this systematic review as well. The author does not compare DON's to non Vycross fillers used in his clinic, which would be a beneficial comparison. There is mention of COVID-19 vaccine triggering inflammatory events for HA filler which is relevant to this topic and something that should be explored.

Sadeghpour, M., Quatrano, N., Bonati, L. M., Arndt, K., Dover, J., & Kaminer, M. S. (2019, August). Delayed-Onset Nodules to Differentially Crosslinked Hyaluronic Acids: Comparative Incidence and Risk Assessment. *American Society for Dermatologic Surgery*, *45*, 1085-1094.

Sadeghpour et al. (2019) completed a retrospective chart review that looked at patients in their clinics that received crosslinked filler over a 12 month period (Sadeghpour et al., 2019). They specifically looked at the Allergan HA crosslinked fillers with trade names, Volbella, Vollure,and Volluma. For reference they also examined a non-crosslinked filler made by Galderma (Restylane Silk), but also commonly used in the lips. In this article N=1029. These patients received Allergan crosslinked filler treatments over a twelve month period. Five of these patients developed DONs. These patients all had received Volbella with an incidence of 1.0%. No DONs were found in Vollure or Voluma (Sadeghpour et al., 2019). Restylane silk was 0.25% per patient.. Only one of these patents reported any potential immune triggers; a dental cleaning 3 weeks prior to treatment.Dental cleaning is thought to potentially introduce bacteria into the bloodstream potentially leading to filler infections; especially newly infected filler around the mouth (Sadeghpour et al., 2019).. All of the nodules resolved with multiple sessions of hyaluronidase; a common filler dissolving agent, and triamcinolone (corticosteroid) injected into the nodules (Sadeghpour et al., 2019). The authors of this article (Sadeghpour et al., 2019) state that Volbella should be associated with higher risk of DONs than other FDA approved fillers. They believe this is due to the lower particle size and the crosslinking technology that trigger pro-inflammatory effects (Sadeghpour et al., 2019).

Turkmani, M. G., De Boulle, K., & Philipp-Dormston, W. (2019). Delayed Hypersensitivity Reaction to Hyaluronic Acid Dermal Filler Following Influenza-like Illness. *Clinical, Cosmetic, and Investigational Dermatology*, *12*, 277-283

This article presents 14 case studies. Each patient had a delayed reaction to HA filler after flu-like illness (Turkmani et al., 2019). The fillers the patients received were crosslinked fillers or a combination of crosslinked and non-crosslinked fillers. In each case the patient started to have a reaction to the filler 3-5 days after a flu-like illness (Turkmani et al., 2019). The immunologic reaction between the viral infection and the fillers is poorly understood. The authors believe this as a type 4 hypersensitivity reaction. There was no histological analysis on these patients and symptoms resolved with a course of oral steroids (Turkmani et al., 2019).

This study will help guide my map to safer injections. Better patient education and consent will be crucial. It contradicts the sources stating that there is some trigger with the breakdown of the filler.

Wu, E. W., Stokar, E., Franks, L., & Meehan, S. A. (2021). Histopathologic reaction patterns to differentially cross-linked hyaluronic acid fillers: A retrospective case series. *Journal of Cutaneous Pathology*, *48*, 758-762.

In this article authors looked at reactions to hyaluronic acid fillers from January 2014 to December 2019 in their dermatology practice (Wu et al., 2021). Fifteen cases of nodules were found. In 11 of the 15 cases where the filler was known, all nodules were after using Allergan's Vycross fillers (Vobella, Voluma, Vollure). The authors looked at biopsy specimens of these granulomas. Results were consistent with an inflammatory response; histopathologic pattern of discrete foci of tightly cuffed palisaded granulomas with

eosinophils (Wu et al., 2021). There was no evidence of bacteria, fungi, or microorganisms found in the samples thus the authors state that with the lack of neutrophils these delayed reactions are unlikely biofilms. Wu et al (2021) state that the increase in DONs is likely related to the crosslinking molecules used by Allergan (which are proprietary) or the low molecular weight of the filler, or a combination of both, to be proinflammatory.