



UNESP - Universidade Estadual Paulista
“Júlio de Mesquita Filho”
Faculdade de Odontologia de Araraquara



Evânio Vilela da Silva

**Imunocaracterização de centros germinativos e subgrupos de células
dendríticas na síndrome de Sjögren primária: um estudo comparativo**

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Tese apresentada à Universidade Estadual Paulista (Unesp), Faculdade de Odontologia, Araraquara para obtenção do título de Doutor em Ciências Odontológicas, na Área de Diagnóstico e Cirurgia

Orientador: Profa. Dra. Andreia Bufalino

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dendríticas na síndrome de Sjögren primária: um estudo comparativo**

Comissão julgadora

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Faculdade de Odontologia de Araraquara, Universidade Estadual Paulista “Júlio de Mesquita Filho”, Araraquara, São Paulo.

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[...]

“A vida é uma corrida
que não se corre sozinho.
E vencer não é chegar,
é aproveitar o caminho
sentindo o cheiro das flores
e aprendendo com as dores
causadas por cada espinho.”

* Bráulio Bessa

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RESUMO

A síndrome de Sjögren (SS) é uma doença autoimune crônica caracterizada por infiltração linfocitária frequentemente afetando as glândulas salivares e lacrimais, causando sintomas de secura, e muitas vezes acompanhada de produção anormal de autoanticorpos. A análise histopatológica das glândulas salivares menores (GSm) é amplamente utilizada nos critérios de classificação da SS primária (SSp), mostrando aglomerados linfocíticos permeando o parênquima glandular, definido como sialadenite linfocítica focal (SLF), com escore focal (EF) ≥ 1 . Em certas situações, a SLF pode se organizar em centros germinativos (CGs). Curiosamente, a SLF também pode ser observada ao avaliar biópsias intraorais contendo parênquima de GSm de indivíduos assintomáticos (pacientes não-SS não sicca). Assim, o objetivo deste trabalho foi avaliar, por imunoistoquímica, os CGs e células dendríticas plasmocitoides (CDp) em biópsias de GSm de pacientes com SSp e pacientes não-SS não-sicca e relatar um caso raro de paciente com SS com extravasamento de muco exibindo reação granulomatosa do tipo corpo estranho (população de macrófagos). Os resultados foram formatados em 3 artigos científicos. No primeiro artigo, focamos na análise comparativa de CGs avaliando SLF em 13 pacientes SSp e 11 pacientes não-SS não-sicca, usando os anticorpos D2-40 e CD57, em correlação com os imunomarcadores Bcl-6 e Ki-67. Os nossos resultados mostram diferenças histomorfológicas (área e diâmetro) e imunofenotípicas (perfil de expressão de D2-40 e CD57) entre os grupos de pacientes, sugerindo interações celulares e/ou moleculares distintas na patogênese de CGs ao avaliar comparativamente a SLF. No segundo artigo, avaliamos o perfil de distribuição de CDp em uma casuística de 50 pacientes SSp e 31 pacientes não-SS não sicca, a partir dos imunomarcadores CD123 e CD303. Como resultado, observamos que as CDp se apresentam com densidade variando de ausente a intensa no grupo SSp e com expressão limitada no grupo não-SS não-sicca ($p < 0,001$ para ambos os marcadores, CD123 e CD303), com distribuição na periferia do SLF e em padrão perivascular. Por fim, relatamos o caso de uma paciente de 63 anos com diagnóstico de SSp apresentando massa nodular na mucosa oral. A análise microscópica revelou SLF e um exuberante infiltrado linfoplasmocitário contendo, na parte central, uma reação de célula gigante do tipo corpo estranho (macrófagos), permeada por numerosos ductos residuais de glândulas salivares, sugerindo uma possível relação com os mecanismos imunes da SS. Concluimos, que os CGs, CDp, bem como populações de macrófagos, apresentam mecanismos celulares importantes na SLF de pacientes com SSp, contribuindo na elucidação da resposta imune e inflamatória dessa doença e de possíveis alvos terapêuticos.

Palavras-chave: Síndrome de Sjögren. Glândulas salivares menores. Centro germinativo. Células dendríticas. Imuno-Histoquímica.

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ABSTRACT

Sjögren's syndrome (SS) is a chronic autoimmune disease characterized by lymphocytic infiltration often affecting the salivary and lacrimal glands, causing sicca symptoms, and often accompanied by abnormal production of autoantibodies. Histopathological analysis of the minor salivary glands (mSG) is widely used in the classification criteria of primary SS (pSS), showing lymphocytic clusters permeating the glandular parenchyma, defined as focal lymphocytic sialadenitis (FLS) with a focal score (EF) ≥ 1 . In certain situations, the SLF can organize into germinal centers (GCs). Interestingly, FLS can also be seen when evaluating intraoral biopsies containing mSG parenchyma from asymptomatic individuals (non-SS non-sicca patients). The aim of this study was to evaluate, by immunohistochemistry, GCs and plasmacytoid dendritic cells (pDCs) in mSG biopsies from patients with SS and non-SS non-sicca patients and to report a rare case of a patient with SS with mucus extravasation exhibiting foreign body-type granulomatous reaction (macrophage population). The results were formatted in 3 scientific papers. In the first, we focused on the comparative analysis of GCs evaluating SLF in 13 pSS patients and 11 non-SS non-sicca patients, using antibodies D2-40 and CD57, in correlation with immunomarkers Bcl-6 and Ki-67. Our results show histomorphological (area and diameter) and immunophenotypic (D2-40 and CD57 expression profile) differences between patient groups, suggesting distinct cellular and/or molecular interactions in the pathogenesis of GCs when comparatively evaluating FLS. In the second, we evaluated the pDCs distribution profile in a series of 50 pSS patients and 31 non-SS non-sicca patients, based on the immunomarkers CD123 and CD303. As a result, we observed that pDCs presented density ranging from absent to intense in the pSS group and with limited expression in the non-SS non-sicca group ($p < 0.001$ for both markers, CD123 and CD303), with distribution in the periphery of the FLS and perivascular pattern. Finally, we report the case of a 63-year-old female patient diagnosed with pSS presenting with a nodular mass in the oral mucosa. Microscopic analysis revealed focal lymphocytic sialadenitis and an exuberant lymphoplasmacytic infiltrate containing, in the central part, a foreign body-type giant cell reaction, permeated by numerous residual ducts of salivary glands, suggesting a possible relationship with the immune mechanisms of SS. We conclude that GCs, CDp, as well as populations of macrophages, present important cellular mechanisms in the SLF of patients with pSS, contributing to the elucidation of the immune and inflammatory response of this disease and possible therapeutic targets.

Keywords: Sjögren's syndrome. Salivary glands, minor. Germinal center. Dendritic cells. Immunohistochemistry.

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1 INTRODUÇÃO

A síndrome de Sjögren (SS) é uma doença autoimune crônica caracterizada por infiltração linfocitária frequentemente afetando as glândulas salivares e lacrimais, causando sintomas *sicca*. Relevantemente, 5-10% dos pacientes com SS podem apresentar manifestações extraglandulares, como mialgia, artralgia e fadiga. Além disso, distúrbios imunológicos, como hipergamaglobulinemia, fator reumatoide, hipocomplementemia, crioglobulinemia, manifestações cardiovasculares, como o fenômeno de Raynaud, e autoanticorpos direcionados contra os autoantígenos SSA/Ro e SSB/La são frequentemente detectados¹. Ainda, apresentam uma maior predisposição a desenvolver linfomas não-Hodgkin de células B, especificamente o linfoma de tecido linfoide associado à mucosa (MALT), com risco de 15 a 20 vezes maior entre pacientes com SS quando comparado à população em geral^{2,3}.

A SS ocorre em uma forma primária (SSp) não associada a outras doenças e uma forma secundária (SSs) que complica ou se sobrepõe a outras condições reumáticas, mais comumente artrite reumatóide (AR), lúpus eritematoso sistêmico (LES) e esclerose sistêmica⁴. A prevalência da SS na população geral varia de 0,5 a 3%, com incidência de 4 casos por 100.000 por ano, e uma proporção de acometimento mulher/homem relatada de 9:1, sendo predominantemente observada em adultos de meia-idade, com pico de incidência por volta dos 50 anos de idade².

A patogênese da SS ainda não está esclarecida; no entanto, acredita-se que a SS se desenvolva quando indivíduos com traços de suscetibilidade genética são expostos a fatores de riscos desencadeadores da doença, incluindo fatores ambientais e hormonais e modificações epigenéticas, levando à ativação da imunidade inata e adaptativa⁵⁻⁷. A ativação imune contra as partículas de ribonucleoproteína Ro/SSA e La/SSB expressas pelo epitélio das glândulas exócrinas pode ser desencadeada por células dendríticas (CDs) presentes também nesse epitélio, que conduzem o acúmulo e a ativação das células do sistema imune, em especial das células T e B⁸. Além disso, as infecções virais, especialmente pelo vírus Epstein-Barr (VEB) e citomegalovírus (CMV), visualizadas em pacientes com SS montam uma assinatura de interferon tipo I (IFN-I) e estimulam as CDs, ativando o sistema de resposta imune inata e contribuindo para a cronicidade do processo autoimune^{5,6}.

Para um diagnóstico de SSp, os atuais critérios da os critérios do Colégio Americano de Reumatologia (ACR) e da Liga Européia Contra o Reumatismo (EULAR) requerem medidas objetivas de diminuição da produção de lágrimas ou saliva, bem como aberrações imunológicas verificadas por meio da detecção de autoanticorpos anti-SSA/Ro ou por achados histológicos de sialadenite linfocítica focal (SLF) em biópsias de glândulas salivares menores (GSm)⁹. Outros exames, como cintilografia, ultrassonografia, sorologia para autoanticorpos anti-La/SSB e fator reumatoide (FR) podem auxiliar no diagnóstico⁸.

Como comentado acima, as biópsias de GSm de pacientes com SS exibem aglomerados linfocitários (focos) permeando o parênquima glandular, definido como SLF. O escore focal (EF) define o número de focos, cada um deles contendo 50 ou mais linfócitos, em uma área glandular de 4mm²¹⁰. Notavelmente, para preencher o critério diagnóstico para SS, a SLF deve apresentar um EF ≥ 1 ^{9,10}. Pela imunistoquímica, a SLF apresenta células TCD4+ e, em quantidades menores, células TCD8+, células B e plasmócitos; e em etapas finais há presença de fibrose, o que contribui com a disfunção do órgão^{8,10}. Em certas situações, a SLF pode se organizar em centros germinativos (CGs)¹¹.

O início dos sintomas relatados pelo paciente muitas vezes precede o diagnóstico em muitos anos, o que pode estar relacionado a um início insidioso da SSp e ao fato de que os sintomas de *sicca* são comuns na população em geral⁷.

As recomendações atuais de tratamento para SSp concentram-se no manejo dos sintomas *sicca* e na estimulação da função glandular residual com agonistas muscarínicos, mas também sugerem o uso de vários DMARDs (drogas modificadoras da doença) sintéticos convencionais, como a hidroxicloroquina, e biológicos, como o rituximabe, para o tratamento de órgãos específicos manifestações¹².

1.1 Centros germinativos

Os CGs que são microambientes especializados dentro de tecidos linfóides, onde as células B sofrem extensa proliferação, hipermutação somática e seleção de afinidade de antígeno, podem ser visualizados ao avaliar biópsias GSm de pacientes com SS^{1,12}. Adicionalmente, também pode ser observado ao avaliar biópsias intraorais contendo parênquima de GSm de indivíduos saudáveis assintomáticos¹.

CGs apresentam compartimentalização de células T e células B, presença de redes de células dendríticas foliculares CD21⁺ (CDFs) e vênulas endoteliais altas^{13, 14}. Essas estruturas organizadas em infiltrados linfóides de GSm é clinicamente relevante, uma vez que a sua presença parece estar associada a doença mais grave, incluindo maior escore focal, redução da produção de saliva e níveis mais elevados de fator reumatoide, anti-SSA/ Ro, anti-SSB/La e mediadores pró-inflamatórios^{11,15}. Também foi sugerido como preditor para o desenvolvimento de linfoma^{16, 17}, embora outros estudos indiquem que os CGs não são preditivos para o desenvolvimento de linfoma em pacientes com SS^{18,19}.

Estudos anteriores em SSp, por coloração de hematoxilina e eosina (H&E) e alguns deles também por imunoistoquímica, mostraram que CGs podem ser detectados em aproximadamente 25% das biópsias de mSG, variando de 16,5 a 54,5%^{1,11}. Por imunoistoquímica, os GCs foram avaliados usando marcadores para superfície de linfócitos (CD3/CD20), redes de CDFs (CD21, CD23, CD35) e o anticorpo Bcl-6^{1,14,15,18,20}, com estudos recentes indicando Bcl-6 como um marcador sensível e específico para identificação inequívoca de CGs em biópsias de GSm obtidas de pacientes com SSp^{1,14}.

Além disso, as CDFs participam da reação do centro germinativo, impedindo a apoptose das células B do centro germinativo e estimulando as interações celulares e a proliferação. A podoplanina, uma glicoproteína transmembranar do tipo mucina, é expressa na rede de CDFs do centro germinativo e em uma variedade de tipos de células humanas, incluindo endotélio linfático, mesotélio e células alveolares tipo I, e pode ser identificada por D2-40. Estudos revelam que este é um marcador sensível para a rede CDFs, que é superior ou igual ao CD21 na avaliação de CDFs reativas e neoplásicas^{21,22}. Notavelmente, a subpopulação de células T CD4⁺ CD57⁺, células T-folicular helper (Tfh), são células críticas para a maturação das células B e estão normalmente presentes nos centros germinativos da amígdala e formam cerca de 5 a 8% de todas as células. Essas células Tfh coexpressam o fator de transcrição Bcl-6, podendo assim ser diferenciadas de outras subpopulações de células T auxiliares²³.

1.2 Células dendríticas plasmocitoides

As células dendríticas (CDs) são derivadas de células-tronco hematopoiéticas na medula óssea, integrantes do sistema de imunidade inata e consideradas como eficientes células apresentadoras de antígenos (APCs). Estas são capazes de estimular uma resposta imunitária celular contra células tumorais, desempenhar um papel importante na ativação e função de linfócitos T e, ainda, auxiliar a manutenção da tolerância periférica²⁴. As CDs, quando sofrem processo de diferenciação são divididas em células dendríticas mieloides (CDmi) e plasmocitoides (CDp)²⁴. Estas, foram sugeridas como candidatas a células mestras na ativação de células T e B na SS, no entanto, sua contribuição tem sido subavaliada²⁵.

As CDp são células derivadas de progenitores linfoides e altamente especializadas na produção e secreção de altos níveis de interferon tipo I (IFN-I), especialmente após ativação por agentes virais (resposta antiviral). Elas expressam constitutivamente receptores semelhantes a endossômicos *toll-like* (TLR), os quais atuam no reconhecimento de RNA e DNA virais e podem se ligar aos ácidos nucleicos endógenos²⁶. Entre os receptores endossômicos, o TLR7 e TLR9 constituem vias cruciais pelas quais as CDp são ativadas em doenças autoimunes sistêmicas²⁷.

Um conjunto de evidências supõe que a secreção de IFN-I pelas CDp desempenha um papel importante na perpetuação do processo inflamatório na SSp, uma vez que estimulam os monócitos a produzirem altos níveis de fator de ativação de células B (BAFF), um mediador da ativação de células B e da produção de autoanticorpos na SSp^{26,28}. Ainda, a ativação das vias secretoras de IFN-I correlacionou-se com o aumento da gravidade da inflamação da glândula salivar e disfunção secretora em pacientes com SS^{27, 29-31}.

Além da produção robusta de IFN tipo I, as CDp também podem secretar várias outras citocinas inflamatórias, como TNF α e IL-6, produzir quimiocinas e expressar moléculas coestimuladoras em suas superfícies, para influenciar as respostas imunes inatas e adaptativas³¹. Em estudo de transcriptoma, demonstrou-se que CDp de pacientes com SS têm um perfil de transcrição mais ativado e preparadas para produzir altos níveis de citocinas pró-inflamatórias, logo, esses achados suportam fortemente um papel patogênico potencial das CDp na doença SS²⁶.

Recentemente, um estudo foi realizado para elucidar o papel das CDp no desenvolvimento da SS usando um modelo animal (camundongos NOD) desta doença, e os resultados mostraram que as CDp contribuem criticamente para o início e desenvolvimento do distúrbio da glândula salivar semelhante à SS, incluindo sialadenite e hipossalivação, nestes ratos. Os resultados também forneceram vários mecanismos celulares e moleculares potenciais subjacentes ao efeito promotor de SS das CDs, que incluem a produção de IFN-1 como as principais fontes celulares e a regulação positiva de vários fatores pró-inflamatórios e quimioatraentes nos tecidos GSM alvo³². Ainda, neste estudo, mostrou-se que a depleção de CDp e a ablação de anti-IFNAR1 causam uma regulação negativa da expressão de IL-7 e BAFF em GSM, dois fatores patogênicos críticos na SS, aumentando as respostas das células T e B, respectivamente³².

Ao avaliar a presença das CDp em GSM de pacientes com SSp, através da expressão fenotípica dos anticorpos CD123²⁷ e CD303^{33, 34}, os autores notaram que as CDp eram expressas em número aumentado nas GSM de pacientes com SSp, com distribuição nos focos linfocíticos e áreas ductais, quando comparado ao controle (GSM saudáveis). Adicionalmente, ao correlacionar a presença dessas células na inflamação glandular e no sangue periférico da SSp, notou-se que, apesar de não haver uma correlação estatística, as CDp estavam aumentadas nas GSM e reduzidas no sangue periférico³⁵.

1.3 Macrófagos

Os macrófagos exibem morfologia diversificada e um expressivo conjunto de funções, estando presente em todos os órgãos e tecido conjuntivo. Dentre as funções naturais dos macrófagos evidencia-se: apresentação de antígenos, fagocitose de restos celulares e recrutamento de outras células imunes como reforço no combate aos corpos estranhos e células neoplásicas³⁶. Os macrófagos M1, são caracterizados por um fenótipo pró-inflamatório, promoção de T helper 1 (Th1) resposta imune, sendo ativados classicamente e com enorme capacidade de fagocitose; já os macrófagos M2 exibem funções regulatórias em reparação tecidual, remodelação e promoção de resposta imune em Th2 onde são aqueles ativados alternativamente, estando

envolvidos na desativação do sistema imune com a produção de citocinas anti-inflamatórias³⁷.

Estudos que avaliam a natureza e expressão dos macrófagos na SLF em biópsias de glândulas salivares labiais na SSp, onde os macrófagos CD68+ apresentaram-se com distribuição abundante na SLF quando comparada aos grupos controle, analisando assim que dentro dos infiltrados inflamatórios focais expressavam COX-1 e IL-18^{38, 39}. Entretanto, no estudo de Yarom e colaboradores⁴⁰, os macrófagos CD68+ estavam presentes isoladamente na SLF, compreendendo apenas 0,04% das células infiltrantes.

Curiosamente, um estudo realizado por de Wilde e colaboradores descreve a presença de células gigantes multinucleadas em estreita associação com ductos das glândulas salivares avaliando GSm de pacientes com SS, e que provavelmente representa uma reação de células gigantes do tipo corpo estranho, sendo observada em 4 de 55 (7%) pacientes com SS⁴¹.

Contudo, a população macrofágica é dinâmica, devido participar da inflamação e na resolução desse processo, contudo, pouco é compreendido sobre o papel dessas células inflamatórias na imunopatogênese da SS.

2 OBJETIVO

Analisar, através de marcadores imunoistoquímicos, centros germinativos e a distribuição de células dendríticas plasmocitoides e macrófagos em biópsias de glândulas salivares menores de lábio inferior em pacientes com SS, visando uma compreensão mais abrangente das funções específicas destes subtipos celulares.

2.1 Objetivos específicos

- Caracterizar os centros germinativos e células dendríticas foliculares através dos imunomarcadores Bcl-6, D2-40, CD57 e Ki-67.
- Avaliar a distribuição de CDs plasmocitoides através dos imunomarcadores CD123 e CD303.
- Comparar o perfil imunoistoquímico de distribuição dos CGs e CDp em pacientes com SS e pacientes não-SS não sicca.
- Avaliar a inter-relação das distintas populações celulares imunes para melhor compreensão dos mecanismos imunopatológicos na SS.

3 PUBLICAÇÕES

Os resultados foram formatados em 3 publicações científicas. No primeiro artigo, focamos na análise comparativa de CGs avaliando SLF em pacientes SSp e pacientes não-SS não-sicca, enquanto no segundo artigo, caracterização imunoistoquímica das CDp, e o terceiro artigo, relatamos um caso de paciente com SS com extravasamento de muco exibindo reação granulomatosa do tipo corpo estranho.

3.1 Publicação 1*

CHARACTERIZATION OF ECTOPIC GERMINAL CENTERS IN SJÖGREN'S SYNDROME (SS) AND NON-SS NON-SICCA PATIENTS

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Keywords: Sjögren's syndrome, non-Sjögren syndrome, non-sicca control; oral reactive lesions; focal lymphocytic sialadenitis, ectopic germinal centers; immunohistochemistry; D2-40, CD57.

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NOTES

Compliance with Ethical Standards

Ethical Approval

This study was reviewed and approved by the Research Ethics Committee of the Ribeirão Preto Medical School, University of São Paulo (Protocol number: 60786216.8.0000.5440) and all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflicts of interest statement: The authors have no conflict of interest in the present manuscript.

Author contributions: All authors were involved in drafting the article or revising it critically for important intellectual content.

CHARACTERIZATION OF ECTOPIC GERMINAL CENTERS IN SJÖGREN'S SYNDROME (SS) AND NON-SS NON-SICCA PATIENTS

Recently, we have published a study in which shown that focal lymphocytic sialadenitis (FLS), an important criterion for Sjögren syndrome (SS) diagnosis only when a focus score (FS) ≥ 1 is detected, can also be observed when assessing intraoral biopsies containing minor salivary gland (mSG) parenchyma from healthy asymptomatic individuals (Silva et al., 2022). In this study, the frequency of germinal centers (GCs) comparing primary SS (pSS) and non-SS non-sicca patients was similar. By immunohistochemical analysis (IHC), all GCs were Bcl-6 and CD23 positive, with variable expression of CD10. However, some foci (without GCs) were also CD23 positive (Silva et al., 2022). Our results, supported by previous study (Nakshbandi et al., 2020), indicate que Bcl-6 is a reliable marker for GC detection in these cases.

In SS patients, the presence of GCs when assessing FLS seems to be associated with more severe disease, including higher levels rheumatoid factor , anti-SSA/B antibodies, proinflammatory mediators, higher FS, reduced saliva production (Carubbi et al., 2019; Risselada et al., 2013), as well as predictor of MALT lymphoma development (Theander et al., 2011; Sène et al., 2018), with some studies not supporting this latter (Haacke et al., 2017; 2019).

In our Oral Histopathology Laboratory (FORP/USP), evaluating the D2-40 and CD57 immunoexpression in palatine tonsil specimens (diagnosed as follicular hyperplasia), we have frequently observed a strong expression of D2-40 within GCs, evidencing follicular dendritic cell (FDC) networks (Marsee et al., 2009; Yu et al., 2007), as well as a homogeneous distribution of strongly stained CD57+ cells inside the GCs, characterizing T-follicular helper (TFH) cells (Sattarzadeh et al., 2015) (**Figure 1A**).

Thus, considering the importance of GC detection, the lack of specificity of routinely used GC markers, as well as the similar frequency of GCs assessing FLS in SS and non-SS non-sicca patients, in the current study, we have focused comparative analysis of GCs, using D2-40 and CD57, in correlation with Bcl-6 and Ki-67 immunomarkers, aiming to highlight differences with diagnostic implications in these two populations.

This study was approved by the Ethics Committee in Human Research from FMRP/USP (statement number: 68748117.0.0000.5440). The clinicopathological features of the patients included in the current study are shown in **Table 1**. Ten of 13 cases (pSS) and 5 of 11 cases (non-SS non-sicca) were published previously by us (Silva et al., 2022). The SS diagnosis followed strict criteria of American College of Rheumatology/European League Against Rheumatism (Shiboski et al., 2017). The 6 cases of non-SS non-sicca (not previously published) were selected after careful microscopical analysis of 500 oral mucosal biopsies (350 females and 150 males; mean age, 50 years), clinically and microscopically corresponding to inflammatory/reactive lesions, which also presented, at the periphery of the specimen and away from the main lesion, typical FLS features (Silva et al., 2022). The antibodies used were Bcl-6 (Clone LN22, 1:500 dilution, Leica Biosystems), D2-40 (Clone M3619, 1:500 dilution, DakoCytomation), CD57 (Clone TB01, 1:600 dilution, DakoCytomation), and Ki-67 (Clone ab16667, 1:500 dilution, Abcam). GC histomorphology, Bcl-6 positivity, as well as inclusion and exclusion criteria, were previously defined (Silva et al., 2022). For D2-40, immunoreactivity was graded semiquantitatively according to the percentage of positivity of FDCs: 0, <5%; 1+, 5%-25%; 2+, >25%-50%; 3+, >50%. The intensity of staining was graded as weak (1+), moderate (2+), or strong (3+). For CD57, the scoring system was: 0, no positive cells; 1+, 1-5 positive cells; 2+, >5-10 positive cells; 3+, >10 positive cells. For Ki-67, the scoring system was: 1+, <10% positive cells; 2+, 10%-35% positive cells; 3+, >35% positive cells. Analysis of variance was applied to assess differences between groups. For categorical data, the G-test and chi-square test was applied. A probability (p) value <0.05 was considered statistically significant (IBM-SPSS 20.0 statistics).

Our results showed the area and largest diameter of GCs, as well as FLS with FS $\geq$ 1, were significantly higher in pSS than non-SS non-sicca group.

On IHC, 45/121 and 28/66 foci in pSS and non-SS non-sicca group, respectively, showed Bcl-6 positivity characterizing (also by histomorphology) GCs. Almost all Bcl-6+ foci were also D2-40 and CD57 positive (**Table 1 and Figure 1B-E**). However, D2-40 and CD57 expression was not restricted to GCs. In fact, D2-40+/Bcl6- foci (39% vs 23%, p=0.062) and CD57+/Bcl-6- foci (11% vs 4%, p=0.24) were detected more frequently in pSS than non-SS non-sicca group. Regarding percentage of positivity and intensity of staining of the immunomarkers, it was greater in pSS than

non-SS non-sicca group, with D2-40 and CD57 expression being statistically significant (**Figure 1, B-E**).

In this study, only a cluster of ≥ 5 Bcl-6+ cells, showing a nuclear staining pattern with adequate morphology and organization, was considered positive and characterizing a GC (Silva et al., 2022; Nakshbandi et al., 2020; Haacke et al., 2017). All these GCs expressed Ki-67 and frequently exhibited a polarized positivity, typical of reactive/benign nature of the lymphoid proliferation (Almeida et al., 2020).

D2-40 antibody recognizes a mucin-type transmembrane glycoprotein, which is expressed in GC FDCs networks, including FDC tumors, as well as in lymphatic endothelium, kidney podocytes, mesothelium, and pulmonary alveolar cells. Moreover, regarding immune cells, macrophages and Th17 cells can express D2-40 (Yu et al., 2007; Marsee et al., 2009). In the current study, D2-40 expression also was observed in myoepithelial cells, lymphatic vessels and basal cell layer of surface epithelium. The D2-40+/Bcl-6- foci probably represent primary lymphoid follicles or isolated FDCs.

CD57 antibody recognizes a 110 kDa protein on a subset of NK and T cells, and neural tissues. Inside GCs, CD57+ TFH cells provide key signals to B cells for GC development, modulating immunoglobulin synthesis. Moreover, TFH cells can also express Bcl-6 (Shane, 2021). In this study, CD57+ cells were observed inside (TFH cells) and outside (NK or T cells) GCs, besides highlighting isolated neural bundles. Inside GCs, Bcl-6+ cells morphologically resembling lymphocytes favored TFH cells (Shane, 2021).

In summary, our results show histomorphological (area and diameter) and immunophenotypical (D2-40 and CD57 expression profile) differences, suggesting distinctive cellular and/or molecular interactions in the pathogenesis of GCs when comparatively evaluating SLF in pSS and non-SS non-sicca patients.

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Table 1. Clinicopathological features of the biopsies from pSS patients and non-SS non-sicca patients included in the current study.

Clinicopathological features	pSS patients (n=13)	Non-SS non- sicca patients (n=11)	p-value
Mean age/range (years)	51/27–74	38/6–78	0.104
Gender			
Female	13	8	0.146
Male	0	3	
Diagnosis			
pSS	13	0	0.009
Inflammatory fibrous hyperplasia	-	7	
Mucocele	-	3	
Ranula	-	1	
Serological parameters §			
Anti-SSA positive, n° (%)	12/13 (92)	0	0.028
Anti-SSB positive, n° (%)	9/13 (69)	0	
RF positive, n° (%)	8/13 (61)	0	
ANA positive, n° (%)	8/13 (61)	0	
Histopathological findings			
FLS with FS≥1	13	6	0.007
FLS with FS<1	0	3	
FLS/CSS with FS<1	0	2	
GCs based on H&E			
Range of GCs (mean)	1 – 12 (3.6)	1 – 7 (2.1)	0.240
Morphometry of GCs			
Area ±SD (mm²)	0.08±0.02	0.05±0.02	
Largest diameter ±SD (µm)	345.5±59.8	268.8±61.1	
Number of foci based on IHC	121	66	
Bcl6 ⁺ GCs (%)	45/121 (37)	28/66 (42)	0.529
D2-40 ⁺ Bcl6 ⁻ (%)	47/121 (39)	15/66 (23)	0.062
CD57 ⁺ Bcl6 ⁻ (%)	13/121 (11)	3/66 (4)	0.240

Abbreviations: ANA, antinuclear antibodies; CSS, chronic sclerosing sialadenitis; FLS, focal lymphocytic sialadenitis; FS, focus score; GC, germinal center; H&E, hematoxylin and eosin; IHC, immunohistochemical analysis; pSS, primary Sjögren's syndrome; RF, rheumatoid factor; SD, standard deviation; SSA, Sjögren's syndrome antigen A; SSB, Sjögren's syndrome antigen B.

§ Anti-SSA titer>25 EU/ml, Anti-SSB titer≥50 EU/ml, ANA titer≥1:320, and IgM RF>12.5 IU/ml titer, were considered positive.

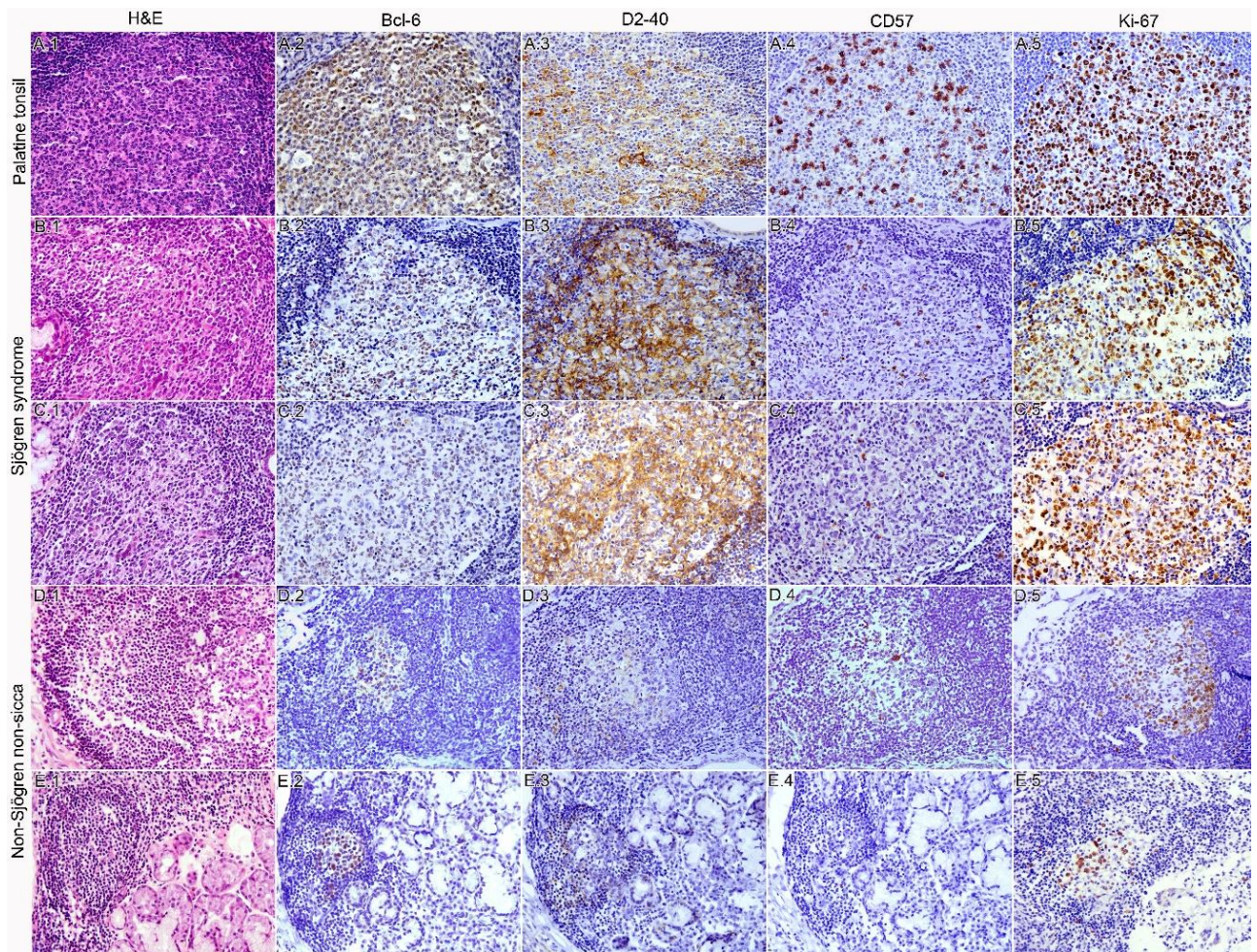
Figure 1:

Figure 1. Histopathological and immunohistochemical analysis, through consecutive (serial) tissue sections, of germinal centers (GCs) in palatine tonsil (diagnosed as follicular hyperplasia) (A) and intraoral focal lymphocytic sialadenitis (FLS) in primary Sjögren syndrome (pSS) (B and C) and non-SS non-sicca (D and E) patients. In the palatine tonsil sample (A), there was homogeneous and strong expression of all markers. In B and C are shown two cases of pSS. Notice the evident cytoplasmic and membranous staining pattern of D2-40 highlighting follicular dendritic cells (FDCs), whereas CD57+ T-follicular helper (TFH) cells appear more scattered, with polarized positivity for Ki-67. In D and E are shown two cases of non-SS non-sicca. It can be appreciated that their findings are less evident with that observed in pSS patients (all figures, x20 original magnification).

3.2 Publicação 2*

IMMUNOCHARACTERIZATION OF PLASMOCYTOID DENDRITIC CELLS IN PRIMARY SJÖGREN'S SYNDROME AND NON-SS NON-SICCA PATIENTS: A COMPARATIVE STUDY

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Keywords: Sjögren's syndrome; non-Sjögren syndrome non-sicca patients; oral reactive lesions; focal lymphocytic sialadenitis, plasmocitoid dendritic cells; immunohistochemistry; CD123, CD303.

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9 and 2022/12760-9) from State of São Paulo Research Foundation (FAPESP) and research grant (304241/2021-0) from National Council for Scientific and Technological Development (CNPq).

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NOTES

Compliance with Ethical Standards

Ethical Approval

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Conflicts of interest statement:

The authors have no conflict of interest in the present manuscript.

Author contributions:

All authors were involved in drafting the article or revising it critically for important intellectual content.

IMMUNOCHARACTERIZATION OF PLASMOCYTOID DENDRITIC CELLS IN PRIMARY SJÖGREN'S SYNDROME AND NON-SS NON-SICCA PATIENTS: A COMPARATIVE STUDY

Sjögren's syndrome (SS) is a chronic autoimmune disease characterized by lymphocytic infiltration, often affecting the salivary and lacrimal glands, causing sicca symptoms and often accompanied by abnormal production of autoantibodies (Silva et al., 2022). Histopathological analysis of minor salivary gland (mSG) is widely used in primary SS (pSS) classification criteria and the histopathological hallmark of the disease is focal lymphocytic sialadenitis (FLS) with focus score (FS) ≥ 1 (Shiboski et al., 2017; Carubbi et al., 2019). Additionally, we showed that FLS can also be observed when evaluating intraoral biopsies containing mSG parenchyma from healthy asymptomatic individuals (non-SS non-sicca patients) (Silva et al., 2022).

Recent evidence indicates that plasmacytoid dendritic cells (pDCs) critically contribute to the development and onset of SS-like salivary gland exocrinopathy (Zhou et al., 2022). This study, using the non-obese diabetic mouse model, provided several potential cellular and molecular mechanisms underlying the promoter effect of pDCs in SS, which include the production of type I interferons (IFNs) as the main cellular sources and the upregulation of a number of pro-inflammatory factors and chemoattractants in the target mSG tissues and that anti-BST2-mediated pDCs depletion and anti-IFNAR1 ablation both cause a down-regulation of IL-7 and BAFF expression in mSG, two critical pathogenic factors in SS, reducing local T and B cell responses, respectively (Zhou et al., 2022).

In addition to robust production of type I IFN, pDCs can also secrete several other inflammatory cytokines such as TNF- α and IL-6, produce chemokines, and express costimulatory molecules on their surfaces to influence innate and adaptive immune responses (Cao et al., 2014; Swiecki & Colonna, 2015; Zhou et al., 2022). Recent transcriptome study conducted by Hillen et al., demonstrated that pDCs from patients with SS have a more activated transcriptional profile and are better prepared to produce high levels of pro-inflammatory cytokines, thus supporting a potential pathogenic role of pDCs in SS disease (Hillen et al., 2019).

Thus, considering the importance of pDCs in perpetuating the inflammatory process of the salivary gland in patients with SS (Gottenberg et al., 2006; Zhou et al., 2022), as well as the presence of FLS and germinal centers (GCs) in mSG biopsies from pSS patients and non-SS non-sicca patients (Silva et al., 2022), in the current study, we focused on the comparative immunohistochemical analysis of pDCs, using the immunomarkers CD123 and CD303, with the aim of characterizing the presence of these cells in glandular inflammation and highlighting differences with diagnostic implications in these two populations.

This study was approved by the Ethics Committee in Human Research from FMRP/USP (statement number: 68748117.0.0000.5440). The clinicopathological features of the patients included in the current study are shown in **Table 1**. These cases were selected from a retrospective study previously published by our group (Silva et al., 2022). The study similarly included 50 pSS patients diagnosis followed strict criteria of American College of Rheumatology/European League Against Rheumatism (Shiboski et al., 2017) and 31 cases of the patients clinically and microscopically diagnosed as mucocoele, inflammatory fibrous hyperplasia or ranula, which presented typical FLS features (non-SS non-sicca patients) (Silva et al., 2022). For the evaluation of pDCs, the antibodies used were CD123 (Clone NCL-L, 1:200 dilution, Leica Biosystems) and CD303 (Clone 124B3.13, 1:300 dilution, Dendritics). Sections for immunohistochemistry were incubated with streptavidin-biotin-peroxidase-conjugated secondary antibodies (K0690; Universal Dako LSAB+Kit, Peroxidase, Carpinteria, California). The reactions were revealed with diaminobenzidine and the sections counterstained with Carazzi's hematoxylin. The slides were analyzed in an optical microscope with a magnification of 400x (Leica DM 2500). Five areas with strong staining were selected for photomicrography (Leica Application Suite – LAS) and cells were counted using ImajJ software. The Shapiro–Wilk's test for assessing the normal distribution was used, and the statistical significance was determined by the Mann–Whitney U-test. A probability (p) value <0.05 was considered statistically significant (IBM-SPSS 20.0 statistics).

Our results showed that pDCs were identified by immunohistochemical staining CD123 and CD303, showing an ovoid to circular morphology, with well-defined cytoplasmic immunostaining, with density ranging from absent to frequent in the pSS

group and with limited expression in the non-SS non-sicca group, with distribution in the periphery of the FLS and in a perivascular pattern (**Figure 1**).

The mean number of CD123+ pDCs was 6.8 (Q1 = 3.75; Q3 = 8.2) in the pSS group (Figure 2-C) and 3.0 (Q1 = 0.4; Q3 = 4.7) in the non-SS non-sicca group (Figure 1-G). The number of CD303+ pDCs was also evaluated, which showed a mean number of immunostained cells in the pSS group of 5.7 (Q1 = 2.75; Q3 = 8.2) and in the non-SS non-sicca group it was of 2.1 (Q1 = 0; Q3 = 3.4) (Figure 1-H). Thus, the expression of pDCs in FLS from pSS patients, for both immunomarkers, was significantly higher when compared to FLS in non-SS non sicca patients ($p < 0.001$ and $p < 0.001$, CD123+ and CD303+ respectively).

Furthermore, our results showed a slightly larger population of CD123+ pDCs compared to CD303+ pDCs, without significant difference.

CD123 is an alpha chain of the IL-3 receptor expressed by pDCs showing higher maturity and ability to secrete cytokines, while CD303, also termed blood-derived dendritic cell antigen 2 (BDCA2) protein, is expressed by immature pDCs (Zhou et al., 2021; Dyduch et al., 2023). The infiltration of these pDCs in to pSS has been related to the presence of a type I IFN signature activated or pro-inflammatory cytokines by endosomal Toll-like receptors (TLR) 7 and 9 signaling, through which they recognize viral RNA and DNA (Hillen et al., 2019; Yura & Hamada, 2023). Additionally, when evaluating the presence of pDCs in mSG of patients with pSS, through the phenotypic expression of antibodies CD123 (Gottenberg et al., 2006) and CD303 (Vogelsang et al., 2010; Shimizu et al., 2019), these studies noted that pDCs were expressed in increased numbers in mSG of these patients, with distribution in FLS and ductal areas compared to control (healthy mSG).

Finally, we hypothesize that the expression of CD123+ and CD303+ pDCs in the mSG of pSS patients may be contributing to the pathogenic mechanism of this disease, resulting in an increased severity of salivary gland inflammation and secretory dysfunction, corroborating previous studies (Bave et al., 2005; Gottenberg et al., 2006; Zhou et al., 2022). Thus, our results show immunophenotypic differences (CD123 and CD303 expression profile) when comparing FLS in pSS patients and non-SS non-sicca patients, suggesting that these have diagnostic and immunomodulatory implications in pSS.

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Table 1. Clinicopathological features of the cases included in the current study.

Clinicopathological features	pSS patients (n=50)	Non-SS non-sicca patients (n=31)
Mean age/range (years)	55/12–81	42.3/9–69
Gender		
Female	46	18
Male	4	13
Diagnosis		
pSS	50	0
Inflammatory fibrous hyperplasia	0	17
Mucocele	0	13
Ranula	0	1
Serological parameters *		
Anti-SSA positive, n° (%)	39/50 (78)	0
Anti-SSB positive, n° (%)	20/50 (40)	0
RF positive, n° (%)	9/30 (30)	0
ANA positive, n° (%)	15/30 (50)	0
Histopathological findings (H&E)		
FLS with FS≥1	50	12
FLS with FS<1	0	10
FLS/CSS with FS<1	0	9

Abbreviations: ANA, antinuclear antibodies; CSS, chronic sclerosing sialadenitis; FLS, focal lymphocytic sialadenitis; FS, focus score; H&E, hematoxylin and eosin stain; pSS, primary Sjögren's syndrome; RF, rheumatoid factor; SSA, Sjögren's syndrome antigen A; SSB, Sjögren's syndrome antigen B.

* Anti-SSA titer >25 EU/ml, Anti-SSB titer ≥50 EU/ml, ANA titer ≥1:320, and gM RF >12.5 IU/ml titer, were considered positive. A significant positive correlation ($p<0.05$) was observed only when comparing anti-SSA with the number of foci and FS.

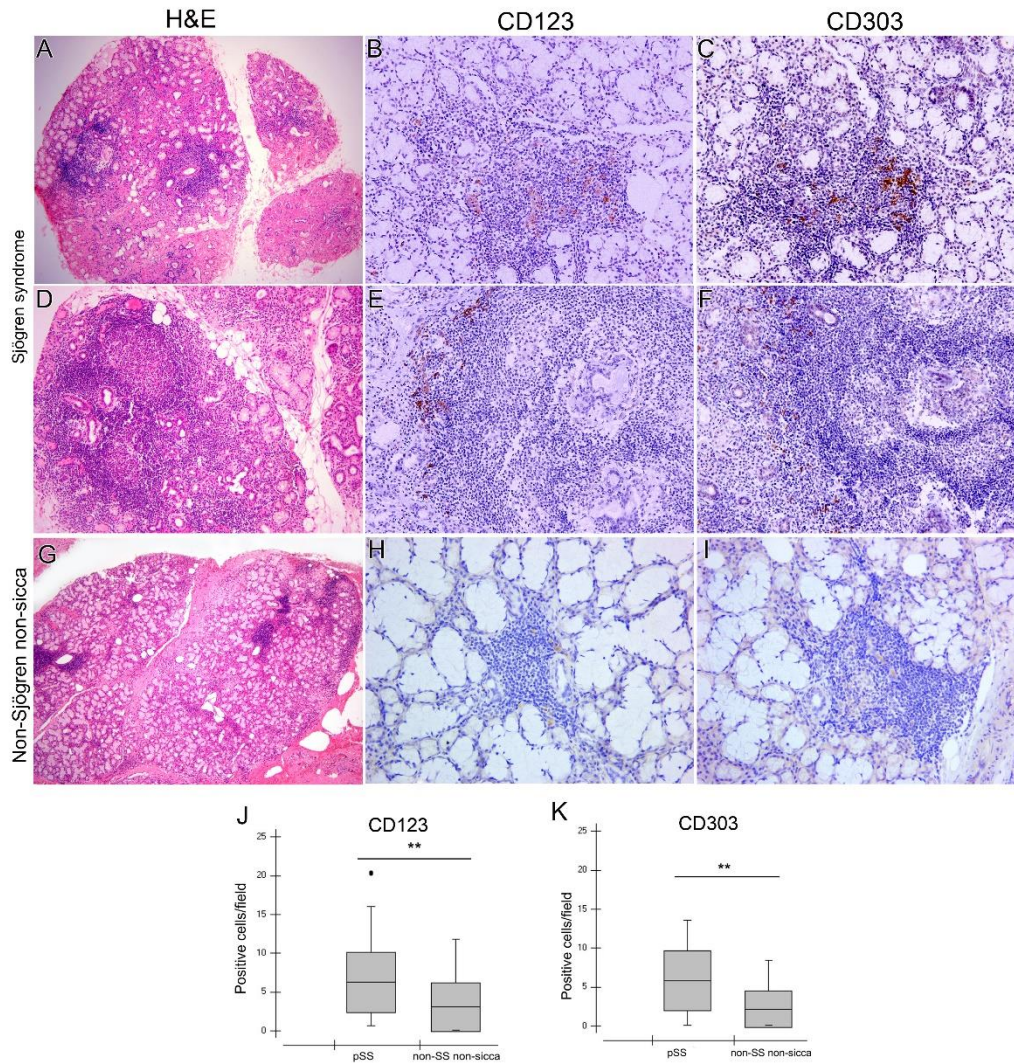


Figure 1. Histopathology of intraoral focal lymphocytic sialadenitis (FLS) (A, D and G) and immunohistochemical analysis, through consecutive (serial) tissue sections, of plasmacytoid dendritic cells (pDCs) in FLS of primary Sjögren's syndrome (pSS) (A-F) and non-SS non-sicca (G-I) patients. In the group of pSS patients, pDCs were identified by immunohistochemical staining of CD123 (B and E) and CD303 (C and F), showing an ovoid to circular morphology, with well-defined cytoplasmic immunostaining with distribution in the periphery of the FLS and in a perivascular pattern. In the group of non-SS non-sicca patients, pDCs CD123 (H) and CD303 (I) were expressed with limited frequency. In J and K, graphs showing the expression of pDCs in FLS from pSS patients, for the immunomarkers CD123 (J) and CD303 (K), was significantly higher when compared to FLS in non-SS non sicca patients. ** $p < 0.001$ (A and D, x5; B, C, E and F, x20 original magnification).

3.3 Publicação 3*

INTRAORAL FOREIGN BODY-TYPE GIANT CELL REACTION IN A SJÖGREN'S SYNDROME PATIENT: AN IMMUNOHISTOCHEMICAL ANALYSIS

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Keywords: Sjögren's syndrome; oral mucocoele; focal lymphocytic sialadenitis; non-infectious granulomatous inflammation; foreign body-type giant cell reaction

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NOTES

Compliance with Ethical Standards

Author contributions: All authors were involved in drafting the article or revising it critically for important intellectual content.

Conflicts of interest statement: The authors have no conflict of interest in the present manuscript.

Patient consent: Written informed consent was received from the patient for the publication of data.

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ABSTRACT

Sjögren's syndrome (SS) is a chronic autoimmune disease characterized by lymphocytic infiltration that mainly affects the salivary and lacrimal glands, causing sicca symptoms. Non-infectious granulomatous inflammation affecting the salivary glands is rare, and it may be caused by duct obstruction associated with mucus extravasation. A 63-year-old female patient, diagnosed 19 years ago with primary SS, was referred presenting a nodular mass on the buccal mucosa 1 week ago. Microscopic analysis revealed focal lymphocytic sialadenitis and an exuberant lymphoplasmacytic infiltrate containing in the central part a foreign body-type giant cell reaction, permeated by numerous residual salivary gland ducts. Polarizing microscopy did not identify foreign material. Grocott-Gomori, acid-fast bacilli and Gram stains were negative. However, periodic acid-Schiff and mucicarmine stains revealed positivity in several multinucleated giant cells (MGC), adjacent macrophages and extracellular environment, consistent com mucus extravasation. Immunohistochemical analysis revealed strong positivity for CD68, CD163 and CD11c, highlighting MGC and macrophages, permeated by numerous T cells, B cells and plasma cells, whereas pan-CK and EMA evidenced residual salivary gland ducts. In the current case, mucus extravasation is a probable cause of foreign body-type giant cell reaction, suggesting a possible relationship with immune mechanisms of SS involved in its pathogenesis.

Keywords: Sjögren's syndrome; oral mucocoele; focal lymphocytic sialadenitis; non-infectious granulomatous inflammation; foreign body-type giant cell reaction.

INTRODUCTION

Sjögren's syndrome (SS) is a chronic autoimmune disease characterized by lymphocytic infiltration often affecting the salivary and lacrimal glands, causing sicca symptoms. Relevantly, 5-10% of SS patients can present extraglandular manifestations, such as myalgia, arthralgia and fatigue. Moreover, immunological disturbances, such as hypergammaglobulinemia, rheumatoid factor,

hypocomplementemia, cryoglobulinemia and autoantibodies directed against SSA/Ro and SSB/La autoantigens are frequently detected [1]. Minor salivary gland (mSG) biopsies showing focal lymphocytic sialadenitis (FLS) with focus score (FS) ≥ 1 , is an important diagnostic criterion for defining SS [1, 2]. Oral lesions in SS patients, such as angular cheilitis, atrophic glossitis, ulcerations, caries, gingivitis and candidiasis, are commonly reported and it have been associated with decreased saliva production [3, 4]. It is interesting to note that, although obstructive phenomenon by periductal lymphocytic infiltrates can be observed in mSGs from SS patients, reactive lesions, such as oral mucocele and ranula, are uncommon [4-6]. However, the presence of multinucleated giant cells (MGC) in close association with salivary gland ducts assessing mSGs from SS patients [7], as well as non-infectious granulomatous inflammation affecting submandibular glands in non-SS patients, with duct obstruction associated with mucus extravasation as major cause [8], have been reported.

Oral mucocele is a common reactive lesion of the oral mucosa, which microscopically exhibits mucus extravasation surrounded by a wall of granulation tissue containing abundant foamy macrophages [9]. Unusual histopathological patterns of oral mucocele have been described, including myxoglobulosis-like change, papillary synovial metaplasia-like change and clear cell and signet-ring cell changes [9].

Here, we describe an intraoral nodular lesion affecting a SS patient, which microscopically exhibited a foreign body-type giant cell reaction, probably associated with mucus extravasation arising from ruptured or damaged salivary gland ducts.

CASE PRESENTATION

A 63-year-old white woman was admitted to the Oral Medicine Service for evaluation of an intraoral nodule 1 week ago. She reported a primary SS (pSS) diagnosis at age of 44 years, presenting at this time mouth dryness, conjunctivitis sicca, positivity for anti-SSA/Ro antibodies (> 25 EU/ml), Schirmer's test (< 5 mm) in both eyes, total non-stimulated saliva flow (< 1.5 mL in 15 min), and mSG biopsy showing FLS with FS > 1 , sufficiently fulfilling currently recommended criteria.² In addition, antinuclear antibodies (ANAs) were positive ($> 1:320$). The sedimentation

rate, rheumatoid factor, anti-SSB/La, anti-Sm, and anti snRNP were negative. In addition to the use of artificial tear and saliva, treatment included stimulation of residual glandular function with a muscarinic agonist to alleviate sicca symptoms.

Intraoral examination revealed a well-demarcated nodular mass on the left side of the buccal mucosa, measuring 1.5 cm on its greatest diameter and covered by normal-appearing mucosa. The lesion was resilient in consistency, painless, fluctuant and lobulated on palpation (Figure 1A). Extraoral examination was unremarkable. The clinical differential diagnosis included mesenchymal neoplasm (neurofibroma, schwannoma, myofibroma), salivary gland neoplasm (pleomorphic adenoma), mucocele, fibrous hyperplasia and subacute necrotizing sialadenitis (SANS). After excisional biopsy, the microscopical analysis showed typical FLS and adjacent an exuberant lymphoplasmacytic infiltrate, containing in the central part a foreign body-type giant cell reaction, surrounded by numerous residual ductal structures (Figure 1 B-C). Polarizing microscopy failed to identify foreign material. Grocott-Gomori, acid-fast bacilli and Gram stains were negative. However, periodic acid-Schiff and mucicarmine stains showed positivity on several multinucleated giant cells, adjacent macrophages and extracellular environment, being consistent with mucus extravasation (Figure 1D). The immunohistochemical analysis showed a strong positivity for CD68, CD163, CD11c, and weak for FXIIIa, highlighting the foreign body-type giant cell reaction. S100 evidenced scarce dendritic-like cells, while CD1a and CD207 were negative. The surrounding lymphoplasmacytic infiltrate revealed to be composed of CD3+ T cells, with predominance of CD8+ than CD4+ T cells, CD20+ B cells, with peripheral sheets of polyclonal (similar kappa/lambda ratio) CD138+/EMA+ plasma cells. The residual salivary ductal structures were exhibited through pan-CK AE1/AE3 and EMA. The Ki-67 proliferation index was <5% (Figure 2). The lesion healed optimally after 1 month and, currently, after 7 years, the patient is doing well, under care by a multidisciplinary team.

DISCUSSION

We report an unusual case of pSS who presented an intraoral nodular lesion, which histopathologically showed an exuberant lymphoplasmacytic infiltrate and foreign body-type giant cell reaction consistent with mucus extravasation. This is rarely reported when assessing oral lesions in SS patients; however, we believe that previous

studies support our findings. In fact, the presence of MGC in close association with salivary ducts assessing labial SGs from SS patients, and which probably represents a foreign body-type giant cell reaction, was observed in 4 of 55 (7%) SS patients [7]. Moreover, in a large series evaluating 469 major salivary glands, 57 (12%) cases were diagnosed as granulomatous sialadenitis. In 34 of 57 cases, all corresponding to submandibular gland, a diagnosis of non-infectious granulomatous sialadenitis was made, with duct obstruction associated with mucus extravasation as major cause [8].

Although mucocèles are common reactive lesions affecting the oral mucosa, its detection is very uncommon in SS patients, with only 3 cases previously reported [4,5]. However, after careful clinicopathological analysis of these cases, we consider that 2 cases represent typical ranulas [5], and 1 case could be an example of superficial ranula [4], such as previously reported [10].

Ranulas are caused by extravasation of mucus from damage or obstruction of the sublingual gland or its duct, causing a clear or bluish lump on the floor of the mouth [11]. It has been hypothesized that extravasation of saliva from damaged or ruptured ducts associated with lymphocytic infiltration in SS patients causes ranulas [6]. In this context, studies have focused on the role of ranulas in early diagnosis of SS [6, 11]. Recently, a study reported a 22-year-old female who developed a unilateral ranula, being diagnosed with pSS 3 years later [11]. The same authors provide a review of SS and ranulas from 1980 to 2020. About 17 pSS patients with ranula were described, being 15 female and 2 male, with mean age of 32.6 years (range, 8 to 66 years). Ranulas were detected before or at the same time of pSS diagnosis in 9 and 6 patients, respectively; with only 3 cases presenting bilateral ranula. The time interval from ranula detection to pSS diagnosis ranged from 0 to 7 years, indicating that pSS occurs insidiously. These findings suggest that ranulas could be early clinical manifestations of SS [11].

Such as commented above, distinctive microscopical patterns of oral mucocèle have been reported [9]. The findings in the current case, not previously reported, strongly suggest that mucus extravasation is a probable cause of the foreign body-type giant cell reaction. However, it should be differentiated from infectious (bacterial, fungal and parasitic origin) and non-infectious (sarcoidosis, granulomatous cheilitis, Crohn disease, vasculitis syndromes) diseases, which typically present epithelioid granulomas variably associated with MGC [12-14]. Moreover, although not detected in

the current case, meticulous analysis applying polarization microscopy to identify exogenous materials by accidental or iatrogenic implantation, is relevant [15].

Considering the clinical presentation and exuberant lymphoplasmacytic infiltrate, the current case should also be differentiated from SANS [15], which is an unusual self-limiting, inflammatory condition affecting mSGs. Clinically, it presents as a painful, non-ulcerated swelling, often involving the hard palate, followed by soft palate, buccal mucosa and tongue [16, 17]. On microscopy, it is characterized by diffuse infiltration of acute and chronic inflammatory cells involving the parenchyma of mSGs, which present ductal atrophy and acinar necrosis, with eosinophilia being a constant finding [16], unlike the current case. By immunohistochemistry, SANS shows the inflammatory infiltrate consisting predominantly of M2 macrophages, in diffuse distribution pattern, and cytotoxic T lymphocytes, with virtually absence of CD20+ B cells and scarce CD138+ plasma cells [16]. Similar immunohistochemical profile was observed in the current case; however, the foreign body-type giant cell reaction was highlighted with macrophage markers.

CONCLUSION

Although 3 cases of oral mucocele have been reported affecting SS patients [4, 5], we believe that these cases could be better diagnosed as ranula. The possibility of ranula as an early clinical manifestation of SS should be considered. In the current case, it is feasible that the lymphocytic infiltration is involved with the mechanisms of ruptured or damaged salivary ducts resulting in mucus extravasation phenomena and consequent foreign body-type giant cell reaction, with a probable involvement of immune mechanisms of SS in its pathogenesis.

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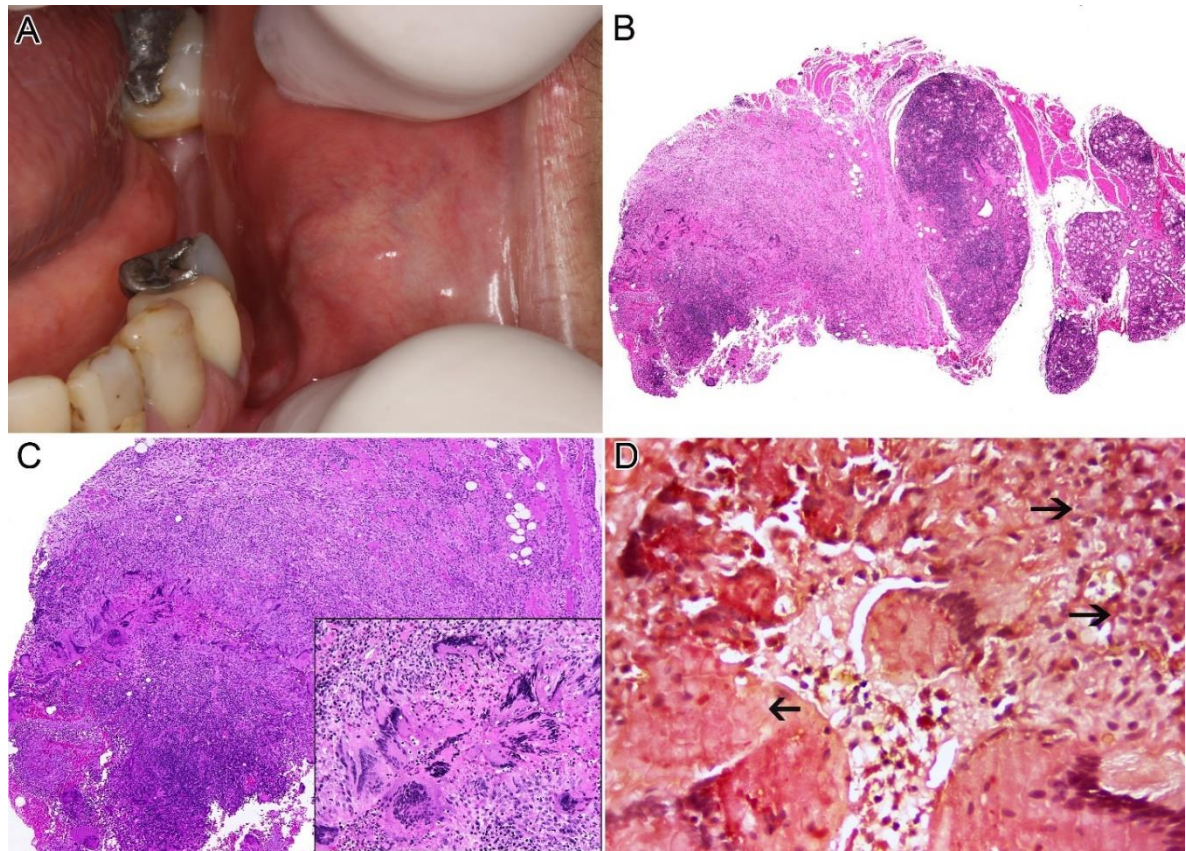
Figure 1:

Figure 1. Clinical presentation of non-ulcerated nodule on the left side of the buccal mucosa (A). Histopathological analysis on hematoxylin and eosin (H&E) stain exhibited typical focal lymphocytic sialadenitis (right side) and an exuberant lymphoplasmacytic infiltrate associated with foreign body-type giant cell reaction (B, x2.5). At high-power magnification showing lymphoplasmacytic infiltrate, multinucleated giant cells and macrophages, admixed with residual salivary gland parenchyma (C, x10. Inset shows cellular details). Strong positivity for mucicarmine stain on extracellular environment, as well as macrophages and multinucleated giant cells (arrows), confirmed the presence of mucus extravasation phenomena (D, x40).

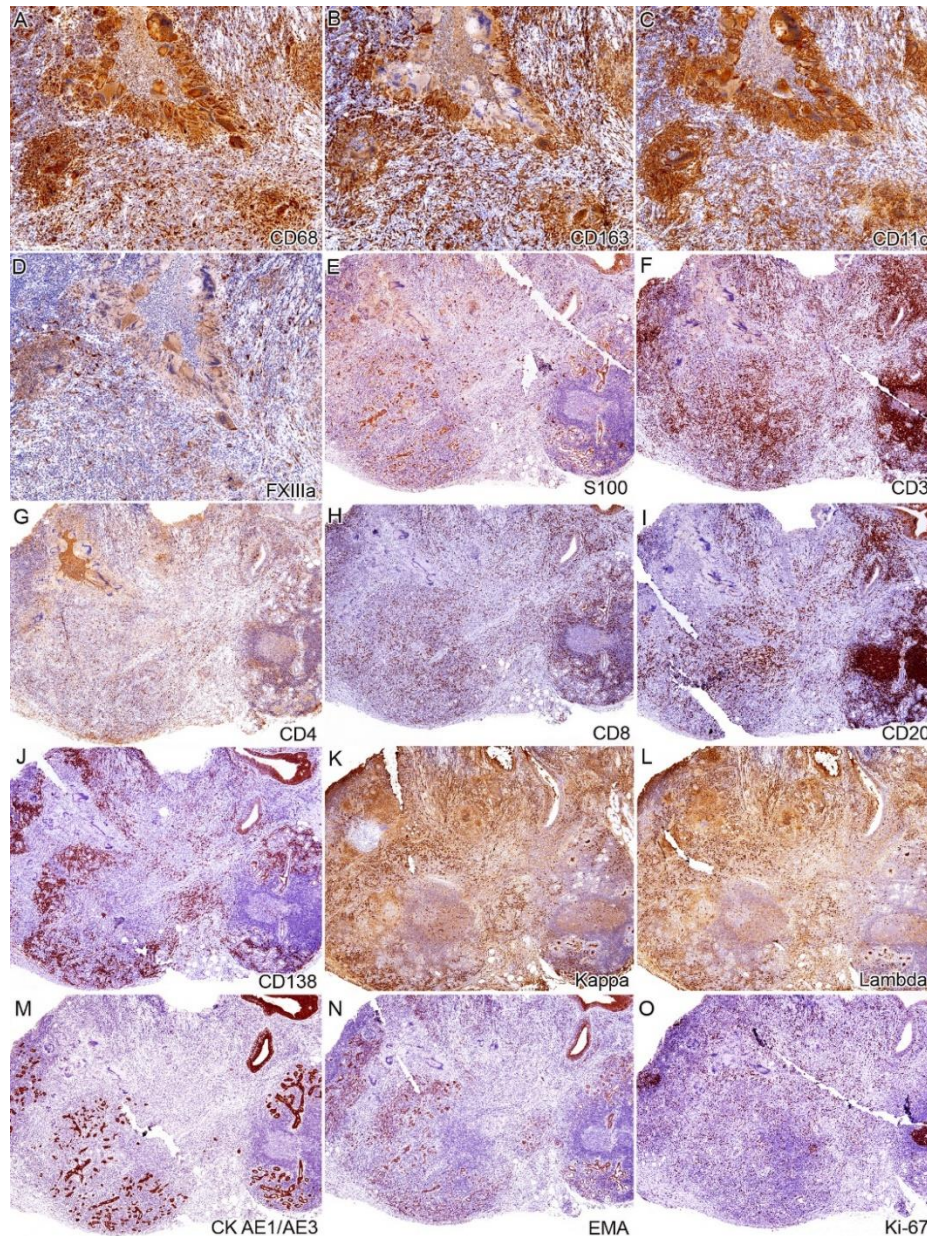
Figure 2:

Figure 2. Immunohistochemical analysis, through consecutive (serial) sections, revealed strong expression of CD68, CD163 and CD11c, with weak positivity for FXIIIa, notably delimiting a central cavity, and supporting a macrophage lineage cells (A-D). Scarce dendritic-like cells and residual salivary gland parenchyma were S100 positive (E). The lymphoplasmacytic infiltrate was constituted by CD3+ T cells, with CD8+ higher than CD4+ T cells, permeated by CD20+ B cells (F-I). At the periphery, sheets of reactive (similar kappa/lambda ratio) CD138+ plasma cells were evidenced (J-L). pan-CK AE1/AE3 and EMA highlighted the residual salivary gland parenchyma involved by inflammatory cell infiltrate (M, N). The Ki-67 proliferation index was <5% (O). Notice the typical focal lymphocytic sialadenitis, including germinal center, at the right side (E-O). (A-D, x10; E-O, x5).

4 CONCLUSÃO

Nossos resultados mostram diferenças histomorfológicas (área e diâmetro) e imunofenotípicas (perfil de expressão de D2-40 e CD57), sugerindo interações celulares e/ou moleculares distintas na patogênese de centros germinativos ao avaliar comparativamente SLF em pacientes com SSp e pacientes não-SS não-sicca.

A expressão de células dendríticas plasmocitoides CD123+ e CD303+ na SLF de pacientes com SSp, com diferenças significativas, ao comparar com pacientes não-SS não-sicca, sugere implicações diagnósticas e imunomodulatórias dessas células na SSp.

A população macrofágica pode contribuir na elucidação da resposta imune e inflamatória da SS e de possíveis alvos terapêuticos.

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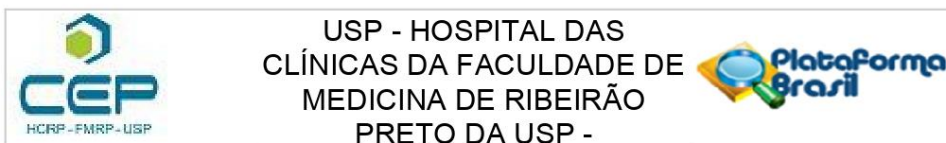
* De acordo com o Guia de Trabalhos Acadêmicos da FOAr, adaptado das Normas Vancouver. Disponível no site da Biblioteca: <http://www.foar.unesp.br/Home/Biblioteca/guia-de-normalizacao-atualizado.pdf>

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ANEXO A - PARECER DO COMITÊ DE ÉTICA EM PESQUISA



PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Caracterização imunofenotípica de células do sistema imune inato e adquirido em biópsias de glândulas salivares menores de pacientes com síndrome de Sjögren

Pesquisador: Jorge Esquiche León

Área Temática:

Versão: 1

CAAE: 43517621.9.0000.5440

Instituição Proponente: UNIVERSIDADE DE SAO PAULO

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.580.321

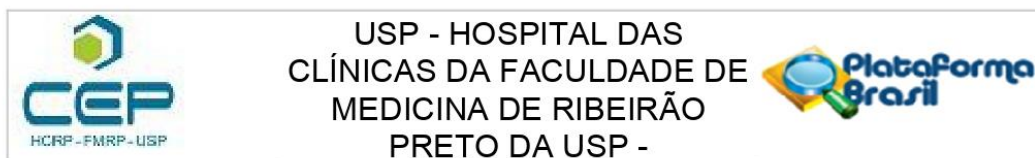
Apresentação do Projeto:

A síndrome de Sjögren (SS) é uma doença autoimune crônica sistêmica que afeta principalmente as glândulas salivares e lacrimais, ocasionando xerostomia e ceratoconjuntivite sicca, respectivamente. Histologicamente, as biópsias de glândulas salivares menores (GSm) de pacientes com SS exibem aglomerados linfocitários (focos) permeando o parênquima glandular, definido como sialadenite linfocítica focal (SLF). O escore focal (EF) define o número de focos, cada um deles contendo 50 ou mais linfócitos, em uma área glandular de 4mm². Notavelmente, GSm mostrando SLF com EF 1, é um critério diagnóstico importante para definir a SS. Pela imunoistoquímica, a SLF apresenta células TCD4+ e, em quantidades menores, células TCD8+, células B e plasmócitos; e em etapas finais há presença de fibrose, o que contribui com a disfunção do órgão. Estudos tem mostrado que além das populações de linfócitos, outras células imunes como macrófagos e células dendríticas podem ser encontradas na SLF, no entanto, precisa ser mais bem definida

Objetivo da Pesquisa:

Analisar, através de marcadores imunoistoquímicos, a localização e distribuição de distintos subtipos celulares em biópsias de glândulas salivares menores de lábio inferior em pacientes com SS, visando uma compreensão mais abrangente das funções específicas destes subtipos celulares.

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Continuação do Parecer: 4.580.321

Avaliação dos Riscos e Benefícios:

Riscos: O estudo será realizado em blocos parafinados de amostras que já foram excisadas como parte do tratamento realizado no paciente. Os espécimes serão devidamente armazenados em local adequado identificados por códigos, garantindo a preservação do material e seu anonimato visando diminuir minimizar os riscos. O material ficará sob a guarda do Prof. Dr. Jorge Esquiche León e do Prof. Dr. Alfredo Ribeiro da Silva, os quais ficarão responsáveis pela autorização do uso do material.

Benefícios: Estudos desta natureza são importantes porque as associações clinicopatológicas que serão realizadas podem esclarecer o papel e participação das células do sistema imune na patogênese desta doença, visando fatores terapêuticos e prognósticos para o paciente.

Comentários e Considerações sobre a Pesquisa:

Trata-se de um estudo retrospectivo, observacional. Os materiais a serem estudados proveem de biópsias emblocadas em parafina realizadas previamente à pesquisa, por indicação clínica e independentemente da mesma. Não haverá novos procedimentos nem outros diagnósticos em função da pesquisa.

Considerações sobre os Termos de apresentação obrigatória:

Documentos devidamente apresentados. Solicita a dispensa de aplicação do Termo de Consentimento Livre e Esclarecido. Tratando-se de um estudo retrospectivo e baseado na análise de espécimes de arquivo, não haverá contato direto com os pacientes. Devido ao período relativamente longo coberto pelo critério de seleção das amostras e pelo tipo de material, não será possível localizar os voluntários e abordá-los para aplicar o TCLE.

Recomendações:

não se aplica

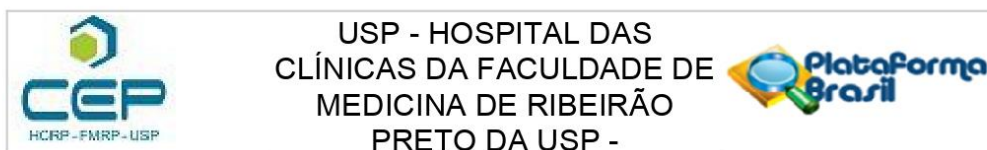
Conclusões ou Pendências e Lista de Inadequações:

Diante do exposto e à luz da Resolução CNS 466/2012, o projeto de pesquisa, assim como a solicitação de dispensa de aplicação do Termo de Consentimento Livre e Esclarecido, podem ser enquadrados na categoria APROVADO.

Considerações Finais a critério do CEP:

Projeto Aprovado: Tendo em vista a legislação vigente, devem ser encaminhados ao CEP, relatórios parciais anuais referentes ao andamento da pesquisa e relatório final ao término do trabalho. Qualquer modificação do projeto original deve ser apresentada a este CEP em nova versão, de

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Continuação do Parecer: 4.580.321

forma objetiva e com justificativas, para nova apreciação.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1702538.pdf	23/02/2021 20:47:30		Aceito
Outros	same.pdf	23/02/2021 20:45:06	Evânio Vilela da Silva	Aceito
Outros	APROVACAO_11512_UPC.pdf	22/02/2021 22:48:03	Evânio Vilela da Silva	Aceito
Declaração de Instituição e Infraestrutura	Autorizacao_instalacoes.pdf	22/02/2021 22:46:23	Evânio Vilela da Silva	Aceito
Folha de Rosto	Folha_de_rostoassinada.pdf	22/02/2021 22:35:21	Evânio Vilela da Silva	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Solicitacao_de_dispenza_do_TCLE.pdf	15/02/2021 16:22:07	Evânio Vilela da Silva	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_de_Pesquisa.pdf	15/02/2021 16:19:50	Evânio Vilela da Silva	Aceito
Cronograma	CRONOGRAMA.pdf	12/02/2021 15:38:17	Evânio Vilela da Silva	Aceito
Orçamento	ORCAMENTO_DETALHADO.pdf	12/02/2021 15:38:02	Evânio Vilela da Silva	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

RIBEIRAO PRETO, 09 de Março de 2021

Assinado por:
MARCIA GUIMARÃES VILLANOVA
 (Coordenador(a))

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Não autorizo a publicação deste trabalho pelo prazo de até 2 anos após a data de defesa

(Direitos de publicação reservado ao autor)

Araraquara, 06 de setembro de 2023.

Evânio Vilela da Silva