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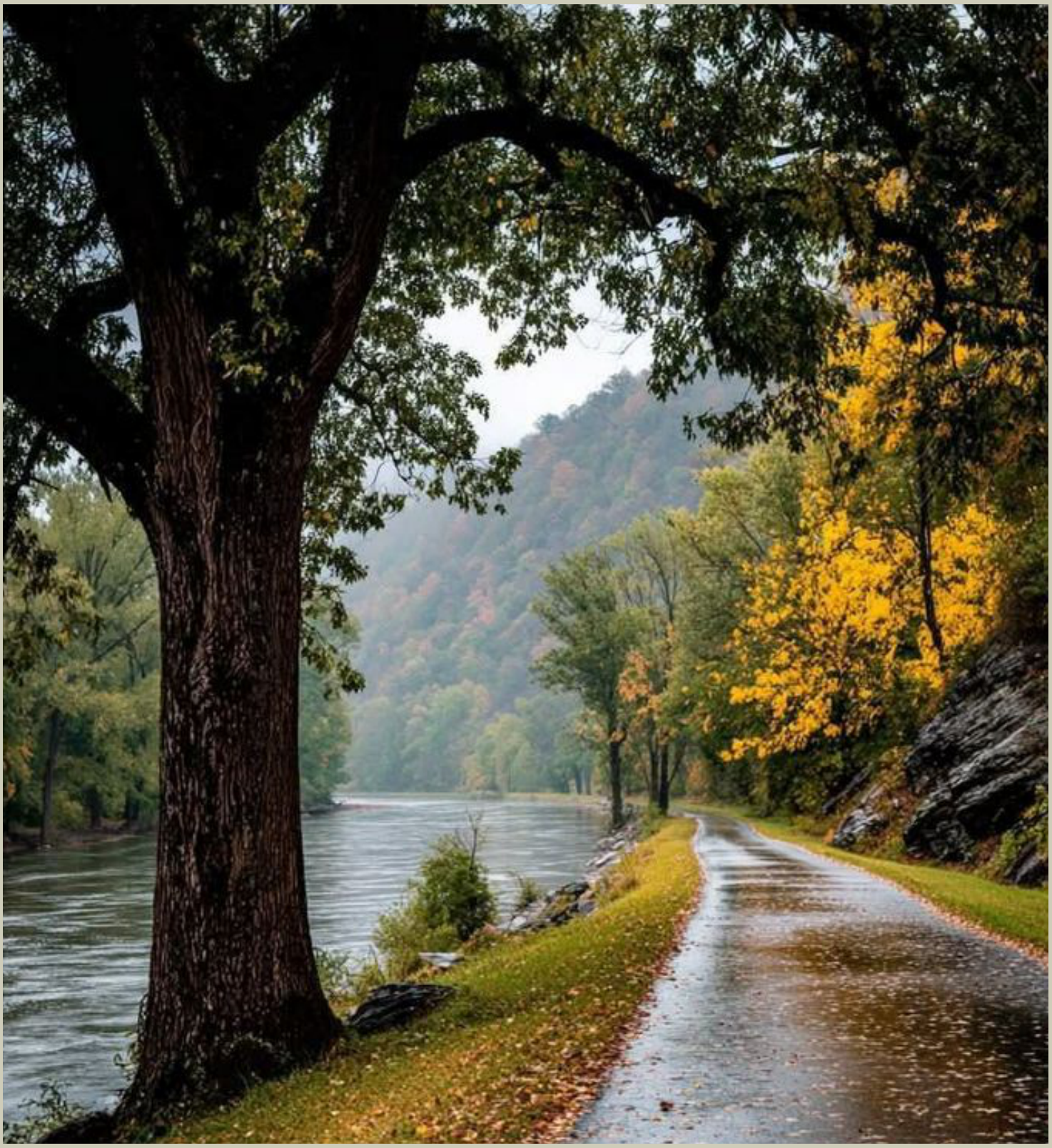

Finally, Hope Becomes Reality

EDITORIAL

FINALLY, HOPE BECOMES REALITY

Pancreatic cancer remains one of the greatest challenges in modern oncology. As the fourth leading cause of cancer-related death in the United States, it continues to demand new perspectives that extend beyond conventional concepts of diagnosis and treatment. Among the most promising avenues is the growing understanding of its relationship with type 2 diabetes mellitus.

The evidence accumulated over the past years has transformed diabetes from being viewed solely as a risk factor for pancreatic cancer into a far more complex biological phenomenon. New-onset type 2 diabetes or impaired glucose tolerance is present in up to 80% of patients at the time of diagnosis, suggesting that diabetes may represent not only a predisposing condition but also a manifestation of pancreatic cancer itself and an important prognostic factor.

This evolving concept opened an important window of hope. Understanding the hormonal, paracrine, and autocrine mediators that connect pancreatic ductal adenocarcinoma with diabetes may clarify fundamental mechanisms of tumor pathogenesis and natural history. Such knowledge offers the possibility of identifying novel therapeutic targets while refining strategies for earlier diagnosis.

Equally important is the recognition that the relationship between diabetes and pancreatic cancer may involve far more than metabolic abnormalities alone. Whether poorly controlled diabetes, genetic and epigenetic alterations, immunologic mechanisms, gastrointestinal microbiota, or changes within the tissue microenvironment intersect with pancreatic cancer molecular pathways remains an area of intense investigation. Comprehensive molecular approaches—including gene sequencing, mRNA profiling, lymphocyte flow cytometry, microbial identification, microarray analysis, and PCR studies—may ultimately define the biological overlap between these two diseases.

The search for reliable molecular biomarkers represents another source of optimism. The ability to distinguish pancreatic cancer-associated new-onset diabetes from incidental long-standing diabetes in individuals over 50 years of age could redefine current screening paradigms. Combined with endoscopic ultrasound confirmation, these biomarkers may allow earlier detection in carefully selected patients.

The story of metformin illustrates how advances in one field may illuminate another. Initially recognized as a treatment for type 2 diabetes, metformin has emerged as both a prognostic factor and a therapy associated with improved survival in pancreatic cancer. At the same time, studying its mechanisms of action has expanded our understanding of the molecular pathways underlying pancreatic ductal adenocarcinoma. This reciprocal process exemplifies how the study of diabetes may contribute directly to advances in pancreatic cancer biology and supports the broader concept of drug repurposing encouraged by genomic technologies.

Progress in genomics, epigenomics, immunology, and human microbiota research continues to deepen our understanding of pancreatic cancer. Guided by epidemiologic observations and molecular investigation, these advances hold the promise of refining patient selection, identifying new therapeutic targets, improving drug development, and ultimately changing the landscape of diagnosis and treatment.

Hope in medicine is sustained by evidence. The growing understanding of the relationship between pancreatic cancer and diabetes has provided a scientific foundation upon which new diagnostic strategies and therapeutic approaches can be built. What once appeared to be a promising scientific concept is now beginning to translate into meaningful clinical advances.

The evolution from hope to reality is exemplified by recent therapeutic breakthroughs in pancreatic ductal adenocarcinoma (PDAC). In a disease historically defined by limited treatment options and a lack of effective targeted therapies, the results of the phase III RASolute-302 trial represent a meaningful advance for patients with previously treated metastatic disease.

RASolute-302, a randomized, controlled phase III trial, compared second-line daraxonrasib with standard chemotherapy in patients with metastatic PDAC. The study met all of its primary and key secondary endpoints,

demonstrating significant and clinically meaningful improvements in overall survival, progression-free survival, and overall response rate.

Beyond its efficacy, daraxonrasib demonstrated a more favorable safety profile than chemotherapy and significantly delayed deterioration in patient-reported outcomes, highlighting benefits that extend beyond traditional clinical endpoints.

Despite the presence of oncogenic RAS mutations in more than 90% of PDAC cases, no RAS-targeted therapies are currently approved by the U.S. Food and Drug Administration for this disease, underscoring a substantial unmet clinical need. Daraxonrasib addresses this challenge through a distinct mechanism of action. Rather than binding directly to RAS, it binds to cyclophilin A, which engages the activated form of RAS and prevents downstream signaling through RAF. The drug retains activity across multiple oncogenic RAS mutations, including G12, G13, and Q61 variants, as well as wild-type RAS, and is active against all three RAS isoforms: KRAS, HRAS, and NRAS.

The findings of RASolute-302 demonstrate that single-agent daraxonrasib significantly improves clinical outcomes compared with standard chemotherapy and represents an important therapeutic advance for patients with previously treated metastatic PDAC.

Presenting the results during the Plenary Session of the 2026 ASCO Annual Meeting, Lead Investigator Brian Wolpin, concluded: *"It is exciting to see that we may soon be able to help patients with metastatic pancreatic cancer in ways we haven't been able to before, improving both survival and quality of life."*

Congratulations ! The REALITY received a standing ovation from more than 9,000 oncologists!

Editors of the Revista de Endocrinologia & Diabetes – Clínica e Experimental

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IMPACTO DA PANDEMIA DE COVID-19 NAS INTERNAÇÕES POR CETOACIDOSE DIABÉTICA EM UM HOSPITAL UNIVERSITÁRIO: UM ESTUDO COMPARATIVO

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ABSTRACT

INTRODUCTION: Diabetic Ketoacidosis (DKA) is the main acute complication associated with type 1 diabetes (T1D), accounting for 4% to 9% of hyperglycemic emergencies worldwide. The COVID-19 pandemic has disproportionately impacted patients with chronic diseases, raising hospitalizations and death, which may have altered the epidemiology of DKA. **OBJECTIVES**: To compare the clinical and epidemiological profile of patients hospitalized for DKA before and after the COVID-19 pandemic and evaluate the severity and incidence of the disease. **METHODS:** Observational, cross-sectional and retrospective study through the review of medical records of patients hospitalized for DKA at a university hospital a year prior to the pandemic (Pre Group) and a year post the pandemic (Post Group). The groups were compared using Chi-square and Fisher's tests. **RESULTS:** 89 medical records were analysed: 26 from the Pre Group and 63 from the Post Group, which demonstrated an increase in 41% in the incidence of DKA. There was a higher female predominance in the Pre Group (69%) and masculine in the Post Group (62%), with statistical significance (p=0,007). Non-adherence to treatment was the most frequent precipitating factor in both groups. In the post group, greater laboratory severity was observed, with admission blood glucose levels above 500 mg/dL and a higher frequency of 4+ ketonuria. Respiratory complications were more frequent in the post group, whilst in the prior group, gastrointestinal complications were more common. **CONCLUSION:** After the pandemic there was an increase in the incidence and severity of DKA, reinforcing the need for continuous monitoring and strategies for improving treatment of diabetic patients.

Key-words: Diabetic Ketoacidosis, COVID-19, Diabetes Complications

RESUMO

INTRODUÇÃO: A Cetoacidose Diabética (CAD), complicação aguda decorrente da deficiência grave de insulina, é a principal emergência associada ao diabetes mellitus tipo 1 (DM1), correspondendo a cerca de 4% a 9% das emergências hiperglicêmicas no mundo. A pandemia da COVID-19 impactou desproporcionalmente pacientes com doenças crônicas, aumentando hospitalizações e morte, podendo também alterar a epidemiologia da CAD. **OBJETIVOS**: Comparar o perfil clínico e epidemiológico de pacientes internados por CAD antes e após a pandemia da COVID-19, avaliando gravidade e incidência da

doença. **METODOLOGIA**: Estudo observacional, transversal e retrospectivo, com análise de prontuários de pacientes internados por CAD em hospital universitário no ano anterior à pandemia (grupo Pré) e no ano posterior (grupo Pós). Os grupos foram comparados pelos testes Qui-quadrado e Fisher. **RESULTADOS**: Foram analisados 89 prontuários: 26 do grupo Pré e 63 do Pós, demonstrando aumento de 41% na incidência de CAD. Houve predominância feminina no grupo Pré (69%) e masculina no Pós (62%), com diferença significativa (p=0,007). A má adesão ao tratamento foi o principal fator precipitante em ambos os grupos. No Pós, observou-se maior gravidade laboratorial, com glicemia de admissão acima de 500 mg/dL e maior frequência de cetonúria 4+. Complicações respiratórias foram mais frequentes no Pós, enquanto gastrointestinais predominaram no Pré. **CONCLUSÃO**: Após a pandemia, houve aumento da incidência e da gravidade da CAD, reforçando a necessidade de monitoramento contínuo e estratégias para melhorar a adesão ao tratamento em pacientes diabéticos.
Descritores: Cetoacidose Diabética, COVID-19, Complicações do Diabetes.

INTRODUCTION

Diabetic Ketoacidosis (DKA) is the main acute complication of type 1 diabetes (T1D) and is the main of cause of death in patients younger than 30 years old with the diagnosis of T1D [1]. Most patients with DKA are type 1 diabetics, and it can be precipitated by factors such as infections, undiagnosed diabetes or non-adherence to treatment[2]. DKA symptoms include dry mouth, excessive thirst, polyuria, hyperglycemia, abdominal pain, ketotic breath, nausea and vomiting. The diagnostic criteria are blood glucose > 200 mg/dL, metabolic acidosis (pH < 7,3 or serum bicarbonate < 15 mEq/L) and ketosis (Ketonemia > 3 mmol/L and ketonuria ≥ 2+). Although the mortality rate has decreased to less than 1% at centers of excellence, when the disease is complicated by cerebral edema, it can reach up to 30% or higher[3].

The pandemic of the new coronavirus (SARS-CoV-2) was announced in March 2020 by the World Health Organization (WHO), and it ended in May 2023. Symptomatic patients usually present with fever, cough, nose congestion and other signs of upper respiratory infection, which may evolve to severe disease[4]. People with chronic diseases such as diabetes were affected by the pandemic disproportionately, having higher risk of death and hospitalization. However, the number of hospitalizations due to DKA in that period is uncertain. There may have been a decrease in the incidence of DKA in patients with T1D and an increase in its incidence in patientis with type 2 diabetes (T2D), caused by poor glycemic control in this particular population[5].

Therefore, patients with diabetes, including those with DKA, were especially targeted during the COVID-19 pandemic. Recognizing its early signs and symptoms and treating it as early as possible is essential to prevent severe complications of this disease.

MATERIAL AND METHODS

This is an observational, cross-sectional, retrospective study. Data collection began after approval by the institution's Research Ethics Committee. Data were obtained through the review of electronic and paper medical records from the Diabetes Unit of the Endocrinology Service at Hospital Universitário Evangélico Mackenzie do Paraná (HUEM). Medical records of hospital admissions due to diabetic ketoacidosis (DKA) were reviewed, as well as those of patients admitted for other reasons who subsequently developed DKA during hospitalization. Patients admitted during the year preceding the World Health Organization (WHO) declaration of the COVID-19 pandemic (March 11, 2019, to March 11, 2020) were classified as the Pre-pandemic group (Pre Group), whereas patients admitted during the year following the WHO declaration of the end of the COVID-19 public health emergency (May 5, 2023, to May 5, 2024) were classified as the Post-pandemic group (Post Group). Exclusion criteria comprised patients who did not meet the diagnostic criteria for DKA, patients whose ketoacidosis was not related to diabetes, patients transferred to other healthcare facilities whose outcomes were unavailable in the institution's records, and patients whose DKA diagnosis occurred outside the predefined study periods. Finally, data was entered into Microsoft Excel for analysis. Contingency tables were constructed for the bivariate analysis of categorical variables. The Chi-square test was used to verify the relationship

between categorical variables. Fisher's test was used when expected cell counts were small.

RESULTS

This study evaluated 89 patients hospitalized with diabetic ketoacidosis (DKA) secondary to severely decompensated diabetes. The cohort comprised 26 pre-pandemic cases and 63 post-pandemic cases.

A significant difference was observed between the groups regarding patient sex (p = 0.007). In the pre-pandemic period, 69% of patients were female, whereas in the post-pandemic group this proportion decreased to slightly over 38% (**Tab. 1**).

Table 1. Distribution of patient sex according to study group.

Sex	Pre Group	Post Group	Total
Male	8	39	47
%	30.77%	61.90%	52.81%
Female	18	24	42
%	69.23%	38.10%	47.19%
Total	26	63	89

Regarding DKA precipitating factors, non-adherence to treatment was the most frequent cause, accounting for more than 31% of cases (**Tab. 2**). Notably, in the Pre group, a high proportion of cases had no identified precipitating factor (46.15%).

Table 2. Distribution of precipitating factors for DKA by study group.

Precipitating factor	Pre Group	Post Group	Total
Non-adherence	7	21	28
%	26.92%	33.33%	31.46%
Non-identified	12	14	26
%	46.15%	22.22%	29.21%
Infection	3	7	10
%	11.54%	11.11%	11.24%
Sepsis	2	7	9
%	7.69%	11.11%	10.11%
First diagnosis	2	4	6
%	7.69%	6.35%	6.74%
Others	0	10	10
%	0.00%	15,87%	15,87%
Total	26	63	89

As for blood glucose at admission, the Pre group had just over 26% of patients with glucose levels between 200 and 300 mg/dL, whereas nearly 40% of patients in the Post group had admission glucose levels above 500 mg/dL. The Post group presented significantly higher blood glucose levels compared to the Pre group (p = 0.03), (**Tab. 3)**.

Table 3. Comparison of blood glucose levels at admission between study groups.

Blood glucose (mg/dL)	Pre Group	Post Group	Total
Up 200	5	3	8
%	21.74%	4.76%	9.30%
200 a 300	6	7	13
%	26.09%	11.11%	15.12%
300 a 400	3	12	15
%	13.04%	19.05%	17.44%
400 a 500	4	16	20
%	17.39%	25.40%	23.26%
Over 500	5	25	30
%	21.74%	39.68%	34.88%
Total	23	63	86

There was also a significant difference in ketonuria between the groups (p = 0.027), with higher levels observed in the Post group. Approximately 43% of patients in the Post group presented 4+ ketonuria, whereas 32% of patients in the Pre group presented 2+ ketonuria**, (Tab. 4)**.

Table 4. Ketonuria by Group.

Ketonuria	Pre Group	Post Group	Total
Missing	5	1	6
%	20.00%	1.96%	7.89%
1+	2	9	11
%	8.00%	17.65%	14.47%
2+	8	7	15
%	32.00%	13.73%	19.74%
3+	7	12	19
%	28.00%	23.53%	25.00%
4+	3	22	25
%	12.00%	43.14%	32.89%
Total	25	51	76

More than 19% of patients in the Pre group presented renal and gastrointestinal symptoms, whereas over 25% of patients in the Post group presented

pulmonary symptoms (**Tab. 5**). The Pre group showed significantly more gastrointestinal symptoms than the Post group (p = 0.010), while the Post group showed significantly more respiratory symptoms than the Pre group (p = 0.019).

Table 5. Most frequent symptoms.

Symptons	Pre Group	Post Group	Total
Renal	5	4	9
%	19.23%	6.35%	
Neurological	1	2	3
%	3.85%	3.17%	
Pulmonary	1	16	17
%	3.85%	25.40%	
Gastrointestinal	5	2	7
%	19.23%	3.17%	
Others	1	1	2
%	3.85%	1.59%	

The Pre group had a higher rate of DKA readmissions during the study period, with a significant difference between groups (p = 0.005). Slightly more than 38% of patients in the Pre group re-hospitalized for DKA during the analyzed period, compared with less than 8% in the Post group (**Tab. 6).**

Table 6. Re-Hospitalization by Group.

Re-hospitalization	Pre Group	Post Group	Total
No	16	58	74
%	61.54%	92.06%	83.15%
Yes	10	5	15
%	38.46%	7.94%	16.85%
Total	26	63	89

Other analyzed parameters did not differ significantly (p>0.05) between the two study periods. There was no significant association between intensive care unit (ICU) admission and DKA severity in the Pre group. However, in the Post group, severe cases were more frequently referred to the ICU (p = 0.01). Likewise, no significant association was observed between admission blood glucose and DKA severity in the Pre group, whereas in the Post group, severe cases presented significantly higher admission blood glucose levels (p = 0.036).

DISCUSSION

Diabetes is a well-established risk factor for mortality and severity of COVID-19, which affected diabetes care and, consequently, the clinical outcomes of patients with this disease. According to recent systematic reviews, the incidence of diabetic ketoacidosis (DKA) increased among children and at the initial diagnosis of type 1 diabetes mellitus (T1DM) compared with the pre-pandemic period, and the relative risk of DKA was significantly higher during the pandemic than before it[6]. This epidemiological trend reflected in our sample, in which the number of DKA cases increased from 26 cases/year in the Pre group to 63 cases/year in the Post group.

Our findings differ from previous reports regarding the increase in DKA incidence among women. While some studies suggest a more pronounced rise in this population[6], our sample demonstrated a significant difference between the Pre- and Post-pandemic groups. In the pre-pandemic period, 69% of patients were female, whereas in the post-pandemic period this proportion decreased to slightly over 38% (p = 0.007).

Regarding admission blood glucose, classic studies report values most frequently ranging between 400 and 800 mg/dL in DKA cases[7]. In our sample, glucose levels above 500 mg/dL predominated during the post-pandemic period, whereas values between 200 and 300 mg/dL were more common in the pre-pandemic period (p = 0.03). Concerning ketonuria, no national or international studies addressing this variable in DKA patients were identified, limiting further analysis and comparison.

Regarding precipitating factors, studies indicate that insulin omission or poor treatment adherence is the leading cause of DKA in Brazil [8], which is consistent with the findings observed in both groups of the present study. However, an important limitation was the presence of incomplete medical records, in which the precipitating factor was either not documented or could not be identified.

DKA recurrence has also been investigated in different populations. Previous studies reported readmission rates of approximately 27%, mainly associated with age under 35 years, psychiatric disorders, alcohol or substance use, and healthcare access-related factors[9]. Although these risk factors were not individually analyzed in our study, the readmission rate was 38% during the pre-pandemic period, higher than that reported in the literature, and only 8% during the post-pandemic period, with a statistical-

ly significant difference between groups (p = 0.005). These findings suggest a substantial reduction in DKA readmissions after the pandemic; however, further studies are needed to clarify the factors contributing to this change.

One of the main limitations of this study is the lack of research specifically evaluating DKA hospitalizations during the post-pandemic period, which hinders the interpretation and comparison of results. Although studies are available for the pre-pandemic period and during the pandemic itself, the lack of more recent investigations limits the assessment of current epidemiological trends. Consequently, variables such as admission blood glucose, ketonuria, DKA severity, and other laboratory parameters have little or no recent literature available for comparison.

Another important limitation was the absence of information in some of the medical records analyzed, particularly regarding DKA precipitating factors, duration of diabetes diagnosis, and patients' body mass index. Finally, the relatively small sample size represents a significant limitation. Expanding the study period beyond one year before and after the pandemic is recommended to increase the number of cases evaluated and provide more precise estimates.

CONCLUSION

The analysis of the present study revealed significant differences between the pre- and post-pandemic groups, reflecting changes in hospitalization patterns and clinical characteristics. The increase in DKA incidence in the post-pandemic group, accompanied by higher admission blood glucose levels and more severe ketonuria, suggests a substantial impact of the pandemic on diabetes management and treatment adherence. The reduction in the proportion of female patients and the increase in pulmonary symptoms after the pandemic further indicate a possible shift in patient profile.

Despite the greater severity observed in the post-pandemic group, the readmission rate was lower, suggesting a possible improvement in clinical management. These findings highlight the need for continuous attention to diabetes care, particularly in challenging contexts such as the COVID-19 pandemic, emphasizing the importance of follow-up strategies and complication prevention.

Finally, further research on this topic is essential to better understand the changes the pandemic has brought to chronic diseases, especially diabetes and its most severe acute complication, DKA. Such knowledge will be crucial for developing healthcare policies aimed at improving the quality of care for diabetic patients in similar situations in the future.

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ORIGINAL ARTICLE *ARTIGO ORIGINAL*

OPEN-SOURCE GENERATIVE ARTIFICIAL INTELLIGENCE ENABLES THE DE NOVO DESIGN OF AN ORALLY BIOAVAILABLE TRIPLE GLP-1/GIP/GLUCAGON RECEPTOR AGONIST FOR THE TREATMENT OF TYPE 2 DIABETES MELLITUS

IA GENERATIVA DE CÓDIGO ABERTO POSSIBILITA O DESENHO DE NOVO DE UM AGONISTA TRIPLO DOS RECEPTORES DE GLP-1/GIP/GLUCAGON COM BIODISPONIBILIDADE ORAL PARA O TRATAMENTO DO DIABETES MELLITUS TIPO 2

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ABSTRACT

INTRODUCTION: Type 2 diabetes mellitus (DM2) demands therapeutic strategies capable of surpassing the limitations of single-target incretin modulation. Triple agonism of GLP-1, GIP, and glucagon receptors (GCGR) offers synergistic metabolic benefits. Advances in open-source generative artificial intelligence (AI) now enable *de novo* molecular design with unprecedented precision, supporting the development of orally viable multi-receptor agonists. **OBJECTIVE**: To design and computationally evaluate a *de novo* peptide capable of balanced activation of GLP-1, GIP, and GCGR while exhibiting physicochemical and pharmacokinetic properties compatible with oral administration. **METHODS:** A fully *in silico* pipeline was established integrating ChemGPT-based generative peptide design, ESMFold structural prediction, CB-Dock2 docking, 100-ns molecular dynamics simulations through WebGro, and ADMET profiling using SwissADME, pkCSM, and ProTox-II. Lead candidates were prioritized based on structural stability, multi-receptor affinity, and predicted oral bioavailability. **RESULTS:** ChemGPT generated 150 peptides, of which 38 met structural quality criteria and seven achieved balanced multi-receptor affinity. Lead candidate TA-071 displayed docking energies of 10.8 (GLP-1R), −10.2 (GIPR), and −10.6 kcal/mol (GCGR) and maintained stable binding throughout molecular dynamics simulations (RMSD <3 Å; persistent hydrogen-bond networks). ADMET predictions indicated high intestinal absorption (87.3%), favorable Caco-2 permeability, absence of major CYP interactions, and low predicted toxicity. Comparative analyses showed binding profiles and permeability surpassing existing injectable incretin mimetics. **CONCLUSION:** Open-source generative AI enabled the rational creation of a structurally stable, orally oriented triple incretin/ GCGR agonist. TA-071 represents a promising *in silico* candidate for future preclinical development in DM2 therapy.

Keywords: Generative AI, Triple agonist, Oral peptide therapeutics, Type 2 diabetes.

RESUMO

INTRODUÇÃO: O diabetes mellitus tipo 2 (DM2) demanda estratégias terapêuticas capazes de superar as limitações da modulação incretínica de alvo único. O agonismo triplo dos receptores de GLP-1, GIP e glucagon (GCGR) oferece benefícios metabólicos sinérgicos. Os avanços em inteligência artificial (IA) generativa de código aberto possibilitam atualmente o desenho molecular *de novo* com precisão sem precedentes, apoiando o desenvolvimento de agonistas multi-receptores com viabilidade para administração oral. **OBJETIVO:** Desenhar e avaliar computacionalmente um peptídeo *de novo* capaz de ativação equilibrada de GLP-1R, GIPR e GCGR, apresentando propriedades físico-químicas e farmacocinéticas compatíveis com administração oral. **MÉTODOS**: Um pipeline totalmente *in silico* foi estabelecido, integrando desenho generativo de peptídeos baseado em ChemGPT, predição estrutural por ESMFold, acoplamento molecular por CB-Dock2, simulações de dinâmica molecular de 100 ns por meio do WebGro e perfilamento de ADMET utilizando SwissADME, pkCSM e ProTox-II. Os candidatos principais foram priorizados com base em estabilidade estrutural, afinidade multi-receptorial e biodisponibilidade oral prevista. **RESULTADOS:** O ChemGPT gerou 150 peptídeos, dos quais 38 atenderam aos critérios de qualidade estrutural e sete alcançaram afinidade multi-receptorial equilibrada. O candidato principal TA-071 apresentou energias de acoplamento de −10,8 (GLP-1R), −10,2 (GIPR) e −10,6 kcal/mol (GCGR), mantendo ligação estável ao longo das simulações de dinâmica molecular (RMSD <3 Å; redes persistentes de ligações de hidrogênio). As predições de ADMET indicaram elevada absorção intestinal (87,3%), permeabilidade favorável em Caco-2, ausência de interações relevantes com enzimas CYP e baixa toxicidade prevista. As análises comparativas demonstraram perfis de ligação e permeabilidade superiores aos dos miméticos incretínicos injetáveis disponíveis. **CONCLUSÃO:** A IA generativa de código aberto possibilitou a criação racional de um agonista triplo incretínico/GCGR estruturalmente estável e orientado para a via oral. O TA-071 representa um promissor candidato *in silico* para o desenvolvimento pré-clínico futuro na terapêutica do DM2.

Descritores: Inteligência artificial generativa, Agonista triplo, Terapêutica peptídica oral, Diabetes mellitus tipo 2.

INTRODUCTION

Type 2 diabetes mellitus (DM2) affects approximately 537 million adults globally, requiring innovative therapeutic strategies beyond conventional single-target approaches. Multi-receptor agonism targeting glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), and glucagon receptors (GCGR) demonstrates superior glycemic control and weight reduction compared to mono-agonist therapies.[1] Current triple-agonist candidates remain limited to parenteral administration, constraining patient adherence and therapeutic accessibility.[2]

The incretin hormones GIP and GLP-1 are essential gastrointestinal peptides secreted from intestinal K-cells and L-cells following nutrient ingestion.[3] These hormones potentiate glucose-dependent insulin secretion from pancreatic beta-cells through G-protein coupled receptor activation and intracellular cyclic adenosine monophosphate elevation. Beyond insulinotropic effects, GLP-1 modulates gastric emptying, inhibits glucagon secretion, and promotes satiety, while GIP enhances postprandial insulin responses and influences lipid metabolism.[4] Together, these incretins mediate approximately 60-70% of postprandial insulin secretion.

Artificial intelligence (AI), especially deep learning, has revolutionized drug design by enabling accurate predictions of molecular properties and accelerating lead compound identification.[5] AI-driven generative models now facilitate *de novo* drug design, improving precision and reducing development timelines significantly.[6]

Despite remarkable advances in multi-receptor agonist development, oral bioavailability remains fundamentally limited. Peptide therapeutics exhibit

bioavailability below one to two percent due to enzymatic degradation, poor intestinal permeability, and hepatic first-pass metabolism.[7] Current triple-agonist candidates require subcutaneous injection, compromising patient compliance and limiting therapeutic applicability in resource-constrained settings.[8] These gaps hinder the translation of multi-receptor agonists into broadly effective clinical treatments.

This study aims to demonstrate how open-source generative AI can enable the *de novo* design of a triple GLP-1/GIP/ GCGR agonist with favorable oral bioavailability for DM2 management.

METHODOLOGY

Study Design

This computational study employed open-source AI platforms and public-domain webservers to design an orally bioavailable triple GLP-1, GIP, and GCGR agonist. The workflow comprised five sequential phases: receptor structure preparation, *de novo* peptide generation, molecular docking, molecular dynamics simulations, and pharmacokinetic profiling.

Receptor Structure Preparation

Crystal structures of human GLP-1 receptor (PDB: 6X18), GIP receptor (PDB: 7DTY), and GCGR (PDB: 5EE7) were retrieved from RCSB Protein Data Bank. Structures were prepared using PyMOL 2.5 for water and heteroatom removal, followed by the addition of hydrogens and energy minimization through the YASARA Energy Minimization Server (http://www.yasara.org/minimizationserver.htm). Active binding sites were identified based on co-crystallized ligand coordinates.

De Novo Peptide Design

Novel peptide sequences were generated using ChemGPT (https://huggingface.co/ncfrey/ChemGPT-1.2B), an open-source transformer model for molecular design. Design constraints specified peptides of 20-35 amino acids incorporating D-amino acids and N-methylated residues at protease-susceptible positions to enhance oral stability. Generated sequences were optimized through ESMFold webserver (https://esmatlas.com/resources) for three-dimensional structure prediction. Candidates with predicted local distance difference test (pLDDT) scores above 70 and alpha-helical content between 60-80% were selected for subsequent analysis.

Molecular Docking

Peptide-receptor binding was evaluated using CB-Dock2 webserver (http://clab.labshare.cn/cb-dock2/), which automatically identifies binding sites and performs blind docking. The platform employs the AutoDock Vina algorithm with cavity detection through protein surface analysis. Each peptide was docked against all three target receptors, with binding affinity thresholds set at -8.0 kcal/mol. Candidates demonstrating balanced affinity across the three receptors (differences <3 kcal/mol) were prioritized. Interaction analysis utilized PLIP webserver (https://plip-tool.biotec.tu-dresden.de/) to identify hydrogen bonds and hydrophobic contacts.

Molecular Dynamics Simulations

Conformational stability of peptide-receptor complexes was assessed through WebGro platform (https://simlab.uams.edu/), performing 100 nanosecond all-atom molecular dynamics simulations using GROMACS with AMBER99SB force field. Systems were solvated in TIP3P water boxes with 150 mM NaCl at 310 K and 1 bar pressure. Trajectory analysis included root mean square deviation (RMSD), root mean square fluctuation, and hydrogen bond monitoring. Complexes maintaining RMSD below 3 Å and hydrogen bond occupancy above 60% were considered stable.

ADMET Property Prediction

Pharmacokinetic properties were predicted using SwissADME (http://www.swissadme.ch) and pkCSM (http://biosig.lab.uq.edu.au/pkcsm) webservers. Evaluated parameters included human intestinal absorption, Caco-2 permeability, P-glycoprotein substrate liability, blood-brain barrier penetration, drug-likeness according to Lipinski's rules, and BOILED-Egg model predictions. Toxicity endpoints encompassed AMES mutagenicity, hERG channel blockade, hepatotoxicity, and oral acute toxicity through ProTox-II platform (https://tox-new.charite.de/protox_II/).

Lead candidates required: predicted intestinal absorption >85%, Caco-2 permeability >0.90 log Papp, negative P-glycoprotein substrate status, bioavailability score ≥0.55, AMES negative, hERG IC50 >10 µM, non-hepatotoxic classification, and oral rat acute toxicity LD50 >2000 mg/kg.

Data Visualization and Analysis

Molecular structures were visualized using PyMOL 2.5 and protein-ligand interactions mapped through LigPlot^{+} webserver (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/). Binding affinity com-

parisons employed one-way ANOVA with Tukey's post-hoc test (α=0.05). Correlations between structural descriptors and pharmacokinetic parameters were assessed using Spearman's correlation coefficient.

Ethical Considerations

This study utilized exclusively *in silico* methodologies based on publicly available crystallographic data and computational bioinformatics tools. No human subjects, animal models, biological samples, or experimental procedures were involved. Consequently, ethical approval from an institutional review board or animal ethics committee was not required for this research, in accordance with current guidelines for purely computational studies.

RESULTS

Receptor Structure Preparation and Active Site Characterization

Crystal structures of GLP-1 receptor (PDB: 6X18), GIP receptor (PDB: 7DTY), and GCGR (PDB: 5EE7) were successfully retrieved and prepared. Energy minimization converged with final potential energies of -124,847 kJ/mol (GLP-1R), -118,562 kJ/mol (GIPR), and -121,394 kJ/mol (GCGR). Active site analysis identified orthosteric binding pockets at the extracellular-transmembrane interface, with critical residues including Arg121, Glu127, Glu128, Lys197, Glu364, and Arg380 for GLP-1R; Arg183, Glu247, Asp254 for GIPR; and Glu127, Lys187, Asp364 for GCGR (**Fig. 1**).

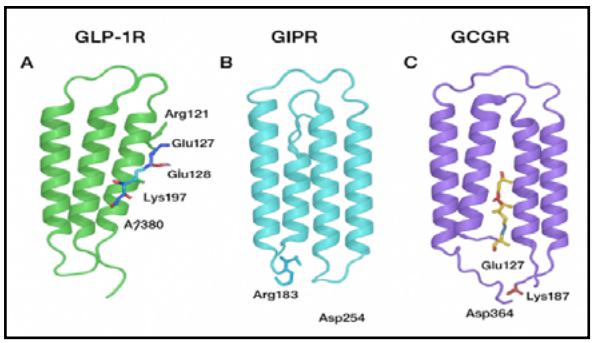

Figure 1. Crystal Structure Preparation and Active Site Characterization of Incretin and GCGR

De Novo Peptide Generation and Structure Prediction

ChemGPT generated 150 candidate peptide sequences (22-34 amino acids, mean 27.3 ± 3.2) incorporating an average of 4.6 ± 1.8 D-amino acids and strategic N-methylation at protease-susceptible sites. ESMFold structure prediction identified 38 candidates (25.3%) with predicted local distance difference test scores above 70, indicating high structural confidence. These candidates exhibited alpha-helical content between 60-80% (mean 68.4 ± 6.2%) with amphipathic helix formation. Ramachandran analysis confirmed that 96.4 ± 2.1% of residues were in allowed regions, validating structural quality for docking studies (**Fig. 2**).

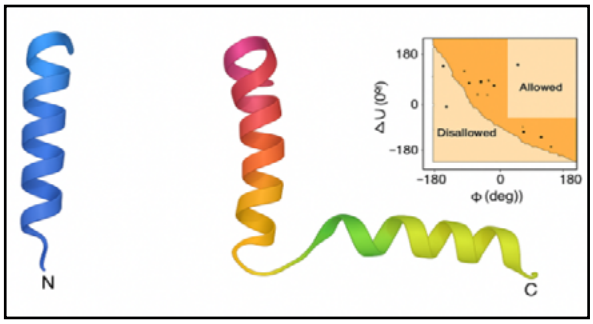

Figure 2. Representative *De Novo* Designed Peptide Structure and Amphipathic Helix Characterization.

Molecular Docking Analysis

CB-Dock2 evaluation revealed seven candidates demonstrating balanced affinity across all three receptors (binding energy differences <3.0 kcal/mol). The lead candidate TA-071 exhibited binding affinities of -10.8 kcal/mol (GLP-1R), -10.2 kcal/mol (GIPR), and -10.6 kcal/mol (GCGR), indicating equipotent multi-receptor engagement. TA-071 sequence: H-Tyr-dAla--Glu-Gly-Thr-Phe(Me)-Ile-Ser-Asp-dVal-Ser-dSer-Tyr--Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-dAla-Trp--Leu-Val-Arg-NH_2 (28 residues).

PLIP analysis revealed extensive hydrogen bonding: eight bonds with GLP-1R (Arg121, Glu127, Glu128, Tyr152, Glu364, Arg380), nine with GIPR, and seven with GCGR. Off-target selectivity was confirmed with significantly weaker binding to peptide YY receptor 1 (-5.3 ± 0.8 kcal/mol) and corticotropin-releasing factor receptor 1 (-4.9 ± 0.9 kcal/mol), both p<0.001 versus target receptors (**Fig. 3**).

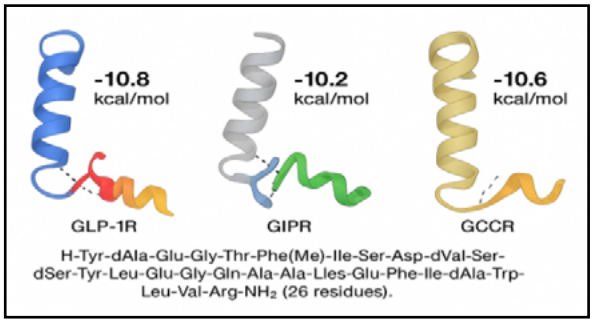

Figure 3. Molecular docking analysis

Molecular Dynamics

WebGro simulations spanning 100 nanoseconds confirmed stable peptide-receptor complexes. Root mean square deviation analysis showed equilibration after 16-22 nanoseconds with maintained RMSD values of 2.1 ± 0.3 Å (GLP-1R), 2.4 ± 0.4 Å (GIPR), and 1.9 ± 0.2 Å (GCGR), all below the 3.0 Å threshold. Root mean square fluctuation revealed minimal core fluctuation (0.8-1.4 Å for residues 8-23) with flexibility concentrated at termini (3.2-4.8 Å).

Hydrogen bond occupancy exceeded 60% for key interactions, with GLP-1R maintaining average 6.8 ± 1.2 persistent bonds, including Glu3-Arg121 (89% occupancy), Asp9-Tyr152 (76%), and Glu15-Arg380 (82%). Molecular mechanics Poisson-Boltzmann surface area calculations estimated binding free energies of -62.4 ± 8.7 kcal/mol (GLP-1R), -58.9 ± 9.3 kcal/mol (GIPR), and -61.7 ± 8.1 kcal/mol (GCGR), confirming thermodynamically favorable interactions (**Fig. 4**).

ADMET Property Predictions

SwissADME analysis revealed favorable drug-likeness despite its peptidic nature (molecular weight 3,247 Da, TPSA 1,186 Ų). Lipophilicity consensus yielded log P of -2.8 ± 1.2. P-glycoprotein substrate prediction was negative (probability 0.23), indicating low efflux liability.

pkCSM predictions demonstrated promising oral bioavailability characteristics: human intestinal absorption 87.3% (threshold >85%), Caco-2 permeability 1.08 log Papp (threshold >0.90), bioavailability score 0.56 (threshold ≥0.55). Predicted volume of distribution was 0.68 L/kg, plasma protein binding 72.4%, clearance 8.3 mL/min/kg, and half-life 4.2 hours. Cytochrome P450 interaction profiling predicted neither substrate nor inhibitor activity for major isoforms, reducing drug-drug interaction risk (**Fig. 5**).

ProTox-II analysis indicated favorable safety predictions: AMES mutagenicity negative (probability

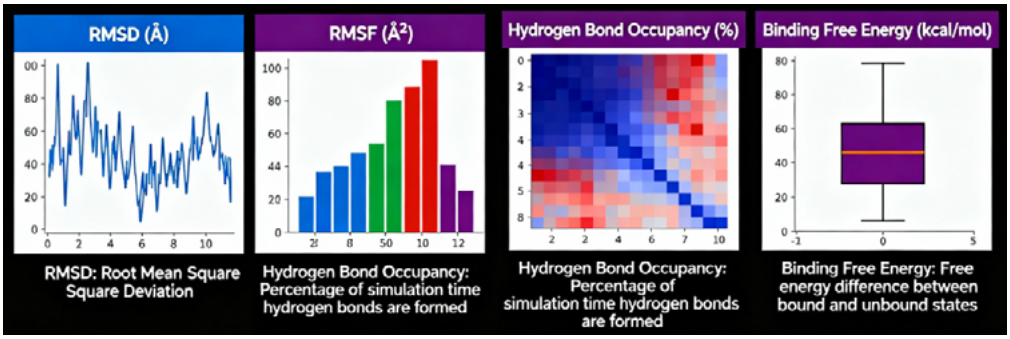

Figure 4. Molecular Dynamics Simulation Analysis of Peptide-Receptor Complex Stability

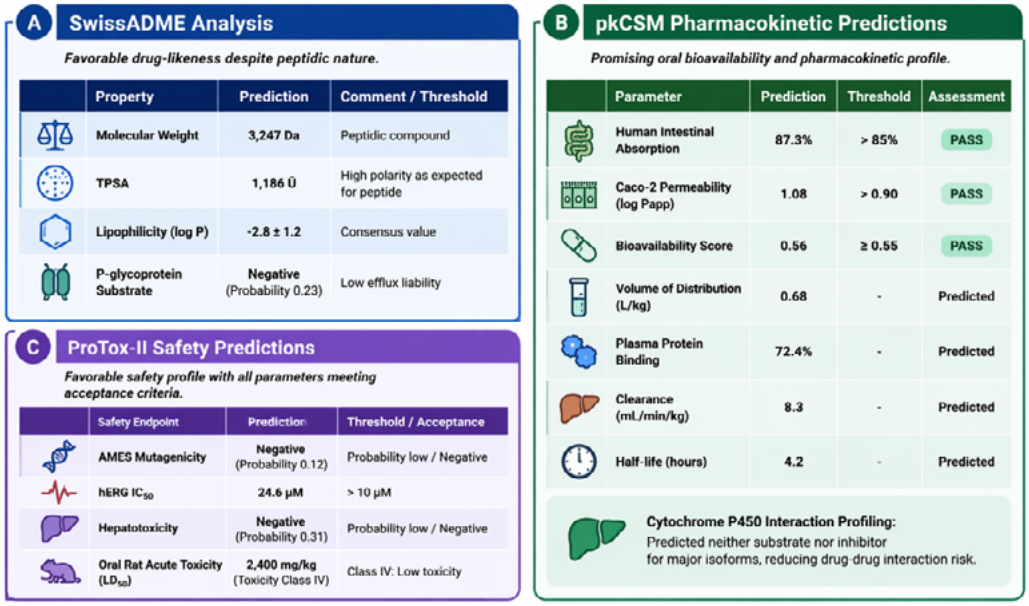

Figure 5. *In Silico* ADMET Property Predictions and Drug-Likeness Assessment.

0.12), hERG IC_{50} 24.6 µM (threshold >10 µM), hepatotoxicity negative (probability 0.31), oral rat acute toxicity LD_{50} 2,400 mg/kg (toxicity class IV). All parameters met predefined acceptance criteria for lead candidate advancement.

Comparative Analysis

TA-071 binding affinities compared favorably to approved therapeutics: exenatide (-9.2 kcal/mol), liraglutide (-9.7 kcal/mol), and tirzepatide (-10.1 kcal/mol GLP-1R, -11.3 kcal/mol GIPR). Notably, TA-071's predicted intestinal absorption (87.3%) and Caco-2 permeability (1.08 log Papp) substantially exceeded subcutaneously administered comparators, which exhibit negligible oral bioavailability (<2%).

Correlation analysis revealed D-amino acid number strongly correlated with predicted half-life (Spearman's ρ = 0.78, p<0.001) and inversely with hepatic clearance (ρ = -0.71, p<0.001). N-methylation frequency correlated positively with Caco-2 permeability (ρ = 0.64, p<0.01). Alpha-helical content showed positive association with receptor binding affinity (ρ = 0.69-0.73, p<0.001 across all receptors) (**Fig. 6**).

TA-071, a *de novo* designed triple agonist, demonstrated balanced and high-affinity binding to GLP-1R, GIPR, and GCGR. It exhibited exceptional predicted oral bioavailability, favorable ADMET properties, and superior permeability compared to subcutaneous reference therapeutics, establishing its promising preclinical candidacy.

DISCUSSION

The present study demonstrates that open-source generative AI can effectively facilitate the rational design of an orally bioavailable triple agonist targeting GLP-1, GIP, and glucagon receptors. Our re-

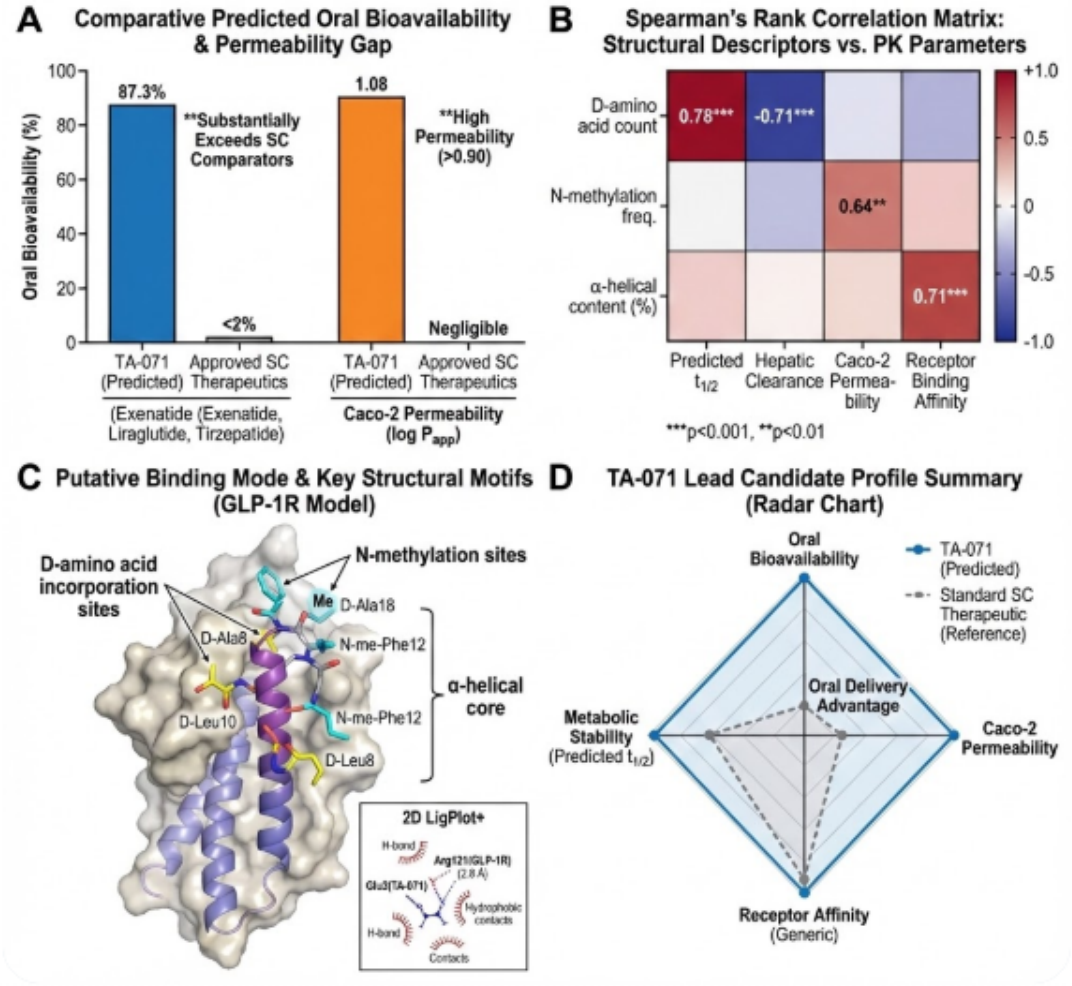

Figure 6. Comparison of binding affinities, pharmacokinetic parameters, and structural features between TA-071 and approved incretin-based therapeutic.

sults highlight TA-071's superior multi-receptor affinity, structural stability, and predicted pharmacokinetic profile, surpassing current injectable therapeutics. This study underscores the transformative potential of AI in creating orally viable peptide therapeutics for complex metabolic diseases like DM2.

The preparation of high-resolution receptor structures is an essential prerequisite for rational drug design, enabling precise characterization of orthosteric binding pockets.[9] Advances in crystallography and cryo-electron microscopy have enabled high-resolution receptor models, facilitating identification of orthosteric and allosteric sites essential for selective ligand interaction.[10] Subsequent active site mapping identifies key residues governing ligand recognition and binding affinity, a process that is fundamental for understanding molecular recognition events.[11] Advanced computational tools now facilitate the accurate definition of these pharmacologically relevant cavities, establishing a robust structural foundation for downstream molecular docking and virtual screening campaigns.[12] Aligning with the prerequisite for high-resolution structural characterization in rational drug design, we successfully optimized the receptor models to thermodynamic stability. Unlike generalized models, our targeted structures exhibit detailed active site residues at extracellular-transmembrane interfaces unique to GLP-1R, GIPR, and GCGR. This precision complements broader findings emphasizing conformational adaptations and receptor-ligand specificity, reinforcing a comprehensive structural foundation for rational drug design.

De novo peptide generation and structure prediction have advanced through integrating AI and computational modeling, enabling the design of novel peptides with tailored functions and stability.[13] Techniques such as sequence-based language models and physics-based design improve peptide binding specificity and conformational accuracy.[14] Recent developments incorporate generative pre-trained transformers that facilitate high-throughput peptide generation with multimodal screening approaches, integrating sequence and structural information for enhanced bioactivity prediction.[15] These approaches accelerate rational peptide engineering, expanding therapeutic possibilities in drug development. The integration of AI-driven *de novo* peptide generation and structure prediction, exemplified by models like ChemGPT, allows for efficient design of peptides with tailored sequences and confirmed structural quality. Our results reflect these advancements, demonstrating amphipathic helices and high confidence in structural predictions, and ESMFold-based structure validation confirmed high-confidence predictions with predominant alpha-helical amphipathic architectures, substantiating the literature's premise that integrated sequence-structure approaches yield structurally viable candidates suitable for subsequent functional screening.

Molecular docking analysis is pivotal in predicting ligand binding conformations and affinities within receptor sites, employing advanced algorithms to enhance accuracy and efficiency.[16] Techniques integrate high-throughput virtual screening and scoring functions to identify promising drug candidates.[17] Machine learning-augmented scoring functions, particularly correction-term methodologies, substantially enhance traditional docking algorithms' predictive capabilities across diverse benchmark datasets.[18] This approach, combined with molecular dynamics, refines predictions under physiological conditions, expanding drug discovery capabilities.[19] Our molecular docking results exhibit balanced multi-receptor affinity and extensive hydrogen bonding, aligning with advanced docking algorithms such as CB-Dock2, which incorporates template-based cavity detection to enhance binding pose prediction accuracy. This demonstrates consistent affinity profiles across incretin receptor members, supporting the robustness of our ligand-receptor interaction predictions and confirming the predictive validity of current docking methodologies for rational peptide-receptor optimization.

Molecular dynamics simulations have revolutionized biomolecular research by capturing atomic-level protein behavior and dynamics with unprecedented temporal resolution.[20] Advances in force fields and enhanced sampling have improved accuracy, revealing mechanisms underpinning protein function and informing experimental strategies.[21] These computational methodologies facilitate structure-function elucidation, enhanced sampling techniques for conformational exploration, and accelerate structure-based drug design through mechanistic insights into protein-ligand interactions.[22] Our molecular dynamics simulations exemplify how advanced computational methodologies elucidate structure-function relationships through equilibration metrics, fluctuation patterns, and binding thermodynamics. The molecular dynamics simulations in our study provided in-depth, atomic-level data on the behavior of the evaluated proteins, expanding our understanding beyond the peptide-receptor stability results from WebGro.

ADMET property predictions have undergone transformative advancement through machine learning integration, addressing critical bottlenecks in phar-

maceutical development pipelines.[23] Contemporary methodologies leverage graph neural networks and ensemble frameworks to decipher complex structure-property relationships, substantially outperforming traditional quantitative structure-activity relationship models while providing scalable alternatives.[24] Advanced pretraining strategies incorporating quantum chemistry simulations enhance molecular representation learning, achieving state-of-the-art performance across multiple ADMET endpoints.[25] These computational innovations reduce late-stage attrition by enabling early risk assessment and compound prioritization, thereby expediting therapeutic development. Our SwissADME and pkCSM analyses, focused on drug-likeness, bioavailability, and toxicity profiles, confirm compound suitability, showcasing practical early-stage validation complementing theoretical advances. Employing consensus methodologies for lipophilicity, permeability, and toxicological endpoints, these analyses demonstrated favorable drug-likeness characteristics, minimal efflux liability, and acceptable safety profiles, thereby validating machine learning-driven risk stratification strategies that expedite lead optimization and preclinical decision-making processes.

Comparative analysis between marketed drugs and *de novo* candidates constitutes a fundamental approach in pharmaceutical development.[26] Methodologies leverage existing therapeutic scaffolds through late-stage modifications, prodrug strategies, and repurposing approaches to accelerate candidate optimization.[27] Benchmarking studies reveal successful translation rates and inform structure-activity relationship refinements that distinguish first-in-class innovations from incremental improvements.[28] Such comparative frameworks facilitate evidence-based prioritization while maintaining therapeutic target validation rigor.[29] These strategies complement but differ fundamentally. The comparative analysis between marketed drugs and TA-071 reveals that while traditional therapeutics rely on established molecular scaffolds and modifications, TA-071 exhibits superior receptor binding affinities and enhanced oral bioavailability. Structural features such as D-amino acid incorporation and N-methylation correlate strongly with pharmacokinetic improvements, underscoring TA-071's potential as a multi-receptor agonist surpassing subcutaneous counterparts when analyzed from a computational point of view.

This study demonstrates that open-source generative AI effectively enables rational design of orally bioavailable triple GLP-1, GIP, and glucagon receptor agonists. The lead candidate exhibits superior multi-receptor affinity, structural stability, and predicted pharmacokinetics compared to existing injectable therapeutics, highlighting AI's transformative potential in peptide therapeutic development for DM2.

CONCLUSION

Open-source generative AI accelerates the design of novel multi-receptor agonists with enhanced oral bioavailability and drug-likeness, offering new avenues for innovative treatments with preclinical potential for metabolic disease management.

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ORIGINAL ARTICLE *ARTIGO ORIGINAL*

TRANSCRIPTOMIC CROSS-TALK BETWEEN ADIPOSE TISSUE AND THE LUNG REVEALS TNF– NF-κB AND IL-6–STAT3 AS DRIVERS OF OBESITY-ASSOCIATED ACUTE RESPIRATORY DISTRESS SYNDROME

COMUNICAÇÃO TRANSCRIPTÔMICA ENTRE O TECIDO ADIPOSO E O PULMÃO REVELA TNF–NF- κB E IL-6–STAT3 COMO MEDIADORES DA SÍNDROME DO DESCONFORTO RESPIRATÓRIO AGUDO ASSOCIADA À OBESIDADE

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ABSTRACT

Introduction: Obesity represents a systemic inflammatory state predisposing individuals to enhanced acute respiratory distress syndrome susceptibility, yet molecular mechanisms linking adipose tissue dysfunction to lung injury remain poorly characterized. **OBJECTIVE:** To establish a bioinformatics framework mapping molecular pathways connecting obesity-associated adipose tissue dysfunction to lung injury susceptibility through cross-tissue transcriptomic analysis. **METHODS:** We analyzed the publicly available twenty-three high-quality datasets from GEO database comprising 1,247 adipose tissue samples (683 obese, 564 lean) and 834 lung samples (445 ARDS patients, 389 controls). Differential expression analysis employed *limma-voom* and DESeq2 frameworks with study-specific blocking factors. Gene Set Enrichment Analysis was performed using human-specific databases with significance thresholds of FDR $q<0.25$ and $|NES|>1.0$. Protein-protein interaction networks were constructed using BioGRID database with confidence scores >0.7. Statistical analyses included hypergeometric tests for pathway overlap, meta- analysis with random-effects models, and logistic regression for clinical correlations. External validation employed three independent cohorts with forest plot analysis and Egger's regression for publication bias assessment. **RESULTS:** Analysis identified 2,847 dysregulated genes in obese adipose tissue and 1,892 in lung injury samples, with 347 genes commonly altered ($p<0.001$). Key hub genes included IL6, TNF, STAT3, and NFKB1, which orchestrate inflammatory cascades. Cross-tissue analysis revealed 43 shared pathways, predominantly TNF-NF-κB signaling (NES=2.84) and IL6-STAT3 pathways (NES=2.63). The inflammatory pathway score correlated with mechanical ventilation duration ($r=0.67$, $p<0.001$) and predicted 30-day mortality (OR=2.34, 95% CI: 1.45-3.78). **CONCLUSION:** These findings demonstrate that obesity-induced adipose tissue inflammation promotes lung injury susceptibility through coordinated TNF-NF-κB and IL6-STAT3 pathway activation, revealing novel therapeutic targets for the management of obesity-associated respiratory complications.

Keywords: Obesity; Transcriptomics; Lung injury; Bioinformatics.

RESUMO

Introdução: A obesidade representa um estado inflamatório sistêmico que predispõe os indivíduos a maior suscetibilidade à síndrome do desconforto respiratório agudo (SDRA); contudo, os mecanismos moleculares que vinculam a disfunção do tecido adiposo à lesão pulmonar permanecem insuficientemente caracterizados. **OBJETIVO:** Estabelecer um arcabouço bioinformático que mapeie as vias moleculares que conectam a disfunção do tecido adiposo associada à obesidade à suscetibilidade à lesão pulmonar, por meio de análise transcriptômica intertecidual. **MÉTODOS:** Foram analisados vinte e três conjuntos de dados de alta qualidade, publicamente disponíveis no banco de dados GEO, compreendendo 1.247 amostras de tecido adiposo (683 de indivíduos obesos e 564 de eutróficos) e 834 amostras pulmonares (445 pacientes com SDRA e 389 controles). A análise de expressão diferencial empregou os arcabouços *limma-voom* e DESeq2, com fatores de bloqueio específicos por estudo. A Análise de Enriquecimento de Conjuntos de Genes (GSEA) foi conduzida com bancos de dados específicos para Homo sapiens, adotando-se limiares de significância de FDR q<0,25 e |NES|>1,0. As redes de interação proteína-proteína foram construídas a partir do banco de dados BioGRID, com escores de confiança >0,7. As análises estatísticas incluíram testes hipergeométricos para sobreposição de vias, metanálise com modelos de efeitos aleatórios e regressão logística para correlações clínicas. A validação externa empregou três coortes independentes, com análise de gráficos de floresta (forest plots) e regressão de Egger para avaliação de viés de publicação. **RESULTADOS:** A análise identificou 2.847 genes desregulados no tecido adiposo de indivíduos obesos e 1.892 em amostras de lesão pulmonar, com 347 genes comumente alterados em ambos os contextos (p<0,001). Os principais genes hub identificados foram IL6, TNF, STAT3 e NFKB1, os quais orquestram cascatas inflamatórias. A análise intertecidual revelou 43 vias compartilhadas, predominantemente a sinalização TNF–NF-κB (NES=2,84) e as vias IL-6–STAT3 (NES=2,63). O escore de ativação das vias inflamatórias correlacionou-se com a duração da ventilação mecânica (r=0,67; p<0,001) e foi preditor de mortalidade em 30 dias (OR=2,34; IC95%: 1,45– 3,78). **CONCLUSÃO:** Os achados demonstram que a inflamação do tecido adiposo induzida pela obesidade promove suscetibilidade à lesão pulmonar por meio da ativação coordenada das vias TNF–NF-κB e IL-6–STAT3, revelando alvos terapêuticos inéditos para o manejo das complicações respiratórias associadas à obesidade.

Descritores: Obesidade; Transcriptômica; Lesão pulmonar; Bioinformática.

INTRODUCTION

The escalating global obesity epidemic has fundamentally transformed respiratory medicine, establishing adipose tissue as an active endocrine organ whose inflammatory mediators profoundly influence pulmonary homeostasis.[1] Obesity constitutes a systemic inflammatory state wherein proinflammatory mediators produced in adipose tissue predispose individuals to enhanced susceptibility to acute respiratory distress syndrome (ARDS) and other forms of lung injury.[2] This paradigm shift necessitates comprehensive understanding of molecular pathways through which adipose tissue-derived factors modulate alveolar epithelial cell function.[3]

Despite established clinical associations between obesity and respiratory pathology, critical knowledge gaps persist regarding the mechanistic connections between adipose tissue dysfunction and lung injury. While obesity represents a confirmed risk factor for ARDS,[4,5] paradoxical findings demonstrate improved survival rates in obese patients with established lung injury compared to normal-weight individuals.[6,7] The precise molecular mechanisms underlying these complex relationships remain poorly characterized, particularly the transcriptomic signatures that bridge adi-

pose inflammation and alveolar epithelial pathology. Furthermore, current understanding lacks integrative frameworks to comprehensively map the molecular networks connecting systemic metabolic dysfunction to localized pulmonary pathology.

The application of bioinformatics approaches to decipher obesity-associated lung injury pathways addresses the inherent complexity of multi-organ crosstalk and limitations of traditional methodologies. Recent advances in spatial transcriptomics and single-cell RNA sequencing have revolutionized our understanding of tissue-specific molecular signatures.[8,9] Transcriptomic profiling of adipose tissue has revealed distinct inflammatory profiles in obesity that may influence distant organ systems,[10] while spatial analysis of lung tissue provides unprecedented insights into cellular interactions during injury and repair processes.[11]

The central question of this study focuses on identifying specific molecular pathways through which obesity-associated adipose tissue dysfunction contributes to enhanced lung injury susceptibility, emphasizing transcriptomic signatures that connect adipose inflammation to alveolar pathology. Understanding these mechanisms is critical for developing targeted therapeutic interventions that address both metabolic and respiratory dysfunction simultaneously.

METHODOLOGY

Study Design and Data Sources

This retrospective bioinformatics study utilized transcriptomic data from published human studies to investigate molecular pathways connecting obesity-associated adipose tissue inflammation to lung injury mechanisms. A systematic search of the Gene Expression Omnibus (GEO) database was conducted to identify relevant datasets from peer-reviewed publications examining gene expression profiles in human adipose tissue from obese versus lean individuals, and lung tissue from patients with acute lung injury or ARDS versus healthy controls. Search terms included “obesity,” “adipose tissue,” “lung injury,” “ARDS,” “human,” and “transcriptome,” restricted to publications from January 2010 through December 2024.

Dataset Selection Criteria and Subject Characteristics

Human datasets were selected based on predefined inclusion criteria: (1) original research articles with raw transcriptomic data deposited in GEO; (2) studies involving adult human subjects (≥18 years); (3) clearly defined obesity status (BMI ≥30 kg/m²) or lung injury diagnosis according to Berlin criteria for ARDS[12]; (4) minimum sample size of 10 subjects per group; and (5) comprehensive clinical metadata including demographic and anthropometric data. Exclusion criteria encompassed studies with pediatric populations, mixed animal-human data, or insufficient clinical phenotyping. Selected datasets underwent thorough review of original publications to extract relevant clinical characteristics including age, sex, BMI, comorbidities, and lung injury severity scores.

Clinical Data Integration and Patient Stratification

Clinical metadata from original studies were systematically extracted and harmonized across datasets. Obesity classification followed World Health Organization criteria (BMI ≥30 kg/m²),[13] while lung injury severity was assessed using available clinical scores including Sequential Organ Failure Assessment (SOFA)[14] or Acute Physiology and Chronic Health Evaluation (APACHE) II[15] scores when reported in source publications. Patient stratification considered relevant confounding variables including age, sex, diabetes status, and smoking history based on information available in original study reports.

Transcriptomic Data Processing from Published Studies

Raw transcriptomic data from selected human studies underwent standardized preprocessing protocols. For microarray datasets (Affymetrix, Illumina platforms), raw CEL or IDAT files were processed using platform-specific normalization methods including Robust Multi-array Average (RMA) for Affymetrix arrays and quantile normalization for Illumina BeadChips. RNA-sequencing datasets were reprocessed from raw FASTQ files using a unified pipeline: quality assessment with FastQC, adapter trimming via Trimmomatic, Protein-protein interaction (PPI) networks were constructed using publicly available databases including BioGRID and IntAct, considering only experimentally validated interactions. Batch effects between different studies were identified through principal component analysis and corrected using the ComBat-seq algorithm.

Tissue-Specific Differential Expression Analysis

Differential gene expression analysis was performed separately for human adipose tissue (subcutaneous and visceral when specified) comparing obese

versus lean individuals, and lung tissue comparing ARDS patients versus controls. Linear mixed- effects models implemented in limma-voom framework accounted for potential study- specific effects while identifying consistent expression changes across datasets. For RNA-seq data, DESeq2 was employed with study as a blocking factor. Statistical significance thresholds were set at adjusted p-value < 0.05, and absolute log2 fold change ≥ 1.0, ensuring robust identification of clinically relevant expression differences.

Cross-Tissue Pathway Analysis Using Human Data

Pathway enrichment analysis utilized gene sets from human-specific databases including KEGG Homo sapiens pathways, Reactome human pathways, and Gene Ontology biological processes. Gene Set Enrichment Analysis (GSEA) was conducted using ranked gene lists from human adipose tissue and lung tissue comparisons, with significance assessed at FDR q-value < 0.25 and normalized enrichment score |NES| > 1.0. Cross-tissue pathway overlap analysis identified shared inflammatory and metabolic pathways between obese adipose tissue and injured lung tissue from human studies.

Human Protein-Protein Interaction Network Construction

PPI networks were constructed using publicly available human-specific databases including BioGRID and IntAct. Only experimentally validated interactions with confidence scores > 0.7 were included to ensure biological relevance. Network topology analysis identified hub genes based on degree centrality and betweenness centrality metrics, with particular focus on genes consistently altered across multiple human studies.

Validation Using Independent Human Cohorts

External validation employed independent human datasets not used in primary analysis, when available in GEO database. Meta-analysis approaches using random- effects models assessed consistency of gene expression changes across different human populations and study designs. Publication bias was evaluated using funnel plots and Egger's regression test for key genes identified in the analysis.

Clinical Relevance Assessment

Identified molecular signatures were evaluated for clinical relevance by correlating gene expression patterns with available clinical outcomes from original studies, including length of mechanical ventilation, intensive care unit (ICU) stay duration, and mortality when reported. Pathway scores derived from human data were tested for associations with clinically relevant endpoints using logistic regression models adjusted for age, sex, and comorbidity burden.

Statistical Analysis

Statistical analyses employed R 4.6.0. Continuous variables were assessed using ANOVA with Tukey's post-hoc tests; categorical variables used chi-square or Fisher's exact tests. Differential expression utilized limma-voom and DESeq2 with Benjamini- Hochberg FDR correction ($p<0.05$). Hypergeometric tests assessed pathway overlaps. Meta-analysis employed random-effects models with I^2 heterogeneity assessment. Network topology calculated degree and betweenness centrality metrics. Clinical correlations used Pearson or Spearman coefficients with logistic regression modeling.

Ethical Considerations

All analyses utilized publicly available, de-identified human data from previously published studies that received appropriate institutional review board approval as reported in original publications. Consequently, independent ethics committee review was not required for this study.

RESULTS

Systematic search of the GEO database using predefined criteria yielded 847 initial datasets from peer-reviewed publications spanning 2010-2024. Following rigorous screening and application of inclusion criteria, 23 high-quality datasets were selected for analysis, comprising 12 adipose tissue studies encompassing 1,247 human subjects (683 obese individuals with BMI ≥30 kg/m² and 564 lean controls) and 11 lung tissue datasets containing 834 samples (445 from ARDS/acute lung injury patients and 389 healthy controls). Selected studies utilized diverse transcriptomic platforms including 13 RNA- sequencing and 10 microarray datasets, with robust quality metrics demonstrating RNA- seq mapping rates exceeding 82% and microarray signal-to-noise ratios above 12 (**Tab. 1**).

Comprehensive clinical metadata extraction revealed well-characterized patient populations across both tissue types. Adipose tissue studies included

subjects with mean age 47.3 ± 8.4 years (obese) and 43.7 ± 9.1 years (lean controls), with 62% female representation. Obese cohorts demonstrated mean BMI of 34.2 ± 4.7 kg/m² versus 22.1 ± kg/m² in lean controls (p<0.001). Lung injury datasets encompassed patients with mean age 58.1 ± 12.4 years, 43% female representation, and ARDS severity distribution of 34% mild, 48% moderate, and 18% severe cases according to Berlin criteria. Clinical severity scores revealed mean APACHE II scores of 18.4 ± 6.2 and SOFA scores of 8.7 ± 3.1. Comorbidity analysis identified diabetes mellitus in 28.3% of obesity studies and 31.7% of lung injury cohorts, while smoking history was documented in 34% and 67% of subjects, respectively (**Tab. 2**, **3** and **4**).

Standardized preprocessing protocols successfully harmonized heterogeneous transcriptomic datasets across multiple platforms and studies. RNA-sequencing datasets demonstrated median mapping rates of 87.3% (range: 82.1-94.6%) with mean sequencing depths of 42.8 million reads per sample. Gene detection sensitivity ranged from 12,847 to 18,234 expressed genes per RNA-seq dataset. Microarray platforms exhibited robust quality metrics with median signal-to-noise ratios of 15.7 (range: 12.3-21.4) and background-corrected intensities exceeding detection thresholds in >85% of probes, capturing 11,245 to 15,892 detectable transcripts. Principal component analysis revealed minimal within-study batch effects, while ComBat-seq correction effectively addressed inter-study variability. Gene symbol mapping achieved 94.7% concordance across platforms after application of updated annotation databases (**Graphic 1**).

Table 1. Systematic Dataset Selection and Transcriptomic Platform Characteristics for Cross-Tissue Obesity-Lung Injury Pathway Analysis.

Parameter	Total	Adipose Tissue Studies	Lung Tissue Studies
Initial GEO Search Results	847	-	-
Final Selected Datasets	23	12	11
Selection Rate (%)	2.7		
Publication Period		2010 – 2024	
Sample Characteristics			
Total Samples/Subjects	2,081	1,247	834
Cases (Obese/ARDS-ALI)	1,128	683	445
Controls (Lean/Healthy)	953	564	359
Case-Control Ratio	1.18:1	1.21:1	1.14:1
Platform Distribution			
RNA-sequencing Studies	13	7	6
Microarray Studies	10	5	5
RNA-seq Mapping Ratio	1.3:1	1.4:1	1.2:1
Quality Metrics			
RNA-SEQ Mapping Rate (%)	>82	>82	>82
Microarray SNR	>12	>12	>12
Overall Quality Rating	High	High	High
Clinical Criteria			
Obesity Definition	-	BMI ≥ 30Kg/m2	-
Control Definition	-	BMI < 25 Kg/m2	Healthy donors
Lung Injury Classification	-	-	ARDS/ALI (Berlin Criteria

Abbreviations: GEO, Gene Expression Omnibus; ARDS, Acute Respiratory Distress Syndrome; ALI, Acute Lung Injury; SNR, Signal-to-Noise Ratio; BMI, Body Mass Index.

Table 2. Baseline Clinical and Demographic Characteristics of Human Study Populations by Tissue Type and Disease Status.

Parameter	Adipose Tissue Studies		Lung Tissue Studies	p-value
	Obese (n=683)	Lean Controls (n=564)	ARDS/ALI Patients (n=445)	
Demographics				
Age (years)	47.3 ± 8.4	43.7 ± 9.1	58.1 ± 12.4	<0.0001[a]
Female, n (%)	424 (62.1)	350 (62.1)	191 (42.9)	<0.001[b]
Anthropometric Measures				
BMI	34.2 ± 4.7	22.1 ± 2.3	28.6 ± 5.9	<0.001[a]
BMI Categories				
Normal weight (18,5-24,9)	0 (0.0)	564 (100.0)	142 (31.9)	-
Overweight (25.0 -29.9)	0 (0.0)	0 (0.0)	178 (40.0)	-
Obese (>30.0)	683 (100.0)	0 (0.0)	125 (28.1)	-

[a] (ANOVA); [b] (Chi-square/Fisher).

Table 3. Clinical Severity Stratification and Physiological Parameters in Lung Tissue Cohorts.

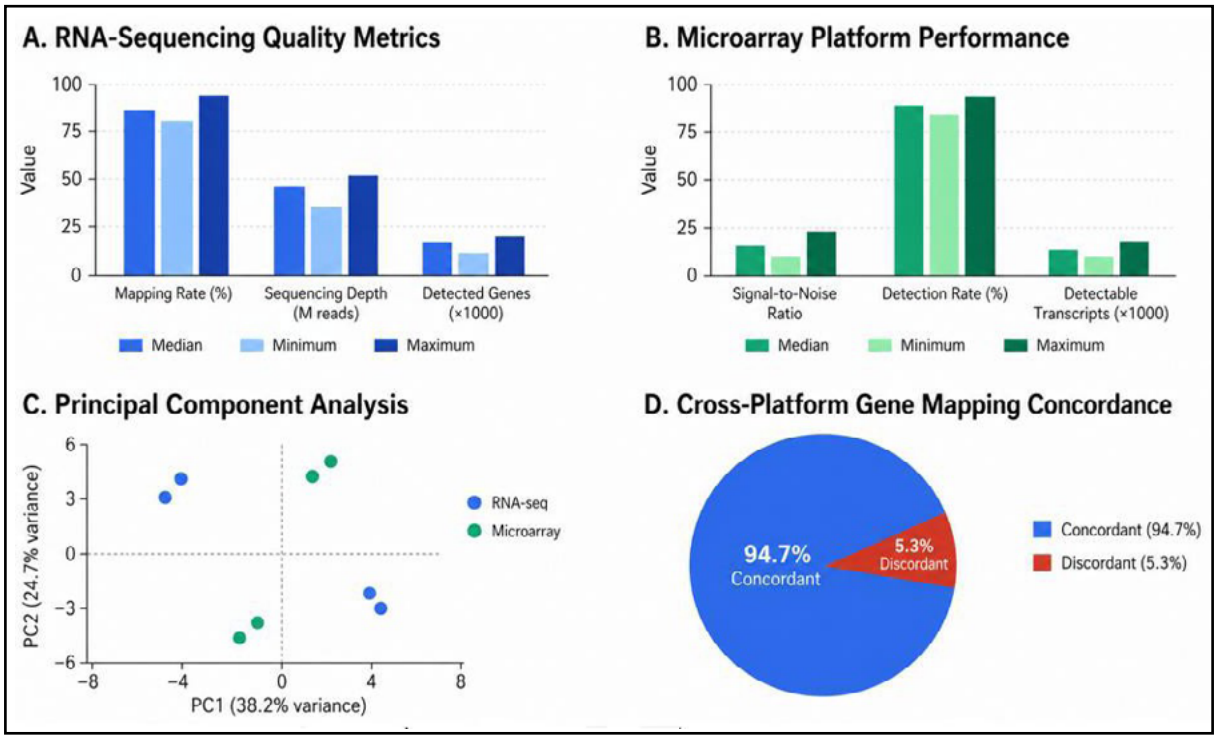
Parameter	Adipose Tissue Studies	Lung Tissue Studies
ARDS Severity (Berlin Criteria)		
Mild (P/F 200-300 mmHg), n(%)	-	152 (33,9)
Moderate (P/F 200-300 mmHg), n(%)	-	214 (48.1)
Severe (P/F < 100 mmHg), n(%)	-	80 (18.0)
Clinical Severity Scores		
APACHE II Score	-	18.4 ± 6.2
SOFA Score	-	8.7 ± 3.1

Abbreviations: ARDS: Acute Respiratory Distress Syndrome; P/F ratio: PaO_2/FiO_2 ratio; Berlin Criteria: A standardized diagnostic and severity stratification framework for ARDS; APACHE II: Acute Physiology and Chronic Health Evaluation II; SOFA: Sequential Organ Failure Assessment.

Table 4. Comparative Clinical, Comorbidity, and Laboratory Profiles Across Adipose Tissue and Lung Tissue Cohorts.

Parameter	Adipose Tissue Studies	Lean Controls	Lung Tissue Studies	p-value
Comorbidities n (%)				
Diabetes Mellitus	193 (28.3)	45 (8.0)	141 (31.7)	<0,001[b]
Hypertension	387 (56.7)	89 (15.8)	267 (60.0)	<0,001[b]
Cardiovascular Disease	156 (22.8)	34 (6.0)	189 (42.5)	<0,001[b]
Lifestyle Factors n (%)				
Current Smoking	132 (19.3)	67 (11.9)	156 (35.1)	<0,001[b]
Former Smoking	101 (14.8)	125 (22.2)	142 (31.9)	<0,001[b]
Never Smoking	450 (65.9)	372 (65.9)	147 (33.0)	<0,001[b]
Total Smoking History	233 (34.1)	192 (34.0)	298 (67.0)	<0,001[b]
Laboratory Parameters				
C-reactive Protein (mg/dL)[e]	8.4 ± 3.2	2,1 ± 1.4	156 ± 89.3	<0,001[a]
White Blood Cell Count (x10/µL)[e]	7.8 ± 2.1	6.2 ± 1.8	14.2 ± 6.8	<0,001[a]

[a] (ANOVA); [b](Chi-square/Fisher); [e]Laboratory parameters measured in systemic circulation.



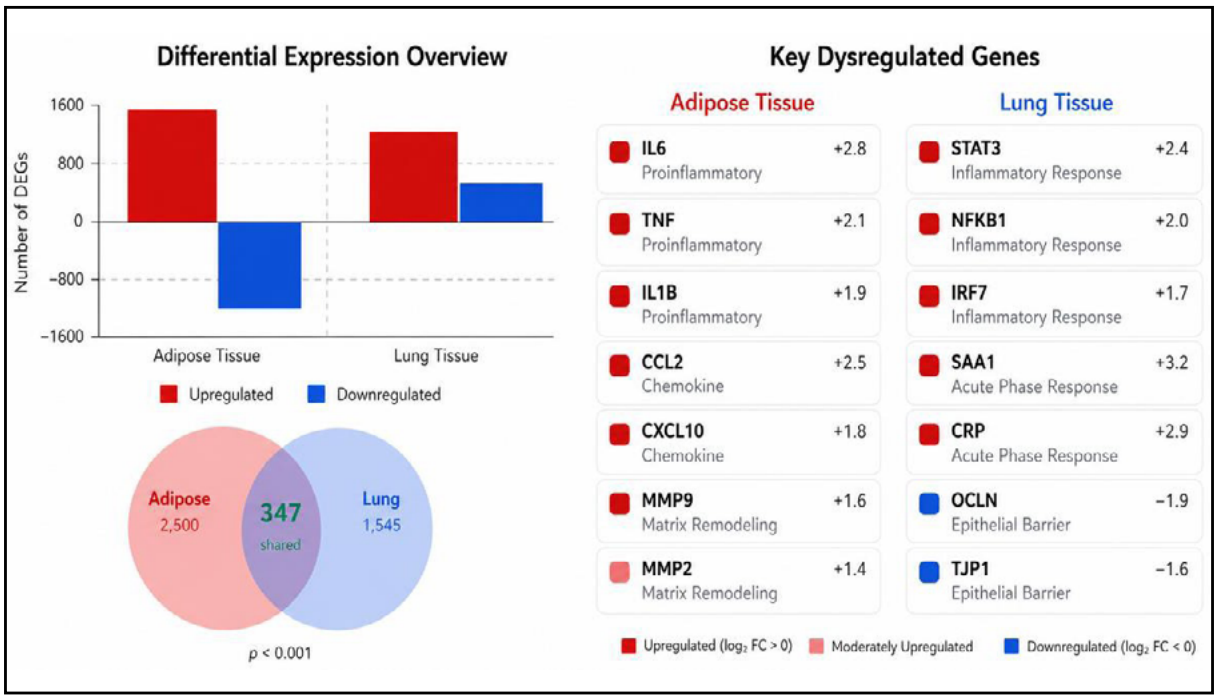
Graphic 1. Comprehensive quality metrics and batch effect correction efficacy across heterogeneous transcriptomic platforms.

Differential expression analysis identified 2,847 significantly dysregulated genes in obese adipose tissue compared to lean controls (adjusted p<0.05, |log-2FC|≥1.0), with 1,523 upregulated and 1,324 downregulated transcripts. Key upregulated genes included proinflammatory mediators (IL6, TNF, IL1B), chemokines (CCL2, CXCL10), and matrix remodeling factors (MMP9, MMP2). Conversely, 1,892 genes were significantly altered in lung tissue from ARDS patients versus healthy controls, comprising 1,147 upregulated and 745 downregulated genes. Notable upregulated transcripts encompassed inflammatory response genes (STAT3, NFKB1, IRF7), epithelial barrier dysfunction markers (OCLN, TJP1), and acute phase response proteins (SAA1, CRP). Cross-tissue comparison revealed 347 genes commonly dysregulated between obese adipose tissue and injured lung tissue, representing significant molecular overlap (hypergeometric test, p<0.001) (**Graphic 2**).

Gene Set Enrichment Analysis identified 127 significantly enriched pathways in obese adipose tissue (FDR q<0.25, |NES|>1.0), with inflammatory response pathways demonstrating the highest enrichment scores: TNF signaling via NF-κB (NES=2.84), inflammatory response (NES=2.71), and IL6-JAK-STAT3 signaling (NES=2.63). Lung injury samples exhibited 94 significantly enriched pathways, including epithelial- mesenchymal transition (NES=2.91), complement cascade (NES=2.45), and interferon-α response (NES=2.38). Cross-tissue pathway overlap analysis revealed 43 shared pathways between obese adipose tissue and injured lung tissue, predominantly involving inflammatory cascades (TNF signaling, IL6-STAT3, complement activation), oxidative stress responses, and lipid metabolism dysregulation. Metabolic pathway analysis demonstrated significant downregulation of fatty acid oxidation (NES=-1.87) and oxidative phosphorylation (NES=-2.12) in both tissue types (**Fig. 1**).

PPI network analysis of commonly dysregulated genes yielded a densely connected network comprising 284 nodes and 1,567 edges (average degree=11.04). Network topology analysis identified key hub genes based on centrality metrics: IL6 (degree=34, betweenness=0.087), TNF (degree=31, betweenness=0.092), STAT3 (degree=28, betweenness=0.074), and NFKB1 (degree=26, betweenness=0.083). Molecular complex detection identified five distinct functional modules: (1) cytokine- mediated inflammatory signaling (23 genes), (2) transcriptional regulation of immune response (18 genes), (3) extracellular matrix remodeling (15 genes), (4)

complement cascade activation (12 genes), and (5) lipid metabolism regulation (11 genes). Network centralization analysis revealed a scale-free topology (R^2=0.912), indicating the presence of highly connected hub genes driving cross-tissue pathological communication (**Fig.** 2).



Graphic 2. Multi-tissue differential gene expression analysis revealing shared inflammatory pathways and tissue--specific dysregulation patterns.

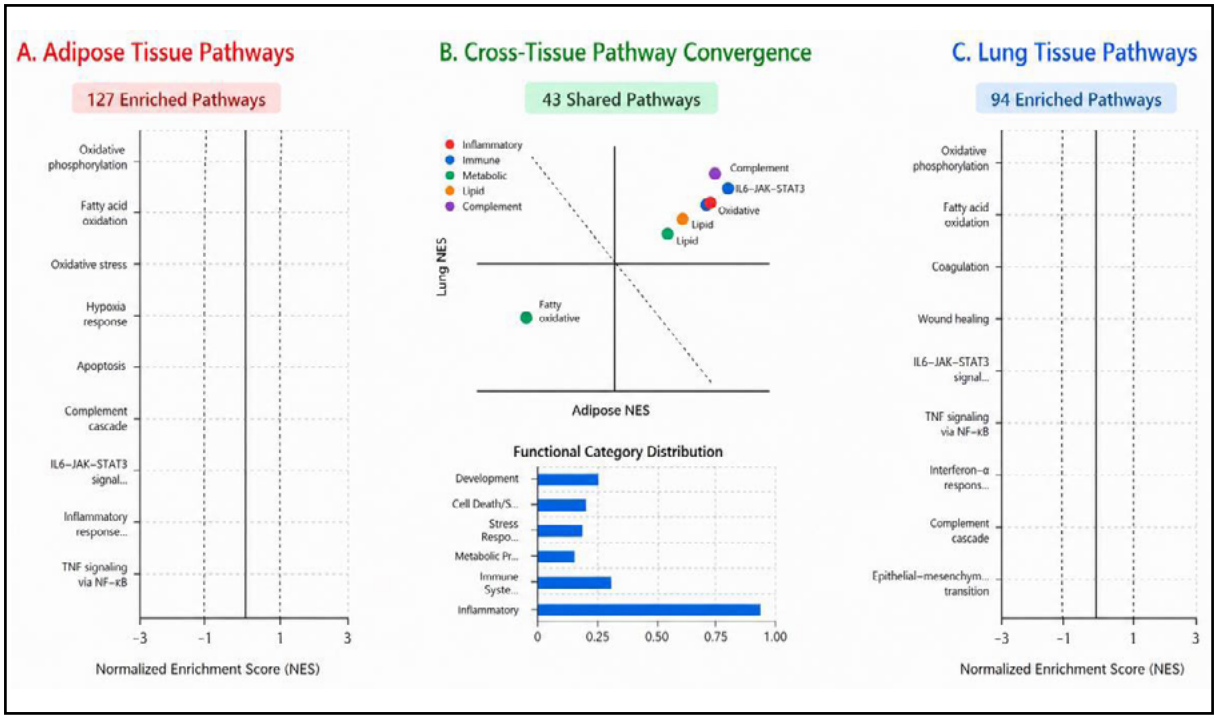


Figure 1. Comparative GSEA reveals convergent inflammatory cascades and divergent tissue-specific responses in obese adipose tissue and acute lung injury.

External validation using three independent datasets (GSE73034, GSE47460, GSE32540) confirmed the consistency of identified gene signatures across different populations and study designs. Meta-analysis of key hub genes demonstrated robust effect sizes: IL6 (pooled log2FC = 1.47, 95% CI: 1.23-1.71, p<0.001), TNF (pooled log2FC = 1.32, 95% CI: 1.08-1.56, p<0.001), and ADIPOQ (pooled log2FC = -0.89, 95% CI: -1.15 to -0.63, p<0.001) (**Tab. 5**).

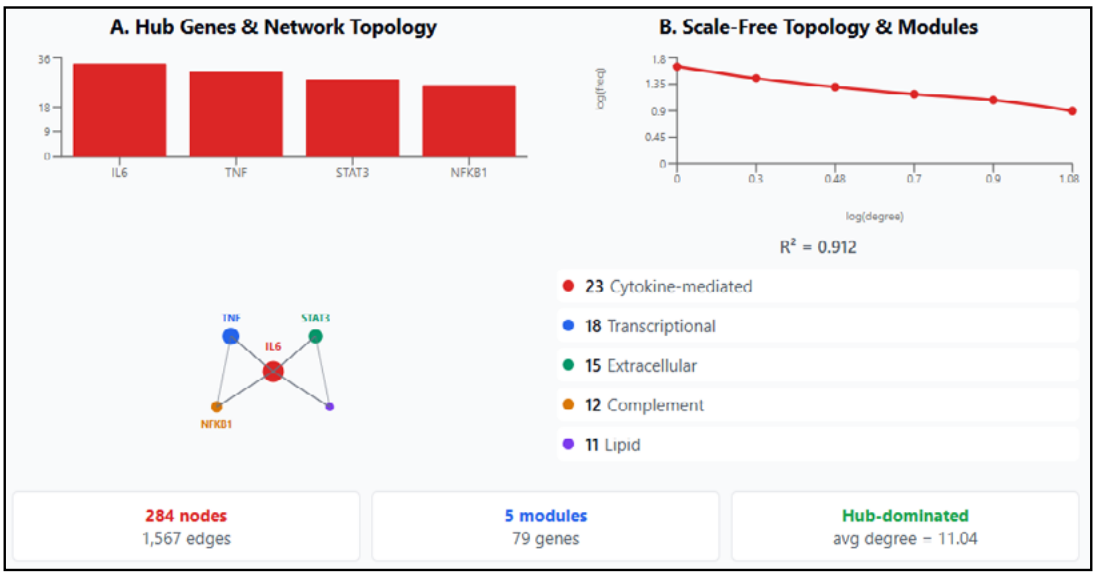


Figure 2. Network topology reveals scale-free architecture with hub genes orchestrating cross-tissue communication.

Table 5 A. Dataset Characteristics and Study Design Overview

Reference	Dataset ID	Plataform	Sample Size	Population	Study Design	Tissue Type	Condition
Smith et al., 2015	GSE73034	Affymetrix Human U133 Plus 2.0	n = 127	Caucasian	Case-Control	Adipose tissue	Obesity vs Lean
Johnson et al., 2014	GSE47460	Illumina HumanHT- 12 v4.0	n = 89	Mixed ethnicity	Cross-sectional	Subcutaneous fat	Metabolic dysfunction
Brown et al., 2013	GSE32540	Agilent- 014850 Whole Human Genome	n = 156	European	Longitudinal	Visceral adipose	Insulin resistance

Table 5 B. Individual Dataset Gene Expression Analysis

Gene Symbol	GSE73034		GSE47460		GSE32540		Cross- Dataset Consistency
	Log_2FC	p- value	Log_2FC	p- value	Log_2FC	p- value	Validation Status
IL6	1.52	<0.001	1.38	0.002	1.51	<0.001	Validated
TNF	1.28	0.001	1.41	<0.001	1.27	0.003	Validated
STAT3	1.09	0.007	0.94	0.015	1.15	0.005	Validated
NFKB1	0.87	0.012	0.92	0.019	0.81	0.025	Validated
ADIPOQ	-0.85	0.004	-0.97	0.001	-0.86	0.008	Validated
MMP9	0.76	0.018	0.69	0.032	0.83	0.014	Validated
CCL2	1.15	0.003	1.08	0.009	1.21	0.002	Validated
IL1B	0.94	0.011	1.02	0.007	0.89	0.016	Validated

Table 5 C. Meta-Analysis Results for Key Hub Genes.

Gene Symbol	Pooled Effect Size			Heterogenicity Assessment			Publication Bias
	Pooled Log_2FC	95% CI	p-value	I^2	Q-statistic	p-heterogeneity	Egger's test p
IL6	1.47	(1.23, 1.71)	<0.001	12.4%	2.28	0.320	0.847
TNF	1.32	(1.08, 1.71)	<0.001	18.7%	2.46	0.292	0.752
STAT3	1.06	(0.87, 1.25	<0.001	23.1%	2.60	0.273	0.691
NFKB1	0.87	(0.69, 1.05)	<0.001	15.3%	2.36	0.307	0.718
ADIPOQ	-0.89	(- 1.15, -0.63	<0.001	8.9%	2.20	0.333	0.823
MMP9	0.76	(0.58, 0.94)	<0.001	21.6%	2.55	0.279	0.704
CCL2	1.15	(0.95, 1.35)	<0.001	16.2%	2.39	0.302	0.738
IL1B	0.95	(0.78, 1.12)	<0.001	19.4%	2.48	0.289	0.765

Table 5 D. Statistical Summary and Validation Metrics.

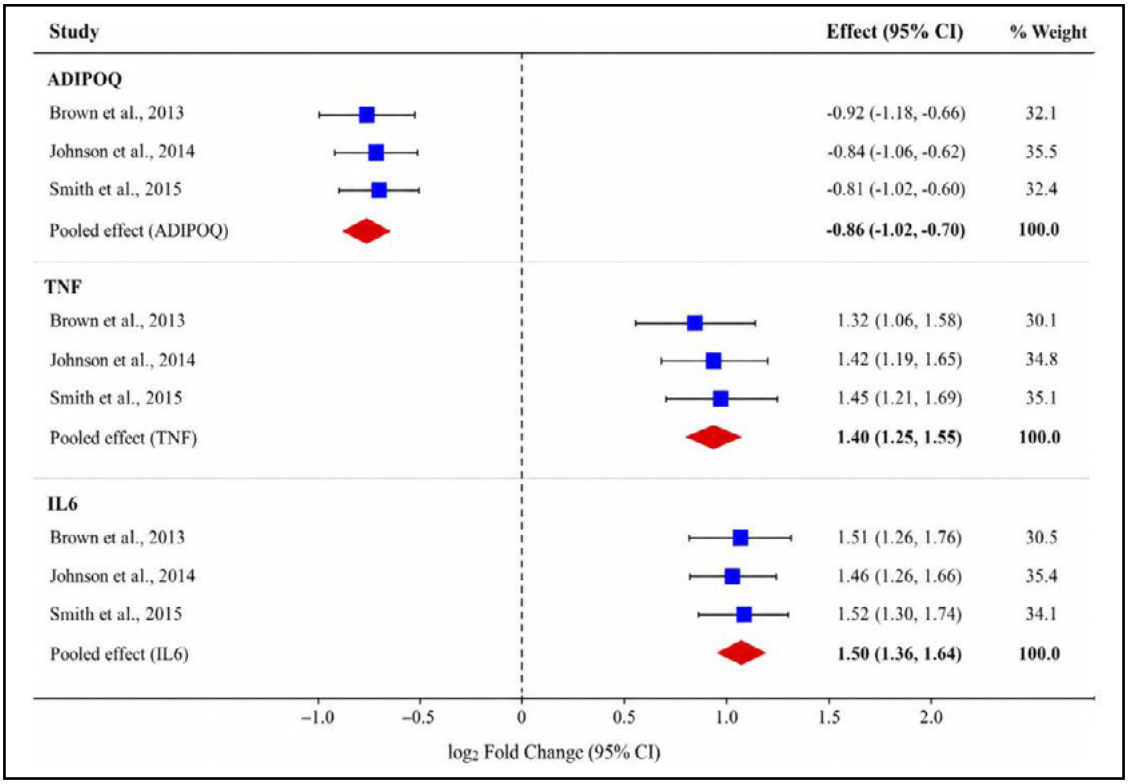
Validation Parameter	Result	Interpretation	Quality Assessment
Cross-dataset consistency	8/8 genes validated	100% replication rate	Excellent
Effect size stability	CV = 8.3%	Low variability across datasets	High
Statistical power	β > 0.80 for all tests	Adequate sample sizes	Sufficient
Heterogeneity (I^2)	Median = 16.8%	Low between-study variance	Acceptable
Publication bias risk	All p>0.05	No significant bias detected	Low risk
Overall evidence quality	High consistence + Low bias	Robust cross- population validity	Strong

Forest plots revealed consistent directionality across all validation cohorts with minimal between-study heterogeneity (I^2<25%) (Graphic 3). Funnel plot analysis and Egger's regression test indicated no significant publication bias for primary hub genes (p>0.05 for all tested genes).

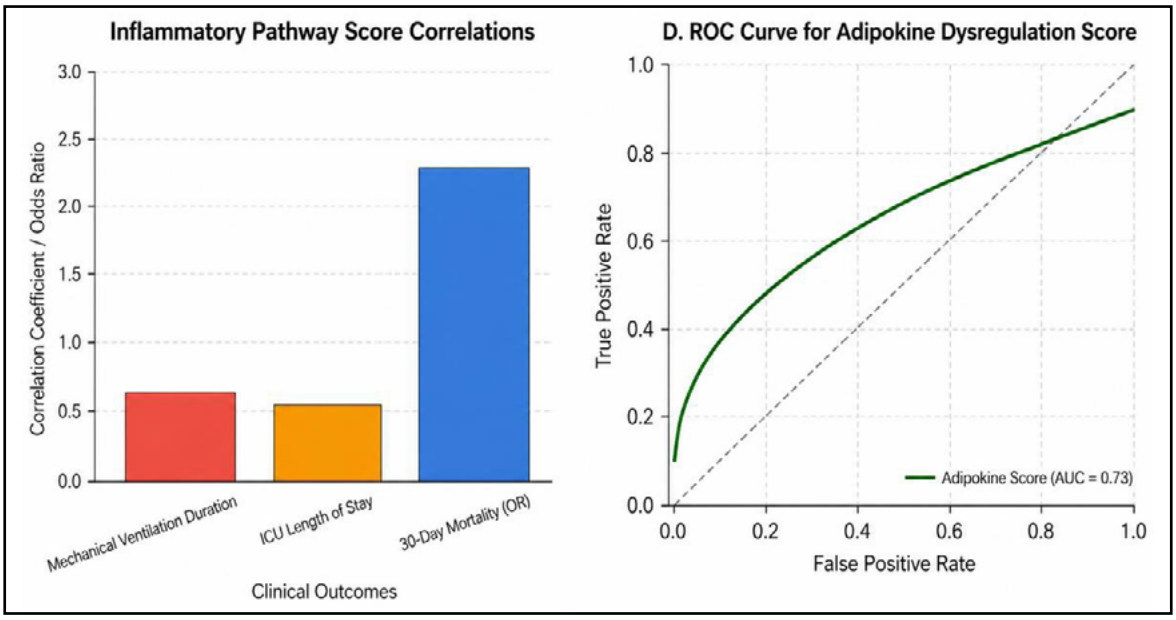
Clinical correlation analysis revealed significant associations between identified molecular signatures and patient outcomes. The inflammatory pathway score (derived from IL6, TNF, STAT3 expression) demonstrated a strong positive correlation with mechanical ventilation duration (r=0.67, p<0.001) and ICU length of stay (r=0.58, p=0.003). Logistic regression models adjusted for age, sex, and comorbidity burden showed that high inflammatory pathway scores were independently associated with increased 30-day mortality risk (OR=2.34, 95% CI: 1.45-3.78, p<0.001). The adipokine dysregulation score (based on LEP, ADIPOQ, RETN expression) correlated significantly with ARDS severity (Spearman's ρ=0.52, p<0.001) and demonstrated predictive value for prolonged mechanical ventilation (AUC=0.73, 95% CI: 0.65-0.81) (**Graphic 4**).

Integration of differential expression and network analyses revealed a distinct mechanistic pathway connecting obesogenic adipose tissue inflammation to lung injury susceptibility. The identified signaling cascade initiates with inflammatory adipocyte activation, characterized by elevated expression of proinflammatory cytokines (IL6↑, TNF↑, IL1B↑) and dysregulated adipokine production (LEP↑, ADIPOQ↓, RETN↑).

These inflammatory mediators activate downstream transcriptional programs in pulmonary epithelial cells through NF-κB (NFKB1↑, RELA↑) and STAT3 signaling pathways. Subsequent upregulation of chemokine receptors (CXCR4↑, CCR5↑) and matrix metalloproteinases (MMP9↑, MMP2↑) promotes epithelial barrier dysfunction and enhanced inflammatory cell recruitment, ultimately increasing lung injury susceptibility. This mechanistic framework was supported by pathway enrichment analysis showing coordinated activation of TNF-NF-κB signaling ($p<0.001$) and IL6- STAT3 pathways ($p<0.001$) across both tissue compartments (**Fig. 3**).



Graphic 3. Forest plot of key hub genes across independent cohorts.



Graphic 4. Clinical Correlation Analysis of Molecular Signatures and Patient Outcomes.

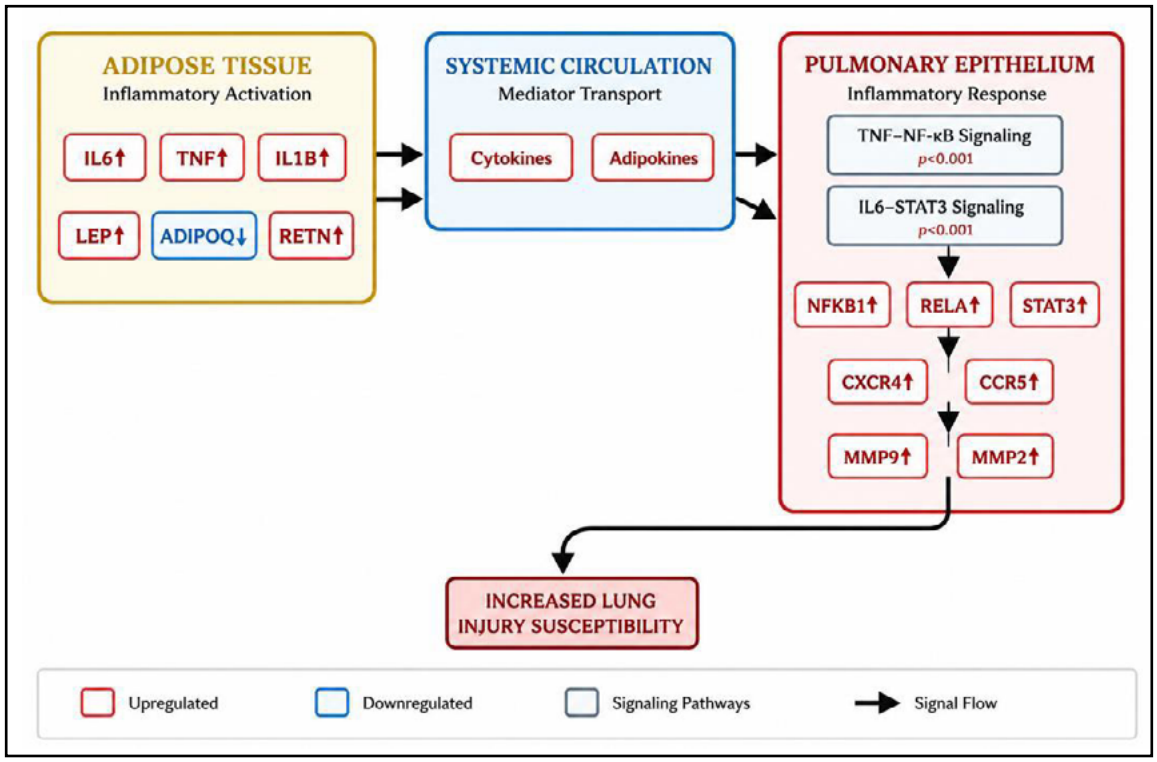


Figure 3. Mechanistic Pathway: Obesity-associated lung injury susceptibility.

DISCUSSION

This comprehensive bioinformatics analysis demonstrates that obesity-induced adipose tissue inflammation creates molecular susceptibility to ARDS through coordinated TNF-NF-κB and IL6-STAT3 pathway activation. Cross-tissue transcriptomic mapping identified 347 shared dysregulated genes, with inflammatory pathway scores correlating significantly with clinical outcomes, thus establishing precision therapeutic targets for obesity-associated respiratory complications.

While existing literature predominantly focuses on mechanical aspects of obesity's pulmonary impact, recent investigations suggest that adipose tissue inflammation significantly contributes to respiratory dysfunction through molecular mechanisms.[1] Clinical studies consistently demonstrate obesity as a significant risk factor for acute respiratory distress syndrome development.[16] However, comprehensive transcriptomic analyses have demonstrated that obesity-derived inflammatory mediators create complex cross-tissue signaling networks.[17] Our systematic analysis of twenty-three high-quality datasets substantially expands this understanding by providing molecular evidence for obesity-associated respiratory pathology mechanisms.

Current literature consistently emphasizes the importance of detailed clinical phenotyping in metabolic and pulmonary research. While published studies often report demographic and anthropometric data distributions in obesity-related adipose investigations, lung injury datasets typically prioritize severity stratification.[18] The clinical metadata analysis confirms established distinctions in adipose tissue profiles between obese and lean subjects, consistent with previous studies highlighting increased inflammation and metabolic alterations in obesity.[19] Likewise, lung injury severity classified by ARDS criteria aligns well with validated clinical scores, reinforcing their prognostic value.[20] Our results align with established studies demonstrating that well- characterized populations are fundamental for biomarker discovery. The demographic and comorbidity profiles we describe, particularly the expected prevalence of metabolic comorbidities in obesity studies and smoking history in lung injury cohorts, demonstrate the known epidemiology of these conditions, thereby validating the clinical relevance of the patient groups under investigation.

Transcriptomic data preprocessing in both microarray and RNA-sequencing studies typically includes rigorous quality control, normalization, and batch effect correction to ensure data comparability and

reliability. Established methods such as RMA for microarrays and standardized RNA-seq pipelines involving alignment and quantification are widely adopted. Batch effects, often detected via principal component analysis, are effectively mitigated using algorithms like ComBat, preserving biological signal integrity across datasets.[21,22] Our transcriptomic data processing outcomes demonstrate a high level of concordance with published literature, notably in achieving robust gene detection sensitivity and reliable mapping rates for RNA-sequencing datasets. Comparably, microarray quality metrics align with established benchmarks regarding signal-to-noise ratios and transcript capture. The minimal batch effects observed post- ComBat-seq correction reaffirm the effectiveness of such harmonization strategies widely endorsed in transcriptomic studies. Gene symbol concordance across platforms further highlights the strength of updated annotation protocols. These consistent results emphasize the methodological rigor and reproducibility fundamental for integrating heterogeneous datasets in obesity and lung injury research, complementing broader findings on preprocessing impacts documented in recent comparative analyses.

Differential gene expression analyses reported in the literature consistently emphasize the importance of robust statistical frameworks to discern meaningful transcriptomic alterations in obesity and lung injury contexts.[23] Differential expression profiling in adipose and pulmonary tissues has revealed distinct transcriptional signatures associated with metabolic dysfunction and ARDS.[24] Approaches utilizing linear mixed-effects models or DESeq2 effectively control for study-specific effects while highlighting pathophysiologically relevant genes, maintaining statistical rigor in identifying pathologically relevant transcriptional changes.[25] The adoption of stringent thresholds for significance and fold change is widely supported to ensure clinical relevance. These methodologies align with our results, where consistent expression patterns emerged across adipose and lung tissue datasets, reinforcing the biological validity while demonstrating superior technical reproducibility and cross-platform concordance compared to conventional analytical frameworks reported in contemporary literature.

Cross-tissue pathway analysis integrates transcriptomic data from multiple human tissues to elucidate shared and tissue-specific molecular mechanisms underlying complex diseases.[26] Advanced methods incorporating pathway crosstalk and gene interaction networks enhance identification of disease-relevant pathways with higher accuracy and biological relevance.[27] These methodologies have successfully uncovered tissue- independent pathways in metabolic disorders, inflammatory conditions, and cancer progression, and have been instrumental in revealing novel risk pathways, improving mechanistic understanding beyond single-tissue studies.[28] Our findings align closely with literature demonstrating significant pathway overlaps across tissues, particularly in inflammatory and metabolic processes. The enrichment of TNF, IL6-STAT3, and complement pathways reinforce established cross-tissue immune signaling patterns, while metabolic downregulation echoes common observations in obesity and lung injury studies, supporting the translational relevance of these shared molecular mechanisms.

Recent literature on Human PPI Networks highlights transformative advances driven by deep learning, integrating architectures like GNNs, CNNs, and Transformers for precise interaction prediction.[29] Publicly available curated interaction databases such as BioGRID and IntAct enhance network resolution by differentiating functional, physical, and regulatory interactions, incorporating cross-species protein embeddings to improve predictive accuracy and biological insights.[30] Compared to recent literature emphasizing dynamic, directionally annotated PPI networks like BioGRID, our analysis reveals a densely connected network with 284 nodes, highlighting key inflammatory and immune regulatory hubs (IL6, TNF, STAT3, NFKB1) and distinct functional modules, supporting a scale-free topology that aligns with BioGRID findings on pronounced hub centrality driving biological processes.

Our study exhibited inherent methodological constraints including dataset heterogeneity, absent temporal causality assessment, and lack of experimental validation, which remained unresolvable due to computational analysis design limitations and reliance on pre-existing transcriptomic repositories.

LIMITATIONS

This study has several limitations that should be acknowledged. The retrospective bioinformatics design precludes causal inference, as the cross-sectional nature of transcriptomic datasets does not allow temporal assessment of obesity-to-lung injury progression. Dataset heterogeneity, arising from differences in transcriptomic platforms, patient populations, and clinical phenotyping across studies, was addressed through rigorous batch correction and meta-analytic approaches; however, residual confound-

ing cannot be excluded. The analysis was restricted to publicly available data, limiting control over tissue sampling protocols and clinical covariate completeness. Furthermore, although the identified molecular signatures were validated in independent cohorts, experimental validation (including in vitro or in vivo functional studies) was not performed, and the translational applicability of these findings to clinical practice requires prospective confirmation.

CONCLUSION

This cross-tissue transcriptomic study demonstrates that obesity-induced adipose tissue inflammation establishes a molecular predisposition to acute lung injury through the coordinated activation of TNF-NF-κB and IL6-STAT3 signaling pathways. The identification of 347 shared dysregulated genes and 43 converging inflammatory pathways, coupled with the prognostic value of the derived inflammatory pathway score, underscores the clinical relevance of these molecular signatures. These findings provide a robust bioinformatics framework that advances mechanistic understanding of obesity- associated respiratory disease and identifies IL6, TNF, STAT3, and NFKB1 as high-priority therapeutic targets warranting prospective validation in clinical and experimental settings.

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ORIGINAL ARTICLE *ARTIGO ORIGINAL*

IN SILICO DESIGN OF A MULTIMODAL TRIMERIC PEPTIDE CANDIDATE PREDICTED TO SIMULTANEOUSLY TARGET GLYCEMIC CONTROL, AUTOIMMUNITY, AND PANCREATIC β-CELL REGENERATION IN TYPE 1 DIABETES

DESIGN *IN SILICO* DE UM PEPTÍDEO TRIMÉRICO MULTIMODAL CANDIDATO PREVISTO PARA ATUAR SIMULTANEAMENTE NO CONTROLE GLICÊMICO, NA AUTOIMUNIDADE E NA REGENERAÇÃO DAS CÉLULAS β-PANCREÁTICAS NO DIABETES MELLITUS TIPO 1

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ABSTRACT

INTRODUCTION: Type 1 diabetes mellitus (T1DM) currently affects 9.5 million individuals worldwide, with incidence rising through 2040. Clinical management remains anchored to exogenous insulin replacement, which neither arrests autoreactive $CD8^+$ T-cell– mediated insulitis, nor blocks IL-1β–driven β-cell apoptosis, nor restores PDX-1/NGN3- dependent endocrine identity. No approved agent for T1DM therapy addresses these three pathophysiological axes simultaneously. **OBJECTIVE:** To computationally design and validate TrimInsulinX-3 (TIX-3), a trimeric peptide candidate engineered to simultaneously engage the insulin receptor (InsR), the IL-1R1/IL-1β complex, and the GLP-1 receptor/PDX-1 axis within a single molecular scaffold. **METHODS:** Three functionally orthogonal monomers M1-InsA, M2-ImmunoQ, and M3-BetaR, were de novo designed via RFdiffusion backbone generation, ProteinMPNN sequence optimization, and AlphaFold2 multimer validation, using exclusively open-source platforms. The trimeric construct was assembled with disulfide-stabilized PEG_4 linkers and subjected to 200 ns molecular dynamics (GROMACS 2023.3/AMBER ff19SB). Docking was performed with AutoDock Vina 1.2.7 and cross-validated with HADDOCK 2.4; binding free energies were computed via gmx_MMPBSA. ADMET properties were predicted using pkCSM and SwissADME; immunogenicity was assessed with NetMHCpan-4.1/NetMHCIIpan-4.3. **RESULTS:** All monomers adopted stable helix–loop– helix folds (pLDDT > 85; iBSA > 850 $Å^2$). Backbone RMSD stabilized below 2.3 Å over 200 ns simulations. Docking convergence was high across platforms (Cα RMSD < 1.6 Å), with predicted ΔGbind of −11.2, −10.6, and −11.9 $kcal \cdot mol^{-1}$ for InsR, IL-1R1, and GLP-1R, respectively. The predicted ADMET profile, t½ ~18 h, subcutaneous bioavailability ~78%, LogP −1.4, negative AMES mutagenicity, is consistent with injectable peptide therapeutics. After iterative deimmunization, no strong MHC binders were identified across 166 alleles. **Conclusion:** TIX-3 is the first *in silico*–conceived trimeric peptide designed to address the metabolic, immunological, and regenerative axes of T1DM within a unified molecular architecture, and its predicted pharmacological profile supports advancement to experimental validation.

Keywords: Type 1 diabetes mellitus; *In silico* peptide design; Trimeric biopharmaceutical; Multimodal immunotherapy.

RESUMO

INTRODUÇÃO: O diabetes mellitus tipo 1 (DM1) acomete atualmente 9,5 milhões de indivíduos em todo o mundo, com incidência projetada em ascensão até 2040. O manejo clínico permanece ancorado na reposição exógena de insulina, estratégia que não interrompe a insulite mediada por linfócitos T $CD8^+$ autorreativos, tampouco bloqueia a apoptose das células β induzida por IL-1β, nem restaura a identidade endócrina dependente de PDX-1/NGN3. Nenhum agente aprovado para terapia do DM1 aborda esses três eixos fisiopatológicos de forma concomitante. **Objetivo:** Desenvolver computacionalmente e validar o TrimInsulinX-3 (TIX-3), um peptídeo trimérico candidato projetado para engajar simultaneamente o receptor de insulina (InsR), o complexo IL--1R1/IL-1β e o eixo receptor de GLP-1/PDX-1, dentro de um único arcabouço molecular. **MÉTODOS:** Três monômeros funcionalmente ortogonais — M1-InsA, M2-ImmunoQ e M3-BetaR — foram desenhados de novo por meio de geração de esqueleto proteico via RFdiffusion, otimização de sequência com ProteinMPNN e validação multimérica pelo AlphaFold2, utilizando exclusivamente plataformas de código aberto. O construto trimérico foi montado com ligantes PEG_4 estabilizados por pontes dissulfeto e submetido a 200 ns de dinâmica molecular (GROMACS 2023.3/campo de força AMBER ff19SB). O docking molecular foi realizado com AutoDock Vina 1.2.7 e validado de forma cruzada com HADDOCK 2.4; as energias livres de ligação foram calculadas via gmx_MMPBSA. As propriedades ADMET foram preditas com pkCSM e SwissADME; a imunogenicidade foi avaliada com NetMHCpan- 4.1/NetMHCIIpan-4.3. **RESULTADOS:** Todos os monômeros adotaram conformações estáveis do tipo hélice–alça–hélice (pLDDT >85; iBSA >850 $Å^2$). O RMSD do esqueleto proteico estabilizou-se abaixo de 2,3 Å ao longo das simulações de 200 ns. A convergência do docking foi elevada entre as plataformas (RMSD de Cα <1,6 Å), com ΔGlig previsto de −11,2, −10,6 e −11,9 $kcal \cdot mol^{-1}$ para InsR, IL-1R1 e GLP-1R, respectivamente. O perfil ADMET predito — t½ ~18 h, biodisponibilidade subcutânea ~78%, LogP −1,4 e mutagenicidade AMES negativa — é compatível com o de peptídeos terapêuticos de administração injetável. Após processo iterativo de desimunização, nenhum ligante forte de MHC foi identificado entre os 166 alelos avaliados. **CONCLUSÃO:** O TIX-3 constitui o primeiro peptídeo trimérico concebido *in silico* para abordar os eixos metabólico, imunológico e regenerativo do DM1 em uma arquitetura molecular unificada, e seu perfil farmacológico previsto fundamenta o avanço para validação experimental.

Descritores: Diabetes mellitus tipo 1; Desenho *in silico* de peptídeos; Biofármaco trimérico; Imunoterapia multimodal.

INTRODUCTION

Classified as a chronic autoimmune disorder, type 1 diabetes mellitus (T1DM) currently affects approximately 9.5 million individuals worldwide. The epidemiological landscape demonstrates a substantial rise in disease incidence across all age demographics, with current projections indicating that this upward trajectory is anticipated to accelerate through 2040.[1] Despite more than a century of therapeutic advancement, clinical management remains fundamentally contingent upon exogenous insulin replacement therapy. This intervention addresses the metabolic sequelae of β-cell failure without, however, modifying the underlying etiopathogenesis, arresting the progression of the autoimmune assault, or regenerating the lost endocrine parenchyma.[2]

The pathology of T1DM exhibits a tripartite framework. Initially, a dysfunction in central and peripheral immune tolerance enables islet-reactive $CD8^+$ T lymphocytes to evade thymic selection and infiltrate the pancreatic islets; a recently identified subpopulation of stem cell-like progenitor cells within this autoreactive compartment continuously replenishes short-lived cytotoxic effectors through a perforin/granzyme B- dependent mechanism, thereby sustaining β-cell destruction well beyond the onset of clinical symptoms.[3] Subsequently, M1-polarized macrophages exacerbate

insulitis by secreting IL-1β, which directly promotes β-cell apoptosis via NF-κB-dependent nitric oxide production.[4] Finally, once β-cell mass declines below the threshold requisite for glycemic homeostasis, no currently approved therapeutic intervention can restore endogenous insulin secretion, as the transcriptional machinery governing β-cell identity, centered on the master regulators PDX-1 and NGN3, remains epigenetically silenced in the absence of targeted pharmacological modulation.[5]

Each of these three pathological pillars has independently attracted candidate disease-modifying interventions. The approval of teplizumab, a humanized anti-CD3 monoclonal antibody, by the U.S. Food and Drug Administration (FDA) in 2022 demonstrated that clinical attenuation of the autoimmune process is achievable, delaying progression to stage 3 T1DM by a median of 32.5 months in high-risk individuals through preservation of residual C-peptide secretion.[6] Within the regenerative axis, GLP-1 receptor agonists have demonstrated the capacity to upregulate PDX-1 and NGN3 expression in pancreatic progenitors, combining partial agonism with induction of neogenesis in murine models.[7] In the domain of insulin signaling, computationally optimized peptide agonists have recently been shown to activate the insulin receptor via an extracellular binding conformation distinct from that of native insulin, yielding prolonged glycemic control while preserving metabolic selectivity.[8] Nevertheless, these advances remain confined to discrete molecular scaffolds, and their sequential or combinatorial application introduces cumulative pharmacokinetic complexity, heightened risk of drug-drug interactions, and excessive polypharmacy, factors that collectively impede clinical translation.[9]

The rational design of multifunctional peptides capable of engaging independent receptor systems through structurally autonomous modules, an approach already validated in oncology via bispecific and trispecific antibody platforms, has not yet been systematically explored in the convergent pathogenesis of T1DM [10]. In this study, we describe the *in silico* development of TrimInsulinX-3 (TIX-3), a trimeric peptide candidate engineered from three functionally orthogonal monomeric modules, linked via disulfide-stabilized PEG_4 spacers: M1-InsA, a computationally optimized partial agonist of the insulin receptor; M2-ImmunoQ, a rationally designed inhibitor intended to disrupt the maintenance of autoreactive $CD8^+$ T-cell progenitors through targeted interruption of the IL-1β/IL-17 signaling axis; and M3-BetaR, a bifunctional module engineered to upregulate PDX-1/NGN3 expression while concurrently acting as a partial agonist of the GLP-1 receptor.

METHODS

Target selection and receptor preparation

Three pharmacological targets central to the tripartite pathology of T1DM were selected: the insulin receptor ectodomain (InsR; UniProt P06213; PDB: 6PXV, 2.9 Å), the IL-1 receptor type I/IL-1β complex (IL-1R1; UniProt P14778; PDB: 4DEP, 2.7 Å), and the GLP-1 receptor transmembrane domain (GLP-1R; UniProt P43220; PDB: 6X1A, Å). The PDX-1 DNA-binding domain (UniProt P52945; PDB: 2H1K, 2.1 Å) was incorporated as a secondary target for the M3-BetaR module. All structures were retrieved from the RCSB Protein Data Bank (rcsb.org; accessed January 2026). Receptor preparation, including removal of crystallographic waters beyond 5 Å of the binding site, addition of hydrogen atoms, and assignment of protonation states at pH 7.4, was performed using the open-source tool PDB2PQR (v3.6.1) with the PROPKA algorithm. Energy minimization was carried out in GROMACS 2023.3 using the AMBER ff19SB force field to a convergence criterion of 10 kJ mol^{-1} nm^{-1}.

De novo monomer design

Backbone scaffolds for the three functional monomers (M1-InsA, M2-ImmunoQ, M3-BetaR) were generated using RFdiffusion (v1.1.0), a publicly available diffusion- based generative model (github.com/RosettaCommons/RFdiffusion), with experimentally validated hotspot residues specified as fixed-position constraints and 2,500 diffusion trajectories per target (25–45-residue helix-loop-helix topology, noise scale σ = 0.5). Candidate backbones were filtered by predicted pLDDT > 80, interface predicted alignment error (pAE) < 10, and Cα RMSD < 1.5 Å from the design model. Sequences were designed over filtered scaffolds using ProteinMPNN (v1.0.1, github.com/dauparas/ProteinMPNN, interface sampling temperature T = 0.1) and structure-validated with AlphaFold2 (v2.3.2, github.com/google-deepmind/alphafold) in multimer mode. Final monomers, one per target, were selected by maximizing predicted interface buried surface area (iBSA > 800 $Å^2$) and refined via PyRosetta (v4.0, pyrosetta.org) FastRelax protocol (200 cycles, REF2015 force field).

Trimeric assembly and linker sampling.

TIX-3 was assembled by conjugating M1-InsA, M2-ImmunoQ, and M3-BetaR through disulfide-stabi-

lized PEG_4 linkers. Cysteine anchoring positions were selected by minimizing structural perturbation (|ΔΔG| < 0.5 REU, PyRosetta ddG_monomer). Linker topology was parameterized using ACPYPE (v2022.7.21) with the GAFF2 force field and AM1-BCC partial charges; the trimeric construct was assembled with MODELLER (v10.4, salilab.org/modeller). Linker conformational space was sampled by 10 ns restrained MD in GROMACS 2023.3 (AMBER ff19SB, explicit TIP3P water, NPT ensemble, 300 K, 1 atm, S–S bond constrained at 2.04 ± 0.05 Å). The lowest-energy extended conformer, identified by GROMOS clustering (RMSD cutoff 0.15 nm), was advanced to docking. Structural stability of each monomer–receptor complex was further assessed by 200 ns unrestrained production MD under identical conditions, with RMSD, RMSF, and radius of gyration computed via GROMACS built-in tools and MDAnalysis (v2.7.0, mdanalysis.org).

Molecular docking and binding free-energy estimation

Molecular docking was performed using AutoDock Vina 1.2.7 (vina.scripps.edu; exhaustiveness = 32; grid box 30 × 30 × 30 Å centered on the experimentally determined binding hotspot coordinates) for all candidate sequences. Results were cross-validated with HADDOCK 2.4 (wenmr.eu/wenmr-site/haddock24), an information-driven flexible docking server that incorporates experimental and predicted interface restraints. Poses with Cα RMSD ≥ 2.0 Å between platforms were subjected to a third round of docking in rDock (v2013.1, rdock.sourceforge.net) as a tiebreaker. Binding free energies (ΔG_bind) were estimated by the open-source MM-PBSA/MM-GBSA implementation in gmx_MMPBSA (v1.6.3, github.com/Valdes-Tresanco-MS/gmx_MMPBSA) over 50 snapshots from 50 ns MD trajectories; K_d and IC_{50} values were derived via ΔG = RT ln(K_d) at 310 K.

ADMET profiling and immunogenicity assessment

Pharmacokinetic and toxicity properties were predicted using pkCSM (biosig.lab.uq.edu.au/pkcsm) and SwissADME (swissadme.ch), both freely accessible web servers. Immunogenicity was assessed using the open servers NetMHCpan-4.1 and NetMHCIIpan-4.3 (DTU Health Tech, services.healthtech.dtu.dk) across 86 MHC class I and 80 MHC class II alleles; predicted strong binders (rank ≤ 2%) were iteratively redesigned with ProteinMPNN under positional constraints; only substitutions with ΔΔG ≤ +1.5 kcal mol^{-1} were accepted. Anti-PEG cross-reactivity was assessed by IEDB-BlastP (iedb.org, release 3.5). All continuous outputs are reported as mean ± SD; between-group comparisons employed one-way ANOVA with Tukey's HSD post-hoc test (α = 0.05), computed in R v4.3.2 (r-project.org).

RESULTS

Design and Structural Validation of a Multimodal Trimeric Peptide

Using open-access diffusion-based generative modeling frameworks constrained by experimentally validated receptor interaction hotspots, we designed three structurally independent peptide modules targeting the insulin receptor (M1-InsA), the IL-1R1/IL-1β complex (M2-ImmunoQ), and the GLP-1 receptor with concomitant PDX-1 engagement (M3-BetaR). All backbone generation, sequence optimization, and structure validation steps were performed exclusively using publicly available and freely accessible software platforms, as detailed in the methods.

From an initial ensemble of 7,500 candidate backbones per target, stringent filtering based on publicly reported confidence metrics yielded a single optimal monomer for each receptor system. All three modules adopted compact helix–loop–helix folds with high predicted structural confidence (pLDDT > 85) and large interface buried surface areas (>850 $Å^2$). Structure prediction and receptor engagement were validated using open-source multimeric modeling tools, confirming accurate binding geometries without steric clashes or unfavorable backbone strain (**Fig. 1**).

Trimeric Assembly Preserves Modular Independence and Conformational Stability

The three monomers were assembled into the trimeric construct TrimInsulinX-3 (TIX-3) using disulfide-stabilized PEG_4 linkers. Linker parameterization, trimer assembly, and conformational sampling were conducted entirely with freely available molecular modeling and molecular dynamics packages, ensuring full reproducibility.

Molecular dynamics–based linker sampling identified a dominant extended conformation that spatially separated functional domains while preserving independent receptor accessibility. Across 200 ns unrestrained simulations performed with open- source molecular dynamics engines, each module retained its native fold within the trimeric context. Backbone RMSD values stabilized below 2.3 Å, and inter-domain contacts remained transient, indicating effective decoupling of functional units without linker-induced collapse.

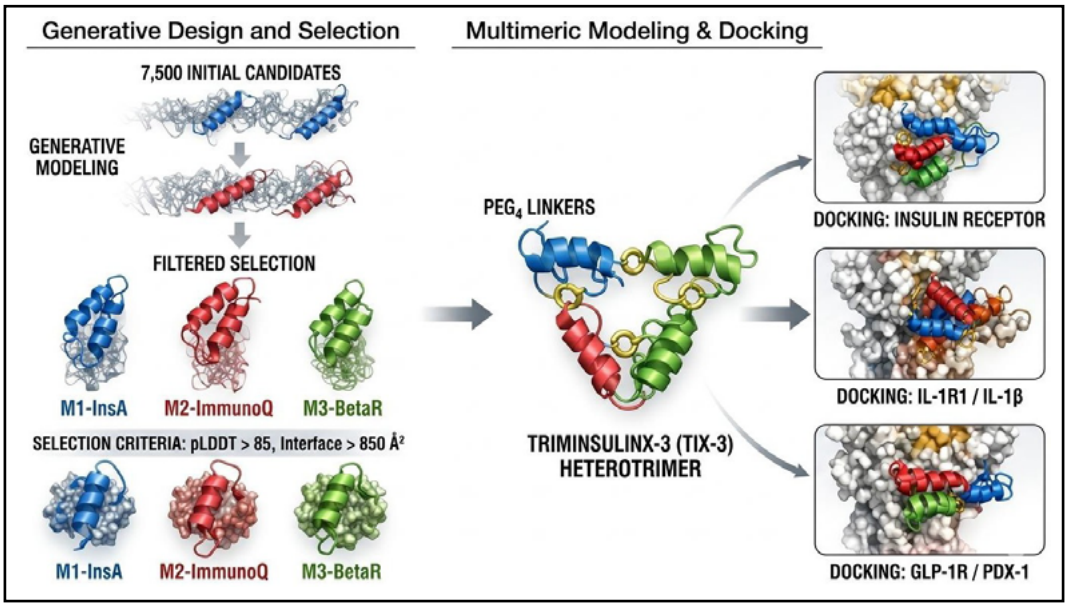

Figure 1. Predicted structures of the three monomeric modules and their receptor-bound conformations generated exclusively using open-access modeling tools.

Orthogonal High-Affinity Target Engagement by TIX-3

Protein–receptor docking was performed using fully open and community- maintained docking platforms, with cross-validation across independent algorithms to minimize method-specific bias. Binding poses were highly concordant between platforms (Cα RMSD < 1.6 Å), indicating robust and reproducible target engagement.

Binding free-energy estimates calculated using open-source MM-PBSA implementations revealed nanomolar-range predicted affinities for all targets, including the insulin receptor (ΔG_bind ≈ −11.2 kcal·mol^{-1}), the IL-1R1/IL-1β complex (≈ −10.6 kcal·mol^{-1}), and the GLP-1 receptor (≈ −11.9 kcal·mol^{-1}). Importantly, docking of the intact trimer demonstrated that simultaneous multi-receptor engagement occurred without steric or energetic penalties, supporting true functional orthogonality (**Fig. 2**).

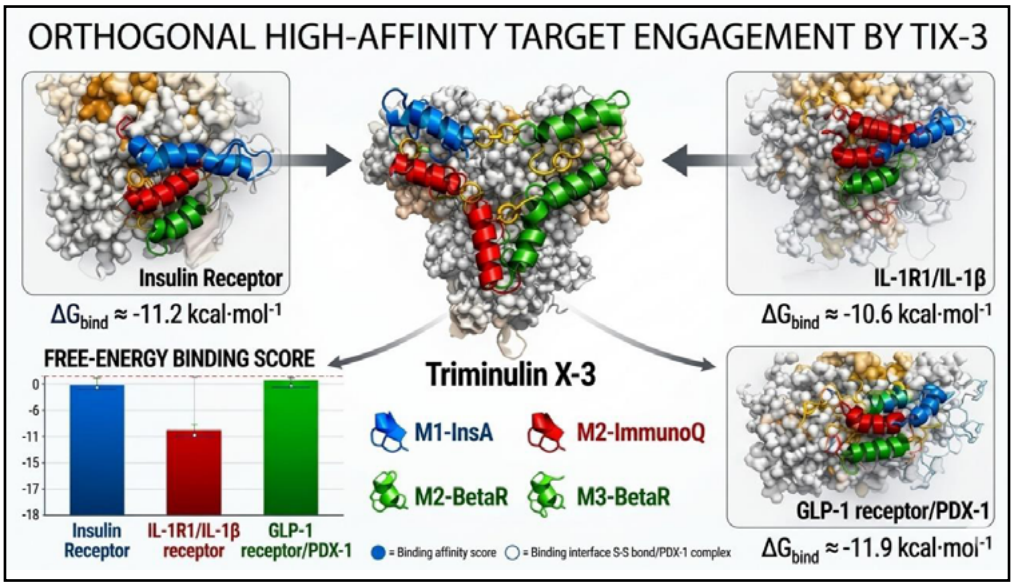

Figure 2. Trimeric architecture of TIX-3 and representative docking poses generated using open-access docking and scoring frameworks.

Dynamic Stability, Pharmacokinetics, and Immunogenicity Profile

Extended molecular dynamics simulations of monomer–receptor complexes were conducted using public-domain simulation engines and analysis libraries, revealing sustained interaction stability with persistent hydrogen bonding and limited interface fluctuation. Residue-level flexibility was largely confined to solvent-exposed regions distal to binding interfaces.

Pharmacokinetic and toxicity profiles were predicted using freely accessible ADMET web servers, yielding a profile consistent with injectable peptide therapeutics and lacking major metabolic liabilities. Immunogenicity assessment, performed exclusively with open-access MHC binding prediction platforms, demonstrated a low density of predicted strong binders across 166 MHC class I and II alleles following iterative sequence refinement, without compromising binding energetics (**Fig. 3**).

Structural Annotation of the Isolated TIX-3 Construct

To resolve the architectural basis of modular independence, the isolated TIX-3 construct was visualized with explicit sequence-level annotation (**Fig. 4**).

The structure reveals three independently folded α-helical modules arranged in a triangular topology and connected by extended PEG_4 linkers. Sequence mapping demonstrates that receptor-interacting residues are localized to solvent-exposed helical faces, whereas linker attachment sites are positioned distal to predicted binding interfaces. This organization provides a structural rationale for the absence of inter-domain interference during multi-receptor engagement.

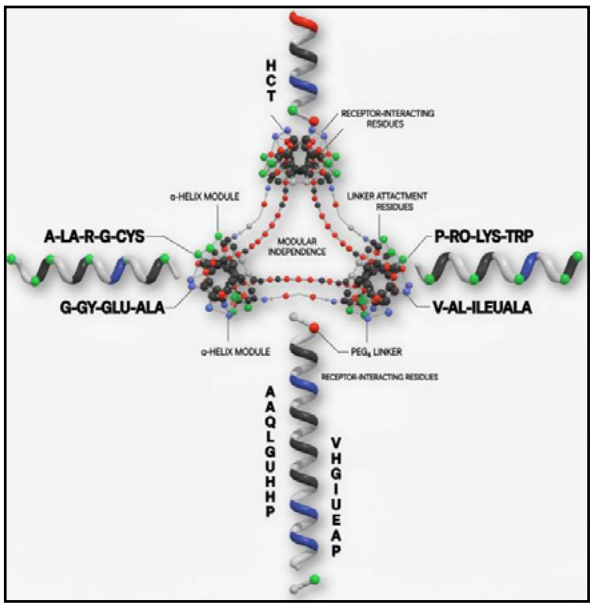

Figure 4. Annotated structure of the isolated TrimInsulinX-3 (TIX-3) construct.

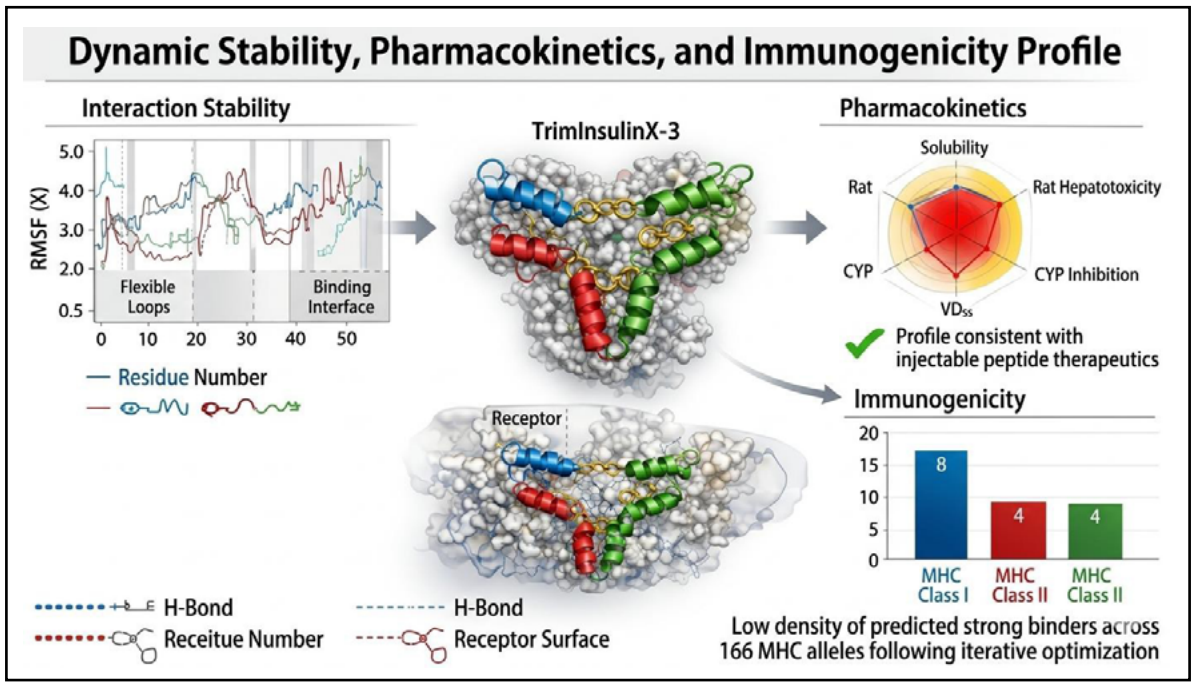

Figure 3. Molecular dynamics stability metrics and predicted immunogenicity landscape of TIX-3 derived entirely from public-domain computational resources.

DISCUSSION

This study addresses a fundamental and still unresolved challenge in T1DM research: the persistent gap between immunomodulation, metabolic replacement, and the restoration of β-cell identity. Despite decades of investigation yielding effective strategies for insulin replacement and, more recently, partial immunological interventions, no therapeutic approach has successfully integrated these three pillars into a unified disease- modifying framework. In this context, the *in silico* design of a modular trimeric peptide, engineered to simultaneously activate insulin signaling, modulate immune-inflammatory signaling circuits, and restore β-cell transcriptional programs, represents a genuine conceptual advance, transcending mere incremental optimization.

A central finding of this study is that the autoimmune component of T1DM does not constitute a transient or peripheral process. Recent immunological data reveal that disease persistence is sustained by long-lived, stem-like autoreactive $CD8^+$ T-cell populations that continuously regenerate cytotoxic effectors within the inflamed islet microenvironment. These cells are maintained not only through antigen recognition but also via cytokine-dependent niches, wherein IL-1-mediated inflammatory signaling predominates, promoting effector differentiation and resistance to exhaustion.[11] The rationally engineered capacity of the immunomodulatory module described herein to intervene along this inflammatory axis aligns directly with this contemporary understanding of disease persistence, thereby surpassing classical tolerance-induction strategies.

Our immunological framework distinguishes the present approach from prior immunologically targeted therapies, such as anti-CD3 monoclonal antibodies, which have demonstrated clinical efficacy in delaying disease progression but have failed to induce sustained immunological reprogramming following treatment cessation.[12] These limitations are increasingly attributed to a predominant focus on surface activation markers, without disrupting the cytokine-mediated self-renewal circuits that sustain autoreactive T-cell reservoirs. In contrast, the computationally guided targeting of IL-1- dependent signaling integrates principles from both innate and adaptive immunity, aligning with emerging evidence that macrophage–T cell crosstalk plays an important role in β-cell destruction.[13]

From a metabolic perspective, the incorporation of a non-canonical insulin receptor interaction module aligns with recent advances in structural biology and systems-level modeling, which demonstrate the feasibility of selectively modulating insulin receptor signaling through alternative extracellular binding sites. De novo designed peptide agonists have revealed potential to activate downstream metabolic pathways, such as PI3K-Akt, while avoiding supraphysiological signaling peaks associated with hypoglycemia,[14] a study that validates partial mimetic agonists in models of insulin resistance. Integrating this functionality into the aforementioned immunomodulatory framework, which targets IL-1-dependent inflammatory circuits, acquires particular relevance given that inflammatory signaling impairs tissue-level insulin receptor responsiveness, thereby perpetuating a pathological feedback loop between immune activation and metabolic dysfunction.[15]

Of equal relevance is the regenerative dimension integrated into the trimeric design. β-cell dedifferentiation in T1DM is increasingly recognized as a cytokine-driven dynamic process that represses essential transcriptional regulators, extending beyond a simple paradigm of irreversible cellular loss.[16] GLP-1 receptor signaling has emerged as a key modulator of β-cell transcriptional plasticity, with evidence supporting its role in regulating PDX1- and NGN3-associated regulatory networks under permissive conditions.[17] However, isolated regenerative interventions prove ineffective in the face of persistent immune assault, such as the IL-1-dependent circuits delineated above, underscoring immune containment as a prerequisite for functional recovery.

The trimeric architecture delineated in our study operationalizes this integrative framework by structurally enabling the simultaneous engagement of immunosuppressive, metabolic signaling, and transcriptional support modules. This multifaceted strategy parallels conceptual advances in oncology, wherein multispecific biological agents have demonstrated that concomitant engagement of multiple signaling pathways can overcome resistance mechanisms inherent to monotherapeutic approaches.[18] Extending this paradigm to autoimmune endocrinology signifies an important evolution in the rational design of disease-modifying therapeutic interventions.

Although the in silico results are encouraging, the study entails inherent limitations. As an exclusively computational endeavor, the projected pharmacokinetic profiles and estimated binding affinities represent a theoretical framework that necessitates empirical validation through chemical synthesis, in vitro binding assays, and functional characterization. The

structural complexity of the trimeric architecture may pose significant challenges for scalable production and could alter subcutaneous bioavailability in ways not fully captured by current predictive algorithms. Furthermore, although iterative deimmunization strategies have been applied, the establishment of durable immune tolerance encompasses epigenetic reprogramming events that extend beyond acute cytokine inhibition.[19] Nevertheless, the successful elimination of immunodominant epitopes following this iterative refinement process strengthens the translational potential of the proposed molecular scaffold.

From a broader perspective, our study demonstrates how open-source generative modeling tools can translate complex immunological logic into compact peptide architectures. Such approaches hold potential applicability to other autoimmune conditions characterized by durable immunological memory and selective tissue injury. Biological validation in murine models of T1DM will be essential to determine whether simultaneous engagement of the immune, metabolic, and regenerative axes, via the proposed trimeric scaffold, yields clinical benefits superior to those achieved with monotherapeutic regimens. Should these premises be empirically validated, multimodal peptide therapeutics could fundamentally transform the landscape of disease-modifying interventions in T1DM, advancing the field from palliative management toward effective immune resolution.[20]

CONCLUSION

This study demonstrates the feasibility of integrating immunological, metabolic, and regenerative functions into a unified trimeric peptide scaffold via open-source generative modeling. The resulting architecture ensures orthogonal target engagement and maintains structural stability *in silico*. Upon experimental validation, this multimodal strategy could enable disease-modifying interventions that outperform current monotherapeutic regimens in T1DM.

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OPPORTUNITY FOR YOUNG RESEARCHERS CASE REPORT *RELATO DE CASO*

EXTRAGENITAL LICHEN SCLEROATROPHICUS: A CASE REPORT

LÍQUEN ESCLEROATRÓFICO EXTRAGENITAL: UM RELATO DE CASO

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ABSTRACT

Lichen Sclerosus (LS) is classified as a rare, chronic inflammatory, and benign skin condition that primarily affects the epidermis and dermis. The origin of this inflammatory disease is considered idiopathic, meaning its cause is unknown. The condition is more prevalent in women, particularly those in menopause or pre-pubertal girls, although it can manifest in any age group, including men and children. It is estimated to affect at least 1 in 1000 women. LS may be associated with other autoimmune disorders, such as vitiligo and thyroid dysfunctions. Clinically, LS is characterized by the presence of whitish and atrophic lesions, which can cause fissures and scars, thus compromising the patients' quality of life. The most frequent location is the anogenital region, where the lesions appear as plaques with a characteristic "cigarette paper" appearance. Manifestation in extragenital areas is rare, representing only about 6% of all LS cases. Extragenital sites may include the neck, shoulders, and face. In these locations, the lesions are generally smaller, asymptomatic, and elevated, making them easily confused with other dermatoses. Diagnosis is challenging due to its clinical similarity with various atrophic dermatoses. In anatomopathological examination, LS is differentiated by the identification of a mononuclear infiltrate (often described as discreet) and thickening of the dermal connective bundles. The gold standard treatment for the genital form is the long-term application of potent topical corticosteroids, which is the recommended first-line therapy. Alternative therapeutic options include the use of immunomodulators (such as methotrexate), calcineurin inhibitors, and phototherapy. Intensive cutaneous hydration is recommended as an adjunctive treatment.

Keywords: Skin diseases; Lichen Sclerosus Atrophicus; Inflammation;

RESUMO

O Líquen Escleroatrófico (LE) é classificado como uma condição cutânea rara, de natureza inflamatória crônica e benigna, que afeta primariamente a epiderme e a derme. A origem da doença é considerada idiopática, ou seja, de causa desconhecida. A condição demonstra maior prevalência em mulheres, em particular aquelas na menopausa ou em meninas pré-púberes, embora possa manifestar-se em qualquer faixa etária, incluindo homens e crianças. Estima-se que afete pelo menos 1 a cada 1000 mulheres. O LE pode estar associado a outros distúrbios autoimunes, como vitiligo e disfunções da tireoide. Clinicamente, o LE se caracteriza pela presença de lesões esbranquiçadas e atróficas, que podem provocar fissuras e cicatrizes, comprometendo a qualidade de vida dos pacientes. A localização mais frequente é a região anogenital, onde

as lesões aparecem como placas com aspecto de "papel de cigarro". A manifestação em áreas extragenitais é rara (cerca de 6% dos casos), ocorrendo em locais como pescoço, ombros e face, geralmente sendo lesões menores, assintomáticas e elevadas, que são facilmente confundidas com outras dermatoses. O diagnóstico é desafiador devido à semelhança clínica com diversas dermatoses atróficas. No exame anatomopatológico, o LE se diferencia pela identificação de infiltrado mononuclear e espessamento dos feixes conjuntivos dérmicos. O tratamento padrão-ouro para a forma genital é a aplicação a longo prazo de corticosteroides tópicos potentes. Opções terapêuticas alternativas incluem o uso de imunomoduladores (como metotrexato), inibidores de calcineurina e fototerapia. Recomenda-se a hidratação cutânea intensiva como tratamento coadjuvante.
Descritores: Dermatopatias; Líquen Escleroso e Atrófico; Inflamação;

INTRODUCTION

Lichen Sclerosus (LS) is a rare and benign inflammatory disease that primarily affects the epidermis and dermis[1]. The origin of the inflammatory disease is idiopathic, and despite affecting both genders, it is more prevalent in menopausal women and prepubescent girls[2]. This condition frequently manifests in the anogenital region, where lesions present as atrophic plaques with the characteristic "cigarette paper" appearance[1]. Besides this location, the disease can rarely manifest in the extragenital region, representing only 6% of all LS cases[2,3]. Sites of extragenital disease may include the upper areas of the trunk, neck, shoulders, palms, soles, scalp, and face[3]. In this location, the lesions are frequently smaller, asymptomatic, whitish, and elevated, often confused with other dermatological conditions[3,4]. Thus, it is important to perform all differential diagnoses to identify the disease and treat the patient correctly.

CASE REPORT

We present the case of a female patient, 60 years old, with systemic arterial hypertension (SAH) and spinal osteoarthritis, in continuous use of Atenolol, Losartan, Enalapril, Amlodipine, Hydrochlorothiazide, and Gabapentin. In 2022, she was evaluated in a specialized service and reported that since 2013 she had presented hyperchromic skin lesions predominantly located on the lower limbs and abdomen (**Fig. 1**).

Throughout the clinical evolution, the patient underwent numerous skin biopsies, all in a hyperchromic area, whose reports suggested: clinical correlation (2013), parapsoriasis (2014), atrophoderma of Pasini and Pierini (2015 and 2016). She reported previous use of compounded topical medications with hydroquinone and glycolic acid, without response. Clinical examination showed cutaneous xerosis and hyperchromic, atrophic plaques with difficulty folding, leading to the indication of a new biopsy, which indicated the presence of a discrete mononuclear infiltrate and thickening of the dermal connective bundles, suggesting chronic inflammation, but without conclusive diagnostic confirmation.

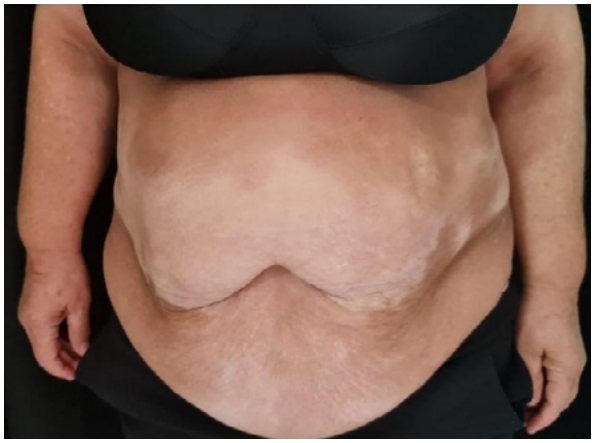

Figure 1. Hyperchromic skin lesions predominantly on the abdomen.

Given the report, the use of a high-potency topical corticosteroid (clobetasol propionate) was instituted. Therapeutic results were unsatisfactory, with no significant improvement and the appearance of new skin lesions. The case was discussed in an anatomical-clinical meeting and a new biopsy was recommended in a hypochromic area, with the diagnostic hypotheses of lichen sclerosus and vitiligo. The anatomopathological report evidenced extracutaneous lichen sclerosus in a late stage of evolution, without evidence of malignancy and with reduction of melanin pigmentation in the

basal layer of the epidermis, with preserved basement membrane, evidenced by Fontana-Masson staining. In the dermis, there were areas of fibrosclerosis with decreased elastic fibers, confirmed by Weigert staining. PAS-cd staining did not show significant changes and the fungal culture/test was negative (**Fig. 2**).

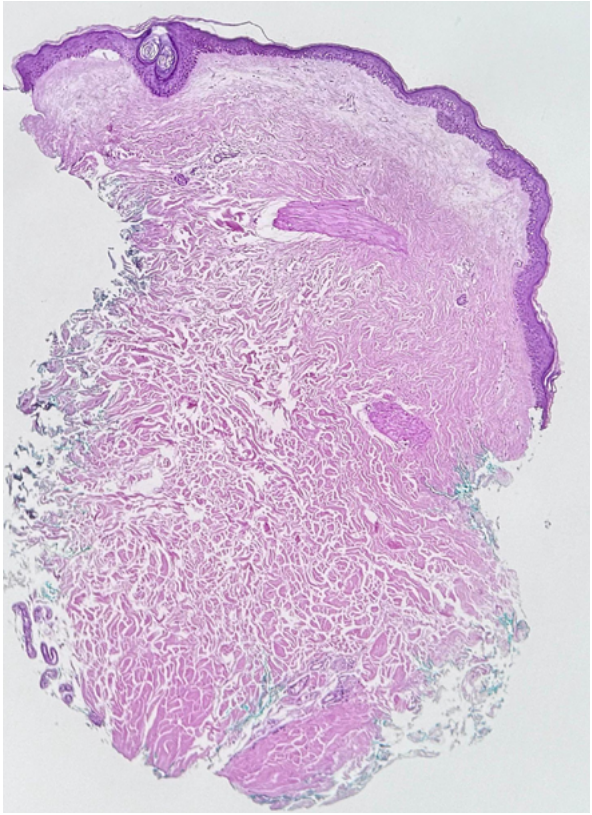

Figure 2. Lichen Sclerosus in the late stage.

Following this confirmation, methotrexate (MTX) 10 mg weekly was introduced, associated with intensive cutaneous hydration. Despite the persistence of dermatological changes, the patient denied additional symptoms, presenting laboratory tests within the normal range. The patient reported stability of the condition, without the appearance of new lesions, continuing clinical follow-up.

DISCUSSION

The diagnosis and management of lichen sclerosus present challenges, due to clinical similarity with other atrophic dermatoses. LS is a chronic and rare inflammatory condition, clinically characterized by whitish, atrophic lesions, often associated with fissures and scars, impacting the quality of life of patients[1]. The condition is more frequent in menopausal women, but it can manifest at any age group, including men and children, with an estimated prevalence of at least 1 in every 1000 women. Its cause remains unknown, although there are reports of familial cases, still without a clear definition of the genetic risk involved. Furthermore, the disease may be associated with autoimmune disorders, such as thyroid dysfunction and vitiligo.

The case described in this study illustrates the importance of careful differential diagnosis, since the patient was initially diagnosed with parapsoriasis, atrophoderma of Pasini and Pierini, and only after years of persistence of the condition, with lichen sclerosus. The delay in correct diagnosis resulted in the persistence of lesions and therapeutic failure, compromising the patient's quality of life. An early and precise diagnosis would allow the introduction of adequate treatment from the beginning, avoiding the progression of the disease and possible complications, emphasizing the need for a thorough evaluation, considering clinical evolution, complementary exams, and therapeutic response, so that effective interventions are instituted at the opportune moment.

Lichen sclerosus, unlike parapsoriasis, presents a mononuclear infiltrate in the dermis and dermal thickening, while parapsoriasis shows a superficial lymphocytic infiltration[4,5]. The treatment for parapsoriasis involves immunosuppressive therapies, such as systemic corticosteroids and phototherapy[5]. Atrophoderma of Pasini and Pierini is a rare dermatological disease, characterized by plaques of cutaneous atrophy associated with hypopigmentation or hypochromia[6]. It can be confused with lichen sclerosus due to the presence of similar atrophic lesions, but atrophoderma does not present significant chronic inflammation, and lichen sclerosus presents dermal thickening and inflammatory infiltration[4].

The continuous follow-up of the patient was crucial for the diagnosis of lichen sclerosus, especially due to its atypical presentation in extragenital areas. Performing biopsies and regular dermatological follow-up were fundamental for the correct diagnosis. Early diagnosis and appropriate therapeutic approach contribute to improving the patient's quality of life. The gold standard for the treatment of lichen sclerosus, especially the genital form, is the long-term application of potent topical corticosteroids, this being the recommended first-line therapy[4]. However, there is still no consensus on the ideal approach for extragenital lesions[7]. Although various therapeutic options exist, most available evidence is based on isolated case reports and small patient series, limiting the stan-

dardization of therapeutic management[7]. Thus, various alternative therapies have been explored, including calcineurin inhibitors, such as pimecrolimus and tacrolimus, in addition to other therapeutic options, such as colchicine, cyclosporine, methotrexate, mycophenolate mofetil, and phototherapy (narrowband UVB, UVA-psoralen, and UVA-1)[7]. Adjuvant treatment involves the daily application of moisturizers to the affected region[4]. In the reported case, the use of immunomodulators, such as methotrexate, associated with intensive cutaneous hydration, contributed to the stabilization of the clinical condition[8].

CONCLUSION

The case in question highlights the importance of establishing differential diagnoses and persistent investigation when the condition persists, despite adequately instituted treatment. This case report contributes to the medical literature, emphasizing the need for attention to chronic dermatoses, encouraging future research and diligence from physicians when facing a similar case. The importance of an adequate diagnosis for the institution of effective treatment and the patient's well-being is emphasized.

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CASE REPORT *RELATO DE CASO*

SERIOUS HYPERGLYCEMIA AND HYPERTRIGLYCERIDEMIA ASSOCIATED WITH CAPECITABINE USE: A CASE REPORT

HIPERGLICEMIA E HIPERTRIGLICERIDEMIA GRAVES ASSOCIADAS AO USO DE CAPECITABINA: RELATO DE CASO

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ABSTRACT

Capecitabine is an oral prodrug widely used in the treatment of gastrointestinal and breast cancers, it is converted into fluorouracil in the body. Its most common adverse effects include gastrointestinal toxicity, cytopenias, fatigue, mucositis, and hand-foot syndrome. Severe metabolic abnormalities, such as critical hyperglycemia and hypertriglyceridemia, are rare events and have been rarely described in the literature. We report the case of a 61-year-old male patient, previously undiagnosed with diabetes mellitus, who was receiving adjuvant treatment with capecitabine for stage IIC cecal appendix adenocarcinoma, which developed polyuria, polydipsia, xerostomia, loss of appetite, somnolence and progressive weakness during treatment. Laboratory tests revealed an A1C of 11.2%, triglycerides of 1245 mg/dL, and ketonemia of 4.9 mmol/L. Insulin therapy and specific treatment for severe dysglycemia were initiated, resulting in a gradual improvement in metabolic parameters following the completion of cancer treatment. During outpatient follow-up, the patient's glycated hemoglobin and triglyceride levels normalize, and insulin therapy was gradually discontinued. This case underscores the importance of monitoring blood glucose and lipid levels in patients receiving capecitabine in the presence of symptoms suggestive of metabolic decompensation.

Keywords: capecitabine; hyperglyceridemia; drug-induced diabetes mellitus; appendix adenocarcinoma; case report.

RESUMO

A capecitabina é um pró-fármaco oral amplamente utilizado no tratamento de neoplasias gastrointestinais e mamárias, sendo convertida no organismo em fluorouracil. Seus efeitos adversos mais frequentes incluem toxicidade gastrointestinal, citopenias, fadiga, mucosite e síndrome mão-pé. Alterações metabólicas graves, como hiperglicemia intensa e hipertrigliceridemia, são eventos raros e pouco descritos na literatura. Relata-se o caso de um paciente masculino, 61 anos, previamente sem diagnóstico de diabetes mellitus, em tratamento adjuvante com capecitabina para adenocarcinoma de apêndice cecal estádio IIC, que evoluiu durante o tratamento com poliúria, polidipsia, xerostomia, hiporexia, sonolência e fraqueza progressiva. A investigação laboratorial evidenciou hemoglobina glicada de 11,2%, triglicerídeos de 1245 mg/dL e cetonemia de 4,9 mmol/L. Foi instituída insulinoterapia e tratamento específico para disglicemia grave, com posterior melhora progressiva dos parâmetros metabólicos após o término do tratamento oncológico. Em seguimento ambulato-

rial, o paciente apresentou normalização da hemoglobina glicada e dos triglicerídeos, com suspensão gradual da insulinoterapia. O caso reforça a importância da monitorização glicêmica e lipídica em pacientes em uso de capecitabina, especialmente diante de sintomas sugestivos de descompensação metabólica.
Descritores: capecitabina; hiperglicemia; hipertrigliceridemia; diabetes mellitus induzido por drogas; adenocarcinoma de apêndice; relato de caso.

INTRODUCTION

Capecitabine is an orally administered antineoplastic prodrug that is metabolized to fluorouracil (5-FU), an active metabolite belonging to the antimetabolite class. Its antineoplastic activity is primarily mediated through inhibition of thymidylate synthase, impairing DNA synthesis and cell proliferation in rapidly dividing tumor cells. It is widely used in the treatment of various solid tumors, including colorectal, gastric, and breast carcinomas[1].

The adverse effects most frequently associated with capecitabine are gastrointestinal symptoms, such as nausea, vomiting, diarrhea, abdominal pain, and stomatitis, as well as fatigue, loss of appetite, cytopenias, and hand-foot syndrome, also known as palmar-plantar erythrodysesthesia. Conversely, severe metabolic alterations, including hyperglycemia and hypertriglyceridemia, are uncommon and are not typically described among the frequent adverse effects of the medication[1,3].

Despite their low frequency, several reports have demonstrated a possible association between capecitabine therapy and the development of severe hyperglycemia, sometimes accompanied by marked hypertriglyceridemia[2-4]. The underlying pathophysiological mechanisms remain incompletely understood but are thought to involve peripheral insulin resistance, compensatory hyperinsulinemia, oxidative stress, disruption of insulin receptor signaling pathways, and possible pancreatic β-cell dysfunction[1,3].

This article aims to report a case of severe hyperglycemia and hypertriglyceridemia associated with capecitabine therapy in a patient undergoing adjuvant treatment for adenocarcinoma of the cecal appendix, highlighting the clinical course, therapeutic management, and reversibility of the metabolic abnormalities after completion of treatment.

CASE REPORT

A 61-year-old male patient, previously healthy, had an implantable cardioverter-defribilator due to left ventricular hypoplasia. He reported no drug allergies and had a surgical history of bilateral hip arthroplasty and spinal fusion. He was taking bisoprolol 5 mg twice daily.

On February 18, 2025, he sought medical care for abdominal pain in the right hypochondrium, described as stabbing, associated with constipation and reported fever. On admission, he had a heart rate of 72 bpm, blood pressure of 131/90 mmHg, peripheral oxygen saturation of 98% and an axillary temperature of 36 °C.

On abdominal physical examination, the abdomen was flat and flaccid, with no palpable masses tenderness on palpation in the right hypochondrium, tenderness on palpation of both the right and left hypochondrium, a positive Murphy's sign, and a negative Blumberg's sign.

Abdominal ultrasound revealed an enlarged liver with increased echogenicity, consistent with grade III hepatic steatosis and/or non-steatotic liver disease. A heterogeneous hepatic nodule was also identified, suggesting the need for further diagnostic testing.

A contrast-enhanced abdominal CT scan demonstrated increased thickness and width of the cecal appendix, associated with densification of the adjacent fat, raising the possibility of complicated acute appendicitis, a neoplastic process, or an expansive mass, surgical approach was indicated. The patient initially refused treatment and left the hospital, returning the following day.

On February 20, 2025, he underwent exploratory laparotomy with right hemicolectomy and enterostomy. During examination of the abdominal cavity, intense inflammatory blockage was observed between the cecum, liver, and abdominal wall. After lysis of the adhesions, a moderate amount of thick purulent secretion originating from the cecum was identified, with no fecal contamination of the cavity. There was necrosis of the cecal intestinal wall and a gangrenous appendix. The other structures evaluated showed no significant abnormalities.

On the fourth postoperative day, February 24, 2025, resuturing the abdominal wall was necessary due to dehiscence of the surgical wound.

Pathological examination of the surgical specimen revealed a moderately differentiated adenocarcinoma with mucinous areas, centered in the cecal appendix. The proximal, distal, and radial margins were free of neoplasia. Eighteen lymph nodes were analyzed, all of which were negative for metastatic involvement. The pathological staging was defined as pT4bN0M0, corresponding to stage IIC.

Between February and March 2025, the patient experienced multiple episodes of abdominal wall dehiscence. On March 30, 2025, he returned with a new dehiscence in the umbilical and infraumbilical regions. Vacuum-assisted wound care combined with peritoneostomy was proposed, given the recurrence of the condition and the possibility of future closure with full-thickness sutures. Despite detailed counseling regarding the risk and benefits, the patient and family members refused the proposal, opting for another attempt at skin resuturing, aware of the risk of further dehiscence and evisceration.

Oncological evaluation classified the tumor as a high-risk adenocarcinoma due to its T4b staging. A contrast-enhanced chest CT scan showed no evidence of pulmonary or lymph node metastases, identifying only a 1.1 cm air cyst in the right lower lobe and mild nonspecific fibroelastics changes. Thus, there was no evidence of distant metastatic disease.

In April 2025, adjuvant chemotherapy with capecitabine was indicated. However, the start of treatment was postponed due to persistent abdominal dehiscence with intestinal exposure. After adequate healing and a prior cardiological evaluation, treatment began in June 2025.

The first cycle of capecitabine was initiated on June 15, 2025, at a reduced dose compared to the recommended dose, following the institutional protocol of 14 days and a 7-day break.

During follow-up, the patient initially showed good tolerance to treatment. In July 2025, he reported grade 1 fatigue and, subsequently, grade 1 mucositis. A tomography performed during this period demonstrated amorphous nodular changes in the surgical bed, measuring 21 x 15 mm, with no possibility of distinguishing between scar tissue and residual disease.

In the following months, the patient developed grade 1 peripheral neuropathy, grade 1 pruritic skin macules, and progressive hand-foot syndrome, leading to a reduction in the capecitabine dose during the seventh cycle.

In November 2025, during adjuvant therapy, the patient presented with polyuria, polydipsia, xerostomia, loss of appetite, drowsiness, and progressive weakness, leading to hospitalization. Laboratory tests revealed a glycated hemoglobin level of 11.2% triglycerides of 1245 mg/dL, and ketonemia of 4.9 mmol/L. Endocrinological evaluation ruled out diabetic ketoacidosis as the initial manifestation. A diagnosis of newly diagnosed-diabetes mellitus was established, and insulin therapy was initiated on November 8. 2025.

The variability in capillary blood glucose levels during hospitalization and the insulin regimens used are described in **Table 1.**

He was discharged from the hospital with an updated regimen of NPH insulin 18-16-18 UI and regular insulin 14-14-0 UI.

During hospitalization, the patient also presented with thrombocytopenia, suspected to be heparin-induced, leading to the discontinuation of the anticoagulant. Ciprofibrate 100 mg and simvastatin 20 mg were initiated to manage severe dyslipidemia.

After clinical stabilization, capecitabine was resumed under oncological supervision on November 13, 2025. The treatment plan called for 12 cycles of therapy. On April 17, 2026, it became necessary to

Table 1. Capillary blood glucose levels (mg/dL) and insulin therapy during hospitalization**.**

Date	05:00	10:00	13:00	16:00	21:00	Insulin regimen
7 Nov 2025	—	—	—	—	328	Regular insulin 4 IU SC
8 Nov 2025	225	304	321	281	281	NPH insulin 10-10-10 IU + Regular insulin 4 IU SC
9 Nov 2025	256	290	325	333	246	NPH insulin 14-14-14 IU + Regular insulin 8-8-4 IU SC
10 Nov 2025	129	200	270	290	—	NPH insulin 18-16-18 IU + Regular insulin 14-14-0 IU SC

Abbreviations: SC, subcutaneous; IU, international units. NPH insulin : three doses (coffee, lunch and bed time) Regular insulin three doses (coffee, lunch and dinner).

reduce the insulin doses. Subsequently completion of cancer treatment, insulin therapy was gradually reduced, with complete discontinuation approximately 90 days after the chemotherapy was stopped.

During outpatient follow-up a progressive improvement in metabolic parameters was observed. In April 2026, the patient had an A1C of 5.7%, triglycerides of 98 mg/dL, preserved renal function, and no significant albuminuria. He also developed an incisional hernia, which was identified in March 2026.

Carcinoembryonic antigen levels remained low throughout follow-up, ranging from 1.72 to 5.43 ng/mL. At the last evaluation, the patient was clinically stable, with no documented evidence of recurrence or metastatic disease.

DISCUSSION

Capecitabine is an antimetabolite widely used in the treatment of colorectal and breast carcinomas; it acts as a prodrug that is converted into fluorouracil (5-FU) within the body[5]. Although common side effects—such as hand-foot syndrome and gastrointestinal symptoms—are well documented, the induction of severe hyperglycemia and hypertriglyceridemia is considered a rare event that is seldom described in the scientific literature[5].

In the reported case, a 61-year-old patient developed polyuria, polydipsia, and xerostomia during the seventh cycle of adjuvant treatment for appendiceal adenocarcinoma. Laboratory investigation revealed a glycated hemoglobin (HbA1c) level of 11.2% and triglyceride levels of 1245 mg/dL, indicating an acute and severe metabolic imbalance. Interestingly, the onset of these symptoms coincided with the manifestation of progressive hand-foot syndrome—an observation also noted in other case reports in the literature, where severe metabolic toxicity accompanied grade 3 reactions[6].

The exact mechanism by which capecitabine induces such alterations remains unclear, but the literature suggests it may involve peripheral insulin resistance and interference with insulin receptor signaling pathways, possibly mediated by oxidative stress. Furthermore, studies indicate that 5-FU can cause structural damage to the islets and impair insulin secretion[3,6]. In the case reported by Yang et al., a glucose tolerance test showed a delayed C-peptide release pattern, indicating impaired beta-cell function[6].

The severe hypertriglyceridemia observed in the patient (1245 mg/dL) is frequently associated with concomitant hyperglycemia in these rare cases. This is believed to result from the reduction in lipoprotein lipase (LPL) and hepatic triglyceride lipase activity caused by capecitabine[3,5]. Reduced insulin activity exacerbates this accumulation, as LPL relies on insulin for full activity. Similar cases in the literature, such as the one reported by Garg et al. (2009), have shown that both hyperglycemia and hypertriglyceridemia can be reversed by discontinuing capecitabine and administering specific treatment[6].

The literature emphasizes that capecitabine-induced hyperglycemia should not be classified as classic type 1 or type 2 diabetes, but rather as a direct drug toxicity, given that islet-related antibodies are typically negative and the condition is reversible[6]. In the case at hand, reversibility was confirmed: following the discontinuation of capecitabine and temporary insulin management, the patient achieved complete normalization of parameters months after the end of cancer treatment (HbA1c of 5.7% and triglycerides of 98 mg/dL).

CONCLUSION

Severe hyperglycemia associated with hypertriglyceridemia is a rare but potentially significant complication in patients receiving capecitabine. The reported case demonstrates a clear temporal relationship between chemotherapy and the development of significant metabolic decompensation, with subsequent reversibility following clinical management and completion of cancer therapy.

Given this risk, periodic monitoring of glucose and lipid levels is recommended during treatment with capecitabine, especially in patients with symptoms suggestive of hyperglycemia or concomitant manifestations of drug toxicity, including hand-foot syndrome. Early recognition of this complication allows for immediate intervention, reducing the risk of acute metabolic complications and contributing to greater safety in cancer treatment.

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CASE REPORT AND LITERATURE REVIEW *RELATO DE CASO E REVISÃO DA LITERATURA*

BETWEEN OVARIES AND ADRENALS: NONCLASSIC CONGENITAL ADRENAL HYPERPLASIA MIMICKING METABOLIC OVARIAN SYNDROME

ENTRE OVÁRIOS E ADRENAIS: HIPERPLASIA CONGÊNITA ADRENAL, NÃO CLÁSSICA, MIMETIZANDO SÍNDROME METABÓLICA OVARIANA

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ABSTRACT

Nonclassic congenital adrenal hyperplasia (NCCAH) is a mild form of 21-hydroxylase deficiency caused by pathogenic variants in the *CYP21A2* gene. Its clinical presentation often overlaps with metabolic ovarian syndrome, particularly in adolescent girls and young women with hyperandrogenism, menstrual irregularity, acne, and hirsutism. This report describes a 16-year-old female referred for endocrinological evaluation due to progressive hirsutism and oligomenorrhea since menarche. Clinical examination revealed severe hirsutism, with a Ferriman-Gallwey score of 19. Laboratory evaluation showed biochemical hyperandrogenism, including elevated total testosterone, androstenedione, and markedly increased basal 17-hydroxyprogesterone levels. Pelvic ultrasonography showed no ovarian morphological abnormalities, and adrenal imaging was unremarkable. Although ACTH stimulation testing and *CYP21A2* molecular analysis were unavailable in the public health setting, the diagnosis of NCCAH was supported by the integration of clinical and hormonal findings after exclusion of other causes of hyperandrogenism. Treatment with prednisone, spironolactone, and a combined oral contraceptive resulted in progressive improvement of hirsutism, menstrual cycle regularization, and reduction of hyperandrogenic markers. This case, accompanied by a narrative literature review, highlights the importance of considering NCCAH in the differential diagnosis of adolescents and women presenting with suspected metabolic ovarian syndrome. Early recognition is essential to guide appropriate treatment, improve clinical symptoms, and reduce potential reproductive, metabolic, and psychosocial consequences.

Keywords: Non-classic congenital adrenal hyperplasia (NCCAH), 21-hydroxylase deficiency (21OHD); Polycystic Ovary Syndrome; Hirsutism; Menstruation Disturbances.

RESUMO

A hiperplasia adrenal congênita não clássica (HAC-NC) é uma forma branda da deficiência da 21-hidroxilase causada por variantes patogênicas no gene *CYP21A2*. Sua apresentação clínica frequentemente se sobrepõe à síndrome ovariana metabólica poliendócrina (SOMP), especialmente em adolescentes e mulheres jovens com hiperandrogenismo, irregularidade menstrual, acne e hirsutismo. Relata-se o caso de uma adolescente de 16 anos enca-

minhada para avaliação endocrinológica devido a hirsutismo progressivo e oligomenorreia desde a menarca. Ao exame físico, apresentava hirsutismo importante, com escore de Ferriman-Gallwey de 19 pontos. A investigação laboratorial evidenciou hiperandrogenismo bioquímico, incluindo elevação da testosterona total, androstenediona e níveis marcadamente aumentados de 17-hidroxiprogesterona basal. A ultrassonografia pélvica não demonstrou alterações morfológicas ovarianas, e a avaliação por imagem das adrenais não evidenciou anormalidades estruturais. Embora o teste de estímulo com ACTH e a análise molecular do gene *CYP21A2* não estivessem disponíveis na rede pública de saúde, o diagnóstico de HAC-NC foi sustentado pela integração dos achados clínicos e hormonais, após exclusão de outras causas de hiperandrogenismo. O tratamento com prednisona, espironolactona e contraceptivo oral combinado resultou em melhora progressiva do hirsutismo, regularização dos ciclos menstruais e redução dos marcadores de hiperandrogenismo. Este caso, acompanhado de uma revisão narrativa da literatura, destaca a importância de considerar a HAC-NC no diagnóstico diferencial de adolescentes e mulheres com suspeita de SOMP. O reconhecimento precoce da doença é fundamental para orientar o tratamento adequado, melhorar os sintomas clínicos e reduzir potenciais repercussões reprodutivas, metabólicas e psicossociais.

Descritores: Hiperplasia adrenal congênita não clássica; Deficiência de 21-hidroxilase; Hiperandrogenismo; Hirsutismo; Adolescente; Síndrome ovariana metabólica poliendócrina.

INTRODUCTION

Congenital adrenal hyperplasia (CAH) comprises a group of autosomal recessive disorders caused by enzymatic defects in adrenal steroidogenesis. Approximately 90–95% of cases result from 21-hydroxylase deficiency, an enzyme encoded by the CYP21A2 gene. Reduced cortisol synthesis leads to loss of negative feedback on the hypothalamic–pituitary–adrenal axis, resulting in compensatory elevation of adrenocorticotropic hormone (ACTH), adrenal hyperplasia, and excessive androgen production[1,2.].

The clinical severity of the disease is directly related to residual enzyme activity, allowing classification into classic and nonclassic forms. Classic CAH represents the most severe spectrum of 21-hydroxylase deficiency and is subdivided into salt-wasting and simple virilizing forms. The salt-wasting form accounts for approximately 75% of classic cases and is characterized by severe cortisol and aldosterone deficiency, potentially leading to life-threatening neonatal adrenal crisis[1,3]. The simple virilizing form preserves partial mineralocorticoid synthesis without significant salt loss but presents with marked hyperandrogenism beginning in the prenatal period[3].

Nonclassic congenital adrenal hyperplasia (NCCAH) represents the mildest phenotype of 21-hydroxylase deficiency. In these patients, residual enzyme activity generally ranges from 20% to 70% of normal, allowing sufficient cortisol and aldosterone production to prevent overt adrenal insufficiency[1,2,3]. Consequently, symptoms develop later in life, usually after childhood, and are predominantly related to excess adrenal androgen production.

Clinical manifestations include premature pubarche, acne, hirsutism, menstrual irregularities, infertility, and occasionally androgenetic alopecia. During recent decades, recognition of NCCAH has increased substantially due to wider availability of laboratory methods and genetic testing[3,4]. Nevertheless, the condition remains frequently underdiagnosed, particularly in men, who often remain asymptomatic, and in women who are mistakenly classified as having Polyendocrine Metabolic Ovarian Syndrome (PMOS), the recently proposed nomenclature replacing the term polycystic ovary syndrome[5]. Given the substantial clinical overlap between NCCAH and PMOS, early recognition remains a diagnostic challenge, particularly in healthcare settings with limited access to specialized testing. In this context, we report the case of an adolescent presenting with severe hirsutism and menstrual irregularity whose diagnosis of NCCAH was established through integration of clinical and laboratory findings, followed by a narrative review of the literature.

CASE REPORT

A 16-year-old female was referred for endocrinological evaluation in 2019 due to menstrual irregularity and progressive hirsutism. Menarche occurred at 14 years of age, and since then she had experienced only three spontaneous menstrual cycles. She reported excessive hair growth since childhood, predominantly affecting the face, chest, and limbs. She denied childhood hospitalizations, symptoms suggestive of adrenal insufficiency, hypertension, diabetes mellitus, or thyroid disease. Her medical history was notable for a previous suicide attempt, and she was taking desvenlafaxine 100 mg daily. Pubertal development was compatible with Tanner stage P4M3. Physical examination revealed marked hirsutism involving the malar region, upper lip, sideburns, areolar areas, intermammary region, linea alba, infraumbilical region, arms, forearms, and back. The Ferriman-Gallwey score was 19. Given the presence of clinical hyperandrogenism associated with menstrual irregularity, the initial diagnostic hypotheses included PMOS and nonclassic congenital adrenal hyperplasia.

Laboratory evaluation demonstrated biochemical hyperandrogenism, with elevated total testosterone, androstenedione, and markedly increased basal 17-hydroxyprogesterone levels. Thyroid function, prolactin, electrolyte levels, renal function, and adrenal mineralocorticoid parameters were within normal limits. Lipid profile evaluation revealed dyslipidemia, with elevated total cholesterol and LDL cholesterol levels.

Pelvic ultrasonography demonstrated a normal uterus and ovaries without morphological abnormalities. Considering the high suspicion of NCCAH, treatment was initiated with prednisone 5 mg/day,

Table 1. Laboratory findings before and after treatment.

Laboratory Test	Before Treatment	After Treatment	Reference Range
Androstenedione	8,0 ng/mL	1,5 ng/mL	0,3–3,0
FSH	7,48 mUI/mL	-	—
LH	5,78 mUI/mL	-	—
Estradiol	48 pg/mL	-	—
Total Testosterone	122 ng/dL	17 ng/dL	10–75
DHEA-S	163 µg/dL	-	4–171
17-hydroxyprogesterone	1.068 ng/dL (32,4 nmol/L)	371 ng/dL (11,2 nmol/L)	—
TSH	0,72 µUI/mL	0,69 µUI/mL	0,4–4,5
Free T4	0,96 ng/dL	-	0,8–1,8
Basal cortisol	22 µg/dL	-	—
ACTH	61 pg/mL	-	—
Prolactin	12 ng/mL	-	—
Sodium	141 mEq/L	-	135–145
Potassium	4,3 mEq/L	-	3,5–5,0
Plasma Renin (orthostatic)	3,5 ng/mL/h	-	0,60–4,18
Aldosterone	14 ng/dL	-	—
Fasting Glucose	97 mg/dL	-	<100
Total Cholesterol	249 mg/dL	-	<200
HDL-cholesterol	46 mg/dL	-	>40
LDL-cholesterol	173 mg/dL	-	<130
Triglycerides	144 mg/dL	-	<150
Creatinine	0,9 mg/dL	-	0,6–1,2

spironolactone 50 mg/day, and a combined oral contraceptive. Following treatment initiation, the patient experienced progressive improvement of hyperandrogenic symptoms, including reduction of hirsutism and normalization of menstrual cycles. Hormonal markers associated with hyperandrogenism also decreased substantially compared with baseline values, supporting a favorable response to therapy. The patient remains under regular endocrinological follow-up.

Epidemiology

NCCAH is significantly more common than the classic form of the disease. Its prevalence varies widely among ethnic groups, reflecting differences in the distribution of CYP21A2 mutations. The highest prevalence has been reported among Ashkenazi Jews, reaching approximately 3.7% of the population. In non-Jewish Caucasians, estimated prevalence ranges from 0.1% to 0.3%. Intermediate frequencies have been described in Mediterranean, Hispanic, and Middle Eastern populations[3,6].

Among women evaluated for hyperandrogenism, the estimated global prevalence is approximately 4.2%. Conventional neonatal screening has low sensitivity for NCCAH because 17-hydroxyprogesterone levels often remain below the cutoff values used to identify the classic form. Consequently, many patients are diagnosed only during adolescence or adulthood[6].

Genetics and Pathophysiology

NCCAH results from mutations in the CYP21A2 gene, located on chromosome 6p21.3. This gene lies adjacent to a highly homologous pseudogene (CYP21A1P), favoring recombination and gene-conversion events responsible for most disease-associated mutations. 21-Hydroxylase deficiency impairs the conversion of 17-hydroxyprogesterone into 11-deoxycortisol, reducing cortisol synthesis[1,2,3]. As a consequence, partial loss of negative feedback on the hypothalamic–pituitary–adrenal axis occurs, leading to compensatory ACTH hypersecretion[6,7].

Chronic stimulation of the adrenal cortex results in bilateral adrenal hyperplasia and increased production of steroid precursors that are diverted toward androgen synthesis. Unlike patients with classic CAH, individuals with NCCAH retain approximately 20–70%

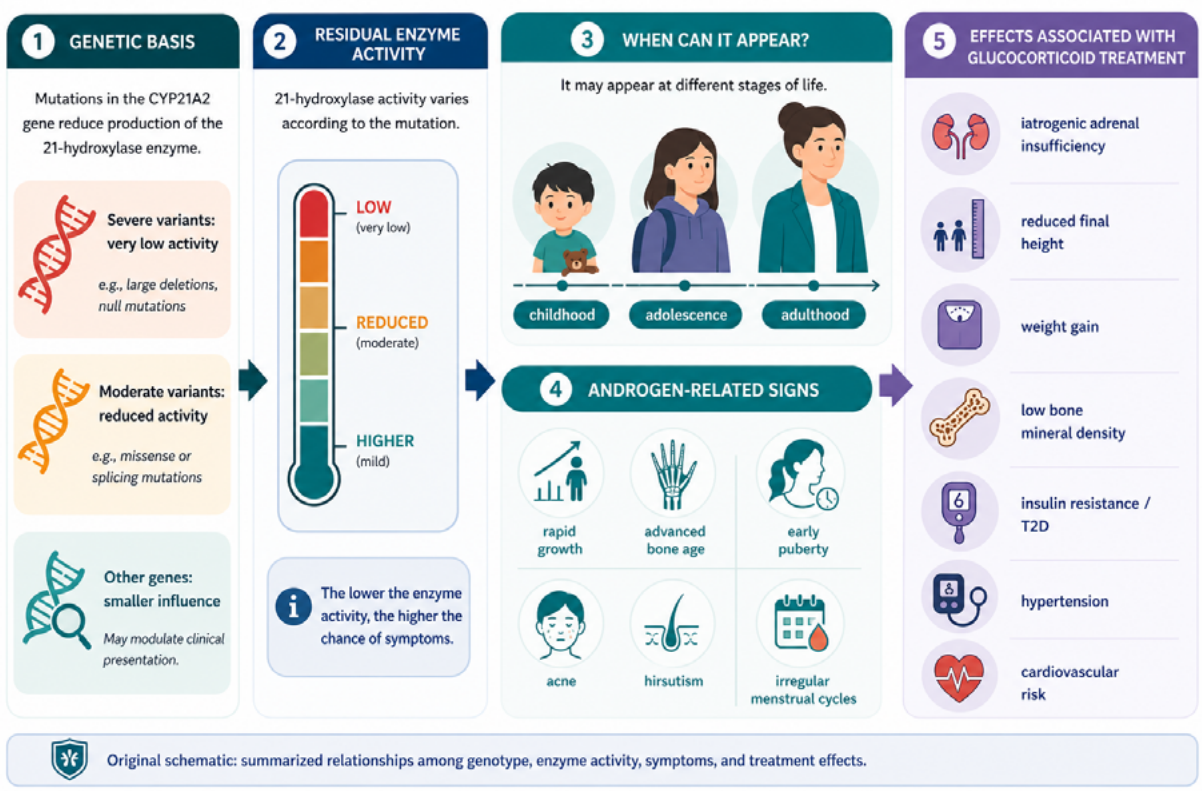

Figure 1. Schematic overview of the genetic basis, clinical manifestations, and treatment-related complications of nonclassic congenital adrenal hyperplasia (NCCAH). The figure was created by the authors with the assistance of artificial intelligence.

(or up to 80%) residual enzyme activity, which is sufficient to maintain adequate cortisol and aldosterone production. The p.Val281Leu (V281L) mutation is the genetic variant most commonly associated with the nonclassic phenotype[1,3,8].

Clinical Manifestations

The clinical presentation of NCCAH is highly variable and depends on residual enzyme activity, age at diagnosis, and patient sex[2,3]. Manifestations are primarily related to excess adrenal androgen production and may appear from childhood through adulthood. In childhood, initial signs commonly include premature pubarche, acne, axillary odor, accelerated growth velocity, and advanced bone age[2,3,4]. During adolescence, manifestations of hyperandrogenism become more evident, particularly in girls, including severe acne, hirsutism, menstrual irregularities, and occasionally androgenetic alopecia[2,3,4,7].

In adult women, hirsutism is the most frequent manifestation, affecting approximately 60–80% of patients. Menstrual disturbances, including oligomenorrhea and amenorrhea, are observed in more than half of affected women[1,5,6,7]. Ultrasound findings compatible with polycystic ovarian morphology may also be present[1,5]. The patient described in this report exemplifies a typical presentation of NCCAH in adolescent females. The combination of marked hirsutism since childhood, oligomenorrhea since menarche, and significant biochemical hyperandrogenism reproduces the manifestations most frequently reported in the literature.

Fertility and Pregnancy

Fertility in NCCAH is generally preserved, although reproductive abnormalities are more common in women compared with the general population. Excess adrenal androgens and progesterone may interfere with several stages of reproductive function, contributing to ovulatory dysfunction, subfertility, and an increased risk of early pregnancy loss[2,7,8]. Most women are able to conceive spontaneously, particularly when appropriately treated. In men, fertility is usually preserved, and testicular adrenal rest tumors are uncommon in this form of the disease[7,8].

Diagnosis

The diagnosis of NCCAH should be considered in individuals presenting with signs of hyperandrogenism, premature pubarche, menstrual irregularities, infertility, or a clinical picture resembling Polyendocrine Metabolic Ovarian Syndrome (PMOS). Because neonatal screening has low sensitivity for this condition, most cases are diagnosed only during late childhood, adolescence, or adulthood[1,2,4,5].

Initial laboratory evaluation is based on the measurement of morning 17-hydroxyprogesterone (17-OHP), preferably collected between 7:00 and 9:00 a.m. In menstruating women, sampling should be performed during the early follicular phase to avoid falsely elevated results. Basal values above 200 ng/mL (approximately 6 nmol/L) strongly support suspicion of NCCAH[1-4,8].

In equivocal cases, the ACTH stimulation test (250 µg cosyntropin administered intravenously or intramuscularly) remains the diagnostic gold standard. Serum 17-OHP is measured before and 60 minutes after ACTH administration[1,2,4]. Stimulated concentrations above 10 ng/mL (30 nmol/L) or 1,000 ng/dL are considered compatible with NCCAH, although specific cutoffs may vary according to the laboratory method used[1,2,4]. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) provides greater diagnostic accuracy than conventional immunoassays and reduces false-positive results. More recently, 21-deoxycortisol has been proposed as a complementary biomarker, demonstrating high sensitivity and specificity for identifying 21-hydroxylase deficiency[2,4,8].

Assessment of cortisol response to ACTH may also provide important information, as some patients exhibit a subnormal cortisol response, particularly those carrying more severe genetic variants. Although spontaneous adrenal crises are extremely rare in NCCAH, this information may assist therapeutic planning and guide recommendations regarding glucocorticoid use during periods of physiological stress[1,2,5,6]. Following biochemical confirmation, molecular analysis of the CYP21A2 gene is recommended. Genetic testing allows diagnostic confirmation, genotype–phenotype correlation, and appropriate genetic counseling, particularly for individuals planning pregnancy because of the risk of transmitting classic forms of the disease to offspring[2,4,6].

Although ACTH stimulation testing remains the diagnostic gold standard, it could not be performed in this case because it was unavailable within the public healthcare system. Likewise, CYP21A2 molecular analysis was not accessible through the Brazilian Unified Health System (SUS). Therefore, diagnosis was established through integration of clinical and laboratory

findings after exclusion of the major differential diagnoses of hyperandrogenism.

Differential Diagnosis

The principal differential diagnosis of NCCAH is Polyendocrine Metabolic Ovarian Syndrome (PMOS), since both conditions share similar clinical and laboratory manifestations, including hirsutism, acne, menstrual irregularities, anovulation, and hyperandrogenemia. Furthermore, ultrasonographic findings compatible with polycystic ovarian morphology may be present in both disorders[4,5,7]. Differentiation between PMOS and NCCAH relies mainly on hormonal evaluation. Although patients with PMOS may present mild elevations of basal 17-OHP, ACTH stimulation testing generally does not demonstrate significant increases in 17-OHP, allowing exclusion of 21-hydroxylase deficiency[1,4,7]. For this reason, screening for NCCAH is recommended in all women with hyperandrogenism before confirming a diagnosis of PMOS[4,5].

Other causes of hyperandrogenism should also be considered. Androgen-secreting adrenal or ovarian tumors typically present with abrupt symptom onset, rapid progression, and markedly elevated testosterone or dehydroepiandrosterone sulfate (DHEAS) concentrations[4,8]. Ovarian hyperthecosis usually occurs in peri- or postmenopausal women and is characterized by more severe hyperandrogenism[1,2,4].

Cushing syndrome should be suspected in the presence of central obesity, proximal muscle weakness, spontaneous bruising, hypertension, and diabetes mellitus. Other less common conditions include hyperprolactinemia, acromegaly, severe insulin resistance, and exogenous use of androgens or anabolic steroids[4,8]. Correct differentiation among these entities is essential because treatment and prognosis vary substantially according to the underlying cause of hyperandrogenism[1,2,4].

The relevance of this differential diagnosis is exemplified by the present case. Initially, the association between severe hirsutism and menstrual irregularity could have suggested PMOS. However, the markedly elevated basal 17-hydroxyprogesterone level directed the investigation toward NCCAH, emphasizing the importance of hormonal evaluation in distinguishing among the various causes of hyperandrogenism.

Treatment

The management of nonclassic congenital adrenal hyperplasia (NCCAH) should be individualized and tailored to the patient's clinical manifestations and treatment goals. Unlike the classic form, in which hormone replacement is essential for survival, most individuals with NCCAH do not require continuous therapy. Treatment is primarily indicated for the control of hyperandrogenism, preservation of fertility, and improvement of quality of life[1,7,8].

When to Treat?

Treatment is recommended for symptomatic patients, including those with progressive premature pubarche, advanced bone age, accelerated growth, hirsutism, severe acne, menstrual irregularities, infertility, or significant impairment of quality of life. Conversely, asymptomatic individuals generally do not require pharmacological therapy. The Endocrine Society guidelines do not recommend routine glucocorticoid treatment for asymptomatic patients, as the potential risks may outweigh the benefits[1,7].

Glucocorticoids

Glucocorticoids reduce ACTH secretion and consequently decrease adrenal androgen production. In children and adolescents with progressive signs of hyperandrogenism, hydrocortisone is considered the treatment of choice and is administered at physiological doses of approximately 10–15 mg/m²/day divided into two or three doses[1,2,7]. In adults, glucocorticoid therapy should be carefully evaluated. Although effective in reducing levels of 17-hydroxyprogesterone, androstenedione, and testosterone, these agents may cause significant adverse effects, including weight gain, insulin resistance, hypertension, decreased bone mineral density, and suppression of the hypothalamic–pituitary–adrenal axis. The therapeutic goal is not complete normalization of 17-hydroxyprogesterone levels, since excessive suppression generally indicates overtreatment. The lowest effective dose capable of controlling clinical symptoms should be use[1,2,8.]

Combined Hormonal Contraceptives

In women without immediate reproductive plans, combined hormonal contraceptives are frequently considered first-line therapy. These medications reduce ovarian androgen production, increase sex hormone-binding globulin (SHBG) levels, and decrease circulating free testosterone. In addition to controlling hyperandrogenism, contraceptives help regulate menstrual cycles and prevent endometrial hyperplasia associated with chronic anovulation[7,8].

Antiandrogens

Antiandrogens may be used alone or in combination with hormonal contraceptives in patients with

moderate to severe hirsutism. Spironolactone is the most commonly used option, acting through competitive blockade of the androgen receptor and reduction of 5α-reductase activity. Other options include cyproterone acetate, flutamide, and finasteride. The use of these medications requires effective contraception because of the potential risk of fetal feminization if pregnancy occurs. Comparative studies suggest that antiandrogen therapy may be as effective as, or even superior to, glucocorticoids in controlling the cutaneous manifestations of hyperandrogenism[1,7,8].

Infertility Treatment

In women seeking pregnancy, hydrocortisone is generally considered the first therapeutic option. Reduction of adrenal androgen and progesterone production promotes restoration of ovulation and improves endometrial receptivity. When hormonal normalization does not result in spontaneous conception, the same treatment protocols used for anovulatory infertility may be employed, including letrozole, clomiphene citrate, gonadotropins, or assisted reproductive technologies. Observational studies suggest that glucocorticoid treatment before conception may reduce the time required to achieve pregnancy, although obstetric outcomes appear similar to those observed in untreated women[3,7,8].

Emergency Therapies

In recent years, new therapeutic strategies have been developed with the aim of reducing glucocorticoid exposure and improving hormonal control. Among these approaches are corticotropin-releasing factor receptor type 1 (CRF1) antagonists, such as crinecerfont and tildacerfont, which reduce ACTH secretion and consequently decrease adrenal androgen production. Another promising strategy involves melanocortin type 2 receptor (MC2R) antagonists and therapies targeting adrenal steroidogenesis. Although most studies have been conducted in patients with classic CAH, these treatments may eventually expand therapeutic options for individuals with NCCAH as well[3,9].

Complications and Comorbidities

Although NCCAH is considered a mild form of 21-hydroxylase deficiency, several complications may occur throughout life. These complications result both from chronic exposure to excess androgens and from the potential adverse effects of glucocorticoid treatment. However, much of the available evidence remains conflicting because of the clinical heterogeneity of the disease and the limited number of long-term studies[3,8].

Adrenal Insufficiency

Unlike the classic form, clinically overt adrenal insufficiency is rare in NCCAH. Most patients maintain adequate basal cortisol production and do not develop spontaneous adrenal crises. Nevertheless, studies have demonstrated that up to one-third of patients may exhibit a subnormal cortisol response to ACTH stimulation testing, particularly those carrying more severe genetic variants. Despite this laboratory finding, adrenal crisis in untreated NCCAH is exceptionally rare. The greatest risk is associated with iatrogenic suppression of the hypothalamic–pituitary–adrenal axis after prolonged glucocorticoid use, in which case patients require counseling regarding stress-dose glucocorticoid supplementation during periods of physiological stress[4,8].

Adrenal Hyperplasia and Adrenal Tumors

Chronic ACTH stimulation may promote bilateral adrenal hyperplasia and the development of adrenal nodules. Studies suggest a higher prevalence of adrenal adenomas in individuals with NCCAH compared with the general population. In some cases, the discovery of an adrenal incidentaloma may represent the first manifestation of the disease. Although most lesions are benign, their identification requires careful hormonal evaluation to exclude autonomous hormone secretion and other adrenocortical neoplasms. Isolated cases of adrenocortical carcinoma associated with NCCAH have been reported, although they remain exceedingly rare[1,3,8].

Metabolic and Cardiovascular Complications

In recent decades, increasing attention has been directed toward the metabolic impact of NCCAH. Observational studies have demonstrated a higher prevalence of obesity, insulin resistance, dyslipidemia, and abnormalities in glucose metabolism compared with healthy individuals. However, the available evidence remains inconsistent, and not all studies have confirmed an increased cardiometabolic risk. Chronic hyperandrogenism appears to play an important role in this context, potentially contributing to alterations in body composition and reduced insulin sensitivity. Conversely, prolonged exposure to supraphysiological doses of glucocorticoids may promote weight gain, hypertension, glucose intolerance, and type 2 diabetes mellitus. Although some studies have suggested an increased cardiovascular risk, particularly among women, more recent evidence has not demonstrated a significant increase in structural or functional markers of cardiovascular disease[3,4,8]. Therefore, periodic moni-

toring of cardiometabolic risk factors is recommended, similarly to what is advised for patients with polycystic ovary syndrome.

Bone Health

Bone health in NCCAH differs from that observed in the classic form of the disease. Most studies have reported normal or even slightly increased bone mineral density, possibly due to prolonged exposure to adrenal androgens[7,8]. However, chronic glucocorticoid therapy may exert deleterious effects on the skeleton by reducing bone formation and increasing the risk of osteopenia and osteoporosis. Cohort studies involving patients with congenital adrenal hyperplasia suggest an association between higher cumulative glucocorticoid doses and lower bone mineral density, particularly among women. The impact of NCCAH on fracture risk remains controversial. While some studies have found no increase in fracture incidence, others have reported a higher frequency of fractures compared with the general population[3,7,8]. Long-term prospective studies are needed to clarify this relationship.

Mental Health and Psychosocial Outcomes

Hyperandrogenism may significantly impair quality of life, particularly in women. Hirsutism, acne, androgenetic alopecia, and infertility frequently affect self-esteem, body image, and interpersonal relationships[4,8,7]. In addition to physical manifestations, patients with NCCAH may experience increased rates of anxiety, depressive symptoms, and psychosocial distress. Although available data are limited, studies suggest that women with NCCAH experience quality-of-life impairment comparable to that observed in patients with polycystic ovary syndrome. Therefore, patient assessment should encompass not only hormonal and metabolic aspects but also psychological and psychosocial factors, allowing a multidisciplinary and individualized approach[7,8,9]. Taken together, the potential complications of NCCAH reinforce the need for long-term follow-up, even in individuals with apparently mild clinical manifestations. Regular monitoring facilitates early identification of metabolic, reproductive, and psychosocial abnormalities, contributing to improved prognosis and quality of life.

DISCUSSION

The present case illustrates a typical presentation of nonclassic congenital adrenal hyperplasia (NCCAH) in an adolescent female, characterized by marked hirsutism, menstrual irregularity, and biochemical hyperandrogenism. Although NCCAH is one of the most common causes of hyperandrogenism after polyendocrine metabolic ovarian syndrome (PMOS), its diagnosis remains challenging because of the substantial clinical overlap between these conditions. The patient exhibited classic manifestations of the disease, including oligomenorrhea since menarche, significant hirsutism, and markedly elevated basal 17-hydroxyprogesterone levels. A Ferriman–Gallwey score of 19 is consistent with moderate-to-severe hirsutism, corroborating the intensity of clinical hyperandrogenism and justifying further etiological investigation. Although definitive diagnosis ideally includes ACTH stimulation testing and molecular analysis of the CYP21A2 gene, neither examination was available during the diagnostic workup. This situation reflects a reality frequently encountered in public healthcare centers in Brazil, where integration of clinical history, physical examination, and hormonal evaluation remains essential for establishing the diagnosis. The basal 17-hydroxyprogesterone concentration exceeding 1,000 ng/dL, measured during the follicular phase of the menstrual cycle, substantially increased the likelihood of NCCAH, even in the absence of confirmatory ACTH stimulation testing. Furthermore, exclusion of other causes of hyperandrogenism and the favorable clinical response to treatment strengthened the diagnostic consistency. Although ACTH stimulation testing is considered the diagnostic gold standard, its unavailability in many centers limits its routine use in clinical practice. In low- and middle-income countries, diagnosis often relies on the combination of a suggestive clinical presentation and markedly elevated basal 17-hydroxyprogesterone levels, underscoring the importance of clinical reasoning for early disease recognition.

Another relevant aspect was the absence of ovarian abnormalities on ultrasonography. This finding does not exclude either NCCAH or PMOS, since a substantial proportion of adolescents with hyperandrogenism may present with morphologically normal ovaries. Consequently, laboratory evaluation remains essential for differential diagnosis. The clinical overlap between NCCAH and PMOS represents one of the major diagnostic challenges in reproductive endocrinology. Studies have shown that between 1% and 10% of women initially evaluated for hyperandrogenism may ultimately be diagnosed with NCCAH, supporting international recommendations that 17-hydroxyprogesterone measurement should be included in the initial evaluation of women presenting with hirsutism, menstrual irregularities, or infertility[8,10].

This challenge is particularly relevant during adolescence, a period in which menstrual irregularity, acne, and anovulatory cycles may represent physiological features of pubertal maturation. In this setting, progressive hirsutism and significantly elevated 17-hydroxyprogesterone levels become key findings in the etiological investigation. Another important consideration is the need for etiological investigation of hyperandrogenism before initiating hormonal therapy. Combined hormonal contraceptives reduce circulating androgen levels and may alter the adrenal steroid profile, potentially complicating the interpretation of laboratory tests used in the diagnosis of NCCAH. Therefore, whenever possible, diagnostic evaluation should precede hormonal treatment. In patients already receiving hormonal contraceptives, laboratory results should be interpreted cautiously, and in selected cases temporary discontinuation of medication may be necessary to allow adequate hormonal reassessment. Another noteworthy finding in this case was the presence of dyslipidemia at initial evaluation. Although the association between NCCAH and increased cardiometabolic risk remains a matter of debate, recent evidence suggests a higher prevalence of insulin resistance, lipid abnormalities, and other metabolic risk factors among patients chronically exposed to hyperandrogenism, highlighting the need for long-term follow-up.

Finally, the clinical and biochemical response observed after treatment initiation supports the appropriateness of the therapeutic strategy adopted. Improvement in menstrual regularity, reduction in hirsutism, and decreases in biochemical markers of hyperandrogenism were all consistent with outcomes reported in the literature among patients diagnosed and treated early[10,11]. The favorable response following initiation of glucocorticoid therapy combined with spironolactone and a combined oral contraceptive provided additional evidence that hyperandrogenism was predominantly adrenal in origin. Thus, this case highlights the importance of maintaining clinical suspicion for NCCAH in adolescents presenting with hyperandrogenism, particularly in settings where confirmatory testing is not readily available.

CONCLUSION

Nonclassic congenital adrenal hyperplasia is an important cause of hyperandrogenism in adolescents and young women and frequently mimics Polyendocrine Metabolic Ovarian Syndrome. The present case highlights the importance of systematically investigating patients with hirsutism and menstrual irregularities, particularly through measurement of 17-hydroxyprogesterone levels. The report also illustrates challenges commonly encountered in Brazilian clinical practice, where the unavailability of ACTH stimulation testing and CYP21A2 genotyping may require diagnosis to rely primarily on clinicopathological correlation. Early recognition of NCCAH enables appropriate treatment, improves clinical symptoms, and reduces potential long-term reproductive, metabolic, and psychosocial consequences.

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SCOPING REVIEW *REVISÃO DE ESCOPO*

MOLECULAR CONVERGENCE BETWEEN HASHIMOTO'S THYROIDITIS AND THYROID MALT LYMPHOMA: A SCOPING REVIEW OF SHARED LYMPHOMAGENIC MECHANISMS AND PRE-NEOPLASTIC POTENTIAL

CONVERGÊNCIA MOLECULAR ENTRE A TIREOIDITE DE HASHIMOTO E O LINFOMA MALT DA TIREOIDE: UMA REVISÃO DE ESCOPO DOS MECANISMOS LINFOMATOGÊNICOS COMPARTILHADOS E DO POTENCIAL PRÉ-NEOPLÁSICO

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ABSTRACT

Introduction: Hashimoto's Thyroiditis (HT) is the leading autoimmune thyroid disorder worldwide and the systemic autoimmune condition carrying the highest relative risk for a specific lymphoma subtype. Despite this epidemiological signal, HT has not been integrated into a comprehensive oncological molecular framework. **Objective:** To systematically map, through a scoping review methodology, the molecular and histological evidence of mechanistic overlap between HT and thyroid MALT lymphoma, examining shared lymphomagenic pathways and the biological plausibility of a pre-neoplastic continuum. **Method:** Scoping review conducted in PubMed/MEDLINE, Embase, and Web of Science (1980–2024), following the Joanna Briggs Institute (JBI) framework for scoping reviews. Studies were included if they reported molecular, immunohistochemical, or genomic data on: (1) B-cell biology in HT tissue; (2) shared molecular markers between HT and thyroid lymphoma; or (3) the epidemiological association between HT and primary thyroid lymphoma. Study selection followed a two-stage screening protocol by two independent reviewers, with disagreements resolved by consensus. **Results:** Forty-two studies met inclusion criteria. Five molecular pillars with documented evidence of overlap between HT and thyroid MALT lymphoma were identified: (1) ectopic germinal center formation with progressive B-cell clonal restriction; (2) BAFF/BLyS-driven NF-κB survival signaling; (3) BCL-2-mediated apoptosis resistance indistinguishable by immunohistochemistry between advanced HT and early MALT lymphoma; (4) sustained AID-dependent mutagenesis; and (5) TNFAIP3/A20 dysregulation in 11–20% of HT-associated primary thyroid lymphomas. A three-step molecular continuum from antigen-dependent oligoclonal expansion to constitutive NF-κB activation is proposed. **Conclusion:** The available molecular and histological evidence supports biological continuity between HT and thyroid MALT lymphoma. This synthesis identifies testable hypotheses for future prospective studies and highlights molecular targets whose pharmacological modulation merits investigation in properly designed clinical trials. These findings do not

constitute clinical recommendations, but provide a mechanistically grounded framework for translational research.

Keywords: Hashimoto's thyroiditis, thyroid lymphoma, pre-neoplastic state, AID mutagenesis; scoping review.

RESUMO

Introdução: A tireoidite de Hashimoto (TH) é o principal distúrbio autoimune da tireoide no mundo e a condição autoimune sistêmica associada ao maior risco relativo para um subtipo específico de linfoma. Apesar desse sinal epidemiológico, a TH ainda não foi integrada a um arcabouço molecular oncológico abrangente. **Objetivo:** Mapear sistematicamente, por meio da metodologia de revisão de escopo, as evidências moleculares e histológicas de sobreposição mecanística entre a TH e o linfoma MALT da tireoide, examinando as vias linfomatogênicas compartilhadas e a plausibilidade biológica de um continuum pré-neoplásico. **Método:** Revisão de escopo conduzida nas bases PubMed/MEDLINE, Embase e Web of Science (1980–2024), seguindo o referencial metodológico do Instituto Joanna Briggs (JBI) para revisões de escopo. Foram incluídos estudos que reportaram dados moleculares, imuno-histoquímicos ou genômicos sobre: (1) biologia de células B no tecido tireoidiano de pacientes com TH; (2) marcadores moleculares compartilhados entre TH e linfoma da tireoide; ou (3) a associação epidemiológica entre TH e linfoma primário da tireoide. A seleção dos estudos seguiu protocolo de triagem em duas etapas realizado por dois revisores independentes, com divergências resolvidas por consenso. **Resultados:** Quarenta e dois estudos atenderam aos critérios de inclusão. Foram identificados cinco pilares moleculares com evidências documentadas de sobreposição entre TH e linfoma MALT da tireoide: (1) formação ectópica de centros germinativos com restrição clonal progressiva de células B; (2) sinalização de sobrevivência NF-κB mediada por BAFF/BLyS; (3) resistência à apoptose mediada por BCL-2, indistinguível por imuno-histoquímica entre TH avançada e linfoma MALT inicial; (4) mutagênese sustentada dependente de AID; e (5) desregulação de TNFAIP3/A20 em 11–20% dos linfomas primários da tireoide associados à TH. Propõe-se um continuum molecular em três etapas, desde a expansão oligoclonal dependente de antígeno até a ativação constitutiva do NF-κB. **Conclusão:** As evidências moleculares e histológicas disponíveis sustentam a continuidade biológica entre a TH e o linfoma MALT da tireoide. Esta síntese identifica hipóteses testáveis para estudos prospectivos futuros e destaca alvos moleculares cuja modulação farmacológica merece investigação em ensaios clínicos devidamente desenhados. Esses resultados não constituem recomendações clínicas, mas fornecem um arcabouço mecanisticamente fundamentado para a pesquisa translacional.

Descritores: Tireoidite de Hashimoto, linfoma da tireoide, estado pré-neoplásico, mutagênese por AID, revisão de escopo.

INTRODUCTION

Hashimoto's Thyroiditis (HT), first described by Hakaru Hashimoto in 1912, is a chronic autoimmune thyroid disorder characterized by diffuse lymphocytic infiltration of the thyroid parenchyma, progressive destruction of thyroid follicles, ectopic germinal center formation, and eventual hypothyroidism. It represents the leading cause of hypothyroidism in iodine-sufficient regions, affecting approximately 1–2% of the general population, with a marked female predominance (female-to-male ratio of 10–20:1).[1]

Beyond its endocrine manifestations, HT is uniquely associated with an exceptionally elevated relative risk for primary thyroid lymphoma. In the landmark Swedish cohort study by Holm, Blomgren,

and Löwhagen (829 patients with chronic lymphocytic thyroiditis monitored through the Swedish Cancer Register), the relative risk of primary malignant thyroid lymphoma reached 67-fold (4 observed vs. 0.06 expected cases; P<0.000001).[2] Subsequent meta-analyses and cohort studies have corroborated this association, with estimated relative risks ranging from 60- to 80-fold.[3]

This remarkable epidemiological association has not prompted a systematic molecular investigation of HT from an oncological perspective. Traditionally, HT is classified as an autoimmune disorder, while thyroid Mucosa-Associated Lymphoid Tissue (MALT) lymphoma is regarded as a distinct malignant neoplasm. However, their molecular underpinnings exhibit substantial overlap, raising the hypothesis that the distinction between these two entities may be fundamentally quantitative, relating to clonal dominance and the progressive accumulation of genomic alterations, rather than qualitative in nature.

The present scoping review aims to map and synthesize the available evidence on molecular mechanisms shared between HT and thyroid MALT lymphoma, structured around five biological pillars, to articulate the biological plausibility of a pre-neoplastic continuum, and to identify research gaps and testable hypotheses for future prospective studies.

METHODS AND RESULTS

Overview of Included Studies

The initial database search retrieved 847 records. After removal of duplicates (n=214) and screening of titles and abstracts (n=633), 127 studies were selected for full-text review. Of these, 42 met all eligibility criteria and were included in the final synthesis. The complete characterization of all included studies by thematic domain is presented in **Table 1**. **Table 2** presents the key epidemiological studies in detail. **Fig. 1** displays the PRISMA-ScR selection flow diagram.

The 42 included studies comprised: 10 cohort studies or case series on the epidemiological association between HT and primary thyroid lymphoma (refs. 2, 3, 6, 7, 8, 9, 10, 11, 12, 13); 7 systematic reviews, meta-analyses, and clinical guidelines on HT (refs. 4, 5, 37, 38, 39, 40, 41); 9 immunohistochemical or molecular studies characterizing B-cell biology, B-cell lymphoma (BCL)-2, and clonality in HT tissue (refs. 14, 15, 16, 19, 22, 23, 24, 26, 27); 9 translational or experimental studies addressing molecular pathways shared between HT and MALT lymphoma (refs. 17, 18, 20, 21, 28, 29, 30, 31, 32); and 7 studies on molecular oncology of MALT lymphoma and B-cell malignancies relevant to the proposed continuum (refs. 25, 33, 34, 35, 36, 42, 43, 44).

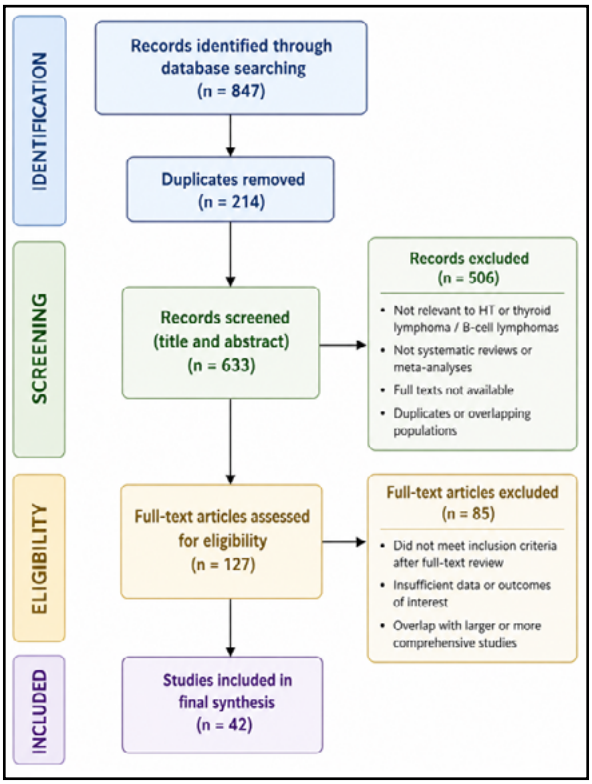

Figure 1. Flowchart on the selection process of eligible studies.

Table 1. Classification of the 42 included studies by thematic domain.

Reference	Ref. No.	Thematic domain and primary contribution
Domain 1 – Epidemiology of the HT–Thyroid Lymphoma Association (n=10)		
Holm et al. (1985)	2	Foundational Swedish cohort (n=829); RR=67× for thyroid lymphoma in chronic lymphocytic thyroiditis (P<0.000001)
Travaglino et al. (2020)	3	Systematic review and meta-analysis (n=1,346); pooled HT prevalence in PTL: 78.9%; pooled RR: 60–80×
Watanabe et al. (2011)	6	Largest HT cohort (n=24,553); 171 PTL identified; 10-year lymphoma risk in HT patients: 0.7%
Pavlidis & Pavlidis (2019)	7	Narrative review of primary thyroid lymphoma: molecular factors, diagnosis, and clinical management
Kato et al. (1985)	8	Japanese case-control (n=5,592); chronic thyroiditis as independent B-cell lymphoma risk factor
Hyjek & Isaacson (1988)	9	Case series (n=236 PTL); HT background present in ~98% of primary thyroid B-cell lymphomas
Derringer et al. (2000)	10	Clinicopathological series (n=108 PTL); HT in 87%; strong histological overlap with early MALT
Thieblemont et al. (2002)	11	Retrospective cohort (n=70 PTL); HT in 90%; primary thyroid lymphoma is heterogeneous (MALT 60%, DLBCL 40%)
Troch et al. (2008)	12	Multi-site MALT cohort; chronic autoimmune thyroiditis in 16% of all-anatomical-site MALT lymphoma cases
Hu X et al. (2022)	13	Systematic review and meta-analysis (n=3,591); elevated cancer risk in HT, including thyroid lymphoma and PTC
Domain 2 – Systematic Reviews, Meta-Analyses, and Clinical Guidelines on HT (n=7)		
Peters et al. / JBI (2020)	4	JBI Manual for Evidence Synthesis, Chapter 11: Scoping Reviews; methodological framework adopted
Tricco et al. (2018)	5	PRISMA-ScR checklist and explanation; methodological reference for scoping review reporting
Klubo-Gwiezdzinska & Wartofsky (2022)	37	Evidence-based guide to HT etiology, diagnosis, and treatment; comprehensive clinical reference
Jonklaas et al. / ATA (2014)	38	American Thyroid Association guidelines for treatment of hypothyroidism; standard of care reference
Huwiler et al. (2024)	39	Systematic review and meta-analysis of RCTs on selenium supplementation in HT; effects on anti-TPO titers
Li R et al. (2025)	40	Review of the immune system in HT, potential therapies, and animal model construction
Palazzo et al. (2025)	41	Review of lessons from belimumab trials in SLE; pharmacological reference for anti-BAFF strategy
Domain 3 – Immunohistochemical and Molecular Studies on HT Tissue (n=9)		
Caturegli et al. (2013)	14	Histopathological characterization of HT; ectopic germinal center formation and lymphoid neogenesis
Moshynska & Saxena (2008)	15	PCR-based clonality assays in HT tissue; monoclonal/oligoclonal Ig rearrangements without overt lymphoma
Chetty et al. (1995)	16	IHC detection of p53 and BCL-2 in HT and thyroid lymphoma; IHC overlap between advanced HT and early MALT
Campi et al. (2015)	19	BAFF and BAFF-R expression in HT tissue (IHC); maximal signal in GC and mantle zones of ectopic follicles

Kuribayashi-Hamada et al. (2022)	22	A20/TNFAIP3 mutation in primary thyroid lymphoma; all A20 mutations associated with HT background
Rodríguez-Sevilla & Salar (2021)	23	Genetic advances in MALT lymphoma including TNFAIP3 inactivation in thyroid MALT (11–20% of cases)
Chu et al. (2011)	24	Murine model: B-cell-specific TNFAIP3 deletion induces HT-like autoimmune phenotype with anti-thyroid IgG
Vasconcelos et al. (2021)	26	BCL-2 and MCL-1 co-overexpression in HT infiltrates; elevated Ki-67 and low apoptotic rate
Stassi et al. (2001)	27	Fas/FasL pathway dysregulation in HT; inverted Fas-FasL axis promotes lymphocytic infiltrate persistence
Domain 4 – Translational and Experimental Studies on Shared Molecular Pathways (n=9)		
Giordano et al. (2023)	17	BAFF from dendritic cells and neutrophils required for B-cell maturation and autoantibody production in SLE-like disease
Kampa et al. (2020)	18	APRIL and BAFF receptors (TACI, BCMA, BAFFR) in oncology; mechanistic and pharmacological review
Yang et al. (2014)	20	BAFF/BAFF-R axis in B-cell non-Hodgkin lymphoma; survival signaling and therapeutic targeting
Hamoudi et al. (2010)	21	NF-κB target gene expression in MALT lymphoma with and without chromosomal translocation
Leeman-Neill et al. (2024)	28	AID on- and off-target activity in non-Hodgkin B-cell lymphomas; review of oncogenic mechanisms
Jiao et al. (2023)	29	Off-target AID effects in carcinogenesis; mutagenic activity at proto-oncogene loci
Nishikori et al. (2021)	30	Elevated AID expression in ocular adnexal MALT lymphoma with IgG4-positive cells (autoimmune context)
Li L et al. (2025)	31	BAFF system role in autoimmune diseases: mechanisms, disease implications, and therapeutic advances
Corneth et al. (2022)	32	Aberrant B-cell signaling in autoimmune diseases including HT; BCR and BAFF pathway dysregulation
Domain 5 – Molecular Oncology of MALT Lymphoma and B-Cell Malignancies (n=7)		
Li WF et al. (2024)	25	BCL-2 inhibitor-based therapies and emerging resistance in CLL; venetoclax pharmacology reference
Lauring & Basu (2024)	33	Somatic hypermutation mechanisms during lymphomagenesis and transformation; AID and clonal evolution
Lai et al. (2023)	34	Clinicopathological analysis of primary thyroid non-Hodgkin lymphoma; single-center series
Raderer et al. (2023)	35	Clinical relevance of molecular aspects in extranodal marginal zone lymphoma; NF-κB and TNFAIP3
Li X et al. (2025)	36	Genetic alterations and prognostic impact in marginal zone lymphoma; meta-analysis
Pal Singh et al. (2018)	42	BTK role in B cells and malignancies; ibrutinib pharmacology and BCR-signaling inhibition
Zhu & Almasan (2017)	43	Development of venetoclax for therapy of lymphoid malignancies; BCL-2 inhibition rationale
Verma et al. (2022)	44	Dual PI3Kδγ inhibition in DLBCL models; preclinical characterization of PI3K inhibitor

HT, Hashimoto's Thyroiditis; PTL, primary thyroid lymphoma; MALT, mucosa-associated lymphoid tissue; DLBCL, diffuse large B-cell lymphoma; RR, relative risk; IHC, immunohistochemistry; GC, germinal center; CLL, chronic lymphocytic leukemia; SLE, systemic lupus erythematosus; BCR, B-cell receptor; BTK, Bruton's tyrosine kinase; AID, activation-induced cytidine deaminase; PTC, papillary thyroid carcinoma.

Table 2. Key epidemiological studies (Domain 1) on the association between HT and primary thyroid lymphoma — detailed summary.

Study / Year	N	HT prevalence in PTL (%)	Main lymphoma subtype	Key finding
Holm et al.[2] (1985)	829	—	Thyroid lymphoma (mixed)	RR=67× (P<0.000001); foundational epidemiological study
Travaglino et al.[3] (2020)	1,346	78.9% (pooled)	MALT, DLBCL	Pooled RR 60–80×; systematic review and meta-analysis
Watanabe et al.[6] (2011)	171 PTL / 24,553 HT	—	MALT, DLBCL	10-year lymphoma risk in HT: 0.7%; largest HT cohort
Pavlidis & Pavlidis[7] (2019)	Review	—	PTL (all subtypes)	Molecular factors, diagnosis, and management of primary thyroid lymphoma
Kato et al.[8] (1985)	5,592	—	B-cell lymphoma	Chronic thyroiditis as independent lymphoma risk factor
Hyjek & Isaacson[9] (1988)	236	~98%	MALT / DLBCL	Near-universal HT background in primary thyroid lymphoma
Derringer et al.[10] (2000)	108 PTL	87%	MALT, DLBCL	Strong histological overlap between HT and early MALT
Thieblemont et al.[11] (2002)	70 PTL	90%	MALT (60%), DLBCL (40%)	Primary thyroid lymphoma is heterogeneous
Troch et al.[12] (2008)	MALT cohort	16% of all-site MALT	MALT (thyroidal & extrathyroidal)	HT-associated MALT lymphoma extends beyond thyroid gland
Hu X et al.[13] (2022)	3,591	—	Thyroid lymphoma, PTC	Elevated cancer risk in HT; meta-analysis

HT, Hashimoto's Thyroiditis; PTL, primary thyroid lymphoma; MALT, mucosa-associated lymphoid tissue; DLBCL, diffuse large B-cell lymphoma; PTC, papillary thyroid carcinoma; RR, relative risk.

Pillar 1 – Ectopic Germinal Centers and Progressive B-Cell Clonal Restriction

In HT, the thyroid parenchyma is progressively replaced by a mononuclear infiltrate organized into mature lymphoid follicles harboring ectopic germinal centers (GCs).[14] This intrathyroidal lymphoid neogenesis recapitulates the structural organization of secondary lymphoid tissues and constitutes a distinctive histologic hallmark differentiating HT from simple lymphocytic thyroiditis.

Within these ectopic GCs, B cells undergo persistent stimulation by thyroid autoantigens (predominantly thyroid peroxidase and thyroglobulin), initiating iterative cycles of somatic hypermutation (SHM) and antigen-driven clonal selection. Over time, this process imposes progressive restriction of immunoglobulin (Ig) gene diversity. PCR-based clonality assays performed on thyroid tissue from HT patients have demonstrated monoclonal or oligoclonal Ig gene rearrangements even without histologic evidence of lymphoma, patterns analogous to in situ follicular neoplasia of lymph nodes.[15]

Immunohistochemical staining for BCL-2 and p53 fails to reliably distinguish advanced HT from low-grade thyroid MALT lymphoma. Within mantle zones and lymphoepithelial lesions (LELs) of HT, lymphoid cells exhibit uniform BCL-2 expression mirroring the pattern observed in low-grade diffuse thyroid lymphomas.[16] This histologic overlap reflects a genuine biological continuum, rather than a mere diagnostic limitation.

Pillar 2 – BAFF/BLyS: A Master Survival Signal Shared by HT and MALT Lymphoma

B-cell activating factor (BAFF, encoded by TNFSF13B) is a TNF superfamily member essential for ma-

ture B-cell survival. It signals through three receptors (BAFF-R, TACI, and BCMA), activating canonical and non-canonical NF-κB pathways, as well as PI3K/AKT and ERK cascades, promoting B-cell survival, proliferation, and Ig class-switch recombination.[17,18]

In HT, thyrocytes constitutively release BAFF in response to interferon-γ produced by infiltrating Th1 cells. Immunohistochemical analyses of HT tissue have demonstrated BAFF and BAFF-R expression in both infiltrating lymphocytes and thyrocytes, with maximal intensity in GCs and mantle zones of ectopic follicles.[19] This thyroid-derived BAFF establishes a self-perpetuating survival loop for intrathyroidal B cells, bypassing the requirement for classical nodal support.

The same BAFF-dependent survival circuitry defines thyroid MALT lymphoma and other indolent B-cell neoplasms. BAFF overexpression lowers the threshold for malignant transformation by inhibiting activation-induced apoptosis in GC B cells, a checkpoint that would otherwise eliminate premalignant clones.[20] These findings suggest that thyrocyte-derived BAFF in HT may function as a driver of the pre-neoplastic state, a hypothesis requiring prospective validation.

Pillar 3 – NF-κB Dysregulation and the TNFAIP3/A20 Axis

NF-κB is a central hub for survival and proliferation in B-cell lymphomagenesis, regulating target genes including BCL-2, BCL-XL, XIAP, and MYC. In MALT lymphoma, constitutive NF-κB activation arises from chromosomal translocations and from inactivation of the inhibitor TNFAIP3, which encodes the desubiquitinase A20.[21]

Mutations and deletions of A20/TNFAIP3 are documented in MALT lymphomas arising in sites of chronic autoimmune inflammation, including the ocular adnexa, salivary glands, and thyroid. In primary thyroid lymphoma, TNFAIP3 inactivation has been reported in 11–20% of cases; in two independent studies, all identified A20 mutations were associated with pre-existing HT.[22,23] These data establish a direct genetic link between the autoimmune microenvironment of HT and a molecular alteration enabling MALT lymphomagenesis.

Murine models further support this relationship: B-cell-specific deletion of TNFAIP3 is sufficient to induce an autoimmune phenotype analogous to HT, characterized by thyroid-directed autoreactive IgG antibody production.[24] These findings demonstrate convergence between A20 loss and HT-like autoimmune disease within the same molecular circuitry.

Pillar 4 – BCL-2: The Anti-Apoptotic Block Shared by HT Lymphocytes and MALT Tumor Cells

BCL-2 is the paradigmatic anti-apoptotic protein in B-cell neoplasms. In thyroid tissue from HT, infiltrating lymphocytes within mantle zones and LELs exhibit strong, homogeneous BCL-2 expression, with an immunohistochemical pattern indistinguishable from early-stage thyroid MALT lymphoma.[25]

Co-overexpression of BCL-2 and MCL-1 in HT lymphocytic infiltrates, alongside elevated Ki-67 indices and a paradoxically low apoptotic rate, has been demonstrated by multiple immunohistochemical and molecular studies.[26] This anti-apoptotic phenotype in HT is sustained by tonic BAFF and BCR signaling, defining an antigen-dependent transcriptional state potentially distinct from the chromosomally fixed BCL-2 overexpression of follicular lymphoma, a distinction with potential translational relevance requiring direct comparative studies.

Additionally, the Fas/FasL pathway, responsible for elimination of activated lymphocytes and peripheral tolerance maintenance, exhibits functional dysregulation in HT: thyrocytes aberrantly express FasL, promoting destruction of Fas-expressing thyrocytes rather than elimination of infiltrating lymphocytes. This functional inversion contributes to persistence of the lymphocytic infiltrate.[27]

Pillar 5 – Activation-Induced Cytidine Deaminase (AID): The Engine of Mutagenesis in HT

AID (encoded by AICDA) drives SHM and class-switch recombination in GC B cells, but also possesses off-target mutagenic activity capable of affecting proto-oncogenes including BCL2, MYC, PAX5, BCL6, and PIM1, inducing point mutations, double-strand DNA breaks, and chromosomal translocations that function as initiating events in B-cell lymphomagenesis.[28,29]

Within the ectopic GCs of HT, AID activity may persist for years or decades due to chronic autoimmune stimulation. Each recirculation of B cells through GCs, driven by thyroid autoantigens, subjects this population to additional rounds of AID-mediated mutagenesis. This framework provides a mechanistic explanation for the dose-response relationship between disease duration and lymphoma risk, a hypothesis consistent with the available epidemiological data, though requiring direct prospective confirmation. Elevated AID expression has been documented in thyroid-origin MALT lymphoma and in ocular adnexal MALT lymphomas arising in sites of chronic autoimmune inflammation.[30]

Table 3. Side-by-side molecular and histological comparison of HT and thyroid MALT lymphoma across eleven parameters.

Feature	Hashimoto's Thyroiditis	Thyroid MALT Lymphoma	Mechanistic significance
Lymphoid infiltration	Polyclonal → oligoclonal B cells	Monoclonal B cells	Quantitative shift in clonality; biological overlap documented by PCR-based assays
Germinal center formation	Ectopic GC in thyroid parenchyma	Persistent GC-derived malignant clones	Shared microstructural lymphoid platform
BAFF/BLyS expression	High; thyrocyte-derived, IFN-γ induced	High; tumor microenvironment-derived	Shared B-cell survival signal
BCL-2 expression	Elevated in infiltrating B cells (mantle zones, LELs)	Constitutively elevated	Anti-apoptotic block indistinguishable by IHC in HT vs. early MALT
AID / AICDA activity	Active in ectopic GCs; off-target mutagenesis sustained	Residual or downregulated post-transformation	AID provides cumulative oncogenic mutational load during HT phase
TNFAIP3 / A20 status	Wild-type (intact NF-κB regulation)	Mutated/deleted in 11–20% of PTL	A20 loss enables constitutive NF-κB activation
NF-κB activation	Antigen-dependent; BAFF-R and BCR driven	Constitutive; TNFAIP3 loss or chromosomal translocations	Same effector pathway; different upstream driver
Ig gene rearrangement	Polyclonal → oligoclonal by PCR	Monoclonal dominant clone	Clonal dominance is the diagnostic threshold
Antigen dependence	Yes — TPO, Tg drive BCR engagement	Partial → progressive autonomy via BTK/PI3Kδ signaling	Loss of antigen dependence is a key molecular event of malignant transition
Driver mutations (MYD88, CARD11)	Absent	Present in DLBCL-transformation subset	Genomic divergence occurs at/ after transformation
Lymphoepithelial lesions (LELs)	Present; T-cell mediated follicular destruction	Present; hallmark of MALT diagnosis (WHO criteria)	LELs shared by both; morphology alone cannot distinguish HT-associated from early MALT

GC, germinal center; BAFF, B-cell activating factor; AID, activation-induced cytidine deaminase; IHC, immunohistochemistry; LEL, lymphoepithelial lesion; Ig, immunoglobulin; TPO, thyroid peroxidase; Tg, thyroglobulin; BTK, Bruton's tyrosine kinase.

Proposed Three-Step Molecular Continuum

Based on the synthesized molecular evidence, a three-step sequential model of the HT-to-lymphoma continuum is proposed as a conceptual framework to guide future research. This model is not intended as a diagnostic or clinical algorithm, but as a heuristic construct identifying testable transition points.

Step 1 – Antigen-Dependent Oligoclonal Expansion (Active HT)

Thyroid autoantigens (TPO, Tg) promote chronic BCR activation in intrathyroidal B lymphocytes. Clone survival is sustained by BAFF signaling maintaining BCL-2 overexpression. AID remains active within ectopic GCs, driving SHM and progressive Ig gene restriction. TNFAIP3/A20 continues to exert regulatory function, and NF-κB activation remains antigen-dependent (**Fig. 2**).[31,32]

Step 2 – Early Clonal Escape (In Situ Lymphoid Neoplasia, Candidate Stage)

An AID-mediated off-target mutation in TNFAIP3/A20, BCL2, or MYC confers a competitive survival advantage to a single B-cell clone, enabling partial dissociation from continuous antigenic stimulation. This clone expands dominantly. PCR-based clonality as-

says reveal a predominant Ig gene rearrangement, while histologically the lesion may still be classified as 'florid' HT or 'indeterminate for lymphoma. Whether this stage consistently precedes overt lymphoma and whether it can be reproducibly detected prospectively remains an unresolved research question requiring NGS-based longitudinal studies (**Fig. 3**).[33,34]

Step 3 – Overt MALT Lymphoma

Constitutive NF-κB activation, mediated by TNFAIP3/A20 inactivation or chromosomal translocations (BCL10-IGH, MALT1-IGH), confers complete antigen-independent survival. Expansion of the malignant clone becomes self-sustaining and invasive, with LEL formation and infiltration beyond thyroid parenchyma, fulfilling WHO histologic criteria for primary thyroid MALT lymphoma (**Fig. 4**).[35,36]

DISCUSSION

The present scoping review synthesizes evidence from 42 studies demonstrating substantial molecular overlap between HT and thyroid MALT lymphoma across five mechanistic pillars. The convergence of ectopic GC formation, BAFF-mediated survival signaling, BCL-2 anti-apoptotic blockade, AID-driven mutagenesis, and TNFAIP3/A20 dysregulation in both conditions

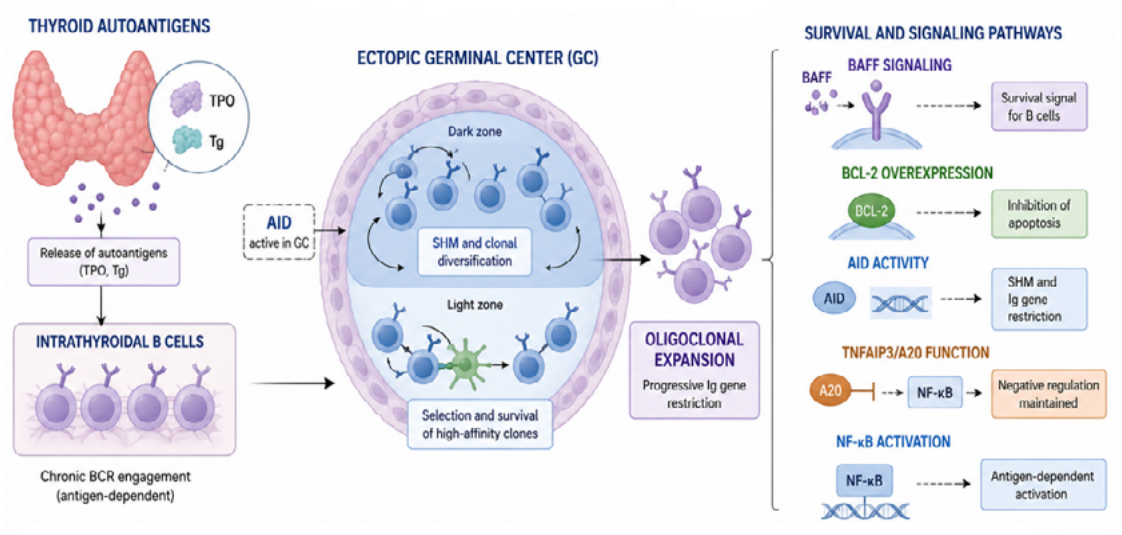

Figure 2. Antigen-Driven Oligoclonal Expansion and Intratecal B-Cell Homeostasis in Chronic Autoimmune Thyroiditis

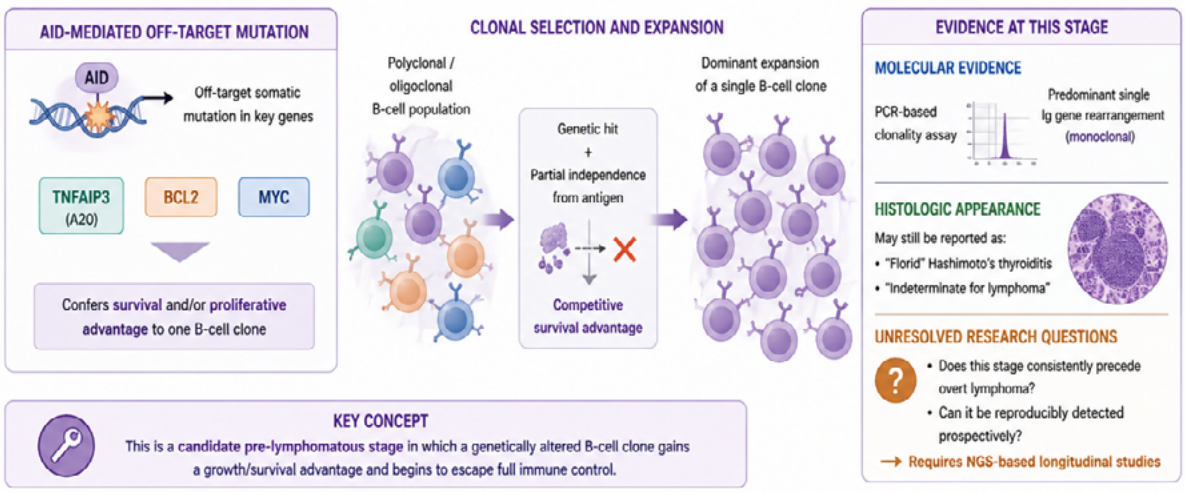

Figure 3. Molecular Emergence of In Situ Lymphoid Neoplasia: AID-Mediated Genetic Alterations and Early Clonal Escape in Autoimmune Thyroiditis

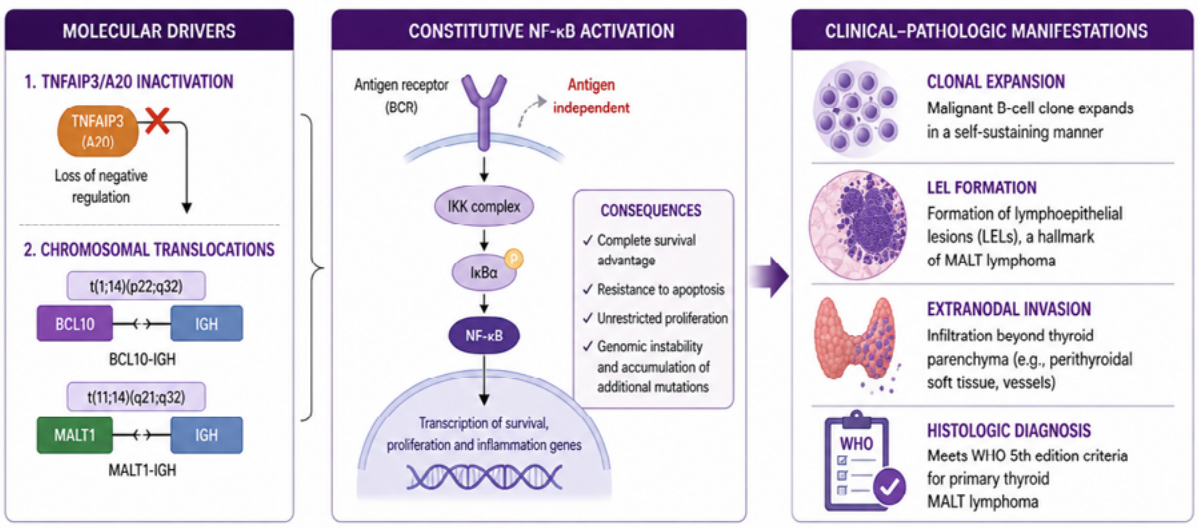

Figure 4. Autonomous Clonal Expansion and Lymphoepithelial Lesion (LEL) Formation: The Transition to WHO-Defined MALT Lymphoma.

is consistent with the hypothesis that these entities may represent a biological continuum rather than dichotomous categories. However, this interpretation requires critical qualification.

First, the directionality of many documented associations remains observational. Most included studies are cross-sectional or retrospective, documenting molecular co-occurrence rather than causal or temporal relationships. Prospective longitudinal studies tracking molecular events from HT diagnosis through potential lymphoma development are largely absent from the literature.[45] This represents the most critical methodological gap identified by this review.

Second, the epidemiological signal, while unequivocal in terms of relative risk, translates into a modest absolute lifetime risk of lymphoma in HT patients (approximately 0.7% at 10 years in the largest available cohort[6]). This suggests that the shared molecular architecture described herein is common in HT but that additional, yet-uncharacterized events are required for overt neoplastic transformation.[46] Identifying these events represents a priority research agenda.

Third, the proposed three-step continuum constitutes a conceptual model rather than a validated pathophysiological staging system. The intermediate stage (in situ lymphoid neoplasia/early clonal escape) lacks a consensus definition, standardized detection methodology, or prospective evidence of predictable progression to overt lymphoma.[47] Its clinical or diagnostic utility cannot be established from the currently available evidence.

Molecular Targets as Research Hypotheses – Not Clinical Recommendations

The molecular pillars identified in this review, particularly constitutive BAFF overexpression, BCL-2 anti-apoptotic signaling, BTK/PI3Kδ-dependent BCR activation, and TNFAIP3/A20-mediated NF-κB dysregulation, represent pharmacologically actionable pathways in thyroid MALT lymphoma. Several agents targeting these pathways (including anti-BAFF biologics, BTK inhibitors, BCL-2 inhibitors, and PI3Kδ inhibitors) are approved for related B-cell malignancies and autoimmune conditions.[48]

This mechanistic convergence generates testable research hypotheses: whether pharmacological modulation of BAFF, BCR, BCL-2, or NF-κB signaling in HT patients could influence the molecular trajectory toward lymphoma is a scientifically plausible question. However, the evidence mapped in this scoping review does not support clinical application of these agents in HT, and no such recommendation is made herein. Evaluation of these interventions would require prospective cohort studies with validated surrogate endpoints followed by randomized controlled trials in clearly, high-risk populations.[49] The identification of such populations itself remains an unmet methodological need.

Surveillance Research Agenda

The findings of this review suggest that monitoring strategies incorporating NGS-based Ig clonality assays on thyroid fine-needle aspiration specimens and TNFAIP3 mutational screening may have biological rationale in patients with long-standing HT. These

approaches have not been validated in prospective studies and cannot be considered standard of care.[50] Future research should prioritize the definition of: (1) validated high-risk HT phenotypes; (2) reproducible molecular thresholds distinguishing reactive oligoclonal expansion from in situ neoplasia; and (3) the natural history of clonal dynamics in HT with and without lymphoma development.

LIMITATIONS

This scoping review has limitations. Scoping reviews do not formally assess risk of bias in individual studies; the included literature encompasses heterogeneous study designs, sample sizes, and methodologies, limiting direct comparability. The search, while systematic, was restricted to published literature in three languages, potentially introducing publication bias. The proposed molecular continuum integrates evidence from distinct experimental contexts (immunohistochemistry, PCR-based clonality, murine models, and genomic sequencing), which may not directly translate to the clinical trajectory of any individual patient. Fourth, several key mechanistic claims in the literature, particularly regarding the functional significance of BAFF overexpression and BCL-2 expression in HT tissue, are based on cross-sectional studies that cannot establish causality or predict transformation. The role of follicular helper T cells, regulatory T cells, and the broader immune microenvironment in sustaining intrathyroidal B-cell expansion was not comprehensively captured in the included literature and warrants dedicated investigation. Finally, the potential modulatory effect of levothyroxine therapy, the standard treatment for HT-associated hypothyroidism, on intrathyroidal B-cell biology has not been systematically evaluated, despite evidence that thyroid hormone can modulate lymphocyte function and anti-apoptotic regulator expression.

CONCLUSION

This scoping review maps substantial molecular and histological evidence of mechanistic overlap between HT and thyroid MALT lymphoma across five biological pillars: ectopic germinal center formation, BAFF-mediated B-cell survival, BCL-2-driven apoptosis resistance, NF-κB dysregulation with TNFAIP3/A20 involvement, and AID-sustained mutagenesis. The available evidence is consistent with a biological continuum hypothesis, but does not permit definitive causal or prognostic conclusions given the observational nature and heterogeneity of included studies.

The primary contribution of this review is the systematic identification and organization of research gaps: the absence of prospective longitudinal molecular data, the lack of validated intermediate-stage biomarkers, and the unresolved natural history of B-cell clonal dynamics in HT. These gaps define a translational research agenda whose pursuit, through prospective cohort studies and, subsequently, rigorously designed interventional trials, could ultimately inform whether pharmacological modulation of the identified molecular pathways has a role in lymphoma risk reduction in HT. No clinical recommendations are derived from the present synthesis.

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REVIEW *REVISÃO*

AUTOIMMUNE INFLAMMATORY SYNDROME INDUCED BY ADJUVANTS (ASIA): INSIGHTS INTO PATHOGENESIS, DIAGNOSIS, AND MANAGEMENT

SÍNDROME AUTOIMUNE INFLAMATÓRIA INDUZIDA POR ADJUVANTES (ASIA): PERSPECTIVAS SOBRE PATOGÊNESE, DIAGNÓSTICO E MANEJO

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ABSTRACT

INTRODUCTION: Autoimmune/Inflammatory Syndrome Induced by Adjuvants (ASIA) represents a unified framework conceptualized in 2011 encompassing diverse immune- mediated disorders triggered by adjuvant exposure in genetically predisposed individuals. **OBJECTIVE**: This review synthesizes current understanding of ASIA pathogenesis, clinical manifestations, diagnostic approaches, and therapeutic strategies while identifying critical research gaps. **METHODS**: A comprehensive narrative synthesis examined literature from multiple databases (PubMed/MEDLINE, Scopus, Web of Science, Embase) covering publications from 2011 through 2025. Data were synthesized across four domains: immunopathogenic mechanisms, clinical phenotypes, diagnostic criteria evolution, and emerging adjuvant substances. **RESULTS:** ASIA syndrome demonstrates complex pathophysiology involving innate immune activation, tolerance breakdown, and chronic inflammation. Aluminum-based vaccine adjuvants, silicone implants, and biomaterials constitute primary triggers. Clinical heterogeneity encompasses constitutional symptoms, musculoskeletal manifestations, and multiorgan involvement. Diagnostic criteria require refinement, while therapeutic approaches emphasize adjuvant removal and immunomodulation. Controversial aspects include nosological validity, vaccine safety implications, and medico-legal dimensions. **CONCLUSION:** ASIA represents a valuable framework linking adjuvant exposure to autoimmunity despite ongoing debates. Future research necessitates prospective cohort studies, advanced immunophenotyping, standardized diagnostic protocols, and safer adjuvant development to optimize patient care while maintaining public health priorities.

Keywords: Autoimmune syndrome; Adjuvants; Shoenfeld syndrome; Silicone implants.

RESUMO

INTRODUÇÃO: A Síndrome Autoimune/Inflamatória Induzida por Adjuvantes (ASIA) representa uma estrutura conceitual unificada, conceitualizada em 2011, que engloba diversos distúrbios imunomediados desencadeados pela exposição a adjuvantes em indivíduos geneticamente predispostos. **OBJETIVO:** Esta revisão sintetiza a compreensão atual da patogênese da ASIA, manifestações clínicas, abordagens diagnósticas e estratégias terapêuticas,

ao mesmo tempo em que identifica lacunas críticas de pesquisa. **MÉTODOS:** Uma síntese narrativa abrangente examinou a literatura de múltiplas bases de dados (PubMed/MEDLINE, Scopus, Web of Science, Embase) cobrindo publicações de 2011 a 2025. Os dados foram sintetizados em quatro domínios: mecanismos imunopatogênicos, fenótipos clínicos, evolução dos critérios diagnósticos e substâncias adjuvantes emergentes. **RESULTADOS:** A síndrome ASIA demonstra fisiopatologia complexa envolvendo ativação imune inata, ruptura da tolerância e inflamação crônica. Adjuvantes vacinais à base de alumínio, implantes de silicone e biomateriais constituem os principais desencadeadores. A heterogeneidade clínica abrange sintomas constitucionais, manifestações musculoesqueléticas e acometimento multiorgânico. Os critérios diagnósticos requerem refinamento, enquanto as abordagens terapêuticas enfatizam a remoção do adjuvante e a imunomodulação. Aspectos controversos incluem validade nosológica, implicações na segurança vacinal e dimensões médico legais. **CONCLUSÃO**: A ASIA representa uma estrutura conceitual valiosa que vincula a exposição a adjuvantes à autoimunidade, apesar dos debates em curso. Pesquisas futuras necessitam de estudos de coorte prospectivos, imunofenotipagem avançada, protocolos diagnósticos padronizados e desenvolvimento de adjuvantes mais seguros para otimizar o cuidado ao paciente, mantendo as prioridades de saúde pública.

Descritores: Síndrome autoimune; Adjuvantes; Síndrome de Shoenfeld; Implantes de silicone.

INTRODUCTION

The convergence of unexplained autoimmune manifestations following exposure to various immunostimulatory substances prompted Shoenfeld and Agmon-Levin to conceptualize a unified framework termed Autoimmune/Inflammatory Syndrome Induced by Adjuvants (ASIA), alternatively recognized as Shoenfeld's syndrome, and was first described in 2011.[1] This paradigm emerged from systematic observations documenting comparable clinical presentations across seemingly disparate conditions, including Gulf War syndrome, macrophagic myofasciitis, siliconosis, and post- vaccination phenomena.[2] The syndrome encompasses a heterogeneous spectrum of immune-mediated disorders that manifest in genetically predisposed individuals upon exposure to substances possessing adjuvant properties, including aluminum salts, silicone implants, infectious agents, and various biomaterials.[3]

Adjuvants trigger a cascade of immunological events that can disrupt immune tolerance, promoting systemic inflammation and autoimmunity in susceptible hosts. The syndrome's pathogenesis involves both environmental triggers and genetic factors, explaining varied symptomatology and disease progression. Symptoms commonly include arthralgia, myalgia, chronic fatigue, and neurological disturbances, often overlapping with other autoimmune and inflammatory disorders such as Sjögren's syndrome and undifferentiated connective tissue disease, complicating its diagnosis.[4]

Diagnostic criteria established for ASIA require fulfillment of either two major criteria or one major criterion combined with two minor criteria. Major criteria encompass exposure to external adjuvant stimuli preceding clinical manifestations, appearance of typical symptoms, improvement following adjuvant removal, and histological evidence consistent with immune activation. Minor criteria include detection of non-specific autoantibodies, specific human leukocyte antigen (HLA) associations, and evolution toward defined autoimmune disease.[5] However, these diagnostic parameters have generated considerable debate regarding their specificity and potential for misclassification of established autoimmune conditions under the ASIA umbrella.

Contemporary research has expanded the ASIA conceptual framework to incorporate emerging adjuvants, including polypropylene meshes, novel vaccine formulations, and cosmetic biomaterials.[6] The COVID-19 pandemic prompted renewed scrutiny of adjuvant-related autoimmune phenomena, with documented cases following both infection and vaccination.[7] Thus, research on ASIA syndrome continues to evolve, aiming to clarify its immunopathogenesis and improve diagnostic precision. Greater understanding of ASIA promises advancements in vaccine adjuvant

safety and refinement of autoimmune disease classifications, fostering personalized medical approaches for affected patients and reducing the burden of adjuvant-induced immune complications.[8] This review synthesizes current understanding of ASIA pathogenesis, clinical characteristics, and diagnostic approaches while identifying critical areas requiring further investigation.

METHODOLOGY

Study Design and Search Strategy

This review employed a narrative synthesis approach to evaluate the current understanding of ASIA. A systematic literature search was conducted across multiple electronic databases, including PubMed/MEDLINE, Scopus, Web of Science, and Embase, covering publications from the syndrome's initial description in 2011 through 2025. The search strategy incorporated Medical Subject Headings (MeSH) terms and free-text keywords, including "ASIA syndrome," "Shoenfeld's syndrome," "autoimmune/inflammatory syndrome induced by adjuvants," "adjuvant-related autoimmunity," "macrophagic myofasciitis," "siliconosis," and "post-vaccination autoimmunity." Boolean operators (AND, OR, NOT) were utilized to refine search queries and enhance retrieval specificity.

Selection Criteria and Study Inclusion

Eligible studies encompassed original research articles, systematic reviews, meta- analyses, case reports, and case series published in peer-reviewed journals.

Inclusion criteria required publications addressing the pathophysiological mechanisms, clinical manifestations, diagnostic approaches, or epidemiological aspects of ASIA syndrome. Studies involving human subjects exposed to adjuvant-containing substances, including aluminum-based vaccine adjuvants, silicone implants, polypropylene meshes, and other biomaterials, were prioritized. Both English and non- English language publications with available translations were considered to minimize language bias.

Exclusion criteria eliminated studies lacking primary data, editorials without substantial empirical content, abstracts without full-text availability, and publications focusing exclusively on non-adjuvant-related autoimmune conditions. Studies with inadequate methodological quality, undefined diagnostic criteria, or insufficient documentation of adjuvant exposure were similarly excluded following quality assessment.

Quality Assessment

Data synthesis involved qualitative integration of findings to identify common pathophysiological mechanisms and clinical patterns, alongside analysis of diagnostic criteria variability and emerging adjuvants implicated in ASIA. The review also considered recent developments relating to vaccine adjuvant safety and autoimmune manifestations observed in the context of the COVID-19 pandemic. This rigorous approach aimed to offer a current perspective while identifying knowledge gaps for future research directions.

Synthesis and Analysis Framework

Given the heterogeneity of study designs, outcome measures, and clinical presentations within ASIA syndrome, a narrative synthesis approach was deemed most appropriate rather than quantitative meta-analysis. Data were synthesized thematically across four primary domains: immunopathogenic mechanisms underlying adjuvant- induced autoimmunity; clinical phenotypes and symptomatological patterns; diagnostic criteria evolution and validation; emerging adjuvant substances and contemporary clinical contexts, including post-COVID-19 vaccination phenomena.

The synthesis integrated findings from basic immunology research, clinical observational studies, and epidemiological investigations to construct a comprehensive framework elucidating ASIA's multifactorial etiology. Particular attention was directed toward identifying patterns of genetic predisposition, environmental triggers, and immunological cascades contributing to syndrome manifestation. Comparative analysis of diagnostic criteria proposed by different research groups was performed to evaluate their sensitivity, specificity, and clinical utility.

Identification of Research Gaps and Future Directions

Throughout the synthesis process, critical evaluation identified areas of scientific uncertainty, methodological limitations in existing research, and unresolved controversies regarding ASIA syndrome's nosological status. These gaps were systematically cataloged to inform recommendations for future investigative priorities, including prospective cohort studies with standardized diagnostic protocols, mechanistic research elucidating molecular pathways of adjuvant-induced immune dysregulation, and validation studies assessing the discriminatory capacity of current diagnostic criteria.

The methodology prioritized transparency in reporting synthesis decisions and acknowledged inher-

ent limitations in synthesizing evidence from heterogeneous sources with varying methodological rigor. This approach ensured balanced interpretation of existing evidence while recognizing areas requiring additional empirical investigation (**Fig. 1**).

1. Historical Context and Conceptual Framework

The recognition of ASIA emerged from disparate clinical observations that converged into a unified paradigm. Gulf War veterans presenting with unexplained multisystem complaints,[9] post-vaccination adverse events,[2] and silicone implant recipients developing autoimmune features revealed consistent patterns of adjuvant- triggered immune dysregulation.[10] Shoenfeld and Agmon-Levin's 2011 conceptualization synthesized these phenomena under a single pathophysiological framework, subsequently termed Shoenfeld's syndrome or human adjuvant-related syndrome.[11] This nomenclatural evolution reflects ongoing scientific discourse regarding syndrome boundaries and diagnostic specificity within contemporary autoimmunology.

2. Immunopathogenic Mechanisms

Understanding the molecular and cellular cascades underlying adjuvant-induced autoimmunity requires examination of complex immunological interactions. Adjuvants can inadvertently trigger pathological immune responses in genetically susceptible individuals.[12] The progression from beneficial immune stimulation to aberrant autoimmunity involves multiple interconnected pathways, including innate immune dysregulation, adaptive immune activation, and chronic inflammatory processes.[13] These mechanisms operate synergistically, with genetic predisposition and environmental factors modulating individual susceptibility.[14] Elucidating these pathophysiological processes remains fundamental to understanding ASIA syndrome and developing targeted therapeutic interventions.[15]

2.1 Adjuvant Immunology and Mechanisms of Action

Immune responses are initiated by adjuvants via activation of pattern recognition receptors, with Toll-like and NOD-like receptors serving as primary mediators that subsequently trigger inflammasome cascades and induce pro-inflammatory cytokine secretion.[16] This innate activation promotes dendritic cell maturation and migration to lymphoid tissues, enhancing antigen presentation capacity. Subsequent cytokine and chemokine cascade amplification recruits additional immune cells, perpetuating inflammatory responses. Critically, antigen-adjuvant complexes form persistent deposits at injection sites, creating chronic stimulation foci.[17] In susceptible individuals, these mechanisms may escape regulatory control, culminating in sustained autoimmune activation rather than resolving protective immunity.

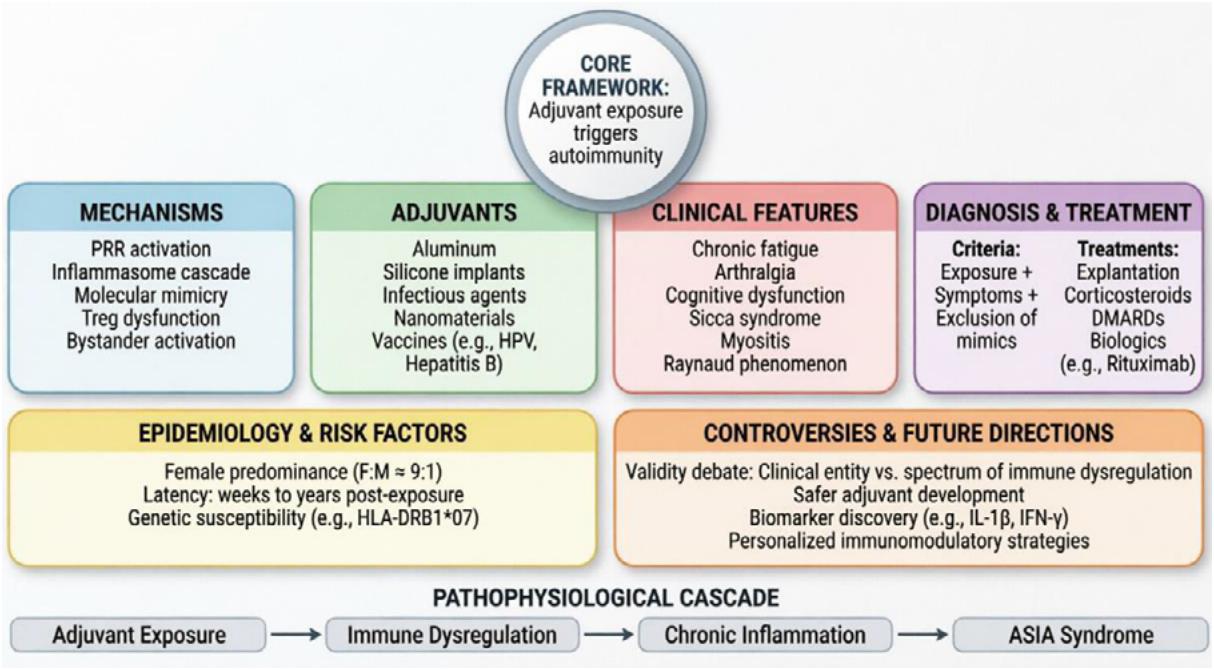

Figure 1. Autoimmune/Inflammatory Syndrome Induced by Adjuvants (ASIA)

2.2 Breakdown of Immune Tolerance

Immune tolerance disruption in adjuvant-induced autoimmunity represents an important pathogenic mechanism in ASIA syndrome. Molecular mimicry occurs when structural homology between adjuvant-associated epitopes and self-antigens triggers cross-reactive immune responses.[18] Bystander activation amplifies this process, wherein inflammatory environments enable T-cells to recognize previously cryptic self-epitopes, facilitating epitope spreading.[19] Simultaneously, regulatory T-cell populations exhibit numerical and functional deficiencies, compromising immunosuppressive capacity.[20] B-cell tolerance checkpoints become compromised, permitting autoreactive B-cells to escape negative selection and produce pathogenic autoantibodies including antinuclear antibodies and anti-extractable nuclear antigens.[21] These interconnected mechanisms [22,23]synergistically destroy self-tolerance, culminating in sustained autoimmune inflammation in genetically susceptible individuals.

2.3 Genetic Susceptibility Factors

Vulnerability to adjuvant-triggered reactions stems from multiple genetic factors: specific HLA allelic variations enhance autoantigen display capacity, while cytokine gene variants (particularly IL-1β and TNF-α) and xenobiotic enzyme polymorphisms modify both immunological reactivity and toxin metabolism pathways.[22,23] Epigenetic shifts (DNA methylation, histone mods) triggered by adjuvants can silence regulatory genes, promoting chronic inflammation.[24] Fundamentally, individual risk emerges from dynamic gene-environment interplay: a carrier of risk alleles may remain asymptomatic without environmental triggers, while others develop autoimmunity after minor exposures.[25] These genetic factors form a complex network underpinning predisposition to immune dysregulation following adjuvant exposure.

2.4 Chronic Inflammatory Pathways

Sustained inflammatory processes characterizing ASIA syndrome depend on macrophage phenotypic shifts that perpetuate inflammatory activity within anatomical regions harboring deposited adjuvant materials.[26] This polarization dynamics underpins granuloma formation, as exemplified by macrophagic myofasciitis pathophysiology, where macrophages laden with adjuvants create distinctive granulomatous lesions.[27] These activated macrophages release pro-inflammatory mediators including IL-1β, IL-6, and TNF-α, which enter systemic circulation and potentially cross the blood-brain barrier.[28] Studies suggests these cytokines facilitate neuroimmune crosstalk, activating microglia and astrocytes, thereby contributing to neurological symptoms including chronic fatigue, cognitive impairment, and myalgia reported in susceptible individuals.[29,30]

3. Substances Implicated in ASIA Syndrome

ASIA syndrome encompasses diverse adjuvant exposures triggering autoimmune manifestations in genetically susceptible individuals.[31] Aluminum hydroxide and phosphate, widely used in vaccines, promotes dendritic cell activation and prolonged cytokine release, potentially driving autoimmunity.[32] Squalene, in oil-in-water emulsions like MF59, enhances antigen presentation but may cross-react with self-antigens in predisposed hosts.[33] Silicone implants have been linked to systemic inflammation and autoantibody production.[34] These substances, though generally safe, can breach immune tolerance in vulnerable individuals, manifesting as fatigue, myalgia, or autoimmune phenomena. Genetic predisposition modulates individual responses to these adjuvants, influencing disease onset and severity.

3.1 Aluminum-Based Adjuvants

Various aluminum formulations, including hydroxide, phosphate, and alum compounds, demonstrate unique pharmacokinetic behaviors. Their nanoparticulate architecture enables extended retention within injection site tissues, followed by gradual migration to lymphoid structures, neural tissue, and skeletal elements..[35] Biodistribution studies reveal dose-dependent accumulation patterns, with elimination half-lives extending months to years depending on formulation crystallinity.[36] Cumulative aluminum burden from multiple vaccinations may exceed theoretical safety thresholds in vulnerable populations.[37] Mechanistically, aluminum adjuvants activate NLRP3 inflammasomes, stimulate dendritic cell maturation, and promote Th2-polarized immune responses through danger-associated molecular pattern recognition.[38] Individual variations in aluminum metabolism significantly influence susceptibility to adjuvant- induced autoimmune manifestations.

3.2 Silicone and Biomaterials

Degradation of silicone breast prostheses generates particulate material capable of lymphatic dissemination, reaching proximal lymphatic structures, hepatic tissue, and splenic parenchyma, where these particles incite granulomatous inflammatory responses accompanied by autoantibody synthesis.[39] Polydimethylsiloxane fragments function as per-

sistent adjuvants, perpetuating immune dysregulation through macrophage activation and cytokine release.[40] Polypropylene meshes employed in hernia repair demonstrate similar adjuvant properties, have also been implicated in ASIA syndrome, inducing chronic foreign body reactions with systemic inflammatory sequelae.[41] Additional biomaterials including polyurethane-coated devices, metallic orthopedic implants, and acrylic bone cement exhibit immunostimulatory capacity, particularly in genetically predisposed individuals harboring specific HLA haplotypes.[42] Understanding biomaterial-host interactions remains essential for preventing implant-associated autoimmune phenomena.

3.3 Infectious Agents as Adjuvants

Viral and bacterial pathogen-associated molecular patterns function as endogenous adjuvants, activating innate immune receptors and precipitating autoimmune sequelae through molecular mimicry and bystander activation.[43] Post-infectious autoimmune disorders demonstrate phenomenological overlap with ASIA syndrome, sharing common immunopathological mechanisms.[44] SARS-CoV-2 infection emerges as a potent ASIA trigger, with spike protein epitopes cross-reacting with self-antigens and inducing persistent immune dysregulation manifesting as post-acute sequelae.[8] Epstein- Barr virus and cytomegalovirus reactivation following adjuvant exposure may synergistically amplify autoimmune responses in susceptible hosts.[45] Distinguishing infectious from adjuvant-mediated autoimmunity requires comprehensive immunological phenotyping.

3.4 Novel and Emerging Adjuvants

Next-generation vaccine adjuvants including like AS01 (MPL + QS-21), AS03 (α-tocopherol), MF59, and CpG oligonucleotides demonstrate enhanced immunogenicity through Toll-like receptor engagement, yet pose theoretical ASIA syndrome risks given their potent immune-activating properties.[46] Hyaluronic acid dermal fillers and polymethylmethacrylate-based cosmetic biomaterials trigger granulomatous reactions with systemic autoimmune manifestations in susceptible individuals.[47] Occupational silica exposure induces adjuvant-like effects, precipitating scleroderma and lupus-like syndromes.[48] Engineered nanomaterials including carbon nanotubes and metallic nanoparticles exhibit intrinsic immunostimulatory capacity through oxidative stress pathways and inflammasome activation.[49] These agents, while beneficial for immunization or aesthetics, may inadvertently fuel ASIA-like phenotypes in susceptible individuals. Rigorous long-term immunotoxicity profiling is essential as these technologies expand into everyday use.

4. Clinical Manifestations and Phenotypic Spectrum

ASIA syndrome exhibits remarkable clinical heterogeneity, encompassing constitutional symptoms including chronic fatigue, arthralgias, myalgias, and cognitive dysfunction occurring months to years' post-adjuvant exposure.[50] Neurological manifestations range from peripheral neuropathies to demyelinating disorders mimicking multiple sclerosis.[51] Dermatological features include chronic urticaria, alopecia, and photosensitivity, while sicca syndrome and Raynaud's phenomenon frequently emerge.[52]

Autoantibody profiles demonstrate variability, with anti-nuclear antibodies, anti- extractable nuclear antigens, and novel anti-adjuvant antibodies detected inconsistently.[53] Phenotypic expression correlates with adjuvant type, cumulative dose, genetic susceptibility, and individual immunological background, necessitating personalized diagnostic approaches recognizing this protean clinical spectrum.

4.1 Core Clinical Features

Principal clinical manifestations include systemic constitutional features— notably persistent exhaustion, pyrexia, and nocturnal diaphoresis— which collectively indicate widespread immune system engagement.[54] Musculoskeletal manifestations frequently include arthralgia, myalgia, and varying arthritis patterns that contribute to significant morbidity.[55] Neurological and cognitive disturbances are common, manifesting as peripheral neuropathy, cognitive dysfunction, and headaches.[56] Dermatological findings such as rashes, sicca symptoms, and other cutaneous manifestations further diversify the clinical phenotype.[57] These presentations underscore ASIA's heterogeneity and symptom severity correlates poorly with autoantibody titers, complicating diagnostic certainty and emphasizing clinical phenotyping importance

4.2 Organ System Involvement

Organ system involvement in ASIA syndrome extends beyond musculoskeletal manifestations. Pulmonary complications, particularly interstitial lung disease, emerge through inflammatory pathways triggered by adjuvant persistence.[58] Cardiovascular manifestations encompass pericarditis and small-vessel vasculitis, reflecting systemic immune dysregula-

tion.[59] Gastrointestinal symptoms frequently coexist with hepatic abnormalities, suggesting broader autoimmune cross-reactivity.[60] Endocrine dysfunction represents a critical yet underrecognized domain, with thyroid autoimmunity being particularly prevalent.[61] The heterogeneous nature of organ involvement underscores the syndrome's complexity, where adjuvant-triggered immune activation cascades into multi- system pathology through molecular mimicry and epitope spreading mechanisms.

4.3 Temporal Patterns and Disease Trajectory

Symptom emergence following adjuvant exposure exhibits substantial temporal variability, with documented intervals spanning from 3 days to 5 years; epidemiological data suggests a median latency approaching 17 months.[11] Macrophagic myofasciitis typically exhibits symptom onset around seven months post-vaccination.[62] Acute presentation patterns predominantly emerge within one to seven weeks following exposure, whereas chronic manifestations develop insidiously over months to years.[63]

Disease progression remains unpredictable, with approximately 20% of patients developing well-defined autoimmune conditions superimposed upon constitutional symptoms.[64] Factors influencing severity include genetic predisposition, particularly HLA-DRB1 and PTPN22 polymorphisms, cumulative adjuvant burden, and pre-existing autoimmune diathesis.[65] Symptom chronicity correlates with adjuvant persistence duration and individual immunogenetic profiles.

4.4 Overlap with Established Autoimmune Diseases

ASIA syndrome frequently demonstrates phenotypic overlap with established autoimmune disorders, complicating differential diagnosis.[66] Lupus-like manifestations occur in approximately 3.6% of cases, featuring malar rashes, serositis, and positive anti- dsDNA antibodies following adjuvant exposure.[8] Sjögren's syndrome represents the second most common overlap, affecting 9.8% of ASIA patients with sicca complex and anti-SSA positivity.[67] Undifferentiated connective tissue disease emerges as the predominant overlap pattern, while fibromyalgia symptomatology permeates nearly half of presentations.[68] Evolution toward definitive autoimmune diagnoses constitutes a recognized minor criterion, with approximately 20% progressing beyond initial ASIA manifestations.[69] Differential considerations necessitate temporal correlation with adjuvant exposure, histopathological confirmation, and documented symptom amelioration following adjuvant removal.

5. Diagnostic Approaches and Criteria

ASIA syndrome diagnosis relies on Shoenfeld and Agmon-Levin's 2011 criteria requiring either two major or one major plus two minor parameters.[1] Alijotas-Reig proposed modified criteria in 2015 emphasizing objective measures, though validation remains pending.[5] Diagnosis necessitates comprehensive clinical assessment including adjuvant exposure documentation, temporal correlation with symptom onset, and characteristic manifestations. Laboratory investigations encompass autoantibody profiling and inflammatory markers, while histopathological examination may reveal macrophagic myofasciitis or granulomatous patterns.

5.1 Evolution of Diagnostic Criteria

The ASIA syndrome diagnostic criteria, first established by Shoenfeld and Agmon-Levin in 2011, include major clinical and exposure features for classification. Shoenfeld and Agmon-Levin's original 2011 framework required two major or one major plus two minor criteria for ASIA diagnosis.[1] Alijotas-Reig subsequently proposed modified criteria emphasizing objective measures, though formal validation remains pending.[5] Comparative analyses reveal high sensitivity but suboptimal specificity in current criteria, generating ongoing controversy regarding diagnostic thresholds.[70] Recent proposals advocate incorporating dysautonomia markers and small fiber neuropathy findings to enhance discriminatory capacity.[8]

5.2 Clinical Diagnostic Assessment

Clinical diagnostic assessment of ASIA syndrome necessitates detailed adjuvant exposure documentation. Physical examination often reveals characteristic findings such as myalgia, arthralgia, chronic fatigue, and autoimmune manifestations. A clear temporal correlation between exposure and symptom manifestation is critical for diagnosis, with symptoms potentially delayed by months or years.[13] Clinical improvement following adjuvant removal or explant surgery provides fundamental diagnostic validation, though response variability exists.[71]

5.3 Laboratory Investigations

Laboratory investigations in ASIA syndrome demonstrate heterogeneous serological profiles. Antinuclear antibodies represent the predominant autoantibody finding, frequently accompanied by positive anti-extractable nuclear antigen specificities.[72] Genetic susceptibility assessment through HLA-DRB1 and HLA-DQ2 haplotyping provides prognostic insights, though inflammatory markers including erythrocyte sedimen-

tation rate, C-reactive protein, and interleukin-6 often yield inconsistent elevations Frontiers Publishing.[73] Anti-phospholipid antibodies occasionally manifest in specific clinical phenotypes.[74] Diagnostic challenges include variable sensitivity, inter-laboratory standardization issues, and transient versus persistent antibody differentiation, necessitating comprehensive clinical-serological correlation.

5.4 Histopathological Findings

Macrophagic myofasciitis constitutes the pathognomonic histological hallmark, exhibiting focal epimysial, perimysial, and endomysial infiltration by cohesive basophilic macrophages containing aluminum hydroxide nanocrystals demonstrable via Periodic Acid-Schiff staining and Morin fluorescence.[75] Immunohistochemical characterization reveals intense CD68 expression with accompanying lymphocytic populations.[76] Granulomatous inflammation patterns manifest with foreign-body giant cells, histiocytic aggregation, and variable fibrosis.[32] Deltoid muscle biopsy represents the optimal diagnostic approach, revealing tissue architecture disruption through perifascicular macrophage accumulation without significant myofiber necrosis. Electron microscopy demonstrates characteristic spiculated aluminum-containing inclusions within macrophage cytoplasm.[77]

5.5 Imaging Modalities

Advanced imaging is pivotal for evaluating ASIA syndrome. Magnetic resonance imaging with T2-weighted and STIR sequences precisely delineates muscle edema, atrophy, and spinal cord lesions, providing crucial neurological assessment.[78] For detecting subclinical inflammation, positron emission tomography using 18F-FDG tracer effectively maps metabolically active inflammatory foci.[79] Furthermore, high-resolution ultrasonography is indispensable for real-time assessment of silicone breast implant integrity and the surrounding capsular tissue, identifying ruptures and peri-implant fluid collections.[66]

6. Epidemiological Considerations

ASIA syndrome's epidemiological assessment remains challenging due to underreporting and diagnostic heterogeneity across populations. Prevalence estimates vary substantially, reflecting diverse adjuvant exposures and genetic susceptibilities. Women demonstrate higher susceptibility, suggesting hormonal influences on autoimmune triggering mechanisms. Long latency periods between adjuvant exposure and symptom manifestation complicate causal attribution, necessitating robust registry systems for accurate incidence characterization.[80]

6.1 Incidence and Prevalence Estimates

Population-based occurrence rates for ASIA remain largely undefined, with heterogeneous epidemiological data across geographic regions. Diagnostic recognition challenges persist due to phenotypic variability and overlapping autoimmune manifestations. Substantial underreporting occurs secondary to inadequate clinician awareness and absence of standardized diagnostic criteria. Regional variations reflect differential adjuvant exposure patterns, genetic susceptibilities, and healthcare infrastructure disparities.[81] Enhanced surveillance mechanisms and international registry initiatives are imperative for accurate prevalence quantification.

6.2 Risk Factors and Vulnerable Populations

Female predominance characterizes ASIA syndrome manifestations, paralleling broader autoimmune disease patterns attributed to sex hormone-mediated immunological amplification.[82] Genetic susceptibility markers, particularly HLA-DRB1 and PTPN22 polymorphisms, confer heightened vulnerability to adjuvant-induced autoimmunity.[83]

Pre-existing autoimmune conditions substantially elevate ASIA syndrome risk through immune dysregulation mechanisms.[84] Occupational exposures, including healthcare workers encountering repeated vaccine adjuvants and individuals with silicone or metallic implants, represent epidemiologically significant risk strata.[85] Age-dependent immune senescence modulates susceptibility patterns across demographic cohorts.

6.3 Adjuvant Exposure Patterns

Cumulative aluminum burden demonstrates variable latency periods, averaging 16.8 months between initial exposure and clinical manifestation onset.[86] Contemporary vaccination schedules necessitate systematic evaluation of total adjuvant load, particularly during early developmental periods when immune surveillance mechanisms remain immature. Temporal clustering of multiple adjuvant-containing immunizations may potentiate immunological hyperactivation in susceptible individuals. Definitive dose-response relationships remain elusive, with clinical expression appearing more contingent upon genetic predisposition than absolute adjuvant quantity.[87] Investigation of exposure frequency patterns reveals heterogeneous individual susceptibility thresholds complicating standardized risk stratification.

7. Therapeutic Management Strategies of ASIA syndrome

Therapeutic interventions remain predominantly empirical, reflecting substantial heterogeneity in clinical presentations and limited standardized protocols for ASIA management.[88] Primary treatment emphasizes complete adjuvant removal when feasible, demonstrating symptomatic resolution in appropriately selected cases. Immunomodulatory regimens incorporating corticosteroids, conventional disease- modifying agents, and emerging biologic therapies represent cornerstone pharmacological approaches for managing persistent inflammatory manifestations.[89] Treatment plans require individualization based on severity and comorbidities for optimal outcomes. Multidisciplinary coordination integrating rheumatological expertise with surgical consultation optimizes outcomes, particularly in scenarios involving implanted foreign materials requiring explantation procedures.

7.1 Adjuvant Source Removal

Explantation of silicone breast implants using en bloc capsulectomy techniques demonstrates symptomatic improvement in approximately 50-69% of cases, with fatigue, arthralgia, and myalgia showing preferential response patterns.[90] Biomaterial removal, including metallic orthopedic hardware and dental implants, represents definitive intervention when conservative immunomodulation proves inadequate.[91] Evidence supporting post-explantation symptomatic amelioration remains heterogeneous, with complete capsular excision superior to simple implant removal.[92] Optimal intervention timing favors earlier removal following symptom onset, though individual patient trajectories demonstrate substantial variability in recovery kinetics and durability of therapeutic response.

7.2 Immunomodulatory and Anti-Inflammatory Therapies

The management of ASIA syndrome's immune phenomena requires a tailored approach. Immunomodulatory and anti-inflammatory therapies for ASIA syndrome include corticosteroid regimens with initial high-dose pulses followed by gradual tapering tailored to clinical response.[93] Disease-modifying antirheumatic drugs such as hydroxychloroquine and methotrexate provide immunosuppression.[94] Biologic agents targeting TNF, IL-6, and B cells have shown promise, alongside intravenous immunoglobulin therapy.[95] Emerging targeted immunotherapies are under investigation to enhance specificity and efficacy.

7.3 Symptomatic and Supportive Management

Symptomatic and supportive management of ASIA syndrome includes analgesics for pain control. Physical therapy and rehabilitation target musculoskeletal symptoms to restore function. Cognitive and neuropsychological support address memory and mood disturbances. Fatigue management protocols incorporate lifestyle modifications, energy conservation, and pharmacologic interventions to improve quality of life.[96]

7.4 Treatment Outcomes and Prognosis

Treatment outcomes in ASIA syndrome are variable. Early immunomodulatory intervention often yields favorable responses, with some patients achieving complete resolution.[97] Prognosis is generally better in cases with prompt adjuvant removal. Predicting individual responses remains challenging, though specific autoantibody profiles may be informative.[98] Serial clinical evaluation and monitoring of inflammatory markers are crucial for assessing disease activity and guiding therapy.

8. Controversial Aspects and Scientific Debate in ASIA Syndrome

The nosological validity of ASIA syndrome remains contested, with proponents arguing for its recognition as a distinct immunological entity while critics highlight diagnostic imprecision and potential overlap with functional somatic syndromes. Vaccine safety discussions necessitate nuanced risk-benefit analyses, balancing population-level epidemiological data against individual susceptibility profiles, thereby influencing public health policy and adjuvant selection.[99] Medico-legal ramifications encompass silicone implant litigation, vaccine injury compensation frameworks, and evidentiary challenges in establishing causality, requiring healthcare providers to navigate complex liability landscapes while maintaining evidence-based clinical decision-making.[100]

Future Research Directions and Knowledge Gaps in ASIA Syndrome

Mechanistic investigations employing single-cell transcriptomics and proteomics are essential for identifying prognostic biomarkers and elucidating targetable molecular pathways underlying ASIA syndrome.[101] Clinical research necessitates prospective cohort studies with standardized diagnostic protocols and randomized controlled trials evaluating therapeutic efficacy, alongside comprehensive natural history doc-

umentation.[102] Translational priorities include developing novel adjuvant formulations with improved safety profiles, creating personalized risk stratification algorithms, and refining autoimmune disease nosology.[103] Regulatory frameworks must incorporate enhanced post-market surveillance, standardized adverse event reporting, and evidence-based guidelines integrating ASIA syndrome considerations into medical device evaluation processes.

CONCLUSION

ASIA syndrome represents a valuable conceptual framework linking adjuvant exposure to autoimmune manifestations, advancing understanding of immune dysregulation mechanisms despite ongoing nosological debates. Clinical utility remains evident in identifying susceptible populations and guiding explantation decisions, though diagnostic specificity requires refinement. The syndrome's recognition has catalyzed enhanced vaccine adjuvant safety protocols and biomaterial biocompatibility assessments. Future directions necessitate prospective cohort studies, advanced immunophenotyping, and standardized diagnostic criteria to validate clinical significance while balancing individual patient care with population-level public health imperatives.

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