UNIVERSIDADE FEDERAL DE MINAS GERAIS
Faculdade de Odontologia
Colegiado de Pós-Graduação em Odontologia

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CARACTERIZAÇÃO HISTOPATOLÓGICA DE REAÇÕES ADVERSAS A MATERIAIS DE PREENCHIMENTO ESTÉTICO EM REGIÃO ORAL E MAXILOFACIAL: *ESTUDO TRANSVERSAL MULTICÊNTRICO*

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Orientadora: Prof.ª Dr.ª Patrícia Carlos Caldeira
Coorientador: Prof. Dr. Lucas Guimarães Abreu

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CARACTERIZAÇÃO HISTOPATOLÓGICA DE REAÇÕES ADVERSAS A MATERIAIS DE PREENCHIMENTO ESTÉTICO EM REGIÃO ORAL E MAXILOFACIAL: ESTUDO TRANSVERSAL MULTICÊNTRICO

ANA CRISTINA TETZNER

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“O que dá o verdadeiro sentido ao encontro é a busca, e é preciso andar muito para se alcançar o que está perto.”

José Saramago

RESUMO

O uso de materiais injetáveis estéticos na região orofacial vem aumentando exponencialmente, assim como as complicações associadas a estes procedimentos. As reações adversas a materiais estéticos podem se confundir com processos neoplásicos, tanto do ponto de vista clínico quanto microscópico. Este trabalho tem como objetivo principal descrever detalhadamente as características clínicas, demográficas, histopatológicas, histoquímicas e imuno-histoquímicas de reações adversas a diferentes preenchedores estéticos na região oral e maxilofacial. Foi realizado um estudo transversal multicêntrico, no qual as amostras foram coletadas de cinco laboratórios de patologia oral no Brasil e no México. Foram realizadas colorações de hematoxilina-eosina, Alcian *blue*, Sirius *red* e azul de toluidina, bem como imuno-histoquímica para CD68, CD3 e CD20. H&E também foi avaliado sob luz polarizada. Estatísticas descritivas foram realizadas. Vinte e três casos foram incluídos. Polimetilmetacrilato foi o material mais comum (n=10), seguido por silicone (n=4), ácido hialurônico (n=3), hidroxiapatita de cálcio (n=3), hidrogel de poliacrilamida (n=2) e ácido poli-L-láctico (n=1). Os pacientes foram principalmente mulheres (91,3%), com idade média de 50,65 anos. A maioria das reações afetou os lábios e foi assintomática, com tempo de evolução variável (7 dias a 10 anos), apresentando-se como nódulos de 58,07 mm em média. Polimetilmetacrilato e silicone apresentaram imagens negativas de formato arredondado, enquanto ácido hialurônico e hidrogel de poliacrilamida apresentaram-se como “piscinas” amorfas. A hidroxiapatita de cálcio apresentou estruturas poliédricas e o ácido poli-L-láctico apresentou formatos semelhantes a fendas. Ambos birrefringentes sob luz polarizada. Células gigantes foram comumente encontradas, exceto em silicone e ácido hialurônico. Granuloma de corpo estranho foi frequente em polimetilmetacrilato. Ácido hialurônico e hidrogel de poliacrilamida apresentaram metacromasia por azul de toluidina. Alcian *blue* foi positivo em todos os casos de ácido hialurônico. Mastócitos foram detectadosem todos os materiais, exceto ácido hialurônico e hidrogel de poliacrilamida. Eosinófilos foram mais raros que mastócitos. Numerosas células CD68 positivas foramvistas em todos os casos. Todos os casos apresentaram células CD3 positivas, com quantidades variáveis. CD20 foi escasso ou negativo na maioria dos casos. Além do artigo científico, foi produzido um *e-book* de histopatologia, enfatizando o diagnóstico diferencial histológico destas lesões. Concluímos que apesar das semelhanças, há características específicas de cada material e da resposta do hospedeiro que auxiliam no diagnóstico histopatológico correto. Formato, tamanho e coloração do material no H&E são características-chave no diagnóstico diferencial. Uma reação intensa de macrófagos é observada em todos os preenchedores estéticos, frequentemente associada à formação de células gigantes. A imuno-histoquímica para CD68 e a coloração por azul de toluidina são as mais abrangentes para auxiliar no correto diagnóstico, sendo que outros marcadores podem ser úteis em casos específicos. Lesões neoplásicas também devem ser consideradas no diagnóstico diferencial histopatológico.

Palavras-chave: preenchedores dérmicos; reação no local da injeção; reação a corpo estranho; microscopia; imuno-histoquímica.

ABSTRACT

Histopathological characterization of adverse reactions to aesthetic filling materials in the oral and maxillofacial region: a multicenter cross-sectional study

The use of aesthetic injectable materials in the orofacial region has been increasing exponentially, which has also led to an increase in complications associated with these procedures. Adverse reactions to aesthetic materials can be confused with neoplastic processes, both from a clinical and microscopic perspective. The main objective of this study is to describe in detail the clinical, demographic, histopathological, histochemical, and immunohistochemical characteristics of adverse reactions to different aesthetic fillers in the oral and maxillofacial region. A multicenter cross-sectional study was conducted, in which samples were collected from five oral pathology laboratories in Brazil and Mexico. Hematoxylin-eosin, Alcian blue, Sirius red, and toluidine blue staining, as well as immunohistochemistry for CD68, CD3, and CD20, were performed. H&E was also evaluated under polarized light. Descriptive statistics were conducted. Twenty-three cases were included. Polymethylmethacrylate was the most common material (n=10), followed by silicone (n=4), hyaluronic acid (n=3), calcium hydroxyapatite (n=3), polyacrylamide hydrogel (n=2), and poly-L-lactic acid (n=1). The patients were predominantly women (91.3%), with an average age of 50.65 years. Most reactions affected the lips and were asymptomatic, with variable evolution times(7 days to 10 years), presenting as nodules with an average size of 58.07 mm. Polymethylmethacrylate and silicone showed negative images with a rounded shape, while hyaluronic acid and polyacrylamide hydrogel presented as amorphous "pools." Calcium hydroxyapatite showed polyhedral structures, and poly-L-lactic acid exhibited fissure-like shapes. Both were birefringent under polarized light. Giant cells were commonly found, except in silicone and hyaluronic acid. Foreign body granuloma was frequent in polymethylmethacrylate. Hyaluronic acid and polyacrylamide hydrogel showed metachromasia with toluidine blue. Alcian blue was positive in all hyaluronic acid cases. Mast cells were detected in all materials except hyaluronic acid and polyacrylamide hydrogel. Eosinophils were rarer than mast cells. Numerous CD68-positive cells were seen in all cases. All cases presented CD3-positive cells, in varying amounts. CD20 was scarce or negative in most cases. In addition to the scientific article, a histopathology e-book was produced, emphasizing the histological differential diagnosis of these lesions. We conclude that despite similarities, there are specific characteristics of each material and host response that aid in the correct histopathological diagnosis. The shape, size, and staining of the material in H&E are key features in the differential diagnosis. An intense macrophage reaction is observed in all aesthetic fillers, often associated with the formation of giant cells. Immunohistochemistry for CD68 and toluidine blue staining are the most comprehensive for assisting in the correct diagnosis, although other markers may be useful in specific cases. Neoplastic lesions should also be considered in the histopathological differential diagnosis.

Keywords: dermal fillers; injection site reaction; foreign-body reaction; microscopy; immunohistochemistry.

SUMÁRIO

1 CONSIDERAÇÕES INICIAIS

Os materiais preenchedores são relatados desde 1830, com a descoberta da parafina pelo químico alemão Baron Karl Ludwig von Reichenbach (Goldwyn, 1980). Em 1899, a parafina foi utilizada para fins estéticos pela primeira vez, quando Robert Gersuny aplicou esse material para preencher uma prótese testicular em um paciente com tuberculose de epidídimo (Kontis; Rivkin, 2009). Entretanto, são nos últimos anos que têm crescido o número de profissionais e pacientes que procuram realizar procedimentos estéticos, por preocupações em atender aos padrões de beleza e amenizar as consequências do processo de envelhecimento. Esses procedimentos são injeções de materiais para preenchimento facial, labial e para o tratamento de rugas (Requena *et al*., 2011). De acordo com a Sociedade Brasileira de Cirurgia Plástica (SBCP, p. 13, 2018), o preenchimento foi o segundo tipo de procedimento não cirúrgico mais utilizado em 2018, correspondendo a 89,6%, atrás apenas da toxina botulínica.

Os materiais de preenchimento são classificados pela FDA (*Food and Drug Administration*) em absorvíveis (biodegradáveis) ou não absorvíveis, de acordo com a biodegradabilidade deles. Os materiais absorvíveis são o ácido hialurônico (Rennova® Lift, Restylane®, Juvederm®, Perlane®), hidroxiapatita de cálcio (Radiesse®) e ácido poli-L-lático (Sculptra®, New Fill®). Enquanto o polimetilmetacrilato (Artefill®, Artecell®), o silicone (Silikon 1000 ®, Silskin ®, PMS 350®, Silicone Medical Grade®) e a poliacrilamida (Aquamid®, Interfall®, Outline® etc.) são classificados como não absorvíveis (Lombardi *et al*., 2004; Vargas-machuca *et al*., 2006, Requena *et al*., 2011). Atualmente, o único material não absorvível aprovado pelo FDA é o polimetilmetacrilato (FDA, 2023). Entretanto, ainda são encontrados casos clínicos na literatura com materiais não aprovados atualmente, provavelmente anteriores à proibição ou ao uso clandestino (FDA, 2020). Esses materiais também podem ser classificados em temporários (absorvíveis) ou permanentes (materiais não absorvíveis), de acordo com a duração deles (Chiang; Pierone; Al-Niaimi, 2017).

Embora a maioria dos materiais preenchedores seja tolerável pelo organismo, ainda não foi disponibilizado um material totalmente seguro e biocompatível. Além disso, injeções inadequadamente muito superficiais também podem provocar reações adversas, bem como materiais de preenchimento

inadequados para a região dos lábios, como a hidroxiapatita de cálcio e o ácido poli-L-lático. Esses materiais não deveriam ser usados para o preenchimento labial, pois podem se aglomerar devido à ação muscular (Haneke, 2019).

As reações adversas a esses materiais podem ocorrer imediatamente após a aplicação, dentro de dias, semanas ou anos após os procedimentos estéticos. As reações com início precoce incluem: eritema, edema localizado, dor, aumento de volume, nódulos (reação mais comum), infecções, reações de hipersensibilidade (geralmente do tipo I), descoloração da pele e oclusão vascular. As reações adversas mais tardias – que ocorrem dentro de semanas ou anos – e graves relatadas são: edema malar, reação de hipersensibilidade (geralmente do tipo IV), insuficiência renal, nódulos inflamatórios, descoloração persistente da pele, migração do material implantado, cicatrizes, náusea, cefaleia, paralisia do lábio superior, trombose da artéria retiniana, mialgia, ulceração, necrose e reações granulomatosas de corpo estranho (Duffy, 2005; Requena *et al.*, 2011; Tamiolakis *et al.*, 2018).

Microscopicamente, o granuloma de corpo estranho é o padrão histológico mais comum dos nódulos e outras manifestações clínicas causadas pelas reações adversas aos materiais de preenchimento estético (El-Khalawany *et al.*, 2015). As propriedades físico-químicas desses materiais impedem uma fagocitose efetiva pelos macrófagos e, então, resulta em uma inflamação granulomatosa (Anderson; Rodriguez; Chang, 2008). “Granuloma” é um tipo histológico específico de inflamação crônica que pode ser classificado em dois grupos: granuloma imunogênico e granuloma de corpo estranho. Independentemente da sua localização, o granuloma é composto por um agrupamento de macrófagos (histiócitos epitelioides), que podem formar células gigantes multinucleadas, associados a uma coroa de linfócitos (Brasileiro Filho; Bogliolo, 2018).

O exame histopatológico das lesões por materiais preenchedores estéticos é o padrão-ouro para identificar o preenchedor responsável pela reação adversa, porque as partículas de cada material têm suas características microscópicas específicas (Alcântara *et al*., 2017; Requena *et al*., 2011). Por isso, o exame histopatológico muitas vezes é imprescindível para um diagnóstico conclusivo, porque as lesões orais por materiais preenchedores podem mimetizar diversas outras doenças. Clinicamente, os nódulos ou aumento de volume causados por esses materiais podem se assemelhar às lesões reacionais, neoplasias de glândulas salivares e mesenquimais (Farahani *et al*., 2012; Anatelli; Chapman; Brennick, 2010).

Outros aspectos clínicos das lesões por materiais cosméticos fazem diagnóstico diferencial com a erisipela, dermatite de contato alérgica, edema facial com eosinofilia, blastomicose, síndrome de Ascher, leishmaniose cutânea, hanseníase e tuberculose (Ficarra; Mosqueda-Taylor; Carlos, 2002; Jham *et al*., 2009).

Casos de reações adversas a materiais cosméticos com o aspecto de granulomas têm como diagnóstico diferencial diversas lesões granulomatosas não infecciosas de lábio superior, como: síndrome de Melkersson-Rosenthal, queilite granulomatosa e outras reações de corpo estranho devido a materiais dentários. Além desses diagnósticos diferenciais, as lesões orais por materiais cosméticos assemelham-se à granulomatose orofacial por causas sistêmicas, como a doença de Crohn e a sarcoidose (Mendoza *et al*., 2022; Müller, 2019). Histopatologicamente, as reações adversas aos materiais de preenchimento podem ter como diagnóstico diferencial o carcinoma mucoepidermoide e, em alguns casos, até clinicamente também (Alli; Murdoch; Meer, 2022; Eversole *et al*., 2013; Karagozoglu; van der Waal, 2008).

As consequências do aumento do uso de preenchedores cosméticos orofaciais têm trazido novos desafios diagnósticos para os patologistas orais e cirurgiões-dentistas, principalmente quando o paciente não relata o uso de preenchedores dérmicos durante a anamnese (Lombardi *et al*., 2004; Owosho *et al*., 2014; Quirino *et al*., 2012). Possivelmente, alguns pacientes não relatam que fizeram procedimentos estéticos por não suspeitarem que sejam a causa das lesões, visto que as reações adversas podem ser tardias. Assim, as reações adversas causam ansiedade e preocupação nesses pacientes, porque eles receiam que possa se tratar de uma lesão neoplásica (Feio *et al*., 2013).

Nesse contexto, o objetivo deste estudo foi analisar os aspectos demográficos, clínicos e histopatológicos dos casos de reações adversas a materiais de preenchimento estético, com especial ênfase nas características histopatológicas, para auxiliar profissionais a melhorar a acurácia do diagnóstico desses pacientes.

1.1 Materiais de Preenchimento Estético

Entre os materiais temporários, alguns são bioestimuladores de colágeno, como o ácido poli-L-lático e a hidroxiapatita de cálcio. O primeiro é composto por um polímero do grupo α-hidroxiácido suspenso em manitol e carboximetilcelulose,

enquanto o último é composto por hidroxiapatita de cálcio (Ca10(PO)6OH2) adicionalmente à carboximetilcelulose e glicerina (Bentklover, 2009; Requena *et al*., 2011).

O ácido hialurônico é amplamente utilizado como material de preenchimento para lábios, rugas e linhas nasolabiais. É um glicosaminoglicano que consiste em unidades repetidas de ácido D-glucurônico e dissacarídeo DN-acetilglucosamina ($C_{28}H_{44}N_2O_{23}$). Devido à abundância natural deste biopolímero (presente em animais e corpo humano), sua biodegradabilidade e biocompatibilidade atraem seus usos versáteis para fins cosméticos (Bukhari *et al*., 2018; Fraser; Laurent; Laurent, 1997).

A poliacrilamida em hidrogel é composta por 2,5% de poliacrilamida e 97,5% de água. Caracteriza-se como um material maleável, uniforme e composto por ligações cruzadas de poliacrilamida, sem micropartículas sólidas (Parada *et al*., 2005). Apesar de semelhante ao ácido hialurônico microscopicamente, pela apresentação de “piscinas” basofílicas, é um material permanente e seu uso foi proibido. O hidrogel de poliacrilamida foi amplamente aplicado para reabilitação de lipoatrofia facial associada ao HIV, além da utilização desse material para o aumento das mamas (de Santis *et al*., 2008; Gao *et al*., 2022; Rauso, 2015). Reações adversas precoces e tardias nesses pacientes têm sido relatadas (Gao *et al*., 2022; Mo *et al*., 2019).

O silicone é composto por polidimetilsiloxano, um grande molécula de unidades $(-[CH_3]_2SiO-)_x$ (Clark; Hanke; Swanson, 1989). Este material pode ser comercializado na forma líquida (óleo), em gel ou na forma sólida (elastômero). As características histopatológicas variam de acordo com a forma comercializada. O silicone líquido tem sido usado para preenchimento de tecidos moles e é o tipo ainda encontrado na região oral, apesar de ter sido proibido pela FDA (Clark; Hanke; Swanson, 1989; Requena *et al*., 2011).

O polimetilmetacrilato é composto por microesferas de polimetilmetacrilato (fase sólida) suspensas em solução de colágeno com cloridrato de lidocaína a 0,3% (Hoffmann *et al*., 1999). A fase líquida é composta por solução de colágeno bovino, utilizado em suturas, agentes hemostáticos e implantes. As partículas de polimetilmetacrilato foram utilizadas previamente para prótese dental, cimento ósseo

ortopédico e placas de craniectomia. Assim, a combinação de colágeno bovino com o polimetilmetacrilato satisfaz questões de biocompatibilidade (Lemperle *et al*., 1991, 2009). Entretanto, colágeno bovino induz reação alérgica em aproximadamente 3 a 4% dos pacientes, por isso, deve-se fazer um teste intradérmicoantes do uso deste preenchedor (Cooperman *et al*., 1985; McClelland *et al*., 1997).

2 OBJETIVOS

2.1 Objetivo geral

Analisar os aspectos demográficos, clínicos e, especialmente, os aspectos histopatológicos, histoquímicos e imuno-histoquímicos das reações adversas a materiais de preenchimento estético na região oral e maxilofacial.

2.2 Objetivos específicos

- Descrever os aspectos histopatológicos dos materiais estéticos observados nacoloração de hematoxilina-eosina, com e sem luz polarizada.
- Descrever o aspecto microscópico dos materiais quando corados pelas técnicas histoquímicas de Alcian *blue* e Azul de Toluidina.
- Descrever o padrão histopatológico da resposta inflamatória aos materiaisestéticos.
- Identificar e quantificar mastócitos e eosinófilos por meio das colorações histoquímicas de Azul de Toluidina e Sirius *red*, respectivamente.
- Identificar macrófagos, linfócitos T e linfócitos B, por meio das colorações imuno-histoquímicas para CD68, CD3 e CD20, respectivamente.
- Descrever dados clínicos das lesões e demográficos dos pacientes acometidos.
- Produzir um atlas de histopatologia da casuística do estudo.

3 METODOLOGIA EXPANDIDA

3.1 Considerações éticas

Este estudo foi realizado de acordo com a Declaração de Helsinque (Belsey, 1978) e aprovado pelo Comitê de Ética em Pesquisa da Universidade Federal de Minas Gerais (UFMG) pelo número do certificado 10723019.0.1001.5149. Os Comitês de Éticade todas as instituições dos participantes foram notificados sobre a aprovação. O relato deste estudo multicêntrico e transversal está em conformidade com a declaração STROBE.

3.2 Desenho de estudo

Estudo transversal, retrospectivo e multicêntrico.

3.3 Seleção da casuística e coleta de dados

Foram revisados os arquivos de biópsia desde o período inicial de registro de cincoserviços de Patologia Oral: Universidade Estadual da Paraíba (2011-2023), Universidade Federal de Goiás (2003-2023), Universidade Federal de Minas Gerais (1998-2023), Universidade Federal de Pelotas (2006-2024) e “Universidad Nacional Autónoma de México” (2019-2024). Foram recuperados casos com diagnóstico histopatológico de reação inflamatória ou granulomatosa por material exógeno para fins estéticos na região oral e maxilofacial. Foram excluídos casos sem blocos ou lâminas de parafina disponíveis e pacientes que apresentavam outras doenças granulomatosas. Os seguintes dados foram coletados dos prontuários de biópsia: dados demográficos (sexo e idade), características da lesão e do material (sintomas, sinais clínicos, tipo de lesão clínica, cor, tamanho, local acometido, tipo de biópsia, exames complementares, evolução da lesão ao longo do tempo, hipótese diagnóstica, marca e tipo de material, localização da injeção, tempo entre a injeção e a biópsia). Pesquisadores de cada centro realizaram buscas e coleta de dados de forma independente, utilizando um modelo de tabela eletrônica previamente padronizado. As tabelas preenchidas foram enviadas à UFMG, onde os dados foram organizados em um único banco de dados para a realização de análises estatísticas.

3.4 Critérios de inclusão e exclusão

Foram incluídos todos os casos com o diagnóstico histopatológico conclusivo,sugestivo ou compatível com reação adversa/ inflamatória/ granulomatosa por material exógeno ou de preenchimento com finalidades estéticas.

Foram excluídos da pesquisa casos sem seus respectivos blocos ou lâminas disponíveis para a avaliação histopatológica, ou casos nos quais os pacientes tinhamoutras doenças granulomatosas.

3.5 Avaliação histopatológica

As lâminas coradas em hematoxilina-eosina (H&E) de todos os casos foram avaliadas por dois examinadores concomitantemente (ACT, PCC) no Laboratório de Patologia Oral e Maxilofacial da UFMG, utilizando um microscópio óptico binocular (Carl Zeiss Microscopy, Jena, Alemanha). As seguintes características histopatológicas foram avaliadas: presença ou ausência de granuloma de corpo estranho, células gigantes e corpos asteroides; forma, tamanho, cor e padrão de distribuição do material; tipo de célula inflamatória predominante. Todos os casos foram polarizados em um microscópio óptico (Opticam® 0600R, modelo LOPT14003, 14,0 Mega Pixels), acoplado a um polarizador de luz, e os materiais foram classificados como birrefringentes ou não birrefringentes. Neste mesmo microscópio óptico, os tamanhos de partículas dos preenchedores cosméticos foram medidos pelo OPTHD *Microscope Imaging Software*®. Foi selecionado o maior diâmetro e medido em micrômetros (µm) através de uma objetiva de 4x e, em casos de partículas muito pequenas, foi utilizada uma objetiva de 10x.

3.6 Histoquímica

As colorações Alcian *blue* (código Histokit EP-11-20018, EasyPath, Indaiatuba, SP, Brasil), Sirius *red* (código 365548-5G, Sigma-Aldrich INC., St. Louis, MO) e azul de toluidina (código 820, Vetec, Rio de Janeiro, RJ, Brasil) foram utilizadas para identificar mucopolissacarídeos ácidos, eosinófilos e mastócitos, respectivamente. Oscortes histológicos de 4µm de espessura foram obtidos a partir dos blocos de parafina originais dos espécimes teciduais.

Para a coloração por Alcian *blue*, realizou-se o seguinte protocolo:

- As lâminas com os cortes dos espécimes teciduais foram imersas três vezes em xilol por 5 minutos cada;
- Após a imersão em xilol, as lâminas foram submetidas à imersão em álcool absoluto duas vezes (5 minutos cada). Em seguida, foram imersasem álcool 95%, seguido de álcool 70%, por 5 minutos cada;
- Com uma pipeta, aplicou-se o Alcian *blue* pH 2,5 nos cortes, que permaneceram em câmara úmida e escura por 30 minutos;
- As lâminas foram lavadas em água corrente por 2 minutos;
- Os cortes foram corados por hematoxilina de Mayer por 5 minutos e,em seguida, lavados em água corrente por 2 minutos;
- As lâminas foram imersas em álcool absoluto duas vezes, por 10minutos cada;
- Em sequência, foram imersas em xilol duas vezes, durante 5 minutos cada.

Para a coloração por Sirius *red*, realizou-se o seguinte protocolo:

- As lâminas com os cortes dos espécimes teciduais foram imersas três vezes em xilol por 5 minutos cada;
- Após a imersão em xilol, as lâminas foram submetidas à imersão em álcool absoluto duas vezes (5 minutos cada). Em seguida, foram imersasem álcool 95%, seguido de álcool 70%, por 5 minutos cada;
- As lâminas foram lavadas em água corrente por 2 minutos e contra-coradas por Hematoxilina de Harris por 1 minuto e 30 segundos. Em seguida, foram lavadas em água corrente por 20 segundos;
- As lâminas foram submetidas à alcool absoluto por 30 segundos;
- O corante Sirius *red* (solução alcalina pH 8-9) foi aplicado nos cortes e mantido por 1 hora e 30 minutos em abrigo protegido de luz;
- Após a coloração, as lâminas foram lavadas em água corrente por 2 minutos;
- Em sequência, foram imersas em álcool absoluto duas vezes; seguida da imersão em xilol duas vezes também, durante 5 minutos em cada imersão de álcool e xilol.

Para a coloração de azul de toluidina, utilizou-se o seguinte protocolo:

- As lâminas com os cortes dos espécimes teciduais foram imersas duas vezes em xilol por 30 minutos cada;
- Em sequência, foram em imersas em álcool absoluto, seguido de álcool 70% e 50% (durante 1 minuto em cada imersão);
- As lâminas ficaram imersas em água 3 vezes, durante 1 minuto emcada banho;
- O corante de azul de toluidina foi aplicado com a pipeta por 1 minuto;
- Em sequência, as lâminas foram imersas em álcool absoluto duasvezes, durante 1 minuto cada;
- Imersão em xilol por 1 minuto e, em seguida, imersão em xilol por 5 minutos.

Todas as lâminas foram montadas com Permount® e lamínulas.

3.7 Avaliação das colorações histoquímicas

As lâminas coradas por Alcian *blue* foram classificadas como positivas quandoapresentaram a cor azul turquesa nos depósitos de material estético.

Para avaliação de Sirius red e azul de toluidina, toda a lâmina foi rastreada emaumento de 100x para identificar as áreas “hot spot” (área contendo o maior número de células positivas). O número de eosinófilos e mastócitos foi contado em 10 campos consecutivos de maior aumento (x400) nas áreas “*hot spots*”, por um examinador treinado (ACT), usando um microscópio de luz com uma grade. Na coloração de Sirius red, os eosinófilos apresentaram coloração citoplasmática vermelha granular com núcleos lobulados azuis. Na coloração de azul de toluidina, os mastócitos apresentaram-se como células fusiformes a ovais, com citoplasma corado em púrpura (metacromasia) e núcleos de coloração azul-celeste. Classificaram-se como mastócitos degranulados quando exibiram numerosos grânulos metacromáticos extracelulares, enquanto os mastócitos não degranulados não mostraram grânulos no espaço extracelular proximal (Ribatti, 2018). As mesmas características e classificações foram aplicadas para os eosinófilos. Os resultados foram expressos como o número total de células positivas para as colorações em cada lâmina.

3.8 Imuno-histoquímica

Cortes de 3µm em lâminas com carga foram submetidos a reações imuno-histoquímicas para CD20 (Dako Cytomation, código M0755, clone L26, 1:200, ácido cítrico pH 6,0), CD3 (Dako Cytomation, código M7254, clone F7.2.38, 1:200, TRIS-EDTA pH 9,0) e CD68 (DBS, código Mob167, clone KP1, 1:1000, ácido cítrico pH 6,0). Foi utilizado o kit EnVision FLEX, seguindo-se as especificações do fabricante, como descrito a seguir:

- Desparafinização com xilol (*overnight*), seguido de imersão em xilol por 15 minutos. Em sequência, foram imersas em álcool duas vezes, durante 5minutos. Seguido de álcool 95% e 80%, por 5 minutos cada; e hidratação com 5 banhos em água destilada;
- Para a recuperação antigênica, as lâminas foram colocadas dentro da solução de recuperação (com o tampão de acordo com cada anticorpo, especificado anteriormente), em uma panela de pressão, por 30 minutos. As lâminas foram retiradas da panela e aguardou- se o resfriamento por 20 minutos em temperatura ambiente;
- Procedeu-se a lavagem das lâminas com 5 banhos em água destilada;
- Em seguida, realizou-se o bloqueio da peroxidase endógena mediante a incubação dos cortes teciduais por 15 minutos;
- Em seguida, as lâminas foram lavadas com 3 banhos do tampão de lavagem, durante 5 minutos em cada banho;
- Os anticorpos primários foram diluídos (nas especificações descritas anteriormente) com o diluente Dako e, em seguida, os cortes teciduais com os anticorpos foram incubados em câmara úmida por 1 hora em temperatura ambiente;
- As lâminas foram desincubadas e lavadas com 3 banhos de tampão de lavagem, por 5 minutos cada;
- Em seguida, aplicou-se a solução de Linker (todos os anticorpos foram do tipo *mouse*) nos cortes teciduais, que foram incubados por 30 minutos;
- As lâminas foram desincubadas e lavadas em tampão de lavagem, com 3 banhos de 5 minutos cada;
- Foi realizada a incubação com polímero por 30 minutos;

- As lâminas foram desincubadas e lavadas com 3 banhos de tampão de lavagem, por 5 minutos cada;
- Para a coloração com o cromógeno diaminobenzidina (DAB), aplicou-se1 gota de cromógeno para 1mL de substrato. O DAB foi aplicado nas lâminas e aguardou-se 2 minutos e 30 segundos. Em sequência, as lâminas foram 5 vezes lavadas em água destilada;
- Realizou-se a contra-coloração com hematoxilina por 2 minutos;
- As lâminas foram lavadas de 8 a 10 vezes em água corrente;
- Realizou-se a desidratação e diafanização, nesta sequência: as lâminasforam imersas em álcool 70% durante 5 minutos; seguido de álcool 90%(5 minutos); imersão em álcool absoluto 3 vezes, por 2 minutos, 5 minutos e 10 minutos cada; imersão em xilol por 5 minutos e, em sequência, imersão em xilol por 10 minutos;
- Montagem das lâminas com Permount® e lamínulas.

3.9 Avaliação da imunoexpressão

A imunoexpressão foi avaliada por dois examinadores concomitantemente (ACT, PCC). Células acastanhadas foram consideradas positivas. A lâmina inteira foi avaliada sob uma ampliação de 200x e a quantidade de células positivas foi classificada como negativa, escassa, moderada ou numerosa.

3.10 Confecção do *e-book*

O e-book foi produzido com imagens e resultados da casuística deste estudo. As fotomicrografias foram obtidas a partir do microscópio óptico (Opticam® 0600R, modelo LOPT14003, 14,0 Mega Pixels) e OPTHD *Microscope Imaging Software*®. Algumas imagens foram escaneadas no Centro de Aquisição e Processamento de Imagens (CAPI) do ICB-UFMG.

4 ARTIGO

Os resultados foram escritos em língua inglesa na forma de artigo científico, submetido ao periódico internacional: **Modern Pathology** (Qualis A1; Fator de Impacto de 7,1 em 2023).

Adverse reactions to cosmetic fillers in the oral and maxillofacial region: clinico-pathological, histochemical, and immunohistochemical characterization

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ABSTRACT

Cosmetic injections are increasing, as their complications, which can be misdiagnosed as neoplastic lesions. This study aimed to detail clinical, pathological, histochemical, and immunohistochemical features of adverse reactions to different cosmetic fillers in the oral and maxillofacial region. Samples were retrieved from five pathology laboratories. Hematoxylin-eosin, Alcian Blue, Sirius Red, and Toluidine blue stains were performed, as well as immunohistochemistry for CD68, CD3, and CD20. H&E was evaluated under polarization. Descriptive statistics were performed. Twenty-three cases were included. Polymethyl-methacrylate was the most common material, followed by silicone, hyaluronic acid, calcium hydroxyapatite, polyacrylamide hydrogel, and poly-L-lactic acid. Patients were mostly women, with a mean age of 50.65 years. Most reactions affected lips and were asymptomatic, with a variable time of evolution (7 days to 10 years), presenting as nodules of 58.07mm on average. Polymethyl-methacrylate and silicone had negative rounded shape, whereas hyaluronic acid and polyacrylamide hydrogel presented as amorphous “pools”. Calcium hydroxyapatite had polyhedral structures and poly-L-lactic acid presented negative cleft-like structures. Giant cells were commonly found, except in silicone and hyaluronic acid. Foreign-body granuloma was frequent in polymethyl-methacrylate. Calcium hydroxyapatite and poly-L-lactic acid were refractile under polarized light. Hyaluronic acid and polyacrylamide hydrogel were metachromatic by Toluidine blue. Alcian blue was positive in all cases of hyaluronic acid. Mast cells were detected in all materials, except hyaluronic acid and polyacrylamide hydrogel. Eosinophils were rarer than mast cells. Numerous CD68-positive cells were seen in all cases. All cases had CD3-positive cells, with variable amounts. CD20 was scant or negative in most cases. In conclusion, an evident macrophage reaction is observed in all esthetic fillers, frequently associated with giant cell formation. Despite similarities, there are specific features of each material and the host response that assist the correct histopathological diagnosis. Immunohistochemistry for CD68 and Toluidine blue stain are useful in doubtful cases.

Keywords: Dermal fillers; Injection site reaction; Foreign-Body Reaction; Microscopy; Immunohistochemistry.

INTRODUCTION

The use of cosmetic fillers has been reported since 1893[1], but an increase of injectable procedures was registered worldwide from 2017 to 2021 according to the latest global survey of International Society of Aesthetic Plastic Surgery[2]. Injections of calcium hydroxyapatite accounted for 290,095 procedures and hyaluronic acid for more than five million in 2021, representing an increase of 73.3% and 60.1%, respectively[2]. There is a great diversity of cosmetic fillers and many of them are used in the oral and maxillofacial region. Calcium hydroxyapatite, hyaluronic acid, and poly-L-lactic acid are classified as non-permanent fillers, as the organism gradually resorbs them[3,4]. On the other hand, the permanent fillers such as polyacrylamide hydrogel, silicone, and polymethyl-methacrylate cannot be resorbed[3]. Polymethyl-methacrylate is the only permanent filler approved by Food and Drug Administration (FDA), when made of polymethyl-methacrylate beads suspended in a solution with bovine collagen[4].

Permanent and non-permanent cosmetic fillers may cause adverse reactions because of inadequate injection technique or by the material itself[5]. Acute or early-onset reactions occur immediately after injection or within days, whereas chronic or late-onset reactions occurs weeks or even years after the procedure. Immediate reactions include erythema, edema, pain, bumps, lumps, bruising. Early-onset reactions include infections by *Staphylococcus* or *Streptococcus*, non-inflammatory nodules, hypersensitivity reactions (usually type I), skin discoloration, vascular occlusion, contour irregularities and soft-tissue necrosis. Late-onset reactions include malar edema, infections caused by *Mycobacterium* species or biofilm, hypersensitivity reaction (usually type IV), inflammatory nodules, filler migration, persistent discoloration, scarring, and foreign body granuloma[6].

When affecting the oral and maxillofacial region, those reactions may cause important esthetic concerns as well as misdiagnosis with other diseases, including mucocele, benign and malignant salivary glands neoplasms, liposarcoma, sarcoidosis, and mucosal cyst[7]. In such cases, a biopsy can be necessary to define the diagnosis.

The microscopic characteristics of the cosmetic fillers and their respective adverse reactions vary according to the type of material[3]. Foreign body granuloma was the most common histologic pattern of inflammation seen in adverse reactions to cosmetic fillers in the face and neck region, representing 87.1% of cases[8,9]. Non-specific chronic inflammation[10] and mast cell-mediated reactions have been described as well[11].

Histological examination is the gold standard to recognize the responsible filler of the adverse reaction because the fillers have specific microscopic features[3,6,7,9,10,11]. However, few studies reported a detailed histopathological description of cosmetic fillers and their inflammatory response[3,12,13]. Therefore, this study aimed to describe in detail the clinical, pathological, histochemical, and immunohistochemical features of adverse reactions to

different cosmetic fillers in the oral and maxillofacial region. This should help pathologists recognize the different cosmetic fillers, whose use has been increasing in the last years.

MATERIALS AND METHODS

This study was performed in accordance with the Declaration of Helsinki and was approved by the Committee of Ethics in Research of Universidade Federal de Minas Gerais (certificate number 10723019.0.1001.5149). Ethics Committees of all participants' institutions were notified about the approval. The reporting of this multicenter, cross-sectional study conforms to the STROBE statement.

Case selection and data collection

Biopsy files were reviewed since the initial period of registration of five Oral Pathology services: “Universidade Estadual da Paraíba” (2011-2023), “Universidade Federal de Goiás” (2003-2023), “Universidade Federal de Minas Gerais” (1998-2023), “Universidade Federal de Pelotas” (2006-2024), and “Universidad Nacional Autónoma de México” (2019-2024). Cases with histopathological diagnosis of inflammatory or granulomatous reaction by exogenous material for aesthetic purposes in the oral and maxillofacial region were retrieved. Cases without paraffin blocks or slides available and patients who had other granulomatous diseases were excluded.

The following data were collected from the biopsy charts: demographic data (sex and age), lesion and material characteristics (symptoms, clinical signs, clinical lesion type, color, size, affected site, type of biopsy, complementary exams, lesion evolution over time, diagnostic hypothesis, brand and type of material, localization of injection, time between injection and biopsy). Researchers from each center performed searches and data collection independently, using a previously standardized electronic table model. The filled tables were sent to “Universidade Federal de Minas Gerais”, where data were organized into a single dataset to perform statistical analysis.

Histopathological evaluation

Hematoxylin-eosin (H&E) slides of all cases were evaluated by two examiners concomitantly (A.C.T. and P.C.C.) at Oral and Maxillofacial Pathology Laboratory of “Universidade Federal de Minas Gerais”, using a binocular optical microscope (Carl Zeiss Microscopy, Jena, Germany). The following histopathological features were evaluated: presence or absence of foreign body granuloma, giant cells and asteroid bodies; shape, size, color and arrangement of the cosmetic filler; predominant inflammatory cell type. All cases were polarized in an optical microscope (Opticam® 0600R, model LOPT14003, 14.0 Mega Pixels), coupled to a light polarizer, and the materials were classified as retractile or non-

retractile. The cosmetic fillers' particle sizes were measured by using the OPTHD Microscope Imaging Software® (Opticam Tecnologia, São Paulo, SP, Brazil), under 40x magnification. In cases of very small particles, 100x magnification was used. The greater diameter of the material particles was measured in micrometers (μm).

Histochemistry

Alcian blue (Histokit code EP-11-20018, EasyPath, Indaiatuba, SP, Brazil), Sirius Red (code 365548-5G, Sigma-Aldrich INC., St. Louis, MO), and Toluidine Blue (code 820, Vetec, Rio de Janeiro, RJ, Brazil) staining were used for identifying acid mucopolysaccharides, eosinophils, and mast cells, respectively. Four micrometers-thick tissue sections were deparaffinized with xylene and hydrated with graded alcohol. After washing in tap water, the slides were stained with alcian blue pH 2.5 (30 minutes), toluidine blue (1 minute), or sirius red pH 8.0 (90 minutes). The slides were then washed with running water and counterstained with Harris hematoxylin, except for the toluidine blue staining. Finally, slides were dehydrated in alcohol, cleared in xylene, and mounted with Permount.

Alcian blue-stained slides were classified as positive when presented the turquoise blue color in the esthetical material deposits. For Sirius red and toluidine blue evaluation, the whole slide was screened at 100x to identify the hot spot areas (area containing the highest number of positive cells). The number of positive eosinophils and mast cells was counted in 10 consecutive high-power fields (400x) in the hot spot area, by one trained examiner (A.C.T.), using a light microscope with a grid. Eosinophils showed a granular red cytoplasmatic staining with blue lobulated nuclei. Mast cells appeared as purple-colored spindle to oval-shaped cells with blue nuclei. They were classified as degranulating mast cells when exhibited numerous extracellular metachromatic granules, whereas non-degranulating mast cells showed no granule in the proximal extracellular space[14]. The results were expressed as the number of positive cells per ten high-power fields (400x).

Immunohistochemistry

Three-μm sections were submitted to immunohistochemical reactions against CD68 (DBS, code Mob167, clone KP1, 1:1000, citric acid pH6.0), CD20 (Dako Cytomation, code M0755, clone L26, 1:200, citric acid pH6.0), and CD3 (Dako Cytomation, code M7254, clone F7.2.38, 1:200, TRIS-EDTA pH9.0). The slides were deparaffinized with xylene, re-hydrated with graded ethanol, and submitted to antigen retrieval using a pressure cook for 30 minutes. Endogenous peroxidase was blocked with a ready-to-use solution (EnVison FLEX, Dako, code K8002). After incubation with primary antibodies and linkers, detection was performed with the ready-to-use polymer. Diaminobenzidine (DAB) chromogen was

applied, and the sections were counterstained with hematoxylin. Positive and negative controls were used in each reaction.

Immunostaining was evaluated by two examiners concomitantly (A.C.T. and P.C.C.). Brown-stained cells were considered positive. The whole slide was evaluated under a 200x magnification and the total amount of positive cells near the esthetical material was graded as negative, sparse, or numerous.

Statistical analysis

Descriptive statistics were performed using the Statistical Package for the Social Sciences (SPSS) software, version 25.0 for Windows (SPSS Inc., Armonk, NY). Quantitative data were analyzed by frequencies, percentage, mean, and standard deviation.

RESULTS

Demographic and clinical features

A total of 23 cases were included in the study. Six different esthetic materials were found, and polymethyl-methacrylate was the most common (43.5%). Table 1 shows demographic and clinical data. The mean age was 50.65 years-old (SD: ±14.708) and ranged from 20 to 75 years-old. The time of evolution ranged from 7 days to 10 years, presenting a mean of 405.63 days (SD: ±857.943; missing data: 4). Ten cases informed the time between the injection of material and the onset of the adverse reaction, which ranged from 10 to 180 months, with a mean of 52.80 months (SD: 67.659). The lesions measured between 2 and 260 mm (mean: 58.07; SD: ± 73.027; missing data: 2).

Case-by-case information can be found in the Supplementary Table. In six cases, the material identified on histopathology differed from the one informed by the clinician. One case (#4) showed more than one material on histopathology, and two cases (#12 and #19) were from the same patient at different times and with different materials.

Histopathological features

Table 2 summarizes the histopathological features of materials, which are illustrated in Figure 1, Figure 2, Figure 3, Figure 4, and Figure 5. Color and shape varied among materials. Polymethyl-methacrylate and silicone were round and negative, but polymethyl-methacrylate had a larger size of particles. Hyaluronic acid and polyacrylamide hydrogel formed amorphous pools, with amphophilic and basophilic colors, respectively. Polyacrylamide hydrogel had a microfoamy appearance, with microfibrils on the periphery, while hyaluronic acid formed uniform deposits. Calcium hydroxyapatite was polyhedral and eosinophilic while poly-L-lactic acid had a cleft-like negative appearance.

Foreign-body granuloma was common in polymethyl-methacrylate samples. Giant cells were found in all materials, though with different frequencies. However, they formed asteroid bodies only in polymethyl-methacrylate and silicone samples. Calcium hydroxyapatite and poly-L-lactic acid were refractile under light polarized microscopy.

Regarding the inflammatory cells seen at H&E, only the polyacrylamide hydrogel had no inflammation in one case, which showed necrosis of the connective tissue. Macrophages were identified in all materials, commonly associated with lymphocytes. Eosinophils, mast cells, and plasma cells were seldom identified in H&E.

Histochemical and immunohistochemical analysis

Hyaluronic acid and polyacrylamide hydrogel showed an intense metachromasia in Toluidine blue staining. Calcium hydroxyapatite showed a light metachromatic staining as well. Alcian blue was positive in all cases of hyaluronic acid and in two cases of calcium hydroxyapatite.

Table 3 shows the results of histochemical and immunohistochemical analysis. Eosinophils were more numerous in polymethyl-methacrylate, hyaluronic acid, calcium hydroxyapatite, and poly-L-lactic acid. Most eosinophils were degranulating in polymethyl-methacrylate, calcium hydroxyapatite, and poly-L-lactic acid.

Mast cells were detected in all materials, except hyaluronic acid and polyacrylamide hydrogel, and they were mostly degranulating cells. Mast cells were more numerous in silicone, followed by calcium hydroxyapatite and polymethyl-methacrylate.

Immunohistochemistry for CD68 evidenced numerous positive cells in all cases. Silicone presented a honeycomb pattern in CD68 staining, except in one case. All cases were CD3-positive, despite the variable amounts. CD20 was scant or negative in most cases.

DISCUSSION

Among the six types of fillers detected in the present study, polymethyl-methacrylate was the most frequent one. The reactions usually appeared as asymptomatic nodules with normal mucosa color. The female predominance has been previously reported, as well as the lip region[6,7,8,10]. Of importance, the material can migrate from the site of injection, and the reaction appears in a surrounding anatomic region[6,13]. Most patients in the current study were above 45 years-old, but it is noticeable that 43.5% were between 20 and 44 years-old. A minimum age of 14-21 years-old has been also reported in other studies[6,7].

The adverse reactions to cosmetic fillers have variable patterns and severity degrees, and clinical manifestations can develop immediately or several months after the injection[13]. In the present study, most patients had late onset reactions, after a mean of 52.80 months. This time lapse usually lead the patients not to relate the esthetic procedure with the complication

onset[15], hindering clinical diagnosis. An interesting finding was that the material informed by the clinician differed from the one observed in H&E slides. Most were reported to be temporary fillers, but the microscopic evaluation revealed permanent materials. Pathologists should keep attention to correctly diagnose the material irrespective of the clinical reports. Moreover, histopathology emerges as a potential medicolegal advice in such cases[15].

Polymethyl-methacrylate and silicone present as negative vacuoles, but some histopathological features help differentiate them. Polymethyl-methacrylate vacuoles are perfectly round, with regular size and shape, and show a larger distance between the vacuoles. In silicone, vacuoles are smaller and grouped together, and a “Swiss cheese” pattern can be found. The adverse reaction to polymethyl-methacrylate has numerous giant cells and often exhibits asteroid bodies and foreign-body granuloma formation. On the other hand, liquid silicone usually shows a mononuclear inflammation with a remarkable presence of multivacuolated bubbly macrophages[3,7,12]. These macrophages show hyperchromatic nuclei, similar to Virchow cells[16] or signet ring-like cells[13], and are arranged in clusters like “grape bunches”. Polymethyl-methacrylate and silicone may mimic spindle cell lipoma and liposarcoma. Immunohistochemical positivity for CD68, and negativity for S-100 and CD34 are supportive of an adverse reaction against polymethyl-methacrylate or silicone rather than a lipoblastic neoplasm[17,18].

Besides these classical histological presentations, some uncommon patterns can be found. In the current series, one case of silicone presented large pseudocystic spaces without bubbly macrophages, as an expected consequence of engulfed silicone[19]. Another case presented foreign-body granuloma next to the classical presentation of bubbly macrophages, with giant cells and asteroid bodies. This foreign-body reaction is common in elastomer silicone used for breast implant, but not in liquid silicone used in the orofacial region[3].

Hyaluronic acid deposits as amorphous amphophilic or basophilic[3] “pools”, which was arranged in three patterns: inside irregular cyst-like spaces, surrounded by palisading macrophages forming duct-like structures, or diffusely scattered through the connective tissue. Depending on the deposition pattern, salivary gland neoplasms can be a reasonable differential diagnosis, especially mucoepidermoid carcinoma[20,21]. In such cases, negative CD68 staining along with positivity for membrane-bound mucins markers, p63, and p40 supports the diagnosis of mucoepidermoid carcinoma[22].

Polyacrylamide hydrogel deposits as “pools”, like hyaluronic acid. However, polyacrylamide hydrogel reveals dark basophilic “pools” of microfoamy appearance[23], with evidence of branches of microfibrils on the periphery of the “pools”, as a contracted material. On the contrary, hyaluronic acid has a homogenous appearance and a less intense staining on H&E. The inflammatory reaction also differs between polyacrylamide hydrogel and hyaluronic acid. Polyacrylamide hydrogel presents a marked granulomatous reaction[23] with

many giant cells. Moreover, there are no palisading histiocytes surrounding polyacrylamide hydrogel, as in hyaluronic acid. Alcian blue and Toluidine blue are very helpful for the identification of hyaluronic acid and polyacrylamide hydrogel deposits[3,16,23]. Interestingly, these histochemical stains evidenced small “pools” of hyaluronic acid intermingling a case of calcium hydroxyapatite, which were not evident on H&E.

Poly-L-lactic acid was refractile under polarization, as previously reported[3,7,13]. Hydroxyethyl-methacrylate, which was not present in our sample, share microscopic features with poly-L-lactic acid, but it is not refractile[3]. The tree cases of calcium hydroxyapatite were refractile in the current study, contrarily to previous reports[3,7,10,13].

In addition to the evident macrophage and giant cell reaction against the fillers, the presence of eosinophils, mast cells, and T lymphocytes was evidenced in most samples of the current study. B-lymphocytes were less common. Silicone and calcium hydroxyapatite were the materials with larger number of mast cells, followed by polymethyl-methacrylate. Eosinophils were rarer than mast cells, and were present in similar amounts in calcium hydroxyapatite, polymethyl-methacrylate, and hyaluronic acid. Eosinophils and mast cells play an important role in allergic reactions. Mast cells have a primary role in hypersensitivity reactions and inflammatory processes[24]. As most mast cells were degranulating, they were considered important effector cells in the adverse reactions herein. The T-lymphocytes predominance over B-lymphocytes points to a cell-mediated adaptive response, which was more evident in polymethyl-methacrylate and calcium hydroxyapatite. Future studies shall investigate the sub-population of these T-lymphocytes. Polyacrylamide hydrogel showed large necrotic areas in the connective tissue and this can explain the scarcity of inflammatory cells in these samples.

Pathogenesis and the natural course of the foreign-body reaction after injection of cosmetic fillers remains unknown[25,26]. Physicochemical properties of the materials, as particle size and chemical composition are the main factors influencing the inflammatory response pathway [25,26,27]. Polymethyl-methacrylate, for example, is a synthetic material composed by medium to large microspheres, as evidenced herein. Greater particles size of this material may facilitate activation of macrophages and the cellular immune system, which usually causes more intense inflammatory responses, such as foreign-body granuloma[27,28].

Calcium hydroxyapatite, poly-L-lactic, and hyaluronic acid are temporary fillers, though the current study evidenced they are not inert. Among all materials investigated herein, hyaluronic acid was the one with the mildest infiltration of mast cells, eosinophils, and lymphocytes, probably due to its composition similar to endogenous components of human tissues[29]. Anyway, patients presented with nodules after hyaluronic acid injection, which prompted the biopsy procedure, evidencing that any exogenous material can lead to an adverse reaction[30]. This reinforces that patients should be aware of risks of adverse reactions

to cosmetic fillers. Importantly, polyacrylamide hydrogel and silicone had been forbidden for cosmetic injections[4]. Calcium hydroxyapatite, polymethyl-methacrylate, and poly-L-lactic acid are FDA-approved[4], however, they are not indicated for injections in lip region as they may clump due to muscle action[31].

The limitations of this study are the limited sample size and the absence of certain types of materials. As strengths, the detailed histopathological description of the cases, with emphasis on differential diagnosis and special staining indications help pathologists to correctly identify these cases.

In conclusion, this study detailed the microscopic features of adverse reactions to six different cosmetic fillers. Despite some similarities, there are specific features of each material and of the host response that assist the correct histopathological diagnosis. Immunohistochemistry for CD68 and Toluidine blue staining are useful in doubtful cases. Polarized light microscopy can be necessary as well. There was an evident macrophage reaction against all fillers, frequently associated with giant cell formation. Hyaluronic acid had the mildest infiltration of mast cells, eosinophils, and lymphocytes, while polymethyl-methacrylate had the most intense infiltration of these cells.

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FIGURE LEGENDS

Figure 1. Polymethyl-methacrylate (PMMA). A. Negative, round, medium to large vacuoles of PMMA, showing foreign-body granuloma (H&E). **B.** Asteroid bodies inside giant cells (H&E). **C.** Eosinophils around PMMA (Sirius red). **D.** Mast cells (Toluidine blue). **E.** CD68 evidencing numerous macrophages and giant cells (HRP-polymer). **F.** CD20 and **G.** CD3 positive lymphocytes (HRP-polymer).

Figure 2. Hyaluronic acid. A. Amphophilic amorphous "pools" forming a cystic-like pattern (H&E). **B.** Palisading macrophages surrounding the "pools", forming a duct-like structure (H&E). **C.** Diffuse pattern of hyaluronic acid dispersed into the connective tissue, with a very pale staining (H&E). **D.** Hyaluronic acid positive for Alcian blue staining. **E.** Hyaluronic acid shows metachromasia with Toluidine blue stain. **F.** Eosinophil nearby hyaluronic acid (Sirius red). **G.** Numerous CD68-positive macrophages in a diffuse pattern (HRP-polymer).

Figure 3. Calcium hydroxyapatite. A. Eosinophilic polyhedral structures of calcium hydroxyapatite, interspersed with giant cells and mononuclear inflammatory cells (H&E). **B.** Same field under polarized light, with refractile particles. **C.** Alcianophilic polyhedral structures of calcium hydroxyapatite (Alcian blue). **D.** Light metachromasia of the material and mast cells (Toluidine blue). **E.** Eosinophils (Sirius red). **F.** CD68 evidencing macrophages and giant cells (HRP-polymer). **G.** A moderate number of CD3-positive cells (HRP-polymer).

Figure 4. Poly-L-lactic acid. A. Cleft-like appearance of poly-L-lactic acid surrounded by with giant cells (H&E). **B.** Same field under polarized light, with refractile particles. **C.** Eosinophils (Sirius red). **D.** Mast cells (Toluidine blue). **E.** Numerous macrophages and giant cells around the material are evidenced by CD68 staining (HRP-polymer). **F.** CD20 and **G.** CD3 positive lymphocytes (HRP-polymer).

Figure 5. Silicone (A-E) and polyacrylamide hydrogel (F-G). A. Negative, round, and small vacuoles of liquid silicone with numerous bubbly macrophages clustered in "grape bunches" (H&E). **B.** Liquid silicone with bubbly macrophages (right), next to foreign-body granuloma reaction (left) (H&E). **C.** Liquid silicone in pseudocystic pattern with large spaces (H&E). **D.** Mast cells (Toluidine blue). **E.** CD68 evidencing a honeycomb arrangement of macrophages (HRP-polymer). **F.** Dark basophilic pools of polyacrylamide hydrogel with contracted periphery and microfoamy appearance. Giant cells surrounding (H&E). **G.** Metachromasia of polyacrylamide hydrogel (Toluidine blue).

Figure 1

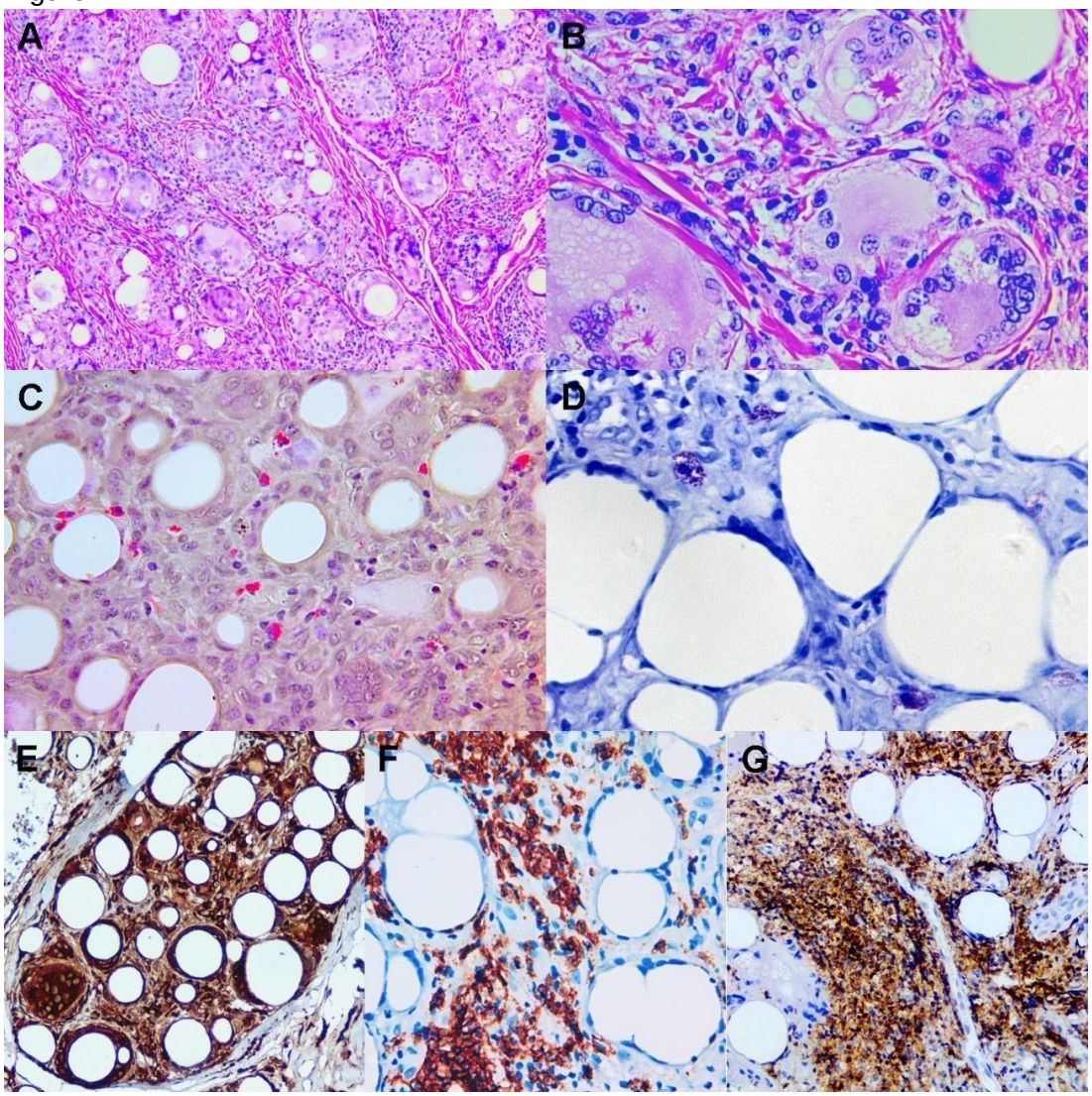


Figure 2

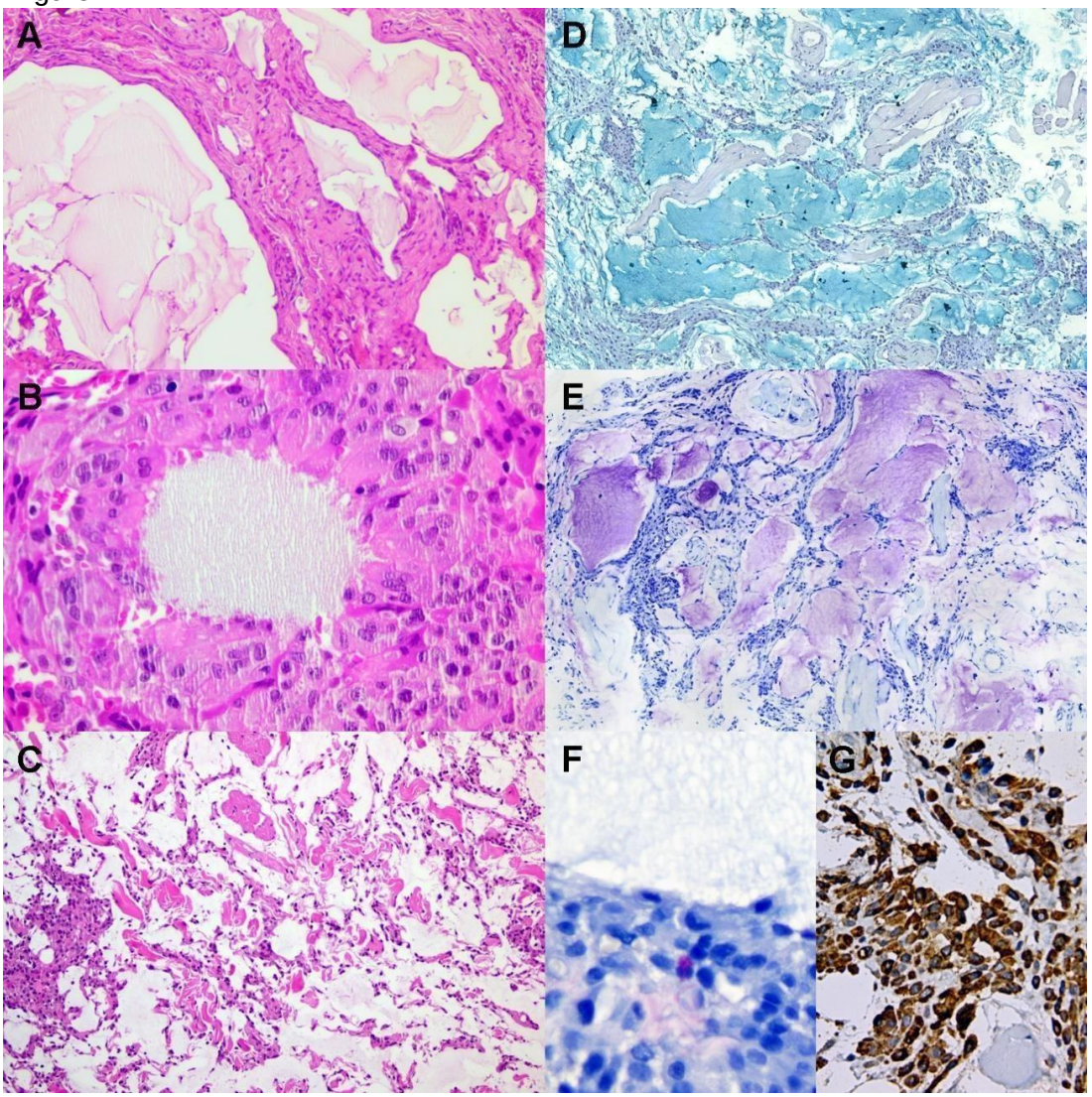


Figure 3

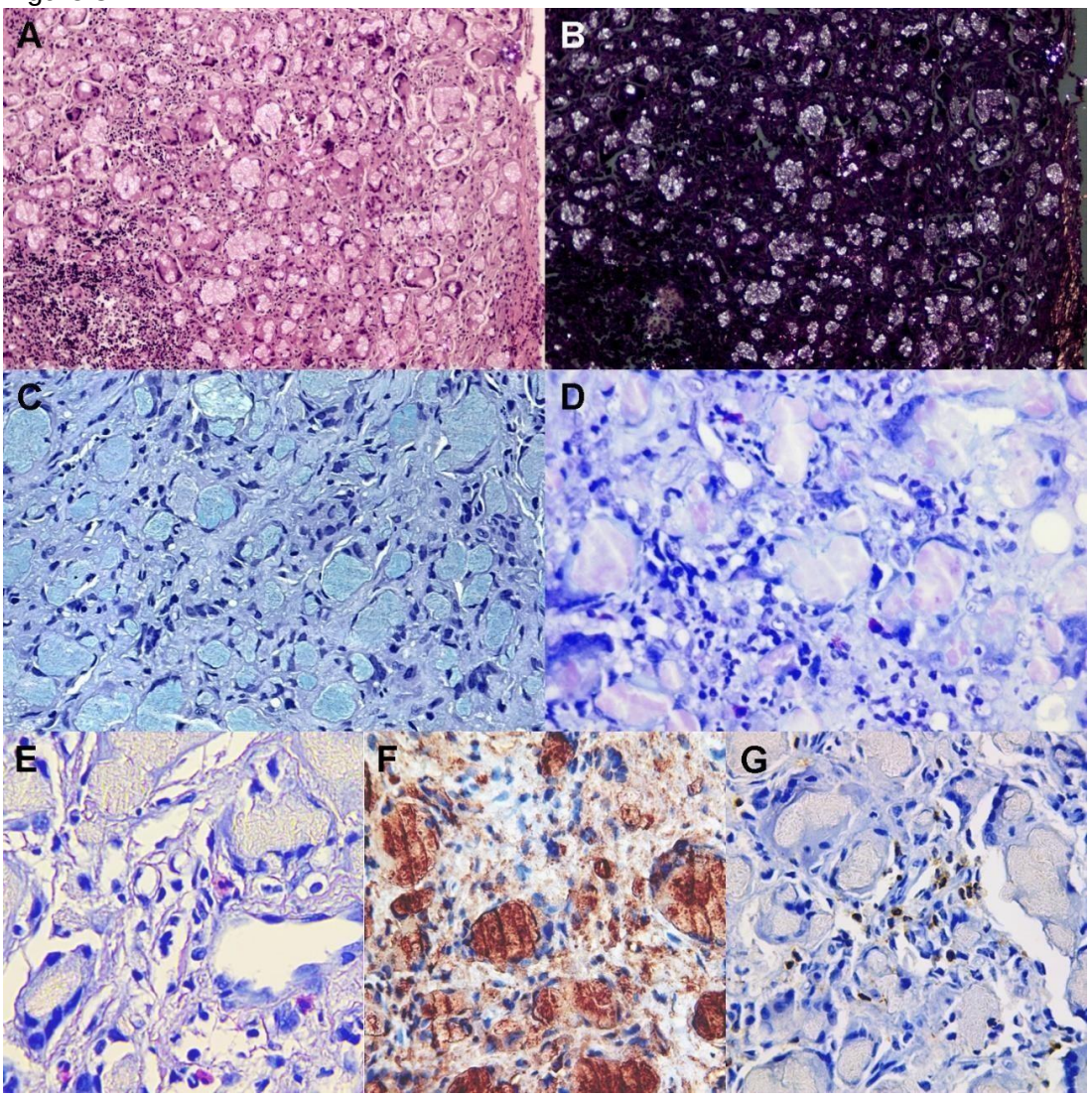


Figure 4

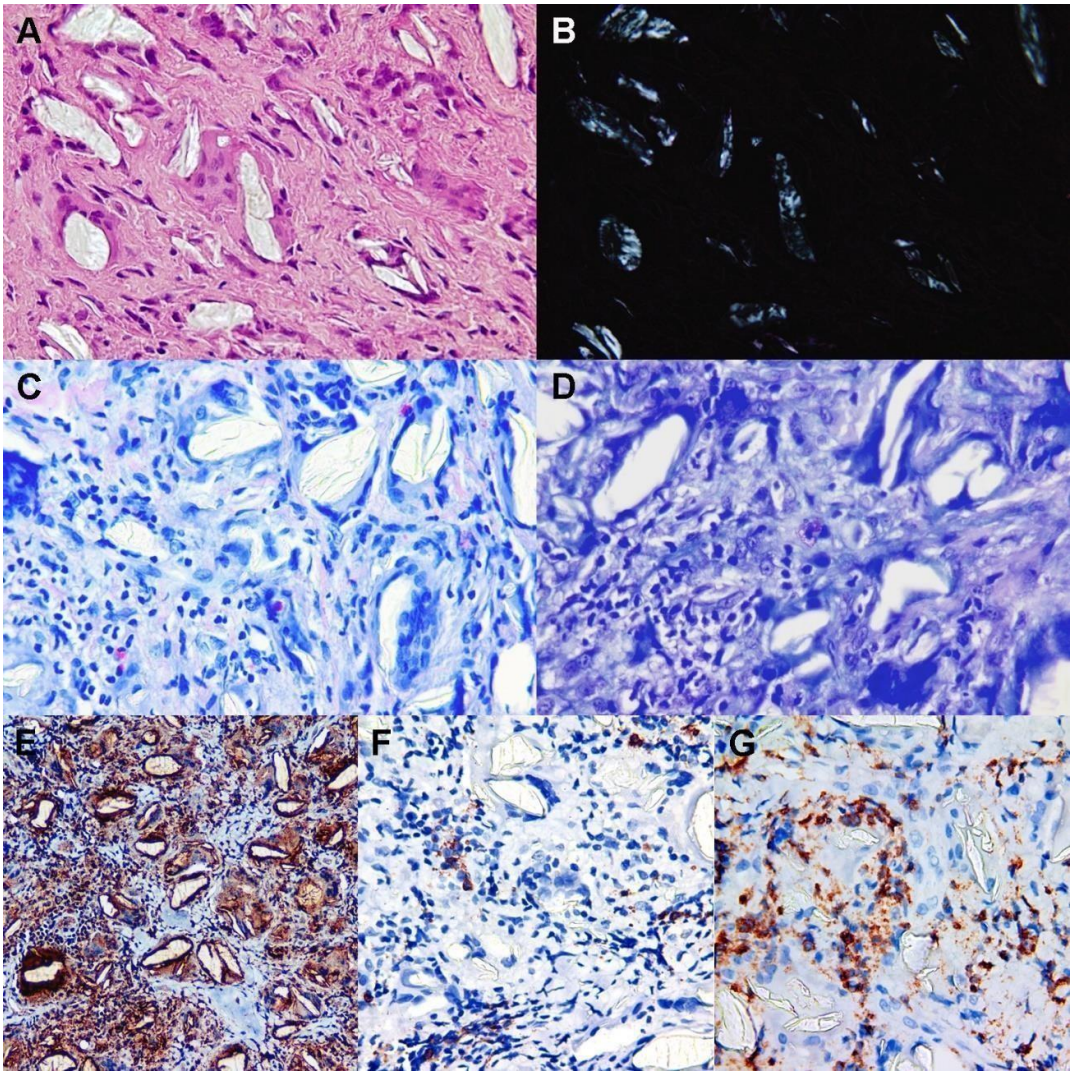


Figure 5

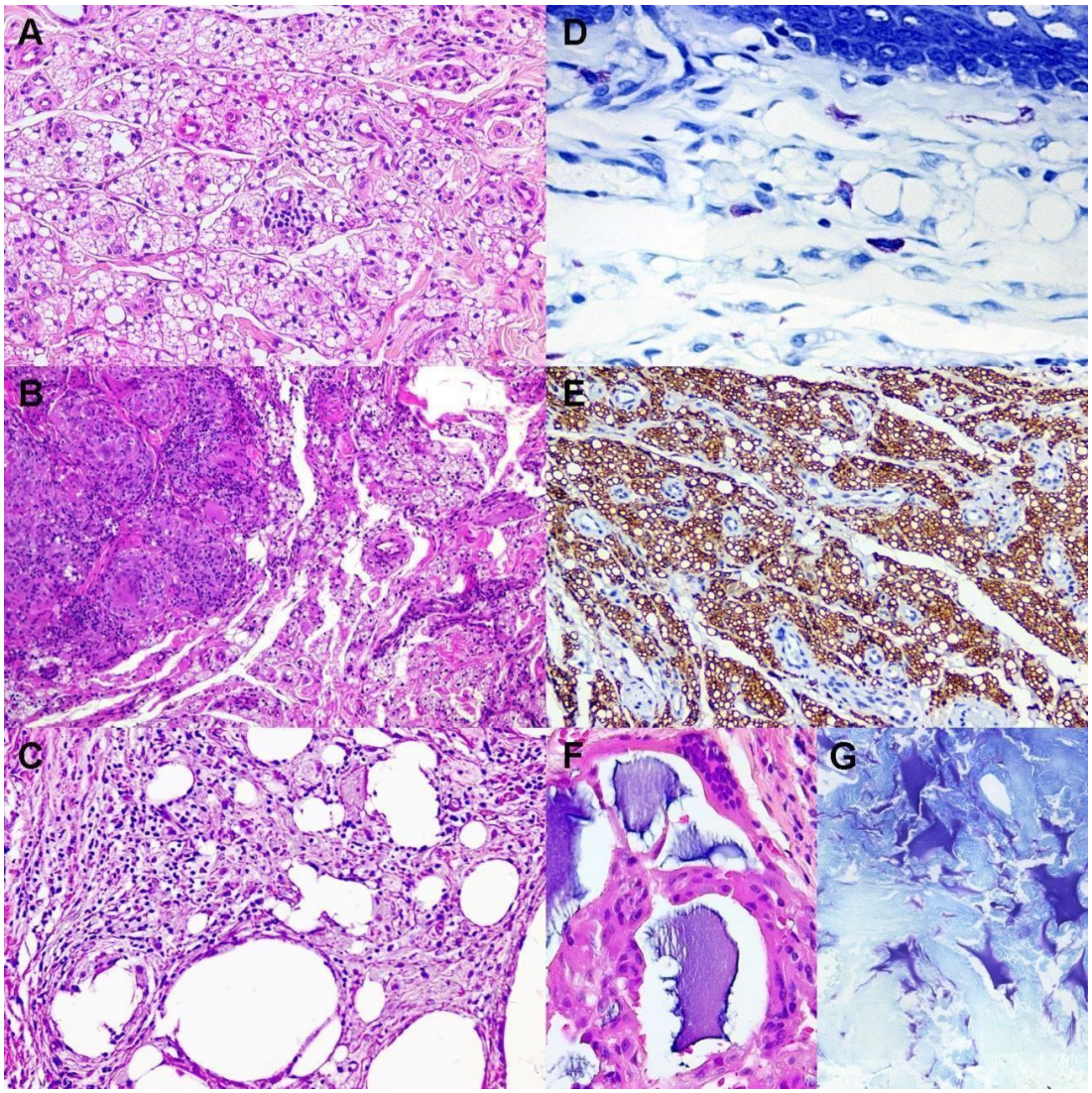


Table 1. Demographic and clinical data of 23 cases of adverse reactions to cosmetic fillers. Characteristic

	Number (%)
Sex	
Female	21 (91.3%)
Male	2 (8.7%)
Age (years)	
20-44	10 (43.5%)
45-58	7 (30.4%)
≥65	6 (26.1%)
Site of lesion	
Upper lip	8 (34.8%)
Lower lip	4 (17.4%)
Buccal mucosa	4 (17.4%)
Multifocal*	3 (13%)
Labial commissure	1 (4.3%)
Upper labial frenulum	1 (4.3%)
Cheek	1 (4.3%)
Retromolar trigone	1 (4.3%)
Symptoms**	
No	19 (90.5%)
Pain on palpation	1 (4.8%)
Weighty face sensation	1 (4.8%)
Clinical presentation	
Nodule	20 (87%)
Papule	1 (4.3%)
Plaque	1 (4.3%)
Blister	1 (4.3%)
Lesion color**	
Normal color of mucosa	15 (75%)
Yellowish	2 (10%)
Purplish	2 (10%)
White	1 (5%)
Clinical hypothesis	
Foreign-body reaction to cosmetic filler	8 (34.8%)
Benign neoplasm	4 (17.4%)
Fibrous hyperplasia	4 (17.4%)
Mucocele or mucus retention cyst	3 (13%)
Pleomorphic adenoma	1 (4.3%)
“Glandular or adipose leukoplakia”	1 (4.3%)
Dermoid cyst or large lymph nodes	1 (4.3%)
Generalized fibrosis	1 (4.3%)
Type of biopsy	
Excisional	21 (91.3%)
Incisional	2 (8.7%)
Injected material (histological diagnosis)	
Polymethyl-methacrylate	10 (43.5%)
Silicone	4 (17.4%)

Table 1 (Continued).

Hyaluronic acid	3 (13%)
Calcium hydroxyapatite	3 (13%)
Polyacrylamide hydrogel	2 (8.7%)
Poly-L-latic acid	1 (4.3%)

*Multifocal: Multiple lesions in upper lip and lower lip; or upper lip and buccal mucosa; or chin, malar region, buccal mucosa, and submandibular region.

**Missing data: symptoms (n= 2); lesion color (n= 3).

Table 2. Histopathological features of 23 cases of adverse reactions to cosmetic fillers.

Histological features	Polymethyl-methacrylate (n=10)	Silicone (n=4)	Hyaluronic acid (n=3)	Calcium hydroxyapatite (n=3)	Polyacryl-amide gel (n=2)	Poly-L-latic acid (n=1)
Material color on H&E						
Negative	10 (100%)	4 (100%)	0	0	0	1 (100%)
Basophilic	0	0	0	0	2 (100%)	0
Eosinophilic	0	0	0	3 (100%)	0	0
Amphophilic	0	0	3 (100%)	0	0	0
Shape						
Round	10 (100%)	4 (100%)	0	0	0	0
Amorphous “pools”	0	0	3 (100%)	0	2 (100%)	0
Polyhedral	0	0	0	3 (100%)	0	0
Needle/ cleft-like	0	0	0	0	0	1 (100%)
Size (µm)						
Mean	97.19	17.98*	1314.27	89.71	768.18	103.55
Range	32.60-169.11	5.35-25.44	68.37-2394.00	50.47-122.71	400.99-1135.36	-
Apparent size						
Small	1 (10%)	3 (75%)	0	1 (33.3%)	0	0
Medium	6 (60%)	0	1 (33.3%)	2 (66.7%)	1 (50%)	1 (100%)
Large	3 (30%)	1 (25%)	2 (66.7%)	0	1 (50%)	0
Arrangement						
Nodular	1 (10%)	0	0	2 (66.7%)	1 (50%)	0
Diffuse	7 (70%)	3 (75%)	3 (100%)	1 (33.3%)	1 (50%)	1 (100%)
Nodular and diffuse	2 (20%)	1 (25%)	0	0	0	0
Foreign-body granuloma						
Present	6 (60%)	1 (25%)	0	0	0	0
Absent	4 (40%)	3 (75%)	3 (100%)	3 (100%)	2 (100%)	1 (100%)
Giant cells						
Present	10 (100%)	1 (25%)	1 (33.3%)	2 (66.7%)	1 (50%)	1 (100%)
Absent	0	3 (75%)	2 (66.7%)	1 (33.3%)	1 (50%)	0
Asteroid bodies						
Present	6 (60%)	1 (25%)	0	0	0	0
Absent	4 (40%)	3 (75%)	3 (100%)	3 (100%)	2 (100%)	1 (100%)
Inflammatory cells						
Absent	0	0	0	0	1 (50%)	0
Macrophages and lymphocytes	8 (80%)	4 (100%)	2 (66.7%)	2 (66.7%)	0	0
Mostly macrophages	0	0	0	0	1 (50%)	0
Mostly macrophages and lymphocytes, with scant eosinophils	2 (20%)	0	1 (33.3%)	0	0	1 (100%)
Many macrophages and lymphocytes, some mast cells, scant eosinophils and plasma cells	0	0	0	1 (33.3%)	0	0
Refractile under polarization						
Yes	0	0	0	3 (100%)	0	1 (100%)
No	10 (100%)	4 (100%)	3 (100%)	0	2 (100%)	0

*Silicone mean was calculated with three representative cases. The excluded case had an unusual histological pattern with large cystic-like spaces and measured 1732.05 µm.

Table 3. Histochemical and immunohistochemical features of 23 cases of adverse reactions to cosmetic fillers

Histochemical and immunohistochemistry Features	Polymethyl-methacrylate (n=10)	Silicone (n=4)	Hyaluronic acid (n=3)	Calcium hydroxyapatite (n=3)	Polyacrylamide hydrogel (n=2)	Poly-L-lactic acid (n=1)
Sirius Red: mean (range)**						
Total eosinophils	5.70 (0-27)	0.75 (0-2)	5.50 (0-11) *	6.67 (2-11)	0	9
Degranulating eosinophils	5.40 (0-27)	0.25 (0-1)	0.50 (0-1)	5.00 (2-8)	0	8
Non-degranulating eosinophils	0.30 (0-2)	0.50 (0-2)	5.00 (0-10)	1.67 (0-3)	0	1
Toluidine Blue: mean (range)**						
Total mast cells	17.70 (6-38)	33 (8-63)	0 *	19.33 (3-34)	0	9
Degranulating mast cells	16.30 (5-38)	31.75 (7-61)	0	18.33 (2-32)	0	8
Non-degranulating mast cells	1.40 (0-4)	1.25 (0-2)	0	1.00 (0-2)	0	1
Immunohistochemistry: n(%)						
CD68						
Numerous	10 (100%)	4 (100%)	2 (100%) *	3 (100%)	1 (100%) *	1 (100%)
CD20						
Numerous	2 (20%)	0	0	0	0	0
Moderate	3 (30%)	1 (25%)	0	0	0	1 (100%)
Scant	5 (50%)	1 (25%)	1 (50%) *	3 (100%)	0	0
Negative	0	2 (50%)	1 (50%) *	0	1 (100%) *	0
CD3						
Numerous	7 (70%)	1 (25%)	0	1 (33.3%)	0	1 (100%)
Moderate	3 (30%)	1 (25%)	1 (50%) *	2 (66.7%)	0	0
Scant	0	2 (50%)	1 (50%) *	0	1 (100%) *	0

***Missing data:** Sirius red, blue toluidine staining and immunohistochemistry had no result in one case of hyaluronic acid, because of scarce tissue, as well immunohistochemistry for polyacrylamide hydrogel.

** number of positive cells per ten high-power fields (400x)

Supplementary Table. Case-by-case information of the 23 samples of adverse reactions to cosmetic fillers.

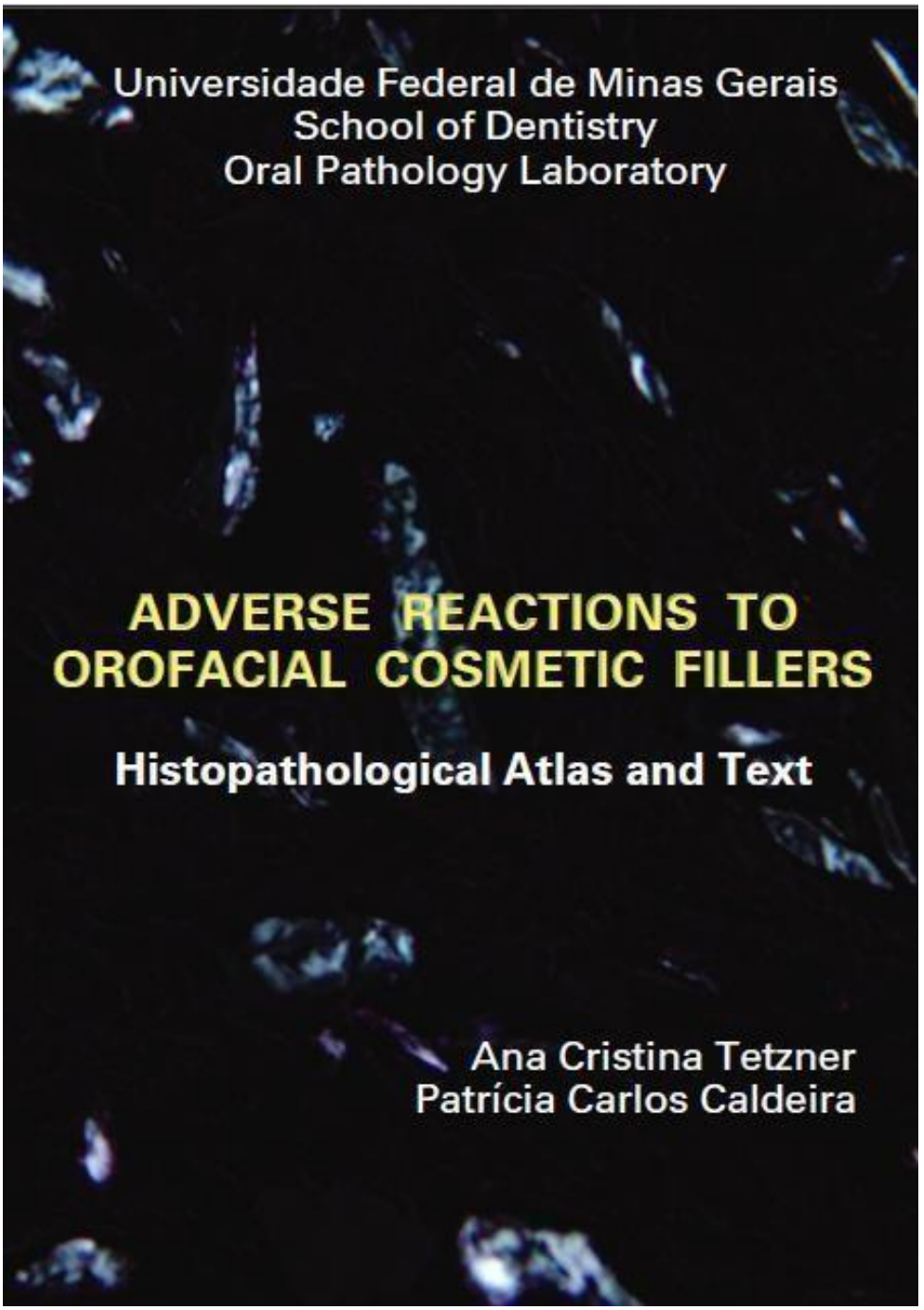
Case	Sex	Age (years)	Site of lesion	Clinical presentation	Reported material	Histological diagnosis
1	F	66	Upper lip	Nodule, normal color of mucosa	Poly-L-latic acid	Polymethyl-methacrylate
2	F	58	Upper lip	Nodule, 50mm	Hyaluronic acid	Silicone
3	F	37	Upper labial frenulum	Nodule, 100mm	Polymethyl-methacrylate	Polymethyl-methacrylate
4	F	31	Buccal mucosa	Nodule, 100mm, normal color mucosa of	Poly-L-latic acid	Calcium hydroxyapatite, mixed with a small quantity of poly-L-latic acid and hyaluronic acid
5	F	43	Buccal mucosa	Blister, 101mm, normal color of Mucosa	Polymethyl-methacrylate	Silicone
6	F	38	Buccal mucosa	Nodule, 80mm, purplish	Polymethyl-methacrylate	Calcium hydroxyapatite
7	M	20	Retromolar trigone	Nodule, 120mm, normal color mucosa of	Hyaluronic acid	Polymethyl-methacrylate
8	F	68	Upper lip	Nodule, 260 mm, normal color of mucosa	Polymethyl-methacrylate	Polymethyl-methacrylate
9	F	39	Vestibular fornix depth	Nodule, 150mm, yellowish	Polymethyl-methacrylate	Polymethyl-methacrylate
10	F	56	Lower lip	Nodule, 5mm, normal color of mucosa	Not reported	Polyacrylamide hydrogel
11	F	75	Upper lip and lower lip	Multiple nodules, 4mm, color not informed	Not reported	Polyacrylamide hydrogel
12*	F	52	Lower lip	Nodule, 5mm, white color	Not reported	Poly-L-lactic acid
13	F	38	Upper lip	Nodule, 2mm, normal color of mucosa	Not reported	Hyaluronic acid
14	F	68	Lower mucosa labial	Nodule, 10mm, normal color of mucosa	Not reported	Polymethyl-methacrylate

15	F	74	Lower and buccal li	Multiples nodules, 50mm, normal color of mucosa	Not reported	Silicone
16	F	37	p mucosa Lower lip	Plaque, 10mm, yellowish	Not reported	Polymethyl-methacrylate
17	M	41	Upper labial mucosa	Nodule, 5mm, purplish	Not reported	Hyaluronic acid
18	F	45	Upper lip	Nodule, 20mm, normal color of mucosa	Not reported	Hyaluronic acid
19*	F	58	Buccal mucosa	Papule, 5mm, normal color of mucosa	Not reported	Calcium hydroxyapatite
20	F	44	Chin, buccal mucosa, malar and submandibular region	Multiple nodules, size not informed, normal color of mucosa	Not reported	Silicone
21	F	56	Buccal mucosa	Submucous nodule, 10mm, normal color of mucosa	Not reported	Polymethyl-methacrylate
22	F	65	Labial commissure	Submucous nodule, 20mm, normal color of mucosa	Not reported	Polymethyl-methacrylate
23	F	56	Upper lip	Submucous nodule, 12.5 mm, normal color of mucosa	Not reported	Polymethyl-methacrylate

*Cases #12 and #19 were from the same patient at different times.

5 E-BOOK

Trata-se de um atlas com imagens histopatológicas e texto escrito em língua inglesa, com a finalidade de auxiliar patologistas no diagnóstico histopatológico destas lesões. O *e-book* será publicado pelo Sistemas de Bibliotecas da UFMG, com atribuição de ISBN. Planeja-se sua distribuição eletrônica gratuita.



ACKNOWLEDGEMENTS

This e-book was elaborated by Ana Cristina Tetzner during her Master's course in Oral Pathology, under the supervision of Professor Patrícia Carlos Caldeira, from 'Universidade Federal de Minas Gerais' (Brazil).

The content of this e-book was based on the analysis of 23 cases and a literature review. The cases were from five oral pathology centers ('Universidade Federal de Minas Gerais', 'Universidade Federal de Goiás', 'Universidade Federal de Pelotas', 'Universidade Estadual da Paraíba', and 'Universidad Tecnológica de México').

The authors are grateful for the people who made this work possible:

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INTRODUCTION

The use of cosmetic fillers has been reported since 1893[1], but a huge increase in injectable procedures was registered worldwide from 2017 to 2021.[2]

A great diversity of cosmetic fillers can be used in the oral and maxillofacial region. Calcium hydroxyapatite, hyaluronic acid, and poly-L-lactic acid are classified as **non-permanent** fillers, as the organism gradually resorbs them.[3,4] On the other hand, **permanent** fillers such as polyacrylamide hydrogel, silicone, and polymethyl-methacrylate (PMMA) cannot be resorbed.[3]

Permanent and non-permanent cosmetic fillers may cause adverse reactions, such as erythema, edema, pain, bumps, lumps, and bruising.[5] When affecting the oral and maxillofacial region, those reactions may cause aesthetic concerns as well as misdiagnosis with other diseases, including benign and malignant salivary glands neoplasms, liposarcoma, sarcoidosis, etc.[6] In such cases, a biopsy can be necessary to define the diagnosis.

This atlas was designed to help pathologists identifying the different aesthetic fillers and the tissue responses against them. Histological, histochemical, and immuno-histochemical pictures are provided. Microscopic differential diagnosis is discussed as well.

ADVERSE REACTIONS TO OROFACIAL COSMETIC FILLERS

ADVERSE REACTIONS

IMMEDIATE ONSET (24 to 48 hours after filler injection)

- Pain, bruising, erythema, and edema (Fig. 1) are the most common immediate reactions against cosmetic fillers injection.[3,5]

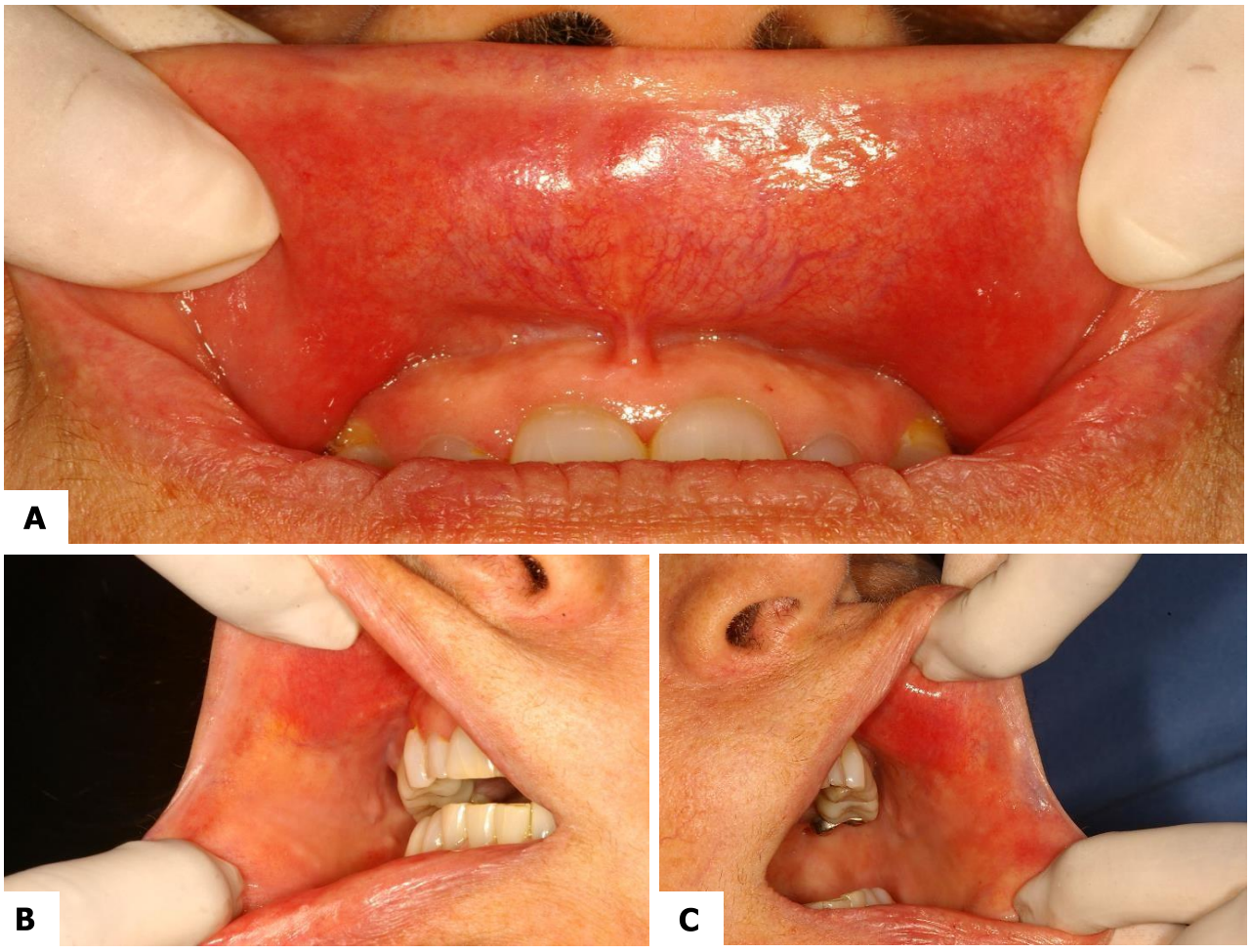

FIG 1. Erythema and discrete edema on lip mucosa (A) and buccal mucosa (B, C) after poly-L-lactic acid injection.

- Lumps and bumps may be the immediate adverse reaction of a wrong technique.[7] Most lumps are only detected under palpation.[8]

ADVERSE REACTIONS

EARLY ONSET (within days)

- Infections (by *Staphylococcus* or *Streptococcus*), non-inflammatory nodules, hypersensitivity reactions (usually type I), skin discoloration/Tyndall effect, vascular occlusion and contour irregularities, local necrosis (uncommon). [3,5,6]

LATE ONSET (after weeks or years)

- Malar edema, infections (by *Mycobacterium* species or biofilm, or biofilm related), hypersensitivity reaction (usually type IV), inflammatory nodules, foreign body granulomatous reactions (Fig. 2), migration of implanted material, persistent discoloration and scarring. [3,5,6]

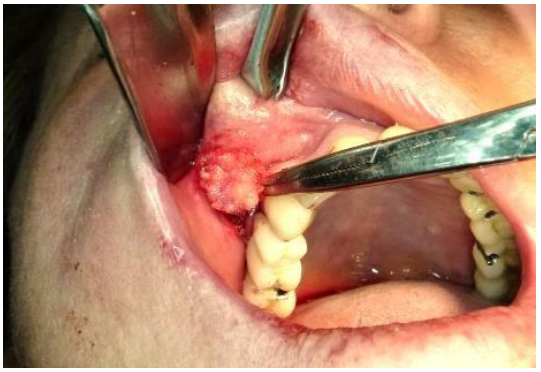

FIG 2. Surgical removal of a lump in the lip submucosal tissue, related to injection of polymethyl-methacrylate.

MICROSCOPIC FEATURES OF THE ADVERSE REACTIONS TO OROFACIAL COSMETIC FILLERS

POLYMETHYL-METHACRYLATE (PMMA)

Artecoll ®, Artefill ®

- Artecoll/Artefill is composed by microspheres of polymethyl-methacrylate suspended in a collagen solution with 0.3% hydrochloride lidocaine.[9] It is the only permanent filler approved by FDA.[4]
- Adverse reactions to PMMA show regular vacuoles with similar shapes and sizes, arranged in a (multi)nodular (Fig. 3) or diffuse pattern.[3]

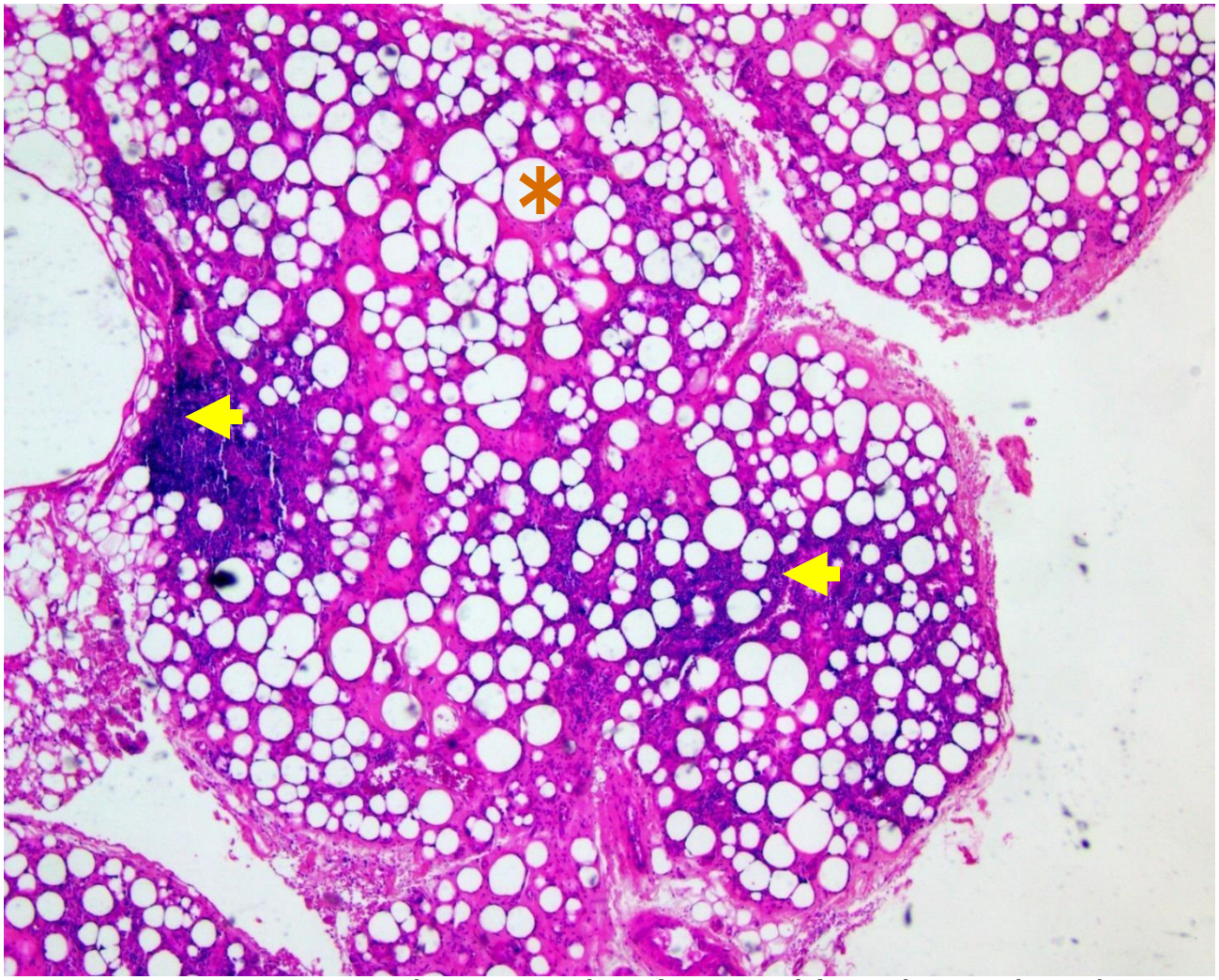

FIG 3. Negative regular vacuoles (asterisk) with similar shapes and size, arranged in a (multi)nodular pattern with lymphocytic inflammation focuses (arrows) (Hematoxylin-eosin staining).

POLYMETHYL-METHACRYLATE (PMMA)

Artecoll ®, Artefill ®

- The vacuoles mimic normal adipocytes but show a perfectly rounded shape in a collagenous background. Macrophages and giant cells usually surround the vacuoles (Fig. 5).

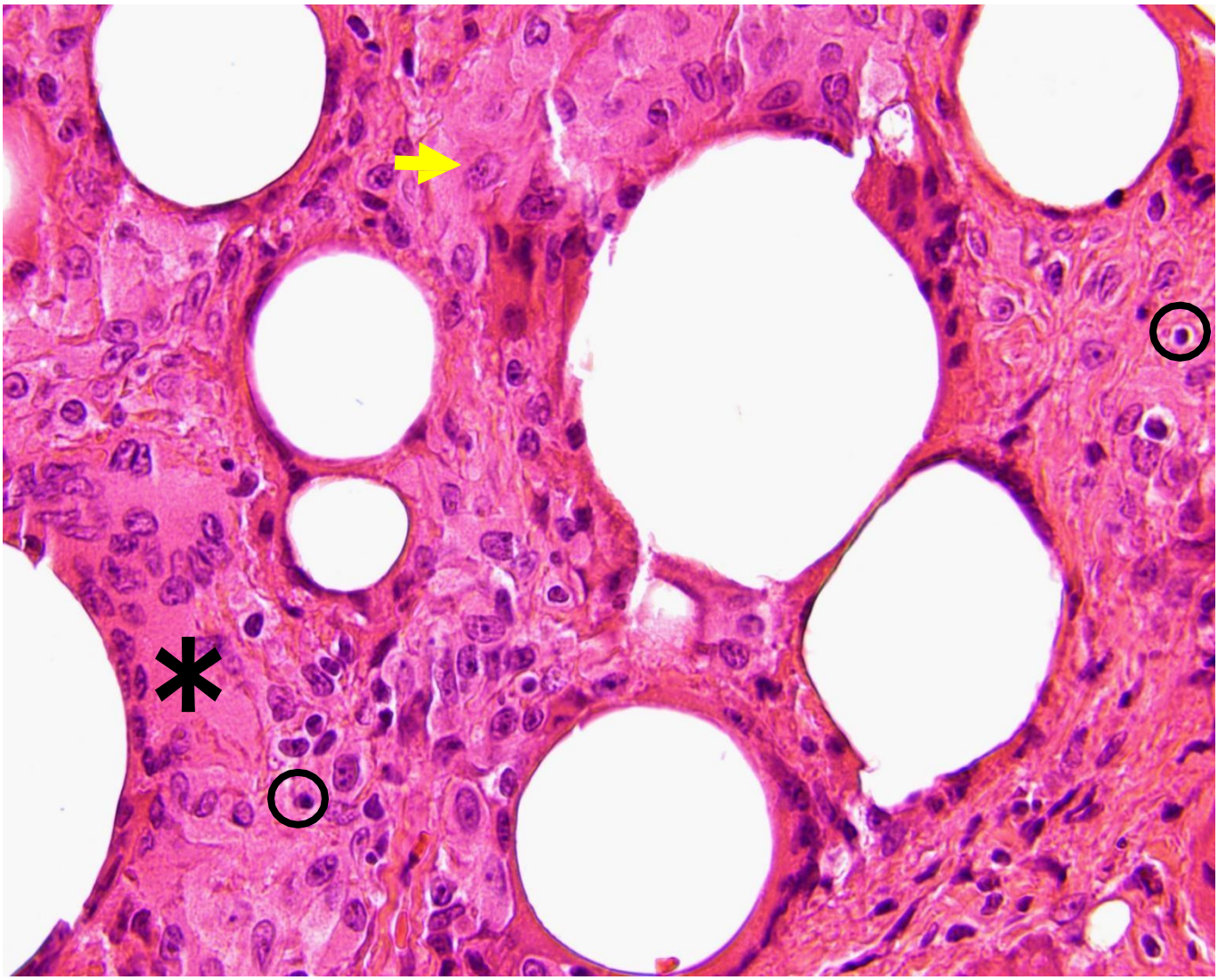

FIG 5. Perfectly rounded vacuoles inserted in a collagenous background, surrounded by many epithelioid macrophages (arrow) and giant cells (asterisk). Few lymphocytes (circles) dispersed (Hematoxylin-eosin staining).

POLYMETHYL-METHACRYLATE (PMMA)

Artecoll ®, Artefill ®

- Host reaction to PMMA shows foreign body granuloma (Fig. 6).

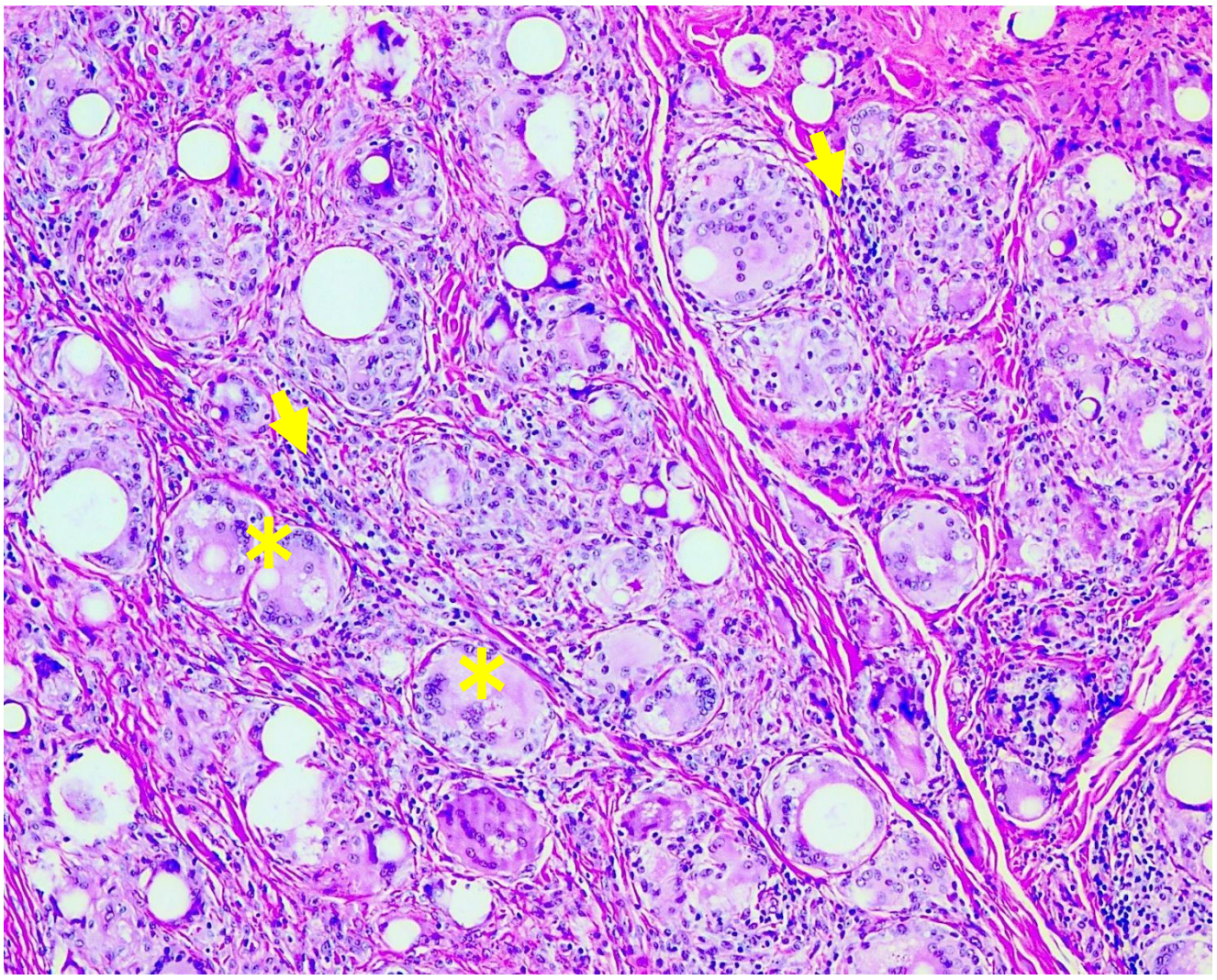

FIG 6. Foreign body reaction against PMMA shows many giant cells (asterisks) and lymphocytic focuses (arrows) surrounding PMMA vacuoles. Septa of dense fibrous connective tissue (Hematoxylin-eosin staining).

POLYMETHYL-METHACRYLATE (PMMA)

Artecoll ®, Artefill ®

- Several giant cells are seen in foreign body reaction to PMMA (Fig. 7A, 7B).

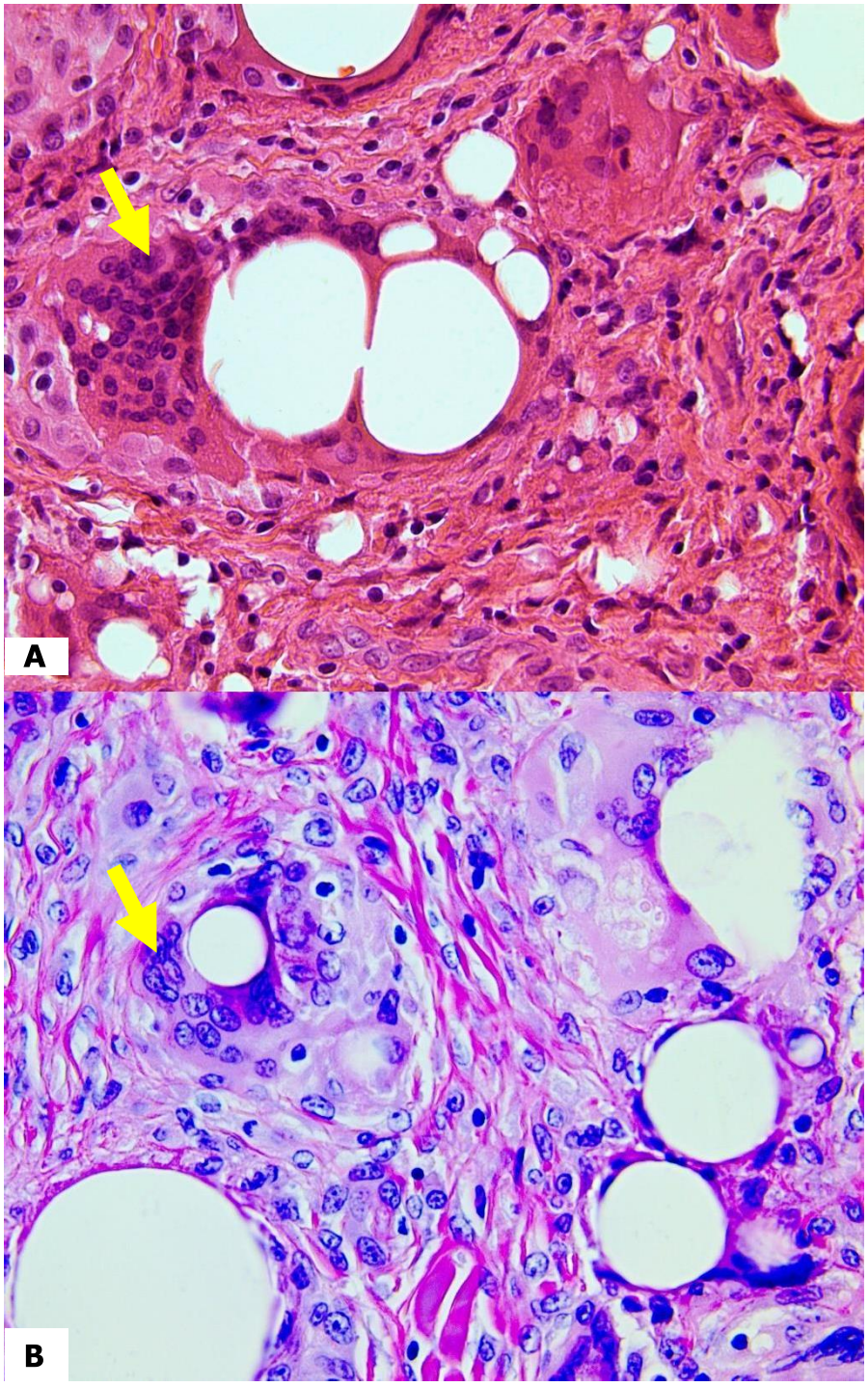

FIG 7 A-B. Giant cells (arrows) surrounding and phagocyting PMMA vacuoles. Notice the dispersed lymphocytes (Hematoxylin-eosin staining).

POLYMETHYL-METHACRYLATE (PMMA)

Artecoll ®, Artefill ®

- Asteroid bodies are often seen in the cytoplasm of multivacuolated giant cells (Fig. 8).

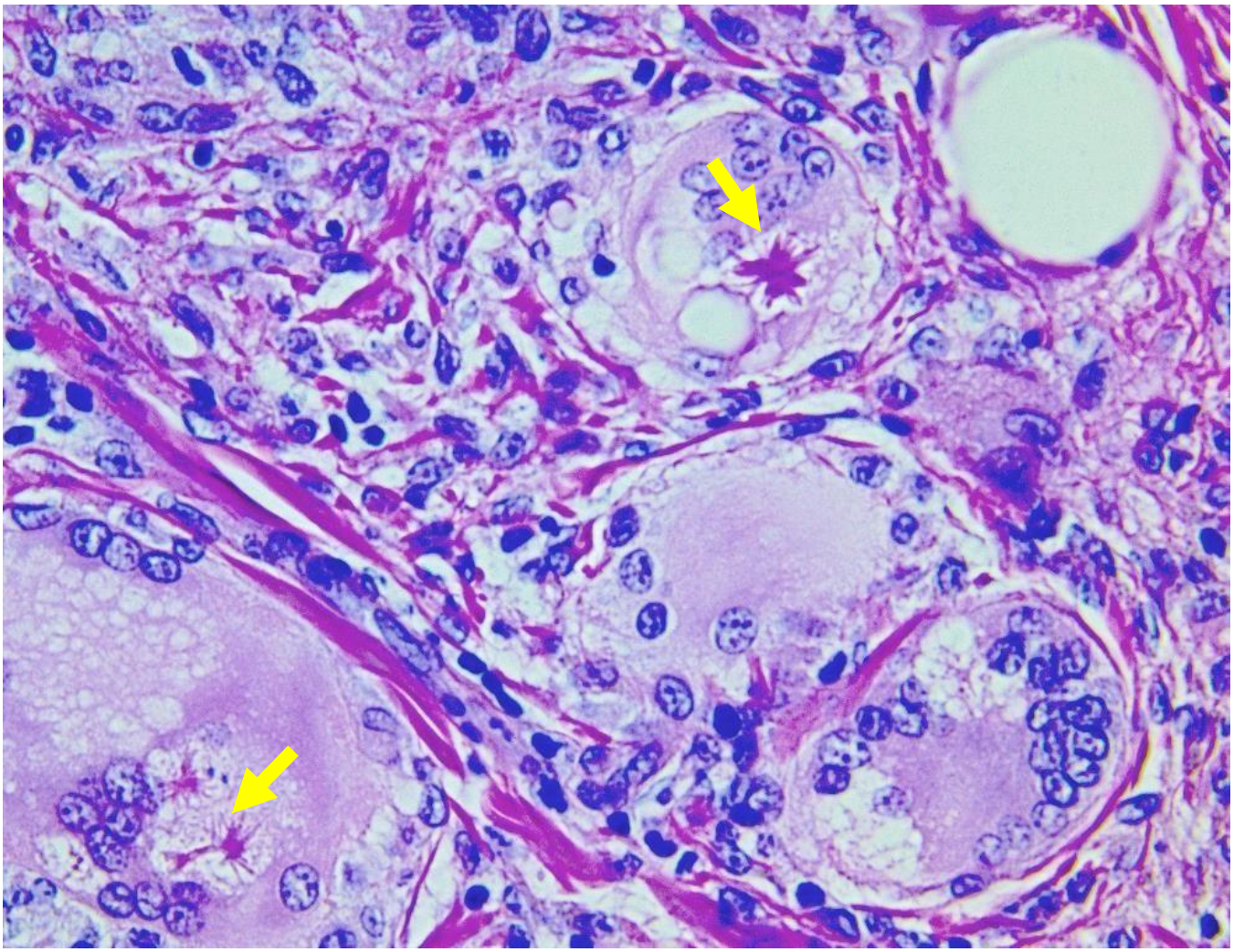

FIG 8. Asteroid bodies (star-shaped spiculated structures) in the cytoplasm of multivacuolated giant cells (arrows) (Hematoxylin-eosin staining).

POLYMETHYL-METHACRYLATE (PMMA)

Artecoll ®, Artefill ®

Alcian blue staining

- Alcian blue staining is negative.

Toluidine blue staining

- Numerous mast cells are often seen in PMMA reactions (Fig. 9).

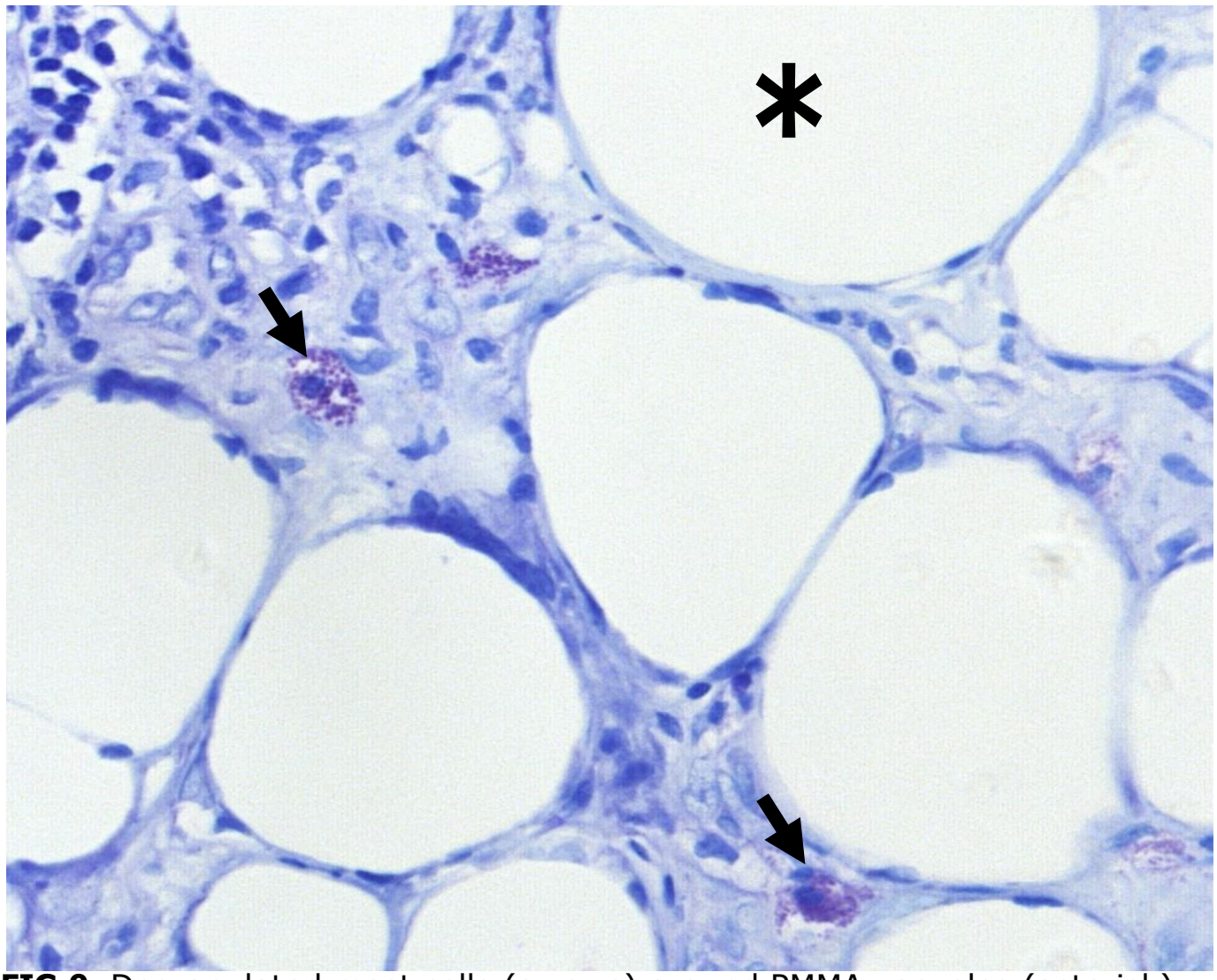

FIG 9. Degranulated mast cells (arrows) around PMMA vacuoles (asterisk) (Toluidine blue staining).

POLYMETHYL-METHACRYLATE (PMMA)

Artecoll ®, Artefill ®

Sirius red staining

- Eosinophils are usually seen in PMMA reactions (Fig. 10).

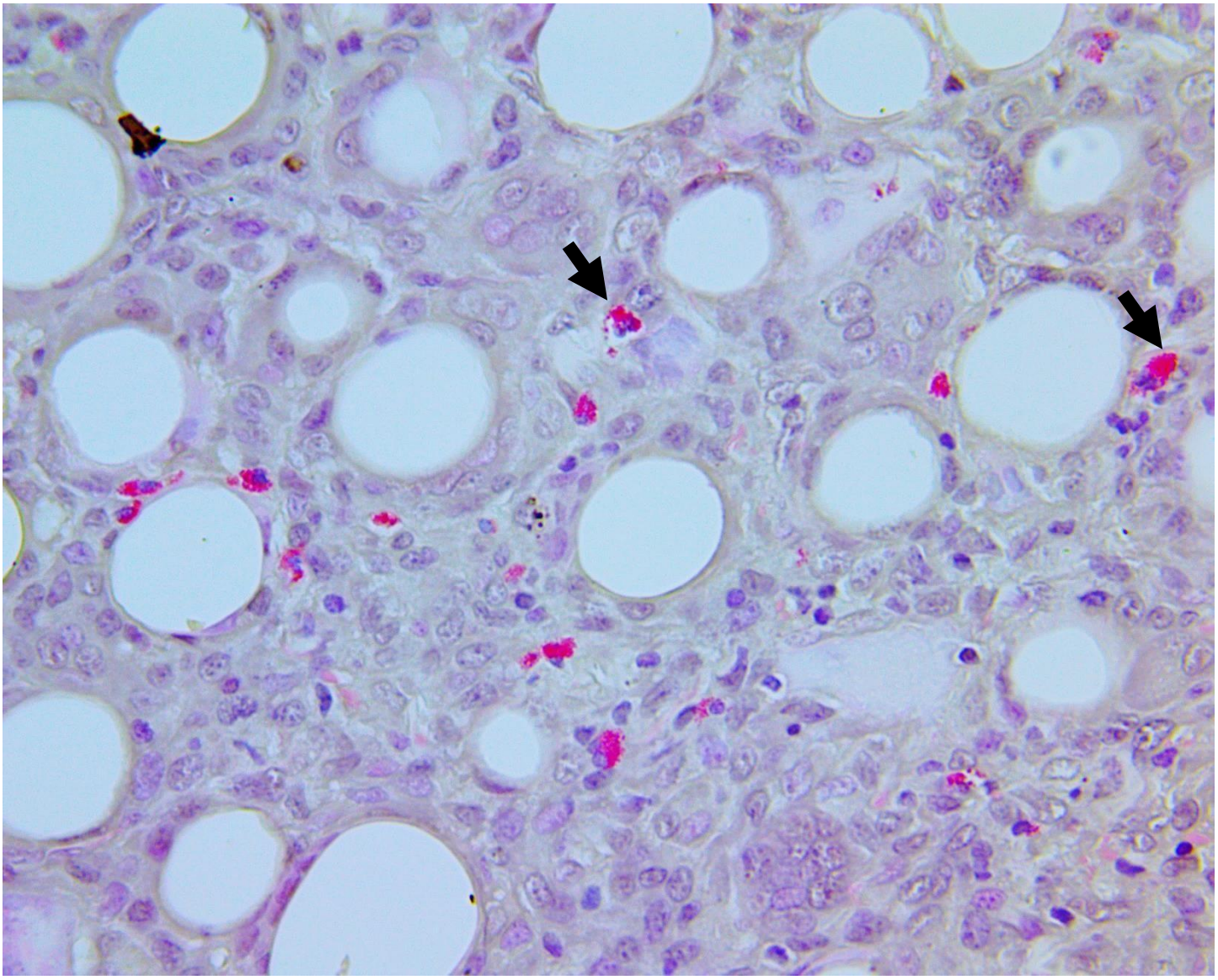

FIG 10. Numerous eosinophils (arrows) surrounding PMMA vacuoles (Sirius red staining).

POLYMETHYL-METHACRYLATE (PMMA)

Artecoll ®, Artefill ®

Immunohistochemistry for CD68

- Macrophages and giant cells are positive for CD68 (Fig. 11).

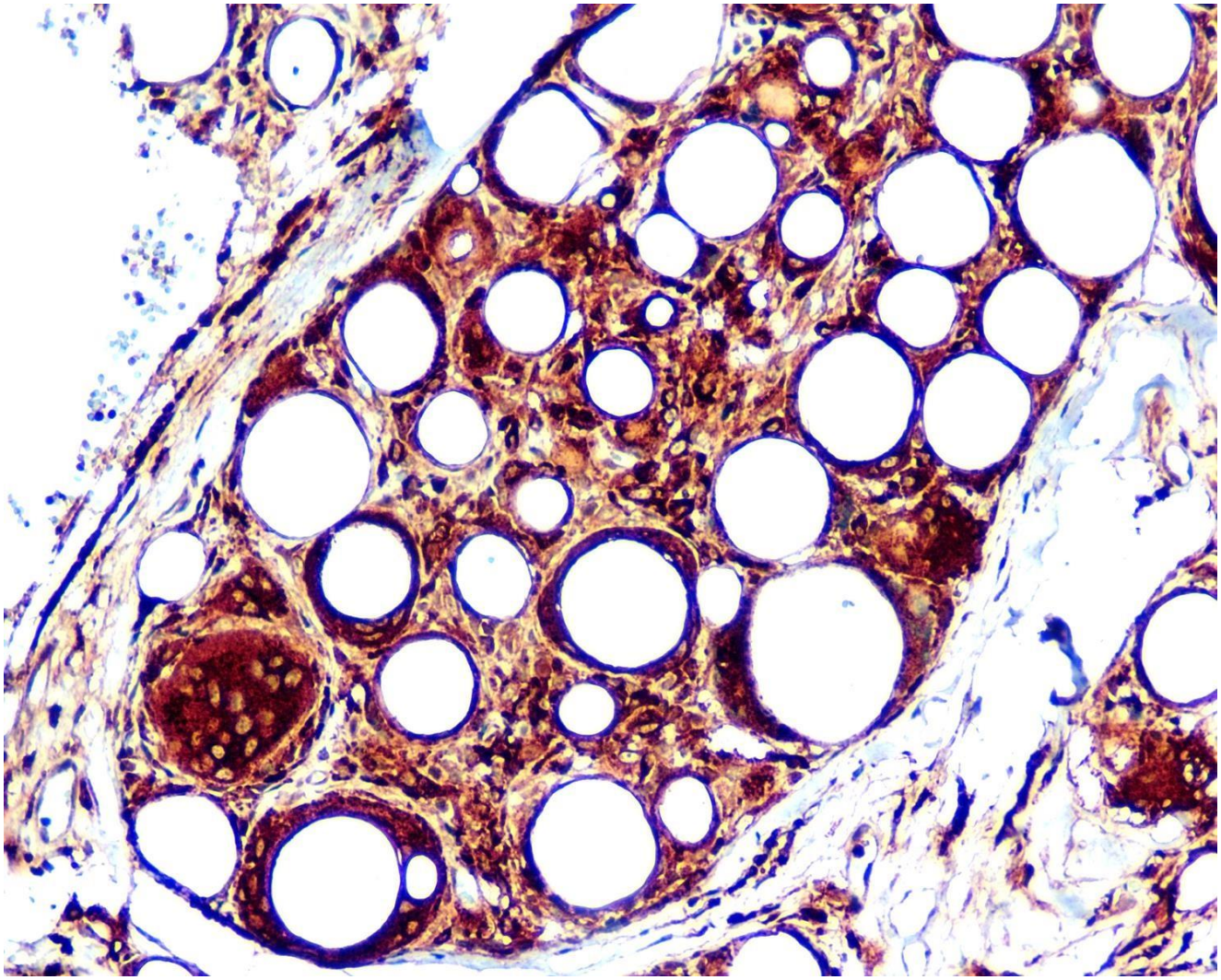

FIG 11. CD68-positive macrophages and giant cells in adverse reaction to PMMA (HRP-polymer immunohistochemistry).

POLYMETHYL-METHACRYLATE (PMMA)

Artecoll ®, Artefill ®

Immunohistochemistry for CD20

- A variable amount (scant to abundant) of CD20-positive lymphocytes is observed in reactions against PMMA (Fig. 12).

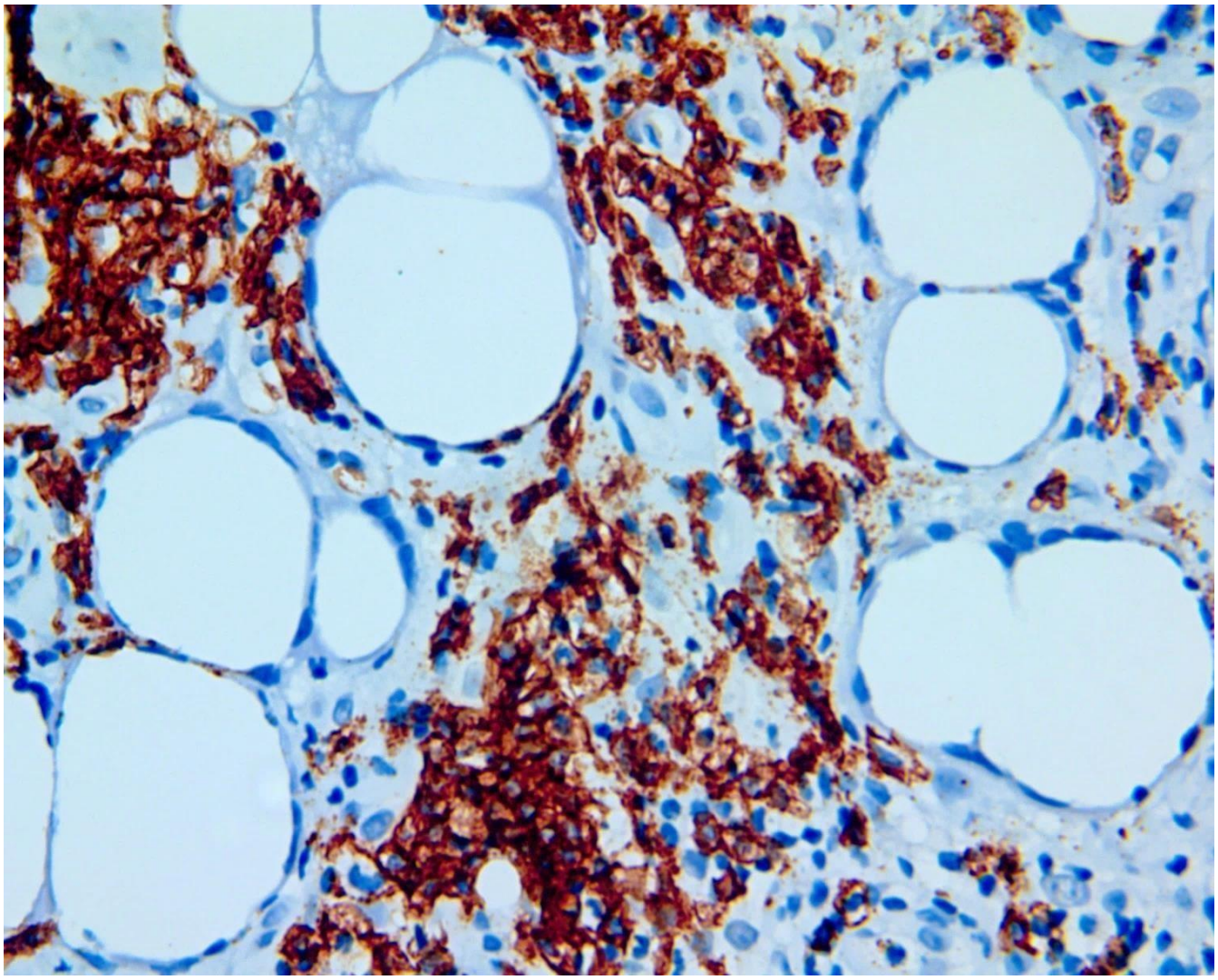

FIG 12. Numerous CD20-positive lymphocytes intermingle PMMA vacuoles (HRP-polymer immunohistochemistry).

POLYMETHYL-METHACRYLATE (PMMA)

Artecoll ®, Artefill ®

Immunohistochemistry for CD3

- Adverse reaction to PMMA shows numerousCD3-positive T lymphocytes surrounding the cosmetic filler (Fig. 13).

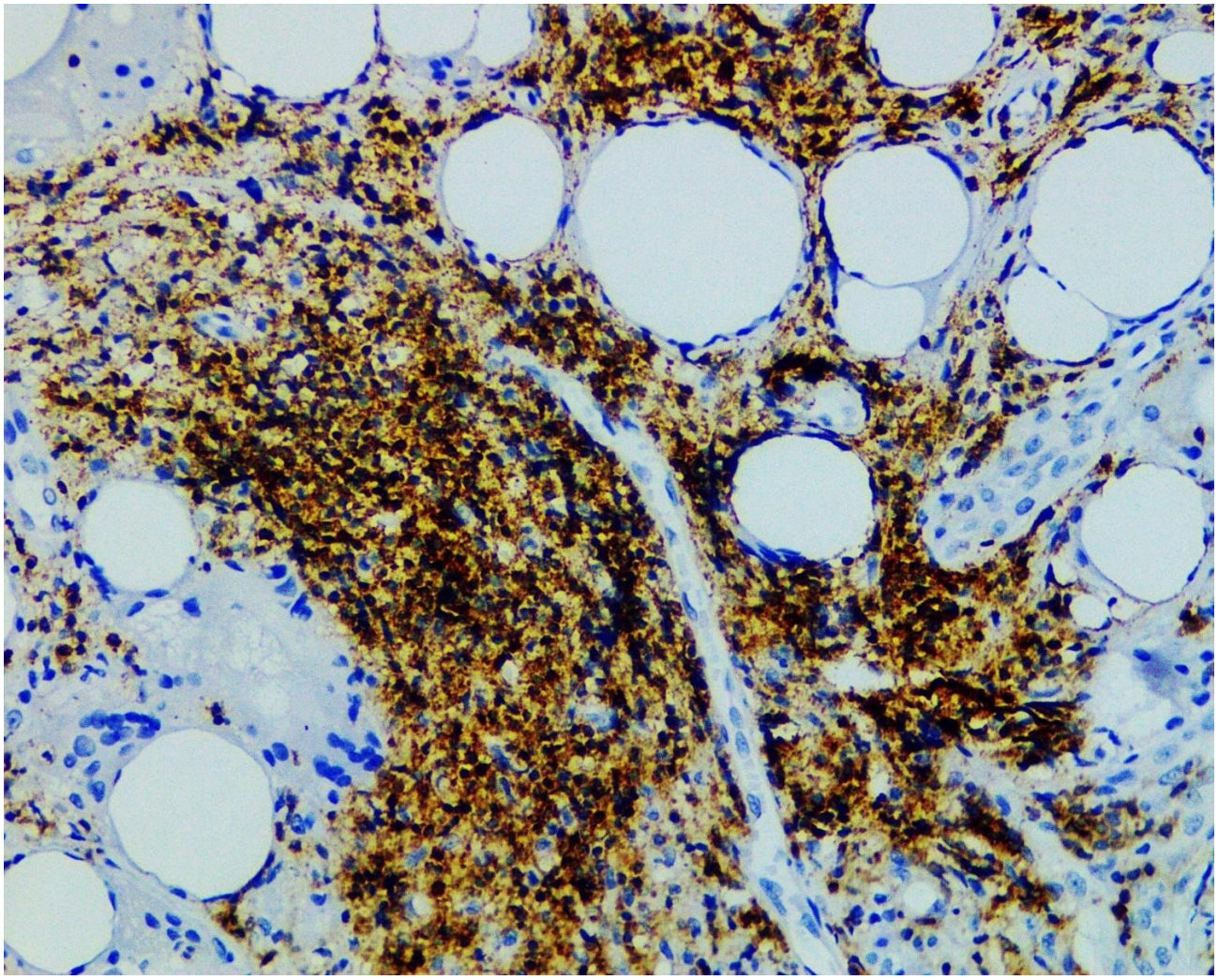

FIG 13. CD3-positive lymphocytes intermingle PMMA vacuoles (HRP-polymer immunohistochemistry).

SILICONE

Silikon 1000 ®, Silskin ®, PMS 350 ®, Silicone Medical Grade®

- Silicone is composed by polydimethylsiloxane, a large molecule of $(-[CH_3]_2SiO-)_x$ units.[10]
- This material is presented as liquid/oil, gel, and elastomer (solid) forms.[3,10] Histopathological findings are variable depending on silicone type.
- Liquid silicone has been used for soft tissue augmentation.[10]
- The arrangement of liquid silicone through tissue is diffuse (Fig. 14A) and seen in the lamina propria (Fig. 14B).

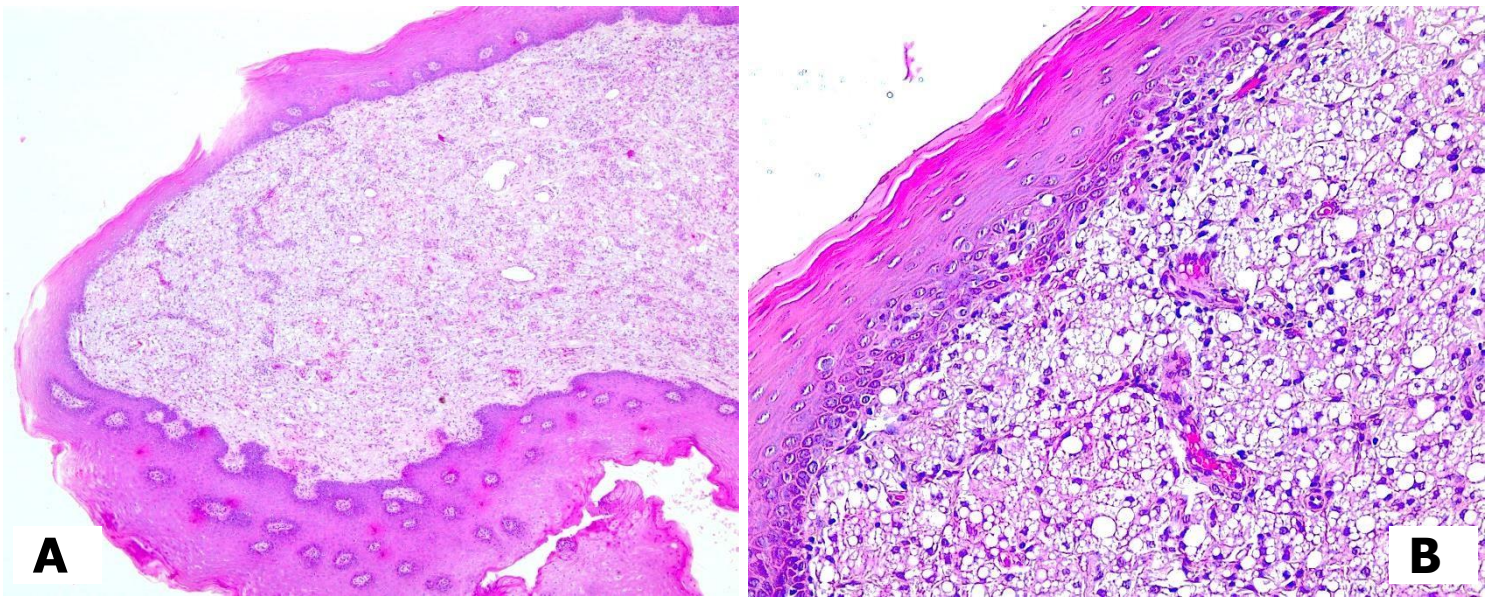

FIG 14. A. Diffuse arrangement of liquid silicone through tissue. **B.** Silicone is dispersed throughout the lamina própria (Hematoxylin-eosin staining).

SILICONE

Silikon 1000 ®, Silskin ®, PMS 350 ®, Silicone Medical Grade®

- Macrophages phagocyte silicone particules, appearing as bubbly macrophages with multivacuolated cytoplasm (Fig. 15).
- Multinucleated giant cells are uncommon.

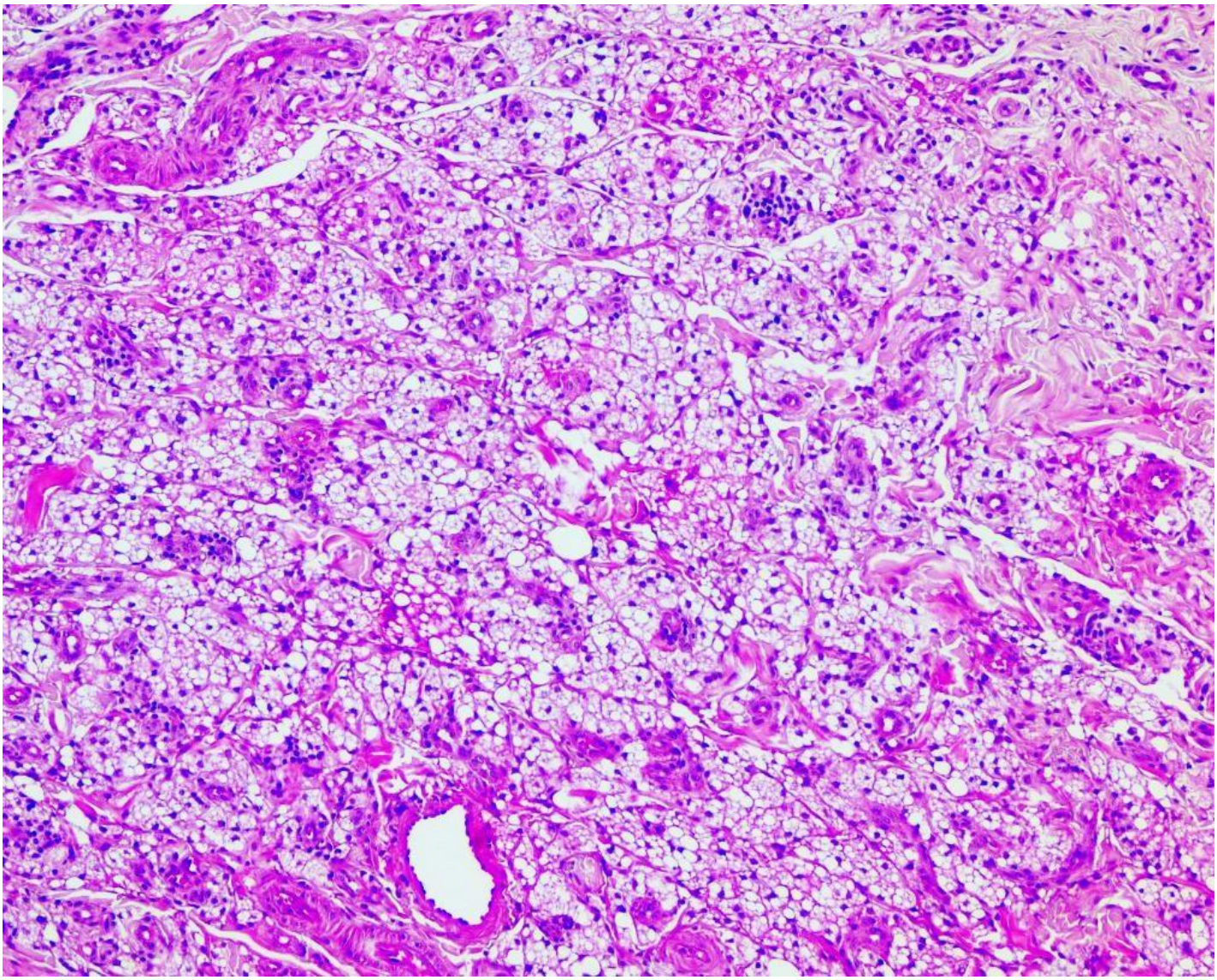

FIG 15. Liquid silicone. Numerous bubbly macrophages as consequence of silicone phagocytosis (Hematoxylin-eosin staining).

SILICONE

Silikon 1000 ®, Silskin ®, PMS 350 ®, Silicone Medical Grade®

- Multivacuolated bubbly macrophages show a hyperchromatic nuclei, similar to Virchow cells[11] or signet ring-like cells[12], and they are arranged in clusters of "grape bunches" of very small size (Fig. 16A-B).

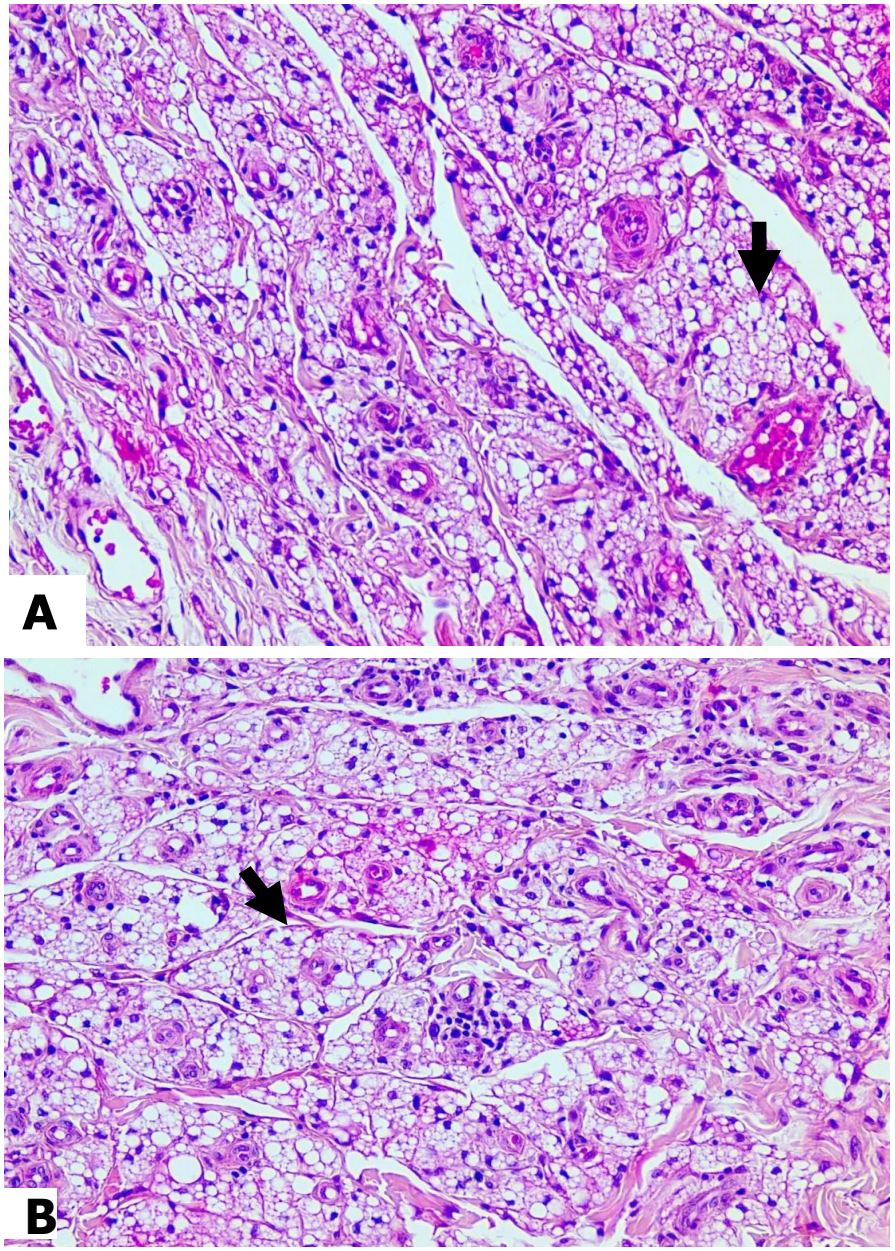

FIG 16. A-B. Liquid silicone. "Grape bunches"(arrows) of bubbly macrophages (Hematoxylin-eosin staining).

SILICONE

Silikon 1000 ®, Silskin ®, PMS 350 ®, Silicone Medical Grade®

- Adverse reactions to silicone may also show varying sizes of vacuoles, forming a Swiss cheese pattern[3,11,13] (Fig. 17).

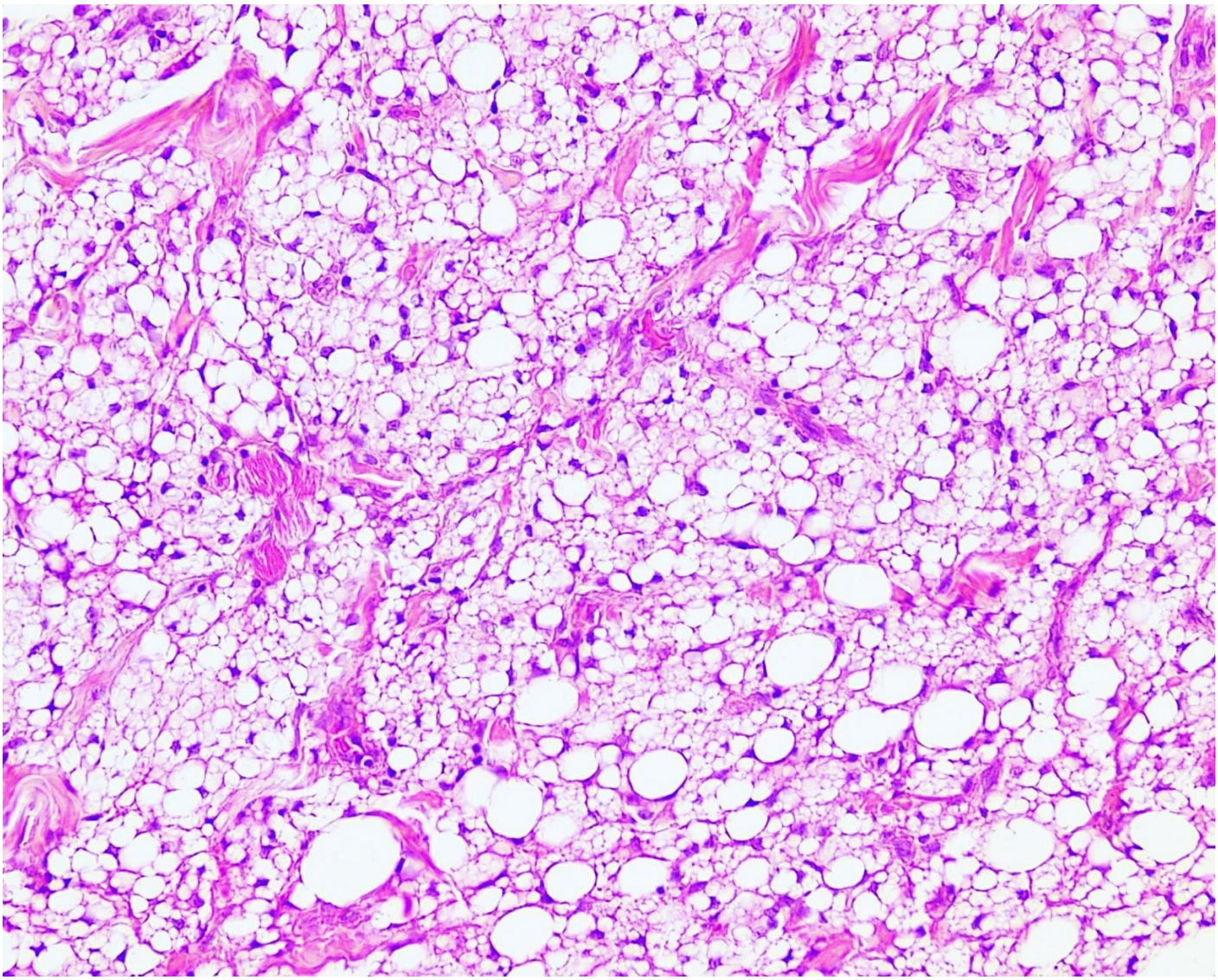

FIG 17. Liquid silicone. Bubbly macrophages showing a Swiss cheese pattern interspersed by some collagen bundles. (Hematoxylin-eosin staining).

SILICONE

Silikon 1000 ®, Silskin ®, PMS 350 ®, Silicone Medical Grade®

- Swiss cheese pattern may also show a great variation of vacuole sizes, mimicking cystic spaces[13] (Fig. 18).

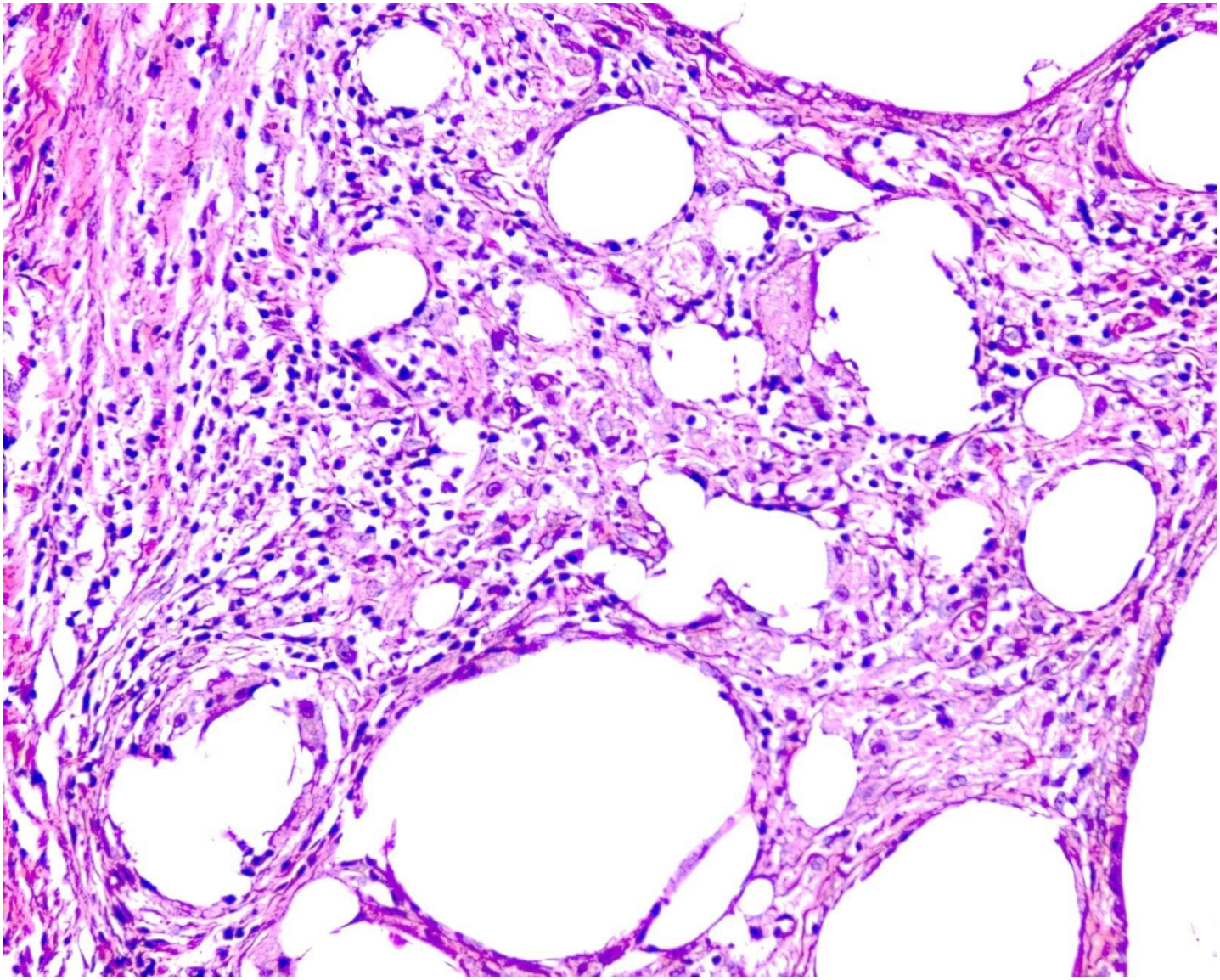

FIG 18. Liquid silicone. Swiss cheese pattern with large vacuoles, mimicking cystic spaces (Hematoxylin-eosin staining).

SILICONE

Silikon 1000 ®, Silskin ®, PMS 350 ®, Silicone Medical Grade®

- Host reaction to silicone can produce foreign body granuloma containing multinucleated giant cells with asteroid bodies (Fig. 19).
- This presentation is common in elastomer silicone[3], but not in liquid silicone.

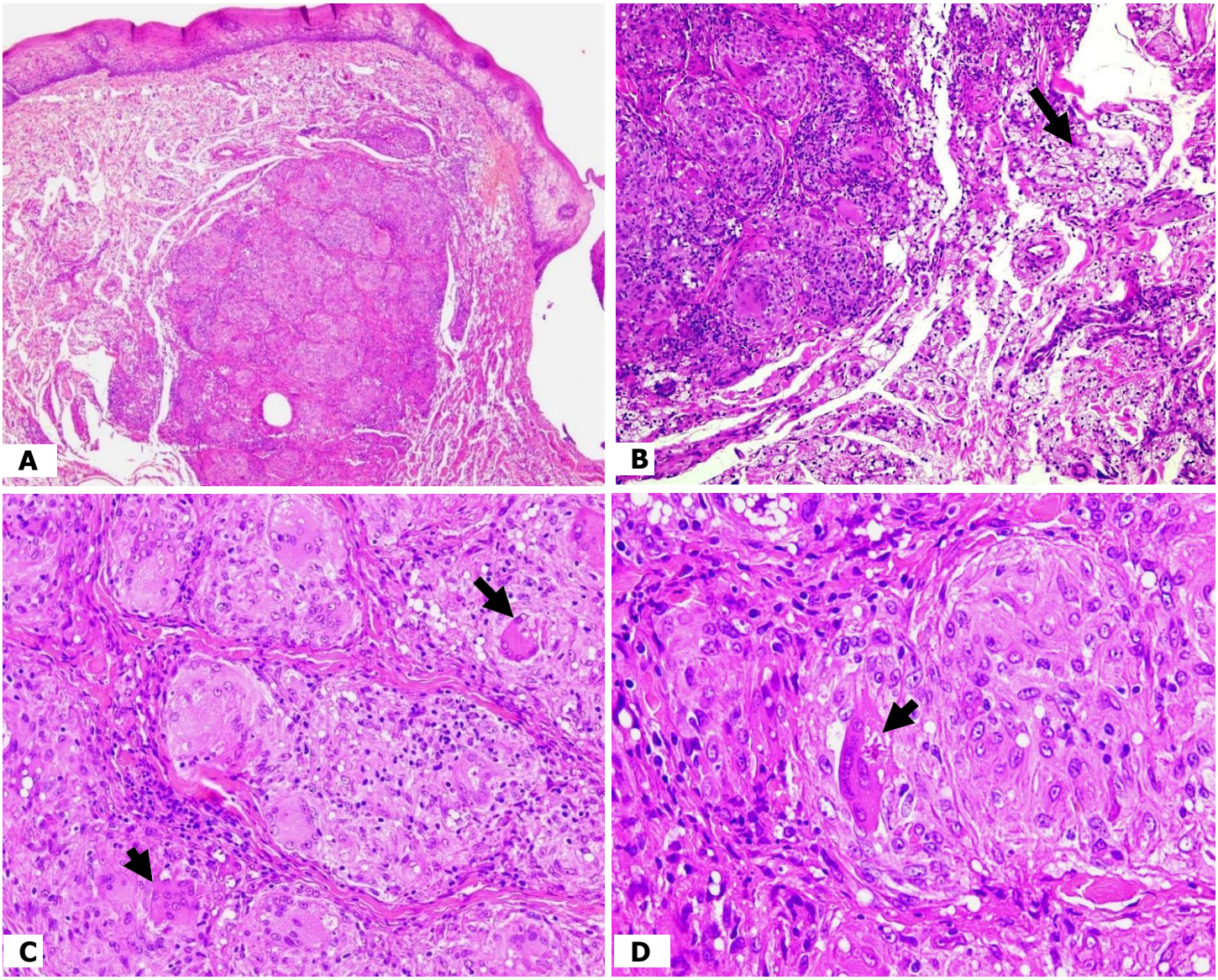

FIG 19. A. Foreign body granuloma with a nodular arrangement. **B.** Bubbly macrophages (arrow) phagocyting silicone near the granuloma. **C.** Granulomas with numerous giant cells (arrows) and lymphocytes. **D.** Asteroid body (arrow) inside giant cell (Hematoxylin-eosin staining).

SILICONE

Silikon 1000 ®, Silskin ®, PMS 350 ®, Silicone Medical Grade®

Alcian blue staining

- Alcian blue staining is negative.

Toluidine blue staining

- Mast cells are usually abundant in reactions to silicone (Fig. 20).

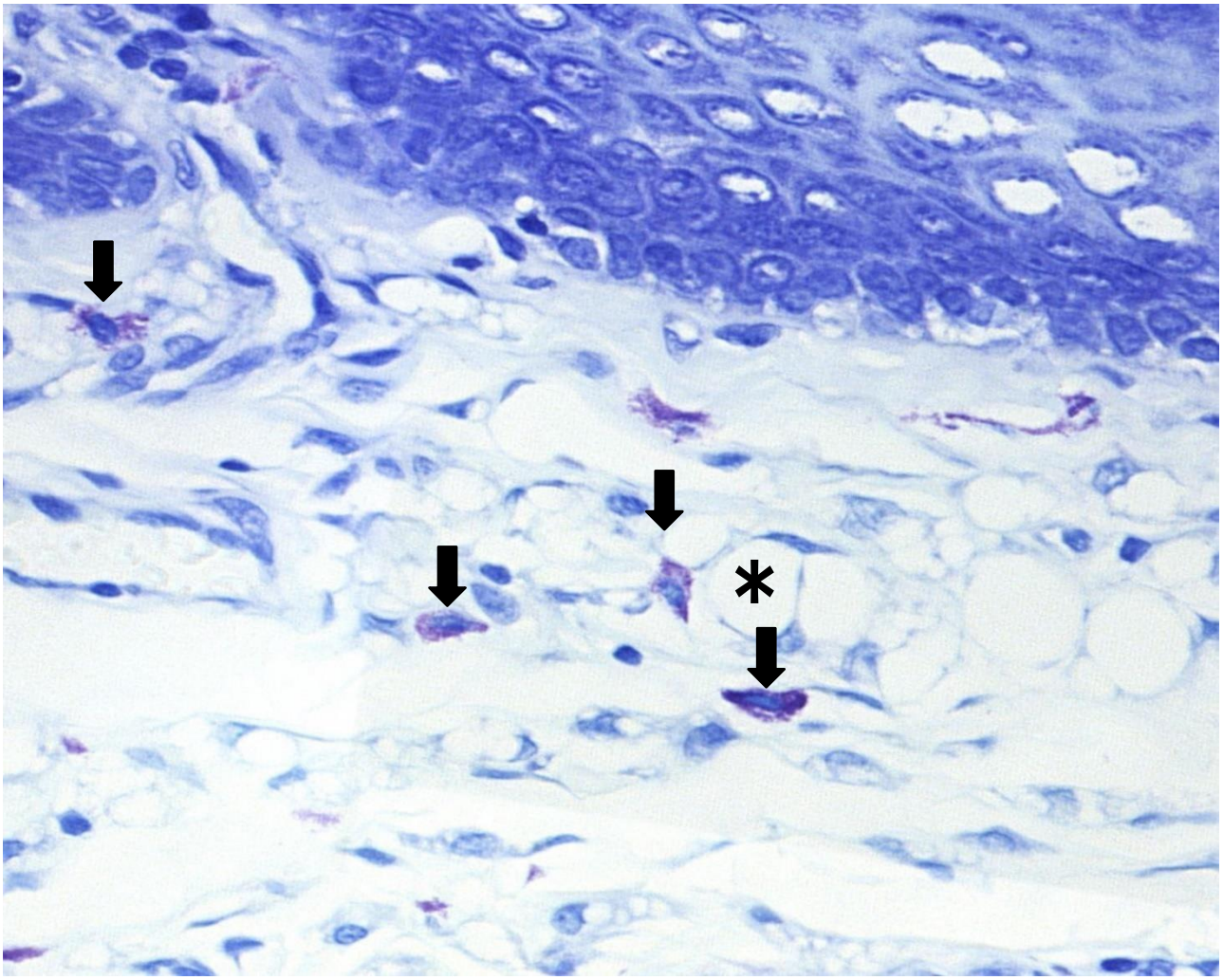

FIG 20. Mast cells (arrows) interspread in liquid silicone (asterisk) (Toluidine blue staining).

SILICONE

Silikon 1000 ®, Silskin ®, PMS 350 ®, Silicone Medical Grade®

Sirius red staining

- Eosinophils are usually not present in adverse reaction to silicone. These cells may be present in rare cases[3].

Immunohistochemistry for CD68

- CD68 is highly expressed. The macrophages that phagocytize the silicone lead to the formation of a honeycomb appearance (Fig. 21).

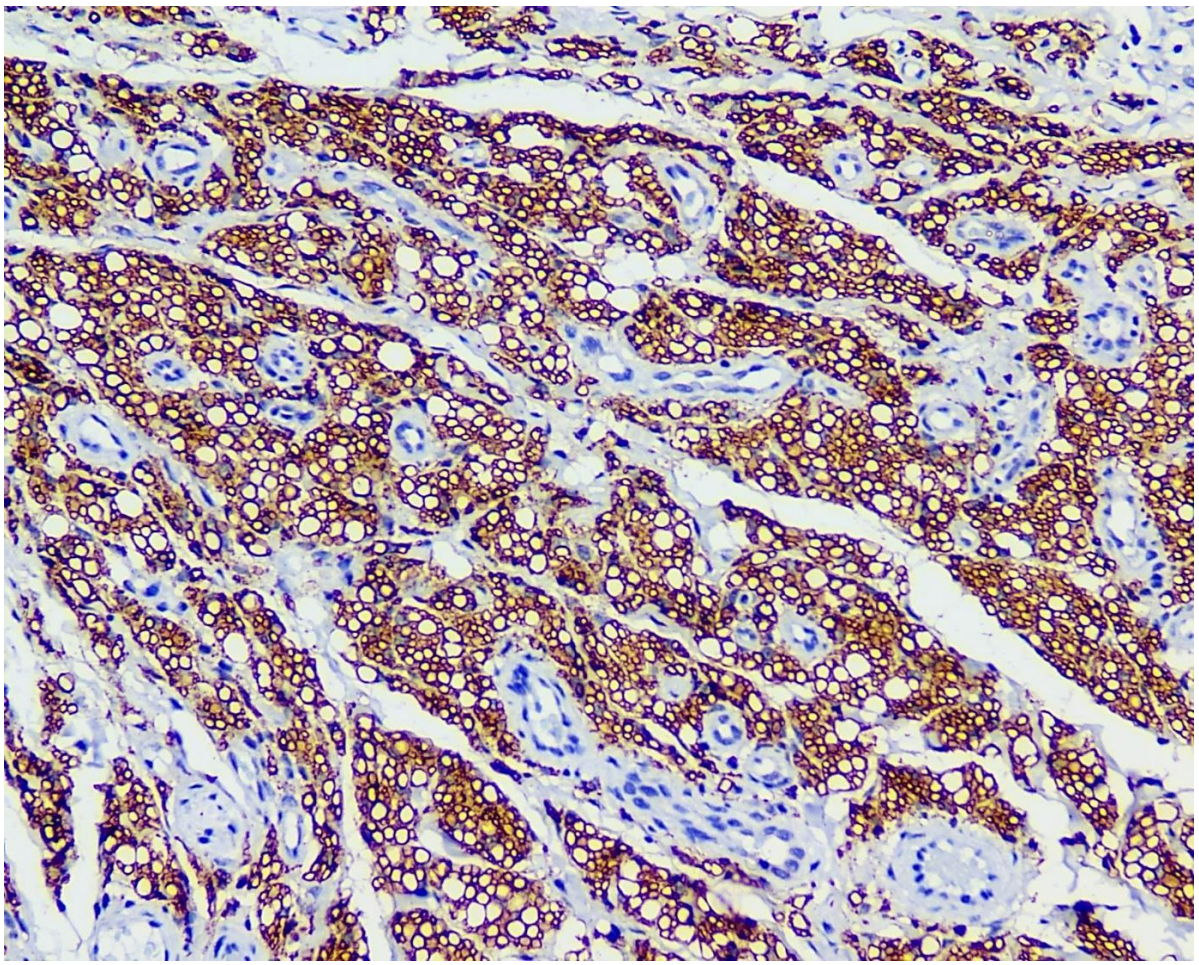

FIG 21. Adverse reaction to liquid silicone exhibiting numerous CD68-positive macrophages (HRP-polymer immunohistochemistry)

SILICONE

Silikon 1000 ®, Silskin ®, PMS 350 ®, Silicone Medical Grade®

Immunohistochemistry for CD20

- CD20-positive cells are scant in reactions against silicone (Fig. 22).

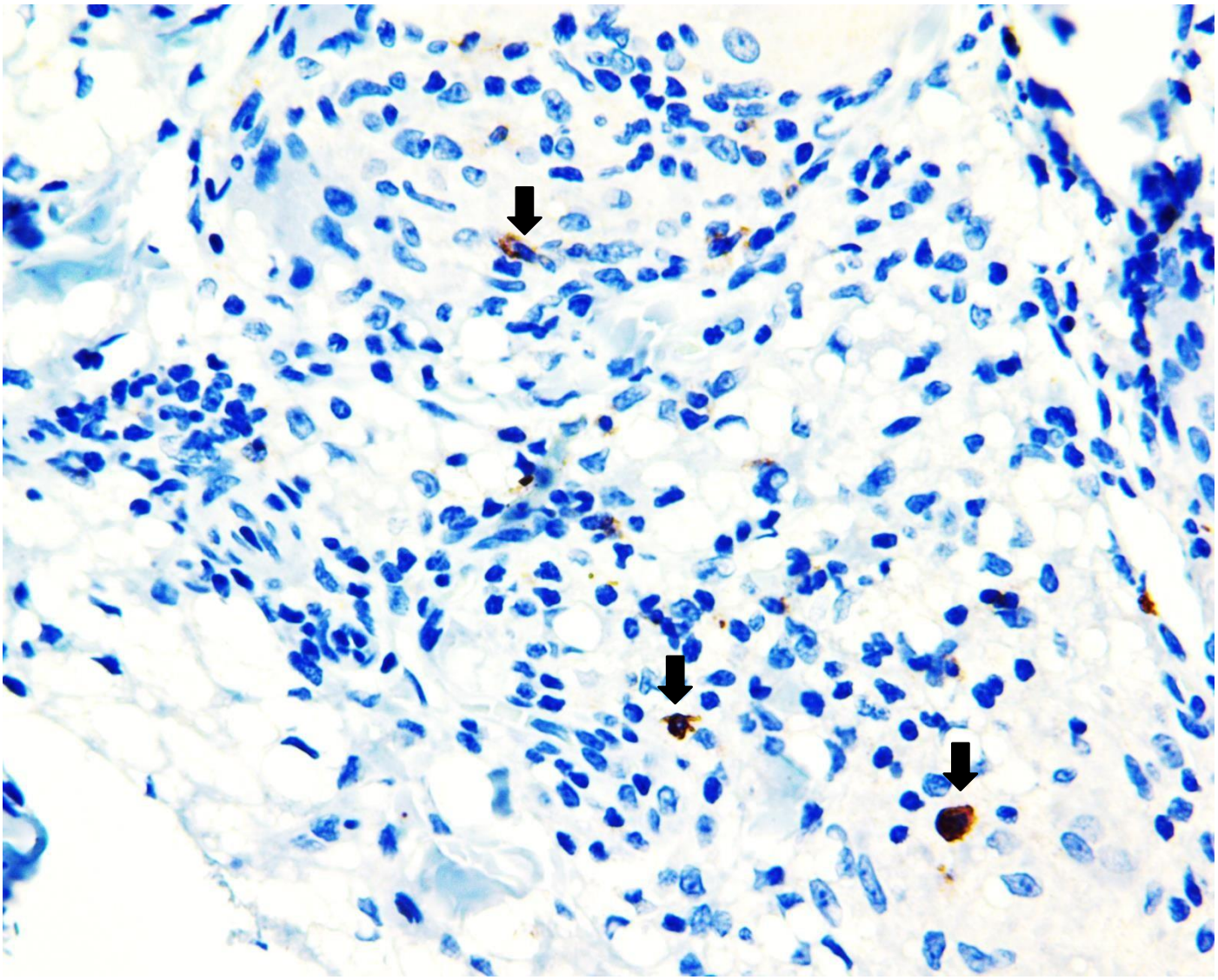

FIG 22. Adverse reaction to liquid silicone exhibit scant CD20-positive lymphocytes (arrows) (HRP-polymer immunohistochemistry).

SILICONE

Silikon 1000 ®, Silskin ®, PMS 350 ®, Silicone Medical Grade®

Immunohistochemistry for CD3

- Few CD3-positive cells can be seen (Fig. 23).

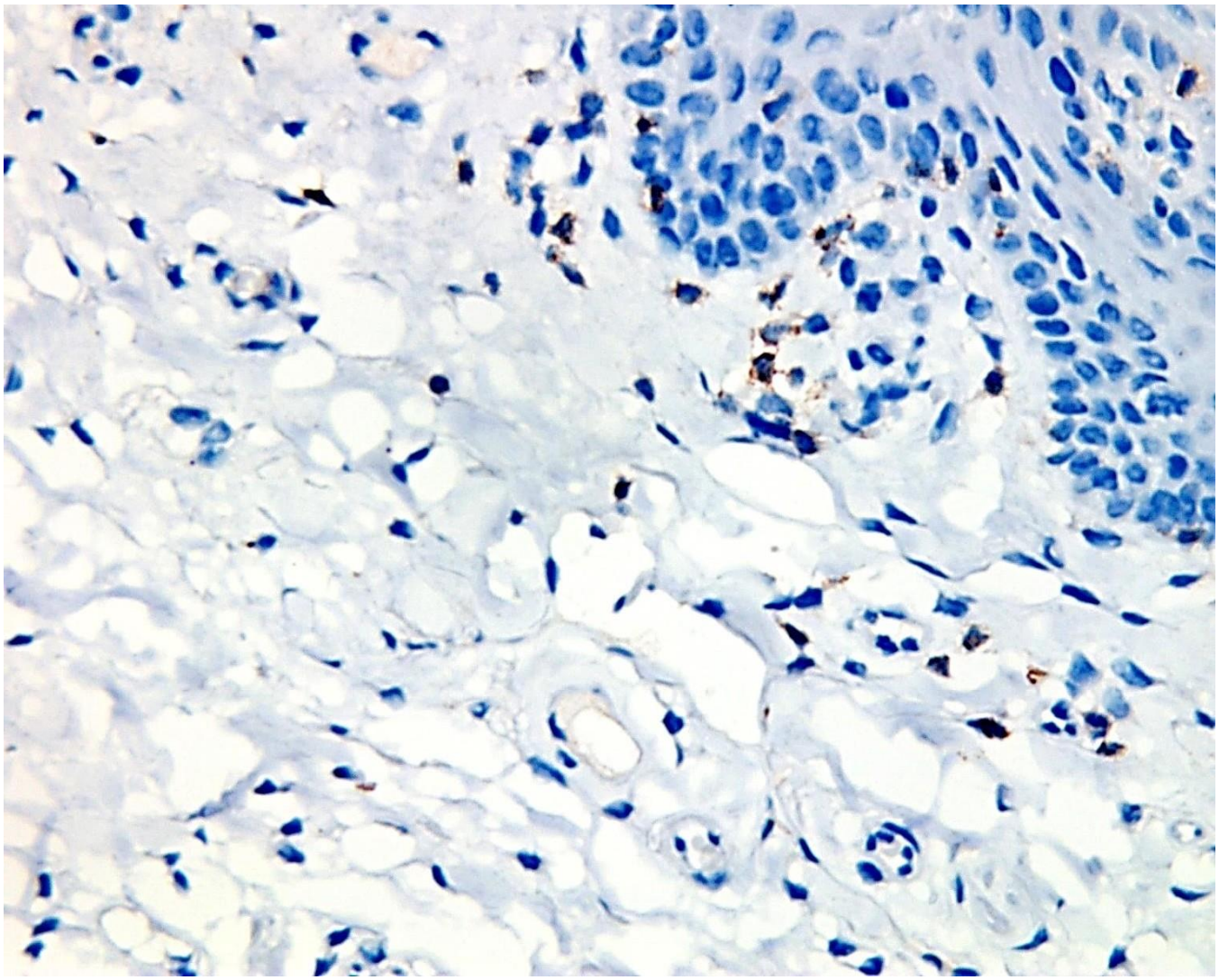

FIG 23. Adverse reaction to liquid silicone exhibit scant T cells positive by CD3 marker (HRP-polymer immunohistochemistry).

PMMA, SILICONE, AND SPINDLE CELL LIPOMA: Differential diagnosis of permanent fillers and neoplasia presenting as round and negative vacuoles

POLYMETHYLMETACRYLATE

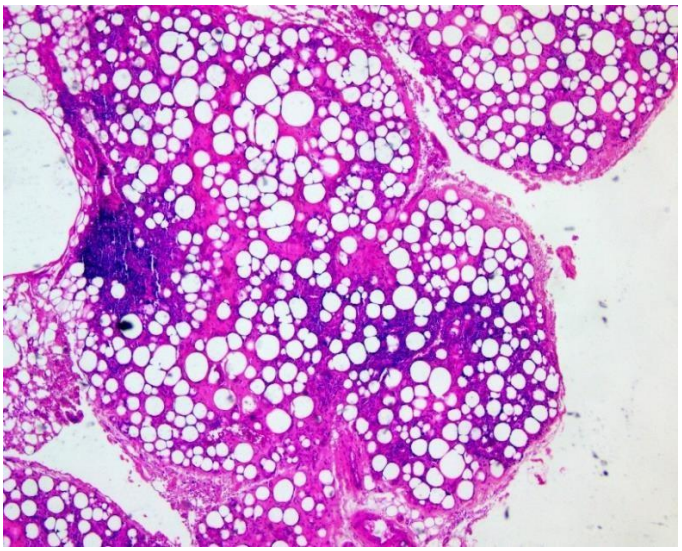

FIG 24. Adverse reaction to PMMA (Hematoxylin-eosin staining).

SILICONE

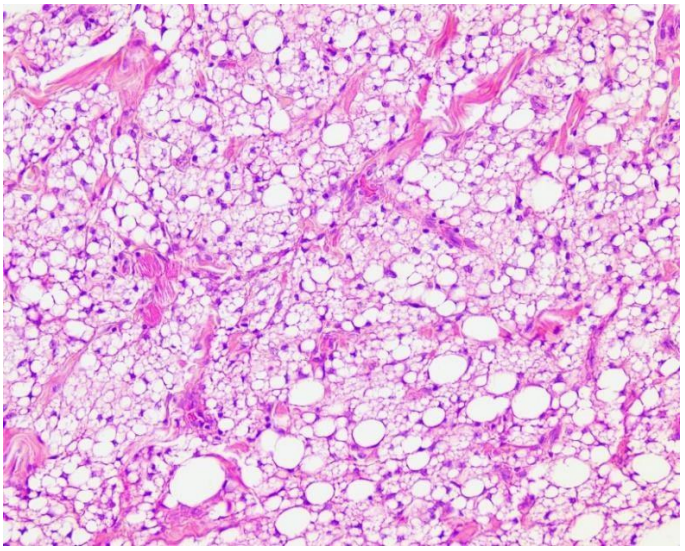

FIG 25. Adverse reaction to silicone (Hematoxylin-eosin staining).

SPINDLE CELL LIPOMA

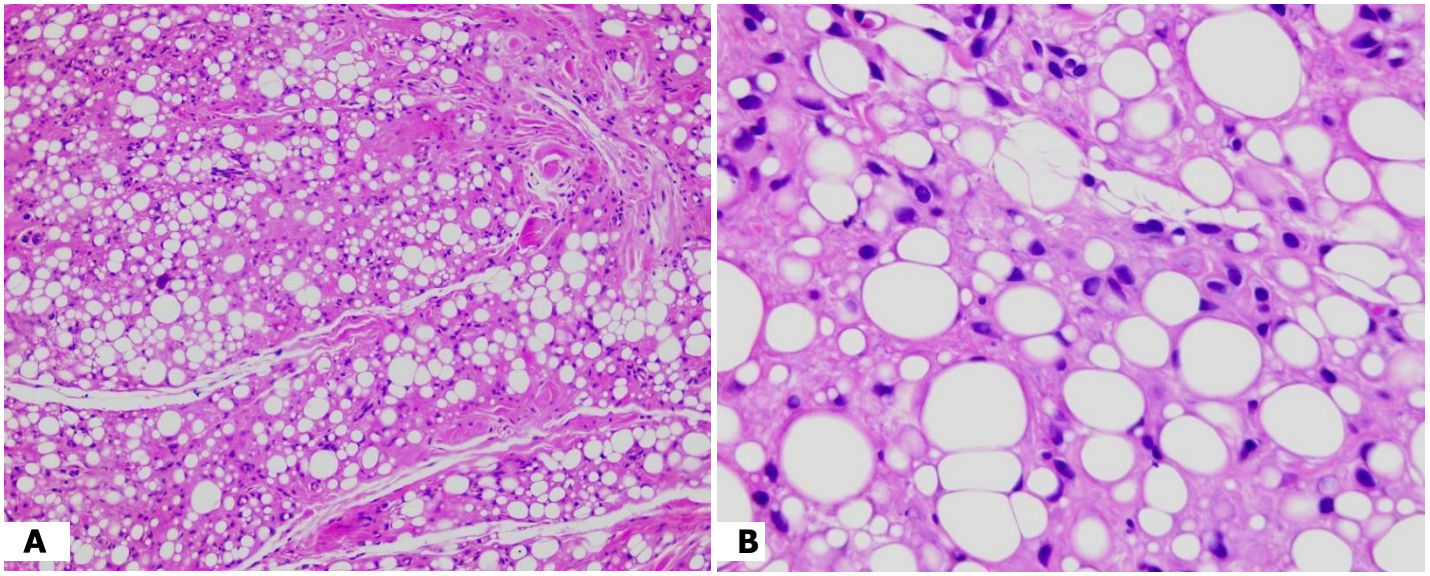

FIG 26 A-B. Spindle cell lipoma. **A.** Low magnification. **B.** High magnification. (Hematoxylin-eosin staining).

PMMA, SILICONE, AND SPINDLE CELL LIPOMA: Differential diagnosis of permanent fillers and neoplasia presenting as round and negative vacuoles

BOX 1. Histopathological differential diagnosis of the permanent fillers.

Features	PMMA	SILICONE (liquid)	SPINDLE CELL LIPOMA AND VARIANTS[14,15]
Shape	Perfectly rounded negative spaces or vacuoles, with a regular contour and similar shape.	Rounded negative vacuoles with irregular contour. Mimicks normal adipocytes more than PMMA does.	Mature rounded adipocytes and spindle cells.
Size	Similar size between vacuoles, that usually has medium size.	Different sizes: depends on type of arrangement (see next page)	Slight variation in size

BOX 1. Histopathological differential diagnosis of the permanent fillers.

Features	PMMA	SILICONE (liquid)	SPINDLE CELL LIPOMA AND VARIANTS[14,15]
Arrangement	• May be nodular, diffuse or mixed (nodular and diffuse arrangement) • There is a distance between vacuoles, whereas in silicone vacuoles are grouped	• Usually diffuse • Macrophages with bubbly or foamy multivacuolate cytoplasm arranged in clusters of "grape bunches": vacuoles of very small sizes. • "Swiss cheese": varying sizes, from small to big spaces. Can mimick cystic spaces.	• Nodular
Type of reaction	• Foreign-body granuloma	• Chronic inflammation, predominantly by macrophages • Foreign body granuloma is rare	• Mast cells are prominent

BOX 1. Histopathological differential diagnosis of the permanent fillers.

Features	PMMA	SILICONE (liquid)	SPINDLE CELL LIPOMA AND VARIANTS[14,15]
Macrophage nuclei	Normal staining	Hypercromatic nuclei	Macrophages are not expected to be numerous
Giant cells	Present, numerous. Surrounding and/or phagocyting vacuoles	Absent	Pleomorphic lipoma: floret-like giant cell
Asteroid bodies	Present	Absent	Absent
Stroma	Often collagenous fibrosis; Dense connective tissue	Loose connective tissue	Bundles of thick collagen fibers in variable proportions. Sometimes myxoid stroma also is seen
Immuno-histochemistry	Positive for CD68 and negative for S100	Positive for CD68 (honeycomb pattern) and negative for S100	• Adipocytes positive for S100 and negative for CD68 • Spindle cells are positive for CD34 and negative for S100

HYALURONIC ACID

Rennova®, Restylane®, Juvéderm®, Perlane®, Hylaform®, Macrolane®

- Hyaluronic acid is a glycosaminoglycan that consists of repeating D-glucuronic acid and DN-acetylglucosamine disaccharide units ($C_{28}H_{44}N_2O_{23}$).[16,17]

- Adverse reactions to hyaluronic acid usually show homogenous "pools" of an amorphous material inside irregular cyst-like spaces. The material can be basophilic or amphophilic (Fig. 27).

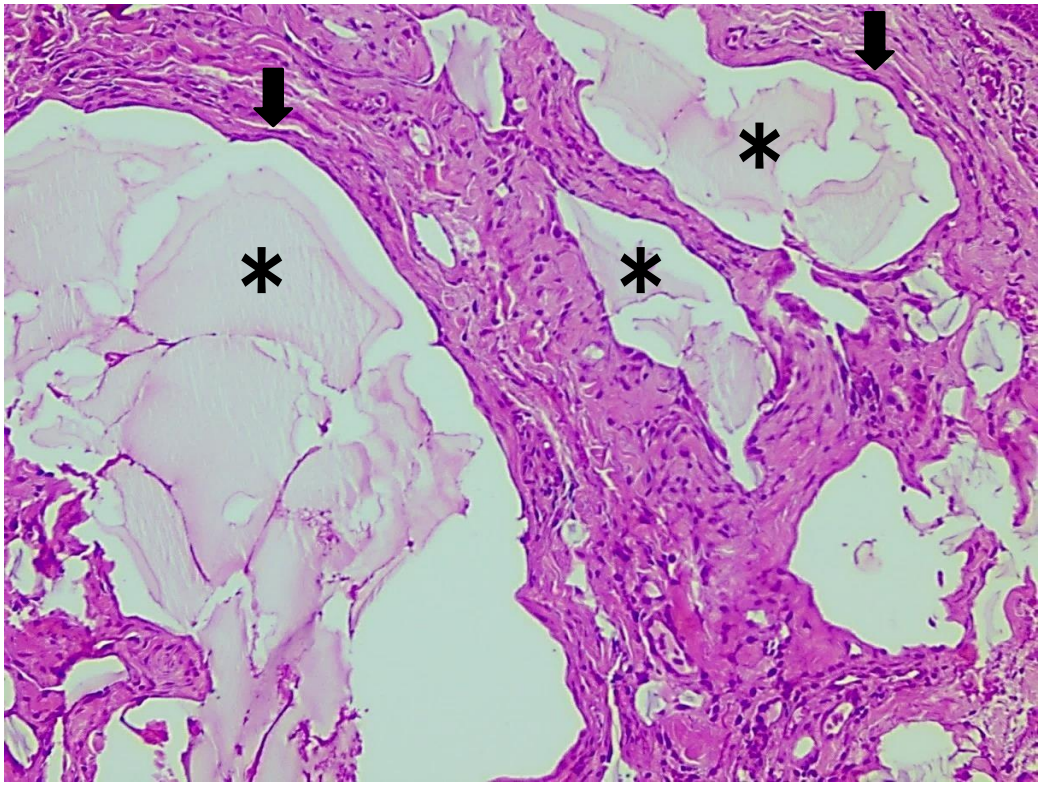

FIG 27. "Pools" of hyaluronic acid inside irregular cyst-like spaces delimited by fibrous tissue (arrows). Note the amphophilic staining of the material (asterisks) (Hematoxylin-eosin staining).

HYALURONIC ACID

Rennova®, Restylane®, Juvéderm®, Perlane®, Hylaform®, Macrolane®

- Palisading macrophages forming duct-like structures surrounding the hyaluronic acid pools are a common finding (Fig 28 A-B).

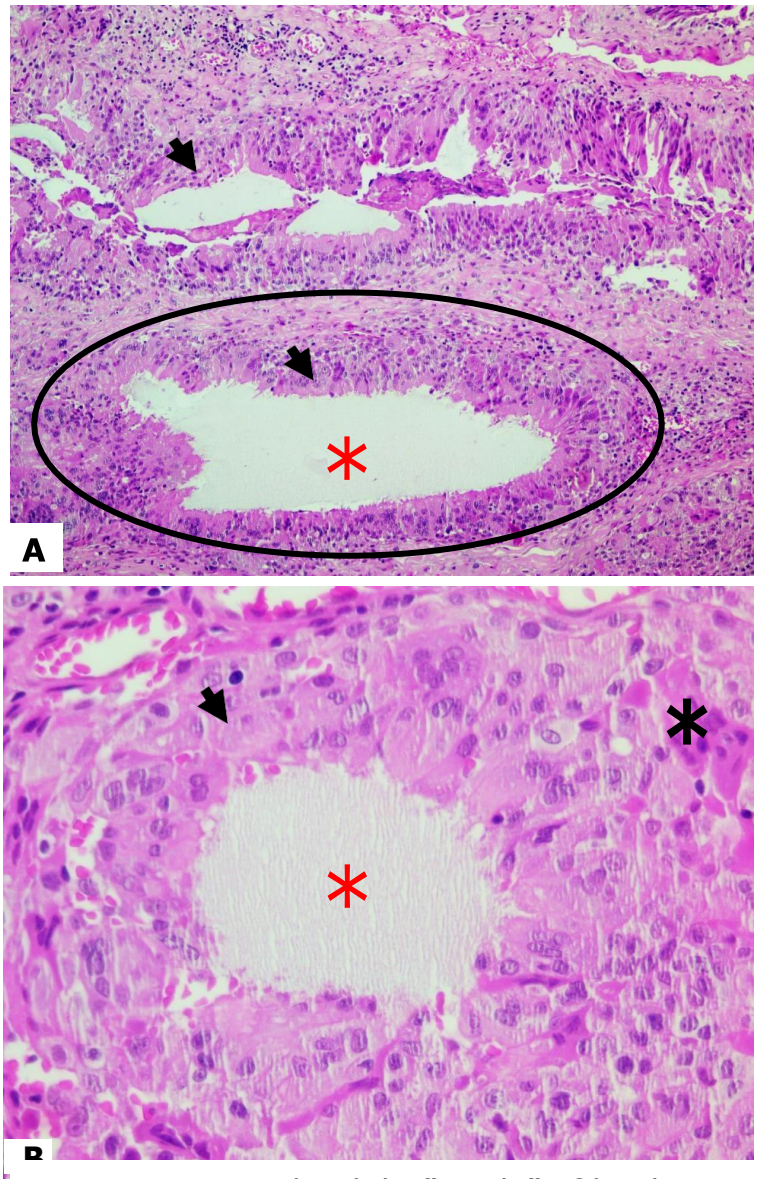

FIG 28. A. Amphophilic "pools" of hyaluronic acid (red asterisks) inside duct-like structures (circle) lined by palisading macrophages (arrows). **B.** High magnification of macrophages (arrow) and giant cell (black asterisk) (Hematoxylin-eosin staining).

HYALURONIC ACID

Rennova®, Restylane®, Juvéderm®, Perlane®,

Hylaform®, Macrolane®

- Early onset reactions may show the uncommon diffuse arragement of hyaluronic acid through the connective tissue (Fig. 29).

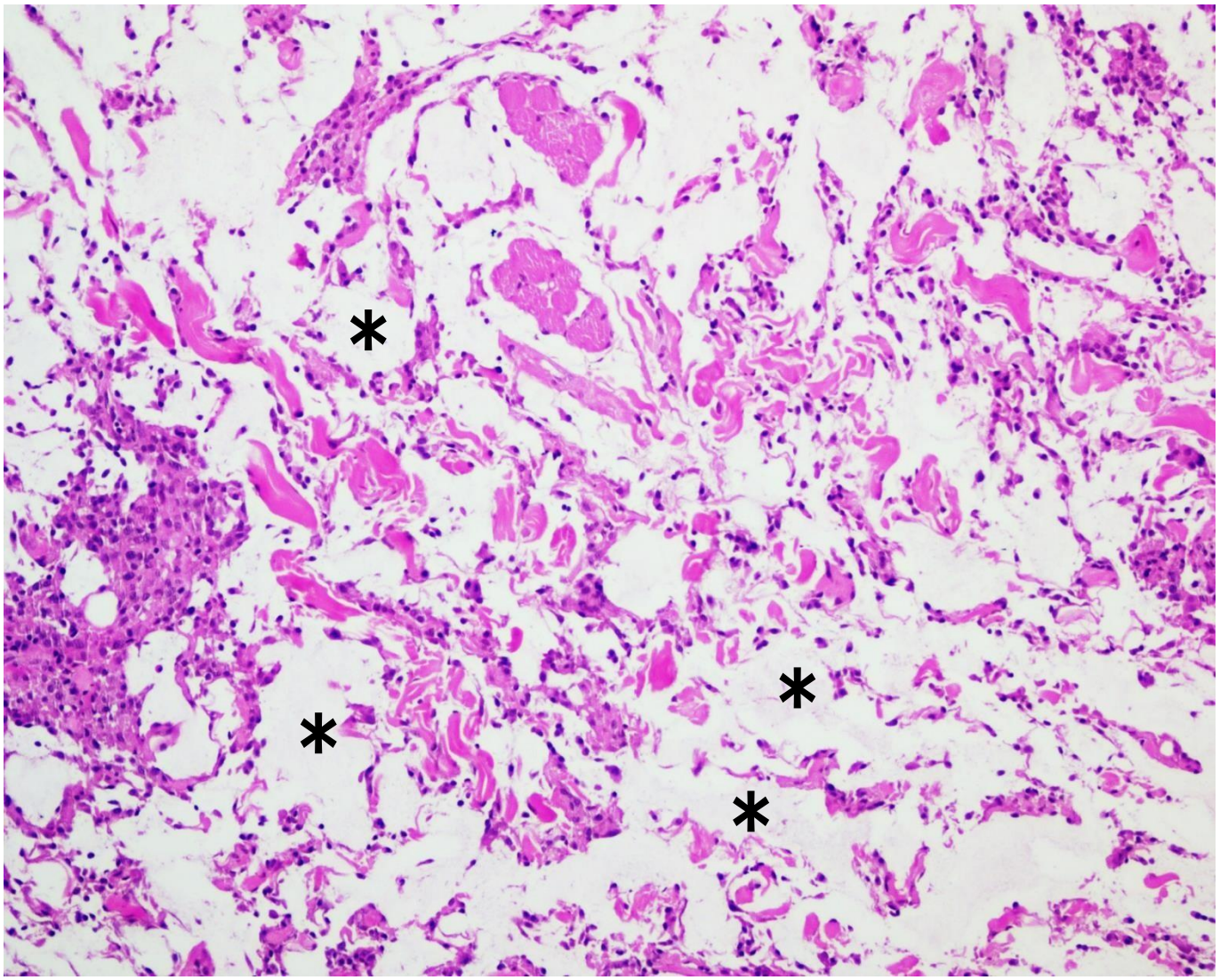

FIG 29. Hyaluronic acid (asterisk) interspersing connective tissue (Hematoxylin-eosin staining).

HYALURONIC ACID

Rennova®, Restylane®, Juvéderm®, Perlane®, Hylaform®, Macrolane®

- Sometimes the acid hyaluronic staining may be very pale (Fig. 30).

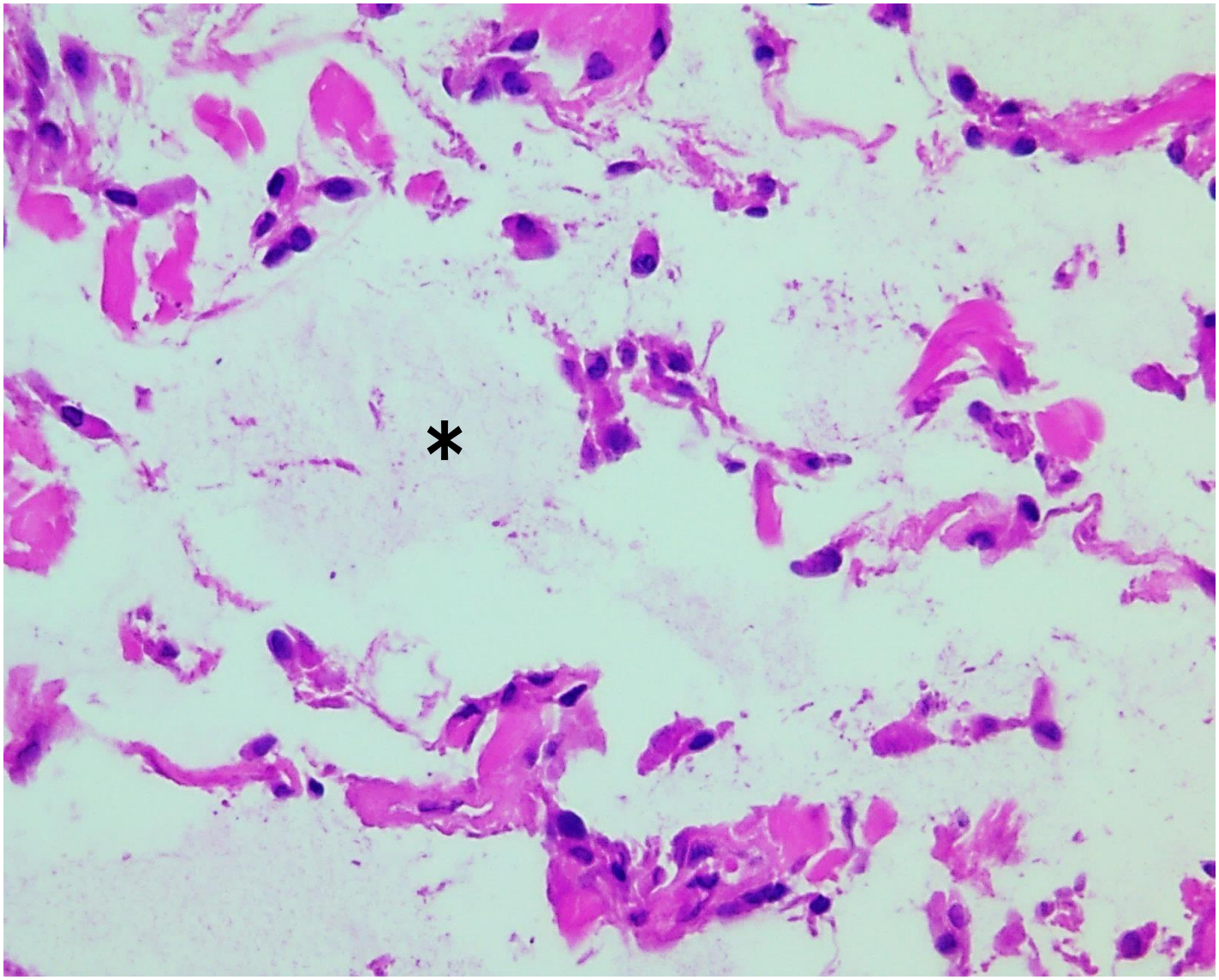

FIG 30. Note the pale amphophilic staining of hyaluronic acid (asterisk) (Hematoxylin-eosin staining).

HYALURONIC ACID

Rennova®, Restylane®, Juvéderm®, Perlane®, Hylaform®, Macrolane®

Alcian blue staining, pH 2.5

- Acid mucopolysaccharides of the hyaluronic acid are positive for Alcian blue, showing a cyan blue/ turquoise shade (Fig. 31).

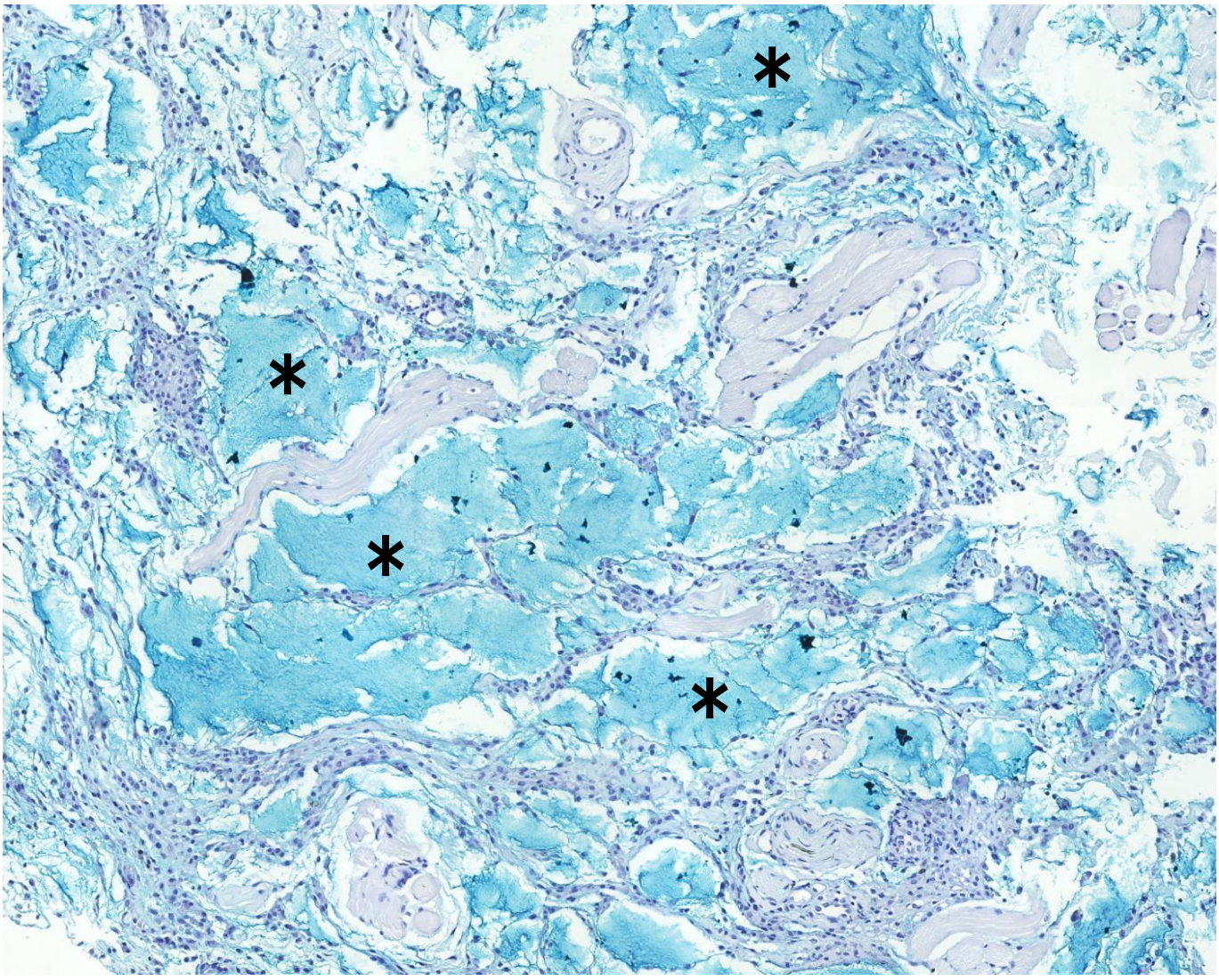

FIG 31. Hyaluronic acid is positive for Alcian blue staining (asterisks).

HYALURONIC ACID

Rennova®, Restylane®, Juvéderm®, Perlane®, Hylaform®, Macrolane®

Alcian blue staining, pH 2.5

- Alcian blue staining is useful in identifying hyaluronic acid between other fillers in the same slide (Fig. 32).

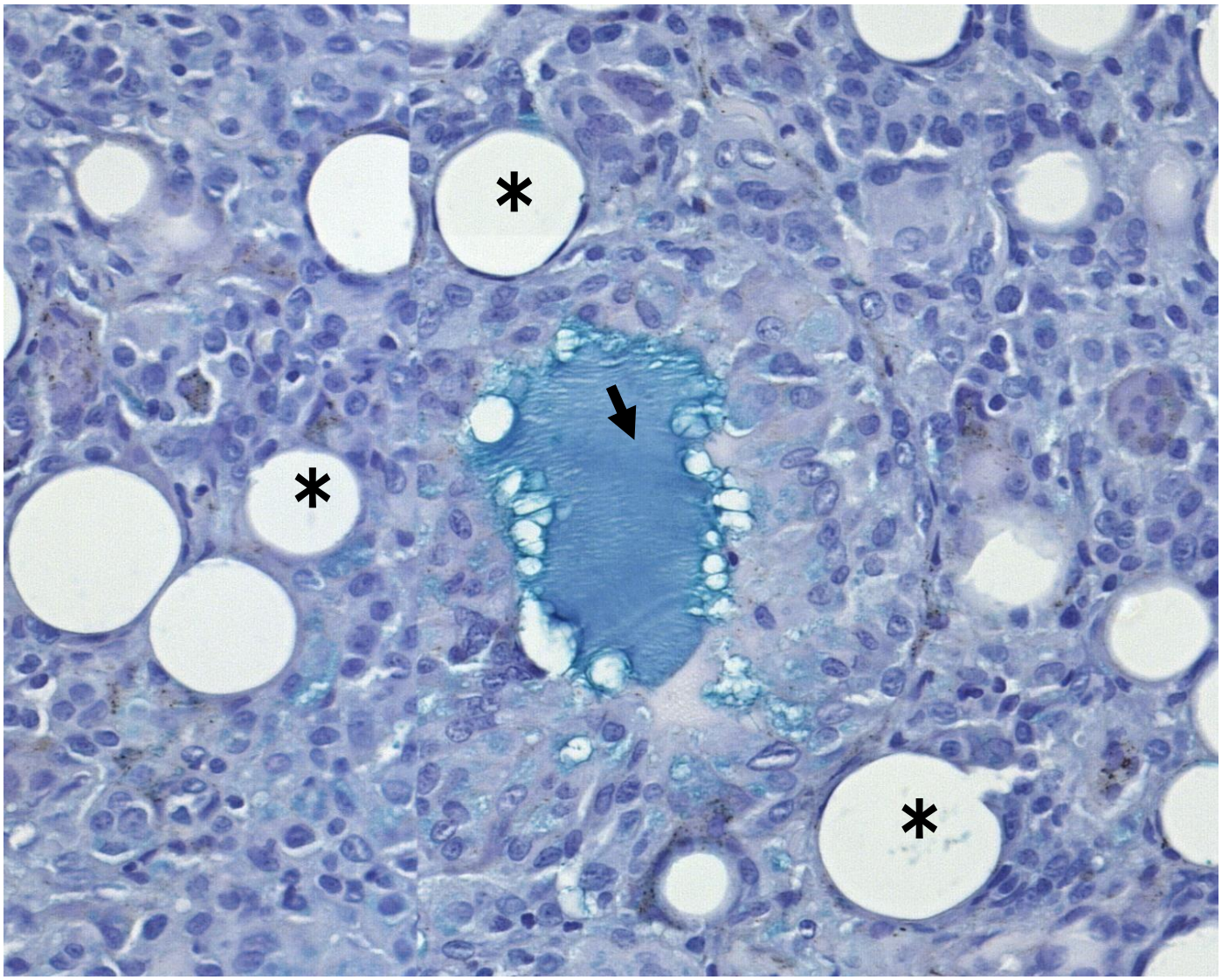

FIG 32. Hyaluronic acid pool positive for Alcian blue (arrow), surrounded by PMMA vacuoles (asterisks).

HYALURONIC ACID

Rennova®, Restylane®, Juvéderm®, Perlane®, Hylaform®, Macrolane®

Toluidine blue staining

- Hyaluronic acid has metacromasia for Toluidine blue, showing a purple/ magenta staining (Fig. 33).

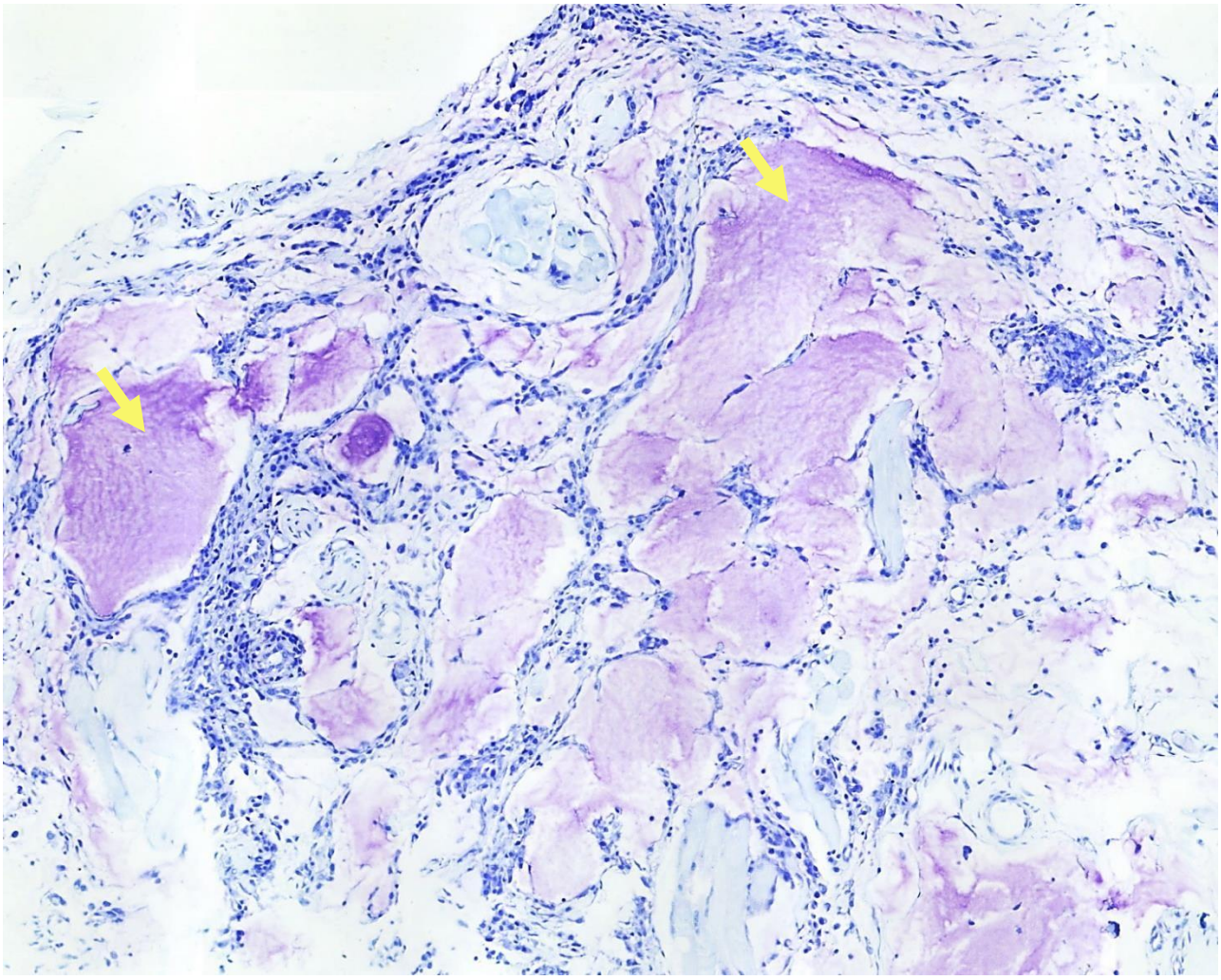

FIG 33. Metachromasia of hyaluronic acid (arrows) (Toluidine blue staining).

- Mast cells were not found in reactions against hyaluronic acid.

HYALURONIC ACID

Rennova®, Restylane®, Juvéderm®, Perlane®, Hylaform®, Macrolane®

Toluidine blue staining

- Toluidine blue staining is also useful in identifying hyaluronic acid between other fillers in the same slide (Fig. 34).

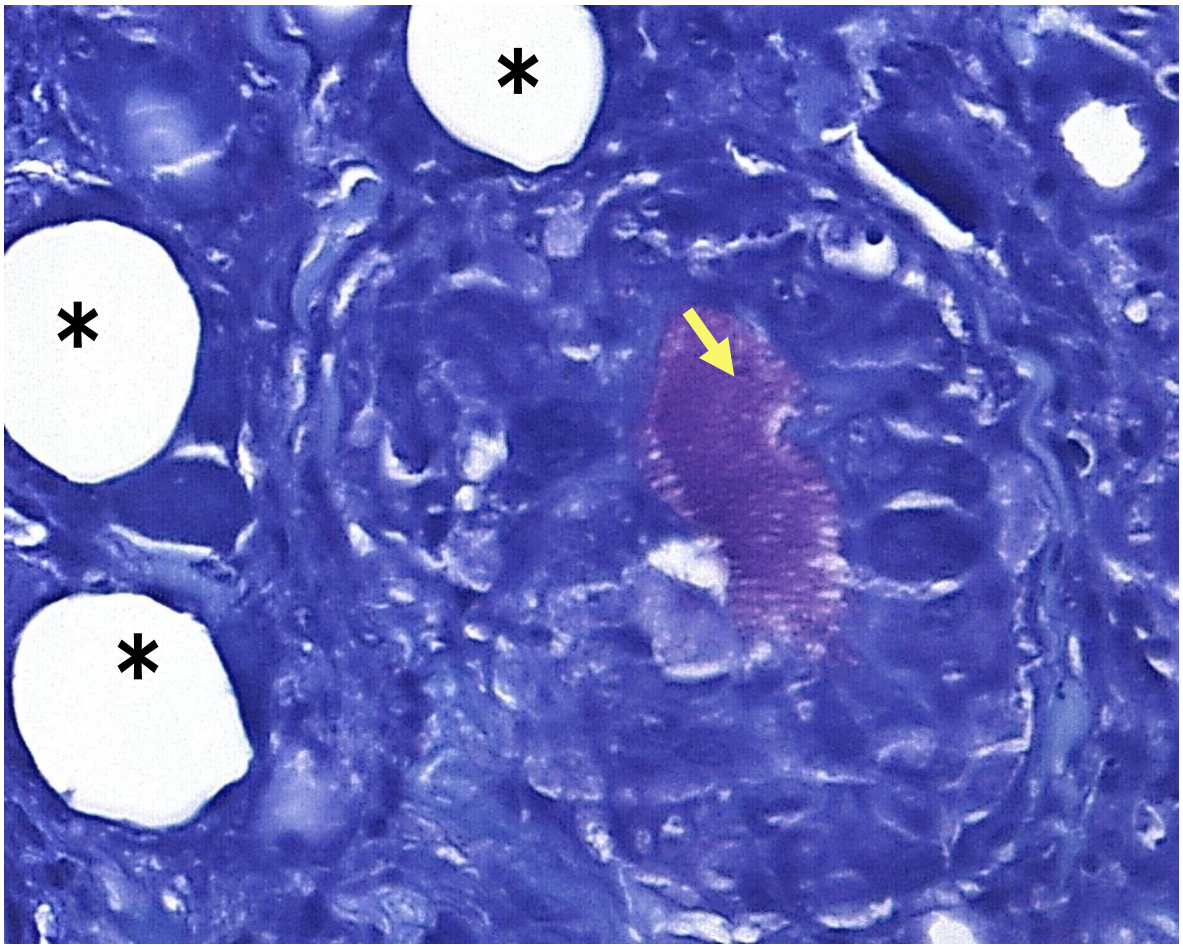

FIG 34. Hyaluronic acid pool (arrow) with metacromasia, surrounded by PMMA vacuoles (asterisks). Same case shown in Fig. 32 (Toluidine blue staining).

HYALURONIC ACID

Rennova®, Restylane®, Juvéderm®, Perlane®, Hylaform®, Macrolane®

Sirius red staining

- Eosinophils are very rare in reactions to hyaluronic acid (Fig. 35).

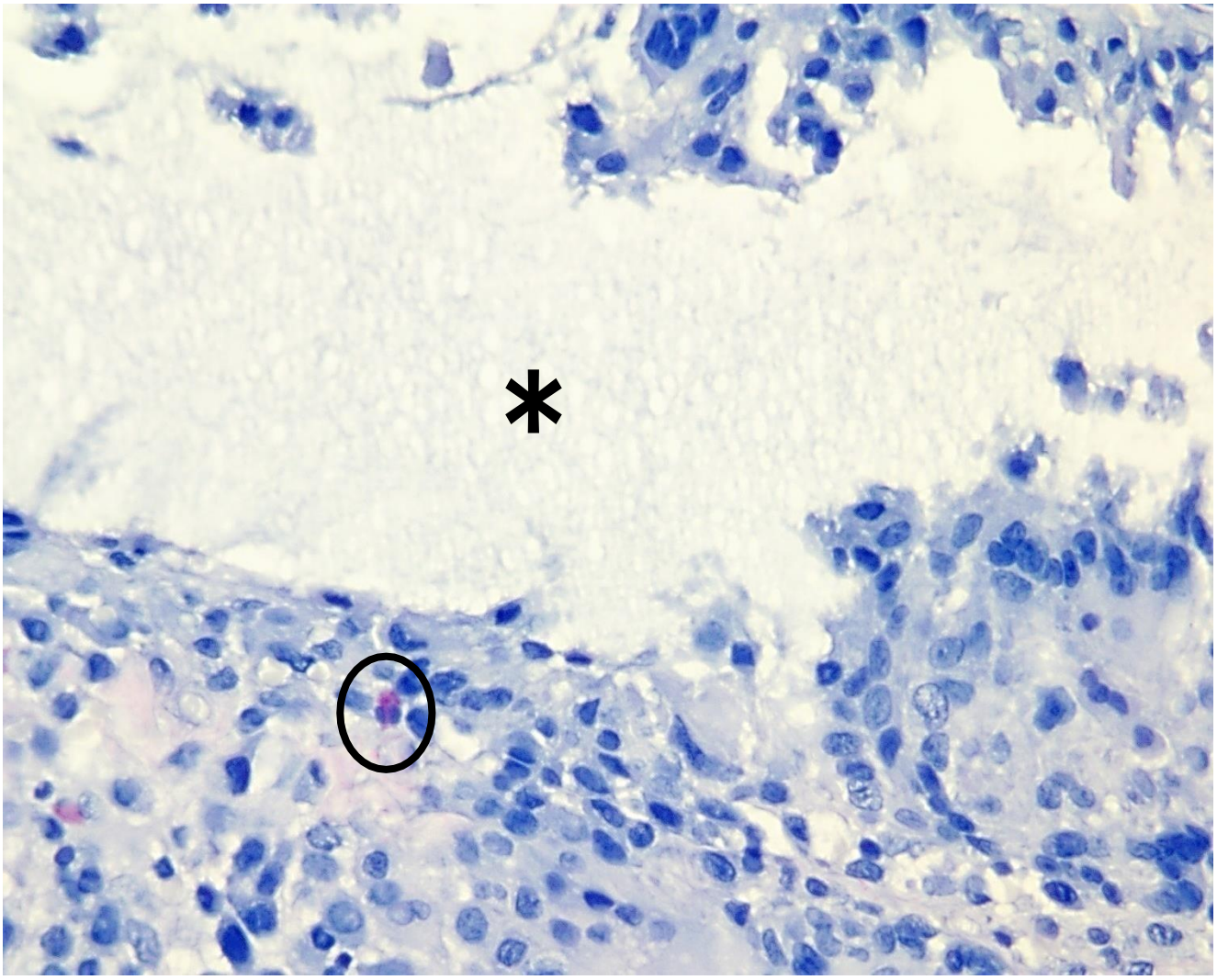

FIG 35. Eosinophil (circle) in adverse reaction to hyaluronic acid (asterisk) (Sirius red staining).

HYALURONIC ACID

Rennova®, Restylane®, Juvéderm®, Perlane®, Hylaform®, Macrolane®

Immunohistochemistry for CD68

- When the reaction forms the cyst-like structures, the palisading macrophages are evidenced by the CD68 staining (Fig. 36).

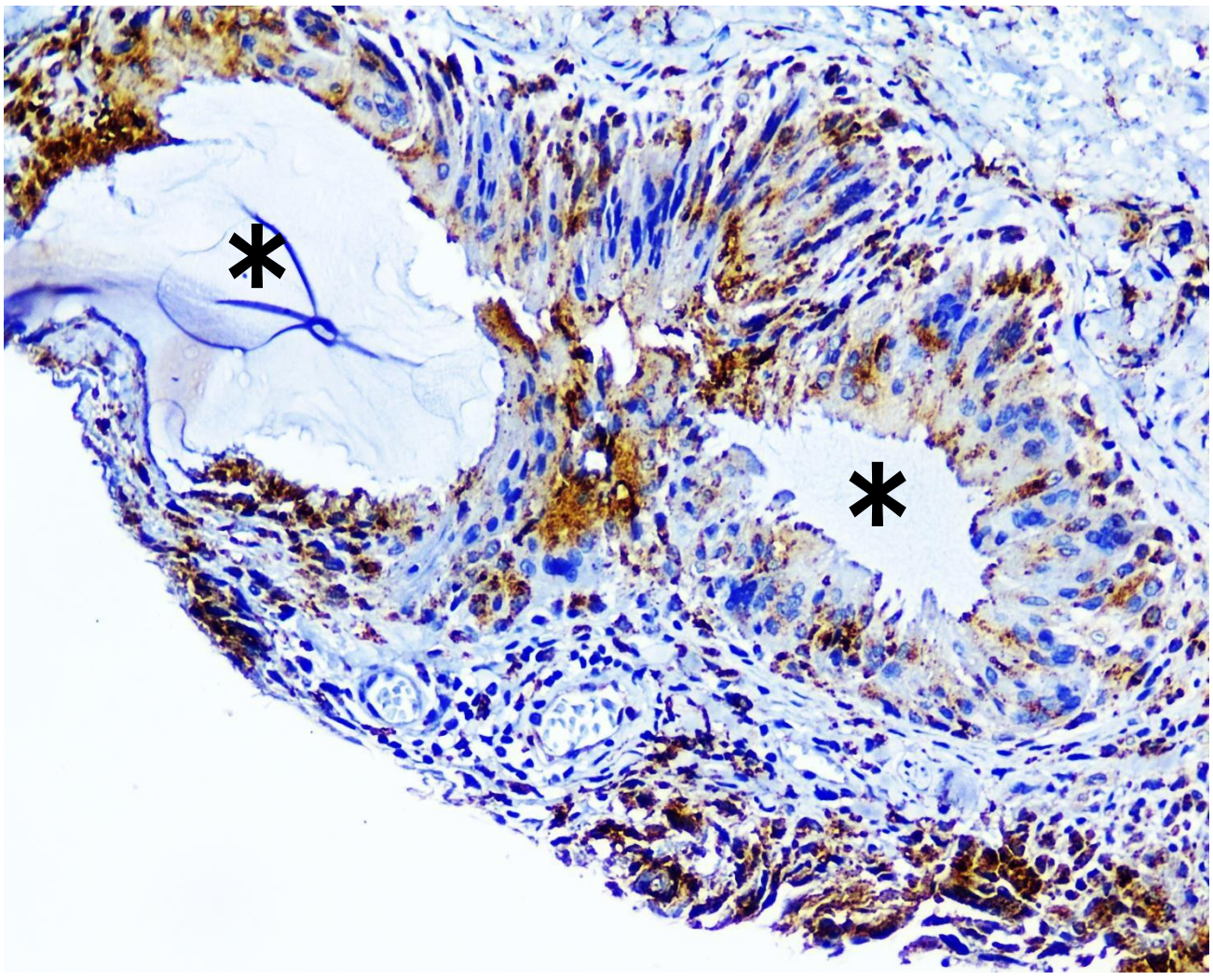

FIG 36. CD68 positivity in palisading macrophages surrounding hyaluronic acid pools (asterisk) (HRP-polymer immunohistochemistry).

HYALURONIC ACID

Rennova®, Restylane®, Juvéderm®, Perlane®, Hylaform®, Macrolane®

Immunohistochemistry for CD20

- CD20-positive lymphocytes are scant next to the material (Fig. 37).

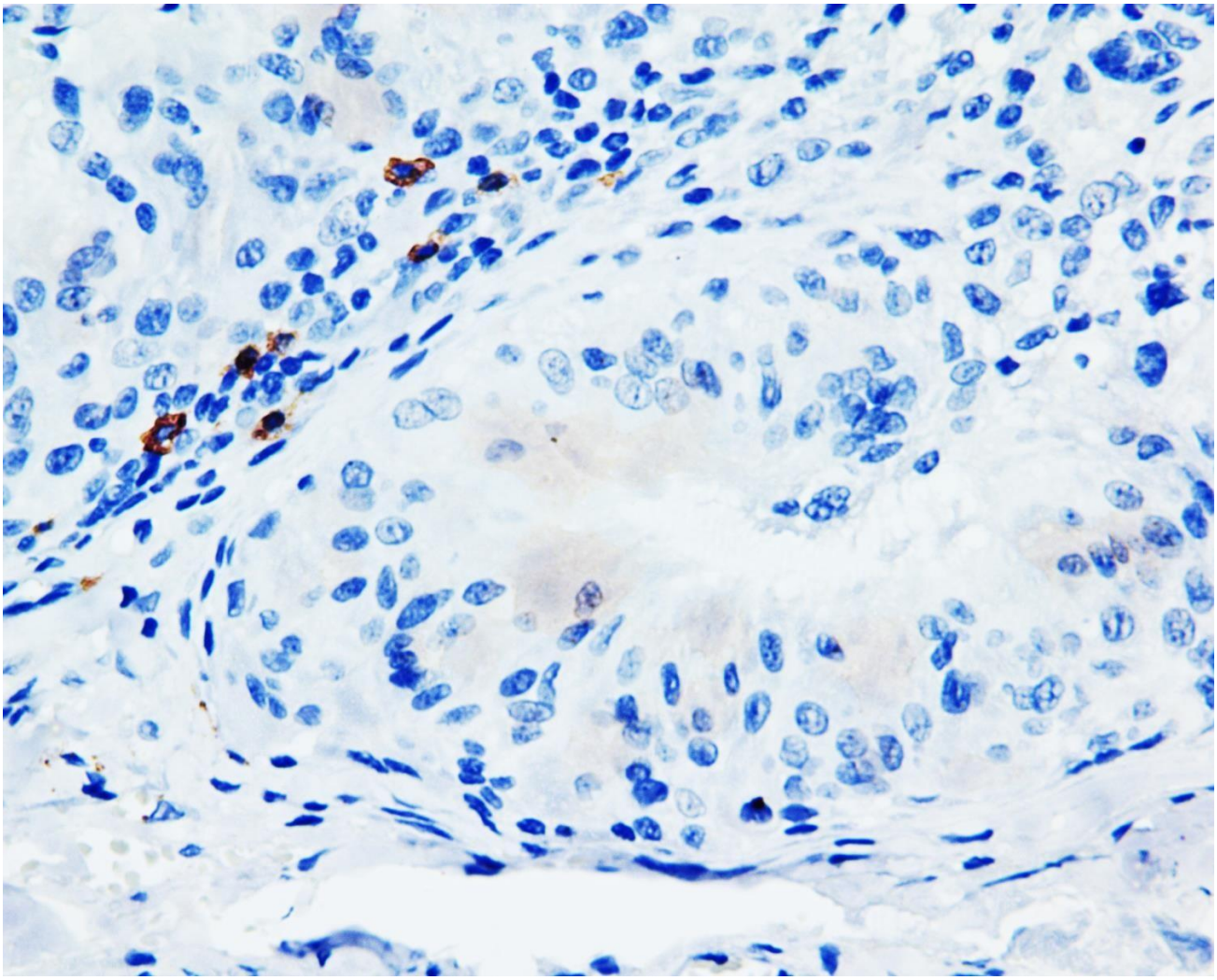

FIG 37. Immunohistochemistry for CD20 marker in adverse reaction to hyaluronic acid (HRP-polymer immunohistochemistry).

HYALURONIC ACID

Rennova®, Restylane®, Juvéderm®, Perlane®, Hylaform®, Macrolane®

Immunohistochemistry for CD3

- A moderate amount of CD3-positive lymphocytes is observed next to the material (Fig. 38).

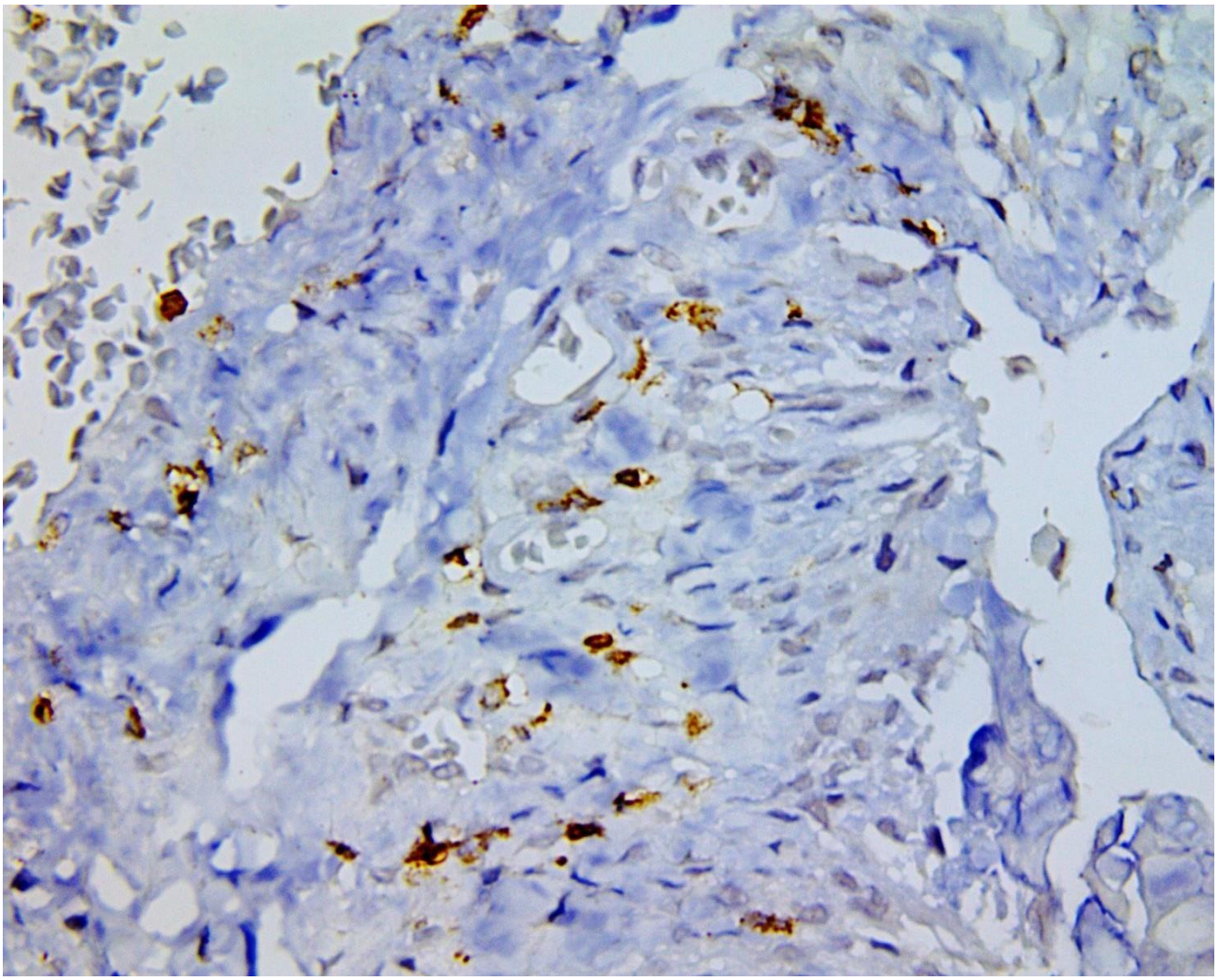

FIG 38. Immunohistochemistry for CD3 marker in adverse reaction to hyaluronic acid (HRP-polymer immunohistochemistry).

HYALURONIC ACID *versus* MUCOEPIDERMOID CARCINOMA: Differential diagnosis

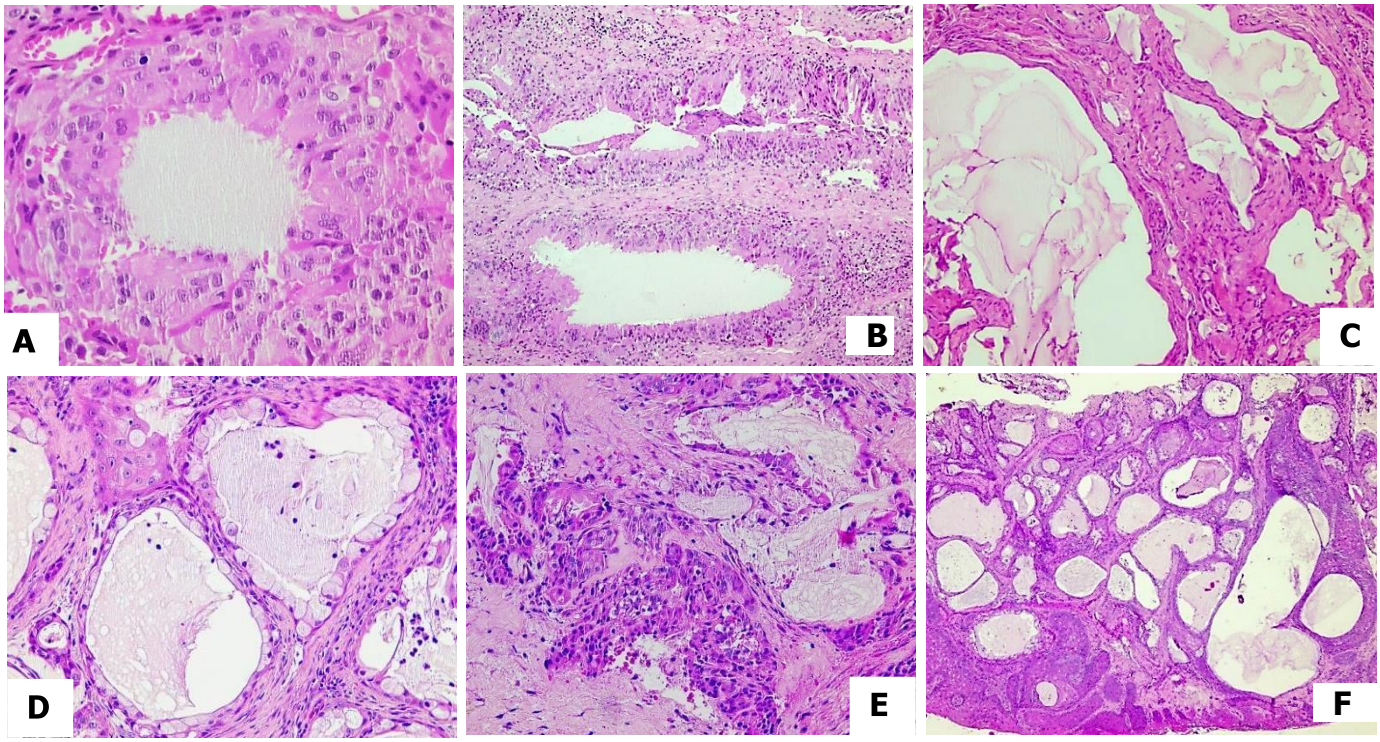

FIG 39. A, B, C: Hyaluronic acid. **D, E, F:** mucoepidermoid carcinoma (Hematoxylin-eosin staining).

- Cystic-like structures (Fig. 40A-B) or "pools" of hyaluronic acid (Fig. 40C) may be misinterpreted as a low-grade mucoepidermoid carcinoma (Fig. 39D-F)[18,19].

- As mucoepidermoid carcinoma is also positive for Alcian blue in mucous cells[20], immunohistochemistry can help differential diagnosis. Mucoepidermoid carcinoma is negative for CD68 and positive for membrane-bound mucins markers, p63 and p40.[20,21]

POLYACRYLAMIDE HYDROGEL

Aquamid®

- Composed by 2.5% polyacrylamide and 97.5% water.[11]
- The material presents as dark basophilic pools.
- Sometimes, microfoaming can be seen in high magnification (Fig. 40).

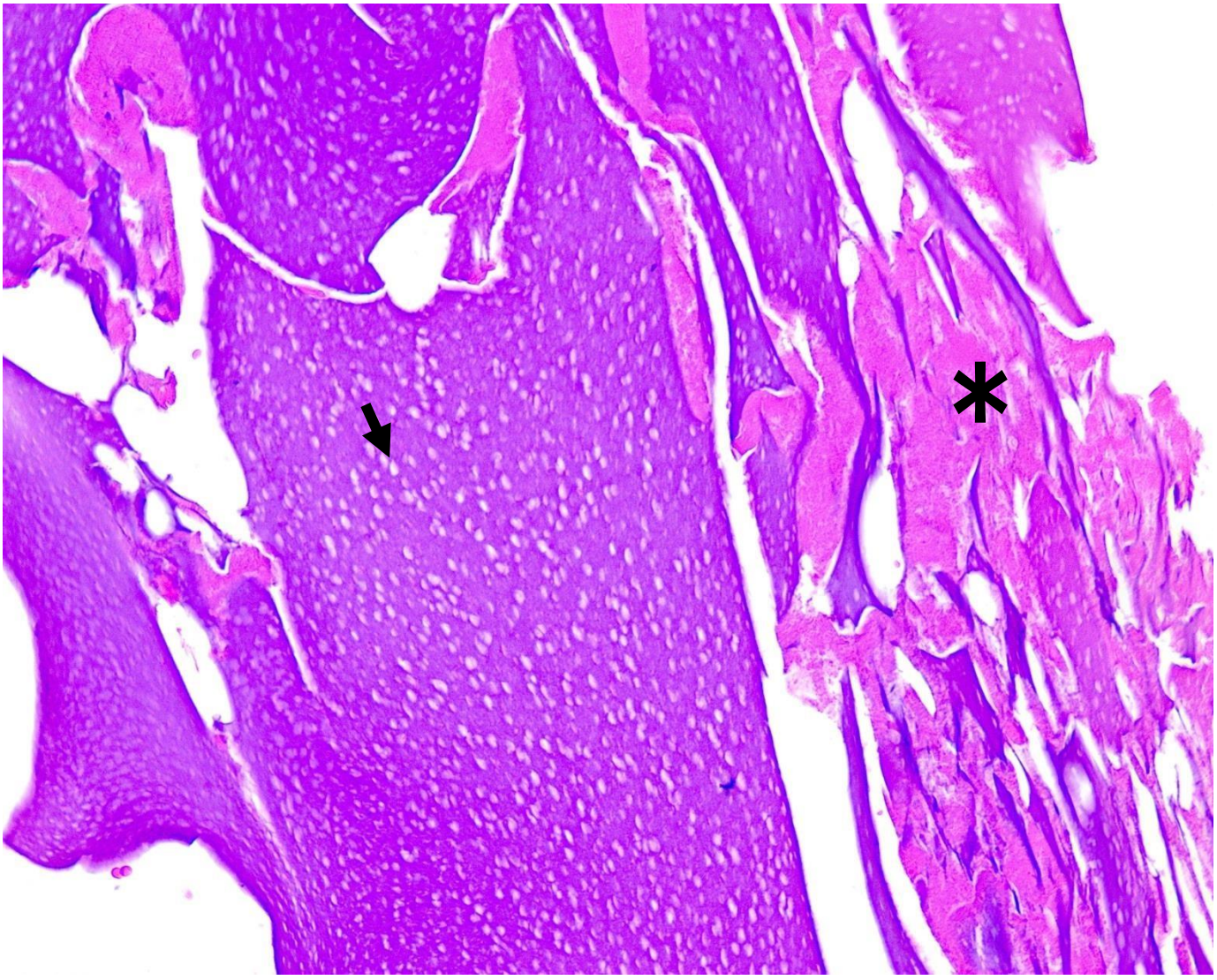

FIG 40. Adverse reaction to polyacrylamide hydrogel showing a microfoaming aspect (arrow), in a necrotic background (asterisk) (Hematoxylin-eosin staining).

POLYACRYLAMIDE HYDROGEL

Aquamid®

- Usually shows branches of microfibrils on the periphery of the material "pools", like a contracted material (Fig. 41A-B).
- Unlike hyaluronic acid, the polyacrylamide hydrogel is usually associated with many giant cells (Fig. 41C-D)

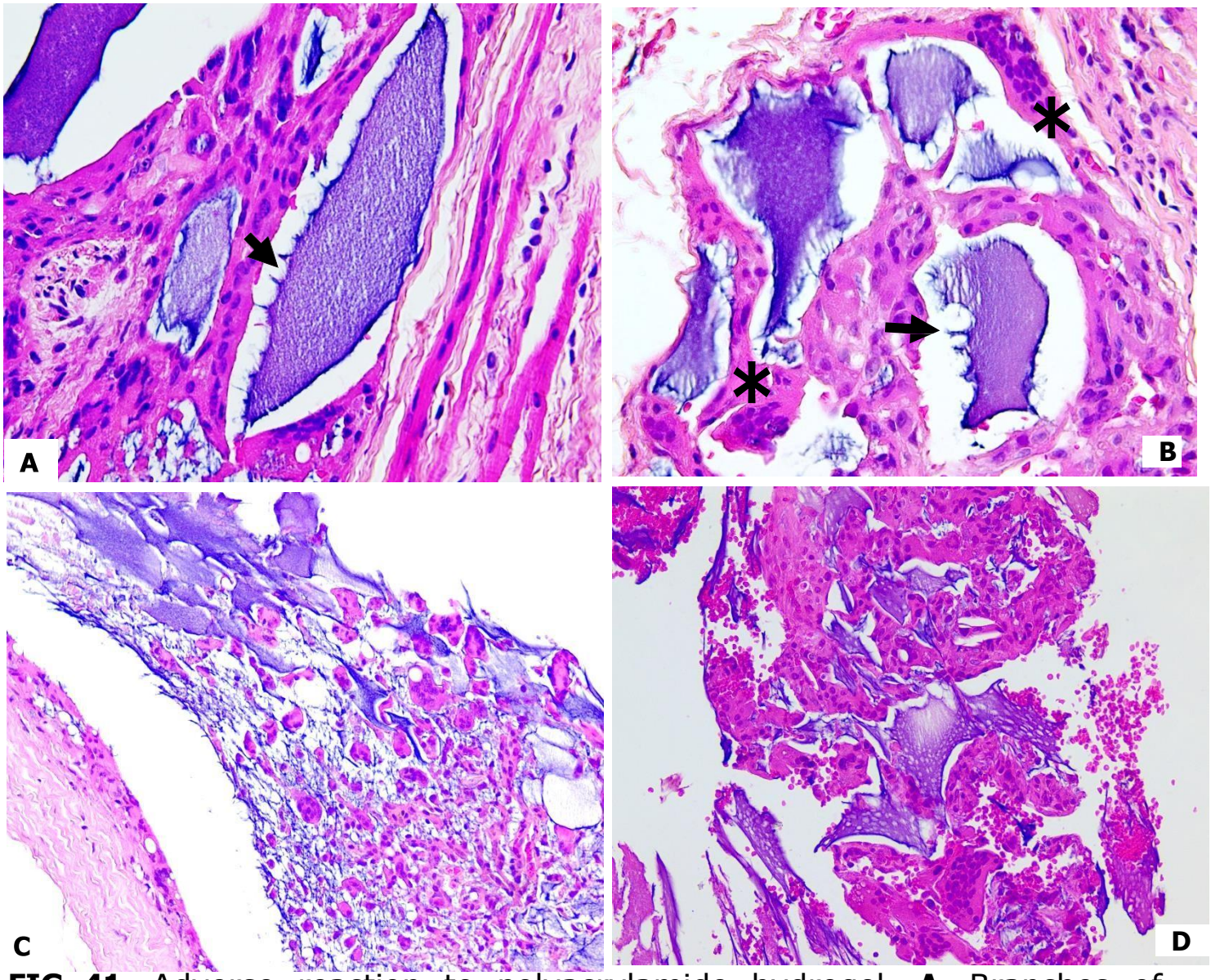

FIG 41. Adverse reaction to polyacrylamide hydrogel. **A.** Branches of microfibrils on the periphery of the material (arrow). **B.** Several "pools" of the material showing microfibribrils (arrow) and giant cells (asterisks) surrounding. **C-D.** Note numerous giant cells **(C,D)** (Hematoxylin-eosin staining).

POLYACRYLAMIDE HYDROGEL

Aquamid®

Alcian blue staining

- Alcian blue staining was negative in the samples evaluated, although other studies reported a positive staining.[11,22]

Toluidine blue staining

- Polyacrylamide hydrogel has metacromasia for Toluidine blue, showing a purple/ magenta staining (Fig. 42).
- Mast cells were not found in reactions against polyacrylamide hydrogel.

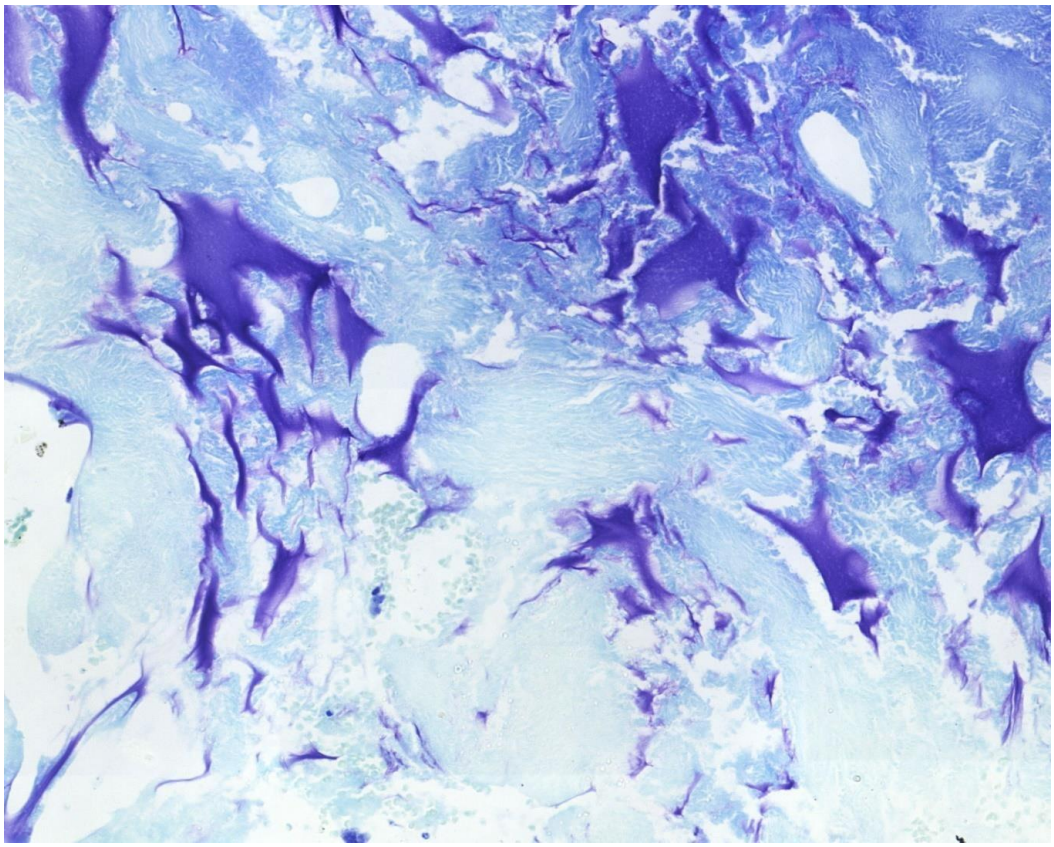

FIG 42. Polyacrylamide hydrogel has metachromasia for toluidine blue staining.

POLYACRYLAMIDE HYDROGEL

Aquamid®

Sirius red staining

- Scant eosinophils are seen in inflammatory infiltrate by polyacrylamide hydrogel (Fig. 43).

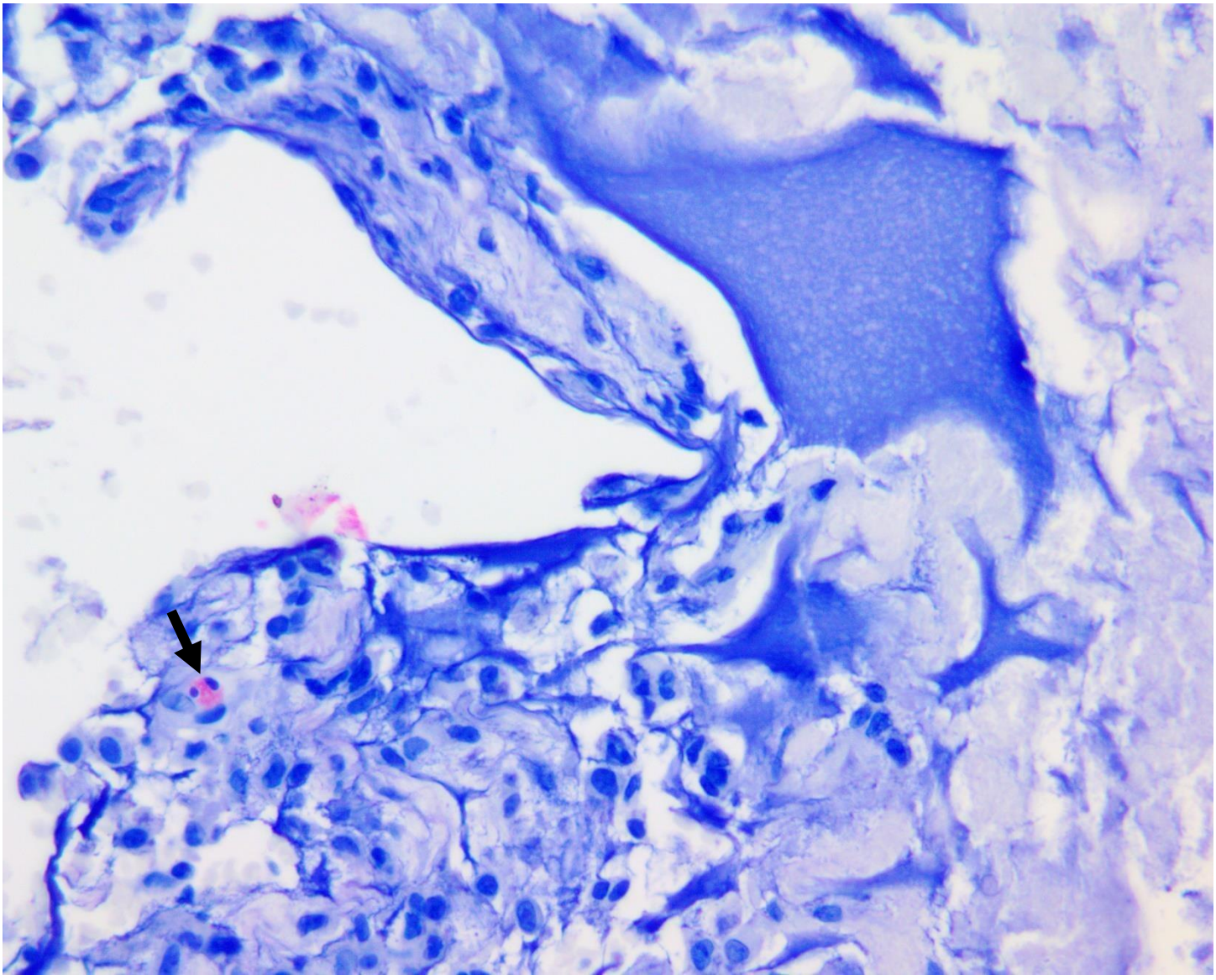

FIG 43. Adverse reaction to polyacrylamide hydrogel showing eosinophil (arrow). (Sirius red staining).

POLYACRYLAMIDE HYDROGEL

Aquamid®

Immunohistochemistry for CD68

- The numerous giant cells and macrophages are evidenced by the CD68 staining (Fig. 44).

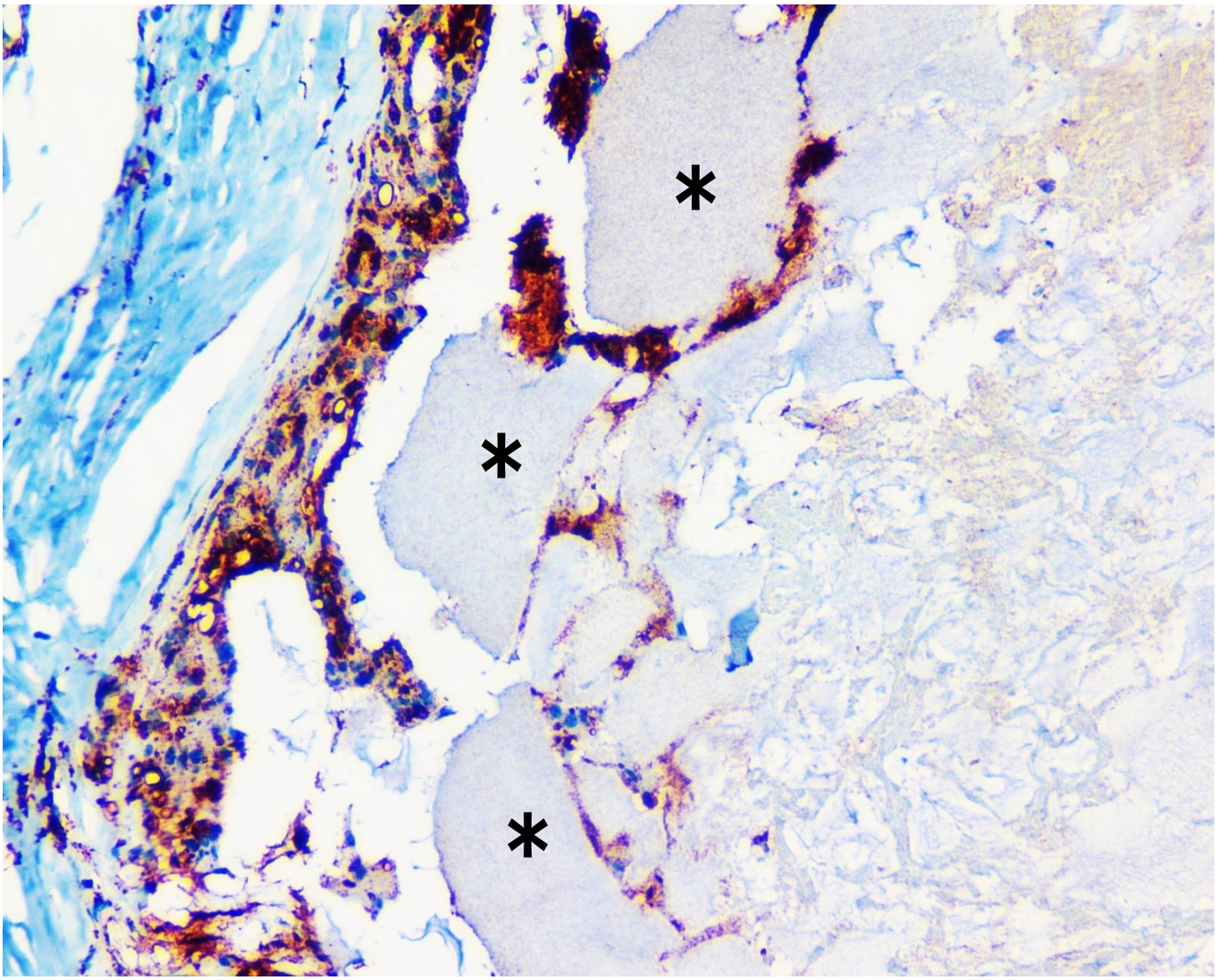

FIG 44. Immunohistochemistry for CD68 in adverse reaction to polyacrylamide hydrogel (arrow) (HRP-polymer immunohistochemistry).

POLYACRYLAMIDE HYDROGEL

Aquamid®

Immunohistochemistry for CD20

- No CD20-positive cells are expected.

Immunohistochemistry for CD3

- There is scant CD3-positive lymphocytes next to the material (Fig. 45).

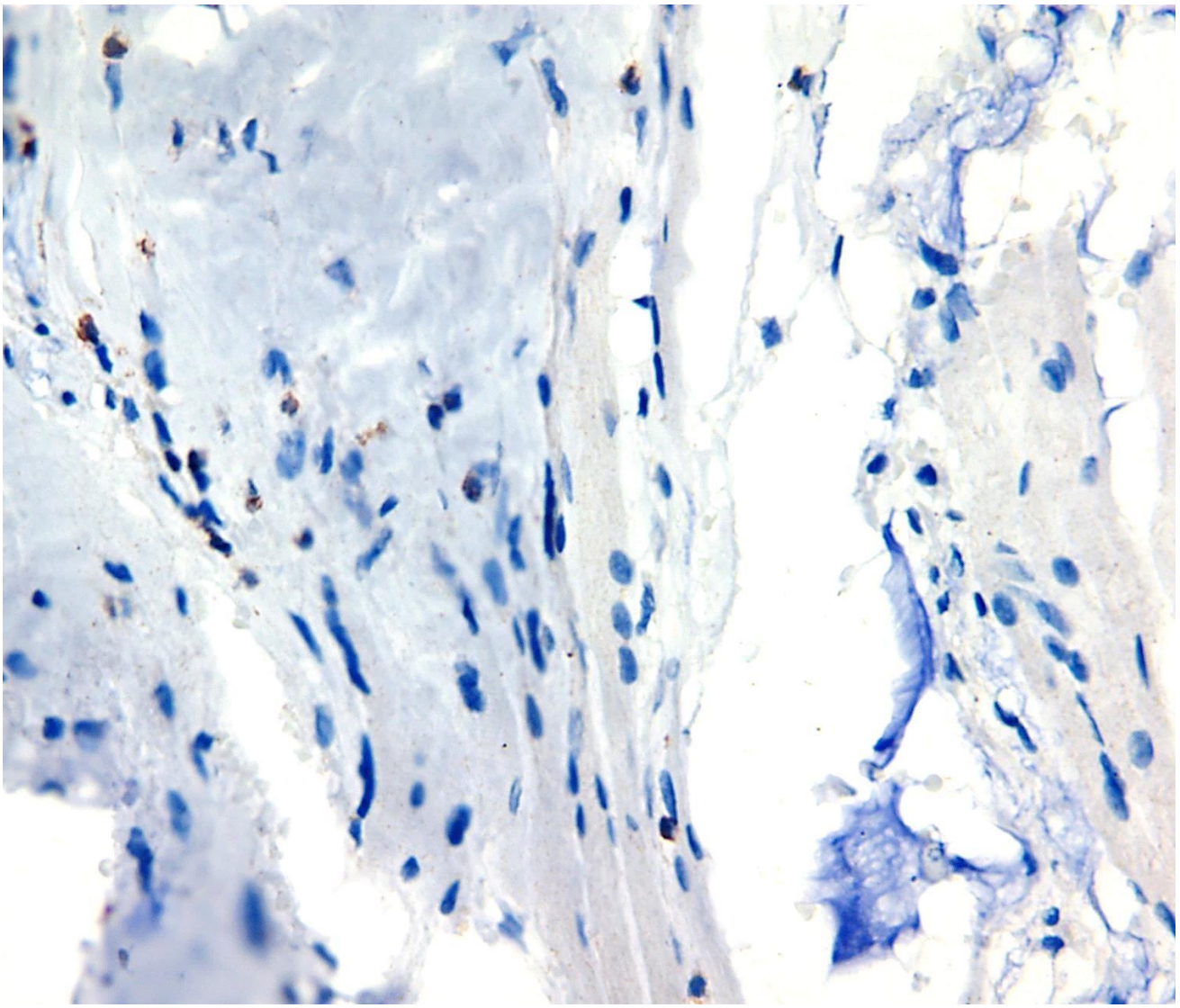

FIG 45. Immunohistochemistry for CD3 marker in adverse reaction to polyacrylamide hydrogel (HRP-polymer immunohistochemistry).

HYALURONIC ACID and POLYACRYLAMIDE HYDROGEL:

Differential diagnosis of fillers deposited as acellular "pools"

HYALURONIC ACID

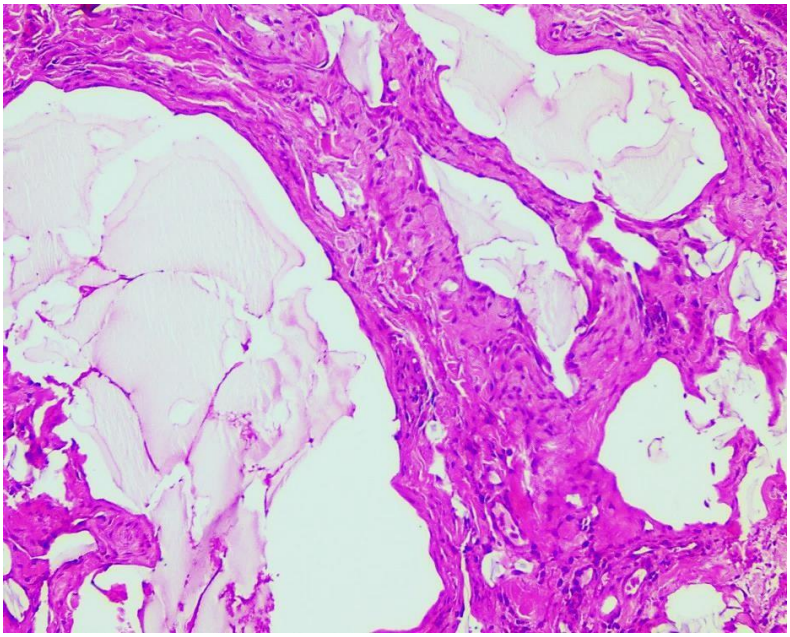

FIG 46. Adverse reaction to hyaluronic acid (Hematoxylin-eosin staining).

POLYACRYLAMIDE HYDROGEL

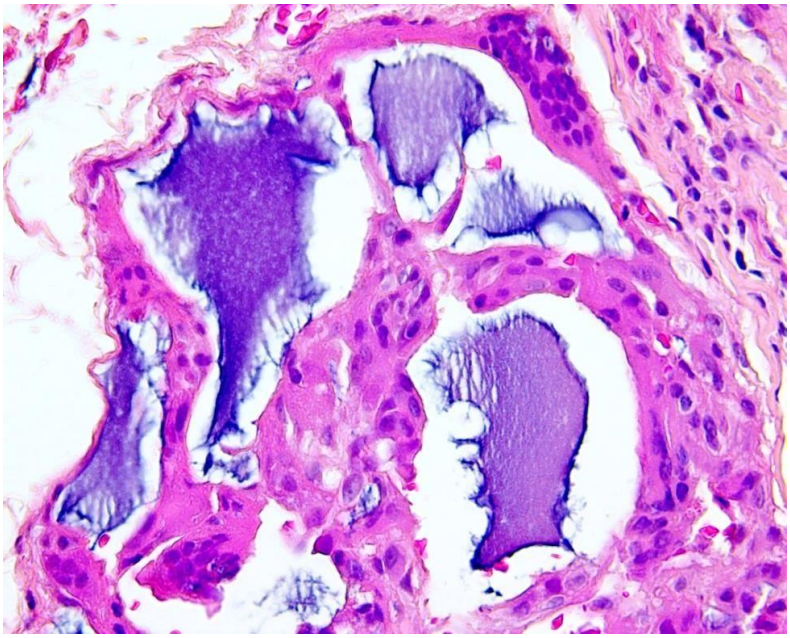

FIG 47. Adverse reaction to polyacrylamide hydrogel (Hematoxylin-eosin staining).

HYALURONIC ACID and POLYACRYLAMIDE HYDROGEL:

Differential diagnosis of fillers deposited as acellular "pools"

BOX 2. Histopathological differential diagnosis between polyacrylamide hydrogel and hyaluronic acid.

HYALURONIC ACID	POLYACRYLAMIDE HYDROGEL
Uniform appearance	Microfoamy appearance (at high magnification)[22]
Usually there is no microfibril branches on the periphery, without contraction effect	Branches of microfibrils on the periphery of the "pools", as a contracted material
Mild inflammatory reaction, with a small number of giant cells[22]	Marked granulomatous reaction with a large number of giant cells[22]
Usually presnts a light staining on H&E	Usually presents dark staining on H&E
May form palisading histiocytes	No palisaded histiocytes
Digestible with hyaluronidase[18]	Not digestible to hyaluronidase
Metachromasia on Toluidine blue staining	Metachromasia on Toluidine blue staining[22]

CALCIUM HYDROXYAPATITE

Radiesse®, Radiance®

- This filler stimulates the endogenous production of collagen and it is composed by calcium hydroxyapatite ($Ca_{10}(PO)_6OH_2$) plus carboxymethylcellulose and glycerine.[3]

- Reactions to calcium hydroxyapatite can show nodular or diffuse arrangement (Fig. 48 A-B).

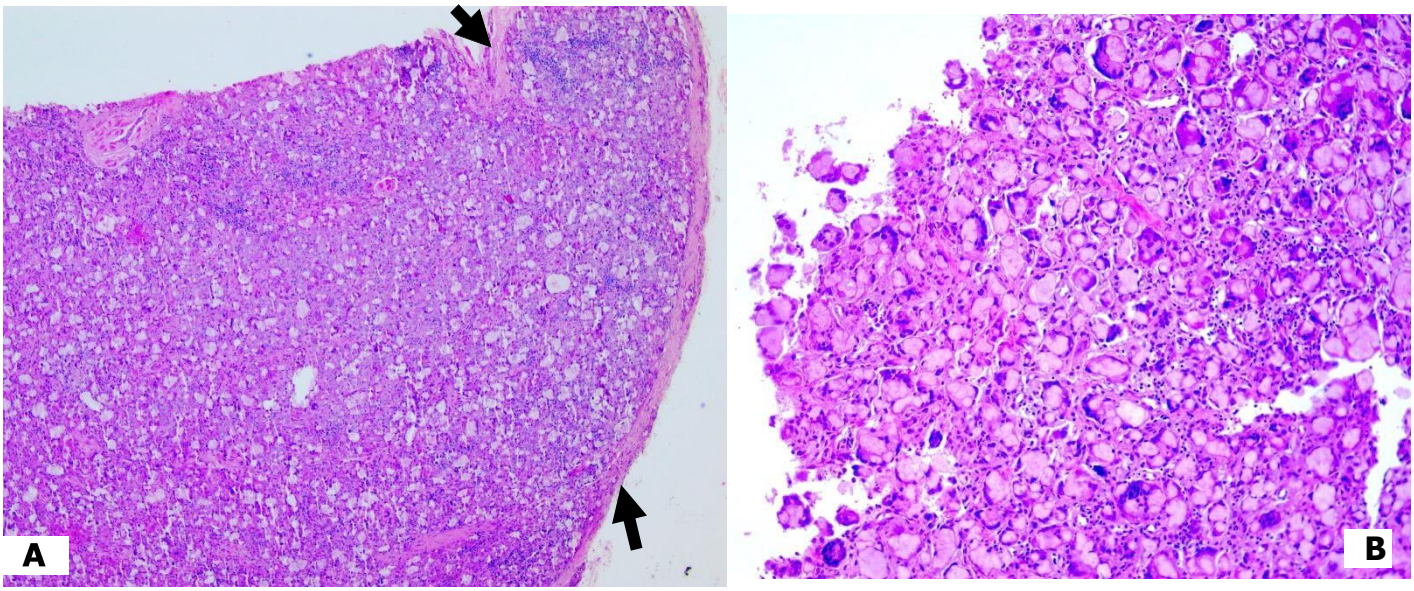

FIG 48. A. Nodular arrangement of adverse reaction to calcium hydroxyapatite. Note the capsule surrounding the tissue (arrows). **B**. Diffuse arrangement of adverse reaction to calcium hydroxyapatite. The particles of calcium hydroxyapatite are scattered through the tissue (Hematoxylin-eosin staining).

CALCIUM HYDROXYAPATITE

Radiesse®, Radiance®

- The material presents as polyhedral or spheroid structures, with amorphous, crackled, and glassy-looking appearance. It has a light eosinophilic coloration on H&E (Fig. 49, 50).

- Typically, there is a chronic inflammation with many lymphocytes and epithelioid hystiocytes around the material (Fig. 49, 50, 55).

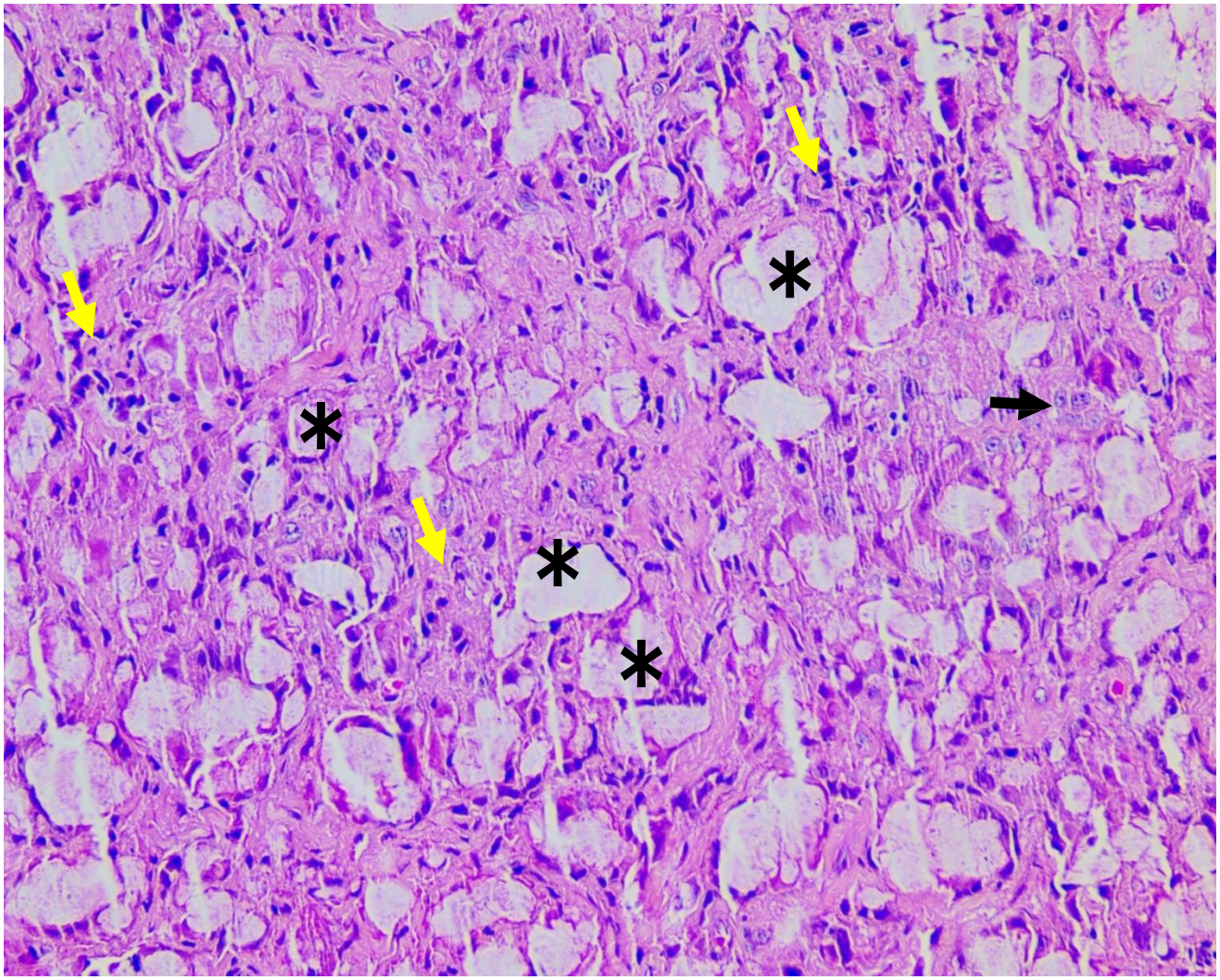

FIG 49. Polyhedral structures (asterisks) in a fibrous connective tissue background with many lymphocytes (yellow arrow) and some epithelioid hystiocytes (black arrow) (Hematoxylin-eosin staining).

CALCIUM HYDROXYAPATITE

Radiesse®, Radiance®

- Giant cells also may be seen (Fig. 50), whereas asteroid bodies were not found in this sample, but was reported by literature.[23]
- A classic foreign body granuloma reaction may be present, though it is not common.[3]

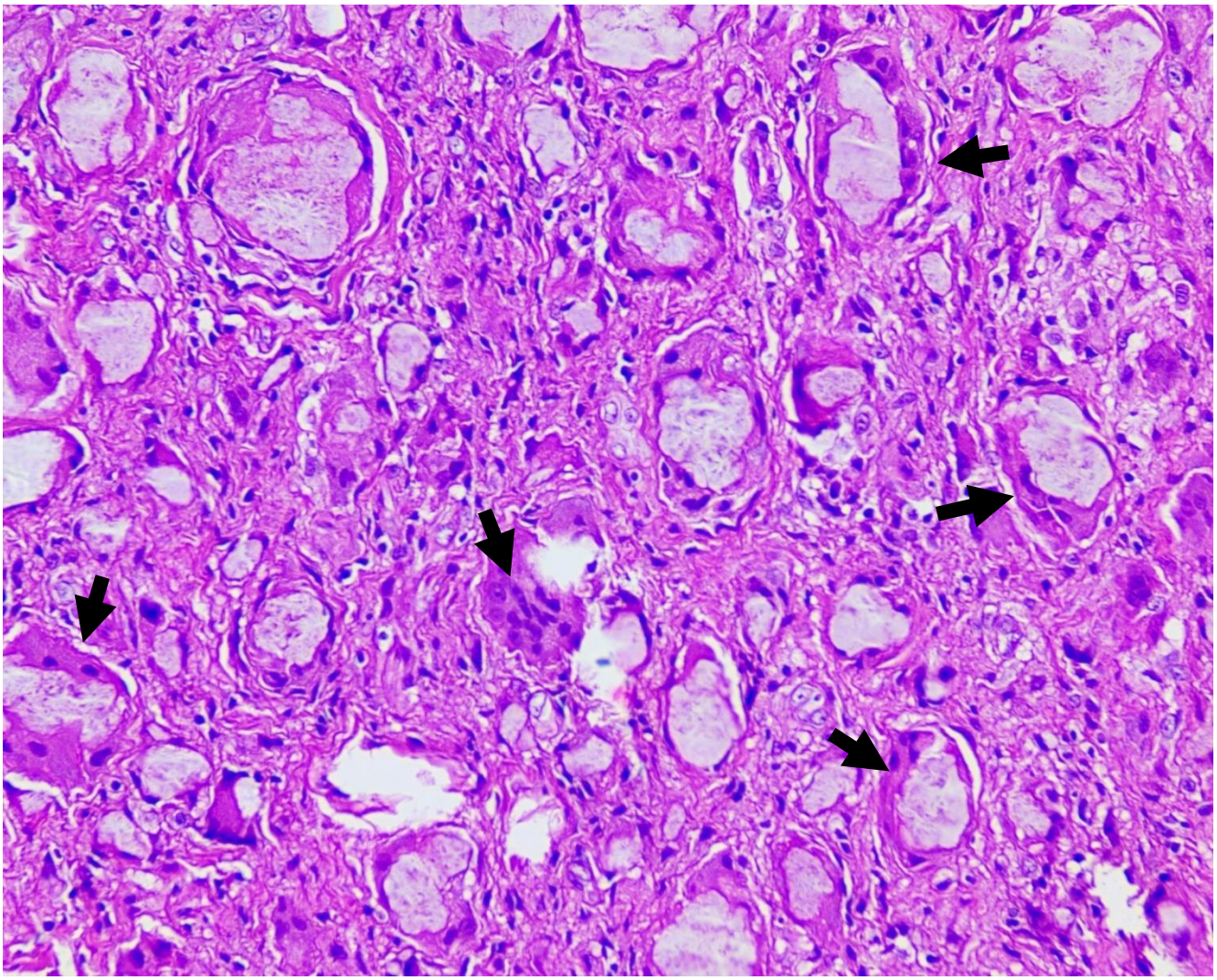

FIG 50. Multinucleated giant cells (arrows) surrounding and phagocyting the material in a fibrous connective tissue background with chronic inflammation (Hematoxylin-eosin staining).

CALCIUM HYDROXYAPATITE

Radiesse®, Radiance®

Alcian blue staining

- Previous studies reported a negative staining for Alcian blue in calcium hydroxyapatite.[3,5,21] In our sample, two cases were positive and one negative.

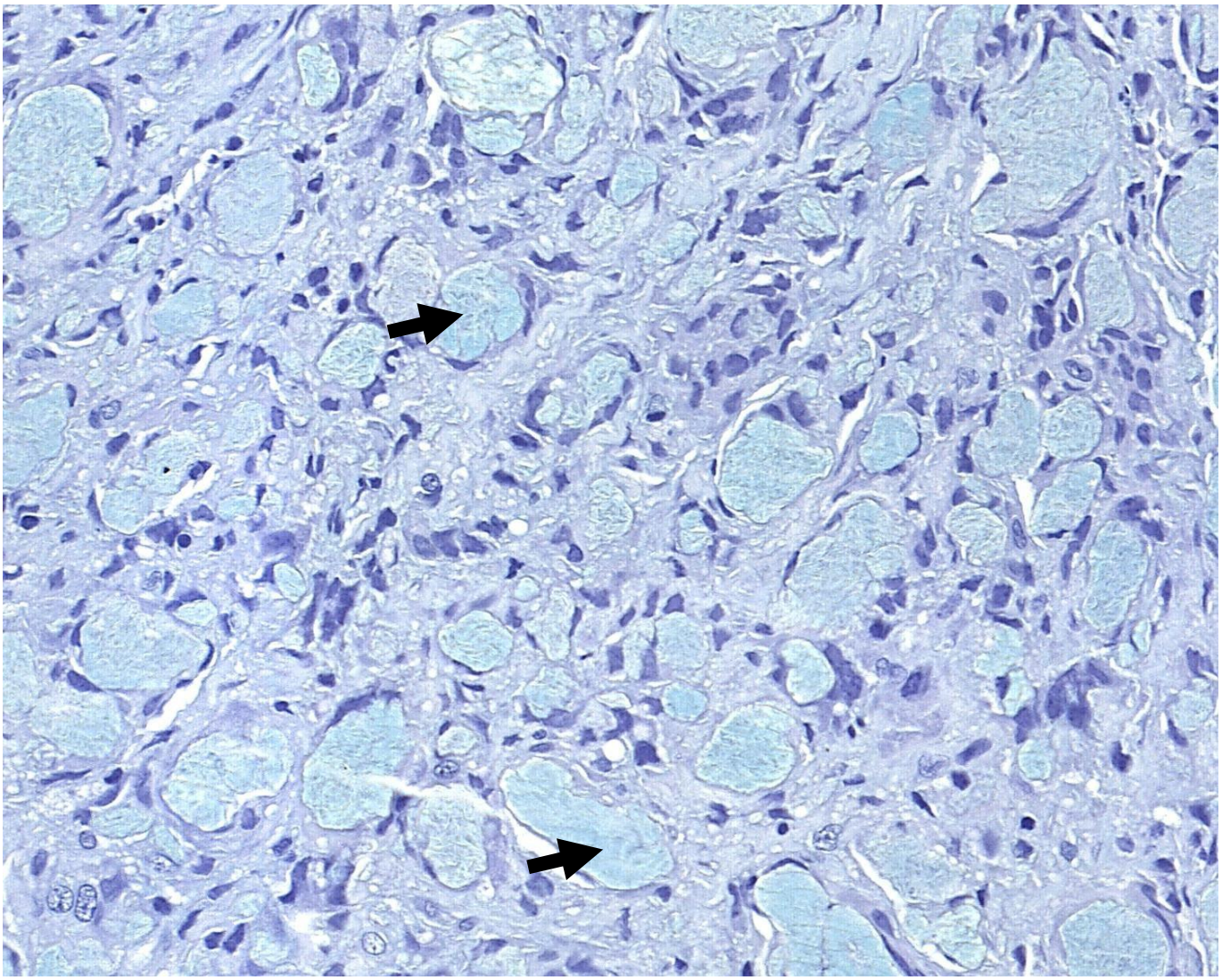

FIG 51. Alcianophilic polyhedral structures of calcium hydroxyapatite (arrows) (Alcian blue staining).

CALCIUM HYDROXYAPATITE

Radiesse®, Radiance®

Toluidine blue staining

- Numerous mast cells are found in reactions to calcium hydroxyapatite (Fig. 52).
- The material itself has a light metachromasia (Fig. 52).

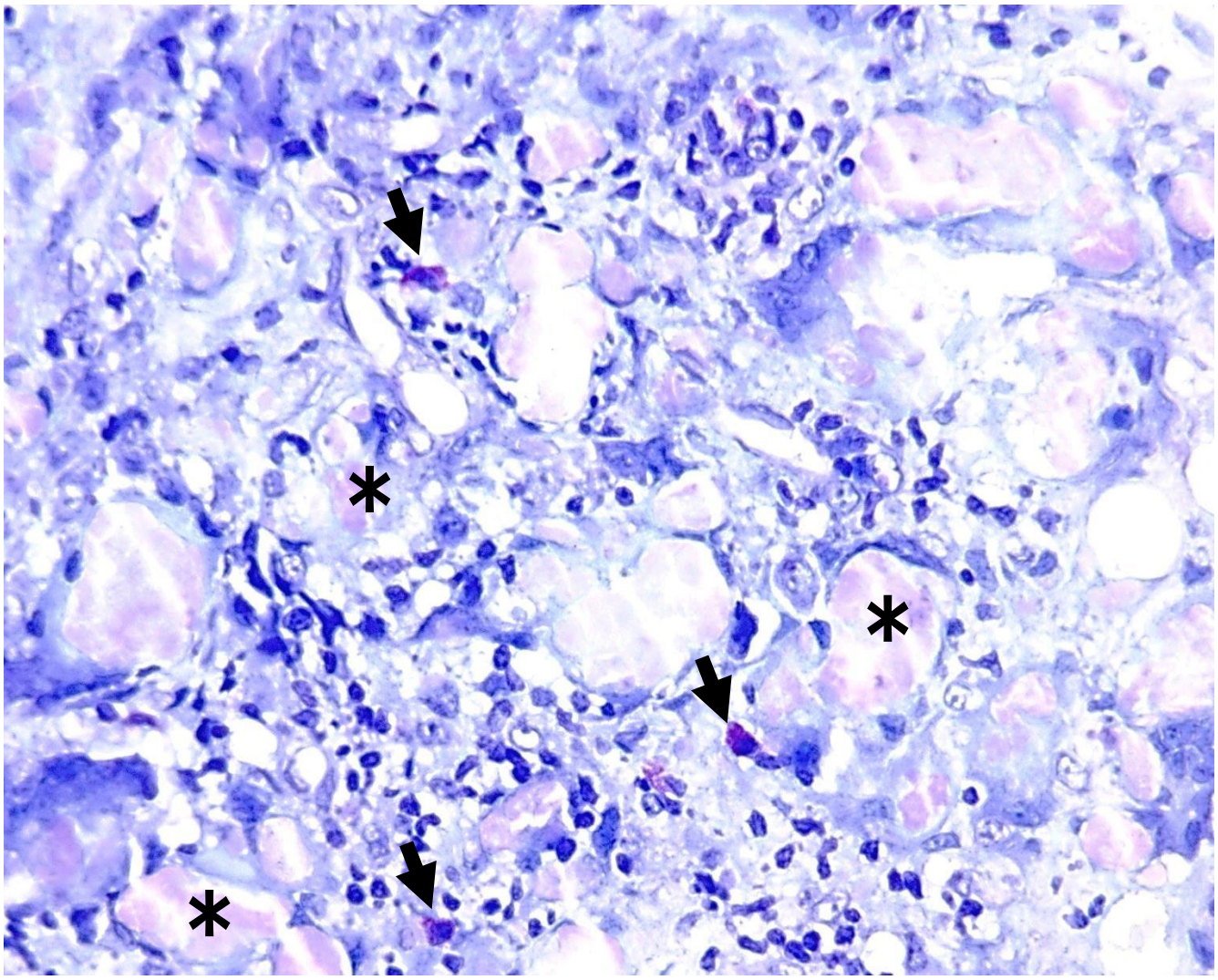

FIG 52. Calcium hydroxyapatite shows a light metacromasia (asterisk). Non-degranulating mast cells (arrows) (Toluidine blue staining).

CALCIUM HYDROXYAPATITE

Radiesse®, Radiance®

Sirius red staining

- A moderate number of eosinophils are seen in inflammatory infiltrate by calcium hydroxyapatite (Fig. 53).

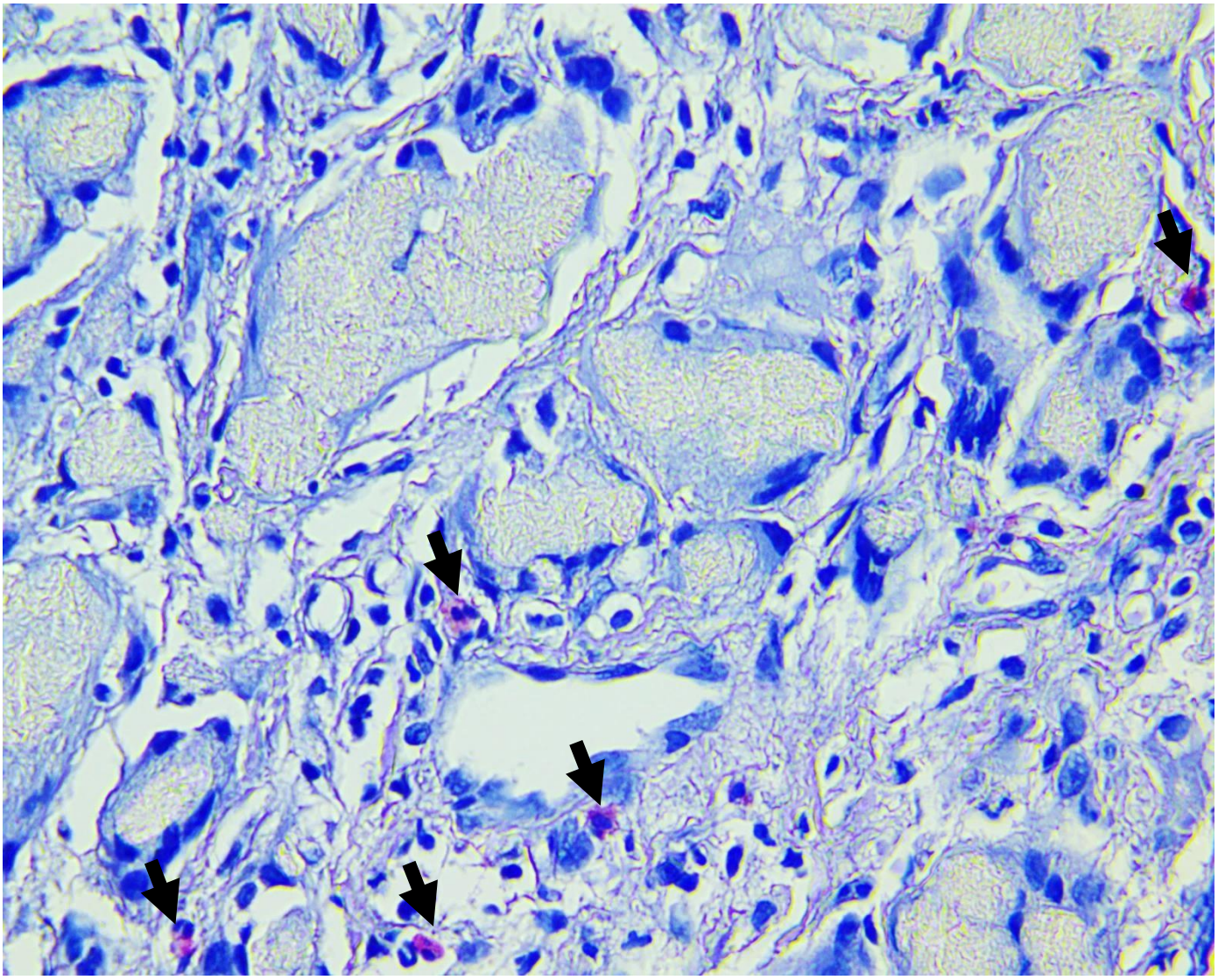

FIG 53. Eosinophils (arrows) in adverse reaction to calcium hydroxyapatite. (Sirius red staining).

CALCIUM HYDROXYAPATITE

Radiesse®, Radiance®

Polarized Light Microscopy

- In our series, the three cases of calcium hydroxyapatite were refractile under polarized light microscopy, revealing a white bright (Fig. 54 A-D). However, other studies reported contrary results.[6,12,24]

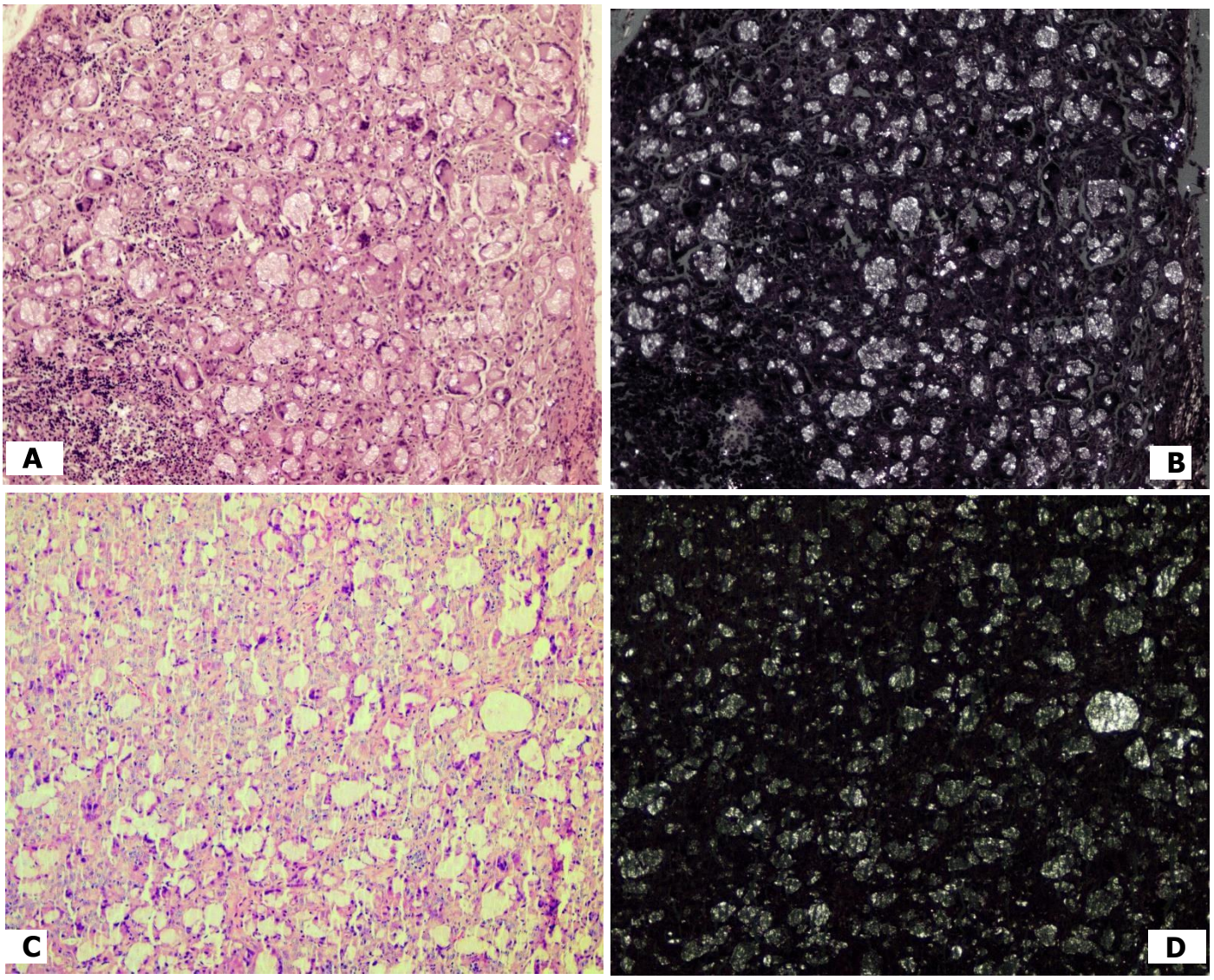

FIG 54 A-D. Two cases (A-B, C-D) of adverse reaction to calcium hydroxyapatite showing a white birefringence under polarized light microscopy (Hematoxylin-eosin staining).

CALCIUM HYDROXYAPATITE

Radiesse®, Radiance®

Immunohistochemistry for CD68

- The giant cells and macrophages are evidenced by the CD68 positivity (Fig. 55).

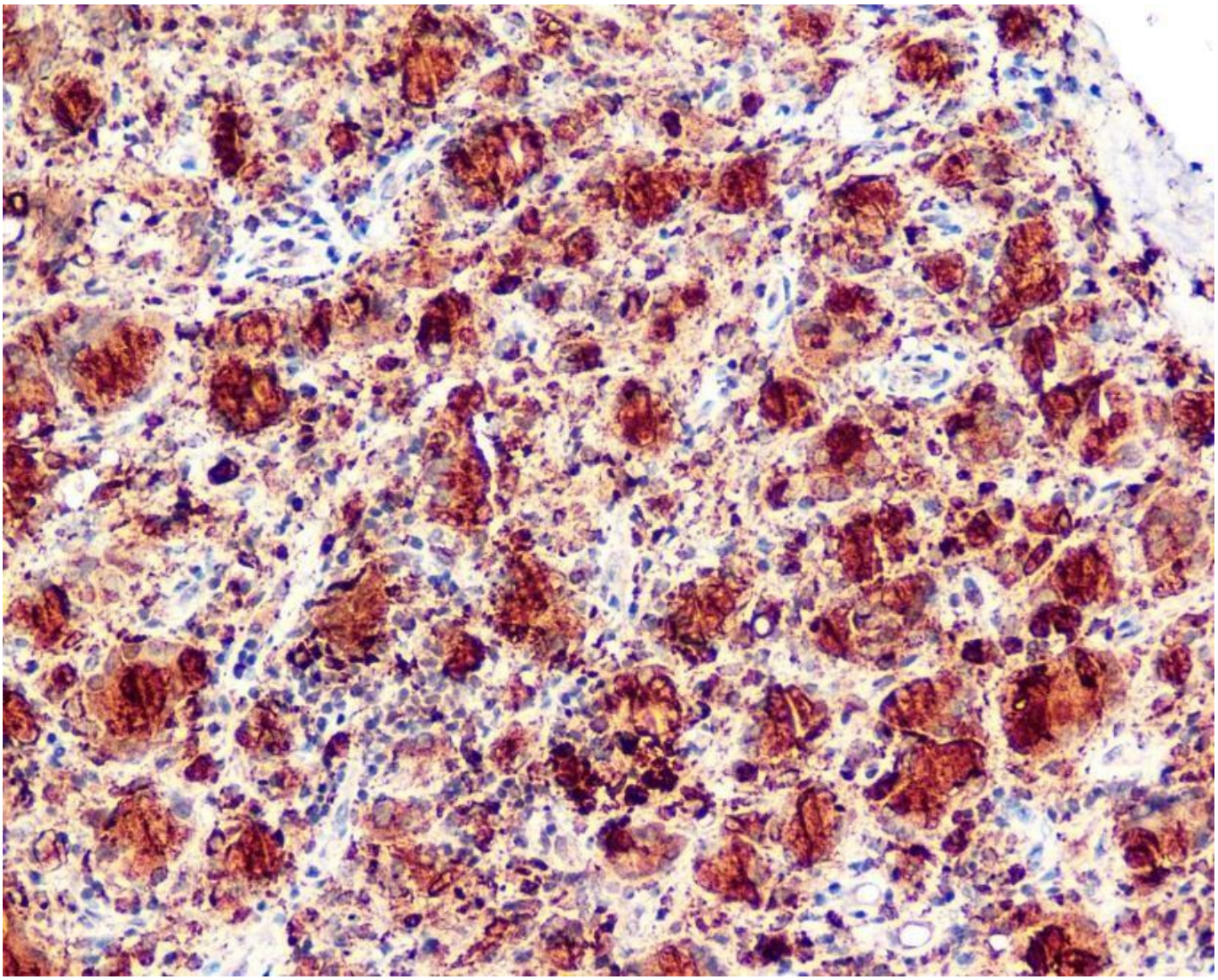

FIG 55. Immunohistochemistry for CD68 shows positivity for macrophages and giant cells in adverse reaction to calcium hydroxyapatite (HRP-polymer immunohistochemistry).

CALCIUM HYDROXYAPATITE

Radiesse®, Radiance®

Immunohistochemistry for CD20

- Adverse reaction to calcium hydroxylapatite shows scant CD20 positive lymphocytes next to the material (Fig. 56).

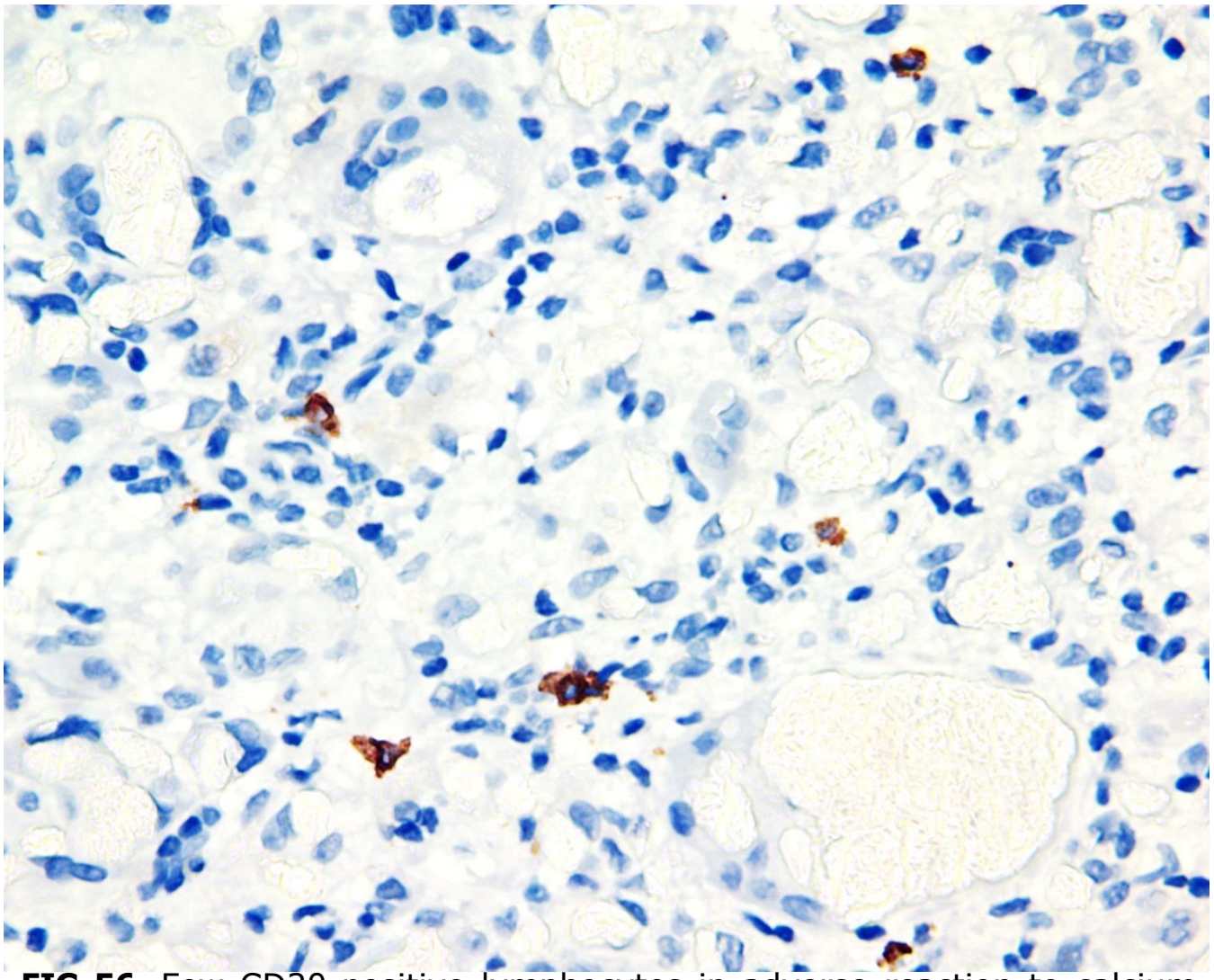

FIG 56. Few CD20-positive lymphocytes in adverse reaction to calcium hydroxyapatite (HRP-polymer immunohistochemistry).

CALCIUM HYDROXYAPATITE

Radiesse®, Radiance®

Immunohistochemistry for CD3

- A moderate number of CD3-positive lymphocytes are found (Fig. 57).

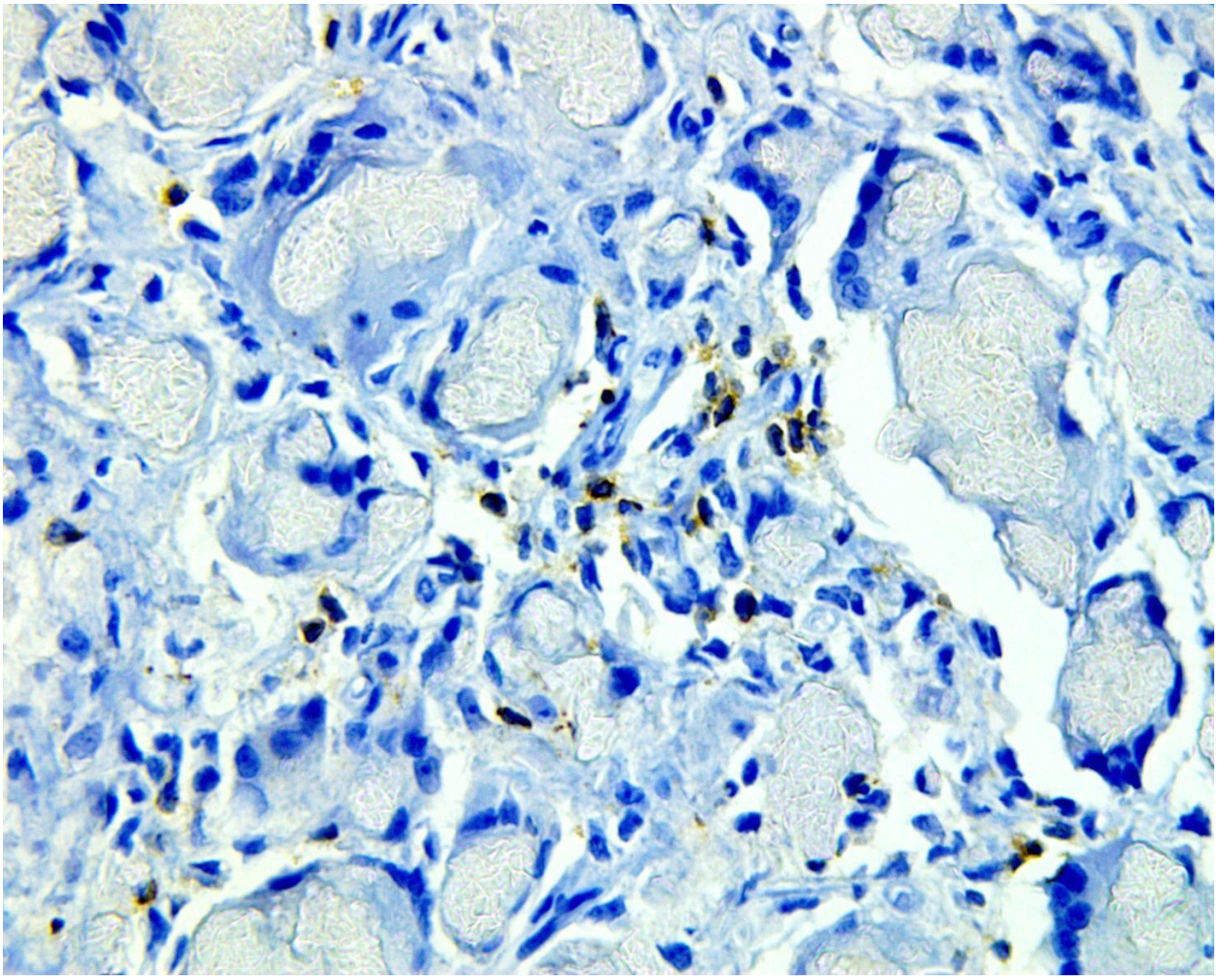

FIG 57. CD3-positive lymphocytes in adverse reaction to calcium hydroxyapatite (HRP-polymer immunohistochemistry).

POLY-L-LACTIC ACID

Sculptra®, Newfill®

- Poly-L-lactic acid stimulates collagen production. It is composed by a polymer of the α-hydroxy-acid group suspended in mannitol and carboxymethylcellulose.[25]

- This material shows fusiform (spiky shape) translucent or glassy-looking particles of different sizes. The particles resembles a "surfboard" and are similar but wider than cholesterol cleft-like material (Fig. 58, 59).
- It has a diffuse arrangement in the tissue.

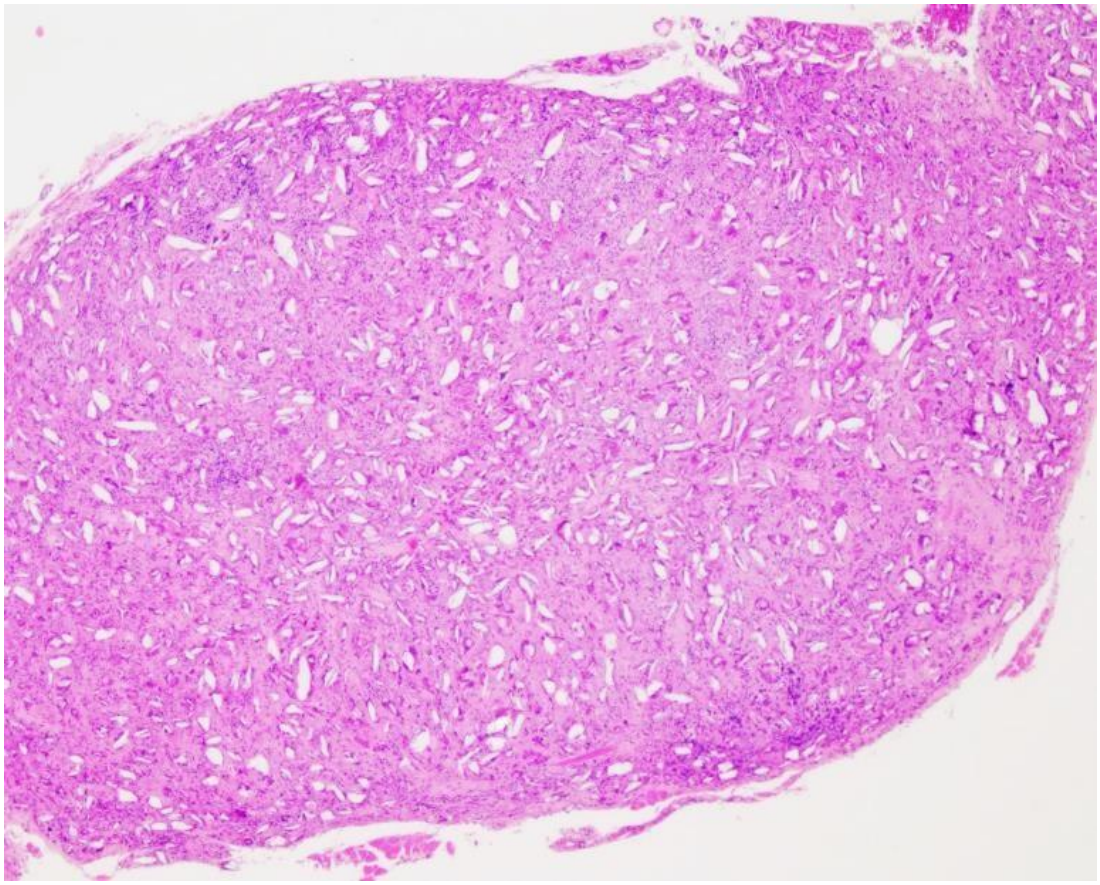

FIG 58. Adverse reaction to poly-L-lactic acid showing cholesterol cleft-like material in a diffuse arragement (Hematoxylyn-eosin staining).

POLY-L-LACTIC ACID

Sculptra®, Newfill®

- Multinucleated giant cells are numerous. They usually surround or phagocyte the material (Fig. 59). Asteroid bodies are common.
- Lymphocytes are numerous (Fig. 59).
- Formation of foreign body granuloma is a common feature.[3]

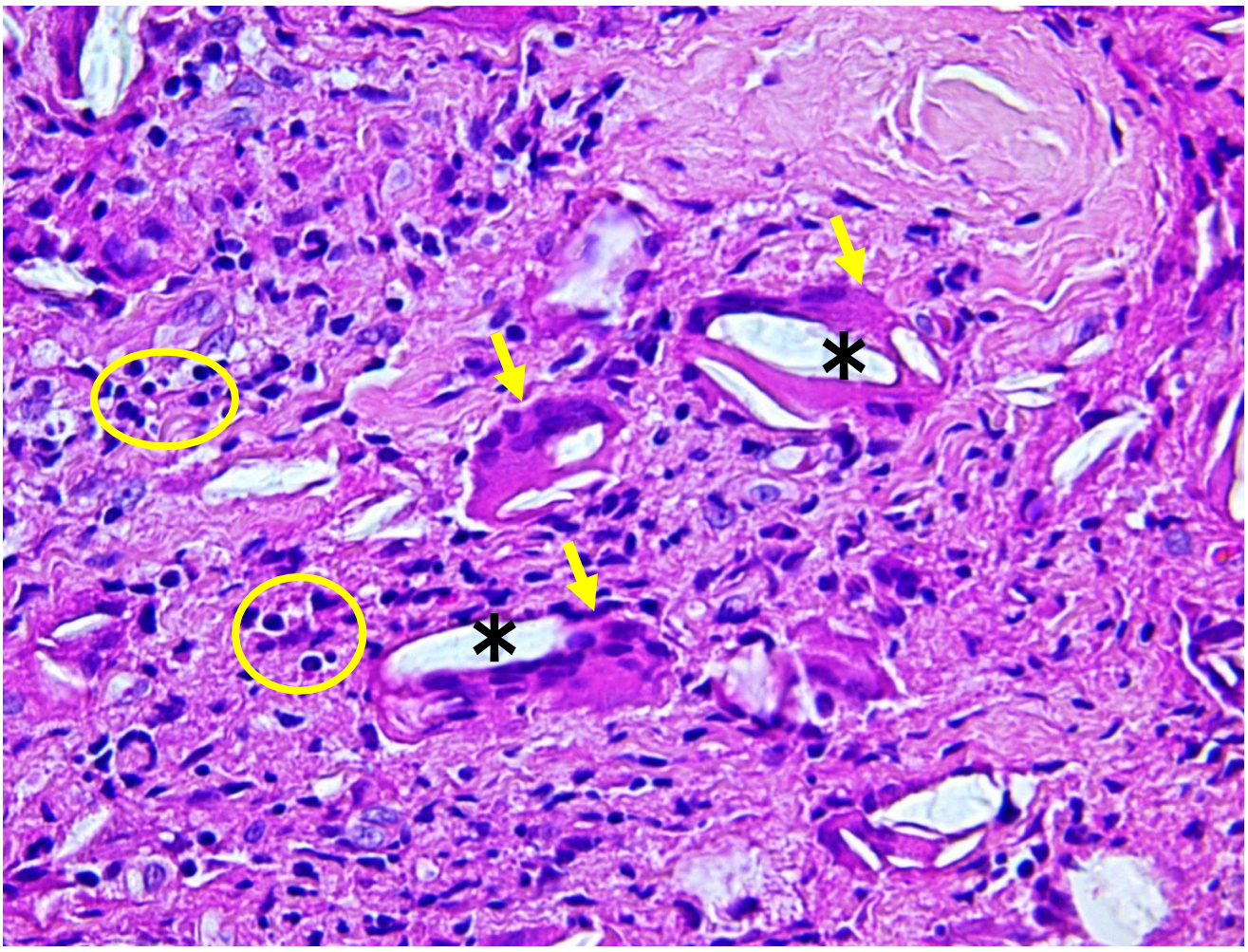

FIG 59. Poly-L-lactic acid reaction with giant cells (arrows) phagocyting the glassy-looking, spiky material (asterisk). Lymphocytes (circles) are dispersed in the connective tissue (Hematoxylyn-eosin staining).

POLY-L-LACTIC ACID

Sculptra®, Newfill®

Alcian Blue Staining

- Alcian blue staining was negative.

Toluidine Blue Staining

- Few mast cells are seen in inflammatory infiltrate associated with poly-L-lactic acid (Fig 60).

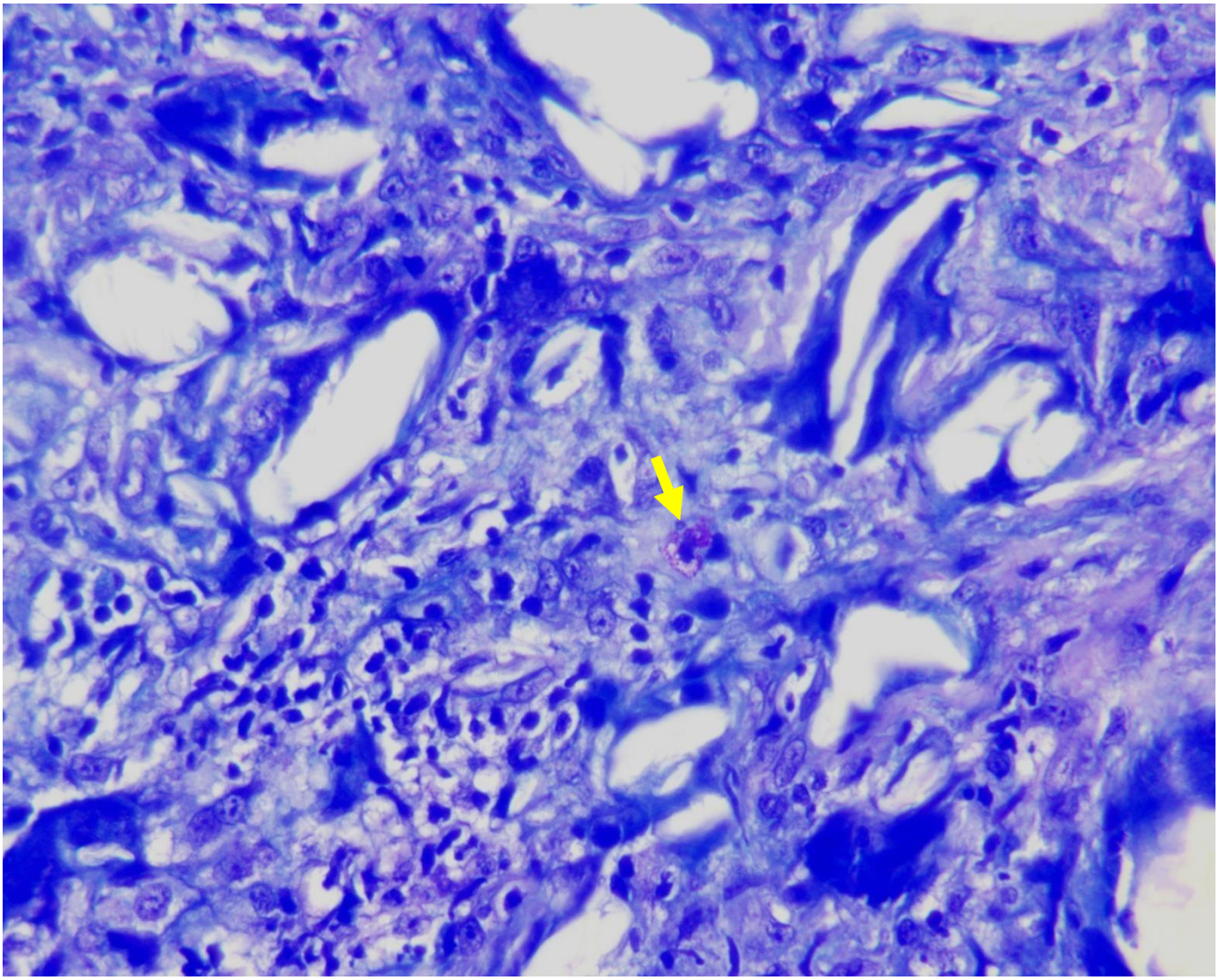

FIG 60. Mast cell (arrow) in adverse reaction to poly-L-lactic acid (Toluidine blue staining

POLY-L-LACTIC ACID

Sculptra®, Newfill®

Sirius Red Staining

- A moderate number of eosinophils are seen in inflammatory infiltrate by poly-L-lactic acid (Fig. 61).

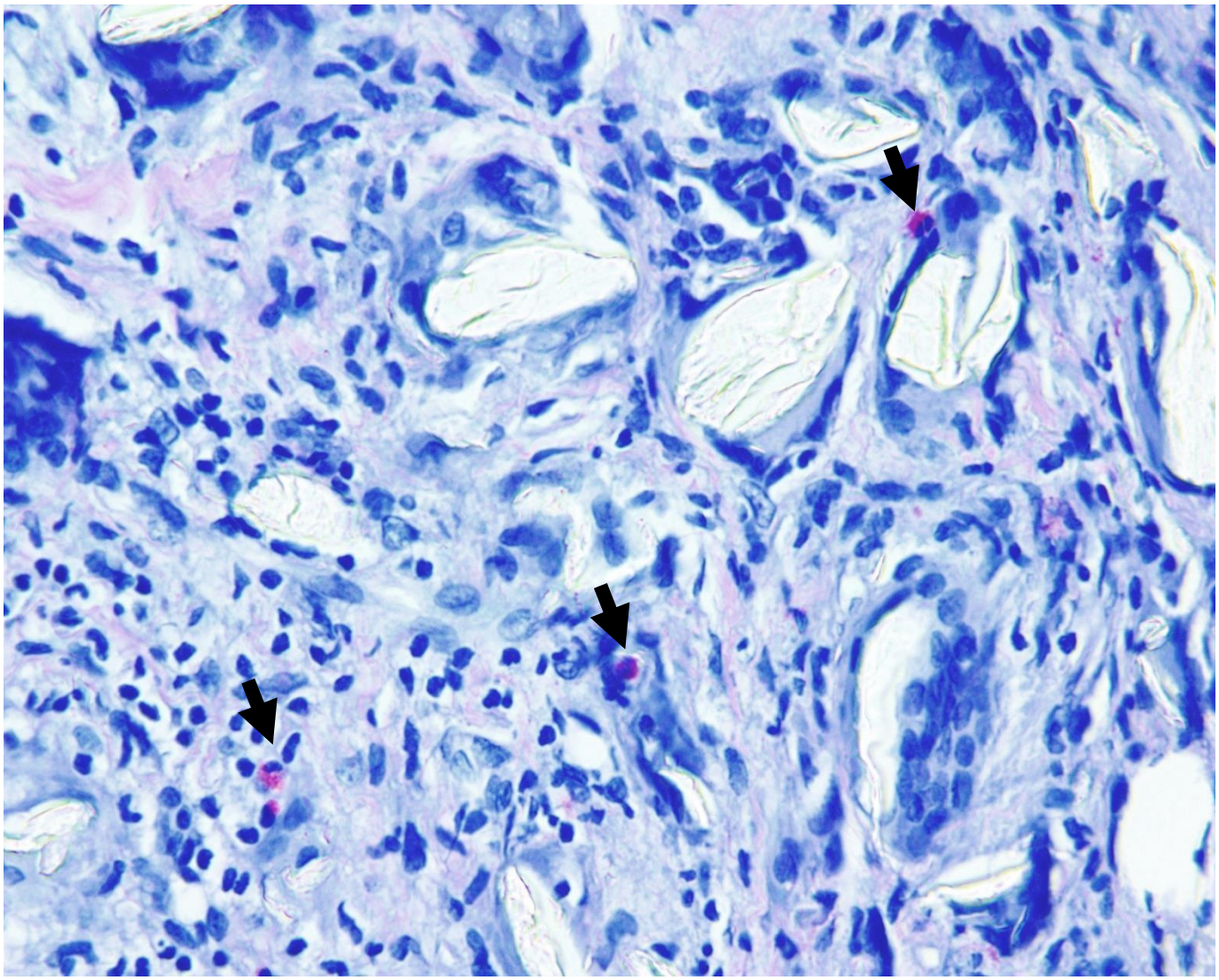

FIG 61. Eosinophils (arrows) in adverse reaction to poly-L-lactic acid (Sirius red staining).

POLY-L-LACTIC ACID

Sculptra®, Newfill®

Polarized Light Microscopy

- Poly-L-lactic is refractile under polarized light microscopy and shows a white bright (Fig. 62 B).

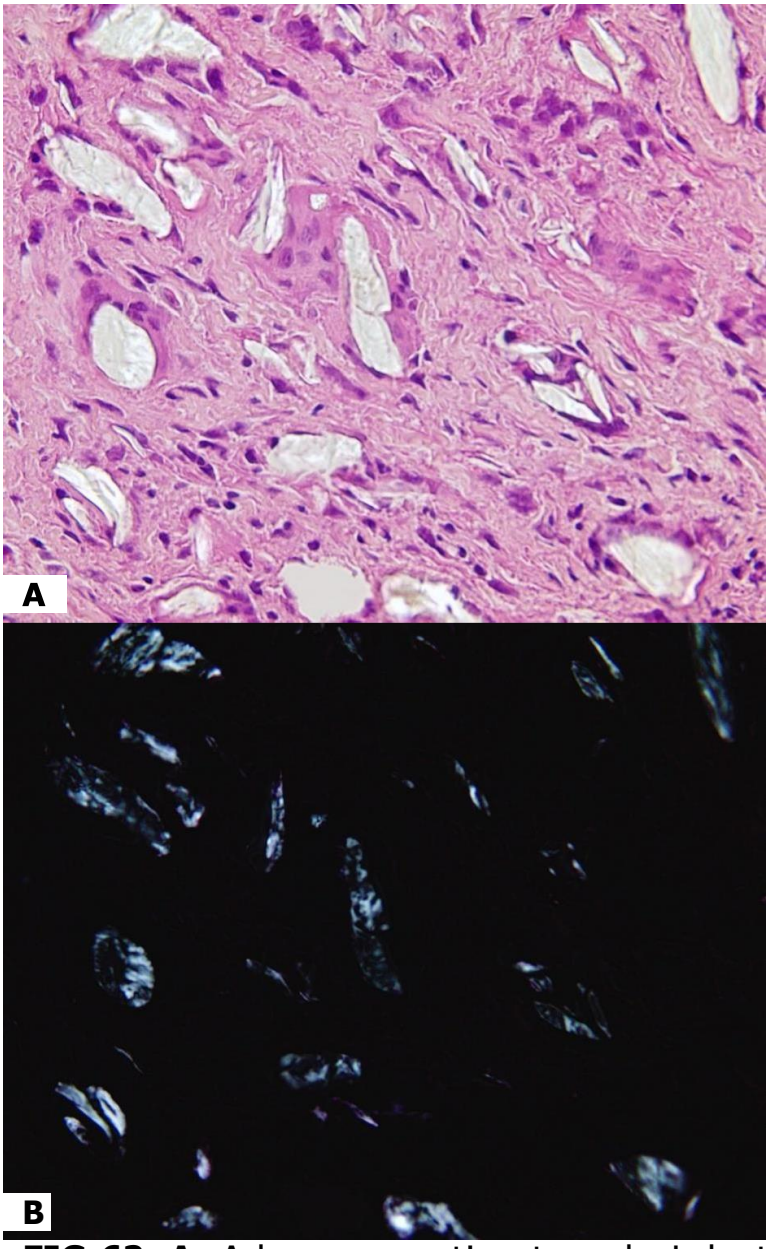

FIG 62. A. Adverse reaction to poly-L-lactic under conventional light microscopy**. B.** Same field under polarized light (Hematoxylin-eosin staining).

POLY-L-LACTIC ACID

Sculptra®, Newfill®

Immunohistochemistry for CD68

- Numerous macrophages and multinucleated giant cells around the material are evidenced in CD68 staining. (Fig. 63).

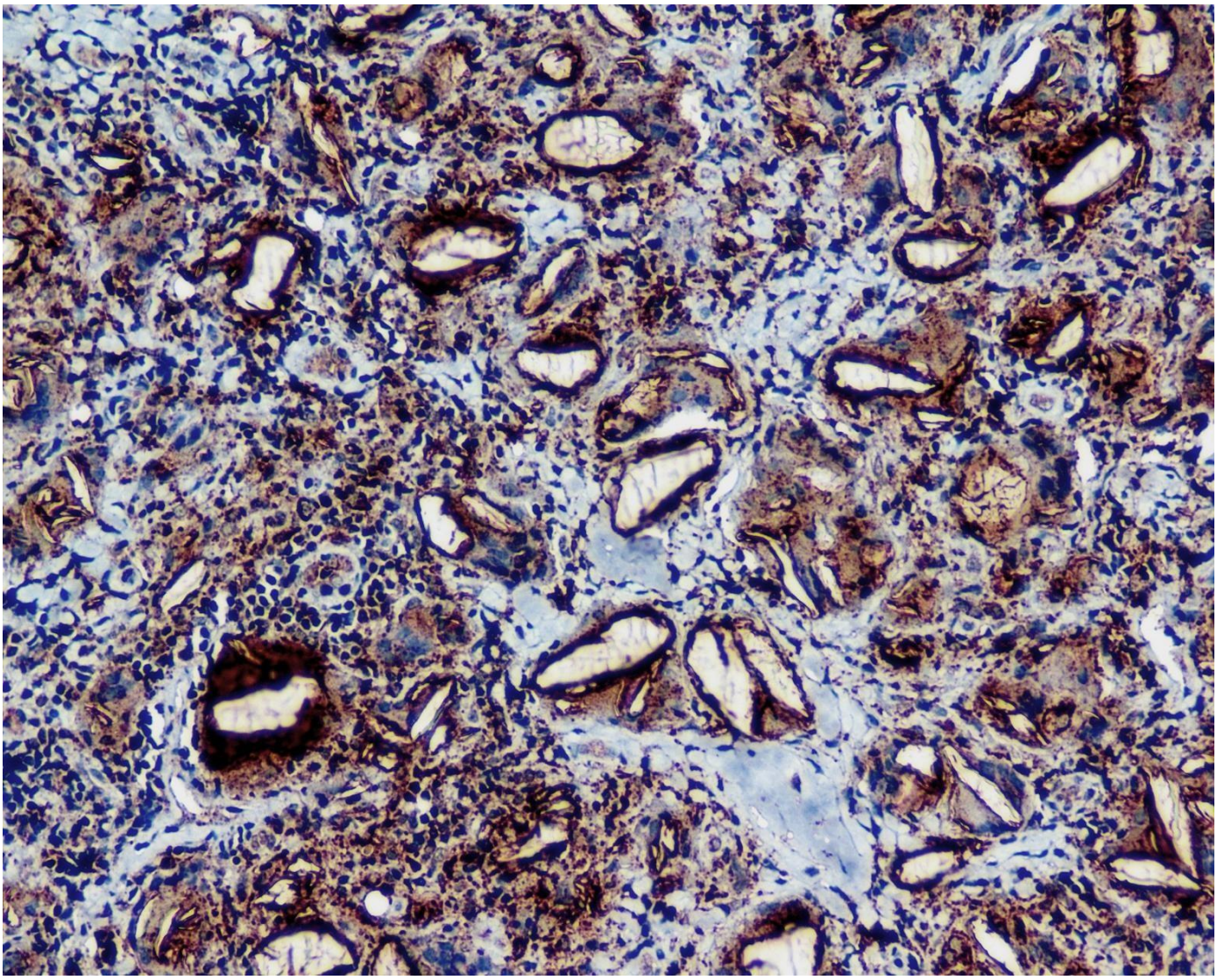

FIG 63. Macrophages and giant cells positive for CD68 (HRP-polymer immunohistochemistry).

POLY-L-LACTIC ACID

Sculptra®, Newfill®

Immunohistochemistry for CD20

- A moderate number of CD20-positive lymphocytes is seen (Fig. 64).

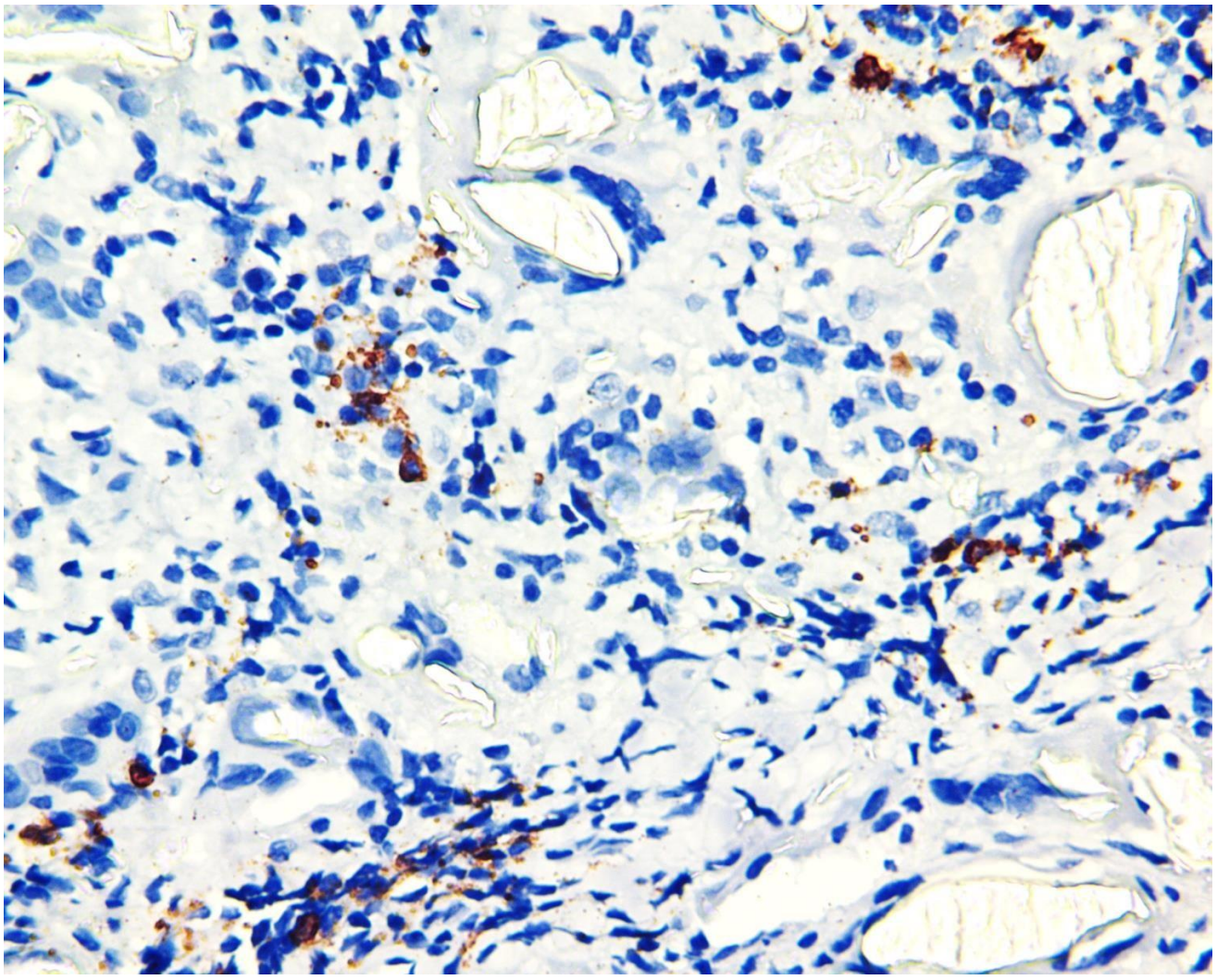

FIG 64. CD20-positive lymphocytes in adverse reaction to poly-L-lactic acid (HRP-polymer immunohistochemistry).

POLY-L-LACTIC ACID

Sculptra®, Newfill®

Immunohistochemistry for CD3

- Numerous CD3-positive lymphocytes are observed surrounding the clefts (Fig. 65).

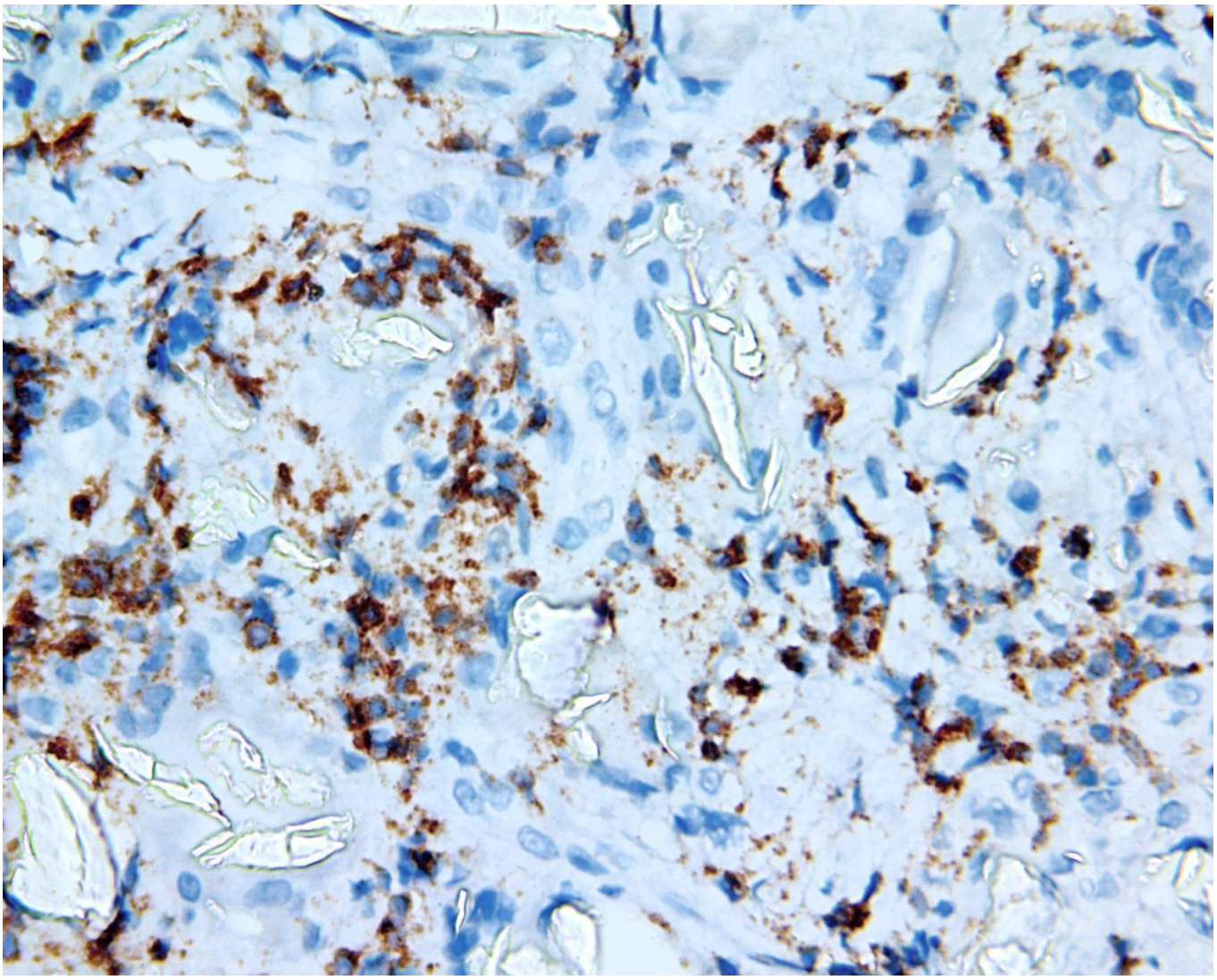

FIG 65. CD3-positive lymphocytes in adverse reaction to poly-L-lactic acid (HRP-polymer immunohistochemistry).

POLY-L-LACTIC ACID, HYDROXYETHYL- METHACRYLATE and CALCIUM HYDROXYAPATITE: Differential diagnosis of glassy-looking materials

BOX 3. Histopathological differential diagnosis between poly-L-lactic acid and hidroxyethymethacrylate.

Features	Poly-L-Lactic Acid (Sculptra®)[3,13]	Calcium hydroxyapatite (Radiesse®)	*Hydroxy-ethyl-methacrylate (Dermalive®) [3,13,23]
Shape	Numerous translucent particles appearing "surfboard" or cholesterol clefts	Numerous polyhedral or spheroid eosinophilic crackled and mineralized material	Numerous pseudocystic structures that looks like"glass cullet" containing polygonal, pink or translucent material
Size	Different sizes of particles	Different sizesof particles	Different sizes and wider than poly-L-latic acid
Foreign body granuloma	Present	Usually absent	Present
Giant cells and asteroid bodies	Present	Giant cells without asteroid bodies	Present
Birefringency	Refractile	Refractile	Non-refractile

*Hydroxyethyl-methacrylate was not illustrated in this e-book.

CONCLUDING REMARKS

- Adverse reactions to cosmetic fillers may present as dispersed inflammatory responses or as foreign-body granulomas.
- Some materials look like each other, but specific histological features of the material and of the host response against it help to correctly classify the cosmetic filler observed in histological slides.
- Neoplastic processes should be considered in the differential diagnosis in some cases.
- Histochemistry may aid in diagnosis, especially Toluidine blue.
- Immunohistochemistry may be useful in differential diagnosis, especially for CD68.
- Cosmetic fillers such as hydroxyethyl-methacrylate (Dermalive®), Polytetrafluoroethylene (Advanta®), and Polycaprolactone (Ellansé®) were not reported in this e-book.

SUMMARY

BOX 4. Summary of all cosmetic fillers reported in this e-book.

Material	Histological features	Host reaction	Birefrin-gence
Poly-methyl-methacrylate (PMMA)	Negative image of round vacuoles with regular shape and size	Foreign body granuloma	Absent
Silicone	Negative image of round vacuoles with varying sizes. Looks like signet-ring cells	Dispersed inflammation	Absent
Hyaluronic acid	Uniform basophilic or amphophilic "pools"	Foreign body granuloma (more common)	Absent
Polyacryl-amide-hydrogel	"Pools" of darker basophilic material with microfoamy appearance	Foreign body granuloma	Absent
Calcium hydroxyapatite (CaHA)	Polyhedral or spheroid eosinophilic crackled material	Dispersed inflammation	Present
Poly-L-lactic acid	Cholesterol cleft-like material	Foreign body granuloma	Present

SUMMARY

BOX 4. Summary of all cosmetic fillers reported in this e-book.

Material	Giant cell / asteroid bodies	Toluidine Blue / Alcian Blue	CD68 immuno-histochemistry
Poly-methyl-methacrylate (PMMA)	Present / Present	Negative / Negative	Positive
Silicone	Absent / Absent	Negative / Negative	Positive (honeycomb arrangement)
Hyaluronic acid	Present / Absent	Positive / Positive	Positive
Polyacryl-amide-hydrogel	Present / Absent	Positive / Positive	Positive
Calcium hydroxyapatite (CaHA)	Present / Absent	Positive / Positive	Positive
Poly-L-lactic acid	Present / Present	Negative / Negative	Positive

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6 CONSIDERAÇÕES FINAIS

Este estudo detalhou as características microscópicas de reações adversas a seis diferentes materiais preenchedores estéticos: polimetilmetacrilato, silicone, ácido hialurônico, hidroxiapatita de cálcio, ácido poli-L-lático e poliacrilamida em hidrogel. Apesar de algumas semelhanças microscópicas, há características específicas de cada material e da resposta do hospedeiro que auxiliam no diagnóstico histopatológico correto. Formato, tamanho e coloração do material no H&E são características-chave no diagnóstico diferencial. A presença de células gigantes e corpos asteroides também podem auxiliar. A imuno-histoquímica para CD68 e a coloração por azul de toluidina são as mais abrangentes para auxiliar no correto diagnóstico, sendo que outros marcadores podem ser úteis em casos específicos. A microscopia de luz polarizada também pode ser necessária em alguns casos. Lesões neoplásicas também devem ser consideradas no diagnósticodiferencial.

Houve uma reação evidente de macrófagos contra todos os preenchimentos, frequentemente associada à formação de células gigantes. O ácido hialurônico foi o material com a resposta inflamatória mais branda de mastócitos, eosinófilos e linfócitos, enquanto o polimetilmetacrilato e o silicone apresentaram a infiltração mais intensa dessas células.

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ANEXO A – Aprovação do comitê de ética em pesquisa

UNIVERSIDADE FEDERAL DE MINAS GERAIS

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: FREQUÊNCIA DE LESÕES ORAIS E MAXILOFACIAIS EM DOIS TIPOS DE SERVIÇO: CLÍNICO E LABORATORIAL

Pesquisador: Ricardo Alves de Mesquita

Área Temática:

Versão: 1

CAAE: 10723019.0.1001.5149

Instituição Proponente: UNIVERSIDADE FEDERAL DE MINAS GERAIS

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.313.670

Apresentação do Projeto:

Os estudos epidemiológicos permitem a melhor compreensão acerca da extensão e nível de acometimento das doenças bucais na população, sendo fundamentais para programas de prevenção, além de fornecerem informações que norteiem o clínico sobre o que ele deve esperar acerca da frequência de ocorrência das doenças e suas associações com fatores de risco.Será realizado um estudo retrospectivo, através do qual se analisará informações contidas em prontuários dos arquivos de dois serviços (clínico e laboratorial), do setor de Estomatologia e Patologia Bucal da Faculdade de Odontologia da Universidade Federal de Minas Gerais (FO-UFMG), Belo Horizonte, Minas Gerais. Informações contidas em prontuários dos arquivos dos serviços (clínico e laboratorial) dos centros de referência da Universidade de Pernambuco, Universidade Federal de Goiás, Universidade Federal do Rio Grande do Sul, Universidade Federal de Pelotas, Universidade Federal do Rio de Janeiro, Universidade Federal do Amazonas, Universidade Federal de Santa Catarina, Universidade de São Paulo e Universidade Estadual da Paraíba também serão analisadas. Os dados serão obtidos a partir dos prontuários disponíveis da Clínica de Patologia e Estomatologia da FO-UFMG, do serviço de Laboratório de Patologia Bucomaxilofacial da FO-UFMG e também dos serviços clínicos e laboratoriais dos outros centros de referência. Informações com relação ao sexo, idade, e cor da pele de crianças/adolescentes, adultos e idosos serão coletadas. Informações com relação ao tabagismo (sim/não), uso de álcool (sim/não) e uso de próteses

Endereço: Av. Presidente Antônio Carlos,6627 2º Ad SI 2005
Bairro: Unidade Administrativa II **CEP:** 31.270-901
UF: MG **Município:** BELO HORIZONTE
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(sim/não) dos adultos e idosos também serão coletadas. Informações com relação a localização da lesão (lábios, lingua, assoalho bucal, gengiva, palato, bochecha, orofaringe, mucosa alveolar, óssea, múltiplas, outras, não apresenta alteração, sem informação) e sintomatologia (sim, não e sem informação) também serão coletadas. As lesões terão seu diagnóstico individual baseado em dois critérios. Neoplasmas benignos e malignos serão classificados de acordo com aclassificação da OMS de 2017.

Objetivo da Pesquisa:

Avaliar e comparar a frequência de lesões orais e maxilofaciais em crianças/adolescentes, adultos e idosos em dois tipos de serviços de referência em Estomatologia e Patologia Bucal (clínico e laboratorial) na Universidade Federal de Minas Gerais e em outros centros de referência brasileiros: Universidade de Pernambuco, Universidade Federal de Goiás, Universidade Federal do Rio Grande do Sul, Universidade Federal de Pelotas, Universidade Federal do Rio de Janeiro, Universidade Federal do Amazonas, Universidade Federal de Santa Catarina, Universidade de São Paulo e Universidade Estadual da Paraíba.

Avaliação dos Riscos e Benefícios:

Riscos:

O risco seria a divulgação de informações (nome e endereço) dos participantes, cujos prontuários vão ser acessados para a coleta de dados provocando constrangimento. No entanto, como descrito no projeto de pesquisa, o anonimato dos participantes será preservado e nenhuma informação pessoal vai ser divulgada.

Benefícios:

Existe uma grande lacuna na literatura científica no que concerne os estudos comparando a frequência de lesões orais e maxilofaciais em serviços clínicos e laboratoriais. Desta forma, este estudo será útil, pois revelará a real frequência destas lesões nos casos de avaliação de dados de origem clínica e dados de origem laboratorial, o que fará com que condições de diagnóstico majoritariamente clínico não sejam subnotificadas ou agravos
que obrigatoriamente necessitam de confirmação laboratorial não sejam descritos de forma imprecisa.

Comentários e Considerações sobre a Pesquisa:

O projeto é exequível. E trata-se de um estudo epidemiológico que auxilia na elaboração de hipóteses diagnósticas e de causalidade, e também na obtenção de dados acerca da prevalência e

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Incidência de moléstias, permitindo o clínico estimar a possibilidade de encontrar determinada doença em sua prática

Considerações sobre os Termos de apresentação obrigatória:

Foram apresentados os seguintes termos: PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1306455.pdf, Ficha_Servico_Laboratorial.pdf, Prontuario_Servico_Clinico.pdf, Justificativa_Ausencia_TCLE.pdf,Projeto_Lesoes_Orais_Maxilofaciais.pdf, Parecer_Institucional.pdf, TCUD_UPE.pdf, TCUD_USP.pdf,TCUD_UFSC.pdf, TCUD_UFRJ.pdf, TCUD_UFPel.pdf, TCUD_UFRGS.pdf, TCUD_UFMG.pdf, TCUD_UFG.pdf, TCUD_UFAM.pdf,TCUD_UEPB.pdf,

Recomendações:

Recomenda-se a APROVAÇÃO do projeto de pesquisa.

Conclusões ou Pendências e Lista de Inadequações:

Somos favoráveis à diligência do projeto FREQUÊNCIA DE LESÕES ORAIS E MAXILOFACIAIS EM DOIS TIPOS DE SERVIÇO: CLÍNICO E LABORATORIAL do Pesquisador Responsável Prof. Ricardo Alves de Mesquita.

Considerações Finais a critério do CEP:

Tendo em vista a legislação vigente (Resolução CNS 466/12), o CEP-UFMG recomenda aos Pesquisadores: comunicar toda e qualquer alteração do projeto e do termo de consentimento via emenda na Plataforma Brasil, informar imediatamente qualquer evento adverso ocorrido durante o desenvolvimento da pesquisa (via documental encaminhada em papel), apresentar na forma de notificação relatórios parciais do andamento do mesmo a cada 06 (seis) meses e ao término da pesquisa encaminhar a este Comitê um sumário dos resultados do projeto (relatório final).

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1306455.pdf	22/03/2019 10:27:49		Aceito
Outros	Ficha_Servico_Laboratorial.pdf	21/03/2019 18:47:48	Anael Sá Costa Borges de Almeida	Aceito
Outros	Prontuario_Servico_Clinico.pdf	21/03/2019 18:47:26	Anael Sá Costa Borges de Almeida	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Justificativa_Ausencia_TCLE.pdf	21/03/2019 18:46:45	Anael Sá Costa Borges de Almeida	Aceito

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UF: MG **Município:** BELO HORIZONTE
Telefone: (31)3409-4592 **E-mail:** coep@prpq.ufmg.br

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Projeto Detalhado / Brochura Investigador	Projeto_Lesoes_Orais_Maxilofaciais.pdf	21/03/2019 18:33:29	Anael Sá Costa Borges de Almeida	Aceito
Declaração de Instituição e Infraestrutura	Parecer_Institucional.pdf	21/03/2019 17:15:47	Anael Sá Costa Borges de Almeida	Aceito
Outros	TCUD_USP.pdf	21/03/2019 17:14:31	Anael Sá Costa Borges de Almeida	Aceito
Outros	TCUD_UPE.pdf	21/03/2019 17:14:05	Anael Sá Costa Borges de Almeida	Aceito
Outros	TCUD_UFSC.pdf	21/03/2019 17:13:38	Anael Sá Costa Borges de Almeida	Aceito
Outros	TCUD_UFRJ.pdf	21/03/2019 17:13:26	Anael Sá Costa Borges de Almeida	Aceito
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Folha de Rosto	Folha_de_Rosto.pdf	21/03/2019 15:59:28	LUCAS GUIMARAES ABREU	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

BELO HORIZONTE, 08 de Maio de 2019

Assinado por:
Eliane Cristina de Freitas Rocha
(Coordenador(a))

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Anexo B – Instruções aos autores do periódico *Modern Pathology*

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Introduction

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Original manuscripts are the main focus of *Modern Pathology*. Our scientific communication is enriched by high impact editorials, reviews, and practice guidelines that relate to all aspects of precision diagnostics in human pathology. As such, *Modern Pathology's* scope encompasses advances in the molecular diagnostics and genomic classifications of disease as well as discoveries in immune-oncology, computational science and applied bioinformatics, and digital pathology.

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Article Description	ABSTRACT	WORD LIMIT	TABLES/FIGURES	REFERENCES
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Review: Review Articles require prior approval by the Editor. Review articles must be of general interest to pathologists and contain original, in-depth analysis of the chosen topic.	Unstructured abstract, max 300 words.	Main body of text (excluding abstract, legends, references) not to exceed 5,000 words	Maximum of 5 tables/figures.	No more than 150 references.
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7. Coon E, Berndt M, Jan A, Svyatsky D, Atchley A, Kikinzon E, Harp D, Manzini G, Shelef E, Lipnikov K, Garimella R, Xu C, Moulton D, Karra S, Painter S, Jafarov E, Molins S. Advanced Terrestrial Simulator (ATS) v0.88 (Version 0.88). Zenodo; 2020, March 25. https://doi.org/10.5281/zenodo.3727209

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Modern Pathology

Adverse reactions to cosmetic fillers in the oral and maxillofacial region: clinico-pathological, histochemical, and immunohistochemical characterization

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Manuscript Number:	MODPATH-D-24-00503
Full Title:	Adverse reactions to cosmetic fillers in the oral and maxillofacial region: clinico-pathological, histochemical, and immunohistochemical characterization
Article Type:	Research Article
Keywords:	Dermal fillers; Injection site reaction; Foreign-Body Reaction; Microscopy; Immunohistochemistry.
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Abstract:	Cosmetic injections are increasing, as their complications, which can be misdiagnosed as neoplastic lesions. This study aimed to detail clinical, pathological, histochemical, and immunohistochemical features of adverse reactions to different cosmetic fillers in the oral and maxillofacial region. Samples were retrieved from five pathology laboratories. Hematoxylin-eosin, Alcian Blue, Sirius Red, and Toluidine blue stains were performed, as well as immunohistochemistry for CD68, CD3, and CD20. H&E was evaluated under polarization. Descriptive statistics were performed. Twenty-three cases were included. Polymethyl-methacrylate was the most common material, followed by silicone, hyaluronic acid, calcium hydroxyapatite, polyacrylamide hydrogel,