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1st Congress of the
Arab Academy of Allergy Asthma and Immunology

الأكاديمية الجزائرية لعلوم أمراض الحساسية والمناعة العيادية

Académie Algérienne d'Allergologie et Immunologie Clinique

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Les auteurs doivent respecter strictement les règles de fond et de forme indiquées dans ces recommandations et doivent adresser leurs textes directement par courriel à l'Académie Algérienne d'Allergologie.

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Sommaire

Prof. Reda DJIDJIK
President of the Algerian Academy of Allergology and Clinical Immunology
President of the Arab Academy of Allergology, Asthmology and Immunology

It is with immense pride and deep emotion that we announce the First Arab Congress of Allergology, Asthmology, and Immunology, to be held in Algiers under the High Patronage of the Prime Minister of Algeria, to whom I express my warmest gratitude for his trust and enduring support of science, medicine, and Arab cooperation in health.

My sincere thanks also go to the Minister of Health for his continued encouragement and attention to this important scientific initiative — a symbol of Algeria's commitment to strengthening biomedical research and regional collaboration in the service of public health.

Organized jointly with the 9th National Congress of Allergology and Clinical Immunology, this event marks a historic milestone in Arab scientific cooperation, bringing together experts, researchers, and clinicians to share knowledge, exchange experience, and advance innovation in allergy, asthma, and immunology.

We are honored to welcome distinguished colleagues and delegates from Mauritania, Tunisia, Libya, Egypt, Jordan, Iraq, Lebanon, Saudi Arabia, Yemen, Kuwait, the United Arab Emirates, Qatar, Syria, Palestine, Sudan, and Bahrain. Their participation reflects the vitality, unity, and excellence of the Arab scientific community.

We are also grateful to the World Allergy Organization (WAO) for its valuable collaboration and support, which grant this congress a truly international dimension and reinforce the visibility of Arab expertise on the global scientific stage.
We are particularly honored by the presence of the President of the World Allergy Organization himself. His participation demonstrates the importance he places on this new Arab initiative in allergology and immunology, and he will deliver a dedicated keynote lecture during the congress.

The congress will address a broad spectrum of topics, including:
- Asthma management in children and adults,
- Innovative biological therapies,
- Drug, food, and respiratory allergies,
- Inborn errors of immunity,
- Autoimmune diseases and immunotherapeutic advances.

Beyond the scientific program, this congress embodies a message of unity, solidarity, and hope.
It aims to establish lasting Arab scientific networks, promote education and research, and prepare a new generation of Arab scientists and clinicians ready to shape the future of allergy and

immunology in our region.

We are convinced that the medicine of tomorrow will rely on a deep understanding of the immune system, and on the principles of precision medicine, innovation, and collaboration.

I extend my heartfelt thanks to all members of the scientific and organizing committees, as well as to our partners and sponsors, whose dedication and efforts have made this vision a reality.

May this congress mark the beginning of a new era of Arab scientific excellence and cooperation, rooted in knowledge, solidarity, and shared ambition.

Welcome to Algiers —
A capital of science, unity, and knowledge.

Prof. Reda DJIDJIK
President of the Algerian Academy of Allergology and Clinical Immunology
President of the Arab Academy of Allergology, Asthmology and Immunology

Pr. Réda DJIDJIK
président de l'AAAIC
président de l'ArAAAI

الأكاديمية العربية
للحساسية والربو والمناعة
Arab Academy of Allergy
Asthma and Immunology

The Algerian Academy of Allergology and Clinical Immunology opens this year the ninth edition of its annual congress with a particular resonance, one that fits within an expanded and dynamic framework. For the first time, the event is held in conjunction with the First Arab Congress of the ArAAAI. This convergence gives the meeting a new dimension: it brings together, within a single scientific space, expertise from the Maghreb, the Middle East, and a broader international network engaged in the rapidly evolving fields of allergology and clinical immunology.

The program highlights the major advances that are redefining our discipline today. The rise of biotherapies in severe asthma and type 2 diseases, the decisive contribution of molecular diagnosis in food and respiratory allergies, the expanding role of genetic screening for primary immunodeficiencies, and the emergence of AI in clinical decision-making all reflect the accelerated evolution of knowledge. These breakthroughs require a refined understanding of immunological mechanisms and the ability to integrate translational research into daily practice.

This edition also places special emphasis on environmental determinants. The impact of climate change, pollinic dynamics, urban and occupational exposures, and the evolving microbiome, as well as challenges related to accessibility and the adaptation of international guidelines to local contexts, highlight the need for a systemic approach. The understanding of allergic and immunological diseases must be rooted in the continuous interaction between the organism and its environment.

The workshops and parallel sessions focused on interpreting immunological tests, managing anaphylaxis, optimizing lung function tests, and analyzing autoantibodies underscore the strong educational dimension of the congress. They emphasize the necessity of continuous skill development, which is essential in disciplines where diagnostic precision directly conditions the quality of patient care.

Finally, this congress reflects a collective commitment: to promote lasting scientific cooperation across the Arab world, to harmonize practices, and to support the emergence of young researchers and clinicians capable of driving innovation throughout the region.

May these days offer a fertile environment for sharing knowledge, strengthening collaboration between teams, and fostering an ambitious vision of allergology and clinical immunology in the service of patients.

Pr. Merzak Gharnaout
Vice president de l'AAAIC
Vice President de ArAAAI

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EDITORIAL

Climate Change and Its Impact on Allergic Disorders in the Middle East

Climate change is a global phenomenon that is having significant impacts on the environment and human health. In the Middle East and North Africa (MENA), the effects of climate change are becoming increasingly evident, with rising temperatures, changing precipitation patterns, and more frequent extreme weather events. One area of particular concern is the impact of climate change on respiratory allergic diseases. In the MENA region, temperatures are rising faster than global average. It is predicted that climatic extremes will worsen in the coming decades1. Respiratory allergic diseases, such as asthma and allergic rhinitis, are already a major public health issue, affecting a significant portion of the population. These conditions are characterized by inflammation of the airways and nasal passages, leading to symptoms such as coughing, wheezing, shortness of breath, and nasal congestion. Climate change is exacerbating these conditions in several ways.

One of the main ways in which climate change is affecting respiratory allergic diseases in the MENA region is through the increasing prevalence of airborne allergens. Warmer temperatures and changes in precipitation patterns are leading to longer and more intense pollen seasons, as well as the proliferation of mold spores and other allergens. This is particularly problematic for individuals with allergies, as exposure to these allergens can trigger asthma attacks and exacerbate symptoms of allergic rhinitis.

In addition to the increased prevalence of allergens, climate change is also contributing to poor air quality. Rising temperatures and changing weather patterns are leading to more frequent episodes of air pollution, which can worsen respiratory symptoms in individuals with allergic diseases. Pollutants such as particulate matter, nitrogen dioxide, and ozone can irritate the airways and exacerbate inflammation, making it harder for people with respiratory allergies to breathe. Recently we conducted a study in Lebanon to explore the impact of climate change on allergen sensitization. We used the Geiger-Köppen climate classification that divides Lebanon in two major zones: a hot and humid coastal plain and a dryer and cooler North and Inland. We found an increase sensitization to trees and molds in the respective climate zones but not to house dust mites and wall pellitory[4].

Furthermore, climate change is also impacting the spread of infectious diseases, which can have indirect effects on respiratory health. As temperatures rise, the range of disease-carrying vectors such as mosquitoes and ticks is expanding, increasing the risk of vector-borne diseases such as dengue fever and Lyme disease. These infections can trigger respiratory symptoms in susceptible individuals and further strain an already overburdened healthcare system.

To mitigate the impact of climate change on respiratory allergic diseases in the MENA, it is crucial for policymakers to take action to reduce greenhouse gas emissions and limit global warming. Additionally, public health measures such as improving air quality, promoting green spaces, and raising awareness about the health effects of climate change can help to protect vulnerable populations from the negative impacts of environmental changes.

In conclusion, climate change is having a significant impact on respiratory allergic diseases, exacerbating symptoms and increasing the burden on individuals with these conditions. By taking proactive measures to address the root causes of climate change and improve respiratory health, we can work towards a healthier and more sustainable future for all.

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REVIEW ARTICLE

NLRP3 inflammasome in different autoimmune diseases

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KEYWORDS

Inflammasome

NLRP3

Abstract

Inflammasome is an intracellular protein composed of a multimeric complex. It is an integral part of the innate immune system targeting the immune cellular homeostasis. The dominant particles/domains in the complex guide its name and function. Researches are ongoing for better understanding of inflammasomes function and their role in different pathologies, therefore the possibility of targeting them with therapeutic agents. NLRP3, specifically, was widely studied and identified in a group of autoinflammatory, autoimmune, degenerative and malignant disorders. Its activation can occur through different exogenous triggers such as microbial agents, or endogenous factors as cellular stress. The latter can explain the participation of NLRP3 in the pathogenesis of autoimmune disorders. Activation of NLRP3 promotes the cleavage of potent proinflammatory cytokines and, hence, forming the functioning form of caspase-1. This process is a part of the innate immune response and, in parallel, the signaling through activating IL-1β and IL-18 is related to the adaptive immunity. In this review, we tried to delineate the importance of NLRP3 inflammasome and its downstream signals in selected autoimmune diseases, and to outline the possible future therapeutic strategy.

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Inflammasomes:

Inflammasomes are multimeric proteins reside in the cellular cytoplasm weighted molecularly 700 KDa. The main 3 components involved in the structure of any inflammasome are sensor or adaptor protein, pattern recognition receptor (PRR) and the proinflammatory caspase-1 [1]. PRR works as an apoptosis-related speck-like component (ASC) with a caspase-recruitment domain (CARD). Inflammasome complex guides the verified function of PRRs in recognizing pathogen-associated molecular pattern (PAMP) and damage-associated molecular pattern (DAMP) [2]. Both external and internal triggers can promote inflammasome activation and initiate the cascade of inflammasome self-cleavage, followed by activation of the proinflammatory cytokines interleukin (IL)-1β and IL-18, and thereafter, promotion of caspase-1 into its active form [3,4]. Inflammasome activation and its downstream proteins function to keep the tissue homeostasis and repair [5].

The first described PRRs as parts of the inflammasome complex were (1) NLRs: nucleotide-binding oligomerization domain (NOD) and leucine-rich repeat (LRR)-containing receptors, (2) ALRs: the absent in melanoma-2 (AIM2)-like receptors and (3) proteins containing a tripartite motif (TRIM), including pyrin. Inflammasome cascade starts upon PAMP or DAMP recognition through one of the aforementioned PRRs, leading to ASC recruitment and generation of a new multimeric complex or 'speck'. Afterwards, cleavage of procaspase-1 into the bioactive caspase-1 follows, leading to stimulation of pro-IL-1β and pro-IL-18 into their active forms. This process completes through activation of the pore-forming gasdermin D (GSDMD) and induction of pyroptosis or inflammatory cell death (Figure 1) [3,6]. Role of inflammasome can be outlined in defending against exogenous pathogens, removing the damaged cell debris, and cross-talking with the adaptive immune cells. Aberrant inflammasome activation is incorporated in many autoinflammatory and autoimmune disorders, infectious diseases and malignancies [3], due to the augmented injurious inflammation, such as in diseases like cryopyrin-associated periodic syndromes (CAPS), diabetes, obesity, atherosclerosis, crystal-related diseases (silicosis and gout), autoimmune diseases (multiple sclerosis, inflammatory bowel disease and lupus), and renal diseases, [acute kidney injury (AKI) and chronic kidney disease (CKD)] [7,8].

Inflammasomes assembly:

Domains of NLRs determine their designation. These domains are 1) a central nucleotide-binding domain (NBD), 2) LRR domain (except in NLRP10), 3) N-terminal (pyrin/CARD), and 4) C-terminal (LRR domain). N-terminal classifies NLRs into NLRP (leading to indirect caspase-1 activation) or NLRC (leading to direct caspase-1 activation) receptors. Inflammasome assembly requires either homotypic CARD–CARD or PYD–PYD interactions, to induce oligomerization [10]. ASC adaptor protein gathers NLRP3, AIM2 and pyrin together in to the inflammasome complex [11]. Alternatively, inflammasome assembly can be mediated through the ALR family instead of LRR and the RIG-I-like receptor (RLR) family [12].

Inflammasome downstream proteins and caspase-1 activation:

NLRP1 inflammasome

Inflammasome containing NLR pyrin domain (NLRP)1 assembly occurs through two main ligands: muramyl dipeptide (MDP), a combined fragment from both Gram positive and negative bacteria, and Bacillus anthracis lethal toxin [13]. NLRP1 oligomerizationrequires the interaction between NLRP1, MDP and adenosine triphosphate (ATP), leading to caspase-1 cleavage. The process is enhanced by the adaptor protein ASC [14].

NLRP3 inflammasome

NLRP3 consists of a sensor PYD, an adaptor (ASC) and an effector (caspase 1). The N-terminal, PYD, is responsible for NLRP3 oligomerization and function. The T-terminal, LRR, promotes autoinhibition by folding back onto PYD. ASC is responsible for the domains' cross-talking [15]. NLRP3 assembly starts with ASC recruitment, followed by cleavage of caspase 1 and motivation of IL-1β and IL-18, leading to pyroptosis. NLRP3 stimulation uniquely depends basically on PAMPs, DAMPs and environmental irritants recognition, through antigen presenting cells, Toll-like receptor (TLR), tumor necrosis factor (TNF)-α, a transcription regulator nuclear factor-B (NF-kβ) [14, 16].

NLRP3 oligomerization occurs through 3 models: the ion flux, the reactive oxygen species (ROS) and the lysosome rupture model. Other noncanonical cascades can

participate in NLRP3 assembly involving caspase-11 cleavage by intracellular lipopolysaccharides (LPS), a mechanism associates the rupture of bacteria-loaded phagolysosomes [12].

In the ion flux model, changes in specific cytosolic cations, such as Kþ, Ca2 þ and Hþ, would activate NLRP3 [16]. Extracellular ATP activates the ATP-gated ion channel P2X7 and triggers rapid Kþ efflux; resulting in caspase-1 cleavage. This model can, additionally, stimulate NLRP1b and NLRC4 assembly [17]. Oxidative stress and release of ROS can activate NLRP3 oligomerization directly and indirectly via ATP, alum, uric acid and Nigericin. However, the precise mechanism remains controversial. It is suggested that ROS inhibitors deactivate NLRP3 through inhibiting NF-kB and pro-IL-1b transcription [18]. Furthermore, the oxidized mitochondrial DNA released from dysfunctional mitochondria and ROS-independent mitochondrial cardiolipin can directly oligomerize the NLRP3 inflammasome [19]. Lysosomal rupture associating phagocytosis of large particulate materials, such as urate crystals, alum, silica, malaria, hydroxyapatite and amyloid-b, can activate NLRP3 inflammasome. Inhibitors of the lysosomal protease Cathepsin B deactivate NLRP3 and NLRP1b [20].

NLRP6 and NLRP12 inflammasomes

Co-expression of NLRP6 or NLRP12 with ASC results in synergistic activation of caspase-1 [21]. NLRP6, unlike NLRP3, is highly expressed in non-hematopoietic cells, mainly intestinal epithelial and goblet cells, to maintain intestinal homeostasis. It contributes in mucus secretion by goblet cells through activating IL-1b- and IL-18, and the downregulation of NF-kB and MAPK signaling [22]. NLRP12 approximates the functional features of NLRP6. It protects against colitis and colon cancer [22], and maintain intestinal homeostasis by negatively regulating inflammatory NF-kB and MAPK.

AIM2-like receptors (ALRs) and RIG-I-like receptors (RLR)

AIM2 and ALRs are members of the pyrin family, containing PYD and DNA-binding domain. These proteins can theoretically bind nucleic acids and recruit ASC to form inflammasomes [23]. AIM2 stimulates inflammasome assembly through recognizing pathogenic or self-DNA [24]. The RIG-I like receptor (RLR), type I interferon (IFN) production inducer, can trigger inflammasome formation [25].

Role of NLRP3 inflammasome in systemic lupus erythematosus (SLE):

In SLE, the increased rate of apoptosis combined with an inefficient clearance of apoptotic cells leads to accumulation of DAMPs and activation of NLRP3. Other DAMPs can activate NLRP3 including nucleic acids, the main self-antigens in SLE, through TLR activation [26].

Neutrophil extracellular traps (NETs) are granule proteins bound to chromatin fibers and produced by activated neutrophils to kill extracellular pathogens. NETs are effective promotors of NLRP3 inflammasome in macrophages. SLE patients are more sensitive to NETs-related inflammasome assembly than healthy controls. Low-density granulocytes (LDGs) are a distinct subset of pro-inflammatory neutrophils detected in patients with SLE. LDGs promote NETosis and the related expression of proteins and enzymes, leading to a higher exposure of immunostimulatory molecules and inflammasome oligomerization. Active IL-18, released by NLRP3 assembly, induces NETosis [27].

Abnormal autophagy is another involved mechanism in NLRP3 stimulation among SLE patients. Physiologically, autophagy is a regulatory mechanism responsible for removing the excess or abnormal cellular components, leading to tuning inflammasome activation. SLE patients have downregulated macrophage autophagy, with hyperactivation of inflammasome in response to the crowded DAMPs and loss of degradation of inflammasome proteins and IL-1 [28]. In cutaneous SLE, NLRP3 assembly contributes to the chronic inflammatory status, through upregulation of IL-1β [29].

NLRP3 is located in chromosome region 1q43-q44 and contains 3 kb upstream of the transcription exons and introns, and 2 kb downstream of the stop codon. Among 60 single nucleotide polymorphisms (SNPs) coding the NLRP3 gene, the five most studied in SLE were rs10754558, rs4612666, rs3806268, rs35829419 and rs4352135 [30]. The rs3806268 GG was associated with low C4 and the rs4612666 TT genotypewas associated with anti-SSA positive rate. SNP rs2043211 increases the risk of SLE among males [31]. In a Brazilian study,rs10754558 was initially associated with SLE development, and rs10754558 G allele was significantly

associated with lupus nephritis (LN) [32]. In an Iranian study, three NLRP3 SNPs were evaluated, two gain of function alleles (rs10754558 and rs4612666) and one loss of function SNP (rs6672995), were associated with underproduction of IL-1. SNP rs10754558 was associated with higher risk of SLE development. Lower age of onset was related to C and G alleles for rs4612666 and rs10754558, respectively. Patients with the C allele for rs461266 had higher levels of ESR, anti-dsDNA, C3 and C4 [33]. However, G allele for rs10754558 caused lower levels of C3 and C4. SNPs rs4612666 and rs10754558 were associated with increased risk of neurological and renal involvement [34].

Role of NLRP3 inflammasome in lupus nephritis (LN):

NLRP3 overstimulation was involved in the pathogenesis and progression of LN and other kidney diseases. Renal mononuclear cells (dendritic cells and macrophages) and parenchymal cells (podocytes, endothelial, parietal and tubular epithelial cells) express the components of NLRP3 inflammasome in response to various PAMPs and DAMPs [28]. Ischemic reperfusion kidney injury can lead to cell damage and renal tubular cell necrosis, followed by release of endogenous DAMPs and NLRP3 cleavage [35]. In pediatric patients with LN, peripheral expression of NLRP3 was significantly correlated to SLE activity index [36].

Role of NLRP3 inflammasome in systemic juvenile idiopathic arthritis (SJIA) and recurrent macrophage activation syndrome (MAS):

An extremely rare heterozygous missense variant, c.482G>A, p.R161H was detected in the CASP1 gene encoding pro-caspase-1 in a patient with SJIA and recurrent MAS (single case report). This gain-of-function allele for both NLRP3 and NF-κB augments the release of IL-6, IL-1β and IL-18. LPS stimulation induces high expression of IL-6 and activation of the NLRP3 inflammasome and capase-1. Expression of the CASP1 variant in an NF-κB reporter luciferase assay stimulates NF-κB activation through RIP2-dependent model [37].

Role of NLRP3 in rheumatoid arthritis (RA):

Rheumatoid arthritis (RA) is associated with overproduction of inflammatory cytokines in the sera and synovia, mainly TNFα, IL-6, and IL-1β [38]. The latter expression in the synovial membrane activates chondrocytes and osteoclasts, leading to cartilage erosion and bone shedding, in addition to over-release of synovial IL-18 [39]. NLRP3 expression in mononuclear cells is upregulated in RA patients [40, 41], but not in the neutrophil, making the contribution of NLRP3 in the inflammatory status of RA debatable. Furthermore, IL-18 levels increase, but not IL-1, denoting that caspase-1 activation is mediated by IL-18 rather than NLRP3 assembly [42].

Role of NLRP3 in systemic sclerosis:

NLRP3 inflammasome is incriminated in the underlying pathogenesis of systemic sclerosis and its associated synovitis, arthritis, fibrosis and vascular damage, through the activation of proinflammatory Th2 cells, profibrotic M2 macrophages, B cells, fibroblasts and endothelial cells, leading to promotion of fibro-necrotic changes [43].NLRP3 and its cascade proteins were expressed profusely in sera and skin biopsies of patients with systemic sclerosis, with significant relevance to cutaneous and pulmonary affection [44]. NLRP3 contribution in systemic sclerosis can be summarized in 2 models: 1) the inhibitory influence on M2 macrophage activation, leading to suppression of the disease progression. It is known that M2 macrophages promote production of profibrotic cytokines including IL-4, IL-13, and transforming growth factor β (TGF-β), leading to overexpression of extracellular matrix (ECM) and fibrosis [45,46]. 2) the effect of NLRP3/IL-1 signaling activation on modulating Th1/Th2 balance and B cells-mediated expression of autoantibodies, pro-fibrosis cytokines and stimulation of fibrosis [46,47]. Notably, B cell activators such as tyrosine phosphatase N22 and BAFF, were reported to activate NLRP3, augment NF-Kβ expression and upregulate ROS and K+ efflux [48].

The expression of NLRP3 in skin biopsies is positively relevant to levels of endothelin (ET)-1 [49]. Suppression of nitric oxide (NO), a potent vasodilator, and increasing ET-1 expression are the key modulators of vascular abnormalities in systemic sclerosis. NO acts as an inhibitor of NLRP3 inflammasome assembly through scavenging dysfunctional mitochondria [50,51]. MiR-155, an activator of NLRP3, is commonly expressed from the fibroblasts of systemic sclerosis patients [52]. Cutaneous NLRP3 was positively correlated with modified Rodnan skin thickness score [49].

Role of NLRP3 in autoimmune renal diseases:

Inflammasomes trigger autoimmune renal diseases using the influence of IL-1β and IL-18 on deviating the T cell response, particularly T helper (Th) cells, towards Th17 and Th1. IL-1 inhibitors and two specific NLRP3 inflammasome inhibitors, MCC950 and β-hydroxybutyrate, showed promising results in the inflammasome-mediated conditions [8].

Puromycin amino-nucleoside (PAN) induces cellular stress in the podocytes through activation of NLRP3 inflammasome. PAN was commonly used to promote nephrotic syndrome (NS) in rodents.The excess of NLRP3, IL-1β and IL-18, can injure the renal tubular cells leading to impaired kidney functions. IL-1β induces inflammation through activation of NF-Kβ, which in turn provokes a potentially fibrinogenic cascade including generation of protein kina, ROS and TGF-β1 [53].

Role of NLRP3 inflammasome in ANCA-associated vasculitis (AAV):

Anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV) can be stimulated by active IL-18 and IL-1β, and the generated ROS by activated neutrophils. ANCA causes TNF-induced neutrophils degranulation and production of ROS. IL-18 is upregulated in the kidney in AAV. In myeloperoxidase glomerulonephritis (GN), Th1 and Th17 hyperstimulation, mediated by IL-1β and IL-18, can induce glomerular injury in experimental anti-myeloperoxidase GN. Exposure to DAMPs or PAMPs in patients with AAV could activate inflammasome directly or through provocation of NETs. Notably, NETs are involved in the renal affection in AAV, rising the risk of GN [8].

NLRP3 inflammasome inhibitors:

NLRP3 inflammasome inhibitors can act directly by blocking the protein function or indirectly by targeting P2X7 receptor, ASC, caspase-1, IL-1 or RIP3. MCC950, a highly-selective NLRP3 inhibitor, can attenuate the severity of autoimmune encephalomyelitis, LN, steroid-resistant asthma and diabetic nephropathy in mice [54-56], through downregulation of NLRP3, caspase-1 and IL-1 production. It was associated with significant improvement in the podocyte effacement and heaviness of proteinuria in short time [57]. MCC950 blocks ATP hydrolysis and inhibits NLRP3 inflammasome formation and activation by directly interacting with the NACHT domain [58,59]. Phase II studies in RA was suspended due to drug toxicity [15].

Similar to MCC950, tranilast can bind to the NACHT domain of NLRP3, showing promising impact on reducing arthritis in mice with minimal side effects [60]. Another NLRP3 inhibitor, CY-09, specifically binds to the ATPase domain hindering the oligomerization of NLRP3, and reducing IL-1β synovial expression in patients with gouty arthritis [61]. It can alsodecrease myocardial fibrosis induced by ischemia [62]. Besides, miRNAs combine with the 3 'UTR sites of NLRP3 transcripts, causing NLRP3 inflammasome downregulation in RA and SLE mouse models [63].

Another NLRP3 direct inhibitor, Tris dibenzylidene acetone dipalladium (DBA), led to marked reduction of proteinuria, lower anti-dsDNA antibodies levels and improved kidney function in mice with LN compared with control mice [23]. Tris DBA acts through regulation of autophagy mediated by the NLRP3 inflammasome and T-cell function along with phosphorylation inhibition of JNK, ERK and p38 MAPK signaling pathways via ROS-mediated inflammation [64].

A20, a TNF-α-induced protein 3, reduces NLRP3-mediated inflammation and pyroptosis, therefore, reducing the risk of pulmonary fibrosis in patients with RA [65]. Another promising NLRP3 suppressor is β-hydroxybutyrate (BHB), an oxidative metabolite of fat, which downstreams NLRP3 activation through inhibiting K+ efflux and ASC speck formation [66].

The ROS inhibitor, Citral (3,7-dimethyl-2,6-octadienal), an active component of the Chinese herbal Litsea cubeba, and M1, a bioactive ginseng, seem to be effective through inhibition of NLRP3 activation, ROS, COX-2 production and Nrf2 activation. M1 significantly suppresses NLRP3 inflammasome activation in podocytes through autophagy induction [67]. ROS-inhibition using chemical scavengers of ROS or inhibitors of NADPH oxidase could down-stimulate NLRP3 inflammasome in response to several stimuli [68].

BBG treatment can reduce the serum levels of IL-1, IL-17 and the Th17/Treg cell ratio. Its efficacy in reducing LN inflammation was confirmed in NZM2328 mice. In kidney tissue, RIP3 activation leads to podocyte necrosis and NLRP3 oligomerization. GSK872, an inhibitor of RIP3,

MLKL and caspase-1, could reduce glomerular and tubulointerstitial inflammation with less IgG and C3 deposition in the kidney tissue [48].

AZD 1208, an inhibitor of PIM-1 in NZb/WF1 mice, suppressed the renal NLRP3 and caspase-1 activation with less IL-1B and proteinuria, through reduction of the calcium influx [69].Caspase-1 blockade is anotherstrategy to downregulate NLRP3 inflammasome. YVAD-CHO is a reversible caspase-1 inhibitor, which reduces IL-1β excretion in systemic sclerosis monocytes. Z-YVAD-FMK blocks overexpression of IL-1β in fibroblasts from skin and lung of systemic sclerosis patients [70]. Sinkihwan-gamibang, a recent Korean therapeutic agent against PAN-induced renal injury and NS, showed promising clinical improvements in patients with chronic nephritis and NS [53].

Conclusion

NLRP3 inflammasome has gained marked interest in the last decade due to its fundamental role with its downstream proteins in autoimmune diseases such as lupus, lupus nephritis, systemic sclerosis, ANCA-related vasculitis among others. Research is lacking as regards the young population; however, a new therapeutic hope has emerged based on the current studies on NLRP3.

Declaration on Interest

Authors declare that they have no conflict of interest.

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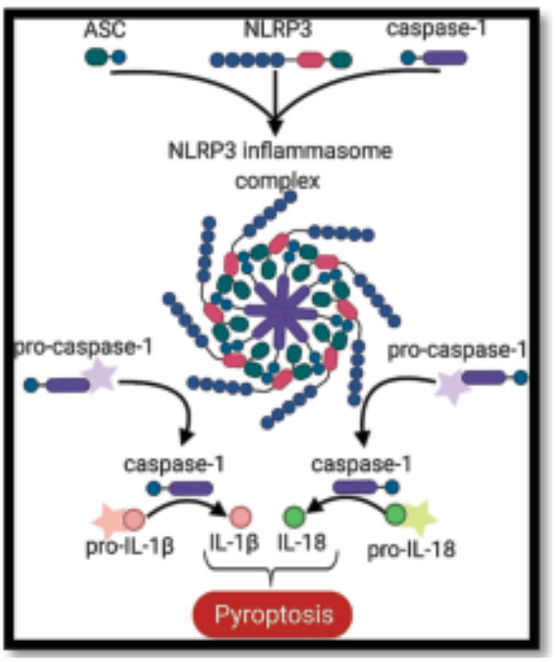

Figure (1): Formation of the NLRP3 inflammasome. NLRP3 inflammasome components (NLRP3, ASC and caspase-1) bind to form NLRP3 inflammasome complex, leading to the cleavage of pro-caspase-1 into its active isomer, caspase-1, which in turn activates pro-IL-1β and pro-IL-18 into IL-1β and IL-18 respectively. The excess in the downstream proteins leads to pyroptosis [9]

Abbreviations:

AAV: ANCA-associated vasculitis
AIM2: absent in melanoma-2
AKI: acute kidney injury
ALRs: AIM2-like receptors
ANCA: anti-neutrophil cytoplasmic antibody
ASC: apoptosis-associated speck-like protein containing a caspase-recruitment domain
ATP: adenosine triphosphate
BHB:β-hydroxybutyrate
CAPS: cryopyrin-associated periodic syndromes
CARD: caspase-recruitment domain
CKD: chronic kidney disease
DAMP: damage associated molecular pattern
DBA: dibenzylidene acetone
ECM: extracellular matrix
ET: endothelin
GN: glomerulonephritis
GSDMD: gasdermin-D
IFN: interferon
IL: interleukin
LDGs: low-density granulocytes
LN: lupus nephritis
LPS: lipopolysaccharides
LRR: leucine rich repeat
MAS: macrophage activation syndrome
MDP: muramyl dipeptide
NBD: nucleotide binding domain
NETs: neutrophil extracellular traps
NF-Kβ: transcription regulator nuclear factor β
NLRP: NLR containing pyrin domain
NLRs: NOD and LRR containing receptors
NO: nitric oxide
NOD: nucleotide-binding oligomerization domain
NS: nephrotic syndrome
PAMP: pathogen associated molecular pattern
PAN: puromycin amino-nucleoside
PRRs: pattern recognition receptors
PYD: pyrin domain
RA: rheumatoid arthritis
RLR: RIG-1 like receptors
ROS: reactive oxygen species
SJIA: systemic juvenile idiopathic arthritis
SLE: systemic lupus erythematosus
SNPs: single nucleotide polymorphism
Th: T helper cells
TLR: toll-like receptors
TNF: tumor necrosis factor
TRIM: tripartite motif

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REVIEW ARTICLE

Syndromes d'atteinte multi-systémique dysimmunitaire : défis diagnostiques et rôle de l'intelligence artificielle

Immune-Mediated Multi-Organ Syndromes: Diagnostic Challenges and the Role of Artificial Intelligence

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MOTS CLÉS
Intelligence artificielle ; Maladies auto-immunes ; Algorithmes diagnostiques ; Syndromes dechevauchement

Résumé
Les syndromes multi-organes d'origine immunitaire, en particulier la connectivite mixte et le syndrome des anti-synthétases, posent un véritable défi diagnostique en médecine interne du fait de leurs manifestations cliniques chevauchantes et souvent atypiques. En brouillant les frontières entre différentes maladies auto-immunes systémiques, ces syndromes entraînent des retards diagnostiques et une incertitude thérapeutique. Dans ce contexte, l'intelligence artificielle apparaît comme un outil prometteur, capable grâce à l'apprentissage automatique d'intégrer des données cliniques et biologiques complexes. Cette revue analyse les difficultés diagnostiques majeures de ces syndromes, met en lumière les limites des classifications actuelles et propose un arbre décisionnel enrichi par l'IA pour améliorer la précision du diagnostic et la précocité de la prise en charge.

KEYWORDS
Artificial Intelligence; Autoimmune Diseases; Diagnostic Algorithms; Overlap Syndromes

Abstract
Immune-mediated multi-organ syndromes, notably mixed connective tissue disease and anti-synthetase syndrome, challenge internists due to their overlapping and atypical features. These conditions blur the lines between systemic autoimmune diseases, delaying diagnosis and complicating treatment. In this context, artificial

intelligence emerges as a valuable ally, using machine learning to integrate complex clinical and biological data. This review discusses the diagnostic complexity of these syndromes, critiques current classification systems, and proposes an AI-assisted decision tree to improve diagnostic accuracy and therapeutic timing.

Introduction

Systemic autoimmune diseases (SADs) such as systemic lupus erythematosus (SLE), systemic sclerosis (SSc), polymyositis (PM), dermatomyositis (DM), and Sjögren's syndrome (SS) often present with overlapping and heterogeneous clinical manifestations [1]. Patients may exhibit signs of more than one autoimmune disorder simultaneously, resulting in the classification of overlap syndromes. Examples include mixed connective tissue disease (MCTD), defined by anti-U1 RNP antibodies, and the anti-synthetase syndrome (ASS), characterized by anti-aminoacyl-tRNA synthetase antibodies such as anti-Jo-1, along with myositis, arthritis, and interstitial lung disease [2,3]. These disorders challenge internists due to their protean presentations and overlapping immunological profiles.

The diagnostic process is further complicated by the absence of universally accepted criteria, the variability in disease evolution, and the frequent occurrence of incomplete or atypical syndromes [4]. While specific autoantibodies provide valuable clues, they are not always definitive or sufficiently sensitive to distinguish between entities. As a result, misclassification and diagnostic delays remain common in clinical practice [5].

In response to these challenges, artificial intelligence (AI) has emerged as a promising tool to enhance diagnostic accuracy. Machine learning algorithms can analyze complex clinical and laboratory data, identify hidden patterns, and propose probabilistic diagnoses, particularly in diseases with overlapping features [6]. Recent applications in rheumatology have demonstrated that AI can support early detection, risk stratification, and treatment planning for autoimmune diseases [7].

This article provides a pedagogical overview of immune-mediated multi-organ syndromes, emphasizing diagnostic strategies in internal medicine and exploring how AI-driven tools can contribute to more accurate and timely clinical decision-making.

Methods

This narrative review was designed to synthesize current knowledge on immune-mediated multi-organ syndromes, particularly focusing on their diagnosis and the potential contribution of artificial intelligence (AI). An extensive search of the PubMed, Scopus, and Web of Science databases was performed, covering the period from January 2015 to April 2025.

The following search terms and combinations were used: "autoimmune overlap syndromes", "connective tissue diseases", "anti-synthetase syndrome", "mixed connective tissue disease", "systemic autoimmunity", "diagnostic delay", "artificial intelligence in rheumatology", "clinical decision support systems", "machine learning", and "internal medicine". Only articles published in English and involving human subjects were considered. The inclusion criteria comprised original research articles, systematic reviews, expert consensus documents, and case series relevant to the diagnosis and classification of systemic autoimmune diseases and their overlap syndromes.

Special attention was given to publications exploring the integration of AI tools in the diagnostic process. Studies addressing algorithms for differential diagnosis, risk prediction models, AI-assisted image interpretation (e.g., HRCT, MRI), and serological profiling using machine learning techniques were included. Articles without sufficient methodological detail or relevance to internal medicine practice were excluded.

In addition to database queries, manual screening of reference lists from included studies was performed to identify potentially relevant literature not captured in the initial search. The information gathered was categorized thematically into four domains: (1) clinical and immunological characteristics of overlap syndromes; (2) diagnostic limitations in internal medicine; (3) AI applications in autoimmune disease diagnostics; and (4) conceptual models integrating AI-assisted decision-making.

Additionally, reference lists of selected publications were screened to identify further relevant sources. Information was synthesized thematically, focusing on diagnostic challenges, the clinical utility of specific autoantibodies, and the integration of AI models in medical diagnostics.

Results

1. Clinical and immunological spectrum of overlap syndromes :

Overlap syndromes represent a diagnostically challenging subgroup of systemic autoimmune diseases, characterized by features shared across multiple well-defined connective tissue disorders. Mixed connective tissue disease (MCTD), for example, involves overlapping features of SLE, SSc, and PM, along with high titers of anti-U1 RNP antibodies [8]. The anti-synthetase syndrome (ASS), on the other hand, is hallmarked by the presence of aminoacyl-tRNA synthetase antibodies (particularly anti-Jo-1) and commonly presents with myositis, interstitial lung disease (ILD), non-erosive arthritis, Raynaud's phenomenon, and mechanic's hands [9,10].

Clinically, these syndromes often exhibit progressive, multi-organ involvement and systemic inflammation, with common presentations including respiratory symptoms, arthralgia, myalgia, fatigue, and cutaneous manifestations [11]. Internists must recognize atypical and incomplete presentations, as patients may not fulfill the full criteria for any single entity. Immunologically, autoantibody profiling remains a cornerstone of diagnosis. Anti-RNP, anti-Jo-1, anti-Mi-2, and anti-PL-7/PL-12 are particularly relevant in classifying these overlaps [12,13]. However, antibody specificity and clinical correlations are not absolute, adding to diagnostic ambiguity [14].

2. Diagnostic challenges:

Diagnosing systemic autoimmune diseases with overlapping features remains a formidable task, especially in early stages where clinical findings are nonspecific. Diagnostic criteria for SLE (EULAR/ACR 2019), systemic sclerosis (ACR/EULAR 2013), and idiopathic inflammatory myopathies (EULAR/ACR 2017) are primarily designed for classification rather than early diagnosis, and often fail to capture overlap syndromes [15,16].

Clinical reasoning is further hampered by variability in disease onset, multi-systemic involvement, and evolving serologies. The presence of only one or two defining features at presentation may lead to underdiagnosis or misclassification [17]. Furthermore, the interpretation of ANA patterns, ENA panels, and HRCT imaging often requires expert consensus, and even experienced clinicians may encounter delays in reaching a definitive diagnosis [18].

The use of algorithms or scoring systems such as the Kahn criteria or the CREST variant in systemic sclerosis can be informative, but remain insufficient in overlap settings. Delays in diagnosis can exceed 12 months in many patients, particularly in low-resource settings or where access to immunological testing is limited [19].

3. Artificialintelligence in autoimmunedisease diagnosis :

AI-based tools, particularly those employing machine learning (ML), have gained traction in recent years as aids for diagnostic decision-making in complex diseases such as SADs. Models using supervised learning algorithms—including random forests, decision trees, and neural networks—have been trained on structured clinical and serological data to differentiate autoimmune diseases with high accuracy [20,21].

CNNs have shown promise in interpreting HRCT scans to distinguish ILD subtypes in ASS patients [22], while other systems have been used to analyze ANA patterns and antibody profiles with greater consistency than human evaluators [23]. A study by Wang et al. (2023) demonstrated that ML models could outperform rheumatologists in classifying early-onset undifferentiated connective tissue disease [24].

Importantly, AI does not replace clinical judgment but augments it by identifying patterns not easily discernible by humans. Integration of electronic health records, real-time laboratory data, and imaging into AI systems is currently under evaluation in several centers, with encouraging preliminary results [25].

4. A Conceptual AI-Supported Diagnostic Tree :

We present a conceptual diagnostic decision tree that incorporates AI logic into clinical reasoning. The proposed framework ingests data such as clinical features (arthralgia, myositis, ILD), laboratory values (autoantibodies, ESR, CRP), and imaging (HRCT findings, musculoskeletal MRI) to produce a ranked list of probable diagnoses. Each branch of the decision tree is modulated by the probabilistic influence of key variables, refined through AI training datasets.

For instance, the presence of symmetric arthritis, positive anti-RNP, and skin involvement would activate branches

toward MCTD, whereas anti-Jo-1 positivity and ILD would reroute toward ASS. The AI module recalibrates the weighting as new data become available (e.g., follow-up labs), allowing for a dynamic diagnostic process.
Such models can be embedded into clinical decision support systems (CDSS) used in hospital information systems. While still largely experimental, early validation studies report increased diagnostic efficiency, reduced inter-observer variability, and improved early detection of complex cases [26,27].

Discussion

Systemic autoimmune overlap syndromes pose persistent challenges in internal medicine due to their variable clinical presentations, evolving serological profiles, and often inconclusive classification frameworks. These syndromes, such as MCTD and ASS, do not always fit within the strict boundaries of existing diagnostic criteria, especially in early or incomplete forms of the disease [28]. The delayed diagnosis in many cases—often extending beyond a year—can lead to suboptimal treatment outcomes, increased organ damage, and diminished quality of life [29,30].
Autoantibody profiling remains central to the diagnosis, yet variability in antibody expression and overlap between specificities (e.g., anti-RNP in both MCTD and SLE) contributes to diagnostic ambiguity. Furthermore, clinical signs such as Raynaud's phenomenon, arthritis, or ILD are not specific and may appear in numerous autoimmune conditions [31]. As such, internists must adopt a comprehensive syndromic approach, integrating clinical, serological, and imaging data over time.
In this landscape, artificial intelligence (AI) offers transformative potential. AI systems can synthesize multidimensional data—ranging from structured lab results to radiological images and clinical narratives—to detect latent patterns, suggest probabilistic diagnoses, and guide clinicians through complex decision trees [32]. Notably, studies have demonstrated that ML algorithms outperform traditional statistical models in distinguishing between overlapping autoimmune diseases and can predict disease progression with increasing accuracy [33,34].
One promising approach involves the construction of decision-support algorithms using supervised learning. These algorithms are trained on annotated datasets comprising thousands of patient records, each labeled with diagnostic outcomes and linked to relevant clinical features. The algorithm learns associations between variables such as autoantibody panels, clinical symptoms (e.g., arthritis, ILD, rash), and imaging findings (e.g., HRCT features of fibrosis), ultimately generating a probability score for differential diagnoses like MCTD, ASS, or undifferentiated connective tissue disease [35].
Advanced models may incorporate ensemble methods (e.g., random forests or gradient boosting) to improve performance and reduce overfitting. Others employ neural networks capable of processing unstructured data such as free-text clinical notes or radiological images [36]. The resulting system can dynamically update diagnostic probabilities as new data points become available—mimicking the iterative reasoning process of experienced clinicians. Importantly, these tools can be implemented in clinical dashboards, offering ranked differential diagnoses alongside recommendations for confirmatory testing.
Despite these advances, several barriers hinder the widespread adoption of AI in clinical practice. These include the need for high-quality, annotated datasets for training, concerns over data privacy and algorithmic bias, and the lack of standardized validation protocols across different healthcare settings [37,38]. Furthermore, there remains skepticism among clinicians regarding the interpretability and transparency of AI-generated outputs—known as the "black box" problem [39].

To ensure clinical relevance, AI tools should be developed collaboratively with clinicians and designed to be interpretable, auditable, and seamlessly integrable into electronic health records. Hybrid models that combine rule-based logic (e.g., existing classification criteria) with machine learning predictions may provide an optimal balance of explainability and performance [40].
In conclusion, the integration of AI into the diagnostic process of immune-mediated multi-organ syndromes holds great promise. By enhancing diagnostic precision, reducing variability, and assisting with early identification, AI can empower internists to manage these complex diseases more effectively. However, future efforts must focus on rigorous clinical validation, interdisciplinary collaboration, and ethical deployment to fully realize this potential.

Conclusion

Immune-mediated multi-organ syndromes, such as MCTD and anti-synthetase syndrome, represent a major diagnostic challenge in internal medicine due to their overlapping features and evolving immunologic profiles. Current classification systems are often insufficient, leading to delayed diagnosis and suboptimal care. Artificial intelligence, particularly through machine learning, emerges as a valuable asset capable of analyzing complex datasets to support early and accurate diagnosis. AI-based decision tools could enhance clinical reasoning, but their implementation must be transparent, ethically sound, and clinically integrated. Future progress depends on interdisciplinary collaboration to develop reliable, validated AI systems that can support more personalized and proactive care.

Conflict of Interest Statement

The authors declare no conflicts of interest in connection with this work.

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ARTICLE ORIGINAL

Vasculitis with antibody to the glomerular basement membrane about 25 cases

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KEYWORDS
Anti MBG
Vasculitis

Abstract

Introduction :

anti-MBG vasculitis is a rare autoimmune disease of small vessels, characterized by autoantibodies targeting the glomerular and alveolar basement membrane, thus defining renal-pulmonary syndrome.

Methods :

We conducted a descriptive, retrospective and monocentric study of anti-MBG vasculitis with renal involvement, diagnosed, treated and followed in nephrology at the CHU Béni-Messous between 2015 and 2025. Demographic, clinical-biological, histological, therapeutic and evolutionary data were collected from medical records.

Results :

We retrospectively studied 25 patients with anti-MBG vasculitis over 10 years, with a sex ratio of 1 and a mean age of 55.2 years (16-100 years). Twenty-two percent were masons. Forty percent had a history of High Blood Pressure. Half consulted for general signs (asthenia and deterioration of the general condition), and 3 for macroscopic hematuria. Typical pulmonary involvement (alveolar hemorrhage) was present in 40% of patients. At the time of diagnosis, 90% of patients had severe renal failure requiring dialysis. Anti-MBG antibodies were positive in 77.3% of cases, associated

with anti-MPO ANCA positivity in 27.3% of cases; 22.7% were negative and diagnosed by renal biopsy. A histological analysis, performed in 77% of patients, revealed extracapillary circumferential necrosis increasing the lesions. Fifty percent of the patients were already at a terminal stage. From a therapeutic point of view, all received corticosteroids (bolus then oral relay), 22/25 cyclophosphamide and 10 plasmapheresis. Three patients died of intra-alveolar hemorrhage before the start of immunosuppressive therapy. The renal prognosis **was** unfavorable, with dialysis in 21 patients, chronic kidney failure in 1 patient and death in 2 patients after 1 year of progression.

Conclusion :

anti-MBG vasculitis is a serious, potentially fatal and functional disease. A rapid diagnosis, based on the search for anti-MBG antibodies and, if necessary, a renal biopsy (in case of negative antibodies), is crucial to initiate emergency treatment.

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Introduction :

Glomerular Basement Membrane Antibody (anti-MBG) disease, also known as Goodpasturesyndrome, is a rare small vessel vasculitis. This autoimmune disease is characterized by the presence of autoantibodies targeting thebasement membrane of renal glomeruli and pulmonary alveoli, which leads to a pneumo-renal syndrome. It manifests as a rapidly progressive glomerulonephritis and sometimes alveolar hemorrhages. In 20 to 60% of cases, anti-neutrophil cytoplasmic antibodies (ANCA) are also observed. The therapeutic management must be rapid and combines plasma exchanges, systemic corticosteroids and an immunosuppressant (cyclophosphamide). The objective of our work is to describe the demographic, clinico-biological and histological characteristics of our population, as well as the therapeutic and evolutionary management, and to assess the overall prognosis of the disease in our population.

Materials and methods

We conducted a descriptive, retrospective, monocentric study in the nephrology department of Beni-Messous university hospital, covering the period from January 2015 to December 2024. Patients with an initial diagnosis of glomerular basal membrane disease (anti-MBG) based on the presence of circulating antibodies were included.MBG and/or by renal biopsy showing linear IgG deposits along the glomerular basal membranes.

Medical records were consulted to gather demographic, clinical (age, sex, history of smoking or occupational or toxic exposure, pulmonary and/or renal presentation), biological (creatinine, glomerular filtration clearance, haematuria, proteinuria, anti-MBG antibody titre, ANCA status) and anatomopathological (percentage of crescent glomeruli, interstitial fibrosis). Patients without complete records were excluded. The primary objective was to assess renal survival (defined as stopping dialysis or maintaining native kidney function at 12 months) and overall survival.

Results

Over a ten-year period (2015-2025), we included 25 patients (sex ratio 1) in our study. The mean age at diagnosis was 55.2 years, with extremes ranging from 16 to 100 years. Among these patients, 22% were in a manual profession (masons) and 40% had a history of high blood pressure. Half of the patients (50%) had presented for general symptoms (asthenia and deterioration of general condition), while 3 patients (12%) had consulted for gross hematuria.

Pulmonary involvement of alveolar hemorrhage type was observed in 40% of patients, and isolated renal involvement in 30%. At the renal level, all patients presented with severe insufficiency: creatinine exceeded 600µmol/l and 80% of them were anuric at the time of diagnosis, requiring hemodialysis. Proteinuria and hematuria were present in all cases. All patients (n = 25)

also had a biological inflammatory syndrome with an increase in CRP, sedimentation rate, and normochromic normocytic anemia

The search for anti-MBG antibodies (glomerular basement membrane) was positive in 77.3% of cases, and among them, 27.7% showed ANCA positivity with anti-MPO specificity.

Renal biopsy, performed in 20 patients, revealed extracapillary glomerulonephritis with linear IgG deposition in 24 patients (note: even though direct immunofluorescence did not show linear IgG depositions, serum antibodies were present). Half of the patients (50%) were already at the scar stage at the time of diagnosis.

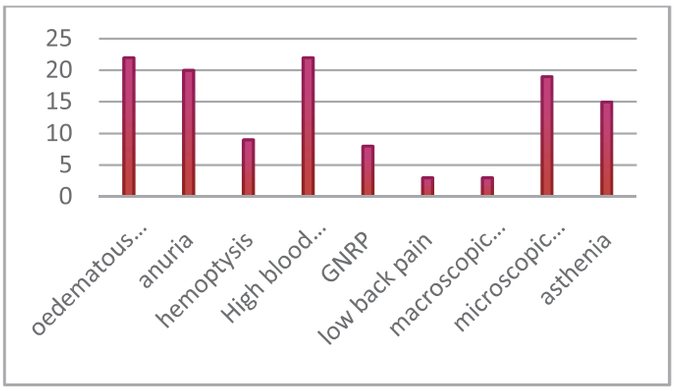


Figure 1. Clinical manifestations

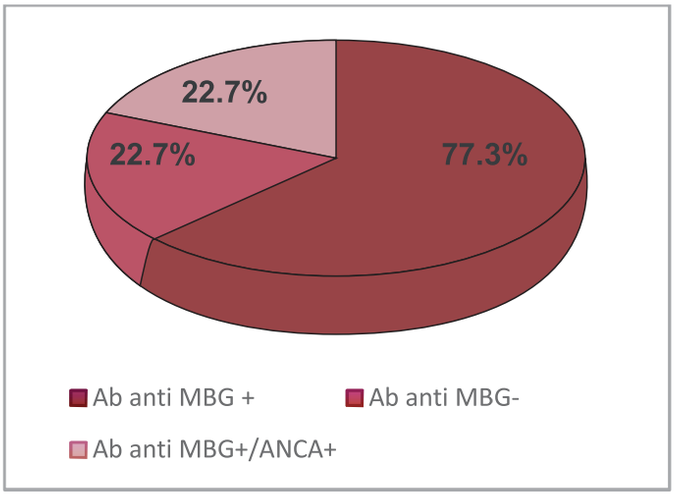
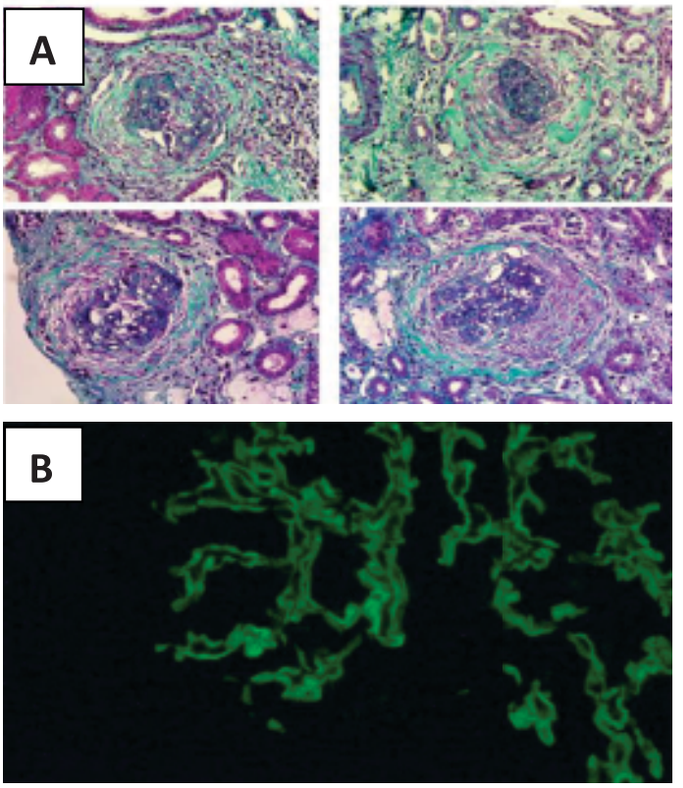


Figure 2. Results of the immunological assessment



Renal biopsy of a 15 year old girl,macroscopic heamaturia, Ab anti MBG negatif.

A: MO: Cortico-medullary 10 to 20 Glomeruli, of which 19 G are undergoing transformation into PAC, showing fairly homogeneous lesions, almost of the same age, sclerous Floccus retracted at the center, surrounded by fibrocellular circumferential crescents, rather fibrous, without fibrinoid necrosis.

B: IF:3 glomeruli: Intense positivity in discontinuous and linear traits along the capillary loops with C3 and IgG.

Therapeutically, corticosteroids were introduced in all patients : 3 bolus of 10 mg/kg/day, then a per os relay at 1 mg/kg/day. Cyclophosphamide induction was used in 22 patients (88%). The other three died of an intra-alveolar hemorrhage before they even received cyclophosphamide. Ten patients (40%) underwent plasmapheresis. After receiving histological results, immunosuppressive therapy was discontinued in 55% of patients who had renal impairment only and advanced stage, and maintained (with plasmapheresis) in 7 patients (28%) presenting with pulmonary involvement despite a poor renal prognosis. Azathioprine (Imurel®) was used as a maintenance treatment in a patient who remained immunologically positive.

The renal prognosis was unfavorable: 21 of the 22 patients followed lost kidney function, only one partially recovered the function. Three patients died after one year of progression: two from intra-alveolar hemorrhage and one from septic shock .

Discussion

We noted in our study that the average age of diagnosis is 55.2 years with extremes of 16 - 100 years. This suggests that the disease with anti-glomerular basal membrane antibody (anti-MBG) can affect both young and elderly subjects, which echoes what is described in the literature on the bimodal distribution of the disease. Sex ratio 1 indicates a nearly equal male/female distribution in our series. This is in line with some studies that do not show very clear sexual bias, especially at a later age [1]

The role of environmental factors in the onset of disease is suspected [2], 22% of our patients were engaged in a manual profession (masons), this may evoke a role of environmental or professional exposure (e.g., dust, solvents) in the onset of anti-MBG disease. The classic presentation of the disease characterized by a pneumo-renal syndrome concerns 60 to 80% of patients, while 20 to 40% of patients have isolated rapidly progressive glomerulonephritis [2], pulmonary involvement (alveolar hemorrhage) was observed in 40% and the isolated renal involvement in 30% shows that the typical presentation «lungs + kidneys» is present but not systematic. On the renal level, all patients had severe insufficiency (creatinine > 600 µmol/L) and 80% were anuric at diagnosis. This indicates that in our series, patients were already at a very advanced stage of the disease at the time of diagnosis. These conditions (very high creatinine, anuria) are of poor prognosis according to the literature.

The diagnosis of the disease is based on the search for an IgG1 isotype against the basal membrane of the circulating glomerular. Their research is a key diagnostic examination and must be carried out within 24 hours of admission to limit any diagnostic delay [3], The anti-MBG antibodies returned positive in 77.3%, of which 27.7% was associated with a positivity of MPO-specific ANCA, which shows that our series covers both the "classic" forms and the so-called "double positive" forms (anti-GBM + ANCA). The coexistence of anti-MBG antibodies and ANCA, or "double positivity", is more common than assumed by chance, with rates ranging from 21 to 60% (37,38). ANCA with anti-myeloperoxidase specificity (MPO) predominate, characterized in 66 to 81% of double-positive patients. The "double positives" (anti-GBM + ANCA) are described as having a more complex profile, sometimes better renal survival if early management, but also increased risk of recurrence [4],.

The renal biopsy performed in 20/25 showed extracapillary glomerulonephritis with linear IgG deposits in 19 patients (1 patient the direct immunofluorescence did not show linear IgG deposits, serum antibodies were present) : this emphasizes that some cases may be atypical or that the technique can be limiting. Half of the patients were already at the scar stage atthe time of diagnosis. All patients receivedcorticosteroidtherapy, the majority (88%) received cyclophosphamide induction treatment, and 40% benefited from plasmapheresis. The treatment was in accordance with the classical recommendations of the anti-GBM disease[5],. The fact that in 55% of patients, immunosuppressive treatment was stopped (in cases of renal disease only and advanced stage) while in 28% it was maintained (pulmonary disease despite poor renal prognosis) reflects a pragmatic strategy taking into account the very bleak prognosis in case of major kidney injury.his confirms the delay in diagnosis and undoubtedly explains the poor recovery of kidney function.

Renal prognosis was very poor: 21 of the 22 patients followed lost kidney function, only one partially recovered. In addition, three deaths (two from intra-alveolar hemorrhage and one from septic shock) after a year of evolution. This rate of kidney function loss is extremely high but consistent with the fact that the majority of patients had a very poor renal condition at diagnosis (creatinine > 600 µmol/l, anuria). The literature indicates that kidney function at diagnosis is the best predictor of recovery. In a review of 79 Swedish patients with anti-GBM, at 6 months 34% were deceased, 41% on dialysis, only 25% alive with functional kidney[6], The best predictor of renal survival was renal function at diagnosis. Your series goes in the same direction, but with even more severe results, probably because the population was already in a very advanced stage.

Conclusion

Our study highlights an often-dreaded clinical reality: despite the implementation of recognized treatments, when anti-MBG disease is diagnosed late, with severe renal failure and anuria, the renal prognosis remains very unfavorable. This reinforces the importance of clinical vigilance, early diagnosis, and possibly increased screening in at-risk situations. Moreover, the significant proportion of forms associated with ANCA or seronegative invites a nuanced look at this autoimmune pathology.

Conflict of interest statement

No conflicts of interest to be disclosed.

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ARTICLE ORIGINAL

Immuno-inflammatory evaluation in patients with psychiatric disorders

Evaluation immune-inflammatoire des patients atteints des troubles psychiatriques

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MOTS CLÉS
Auto-antibodies ;
Psychosis ;
Inflammatory ;
Biomarkers.

Abstract

The molecular mechanisms underlying psychiatric disorders are complex, involving interactions between genetic, environmental, inflammatory, and epigenetic factors, as well as dysfunctions at the level of neurotransmitters, synapses, and neural networks. Currently, neuroinflammation plays an increasingly recognized role in the development and worsening of psychiatric disorders such as depression, schizophrenia, and anxiety disorders. This chronic inflammation can disrupt the normal functioning of the brain, affecting neural circuits and contributing to

symptoms specific to each disorder.
This study aims to evaluate the link between psychotic disorders and immuno-inflammatory processes. It is based on the analysis of 95 patients with psychiatric disorders, compared to 30 healthy controls. The results highlight a significant increase in inflammatory markers and a notable presence of autoantibodies in a subset of patients. These findings suggest the existence of an immune-mediated subtype of psychosis, paving the way for a personalized therapeutic approach.
In summary, inflammation is increasingly recognized as an important factor in psychiatric disorders. Understanding of the underlying mechanisms of inflammation will help improve patient management and quality of life.

Introduction :

Inflammation, long integrated as part of the innate immune response and considered a defensive reaction of the body [1], is now increasingly recognized as playing a key role in the emergence and development of various psychiatric disorders such as depression, anxiety, and schizophrenia [1, 2, 3, 4]. The concept of immunopsychiatry, as an emerging discipline, studies this complex link, to explore possible therapeutic strategies, particularly those based on modulation of the immune system to achieve better control of psychiatric pathologies, especially treatment-resistant forms [5].

Over the past few years, many studies have highlighted a close association between immuno-inflammatory processes and the onset of psychiatric disorders, particularly psychoses [5]. In addition, several observations have established a strong link between autoimmune diseases (AID) and psychiatric disorders, showing not only an increased risk of mental health, but also that psychological factors may play a role in the development or exacerbation of AID [5, 6].

The pathophysiology of the concept AID, which often exhibit a higher prevalence of psychiatric symptoms, suggests a pathogenic interaction between systemic inflammation and brain dysfunction [5, 6]. This relationship is reinforced by the identification of a shared immunogenetic background and by the presence of autoantibodies (auto-Abs) in the serum of some psychotic patients [7, 8]. During neuroinflammation, pro-inflammatory cytokine levels increase significantly, including interleukin-6 (IL-6), which has a proven neurotoxic role. This increase is observed more markedly in individuals experiencing their first psychotic episode compared to healthy subjects. Elevated levels have been found not only in peripheral blood but also, surprisingly, in cerebrospinal fluid, indicating that inflammation is also present in the brain [9, 10]. similary Post-mortem studies have also shown significantly increased levels of IL-6-inducing proteins and NF-κB in the brains of patients with schizophrenia, indicating activation of the inflammatory network within the brain [10, 11].

This inflammation can alter the functionality of neural circuits responsible for emotions and behavior, leading to depressive syndromes observable through functional imaging (fMRI), particularly in patients with elevated levels of C-reactive protein (CRP) [12]. This disruption of communication between different regions can, by itself, cause decreased energy, fatigue, anhedonia, slowed thinking, and even difficulty following conversations or remembering things [12].

In addition, Recent studies have also identified new auto-Abs targeting specific brain receptors, especially in subgroups of patients with schizophrenia or other psychotic disorders, which can in some cases impair synaptic plasticity [6, 9]. These auto-Abs may serve as stratification biomarkers, offering not only a better understanding of the underlying pathophysiological mechanisms but also the possibility of developing targeted therapeutic strategies [8].

Today, research increasingly indicates that major psychiatric illnesses such as schizophrenia, depression, or bipolar disorders should also be considered immuno-inflammatory diseases. It turns out that all of these diseases do not only reside in the brain, but they have a systemic component, that is to say, the immune system. [6, 7, 8, 9].

Within this context, the present study aimed to assess the frequency of autoantibodies in a population of patients

with psychiatric disorders, study the inflammatory status of these patients through CRP measurement and serum protein electrophoresis, and evaluate the clinico-biological correlation between inflammation, autoimmunity, and clinical expression.

Material andmethods

This was a prospective descriptive study conducted between 2020 and 2024 at the Immunology Unit of the Department of Medical Biology, Blida University Hospital. Patients included in our study were recruited from the Psychiatry Departments (EHS Frantz Fanon), the Neurology Department (Blida University Hospital), and other hospitals across different regions of the country. Psychiatric disorders were diagnosed according to the DSM-5 classification criteria.

Some patients also underwent consultations in the Rheumatology Department of the "Ibrahim TERICHINE" Hospital and the Internal Medicine Department of Blida University Hospital.

Out of 125 recruited patients, 95 were selected for the study, all presenting syndromic psychiatric disorders with somatic rheumatologic or neurological manifestations. Additionally, 30 healthy controls were included, with no history of psychiatric, inflammatory, or autoimmune disorders.

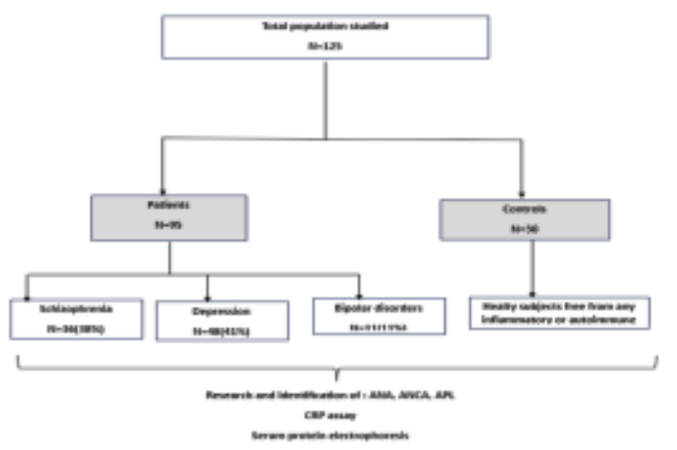

Figure 1. Flow diagram of study population recruitmen

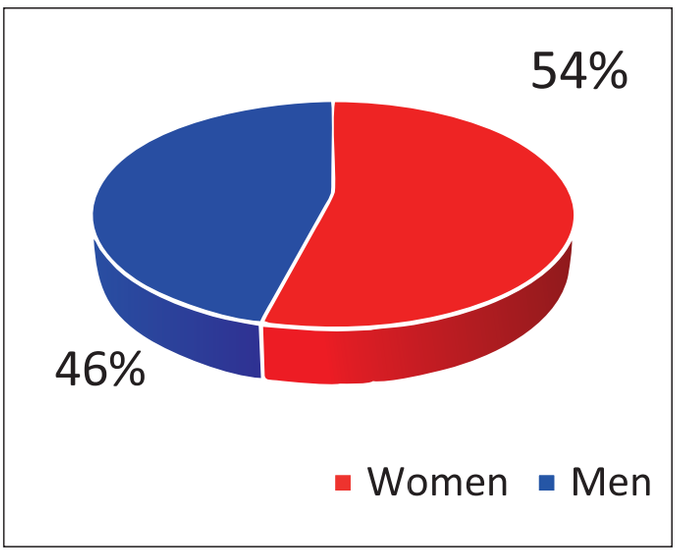

Figure 2. Distribution of patients by sex

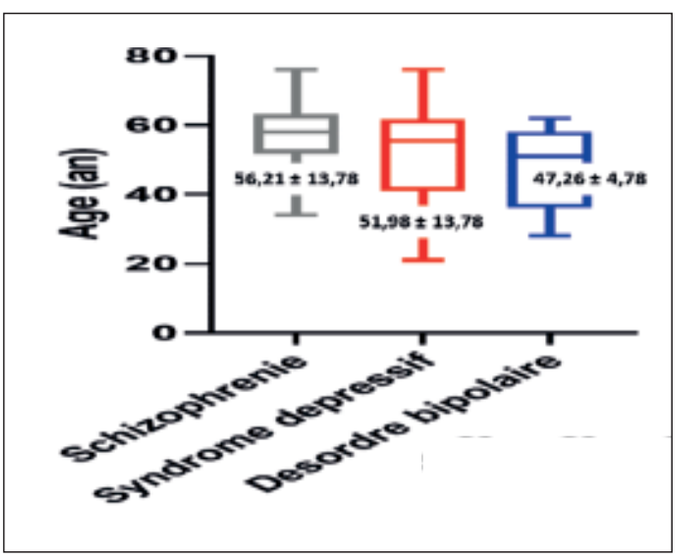

Figure 3. Age distribution of patients with psychiatric disorders

Distribution of patients according to CRP levels

Analysis of CRP levels, a classical marker of systemic inflammation, revealed elevated values in 46 patients (48%). A significant difference was found between patients with psychiatric disorders and the control group (17.44 ± 5.02 g/L vs. 4.1 ± 1.3 g/L, p = 0.002, OR = 3.1).

By psychiatric diagnosis, mean CRP concentrations were significantly higher in patients with schizophrenia compared to those with depression and bipolar disorders (21.19 ± 12.3 g/L vs. 18.22 ± 10.7 g/L vs. 9.63 ± 4.7 g/L, p = 0.002). **(See Figure 4)**

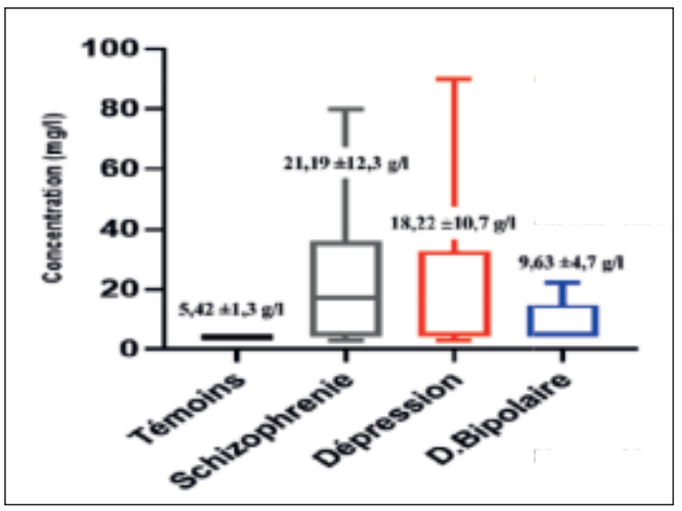

Figure 4. Distribution of CRP levels among patients by diagnosis (Schizophrenia, Depression, Bipolar Disorder).

Distribution of patients according to serum protein electrophoresis profile

The electrophoretic profile in patients with psychiatric disorders revealed notable inflammatory abnormalities compared to the control group.

In controls, all individuals (100%) showed a normal electrophoretic profile, consistent with the absence of inflammatory pathology.

In parallel, we observed, depending on the psychiatric pathology, the serum electrophoretic profile was inflammatory in 35% of patients with schizophrenia (23% CIS, 10% AIS and 2% PCIS), 45% of patients with depressive symptoms (26% AIS, 11% CPIS and 8% CIS), and 21% of patients with bipolar disorders (13% CIS, 6% AIS and 2% PCIS). **(See Figure 5)**

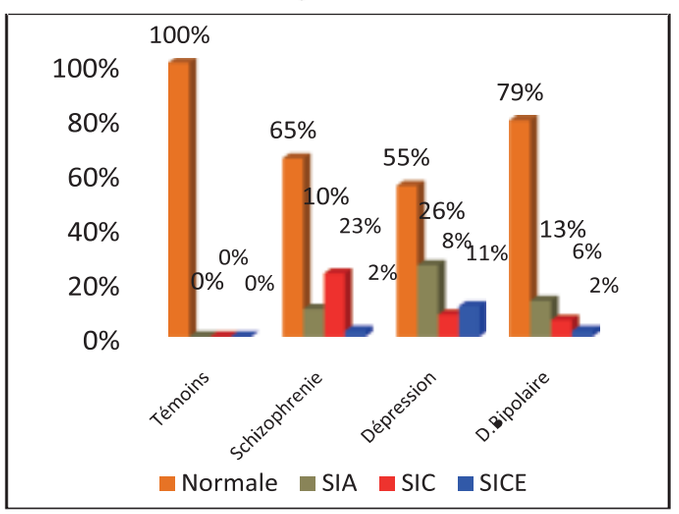

Figure 5. Distribution of serum electrophoretic profiles by diagnosis (Schizophrenia, Depression, Bipolar Disorder).

Distribution of patients according to suspected autoimmune diseases (AID)

Symptoms suggestive of AID were remarkably more frequent in patients with depressive symptoms (33/48; 68%), followed by schizophrenia (56%) and bipolar disorders (27%).

Table 1. Distribution of patients according to suspected autoimmune diseases

	Suspected AID*	No suspected AID
Healthy controls (N = 30)	0	30 (100%)
Schizophrenia (N = 36)	20 (56%)	16 (44%)
Depression (N = 48)	33 (68%)	15 (32%)
Bipolar disorders (N = 11)	3 (27%)	8 (83%)
Total patients (N = 95)	56 (59%)	39 (41%)

*Based on clinical and biological signs such as joint pain, asthenia, alopecia, fever, rapidly developing obesity, anemia, leukopenia, thrombocytopenia, etc.

Distribution of patients according to ANA detection

The search for ANA revealed variable frequencies of positive cases according to the type of psychiatric disorder, with an estimated positivity rate of 75% in patients with depressive symptoms (49% with significant titers), 50% in patients with schizophrenia (30% with significant titers), and 36% in patients with bipolar disorders (13% with significant titers). **(See Figure 6)**

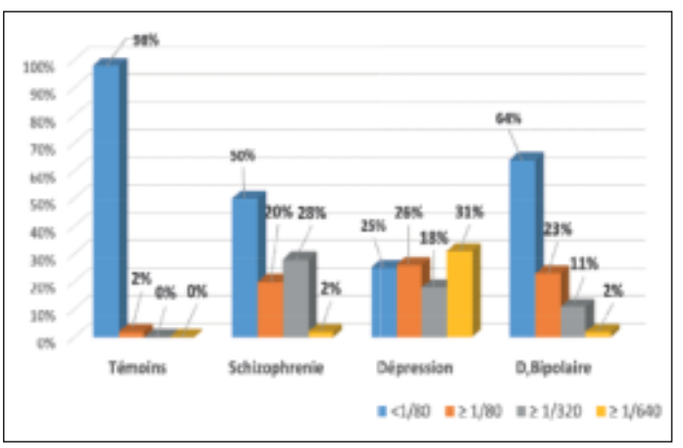

Figure 6. Distribution of ANA positivity by diagnosis

Distribution of patients according to ANA target specificity

In our study, we found that 15 out of 95 patients were diagnosed with SLE, with the presence of multiple targets including anti–native DNA, anti-Sm, and anti-ribosomal antibodies. At lower frequencies, other diagnoses were established: 10 cases of autoimmune thyroiditis, 8 cases of primary or secondary antiphospholipid syndrome (presence of anti-cardiolipin and anti-β2GP antibodies), 4 cases of ANCA-associated vasculitis, 2 cases of Sjögren's syndrome (presence of anti-SSA/SSB autoantibodies), and one case of scleroderma (presence of anti-Scl70 autoantibodies). Interestingly, autoimmune diseases were more pronounced among patients with depressive syndrome, with 10 confirmed cases of SLE (with or without associated APS) and an equal number of autoimmune thyroiditis.

Identified targets (autoantibodies)	Schizophrenia N = 36	Depression N = 48	Bipolar D N = 11	Systemic diagnosis
Anti-DNA	5	5	1	SLE
natif	1	3	1	SLE
Anti-Sm,	0	2	0	SLE
Anti-Sm/RNP,	0	2	0	Mixed
Anti-RNP	0	1	0	Mixed
Anti-SSA/Ro	0	1	0	SS
Anti-SSB,	0	1	0	SS
Anti-Scl70,	0	1	0	SLE
Anti-Jo-1,	1	5	1	PM
APL	2	6	0	SAPL
Anti-TPO	1	5	4	AIT
Anti-tg	0	2	2	AITVasculitis
ANCA	0	1	3	

Table 2. Distribution of patients according to autoantibody specificity and AID diagnosis

Discussion

Autoimmune diseases (AID) with psychiatric manifestations mainly affect women, with an incidence peak during adolescence and early adulthood, although they may occur at any age. This predominance appears to be multifactorial, involving genetic predisposition to develop inflammatory rheumatism with psychiatric expression, in an environment favoring viral infections [6, 7, 13, 14]. AID can manifest with a variety of mental disorders such as mood disorders (depression, anxiety), psychotic disorders (psychosis, delusions), neurodevelopmental disorders (autism spectrum disorders, attention deficit hyperactivity disorder), obsessive-compulsive disorders, fatigue, sleep disturbances, and finally the pains, all of which may influence the perception of symptomatology [9, 15].

Several studies have confirmed this female predominance [6, 13, 14], and have identified the coexistence of multiple comorbid factors, including genetic predisposition common to both AID and psychiatric disorders, stress (trauma, grief, separations), and the inflammatory background characteristic of AID [6, 15].

In our study, we also observed a female predominance across the three groups of pathologies despite the relatively small sample size,regarding age, our patients tended to be older, suggesting that the development of psychiatric symptoms may be linked to chronic exposure to systemic inflammatory flares. A more in-depth etiological investigation would be necessary to identify the main factors underlying this chronic inflammation.

With regard to biological inflammatory markers in autoimmune-related psychotic disorders, CRP remains the most accessible biomarker of inflammation in medical biology services. Produced by the liver, elevated CRP indicates acute or chronic inflammation [16, 17]. In addition to the existence of a link between AID, elevated CRP, and the risk of psychiatric symptoms, it seems that the chronicity of inflammation that characterizes AIDs can affect the central nervous system, thus increasing the risk of psychiatric disorders such as depression, anxiety, and even psychotic symptoms. [16, 17, 18].

Several studies have also demonstrated associations between increased cytokines and inflammatory proteins such as CRP, and that these biomarkers were associated with risks of occurrence of deficits in verbal memory, working memory, processing speed, mental flexibility and reasoning [19, 20, 21]. Most of these studies focused specifically on inflammatory abnormalities and cognitive dysfunction in schizophrenia [16, 17, 21, 22].

In our cohort, CRP values were variable, reflecting heterogeneity of the inflammatory response. Although the mean CRP was slightly lower than in schizophrenia patients, the inflammatory profile remains notable across all three groups studied.

The chronological assessment of inflammatory syndromes in our patients, through serum protein electrophoresis, allowed us to identify underlying inflammatory activity in psychotic disorders with autoimmune features. However, psychiatric symptoms can be complex and multifactorial causes, and electrophoresis is only one part of a broader diagnostic

workup. This examination can also screen for complications such as renal involvement in neurolupus [23, 28].

Other research teams have confirmed the usefulness of serum protein electrophoresis in detecting inflammation and renal involvement since the method was first described, and it is now mainly recommended for diagnosing monoclonal gammopathies [23, 28].

Others have mentioned, in particular,alterations in social cognition and heightened emotional reactivity that may be aggravated by an underlying inflammatory background. This supports the possible existence of a specific inflammatory phenotype, which may be correlated with behavioral dimensions such as impulsivity or violence [23, 28].

In our population, sensitivity in the identification of inflammatory syndromes has not been shown, this can be explained by the fact of the clinical entity with neurological expression, which requires an electrophoretic study on the cerebrospinal fluid (CSF), in order to better understand the inflammatory mechanisms at the level of the nervous system

After evaluating inflammation, we examined the risk of triggering a breakdown in immune tolerance. According to the literature, this risk is variable, estimated between 45% and 55% for the occurrence of psychosis in AID and vice versa [16].

In our population, a variable frequency of positive ANA titers was noted depending on the psychiatric pathology, higher in patients with depression and schizophrenia. This strongly suggests autoimmune pathologies, compared to 2% in our population of healthy controls. Notably, 15 ANA-positive patients exhibited one or more antigenic specificities. This may be explained by multiple factors contributing to neuropsychiatric autoimmune symptomatology, such as medications that induce ANA production, concurrent active infections notably viral or Mycoplasma pneumonia, and hematologic malignancies [14].

The literature remains contradictory. Some studies have established significant associations between connective tissue diseases and autoimmune enteropathies on the one hand, and autoimmune psychoses on the other [24, 25, 26, 27]. Others have found no significant results [28].

The most widely accepted hypothesis is the coexistence of low-grade inflammation and autoimmunity, which, through various molecular and psychological mechanisms, may trigger genuine psychotic syndromes in predisposed individuals [28].

The value of immunopsychiatric analyses will become more evident with the exploration of antineuronal antibodies in larger samples, to clarify the neuronal processes underlying autoimmune psychoses.

In our cohort, neuro-lupus, antiphospholipid syndrome, and autoimmune thyroiditis were the autoimmune diseases most associated with psychosis, particularly schizophrenia and depressive syndromes, and this does not allow us to confirm the link like some research teams [27] or, on the contrary, to deny it like others [23, 24, 25, 29, 30].

Our results confirm the presence of significant inflammatory activation in patients with psychiatric disorders, especially schizophrenia and depression. Elevated CRP and the presence of autoantibodies suggest involvement of the peripheral immune system in the pathophysiology of these disorders. These findings are consistent with the growing body of literature emphasizing the role of pro-inflammatory cytokines and autoimmune processes in psychiatric symptoms.

All the data from our study highlights importance of immuno-inflammatory mechanisms in the understanding of major psychiatric disorders. Elevated CRP, altered electrophoretic profiles, and the presence of auto-Abs in a significant proportion of patients indicate that a non-negligible subset of psychotic, depressive, and bipolar disorders may fall within an immunological framework.

Cross-referencing our results with recent studies, particularly those of Mezoued, Jeppesen, and Severance, it appears that inflammation is not merely an episodic marker but a persistent biological trait in some psychotic patients, particularly associated with negative symptoms, treatment resistance, and atypical clinical forms [27, 28, 30].

These observations strengthen the case for early, targeted immuno-inflammatory screening in psychiatric disorders, aiming to stratify patients according to their inflammatory profile. Such an approach opens the way to personalized precision medicine, still insufficiently applied in this field, integrating immunomodulatory adjuvant treatments, still little used in psychiatry today.

Finally, further longitudinal and mechanistic research is needed to better characterize the links between peripheral inflammation, brain dysfunction, and clinical expression. Identifying reliable and reproducible biomarkers will ultimately enable the development of more precise and effective therapeutic strategies, tailored to each patient's biology.

Conclusion

This study highlights the importance of integrating the evaluation of immuno-inflammatory markers into the management of psychiatric disorders. Such an assessment would allow better identification of patients with an immune-mediated form of psychosis, thereby justifying the use of adjunctive immunomodulatory therapies.

It is crucial that healthcare professionals remain attentive to psychiatric symptoms in individuals with autoimmune diseases, as early screening and timely management can significantly improve patients' quality of life.

Déclaration d'intérêts

The authors have no conflicts of interest to declare.

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ORIGINAL ARTICLE

Demographic, clinical, and laboratory features of Patients with Seronegative Primary Sjögren's Syndrome in Western Algeria

Les caractéristiques démographiques, clinico-biologiques et histopathologiques des patients atteints du Syndrome de Sjogren Primitif Séronégatif dans l'Ouest algérien

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KEYWORDS
Primary Sjogren's syndrome; Seronegative; Autoimmune thyroiditis; Hypergammaglobulinemia; Erythrocyte Sedimentation Rate; Minor salivary gland biopsy

Abstract

Introduction: To investigate the clinical and immune features of patients with primary Sjögren's syndrome (pSS) negative for auto antibodies: anti-SSA/Ro, anti-SSB/La, Anti-Nuclear Antibodies (ANA) and Rheumatic Factor (RF) in a population of Western Algeria.

Studied population and Methods: 65 patients with pSS in western Algeria were retrospectively examined between 2021 and 2023. Patients with negative anti SSA/Ro, anti SSB/La, ANA, and RF antibodies comprised the seronegative group, while patients with at least one positive antibody were included in the seropositive group. Clinical manifestations, laboratory and histopathological findings were

compared between the two groups.
Results: Among the 65 patients with pSS, 16 (24.6%) were seronegative. The most common clinical manifestations in the seronegative patient group are: xerostomia (87.5%), xerophthalmia (81.3%) and joint involvement (68.8%). All of these patients have positive salivary gland biopsies showing a focus score ≥1 (100%). Compared to seropositive pSS, seronegative pSS has a low proportion of female gender (p= 0.029). Among clinical manifestations, Endocrine involvement and Association with a specific auto immune disease exhibited a lower prevalence within the seronegative group (p= 0.006 and p = 0.014 respectively). Among biological and immunological characteristics, elevated sedimentation rate was more frequent in seronegative group (p=0.03) whilehypergammaglobulinemia was more frequent in seropositive group (p=0.025).

Conclusion:
The seronegative pSS is distinct from seropositive pSS, indicating possible different underlying pathogenesis mechanisms. The absence of conventional serologic markers may reflect limitations in current tools, rather than true immunological silence. Novel (not part of diagnostic criteria) antibodies may play a role in identifying this group of pSS patients.

MOTS CLÉS
Sydnrome de Sjogren primitif ;
Séronégatif ;
Thyroïdite auto-immune ;
Hypergammag-lobulinémie ;
Vitesse de sédimentation ;
Biopsie des glandes salivaires mineures

Résumé
Introduction: Étudier les caractéristiques démographiques, clinico-biologiques, histopathologiques des patients atteints du syndrome de Sjögren primitif (SSp) négatifs aux autoanticorps : anti-SSA/Ro, anti-SSB/La, anticorps antinucléaires (ANA) et facteur rhumatoïde (FR) dans une population de l'Ouest algérien.
Population étudiée et Méthodes : 65 patients atteints de SSp dans l'Ouest algérien ont été examinés rétrospectivement entre 2021 et 2023. Les patients présentant des anticorps anti-SSA/Ro, anti-SSB/La, ANA et FR négatifs constituaient le groupe séronégatif, tandis que les patients présentant au moins l'un deces autoanticorps positif étaient inclus dans le groupe séropositif. Les données démographiques, les manifestations cliniques, les résultats biologiques et histopathologiques ont été comparés entre les deux groupes.

Résultats : Parmi les 65 patients atteints de SSp, 16 (24,6 %) étaient séronégatifs. Les manifestations cliniques les plus fréquentes chez les patients séronégatifs sont : la xérostomie (87,5 %), la xérophtalmie (81,3 %) et l'atteinte articulaire (68,8 %). Tous ces patients avaient une biopsie des glandes salivaires positive, montrant un score focus ≥ 1 (100 %). Comparativement au SSp séropositif, le groupeSSp séronégatif présente une faible proportion de femmes (p = 0,029). Parmi les manifestations cliniques, l'atteinte endocrinienne et l'association à une maladie auto-immune spécifique présentaient une prévalence plus faible au sein du groupe séronégatif (p = 0,006 et p = 0,014 respectivement). Parmi les caractéristiques biologiques et immunologiques, une vitesse de sédimentation élevée était plus fréquente dans le groupe séronégatif (p = 0,03) tandis que l'hypergammaglobulinémieétait plus fréquente dans le groupe séropositif (p = 0,025).

Conclusion :
Le SSp séronégatif se distingue du SSp séropositif, ce qui suggère des mécanismes pathogéniques sous-jacents différents. L'absence de marqueurs sérologiques conventionnels pourrait refléter les limites en outils d'exploration actuels, plutôt qu'un véritable silence immunologique. De nouveaux anticorps (ne faisant pas partie des critères diagnostiques) pourraient jouer un rôle dans l'identification de ce groupe de patients atteints de SSp.

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Introduction

Primary Sjögren's syndrome (pSS) is a systemic rheumatism autoimmune disorder that predominantly affects middle-aged women [1].Patients with SS present have a spectrum of clinical manifestations from sicca symptoms (eye and/or mouth dryness), constitutional symptoms (fatigue, myalgia, and arthralgia) to potentially severe extra glandular and/or systemic features, such as interstitial pneumonia, neurological dysfunction, renal disease, autoimmune cytopenia, cutaneous vasculitis, synovitis, myositis and lymphoma as a rare and severe complication [1,2,3].
The typical cases of pSS have emphasized the presence of dry eyes and mouth, the presence of anti-SSA/Ro antibodies and/or lymphocytic infiltration of salivary gland as main criteria to support diagnosis. Because of its nonspecific symptoms, diversity of clinical manifestations and lack of standardized diagnostic methods, the most appropriate diagnostic criteria are still being debated, and misdiagnosis rates are high in the early stages of the disease. Therefore, many patients with SS are not accurately diagnosed until the onset of more serious complications[4].
The hallmark of pSS pathogenesis is B cell hyperactivity and consequently, autoantibodies production such as antinuclear antibodies (ANA; including anti-Ro60/Ro52/La) and rheumatoid factor (RF). Patients fulfilling pSS criteria who do not express classic serum antibodies are called patients with seronegative pSS[5].

In line with the possible relationship between the presence of autoantibodies and the clinical and laboratory findings, the question arose whether seronegative pSS patients may have different characteristics. This study was designed to examine whether disparities exist in the demographic, clinical, and laboratory features of patients with pSS who exhibit quadruple (ANA, anti-SSA/Ro, anti-SSB/La, and RF) antibody negativity.

On a research level, understanding why a subset of patients with pSS do not produce serum autoantibodies would be instrumental in understanding its immunopathology and developing targeted therapeutic strategies.

Studied population and Methods

Studied Population

Our study was conducted at the University Hospital Center (EHU) 1st November 1954 in Oran, from 2021 to 2023. This study included 65 patients diagnosed with primary Sjögren's syndrome (pSS) according to the 2016 ACR/EULAR classification criteria.
pSS Patients who presented with an organ-specific autoimmune disease were included in the study. Patients with secondary Sjögren's syndrome or those with another systemic autoimmune disease were excluded.

Epidemiological, clinical, and histopathological data were collected using a questionnaire filled out by clinicians from the relevant departments, including internal medicine, nephrology, and rheumatology.

Methods

The immunological workup of the patients was carried out in the Immunology Department. The detection of antinuclear antibodies (ANA), anti-SSA/Ro and anti-SSB/La antibodies, as well as the rheumatoid factor (RF), was performed respectively by indirect immunofluorescence on Hep-2 cells, immunodot assay, and latex agglutination.

Serum protein electrophoresis was performed for all patients. Cryoglobulins were only searched for, quantified, and typed in patients with pSS presenting symptoms suggestive of cryoglobulinemic disease.
Patients were classified as having seropositive or seronegative primary Sjögren's syndrome (pSS) based on autoantibodies results. Seropositive pSS was defined by a positive result for at least one of the four autoantibodies (anti-Ro, anti-La, ANA, and RF). Seronegative pSS was defined by a negative result for all four autoantibodies.

Statistical Analysis

All statistical analyses were performed using SPSS software, version 26. Categorical data were expressed as counts and percentages (%), while continuous variables were expressed as means ± standard deviation (SD).
For comparisons between the two groups—seronegative and seropositive—the Chi-square test (χ^2) (2x2 contingency tables) was used. The classical evaluation of χ^2 is valid when expected frequencies exceed 5. Otherwise, a corrected χ^2 must be applied, using either Yates' correction (if expected frequency is <5) or Fisher's exact test (if <3).
Odds ratios (OR) were reported with 95% confidence intervals (CI). A two-tailed p-value < 0.05 was considered statistically significant.

Results

Descriptive analysis of studied population

Among the 65 pSS patients included in this study, 49 (75.4%) were seropositive and 16 (24.6%) were seronegative (Figure 01).

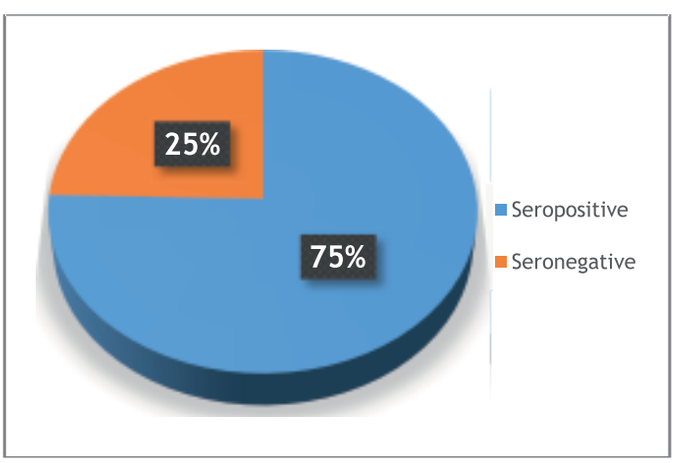

Figure 1: Distribution of patients according to autoantibody status

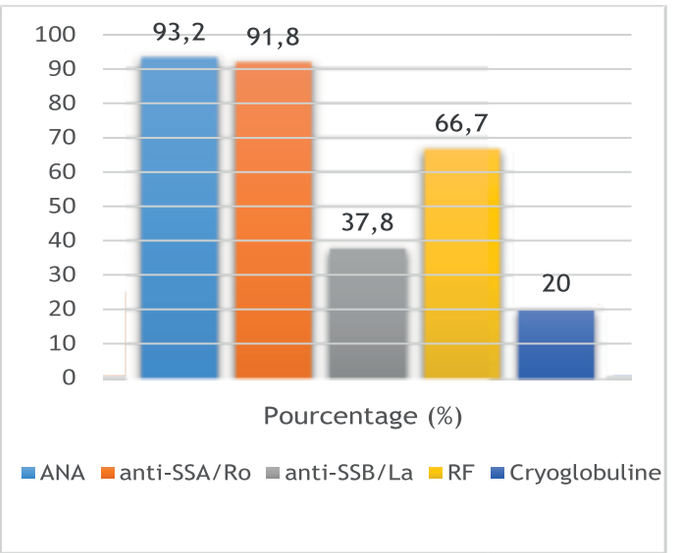

Figure 2: Autoantibody distribution among seropositive patients

88.8% of these patients had a positive salivary gland biopsy, with 34.4% at stage III and 53.1% at stage IV according to Chisholm and Mason score.
In the seronegative group, the most frequent clinical manifestations were: xerostomia (87.5%), xerophthalmia (81.3%) and articular involvement (68.8%). All seronegative patients had a positive salivary gland biopsy with a focus score ≥1 (100%).

Comparative analysis between seropositive and seronegative groups

Compared to seropositive pSS, seronegative pSS had a lower proportion of female patients (p = 0.029) (Table 01). Regarding clinical manifestations, endocrine involvement was more frequent in the seropositive group (43.8%) than seronegative group (6.3%) with an Odds ratio of 7 (CI=1.02-48). This difference is statistically significant (p = 0.006). Seronegative patients are less susceptible to develop a specific autoimmune disease than seropositive patients (0% vs 28.9% respectively). This difference is statistically significant (p= 0.014). All other clinical characteristics are not statistically different between the two groups (Table 01).
For biological and immunological parameters, elevated erythrocyte sedimentation rate (ESR) was more common in the seronegative group (46.2%) than seropositive group (16.2%) with a statistically significant difference (p = 0.03, OR= 1.56 (CI=0.92-2.63). However, hypergammaglobulinemia was more frequent in the seropositive group (55.1%) compared to the seronegative one (18.8%). The difference is statistically

significant and is multiplied by 2.9 (CI=1.03-8.4). All other laboratory findings are not statistically different between the two groups (Table 01).
The frequency of seronegative pSS patients with a lymphocytic focus score of ≥ 1 in the minor salivary gland biopsies (100%) was higher than that of seropositive pSS (88.9%) patients but without significant difference (p=0.3) (Table 01).

Discussion

Patients with autoimmune diseases (AID) may not show any autoantibody, a condition that is referred to as seronegative AID. Although relatively rare, seronegative AID poses a peculiar challenge in clinical practice and may lead to false or delayed diagnoses. In case of seronegativity, the diagnostic approach is usually based, when feasible, on histological evidence of tissue damage related to immune cell infiltration, or may rely on other clinical, laboratory, and instrumental criteria. In some cases, seronegative AID is a mere diagnosis of exclusion [6].
There is no universally accepted definition of which autoantibodies should define seropositive from seronegative pSS[5].While certain studies exclusively focused on the absence of anti-SSA/Ro and anti-SSB/La antibodies [7, 8, 9].Few other studies exploring the concept of quadruple negativity [10, 11, 12, 13, 14].

Proportion of seronegative pSS patients

The proportion of seronegative patients in our study is 24.6%. This proportion is elevated than the proportion found in Yalya et al (10.9%), Yaziris et al (15.5%), Karahan and Karabulut (16.5%) and Chatsiz et al (4.6%) studies. It is included in rang of 8% to 37% reported in literature. This rang include all studies on seronegative pSS no matter how it is defined [5].

Demographic features

In term of demographic findings, feminine gender is statistically less frequent in seronegative group in our study (p=0.029). Chen et al were reported a similar result in their study of comparison between double seronegative and seropositive pSS patients (anti-SSA/Ro and anti-SSB/La) (75.3% vs. 90.6% respectively, p < 0.001) [7]. The pSS is generally a disease with feminine predominance. The lower proportion of women in the seronegative group may reflect a different clinical phenotype of pSS, possibly less influenced by hormonal factors.

No difference in age of onset of symptoms or diagnostic has been reported in our study. Contrary to the study of Karahan and Karabulut, which reported that patients with seronegative pSS were diagnosed later (p=0.026) [11]. The same result has been reported in Bodakci's study, the age at diagnosis of quadruple-seropositive pSS patients was significantly younger than that quadruple-seronegative patients. The interval between the onset of sicca symptoms and diagnosis was the shortest in quadruple-seropositive patients, averaging 23 months, whereas it was the longest in quadruple-seronegative patients, averaging 60 months (p < 0.0001). This shows that the presence of autoantibodies in patients with pSS aids early diagnosis [14].

Clinical features

In our study, mouth, eye and cutaneous dryness are distributed with the same frequency between the two groups. However, urogenital dryness and parotid swelling are most prevalent in seronegative pSS without significant difference. This result is discordant with result reported by Yayla et al, in which dry mouth was found to be more prevalent among seronegative patients (p = 0.003)[12]and Chatsiz et al, in which quadruple seronegative patients presented a high frequency of dry eyes (98.6% vs. 87.5% p=0.01) [13]. This suggests that seronegative patients had a limited glandular symptomatology.
In our study, the most clinical manifestations had not a different distribution between the two groups. This is similar to results of Karahan and Karabulut [11] and Chatsiz et al [13] studies. Autoantibody status is considered as the main factor driving the phenotypic expression of pSS and may help identify subgroups of patients with poor prognosis. Autoantibody-related and circulating immune complexes mechanisms play a major role in organ damage such as vasculitis, Raynaud's phenomenon, and cryoglobulinemia. This suggests that seropositive had more systemic manifestations than seronegative patients [14]. This suggestion is discordant with our finding and findings of another studies (no significant difference were found in extra glandular manifestations between the two groups). It may be explained by the fact that the definition of seronegative group is not correct. Another autoantibodies not researched in routine may explain the similarity in frequency of systemic manifestations between seronegative and seropositive groups [6, 15]. In addition, the clinical expression of the disease should not be

interpreted only from the side of the effector arm (e.g. autoantibodies) but the counter immunoregulation should be also considered.

In our study, the only extra glandular clinical manifestations which demonstrated a statistically significant difference between the two groups are endocrinal involvement (p=0.006) and association with specific autoimmune diseases (autoimmune thyroiditis was the most prevalent) (p=0.014). In the study of Karahan and Karabulut, Thyroid dysfunction is more frequent in seronegative group (26.7% vs 17.5%) but without significant difference (p=0.154) [11]. Similar to our result, Quadruple seronegative patients presented a high frequency of autoimmune thyroiditis (44.1% vs. 17.1% p=0.02) in Chatsiz et al study [13].
Sjögren's syndrome is a complex and heterogeneous autoimmune disease that frequently co-occurs with organ-specific autoimmune diseases. Autoimmune thyroid disease and SS can be considered as pathogenetically correlated. First, these two disorders are characterized by similar histological features with a tissue infiltrate that consists primarily of CD4+ T lymphocytes and the possible formation of germinal center-like structures unrevealing B cell activation [8]. Second, the two conditions present a similar genetic background with thyroid and epithelial cells expressing the same HLA molecules class II: HLA-B8 and HLA-DR3. Another point reinforcing the pathogenetical link between this two diseases is represented by the crucial role of the epithelial cells in orchestrating the tissue inflammation [16]. The finding that seronegative patients presented less thyroid dysfunction and association with autoimmune thyroiditis might unleash strong local and systemic immunoregulatory mechanisms, capable of restricting the autoimmune response confined to the epithelial structures of exocrine glands avoiding in this way wide-spread immune responses against another self-antigens.

Laboratory features

In terms of biological findings, hypergammaglobulinemia was identified more frequently in the seropositive group (55.1% vs 18.8% respectively, p=0.025). This result is concordant with Yazisiz et al (p=0.05)[10], Yalya et al (p=0.004)[12], Bodakci (0.0001) [15]but different from the finding of Karahan and Karabulut (p=0, 76)[11]. While classic pSS involves B-cell hyperactivity and autoantibody production, seronegative patients may exhibit more subtle T-cell-driven inflammation, local cytokine activation and neuroimmune dysregulation. Histologic evidence of focal lymphocytic sialadenitis supports an immune-mediated process, even in the absence of detectable autoantibodies. Non-immune mechanisms such as autonomic dysfunction or acinar cell apoptosis may predominate [17].
Another reasons which may explain the difference between seronegative and seropositive patients is underlying etiology as genetics and epigenetics. Several variants in the HLA region were found to be unique to patients with seropositive pSS[18].Some observations on epigenetic regulation have also reported the upregulated expression of IFN-induced genes, for example, the IFN signature, was mainly seen in patients with seropositive pSS[18,19].The HLA class II and interferon may participate in the initiation and perpetuation of an autoimmune response respectively in anti-SSA/Ro positive pSS patients.
Another biological result which is found significantly different between the two groups is the elevated sedimentation rate (seronegative 46.2% vs seropositive 16.2%, p=0.03) with an odds ratio of 1.56 (CI=0.92-2.63). In Literature, the difference was be statistically significant but in the inverse sense (seronegative 16.5% vs seropositive 33%, p= 0.044)[8], (seronegative 39.5% vs seropositive 54.4%, p < 0.001)[7].
To explain this discrepancy, the seronegative group may be subdivided into subtypes, some are marked by pronounced inflammatory response and others with a modest one. Although seropositivity is generally associated with systemic manifestations, the elevated erythrocyte sedimentation rate in some seronegative patients may suggest alternative inflammatory pathways unrelated to classical autoantibody mediated mechanisms such as cytokine production and epithelial cells activation. Implication of innate immunity in seronegative pSS is proven in Han's study [9].

In pathological examination, our seronegative group had a high frequency of score focus ≥1 compared with seropositive group (100% vs 89% respectively) but without significant difference (p=0.3). This finding is different from Karahan and Karabulut's study, in which the Focus score ≥1 was statistically more prevalent in seronegative group (100%vs 68.9% respectively, p=0.005) [11]and Yaziris's study (96.6% vs 68.4% respectively, p < 0.001) [10]. This discordance may be

explained by the fact that autoantibodies and/or focal lymphocytic infiltration are the two main criteria for pSS diagnosis. When autoantibodies are present (seropositive group), histopathological examination is no longer needed. However, in absence of classical autoantibodies, especially anti-SSA/Ro (seronegative group), the diagnosis of pSS can't be established without positive histological result. This may create a selection bias. This discordance may be caused by the heterogeneity of the definition of a positive result, the collection conditions and interpretation expertise.

Conclusion

Seronegative and seropositive patients with SS appear to display some different demographic (low female prevalence), clinical features (the low rate of association with autoimmune thyroiditis), laboratory features (normal gammaglobulinemia, elevated sedimentation rate). It may be explained by different immunoregulatory, underlying pathogenesis pathways or etiology.

There were several limitations in our study. Firstly, this study was performed in Western Algeria and the sample size included was relatively small, especially the seronegative group of patients pSS. More multi-center studies are needed to validate our results and clarify the features of this distinct subtype.

Secondly, limited by retrospective nature of this study, our results could have been compromised by the bias due to the missing of some clinical and laboratory information.

Thirdly, the retrospective nature of this study and the short median follow-up period of the patients prevented an investigation into the development and frequency of malignancy. Therefore, the effect of seronegativity on lymphoma development could not be explored.

Fourth, we defined our seronegative patients by the absence of the four autoantibodies, so, our discussion was restricted to studies witch used the same definition.

In term of perspectives, unknown antibodies may play a role in autoimmune diseases included pSS. Consequently, it can be speculated that the prevalence of seronegative pSS will diminish in time paralleling the increase in the knowledge of autoantibodies associated [6]. Antibodies Anti-Carbonic Anhydrase 6 (CA6), Anti Parotid Secretory Protein (PSP) and Anti-Salivary Gland Protein (SP1) have been identified in a mouse model and confirmed in SS patients and in patients with idiopathic sicca syndrome [15]. Another autoantibodies (anti-BTBD7, anti-CCL4, anti-M5, anti-HNRNPA1, anti-KDM6B, anti-TMPO, anti-TONSL and anti-OAS3) are found in 53% of anti-SSA/Ro negative patients in the recent study of Engelke et al [20].This autoantibodies may serve as immunological biomarkers to help in identifying and diagnosing pSS seronegative patients.

Although anti-SSA antibodies are a major component of conventional serology, other immune dysregulations, both humoral and cellular, may also be at play. Finding new genetic biomarkers that could aid in the stratification of seronegative patients and offer insight into possible disease mechanisms is in perspective.

Lymphoma development is the most serious complication of pSS. The progression towards B-cell lymphoma is a multistep process related to local chronic antigenic stimulation of B cells. These neoplastic B cells in SS frequently derived from autoreactive clones, most commonly RF-producing B cells, which undergo uncontrolled proliferation and malignant escape [21]. Autoantibodies in pSSare considered as predictive factors of the development of lymphoma[22].A comparative study must be conducted in a population of lymphoma associated pSS patients to investigated prevalence of lymphoma and/or its pathogenesis pathways in seronegative pSS patients.

Declaration of Interest

The authors declare that they have no competing interest.

Acknowledgments

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Table 01: Comparison of demographic, clinical and laboratory features between seronegative and seropositive pSS patients.

	Seropositive (n=49)	**Seronegative (n=16)**	***p* value**
Demographic features			
Age	49.3 ans+/- 11.8 [26-82]	48.1 ans +/- 15.6 [22-74]	*p*= 0.47
Age of onset of symptoms	45.8+/- 11.9	45.5 +/- 15.3	*p*= 0.3
Sex (Women/Men)	47W/2M 95.9% des femmes	12W/4M 75% des femmes	***p*= 0.029**
Clinical features			
Xerostomia (mouth dryness)	41/47 (87.2)	14/16 (87.5)	*pF*=1
Xerophtalmia (eye dryness)	40/47 (85.1)	13/16 (81.3)	*pY*=1
Cutaneous dryness	20/47 (42.6)	7/16 (43.8)	*p*=0.93
Urogenital dryness	15/47 (31.9)	7/16 (43.8)	*p*=0.39
Parotid swelling	9/47 (19.1)	4/16 (25)	*p*=0.62
Articular involvement	36/47 (76.6)	11/16 (68.8)	*p*=0.53
Kidney involvement	7/48 (14.6)	1/16 (6.3)	*pF*=0.67
Lung involvement	6/47 (12.8)	3/16 (18.8)	*pY*=0.86
Endocrine involvement	21/48 (43.8)	1/16 (6.3)	***pF*=0.006 OR=7 (1.02-48)**
Muscular involvement	18/47 (38.3)	6/16 (37.5)	*p*=0.96
Raynaud phenomenon	13/48 (27.1)	5/16 (31.3)	*p*=0.75
Sensitive Neuropathy	7/47 (14.9)	3/16 (18.8)	*pY*= 1
Vasculitis	3/47 (6.4)	1/16 (6.3)	*pF*=1
Purpura	6/47 (12.8)	4/16 (25)	*pY*=0.45
pSS association with specific autoimmune diseases	13/45 (28.9)	0/16 (0)	***pF*=0.014**
Biological features			
Hematological involvement	21/39 (53.9)	4/13 (30.8)	*pY*=0.26
Anemia	14/39 (35.9)	4/13 (30.8)	*pY*=1
Thrombopenia	2/39 (5.1)	0/13 (0)	*pF*=1
Lymphopenia	5/39 (12.8)	0/13 (0)	*pF*=0.31
Elevated sedimentation rate	6/37 (16.2)	6/13 (46.2)	***p*=0.03 OR=1.56 (0.92-2.63)**
Hypergammaglobulinemia	27/49 (55.1)	3/16 (18.8)	***pY*=0.025 OR=2.9 (1.03-8.4)**
Histopathological features			
Positive MSGB	32/36 (88.9)	16/16 (100)	*pF*=0.3
MSGB stage III	11/32 (34.4)	5/16 (31.2)	*p*=0.83
MSGB stage IV	17/32 (53.1)	11/16 (68.8)	*p*=0.3

OR: *Odds Ratio,* ***PF:*** *Fischer adjusted p value,* ***PY:*** *Yates ajusted p value,* ***CI:*** *Confiance Interval,* ***MSBG:*** *Minor Salivary Gland Biopsy,* ***W:*** *Women,* ***M:*** *Men*

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ORIGINAL ARTICLE

Unraveling a Complex Pediatric Food Allergy: CMPA, Alpha-Gal, and PR-10–Mediated PFAS

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MOTS CLÉS
Allergie aux protéines du lait de vache
Syndrome pollen–aliment médié par les PR-10
Sensibilisation à l'alpha-gal
Diagnostics moléculaires (CRD)
Réactivité croisée
Polyallergie pédiatrique

Résumé

Introduction : es polyallergies pédiatriques complexes impliquent souvent des sensibilisations primaires chevauchantes et des mécanismes de réactivité croisée, rendant le diagnostic et la prise en charge particulièrement difficiles. Les diagnostics moléculaires fondés sur les composants (CRD) améliorent considérablement la précision diagnostique dans ces situations.

Description du cas : Nous décrivons un enfant présentant un phénotype allergique exceptionnel associant une allergie sévère aux protéines du lait de vache (APLV) médiée par les IgE, une sensibilisation à l'alpha-gal et un syndrome pollen–aliment médié par les protéines PR-10. Les CRD réalisés à l'âge de 3 puis 5 ans ont montré des taux élevés et persistants d'IgE dirigées contre le lait entier et la caséine (jusqu'à 78 kUA/L), une réactivité étendue aux protéines PR-10 (rBet v 1, rPru p 1, rMal d 1, rCor a 1, rGly m 4 toutes >100 kUA/L) ainsi qu'une positivité pour l'α-Gal (3,6 kUA/L). Cliniquement, l'enfant présentait des réactions systémiques après ingestion de lait de vache, un syndrome d'allergie orale aux aliments contenant des PR-10, et un épisode

d'anaphylaxie sévère après consommation de yaourt et de pêche. La prise en charge a reposé sur l'éviction stricte du lait et du bœuf ainsi que l'instauration d'une immunothérapie spécifique au pollen de bouleau.

Conclusion : Ce cas illustre le rôle essentiel des CRD pour démêler des profils de sensibilisation complexes, distinguer une allergie alimentaire primaire d'une réactivité croisée induite par les pollens et guider une prise en charge personnalisée. L'association d'une APLV sévère, d'un PFAS médié par les PR-10 et d'une sensibilisation à l'alpha-gal souligne l'importance d'une évaluation individualisée dans les polyallergies pédiatriques.

KEYWORDS
Cow's milk protein allergy
PR-10–mediated pollen–food allergy syndrome
Alpha-gal sensitization
Component-resolved diagnostics
Cross-reactivity
Pediatric polyallergy

Abstract

Background: Complex pediatric polyallergy often involves overlapping primary sensitizations and cross-reactive pathways, making diagnosis and management challenging. Component-resolved diagnostics (CRD) significantly improves diagnostic accuracy in such settings.

Case Presentation: We report a child with an uncommon triple-allergy phenotype combining severe IgE-mediated cow's milk protein allergy (CMPA), alpha-gal sensitization, and PR-10–mediated pollen–food allergy syndrome (PFAS). CRD performed at ages 3 and 5 revealed persistently high whole-milk and casein-specific IgE levels (up to 78 kUA/L), extensive PR-10 reactivity (rBet v 1, rPru p 1, rMal d 1, rCor a 1, rGly m 4 all >100 kUA/L), and positive α-Gal (3.6 kUA/L). Clinically, the child experienced systemic reactions to cow's milk, oral allergy syndrome to PR-10–containing foods, and a severe anaphylactic episode after ingestion of yogurt and peach. Management included strict exclusion of cow's milk and beef and initiation of birch pollen allergen immunotherapy. Follow-up over nine months showed good clinical stability.

Conclusion: This case illustrates the pivotal role of CRD in disentangling complex sensitization networks, distinguishing primary food allergy from pollen-driven cross-reactivity, and guiding precision-based management. The coexistence of severe CMPA, PR-10–mediated PFAS, and alpha-gal sensitization underscores the importance of individualized assessment in pediatric polyallergy.

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Introduction :

Complex polyallergies in children represent a growing challenge in clinical immunology, where multiple coexisting sensitizations and extensive cross-reactivity obscure the underlying immune mechanisms [1,2]. Among these conditions, cow's milk protein allergy (CMPA) stands out as the leading food allergy in Tunisia [3], which likely reflects the broader North African epidemiological pattern. It is a clinically significant IgE-mediated allergy of early childhood, frequently associated with severe systemic reactions and a substantial nutritional and psychosocial burden [4,5]. In

contrast, pollen–food allergy syndrome (PFAS), characteristic of the classic northern Mediterranean pattern, is most commonly associated with sensitization to PR-10 proteins (Bet v 1 homologues). It represents a prototypical form of cross-reactivity between inhalant and food allergens and typically presents with milder oropharyngeal symptoms [6–9].

Disentangling such overlapping allergic pathways remains difficult when relying solely on extract-based diagnostic tools. These conventional assays cannot reliably distinguish primary sensitizations from cross-reactive IgE responses, which limits diagnostic precision and therapeutic decision-making. The emergence of molecular allergology—particularly component-resolved diagnostics (CRD)—has enabled unprecedented resolution in the characterization of sensitization profiles. By identifying IgE responses to individual allergenic molecules, CRD refines diagnostic accuracy, predicts clinical relevance, and guides personalized management [10–13].

Here, we report a unique pediatric case marked by severe IgE-mediated cow's milk protein allergy, concomitant alpha-gal sensitization, and PR-10–mediated PFAS. CRD played a central role in delineating the dual molecular mechanisms that explained the patient's complex clinical phenotype. This case exemplifies the translational value of molecular allergology in elucidating intricate sensitization networks and optimizing precision-based management in pediatric allergy.

Case Report

A 3-year-old boy, born at term with normal growth and psychomotor development, was referred for evaluation of suspected food allergy. Family history was positive for atopy: his mother had allergic asthma and house dust mite-induced allergic rhinitis. The child had been mixed-fed from the age of two months and was fully vaccinated. Beginning at six months of age, he developed recurrent episodes of urticaria treated with antihistamines, episodic wheezing with cough, and gastrointestinal manifestations such as vomiting and loose stools, typically occurring within minutes to hours after ingestion of cow's milk or dairy products. Physical examination between episodes was unremarkable, except for mild xerosis, with no evidence of malnutrition or respiratory compromise.

Skin prick testing (SPT) showed a strongly positive wheal to cow's milk extract (Figure 1). Serum allergen-specific IgE (sIgE) measured by ImmunoCAP® fluoroimmunoassay (PhadiaTM 100, Thermo Fisher Scientific®, Uppsala, Sweden), revealed elevated IgE levels to whole cow's milk (f2 = 56.9 kUA/L) and its molecular allergenic components: β-lactoglobulin (nBos d5 = 13.4 kUA/L), α-lactalbumin (nBos d4 = 5.9 kUA/L), and casein (nBos d8 = 62 kUA/L). All specific IgE measurements are summarized in Table 1.

The marked sensitization to the thermostable casein fraction supported a diagnosis of persistent IgE-mediated CMPA, predicting reactivity even to baked milk. A strict milk-free diet and an emergency action plan including self-injectable epinephrine were prescribed.

At age five, after residing in Sweden for two years, the patient re-presented during a visit home with persistent allergic rhinitis and recurrent episodes of urticaria, as well as gastrointestinal and respiratory symptoms triggered by multiple foods. Ten minutes after consuming yogurt and a quarter of a peach, he developed acute angioedema requiring intramuscular epinephrine and emergency care. Given the severity of this IgE-mediated event (anaphylaxis, Grade IV), SPT was deferred, an avoidance diet was initiated, and a comprehensive panel of serum sIgE measurements was prescribed. Because of respiratory involvement, PhadiatopTM testing was performed to screen for inhalant allergen sensitization.

The allergy-focused history revealed prior episodes of oral allergy syndrome to apple and tree nuts; a first-ever known ingestion of peach at the time of the acute reaction; and a prior symptomatic episode after rare beef consumption. Based on these findings, repeat testing for cow's milk proteins, soybean, and beef was performed to evaluate potential cross-reactivity. Additional testing for apple, hazelnut, and peach was guided by the clinical history. Considering the patient's frequent outdoor activities with potential tick exposure, alpha-gal syndrome was evaluated as well. Although no clear tick bites were documented, testing for α-Gal was warranted to exclude delayed mammalian meat allergy.

In the context of deferred skin testing, PhadiatopTM was complemented with the most prevalent inhalant allergens in Tunisia and birch pollen, given the classic birch–apple association and the patient's recent residence in Sweden.

Repeat testing confirmed persistent CMPA, with whole cow's milk sIgE of 59 kUA/L. Molecular analysis again showed sensitization to β-lactoglobulin (13.4 kUA/L), α-lactalbumin (6 kUA/L), and casein (78 kUA/L), consistent with severe and thermostable profile. Sensitizations to

soybean and beef were detected : whole soybean sIgE was 27 kUA/L, Gly m4 (PR-10) >100 kUA/L; beef sIgE was 2.38 kUA/L; and α-Gal (Galactose-α-1,3-galactose) was 3.6 kUA/L. These findings supported continued clinical vigilance regarding potential mammalian meat reactions.

Food allergen testing revealed marked sensitization to peach, with whole allergen sIgE of 18.5 kUA/L, PR-10 component rPru p 1 >100 kUA/L, with negative IgE to LTP (rPru p 3 <0.1 kUA/L) and profilin (rPru p 4 <0.1 kUA/L).

Similarly, sensitization to apple was confirmed with whole allergen IgE of 12.8 kUA/L and PR-10 component rMal d 1 >100 kUA/L. Hazelnut sensitization was also present, with whole allergen IgE >100 kUA/L and PR-10 component rCor a 1 >100 kUA/L.

Taken together, these findings strongly supported a PR-10–mediated PFAS rather than an LTP-driven systemic allergy and emphasized the importance of CRD in defining the patient's molecular sensitization profile.

Phadiatop TM was positive. Extended aeroallergen testing revealed sensitization to olive pollen (t9 = 8.9 kUA/L) and birch pollen (rBet v 1, t215 >100 kUA/L). CRD confirmed broad IgE reactivity to PR-10 homologues :rBet v 1, rPru p 1, rCor a 1, rMal d 1, and rGly m 4 (all >100 kUA/L), with negative IgE to LTPs, profilins, and gibberellin-regulated proteins (GRPs).

This molecular pattern indicated a PR-10–driven sensitization consistent with oral allergy syndrome to fruits and tree nuts, alongside systemic reactions to cow's milk. Given the patient's lifestyle and potential tick exposure, alpha-gal sensitization was considered clinically relevant, even without documented tick bites. Ongoing clinical surveillance was recommended. All sIgE measurements are detailed in Table 2.

Allergic Phenotype and Management

This molecular profile established a dual allergic phenotype:

Persistent, primary IgE-mediated CMPA, associated with mild cross-reactivity to beef (α-Gal = 3.6 kUA/L).

PR-10–related PFAS secondary to birch pollen sensitization, explaining oral and gastrointestinal reactions to PR-10–containing foods (peach, apple, hazelnut, soybean).

The patient was started on birch pollen allergen immunotherapy to target inhalant sensitization and mitigate PR-10–mediated cross-reactivity. Dietary recommendations included complete elimination of cow's milk and beef, with cautious reintroduction of PR-10–containing fruits only when thermally processed. During a 9-month follow-up, the patient remained clinically stable with no recurrence of symptoms. This case illustrates the critical value of component-resolved molecular diagnostics in disentangling complex sensitization patterns, distinguishing true primary food allergy from pollen-related cross-reactivity, and guiding precision-based management in pediatric polyallergy.

Discussion

Polyallergy in pediatric patients represents a multifaceted immunologic challenge in which primary sensitizations, secondary cross-reactivity, and diverse clinical manifestations interact to produce complex and evolving phenotypes. In this case, primary CMPA coexisted with PR-10–mediated PFAS, illustrating the intricate overlap between systemic IgE-mediated food allergy and pollen-related cross-reactive responses. PFAS arises from secondary IgE cross-reactivity between structurally homologous proteins shared by pollens and plant-derived foods. In Northern and Central Europe, birch pollen sensitization to Bet v 1 is the classical trigger and accounts for cross-reactive responses to apple (Mal d 1), hazelnut (Cor a 1), and peach (Pru p 1) [1,8,9,12,14,15]. Epidemiologic data indicate that 5–10% of school-age children in endemic regions experience PFAS, with prevalence increasing to 30–50% among birch-sensitized children. In Sweden, PFAS affects 70% of individuals with birch pollen allergy and 19% of those sensitized to other pollens. In the United Kingdom, prevalence ranges from 17% in children younger than five to 78% in those older than ten. Korean studies report a prevalence of 42.7% in pollen-allergic children and up to 36.6% in preschoolers sensitized to birch pollen [14,16–18]. These observations highlight the strong link between birch sensitization and secondary food allergy in pediatric populations, emphasizing the utility of molecular allergology in characterizing sensitization patterns and guiding individualized management. To date, no peer-reviewed epidemiological study has specifically evaluated the prevalence of PR-10 (Bet v 1)–related PFAS in North African countries, where birch sensitization is rare. No pediatric cohort in the region has applied CRD to quantify PR-10–mediated PFAS. In Tunisia, pollen sensitization profiles are dominated by herbaceous and olive pollens rather than birch [19,20]. Data from southern Europe similarly indicate lower PFAS prevalence or involvement of alternative cross-reactive allergen patterns compared to Northern Europe [21]. In

fact, the prevalence of PFAS in the Mediterranean region, where birch pollen is largely absent, is estimated at approximately 20% [22]. Reported pediatric PFAS prevalences include 35.9% in an Italian cohort, 27% among Italian children with seasonal allergic rhinitis [23,24], 29.7% in Croatia [25], 16% in French children with asthma [26], and 3.3% in Turkish children with respiratory allergies [27]. These variations highlight the need for region-specific studies to better define local PFAS prevalence and molecular profiles in Mediterranean populations.

The mechanistic basis of PR-10 cross-reactivity lies in the conserved tertiary structure of Bet v 1–related proteins [15]. These proteins share a hydrophobic pocket and surface-exposed IgE epitopes recognized by B cells, while T-cell recognition occurs through linear epitopes with variable sequence identity. This dual epitope architecture may contribute to epitope spreading and amplification of clinical symptoms.

In this case, IgE recognition of Bet v 1 and multiple PR-10 homologues explained the constellation of symptoms and justified birch allergen immunotherapy (AIT), which may modulate B- and T-cell responses, reduce epitope spreading, and mitigate PFAS symptoms. The thermolability of PR-10 proteins explains why symptoms are generally restricted to raw foods, in contrast to heat-stable proteins such as casein or LTPs, which can induce systemic reactions [28,29]. PFAS typically presents as oral allergy syndrome (OAS), with localized oropharyngeal symptoms such as itching or mild angioedema [8,30,31]. However, systemic reactions do occur: up to 20% with Rosaceae fruits and 40–50% with soybean, underscoring the need for vigilance even with labile allergens [32]. Recent evidence suggests that PR-10 IgE profiles and quantitative IgE thresholds can differentiate silent sensitization from clinically relevant phenotypes and help predict the risk of systemic reactions, including anaphylaxis. Elysuitina et al. [33] demonstrated that asymptomatic children exhibit significantly lower IgE to Bet v 1 and PR-10 allergens than symptomatic patients. A threshold of 6.8 ISU for Bet v 1–specific IgE was strongly associated with OAS. Broader PR-10 reactivity correlated with multimorbidity. Similarly, Litovkina et al. [34] showed that symptomatic patients have significantly higher IgE levels and broader reactivity to PR-10 allergens, with a cutoff of 70 ISU differentiating isolated OAS from systemic manifestations.

These findings align with our case, in which high-magnitude PR-10 sensitization was associated with clinically significant OAS and an acute angioedema episode. While PR-10 IgE titers can aid risk stratification, symptom severity remains multifactorial and influenced by allergen dose, cofactors, and individual immunologic parameters. Primary CMPA results from IgE sensitization to heat-stable cow's milk proteins, particularly casein (Bos d 8), which predicts persistent allergy and potential anaphylaxis [35].

In this patient, markedly elevated casein-specific IgE indicated high risk for reactions to both raw and heated milk. Specific IgE levels are key predictors of CMPA persistence and severity, as shown in Tunisian and European cohorts [3,36,37]. A Tunisian study identified thresholds of 4.2 kU/L for whole milk and 0.37 kU/L for casein as predictive of persistent CMPA [3]. German data indicate faster tolerance acquisition in children with milk IgE <5 kU/L [36]. Payot et al. similarly found that tolerant children had significantly lower IgE levels to whole milk and casein [37]. Although such thresholds vary by region and technique, levels above 5 kU/L often predict persistent allergy [38,39].

In our patient, milk-specific IgE at 59 kUA/L and casein-specific IgE at 78 kUA/L were highly suggestive of persistence. Beef allergy, though uncommon in the general pediatric population (0.3%) [40], coexists in 13–20% of infants with CMPA and is associated with cross-reactivity to bovine proteins such as bovine serum albumin [41,42]. In our patient, sensitization to beef and α-Gal required careful dietary counseling given the potential for delayed mammalian meat reactions.

The coexistence of severe CMPA and PR-10–mediated PFAS with distinct molecular profiles underscores the importance of CRD in evaluating complex pediatric allergy. CRD allows precise identification of the responsible allergenic components, distinguishing primary systemic allergens from labile cross-reactive proteins. This distinction is essential for risk assessment, dietary recommendations, and therapeutic decision-making. One major advantage of CRD is risk stratification. Heat-stable molecules (e.g., casein, LTPs) are associated with systemic reactions, whereas PR-10 allergens generally induce localized OAS. This molecular precision guides both acute management and preventive strategies [12,43].

CRD also enables targeted nutrition management, avoiding unnecessary restrictions and supporting safe reintroduction of tolerated foods such as cooked fruits in PR-10–mediated PFAS [44–46]. CRD additionally guides AIT decisions, as in this case where birch pollen was

identified as the primary sensitizer [47–50]. Finally, CRD facilitates longitudinal monitoring by tracking evolving sensitization patterns and preventing misinterpretation of extract-based cross-reactivity [11,51–53].

In this child, CRD clarified a dual phenotype: persistent, systemic CMPA requiring strict avoidance of cow's milk and beef, and PR-10–mediated PFAS characterized by high-magnitude sensitization, supporting birch AIT and cautious consumption of cooked fruits. This case illustrates the clinical utility of molecular allergology in diagnosing, stratifying, and managing polyallergic pediatric patients. Overall, CRD provides a powerful translational tool that refines diagnosis, predicts risk, and enables personalized management in complex pediatric allergy. This case highlights its added value in disentangling overlapping sensitization pathways and guiding precision-based therapeutic strategies.

Conclusion

The coexistence of severe CMPA, beef allergy, PR-10–mediated PFAS, and alpha-gal sensitization in a single pediatric patient highlights the complexity and uniqueness of this triple allergy phenotype. This case underscores the critical importance of individualized evaluation in children with multiple coexisting allergies. Component-resolved diagnostics (CRD) proved indispensable in distinguishing primary IgE-mediated sensitizations from secondary cross-reactive responses, guiding precise dietary management, informing allergen-specific immunotherapy, and enabling risk stratification for potentially severe systemic reactions. While PR-10–mediated PFAS is often perceived as mild, its clinical significance increases in the context of overlapping sensitizations, emphasizing the need for vigilant assessment. Ultimately, a CRD-guided precision medicine approach optimizes both safety and quality of life for pediatric patients with complex polyallergic profiles, illustrating the tangible translational value of molecular allergology in specialized pediatric care.)

Conflict of interest

The authors declare that the research was conducted in the
absence of any commercial or financial relationships that could
be construed as a potential conflict of interest.

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Table 1: Specific IgE profile at age 3 years (Whole allergen extract and component-resolved allergens)

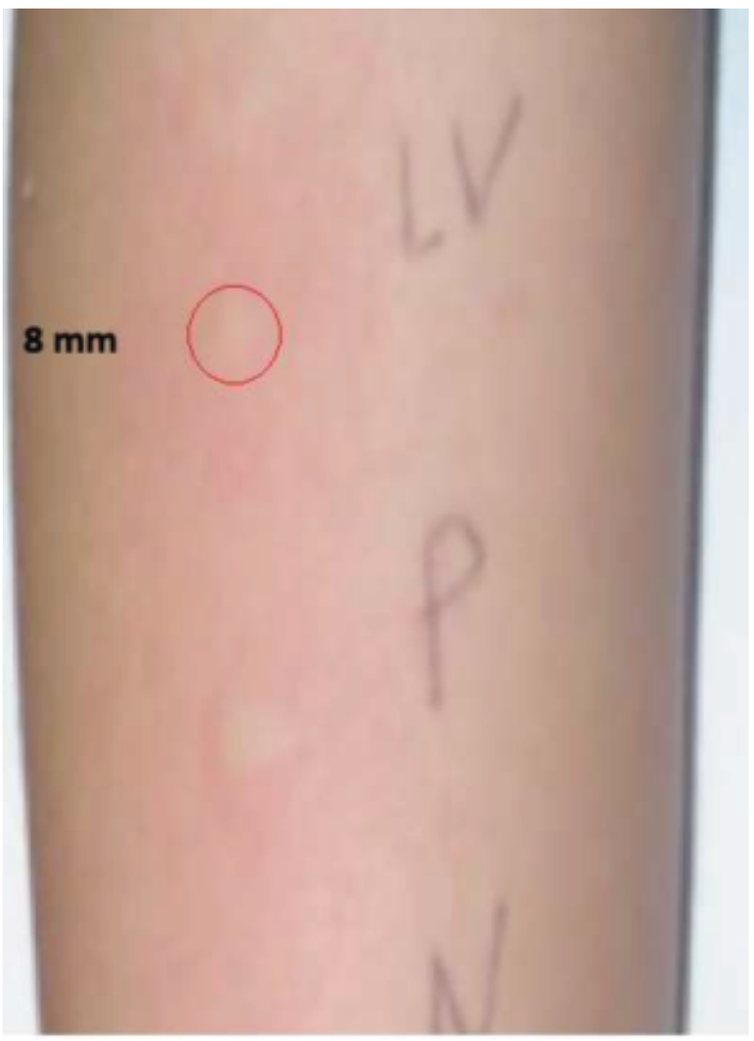
Allergen	Type	Specific IgE (kUA/L)
Cow's milk	Whole allergen	56.9
β-lactoglobulin (nBos d5)	Molecular	13.4
α-lactalbumin (nBos d4)	Molecular	5.9
Casein (nBos d8)	Molecular	62

Table 2: Specific IgE profile at age 5years (Whole allergen extract and component-resolved allergens)

Allergen Category	Allergen	Type	Specific IgE (kUA/L)
Food	Cow's milk	Whole allergen	59
	β-lactoglobulin (nBos d5)	Molecular	13.4
	α-lactalbumin (nBos d4)	Molecular	6
	Casein (nBos d8)	Molecular	78
	Peach	Whole allergen	18.5
	Peach PR-10 (rPru p1)	Molecular	>100
	Peach LTP (rPru p3)	Molecular	<0.1
	PeachProfilin (rPru p4)	Molecular	<0.1
	Apple	Whole allergen	12.8
	Apple PR-10 (rMal d1)	Molecular	>100
	Hazelnut	Whole allergen	>100
	Hazelnut PR-10 (rCor a1)	Molecular	>100
	Soy	Whole allergen	27
	Soy PR-10 (rGly m4)	Molecular	>100
	Beef	Whole allergen	2.38
	α-Gal (Galactose-α-1,3-galactose)	Molecular	3.6
Respiratory	Olive pollen	Whole allergen	8.9
	Birch pollen	Whole allergen	>100
	Birch PR-10 (rBet v1)	Molecular	>100
	Cypress pollen	Whole allergen	<0.1
	Cat dander	Whole allergen	<0.1

PR-10: pathogenesis-related protein family 10; LTP: lipid transfer protein.
All other molecular components tested were negative.

Figure 1 Prick test



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CASE REPORT

Bird Fancier's Lung in an 8-Year-Old Girl: A Pediatric Diagnostic Challenge

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KEYWORDS
Hypersensitivity pneumonitis; Bird fancier's lung; Pigeon exposure; Child; Interstitial lung disease; Asthma misdiagnosis

Abstract

Background:
Hypersensitivity pneumonitis (HP) is an uncommon immune-mediated interstitial lung disease in children, frequently misdiagnosed as asthma. Avian antigens are the predominant cause.

Case presentation:
An 8-year-old girl presented with an 8-month history of dry cough, exertional dyspnea, and weight loss, unresponsive to inhaled therapy. HRCT revealed diffuse ground-glass opacities. Bronchoalveolar lavage showed lymphocytic alveolitis, and serology confirmed elevated pigeon-specific IgG. Chronic exposure to pigeons on an adjacent balcony established the diagnosis of bird fancier's lung. Strict antigen avoidance and systemic corticosteroids (intravenous pulses followed by oral prednisone) resulted in rapid clinical improvement and near-complete radiologic resolution at 12 months.

Conclusion:
Early recognition through targeted environmental inquiry, HRCT, and immunologic testing is critical in children with refractory respiratory symptoms. Antigen avoidance and timely corticosteroid therapy ensure an excellent prognosis

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Introduction

Hypersensitivity pneumonitis (HP), also known as extrinsic allergic alveolitis, is a diffuse interstitial lung disease of immunologic origin triggered by recurrent inhalation of organic or chemical antigens [1]. The condition results from a complex interplay of type III (immune complex–mediated) and type IV (T-cell–mediated) hypersensitivity reactions, leading to alveolitis, granulomatous inflammation, and—when exposure persists—progressive fibrosis [2].

HP is uncommon in children, with an estimated prevalence of four cases per million and an annual incidence of two cases [3]. However, the number of pediatric cases reported worldwide has been steadily increasing, reflecting heightened clinical awareness and improved access to high-resolution imaging [4]. Avian antigens remain the leading cause, with bird fancier's lung accounting for approximately two-thirds of cases[5,6].

We report a pediatric case of HP secondary to chronic pigeon exposure. This case highlights the diagnostic complexity of HP in children and integrates recent advances in diagnostic and therapeutic approaches from international guidelines.

Case Presentation

An 8-year-old girl, living in an urban environment and born to non-consanguineous parents, was referred for progressive exertional dyspnea and weight loss. Her symptoms had begun eight months earlier with a persistent dry cough, initially managed as asthma with inhaled corticosteroids and bronchodilators, without clinical improvement. Over time, asthenia, anorexia, and exercise intolerance progressively worsened. There was no history of tuberculosis exposure, atopy, or recurrent respiratory infections.

On examination, the child was underweight (–3 SD), tachypneic (35 breaths/min) with mild suprasternal retractions, and had an oxygen saturation of 90% at rest and 85% on exertion. Digital clubbing and fine bilateral crackles were present, while cardiovascular examination was unremarkable.

Chest radiography revealed bilateral reticulonodular opacities (Fig. 1). High-resolution computed tomography (HRCT) demonstrated diffuse ground-glass opacities consistent with active alveolitis (Fig. 2). A diagnosis of diffuse interstitial lung disease (ILD) was considered, prompting further evaluation.

Pulmonary function testing showed a mixed restrictive–obstructive pattern (FVC 70% predicted, FEV_1 65% predicted, FEV_1/FVC 80%, TLC 68% predicted). Bronchoalveolar lavage (BAL) revealed lymphocytic alveolitis (36% lymphocytes). Tests for Mycobacterium tuberculosis, primary immunodeficiency, and sarcoidosis were negative.

A detailed environmental history disclosed chronic exposure to pigeons kept on the balcony adjacent to the child's bedroom, with daily contact over several years. Serologic testing demonstrated markedly elevated pigeon-specific IgG (141 mgA/L; hemagglutination titer 1:6400) and three precipitin arcs to pigeon dust antigens, confirming sensitization.

The convergence of clinical, radiologic, immunologic, and environmental findings established the diagnosis of hypersensitivity pneumonitis secondary to avian exposure (bird fancier's lung).

Immediate and strict antigen avoidance was implemented, including removal of the birds and thorough environmental cleaning. Treatment consisted of intravenous methylprednisolone pulses followed by oral prednisone (0.8 mg/kg/day) tapered over six months. Clinical improvement was rapid: within two months, oxygen saturation and exercise tolerance normalized, weight gain resumed, and dyspnea resolved. At 12 months, follow-up HRCT showed near-complete resolution with only minimal residual changes.

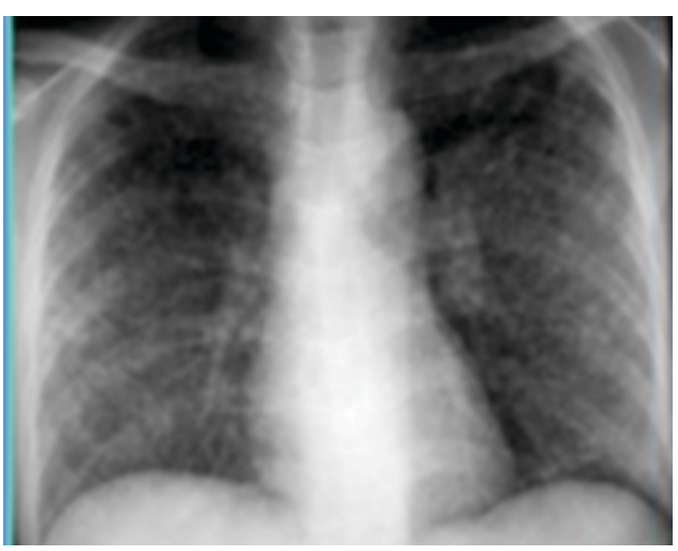

Figure 1. Chest radiograph showing bilateral reticulonodular opacities.

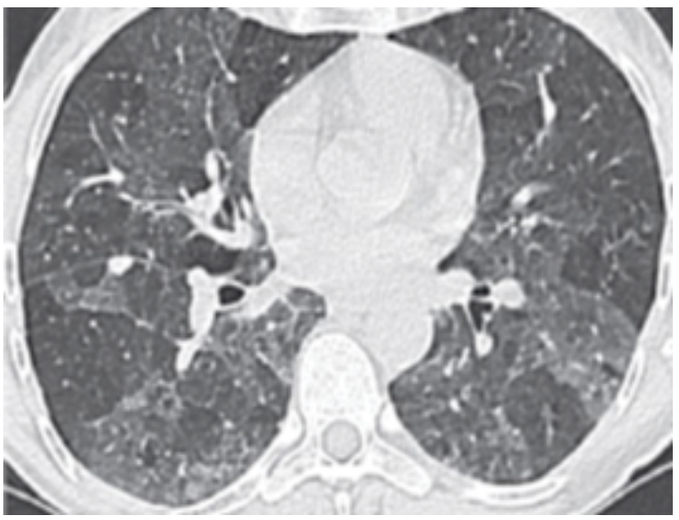

Figure 2. High-resolution computed tomography (HRCT) demonstrating diffuse ground-glass opacities.

Discussion

Pediatric HP is rare but likely underdiagnosed. The median age at onset ranges from 8 to 10 years, with no clear sex predominance [4,5]. The main antigenic sources include avian proteins, thermophilic actinomycetes, and molds such as Aspergillus species [4–8].

The clinical presentation is heterogeneous and depends on the intensity and duration of antigen exposure [7, 8]. The traditional classification into acute, subacute, and chronic forms has been replaced by the clinico–radiologic–pathologic classification proposed by Vasakova et al. [1], which distinguishes two main entities: (a) an acute/inflammatory form (<6 months, potentially reversible), characterized by dyspnea, dry cough, and weight loss; and (b) a chronic/fibrotic form, which is irreversible and carries a poorer prognosis. In children, the non-fibrotic inflammatory phenotype predominates, and the prognosis is excellent when diagnosis and antigen avoidance occur early [4–6].

Host susceptibility factors—including HLA-DRB1 polymorphisms, MUC5B mutations, and telomeric abnormalities—as well as environmental cofactors such as viral infections and air pollution, may modulate fibrotic progression risk [7,8].

According to current international recommendations [4–8], diagnosis relies on a combination of: (1) a compatible clinical presentation; (2) documented antigen exposure; (3) suggestive HRCT features; (4) BAL lymphocytosis; and (5) supportive histopathology when necessary. HRCT remains the imaging modality of choice. In pediatric cases, subacute HP typically shows ground-glass opacities, centrilobular nodules, or mosaic attenuation with air trapping [1,4–8]. Fibrotic forms are characterized by reticulation, traction bronchiectasis, and honeycombing, often with upper-lobe predominance [1,4–8].

The presence of avian-specific IgG antibodies confirms exposure but is not diagnostic in isolation, as elevated titers may persist after antigen avoidance [4–8].

A BAL lymphocytosis >30% provides strong diagnostic support [6,8]. HP frequently mimics other respiratory disorders such as asthma, atypical pneumonia, sarcoidosis, cystic fibrosis, or connective tissue–associated interstitial pneumonias [4–6]. In our case, the initial misdiagnosis of refractory asthma delayed recognition for eight months—a common pitfall in pediatric practice [4–6].

Antigen avoidance remains the cornerstone of management [4–8]. Beyond this, corticosteroids (prednisone 0.5–1 mg/kg/day for 4–8 weeks) hasten recovery [4,5]. Intravenous corticosteroid pulses should be considered in cases of persistent symptoms or severe impairment on pulmonary function tests and HRCT [4–6]. Immunosuppressive agents such as azathioprine or mycophenolate mofetil are reserved for relapsing or steroid-dependent disease [4–6]. In adults, antifibrotic agents such as nintedanib have demonstrated benefit in progressive fibrosing HP [7,8], though pediatric data remain limited. Supportive measures include vaccination, pulmonary rehabilitation, and nutritional follow-up [4–6].

The prognosis of non-fibrotic pediatric HP is generally excellent, with complete clinical and radiologic recovery in most cases [4–6].

Diagnostic delay and ongoing antigen exposure are the principal risk factors for fibrotic progression and chronic respiratory failure [4–6]. The favorable outcome in our patient after antigen avoidance and corticosteroid therapy confirms the reversibility of early inflammatory forms.

Our case aligns with recent pediatric reports. Maggiolino et al. (2024) described a 5-year-old girl exposed to doves who achieved complete recovery after corticosteroid therapy and antigen avoidance [9]. Zahraldin et al. (2023) reported an adolescent with severe HP secondary to parrot and pigeon exposure, requiring high-flow oxygen and intravenous methylprednisolone pulses, with multiple avian precipitins identified [10]. These cases, together with ours, emphasize the critical importance of

a systematic and repeated environmental assessment in children with chronic respiratory symptoms unresponsive to standard asthma treatment. Early recognition and prompt intervention ensure full reversibility of the disease.

Conclusion

Although uncommon, HP should be considered in any child presenting with chronic cough or exertional dyspnea unresponsive to standard asthma therapy.

Systematic environmental assessment, together with high-resolution computed tomography, bronchoalveolar lavage, and serologic testing, is essential for accurate diagnosis.

Our case of Bird Fancier's Lung highlights the diagnostic pitfalls and underscores the critical importance of thorough environmental evaluation.

When recognized early and managed with strict antigen avoidance and timely systemic corticosteroid therapy, pediatric HP carries an excellent prognosis.

CONFLICT OF INTEREST STATEMENT

No conflicts of interest to be disclosed.

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CASE REPORT

The Contribution of Molecular Allergy Diagnostics to Identifying Cross-Reactive Allergies involving Profilin and nsLTP : A Case Report

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KEYWORDS
CRD
Co-sensitization
Profilin
nsLTP
Molecular Allergy Diagnostics

Abstract

Background: The advent of molecular diagnostics, particularly Component Resolved Diagnosis (CRD), has been a significant advancement in the understanding of the underlying mechanisms of allergies. CRD is based on the identification of specific molecular allergens.

Purpose: This case aims to illustrate the value of molecular diagnostics in identifying co-sensitization and cross-reactivity profiles, as well as to discuss the practical implications for management, particularly in terms of prevention and patient education.

Methods: This report describes the case of a 25-year-old Algerian patient with pollen-food allergy syndrome, as well as co-sensitization to profilin and nsLTP.

Results : The patient presented with a history of allergic rhinitis and conjunctivitis during the spring. He reported experiencing dyspnea and urticaria immediately after ingesting peanuts and nuts, though sometimes he had no symptoms. He also experienced oral allergy syndrome, immediately after eating certain fruits, such as apples, peaches, and tomatoes. The results of specific IgE antibodies for allergen extracts indicated that the patient had a very broad sensitization profile. Subsequently, molecular allergen testing was performed using a customized allergen-chip based on the ISAC technology. Specific IgE against molecular

components were positive, with most antibodies belonging to two molecular families: profilins and nsLTP. Profilin is often associated with moderate allergic reactions. In contrast, nsLTPs proteins are linked to more severe clinical manifestations. Co-sensitization to Profilins and nsLTP can modulate clinical severity. Additionally, co-factors strongly influence the variable clinical expression of nsLTPs.

Conclusion: This case clearly illustrates the importance of an allergen-specific diagnosis, based on allergenic molecules (CRD-component-resolved diagnosis), for identifying cross- and co-sensitization to multiple allergens. This approach allows for personalized, accurate diagnoses and optimized management of allergic diseases.

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Introduction :

Pollen-food allergy syndrome (PFAS) is an IgE-mediated allergic reaction that occurs after ingesting fruits, vegetables, nuts, and seeds (1). It usually results from initial sensitization to a pollen allergen whose molecular structure is sufficiently similar to that of a food allergen to induce a cross-reaction (2). These proteins share common epitopes called pan allergens, which are mistakenly recognized as identical by the immune system, triggering an allergic response in sensitized individuals (3). The main respiratory sources involved are tree pollens such as birch, olive, cypress, and plane tree (2).

The advent of molecular diagnostics, particularly Component Resolved Diagnosis (CRD), has been a significant advancement in the understanding of the underlying mechanisms of allergies. CRD is based on the identification of specific molecular allergens. This approach allows for the distinction between co-sensitization and cross- sensitization, facilitates the assessment of clinical risk, and supports the development of targeted treatment strategies for each patient (4).

Of the 151 allergenic protein families that have been described, only a few contain cross-reactive allergens, some of which are well characterized from a molecular and clinical perspectives. Key pan allergen families involved in PFAS include pathogenesis-related protein class 10 (PR-10) proteins, profilins, nonspecific lipid transfer proteins (nsLTPs) and gibberellin-regulated proteins (GRPs) (5), each contributing to distinct patterns of cross-reactivity(1).

Profilin is involved in multiple pollen sensitivities and pollen-food-latex syndromes, and is often associated with moderate allergic reactions (4). In contrast, nsLTPs proteins are among the most common triggers of anaphylaxis and are linked to more severe clinical manifestations, particularly in monosensitized patients. However, when concomitant sensitization occurs with pattern allergen such as profilin or PR10, allergic symptoms tend to be less intense –(68).

Co-sensitization to different allergenic families can modulate clinical severity, broaden the range of affected foods, and complicate management. Here, we present an illustrative case of PFAS involving co-sensitization to the nsLTPs and profilin families. This case aims to illustrate the value of molecular diagnostics in identifying co-sensitization and cross-reactivity profiles, as well as to discuss the practical implications for management, particularly in terms of prevention and patient education.

Results:

Case Presentation:

A 25-year-old male living in Algiers, with no significant family history of allergic disease, was referred to the clinical allergology department at Beni Messous University Hospital for evaluation. The patient presented with a history of allergic rhinitis and conjunctivitis during the spring. He reported experiencing dyspnea and urticaria immediately after ingesting peanuts and nuts, though sometimes he had no symptoms. He also experienced oral allergy syndrome, which is characterized by a tingling sensation in the palate
and lip swelling immediately after eating certain fruits, such as apples, peaches, and tomatoes.

Following the clinical evaluation, the patient was referred

to the immunological department for biological assessment. The patient's serum was tested for specific IgE antibodies to the relevant airborne and food allergen extracts, using the IMMULITE® 2000 XPi system (Siemens).

The results indicated that the patient had a very broad sensitization profile **(Table 01)**, with positive results for pollens from **timothy grass, olive, *Parietaria judaica*,** as well as **peach, apple, tomato, peanut, sesame, walnut, hazelnut and almond.**

Table 01: Specific IgE results for airborne and food allergen extracts.

Due to the patient's extremely broad sensitization profile, he can be defined as a highly atopic, polysensitized subject.

The patient's serum was analyzed to exclude cross-reactivity due to Cross-reactive Carbohydrate Determinants (CCD) and was found to be negative for nAna c2 (Bromelain = 0.194 kU/L (class 0)).

Subsequently, molecular allergen testing was performed using a customized allergen-chip based on the ISAC technology, which includes a wide range of recombinant and native allergens. **Table 02** summarizes the positive specific IgE results, along with the sources and families to which each molecular allergen belongs.

Code	Airborne allergens	Specific IgE (kU/L)	Class
D1	Dermatophagoïdes pteronyssinus	< 0.1	0
D2	Dermatophagoïdes farinae	0.147	0
E1	Cat dander	0.224	0
E5	Dog dander	< 0.1	0
W19	**Parietaria Judaica pollen**	**3.43**	**II**
G6	**Timothy grass pollen**	**41.69**	**IV**
T9	**Olive pollen**	**36.92**	**IV**
	Food allergen		
F49	**Apple**	**64.2**	**V**
F95	**Peach**	**145**	**VI**
F25	**Tomato**	**51.3**	**IV**
F13	**Peanut**	**23.9**	**IV**
F17	**Hazelnut**	**2.09**	**II**
F20	**Almond**	**1.37**	**II**
F256	**Walnut**	**2.11**	**II**
F10	**Sesame**	**8.62**	**III**

Table 02: Positive specific IgE results to recombinant molecular allergens

ISU-E: ISAC Standard Unit IgE

Specific IgE against molecular components were positive, with most antibodies belonging to two molecular families: **profilins** (rHev b 8 [latex], rPhl p 12 [timothy grass], rBet v 2 [birch], rSola l 1 [Tomato], rOle e 2 [olive], rAmb a 8 [ragweed]) and **nsLTPs** (rAra h 9 [peanut], rPru p 3 [peach], rArt v 3 [mugwort])

The patient's serum was also tested for IgE antibodies to other foods containing nsLTPs and profilins, and all tests returned positive results: Banana: 12,70 KU/l , Cashew nut: 5,29 KU/l , Pineapple: 4,26 KU/l , Avocado: 8,48 KU/l, Apricot: 3,38 KU/l, Kiwi: 15,91 KU/l, Orange: 9,39 KU/l.

Code	Results (ISU-E)	Allergen source	Molecular family
rAmb a 8	**0.83**	Ragweed (*Ambrosia artemisiifolia*)	**Profilins**
rArt v 3	**1.52**	Mugwort (*Artemisia vulgaris)*	**nsLTP**
rBet v 2	**9.2**	Birch (*Betula pendula*)	**Profilins**
rHev b 8	**12.93**	Latex (*Hevea brasiliensis*)	**Profilins**
rOle e 1	**13.55**	Olive (*Olea europaea*)	Common olive group 1
rOle e 2	**5.36**	Olive (*Olea europaea*)	**Profilins**
rPhl p 1	**1.33**	Timothy grass (*Phleum pratense*)	Grass group 1
rPhl p 2	**0.64**	Timothy grass (*Phleum pratense*)	Grass group 2
rPhl p 12	**9.87**	Timothy grass (*Phleum pratense*)	**Profilins**
rAra h 9	**8.63**	Peanut (*Arachis hypogaea*)	**nsLTP**
rPru p 3	**1.94**	Peach (*Prunus persica*)	**nsLTP**
rSola l 1	**6.78**	Tomato (*Solanum lycopersicum*)	**Profilin**

Discussion:

This report describes the case of a 25-year-old Algerian patient with pollen-food allergy syndrome, as well as co-sensitization to profilin and nsLTP.

Profilin is a protein of 12- 15 kDa in size present in all eukaryotic cells and involved in the organization of cytoskeleton as well as in signal transduction (4). Profilins from higher plants constitute a family of highly conserved proteins showing sequence identities of at least 75% even between members from distantly related organisms. In view of the high sequence homology, cross- reactivity between profilins is extremely common and involves virtually every plant source (**Figure 01**). Thus, profilin can be considered the archetypal pan-allergen (4). They are widely distributed across the plant kingdom, found in both pollen and plant foods sources.

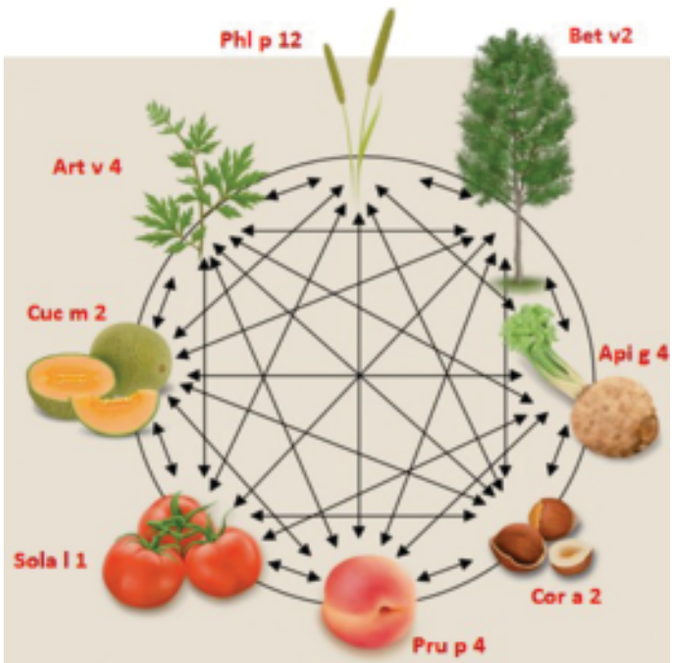

Fig 01 : Cross- reactivity between profilins from different pollen sources and plant foods (4)

Among pollen sources, profilins are present in various botanical families, including *Fagales* (birch, hazel tree, alder, hornbeam, oak, beech), *Graminae* (timothy grass and other grass species), *Asteraceae* (mugwort, ragweed), *Urticaceae* (pellitory), *Oleaceae* (olive, ash), *Cupressaceae* (cypress), *Euphorbiaceae* (annual mercury, date palm). Each species is associated with specific profilin allergens, such as *Bet v 2* for birch or *Phl p 12* for grasses (4). Up to 50% of pollen allergic patients are sensitized to profilin (4). As a minor pollen allergen, sensitisation to profilins occurs after sensitisation to a primary and major pollen source. Grass pollen is usually responsible for this hypersensitivity, but depending on geographical location, birch, ragweed or mugwort pollen may also act as primary sensitizers (9). The clinical significance of profilins as an airborne allergen remains unclear. Provocation tests (conjunctival, nasal and bronchial) have shown that profilins could act as an aeroallergen (4,10,11), but their real impact seems to be limited to the season of the main sensitising pollen –(12). However, in some regions, it may indicate more severe respiratory allergies (13).

In our case, the patient's primary sensitization appears to be to olive and Timothy grass pollens, as their major allergens (**rOle e 1** and **rPhl p 1**, respectively) tested positive. These sensitizations are consistent with the patient's symptoms, which occurred exclusively during the spring season, corresponding to the pollination periods of olive trees (April-June) and Timothy grass (May-August). Additionally, the patient's serum showed strong reactivity to molecular allergens of the profilin family, particularly **Phl p 12** (Timothy grass) and **Ole e 2** (olive tree). The additional positivity to birch profilin (**Bet v 2**), despite the absence of birch pollen in Algeria, indicates a cross-reactivity mechanism mediated by profilins, which are highly conserved panallergens shared among different pollen sources. This pattern reflects sensitization to a common profilin epitope rather than to species-specific pollen allergens.

In addition to pollen profilin sensitization, biological sensitization was also observed to profilins present in Tomato (**rSola l 1**), thus defining the framework of pollen-food allergy syndrome (PFAS) –(14). Profilins are widely distributed across numerous botanical families, including *Rosaceae* (apple, peach, pear), *Cucurbitaceae* (melon), *Actinidiaceae* (kiwi), *Apiaceae* (celery, carrot), *Rutaceae* (orange), *Leguminosae* (peanut, soybean), *Solanaceae* (tomato), *Bromeliaceae* (pineapple), *Corylaceae* (hazelnut), *Brassicaceae* (yellow mustard), *Asteraceae* (sunflower), and *Moraceae* (fig). Although present in virtually all plant-derived foods, profilins have long been underestimated as allergens. It has been reported that up to 50% of profilin-sensitised patients may have food allergies, especially those allergic to foods such as melon, watermelon, citrus fruits, bananas, pineapple, persimmons, zucchini, and tomatoes (14). Clinically, profilin sensitization most often manifests as OAS, characterized by itching and swelling of the lips, mouth, and throat immediately after ingestion of raw plant-derived foods (14). This was also the case for our patient, whose symptoms were limited to OAS and disappeared when the same foods were consumed after cooking. This pattern reflects the intrinsic lability of profilins, which are highly sensitive to heat, pepsin, and enzymatic digestion, leading to denaturation during food processing and explaining the general tolerance of cooked or processed foods (15). In most individuals, profilin-related reactions remain mild and localized; however, in rare cases, severe systemic responses have been described following ingestion of small quantities of purified profilin, particularly in regions with high grass pollen sensitization (16). Such responses may also occur under specific conditions, notably in the presence of particular cofactors (17).

The patient also tested positive for **Hev b 8** profilins in latex. This could be regarded as a potential risk for intraoperative anaphylactic reactions. However, it has been reported that patients with specific IgE reactivity only to profilin in natural latex can undergo surgery or other medical procedures without any risk(15).

The second molecular family to which our patient was sensitized corresponds to nonspecific lipid transfer proteins. Among the five nsLTP components present on the allergen chip (Ole e 7, Art v 3, Par j 2, Ara h 9, and Pru p 3), the patient exhibited specific IgE reactivity to three of them: **Art v 3** (mugwort), **Ara h 9** (peanut), and **Pru p 3** (peach).

The International Union of Immunological Societies (IUIS) lists 46 allergenic nsLTPs from fruits, pollens, vegetables, nuts, seeds, and latex (4). They are the main allergens in the *Prunoideae* subfamily (peach, apricot, plum, cherry…) and exhibit strong IgE cross-reactivity within the *Rosaceae* family, as well as with citrus fruits, grape, tomato, vegetables (such as asparagus and lettuce), nuts (hazelnut, walnut, peanut), maize, onion, carrot, rice, and spelt (with partial cross-reactivity) (16). Their concentration varies depending on the fruit's ripeness, storage, and cultivar(17).

Some pollens (Parietaria, Artemisia, Olea) also contain nsLTPs (**Par j 1, Art v 3, Ole e 7**) but their cross-reactivity with Pru p 3 is variable, depending on sequence homology and structural similarity (**Figure 02**).

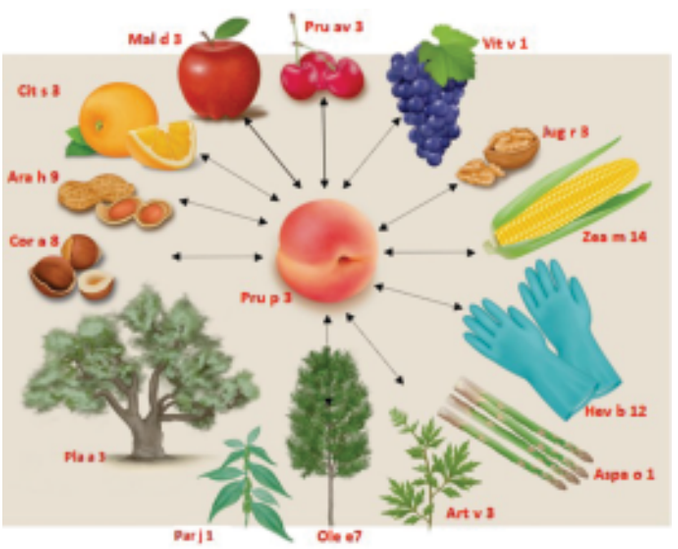

Fig 02: Cross- reactivity between nsLTP from different allergenic sources. Continuous lines indicate a high degree of cross- reactivity among the Rosaceae family. Dashed lines indicate partial cross- reactivity (4).

Our patient tested positive for Art v 3 but negative for Par j 1 and Ole 7. These results are consistent with known cross-reactivity patterns: Par j 1 shows no cross-reactivity with nsLTPs from other sources, Ole e 7 shares less than 20% amino acid sequence identity with Pru p 3, while Art v 3 exhibits partial cross-reactivity with Pru p 3.

In Mediterranean regions, sensitization to nsLTPs is often initiated by peach Pru p 3, and patients subsequently tend to develop cross-sensitization to nsLTPs from other plant foods. Pru p 3, the major allergen in peach, was the first nsLTP identified as a clinically relevant food allergen. It is mainly found in the pericarp of fruits, which contains about 220 times more Pru p 3 than the pulp. It is a small basic protein (91 amino acids, 9.2 kDa) belonging to the prolamin superfamily, and it contains a highly conserved domain composed of alpha-helices that harbor eight cysteine residues forming four intramolecular disulfide bridges. This compact structure provides great stability and resistance to heat (in vitro, its ability to bind to IgE is maintained after 30 minutes at 121°C and 160 minutes at 100°C), pH variations, and proteolytic digestion (approximately 35% of the molecule remains intact after treatment with trypsin) –(18). This stability explains its ability to maintain binding capacity to IgE and its high allergenic potential (19). As part of adapting avoidance measures, peeling these fruits significantly reduces the intake of nsLTP in sensitized individuals.

Following primary sensitization to Pru p 3, cross-reactivity can extend to homologous nsLTPs from other plant sources, including peanut Ara h 9, as observed in our patient. Among the peanut molecular allergens tested (Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 8, and Ara h 9), only Ara h 9 was positive, highlighting the relevance of specific IgE to nsLTP. Determination of Ara h 9 is therefore particularly important in patients from Mediterranean regions, where nsLTP sensitization represents a predominant allergic phenotype, and should be systematically included in the diagnostic workup of peanut allergy.

Since Ara h 9, like Pru p 3, possesses thermal and digestive stability, affected patients can develop systemic symptoms. The clinical manifestations are highly heterogeneous, ranging from local oropharyngeal symptoms to anaphylaxis (8). Our patient reported dyspnea and urticaria immediately after ingesting peanuts and nuts, and only local oropharyngeal symptoms after ingesting peaches. According to I. Mota et al. –(20), the clinical expression of allergy in individuals sensitized to nsLTPs may vary depending on the plant food consumed.

In addition, reactions are generally more severe in patients who are monosensitized to nsLTPs and tend to be milder in cases of concomitant sensitization to profilin or PR-10 (6,7). One plausible explanation for this observation is that, in nsLTP mono-sensitized patients, the high density of nsLTP-specific IgE on effector cells allows Pru p 3 to efficiently trigger mediator release, which is associated with a higher risk of severe reactions(21). Also, the ratio of nsLTP-specific IgE to total IgE may modulate cell activation and the severity of clinical reactivity (22). However, in cases of co-sensitization to nsLTPs along with profilins or the PR-10 protein family, IgE directed against nsLTPs is often accompanied by IgE targeting other allergens. Co-sensitization to unrelated allergens, from the same or different sources, therefore appears to exert a "protective"effect against the clinical expression of nsLTP allergy(21). (Fig03)

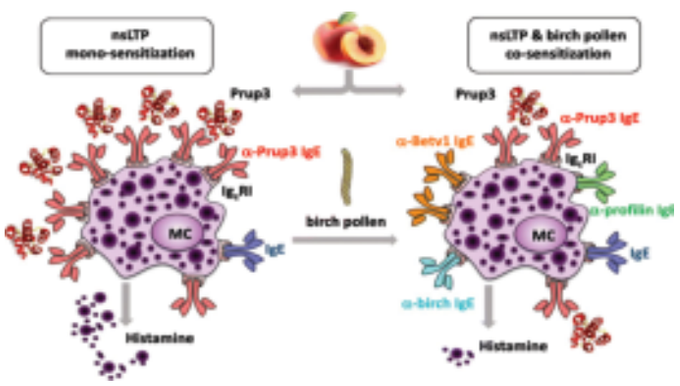

Fig03: Model of nsLTP mono-sensitization (left) vs. co-sensitization to unrelated allergens (right). (22)

Furthermore, the clinical expression is also often modulated by cofactors such as physical exercise, nonsteroidal anti-inflammatory drugs or alcohol intake (25) . The presence or absence of these cofactors may explain why our patient tolerated nsLTP-containing foods on some occasions but experienced severe reactions on others.

The patient's serum was also tested for IgE antibodies to other foods containing nsLTPs, and all tests returned positive results for Banana , Cashew nut , Pineapple , Avocado, Apricot, Kiwi and Orange. Although the patient did not show any symptoms after ingesting these foods. This positivity is likely due to cross-reactivity between nsLTPs from different allergenic sources. It should be noted that sensitization is not synonymous with allergy, and the presence of nsLTPs in a plant-based food does not justify its elimination if it has been tolerated, since contact with the intestinal immune system may contribute to maintaining tolerance (4). In such cases, the oral food challenge remains the gold standard for confirming clinical relevance and, in some instances, should be performed in the presence of cofactors.

Conclusion:

This case clearly illustrates the importance of an allergen-specific diagnosis, based on allergenic molecules (CRD — component-resolved diagnosis), for identifying cross- and co-sensitization to multiple allergens. This approach allows for personalized, accurate diagnoses and optimized management of allergic diseases. It also improves our understanding of the clinical manifestations associated with nsLTP/profilin co-sensitization, which appears to reduce the risk of severe allergic reactions to nsLTP. Finally, co-factors strongly influence the variable clinical expression of nsLTPs.

Declaration of Interests

The authors declare that they have no conflict of interest.

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Hook Effect in Indirect Immunofluorescence Assay: Always on Alert

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KEYWORDS
Prozone effect
Indirect immune
-fluorescence assay
anti-mitochondrial
antibodies
anti-liver cytosol
antibodies

Abstract

Introduction: Indirect immunofluorescence assay (IIFA) represents the most effective method for screening autoantibodies,it has been known to hold the standard over other techniques for its low report of false negatives,although observed rarely in IIFA a marked prozone effect occurredleading to erroneous results.by reporting these observations, we aim to put this phenomenon in the spotlight mainly in AMA2 and anti-LC1 antibodies detection.

Methods: This retrospective study includes four patient's laboratory findings that indicate the prevalence of false-negative anti-mitochondrial (AMA) and anti-liver cytosol (anti-LC1) antibodies resulting from excess antibodies in serum.

Results: Absence of immunofluorescence at certain dilutions, its appearance at others highly suggest the presence of prozone phenomenon in these cases

Conclusion: All laboratories providing IIF assays should be alert to the continuing possibility of hook effect,excellent communication between laboratory and clinical staff is key to minimize the risk due toanalytical errors.

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Introduction:

Indirect immunofluorescence assay (IIFA) represents the most effective method for screening autoantibodies since it allows the detection of most antibodies that are useful for diagnosis, prognosis and monitoring of organ-specific and non-specific autoimmune diseases, The observed fluorescence images provides initial information on the identity of the target, Other methods will allow the precise identification of the molecular nature of the antigen against which the detected antibody is directed.[1]

The technique has been known to hold the standard over other methods for its low report of false negatives. A prominent explanation of false negative reports has been the occurrence of the hook effect, also known as theprozone effect.a phenomenon where a decreased or absent fluorescent signal are observed, even when the antigen is present because of the interference of high antibody concentration with the labeled antibody's binding (secondary antibody) to the primary antibody, which is essential for visualizing the target. [2]

This retrospective study includes four patient's laboratory findings that indicate the prevalence of false-negative anti-mitochondrial (AMA) and anti-liver cytosol (anti-LC1) antibodies resulting from excess antibodies in serum.

Materials and methods

The samples were delivered to the immunology laboratory of Constantine's university hospital center from three separate divisions: Hepato-gastro-enterology, Internal medicine and pediatrics for testing. The brief medical history with the patient's personal information were received along with the Four samples and were specifically ordered to test for autoimmune liver diseases panel.

All case samples were in the form of blood sera; all samples were stored at 4°celsius until use.

The samples were tested with the following techniques: indirect immunofluorescence assay (IIFA) and Immunoblot using the kits provided by Euroimmun, Germany. The assays were performed following the manufacturer's instructions.

The semi-quantitative detection of human antibodies of immunoglobulin class IgG against mitochondria (AMA), smooth muscles (ASMA), liver kidney microsomal type 1 (LKM 1) and liver cytosol antigen type 1 (LC-1) using slides containing a combination of three rat tissue sections: Liver, Kidney and stomach while slides coated with HEp-2 cells acted as substrate for the determination of those against cell nuclei.

For preparation, each sample was incubated with its respective BIOCHIPs slides for 30 minutes at room temperature before applying its conjugate, if the reaction is positive, the bound antibodies are stained with FITC-labelled anti-human antibodies and made visible with the fluorescence microscope. Positive and negative controls were used on each slide.

For monospecific antibody detection ,The EUROLINE test kit provided a qualitative determination of human autoantibodies of immunoglobulin class IgG to 9 different antigens :**AMA-M2**(pyruvate dehydrogenase complex),**M2-3E** (BPO,fusion protein of the E2 subunits of the alpha-2-oxoacid dehydrogenases of the inner mitochondrial membrane),**sp100** (nuclear granula protein,) ,**PML** (promyelocytic leukaemia protein,),**gp210**(integral protein of nuclear membrane,),**LKM-1**(liver-kidney microsomes,),**LC-1**(cytosolic liver antigen type 1),**SLA/LP** (soluble liver antigen /liver-pancreas antigen) and **Ro-52**.

The test kit contains immunoblot strips coated with parallel lines of purified antigens, which are incubated with diluted patient sample(1/101 dilution), in case of positive samples, the specific IgG antibodies will bind to the corresponding antigenic site, the bound is detected using an enzyme-labelled anti-human IgG catalyzing a color reaction.

It is important to mention that in our laboratory we follow the recommendations issued in 2004 by the Committee for Autoimmune Serology of the International Autoimmune Hepatitis Group (IAIHG) regarding serum dilutions on rat tissue sections substrate.

IAIHG states that the conventional starting serum dilution is 1/10. In adults, the positivity cut-off is 1/40, whereas in children and adolescentstiters from 1/10 for anti-LKM1 and for anti-LC1 are considered positive, since autoantibodies are rare in healthy subjects of these age groups[3].Whereas initial serum dilution on HEp-2 cells is 1/80. [4]

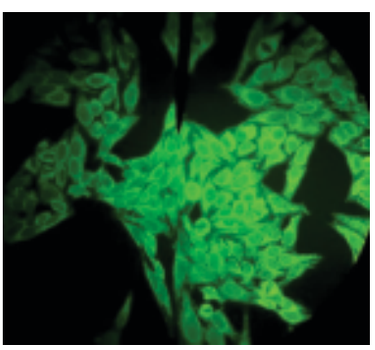
Nevertheless, the kit manufacturer instructs an initial dilution of 1/100 on both substrates.

In these four cases both dilutions were tested, those recommended by IAIHG and those instructed by the manufacturer.

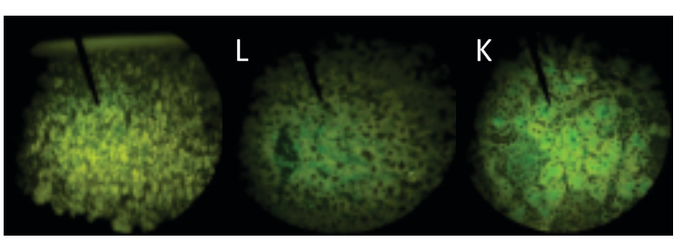
Résultats

Case 1:

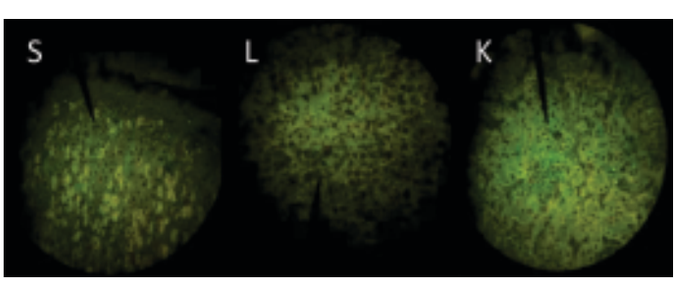
A 47 years old female with a confirmed Primary biliary cirrhosis (PBC) since 2023, treated with AUDC(acideursodésoxycholique,cholestyramine,avlocardyl), she presents an important biological liver cytolysis (Alanine transaminase (ALT) and Aspartate transaminase (AST) levels were 5 times the standard upper limit) and cholestasis (alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) levels were 5 and 3 times the standard upper limit, respectively). along with xerostomia and xerophthalmia. sent from hepato-gastro-enterology division for a suspicion of an association of Sjögren's disease and autoimmune hepatitis.the staining patterns obtained on both substrates for this case are presented in Fig.1.



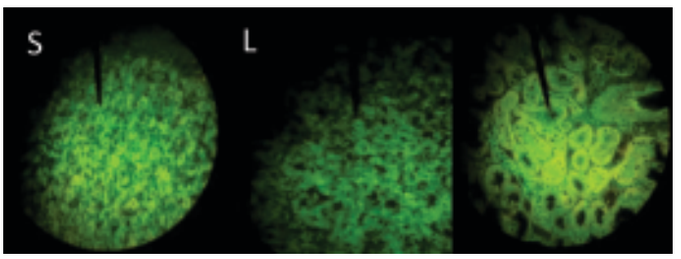
(A): 1/80 dilution 40X



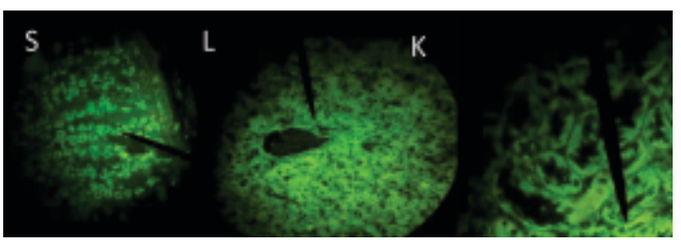
(B) :1/40 dilution 40X



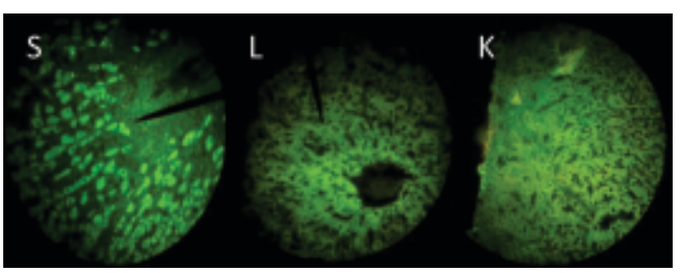
(C) :1/100 dilution 40X



(D) : 1/160 dilution 40X



E: 1/320 dilution 40X



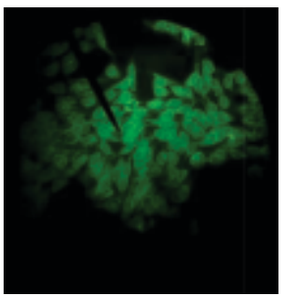
(F) : 1/640 dilution 40X

Fig. 1. Indirect Immunofluorescence staining of anti-mitochondria antibodies on HEp-2 cells (A: distinctive cytoplasmic reticular or granular pattern) and on rat tissue sections: stomach (S), Liver (L) and Kidney (K) showing: With initial dilutions, there is no staining: B (1/40), C (1/100) and with subsequent dilutions staining becomes evident: D (1/160), E (1/320), F (1/640) (Hook effect in case one).

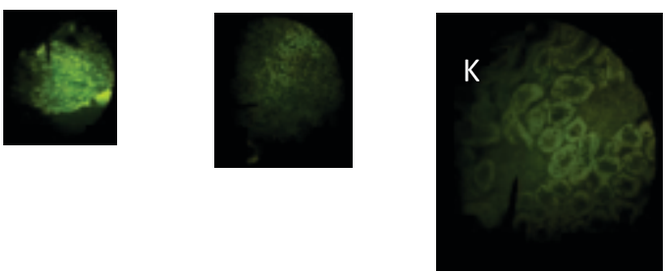
Case 2

A 55 years old female, with history of PBC and rheumatoid arthritis (RA) 4 years ago, high blood pressure 6 years ago and hypercholesterolemia, she began reporting symptoms for 2 months followed the previous diagnosis for which patient was receiving treatment (AUDC 1200 mg /j,CTC 40 mg/j,Imurel 100 mg/j, Exforge ,crestatine,Aspegic),she complained of nausea ,pruritus ,anorexia and general malaise.

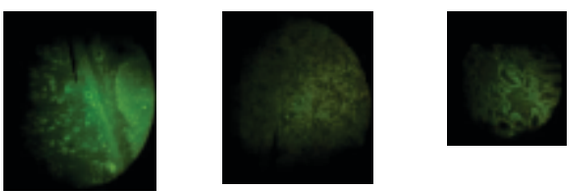
On consideration of an overlap syndrome, serum sample was sent from hepato-gastro-enterology division to our laboratory for testing.the staining patterns obtained on both substrates for this case are presented in **Fig.2**.



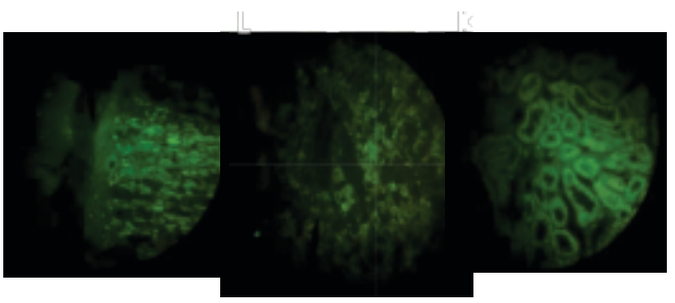
A : 1/80 dilution 40X



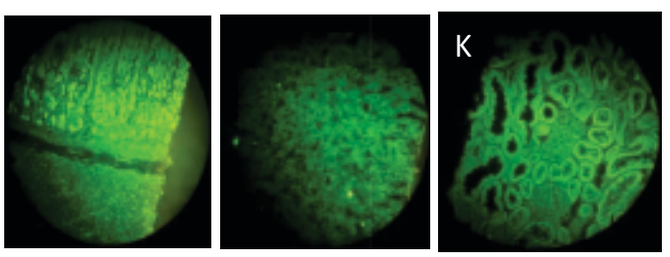
B :1/40dilution 40X



C :1/100 dilution 40X



D : 1/160 dilution 40X



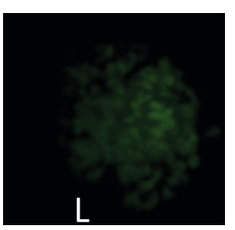
E: 1/320 dilution 40X

F: 1/640 dilution 40X

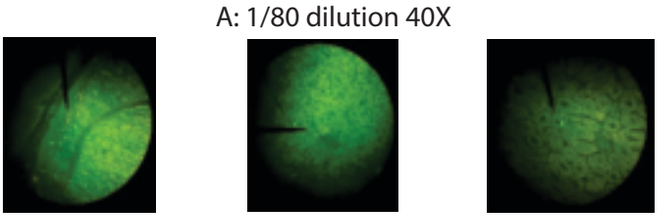
Figure. 2. Indirect Immunofluorescence staining of anti-mitochondria antibodies on HEp-2 cells (A: distinctive cytoplasmic reticular or granular pattern) and on rat tissue sections: stomach (S), Liver (L) and Kidney (K) showing: With initial dilutions, there is no staining: B (1/40), C (1/100) and with subsequent dilutions staining becomes evident: D (1/160) and E (1/320), and then disappears at F (1/640) (Prozone phenomenon in case 2).

Case 3

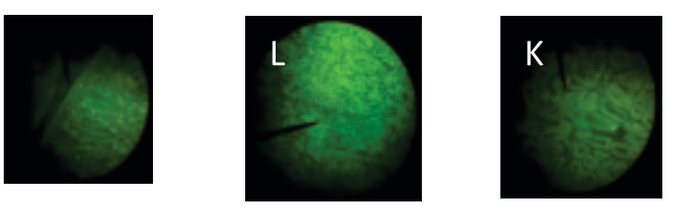
A three years old female child presented with a 3 months history of progressive jaundice and pruritus. Laboratory examinations revealed elevated levels of aspartate aminotransferase and alanine aminotransferase, sent from pediatrics division for further tests due to a suspicion of autoimmune hepatitis.the staining patterns obtained on both substrates for this case are presented in **Fig.3**.



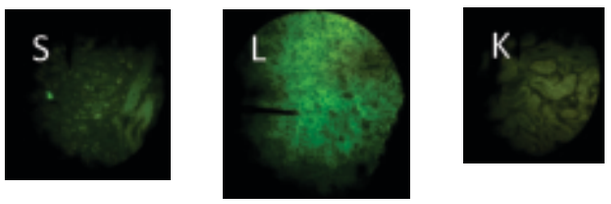
A: 1/80 dilution 40X



B: 1/10 dilution 40X

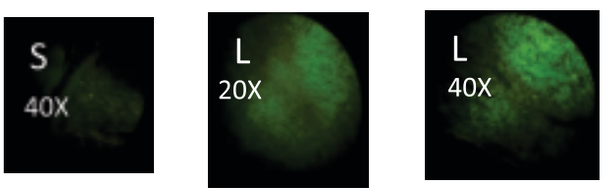


C: 1/40 dilution 40X

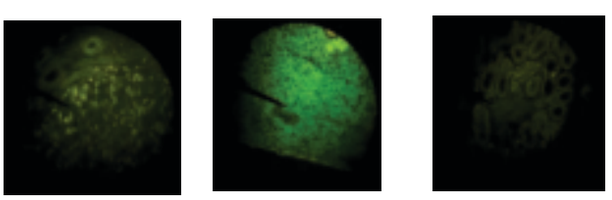


D : 1/100 dilution 40X

E: 1/160 dilution



F: 1/320 dilution

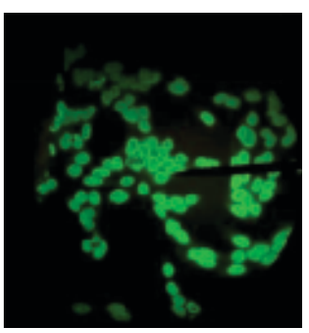


G: 1/640 dilution 40X

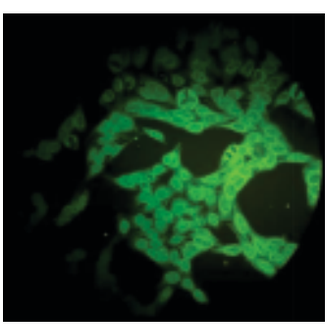
Figure. 3. Indirect Immunofluorescence staining ofanti-LC1 antibodies on rat tissue sections: stomach (S), Liver (L) and Kidney (K) showing: With initial dilutions, there is no LC-1 specific staining: B (1/10), a suspicious one on C (1/40)and with subsequent dilutions staining becomes evident: D (1/100),E(1/160),F(1/320) and G (1/640), the test on HEp-2 cells was negative (A:1/80) (Prozone phenomenon in case 3).

Case 4

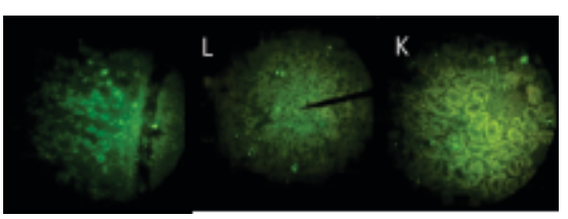
The patient is a 52 years old female sent from internal medicine division known to have Systemic lupus erythematosus, diagnosed approximately 10 years ago. She presented with complaints of fatigue, dry skin, and pruritus that started almost three years prior, she had a liver biopsy due to chronic cholestasis, the alkaline phosphatase and gamma-glutamyl transpeptidase levels were 3 and 7 times the standard upper limit, respectively. Autoantibody testing results are showed in **Fig.4**.



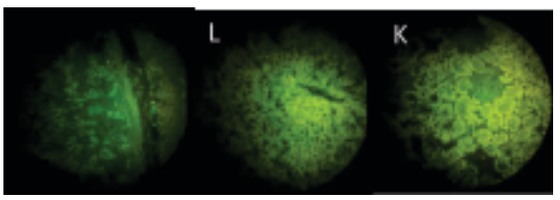
A : 1/80 dilution (40X)



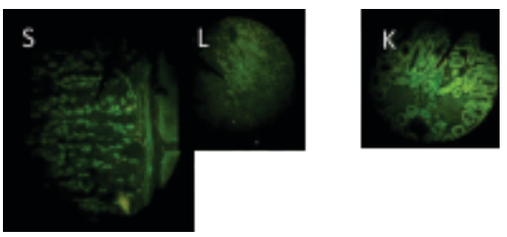
B :1/320 dilution (40X)



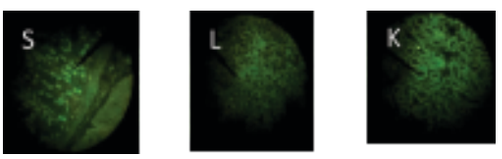
C : 1/40 dilution (40X)



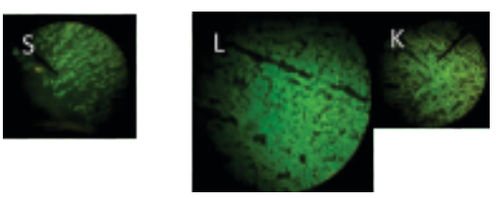
D : 1/100 dilution (40X)



E: 1/160 dilution (40X)



F: 1/320 dilution (40X)



G: 1/640 dilution (40X)

Figure. 4. Indirect Immunofluorescence staining on HEp-2 cells (A: absence of cytoplasmic granular pattern, B: appearance of a distinctive cytoplasmic granular pattern), Indirect Immunofluorescence staining of AMA on rat tissue sections: stomach (S), Liver (L) and Kidney (K) showing: With initial dilutions, a suspicious staining: C (1/40), D (1/100) and with subsequent dilutions staining becomes evident: E (1/160), F (1/320), and G (1/640), (Prozone phenomenon on both substrates in case 4).

Overall, the initial investigation of case 1 and case 2 sera with the standard 1:100 dilution as per the diagnostic kit manufacturer's instructions resulted as negative for anti-mitochondria antibodies on rat tissue sections (stomach, Liver and Kidney) and positive on HEp2 cells at a dilution rate of 1/80 showing a coarse granular filamentous staining extending throughout the cytoplasm.

These two patients are already known for PBC so we were strongly expecting a positive AMA2 antibody, we decided to perform the immunoblot assay that tests for antibodies against 9 antigens related to auto immune liver diseases. The immunoblot test returned positive for both cases, the first one was positive for anti- AMA-M2+++, M2-3 E+++, gp210+++ and Ro-52+++, the second one tested positive also for AMA-M2+++, M2-3 E+++, gp210+++, Ro52+and also Sp100+++ PML+++ at a serum dilution of 1:101.

Indirect immunofluorescence test was repeated serially with doubling dilution, increasing from 1/40 to 1/640 dilution on liver, kidney, and stomach tissue sections (Fig.1 and Fig.2) and from 1/80 to 1/1000 dilution on HEp-2 cells.

The first strong fluorescence was observed at a dilution of 1:160 for both cases with staining pattern indicating AMA antibodies. (cytoplasmic granular fluorescence in liver hepatocytes, distal and poorly proximal tubules of the kidney and stomach gastric parietal cells).

In addition of the characteristic cytoplasmic pattern of AMA antibodies on HEp-2, punctate staining of the nuclear envelope in interphase cells (with accentuation of fluorescence at the points where adjacent cells touch each other, no staining of the metaphase and anaphase chromatin plates) was observed in both cases suggesting the presence of anti-gp210 antibodies, countable discrete nuclear speckles (6 to 20 nuclear dots/cell) were also detected in case 2 witch usually indicates the presence of anti-sp100 antibodies.

Case 3 was a delicate one due to the age of the patient and the strong suspicion of HAI, taking in consideration her critic state an immunoblot was performed immediately which came back highly positive (+++) for anti-LC1 antibodies.

the starting dilution recommended for children on rat tissues :1/10 was negative, but, multiple serum dilutions were performed on rat tissues increasing from 1/40 to 1/640 dilution, an anti LC1 antibody pattern with a brightly staining of the hepatocyte cytoplasm, sparing the centrilobular zone appeared clearly at a serum dilution of 1/100 and sustained until 1/640 dilution.

Case 4 followed a similar procedure. this time no cytoplasmic staining was observed on the standard screening dilution on HEp-2 cells (dilution 1/80) and a suspicious pattern of AMA antibodies on rat tissues was detected. Again in this case subject was already diagnosed for PBC, sample was retested for AMA2 antibodies based on suspicion of a false negative result.

The serum was tittered serially using the doubling dilution method starting at 1/40 to 1/640 dilution on rat tissues and from 1/80 to 1/1000 dilution on HEp-2 cells.
A remarkable staining with characteristic recognizable patterns of AMA antibody specificity on both substrates was detected, starting from 1/320 dilution on HEp-2 cells and from 1/160 on rat tissues. The presence of AMA2 antibodies was confirmed by an immunoblot that returned highly positive (+++) for AMA-M2 and M2-3E.
The absence of immunofluorescence at certain dilutions, its appearance at others highly suggest the presence of prozone phenomenon in these cases which is usually believed to be seen less in the indirect immunofluorescence technique.

Discussion

The suspicion of a prozone effect was embarked upon due to the prior knowledge from laboratory investigators of patient's medical history in case 1,2 and 4 , and the insistence of an urgent testing from clinicians in case 3 , The lack of staining when using the standard dilution (both, those recommended by IAIHG and those as prescribed by the manufacturer) is accounted by the presence of excess antibodies ,a falsely negative result is then seen with the starting dilution.
The occurrence of false negatives was observed in 1/40 and 1/100 dilutions in case 1 and 2 for AMA antibodies, in 1/10 and 1/40 dilutions in case 3 for anti-LC-1 antibodies.
Case 4 showed a false negative AMA in 1/80 dilution on HEp-2 cells and a suspicious pattern of AMA in 1/40 and 1/100 dilutions on rat tissue sections: Stomach, Liver and Kidney.
On high-tittered sera (1/160,1/320 and 1/640 dilutions on rat tissue sections) and 1/320 dilution (on HEp-2 cells), it was evident that the conjugate binding fluorescence confirms the concentration of antibodies bound to target antigens. The immunoblot assay procedure was ideal for confirming the antibody specificity, and the prozone effect. However, it cannot replace IIFA as a primary evaluation[5].
Prozone phenomena are seen in many secondary manifestations of antigen-antibody interactions. The precipitation reaction is the classical example [6], it can also be demonstrated in other systems, notably in bacterial agglutination and hemagglutination assays.
Prozone phenomena in IIFA have received little attention, even though the phenomenon has occasionally been noted in tests for antinuclear antibodies [7].
Immunofluorescence visualizes a primary antibody-antigen interaction. The absence of fluorescence in IIFA staining indicates that the conjugate did not bind. Such a lack of binding is usually due to failure of the primary antibody to bind to the target antigen.In the prozone effect observed in our cases, absence of binding of conjugate molecules occurred despite the binding of primary antibodies to target antigens.
E. UNDER & A. MIETTINEN observed in their study a marked prozone effect in indirect immunofluorescence with rabbit antisera against rat renal proximal tubular epithelial brush border (BB) antigens, they concluded that the antigenic sites of the attached antibodies somehow become inaccessible to the anti-immunoglobulin conjugates and that diluting the specific tissue-reactive antibodies involved exposure of these sites to the conjugate. The observed concentration-dependent inaccessibility may depend on tight packing of antibody molecules attached to the tissue and the space between bound immunoglobulin molecules could become insufficient to allow interposition of anti-immunoglobulin conjugates[8].
Sundqvist. in an extensive microfluorometric study of factors influencing cell surface membrane staining in indirect and direct immunofluorescence, also observed a prozone phenomenon using IIFA. In discussing the mechanism of the prozone effect, he concludes that membrane-attached antibodies in concentrations corresponding to the prozone do not disappear from the cell surface but become inaccessible to the conjugate molecules[9][10].
As it has been mentioned earlier, our tests were performed on diagnosis kits of the same manufacturer: EUROIMMUN,Germany,there is a possibility that the prozone effect observed in these cases is due to the quality of its substrates or conjugates, it would be interesting to test the same serum dilutions of the same patients on other substrates proposed by other manufacturers.
Owing to the fact that only these kits are available in our laboratory, we were unable to investigate this possibility.
As we can see, the mechanisms of prozone effect in general has been well investigated in multiple studies: [11], [12]and[13].But the occurrenceof thisphenomenon in IIFAhave not been given much attention, by reporting these observations we managed to put this phenomenon in the spotlight mainly in AMA2 and anti-LC1 antibodies detection.
These cases illustrate that all laboratories providing IIF assays should be alert to the continuing possibility of

hook effect. Indirect immunofluorescence is generally touted for its higher sensitivity, reliable pattern recognition and simplicity in routine use. However, it is imperative that testing involves caution of possible false-negative results. Serial dilutions of these samples is recommended in order to ensure that the reported result is accurate. These cases also reinforce the critical importance of exchange of information and excellent communication between clinical and laboratory staff in order to minimize the risk of clinical errors arising from erroneous analytical results.

Déclaration d'intérêts

None.

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ARTICLE ORIGINAL

Clinicobiological and immunologicalcharacteristics of monoclonal gammopathies associated with autoimmunediseases: aretrospective single-center analysis of 162 cases

Profil clinicobiologique et immunologique des gammapathies monoclonales associées aux maladies auto-immunes: étude rétrospective monocentrique sur 162 cas

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MOTS CLÉS
Gammapathies monoclonales (MGUS, myélome multiple) ; Maladies auto-immunes (MAI); Polyarthrite rhumatoide (PR) ; Lupus érythémateux systémique; Profil immunologique ; Caractéristiques cliniques ; Scores pronostiques (Mayo Clinic, ISS-R).

Résumé

Introduction : Les gammapathies monoclonales sont fréquentes chez le sujet âgé, toutefois leur association aux maladies auto-immunes (MAI) reste mal caractérisée. Cette étude vise à décrire les caractéristiques cliniques, immunologiques et pronostiques des patients présentant une gammapathie monoclonale associée à une MAI, et à les comparer à la population générale des gammapathies suivie dans notre service.

Méthodes : Étude rétrospective monocentrique incluant 162 patients ayant à la fois une gammapathie monoclonale (MGUS ou myélome multiple – MM) et une MAI, entre 2015 et 2025. Les données ont été comparées aux 5020 cas de gammapathies recensés sur la même période dans notre service. Les caractéristiques démographiques, les types de MAI, les isotypes, la concentration du composant monoclonal (CM) et les scores pronostiques (Mayo Clinic, ISS-R) ont été analysés.

Résultats : La MGUS représentait 73.45 % des cas. L'âge moyen au diagnostic était de 54,75 ans, inférieur à celui des gammapathies isolées (63–65 ans). Une prédominance féminine marquée (69,75 %) a été observée. Les MAI les plus fréquentes étaient la polyarthrite rhumatoïde (30,86 %), le syndrome de Gougerot-Sjögren (11,11 %) et le lupus érythémateux systémique (7,4 %). L'isotype IgG Kappa était majoritaire (37,65 %), avec une concentration moyenne de CM modérée (4,12 g/L). Les scores pronostiques classaient la majorité des MGUS en faible risque évolutif. Des pics monoclonaux transitoires ou fluctuants étaient plus fréquents que dans les gammapathies isolées.

Conclusion : Les gammapathies monoclonales associées aux MAI présentent un profil épidémiologique et immunologique distinct, avec un diagnostic plus précoce, une prédominance féminine et des taux globalement modérés de CM. Bien que les données pronostiques soient rassurantes, le contexte auto-immun impose un **suiviprolongé**, en raison d'un risque évolutif potentiellement modulé mais encore mal défini.

KEYWORDS
Monoclonal gammopathy (MGUS, multiple myeloma); Autoimmune diseases (AID); Rheumatoid arthritis; Sjögren's syndrome; Systemic lupus erythematosus; Immunological profile; Clinical features; Prognostic scores (Mayo Clinic, ISS-R).

Abstract

Introduction: Monoclonal gammopathies are common in elderly individuals, but their association with autoimmune diseases (AID) remains poorly characterized. This study aims to describe the clinical, immunological, and prognostic features of patients with monoclonal gammopathy associated with an AID, and to compare them with the general population of gammopathies followed in our department.

Methods: This is a single-center retrospective study including 162 patients with both monoclonal gammopathy (MGUS or multiple myeloma – MM) and an AID, between 2015 and 2025. Data were compared with 5,020 cases of gammopathies recorded during the same period in our department. Demographic characteristics, types of AID, isotypes, monoclonal component (MC) concentration, and prognostic scores (Mayo Clinic, ISS-R) were analyzed.

Results: MGUS accounted for 73.45% of cases. The mean age at diagnosis was 54.75 years, lower than that of isolated gammopathies (63–65 years). A marked female predominance (69.75%) was observed. The most frequent AIDs were rheumatoid arthritis (30.86%), Sjögren's syndrome (11.11%), and systemic lupus erythematosus (7.4%). The predominant isotype was IgG Kappa (37.65%), with a moderate mean MC concentration (4.12 g/L). Prognostic scores classified most MGUS cases as low risk of progression. Transient or fluctuating monoclonal spikes were more frequent than in isolated gammopathies.

Conclusion: Monoclonal gammopathies associated with AID show a distinct epidemiological and immunological profile, with earlier diagnosis, female predominance, and overall moderate MC levels. Although prognostic data are reassuring, the autoimmune context requires long-term follow-up, due to a potentially modulated but still poorly defined risk of progression.

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Introduction:

A monoclonal immunoglobulin is the secretory product of a single clone of B lymphocytes or plasma cells, secondary to a clonal proliferation. This immunoglobulin may be complete, consisting of two heavy chains and two associated light chains, or incomplete, containing only light chains (kappa or lambda), or more rarely, only heavy chains. Monoclonal gammopathies (MG) are clonal proliferations of plasma cells or lymphoplasmacytic cells, responsible for the secretion of a monoclonal protein or paraprotein. An MG may reveal a malignant hematologic disease (multiple myeloma – MM, Waldenström's disease, lymphoma, etc.) or a benign condition, since monoclonality is not synonymous with malignancy (monoclonal gammopathy of undetermined significance, MGUS). (1)

Multiple myeloma (MM) is a malignant hematologic disorder characterized by the clonal proliferation of

plasma cells, leading to bone marrow infiltration and secretion of a monoclonal immunoglobulin in most cases. According to the International Myeloma Working Group (IMWG), MM is defined by the presence of clonal bone marrow plasma cells ≥10% (or a histologically proven osseous or extraosseous plasmacytoma) and at least one of the following criteria:

- **CRAB criteria** (end-organ damage attributable to plasma cell proliferation):
  - Hypercalcemia: serum calcium >0.25 mmol/L (>1 mg/dL) above the upper limit of normal, or >2.75 mmol/L (>11 mg/dL).
  - Renal insufficiency: creatinine clearance <40 mL/min or serum creatinine >177 µmol/L (>2 mg/dL).
  - Anemia: hemoglobin <10 g/dL or >2 g/dL below the lower limit of normal.
  - Bone lesions: at least one osteolytic lesion detected by skeletal X-ray, CT, or PET-CT.
- **Additionalcriteriaintroducedin2014:**
  - Bone marrowplasmacytosis ≥60%.
  - Serum free light chain ratio ≥100 (measured with the Binding Site assay, with the involved light chain ≥100 mg/L).
  - 1 focal lesion ≥5 mm on MRI. (2)

Prognosis is based on the revised International Staging System (R-ISS), the gold standard for newly diagnosed MM, which incorporates serum β2-microglobulin (Sβ2M), serum albumin, cytogenetic abnormalities by iFISH, and LDH levels:

- **Stage I:** Sβ2M <3.5 mg/L, serum albumin ≥3.5 g/dL, standard-risk cytogenetics by iFISH, and normal LDH.
- **Stage II:** Not fitting criteria for stage I or III.
- **Stage III:** Sβ2M ≥5.5 mg/L plus either high-risk cytogenetics by FISH or elevated LDH. (3)

MGUS is definedby:

- Absence of CRAB criteria or amyloidosis signs.
- Plasma cellproliferation ≥10% and <60%.
- And/or serum monoclonal protein (IgG or IgA) ≥30 g/L.
- And/or Bence Jones proteinuria ≥500 mg/24 h. (1)

Each year, about 1% of patients with MGUS progress to malignant hematologic disorders such as MM, light-chain amyloidosis, Waldenström macroglobulinemia, or lymphoma. This progression risk depends on several parameters (Mayo Clinic model criteria):

- Type of monoclonal immunoglobulin: IgA or IgM MGUS carries a higher risk of progression than IgG MGUS.
- Serum monoclonal protein concentration: levels >15 g/L are associated with higher progression risk.
- Free light chain ratio (FLC ratio): an abnormal ratio (either too high or too low) indicates higher progression risk.

Combining these factors allows risk stratification:

- **Low risk:** No risk factors or only one low-intensity factor.
- **Intermediate risk:** Two risk factors.
- **High risk:** All three risk factors. (4)

Autoimmune diseases (AID) frequently occur with monoclonal gammopathies, particularly MGUS. AIDs are characterized by increased immune reactivity against autoantigens, causing tissue damage. Although their etiology remains largely unknown, genetic, microbial, environmental, and psychological factors are known contributors. Clinical presentations vary, including organ-nonspecific autoimmune diseases (e.g., rheumatoid arthritis – RA, Sjögren's syndrome, systemic lupus erythematosus – SLE), and organ-specific diseases (e.g., pernicious anemia). (5)

From a pathophysiological perspective, monoclonal gammopathies result from the clonal emergence of abnormal plasma cells, promoted by several immunological and genetic mechanisms. In the setting of AID, chronic antigenic stimulation and prolonged B-cell activation play central roles. These repeated stimulations promote the expansion of potentially monoclonal plasma cell clones within a proinflammatory environment.

Cytokines such as IL-6, IL-13, and TNF-α are involved in plasma cell proliferation by inhibiting apoptosis, promoting survival, and maintaining chronic activation. This inflammatory state increases the risk of accumulating genetic alterations and malignant transformation into disorders such as MM. (6, 7)

Myeloma plasma cells differ from normal plasma cells by overexpression of Toll-like receptors (TLRs), especially TLR7, TLR9, and TLR4. This abnormal expression alters responses to physiological immune signals, contributing to prolonged survival and abnormal proliferation of tumor cells. Moreover, overexpression of the IL-6 receptor favors autocrine stimulation, enhancing expansion and resistance to pro-apoptotic signals. (8)

At the genomic level, even at early stages such as MGUS, cytogenetic abnormalities are frequently detected. The most common include translocations involving region 14q32 (IgH heavy chain gene), trisomies of odd

chromosomes (hyperdiploidy), and deletions of 17p (TP53). (9)
Hyperdiploidy, present in most MGUS cases, is generally associated with a favorable prognosis, except for certain abnormalities such as trisomy 21, which correlates with higher progression risk. In contrast, deletion of chromosome 17p13 is recognized as the most unfavorable prognostic factor, predictive of progression to high-risk MM. (10)
Thus, the occurrence of monoclonal gammopathies in the context of AID lies at the intersection of persistent immuno-inflammatory abnormalities and early cytogenetic disturbances, justifying strict longitudinal monitoring even in the absence of clinical signs of progression.
Progression from MGUS to MM is characterized by bone lesions, osteoclast activation, and osteoblast inhibition. Osteoclast activation is triggered by increased RANKL expression by osteoblasts and possibly plasma cells, along with inhibition of osteoprotegerin (OPG) by CD138. Release of macrophage inflammatory proteins MIP-1α and MIP-1β, as well as expression of stromal cell-derived factor 1α, also contribute to osteoclast activation and bone resorption. (11)
The frequency of monoclonal gammopathies increases with age, affecting 3% of individuals over 50 years and 5% after age 70 (7). The overall prevalence of a monoclonal component (MC) among patients with autoimmune diseases is estimated at around 2%. However, some diseases show much higher rates. For example, Sjögren's syndrome is associated with an MC in 43.9% of cases, with a risk of progression to lymphoma. In SLE, MC is found in 14.6% of patients, a frequency correlated with lymphoproliferative disorders. Finally, a prevalence of 19.5% is observed in patients with rheumatoid arthritis (RA). (6)

Study objective:

The aim of our work is to analyze the frequency of autoimmune diseases associated with monoclonal gammopathies (MM and MGUS) and to assess their prognostic impact within our cohort. We also aim to describe the clinicobiological and immunological profiles of these patients.

Materials and methods:

Statistical analysis was performed using Excel 2016 and SPSS software. For quantitative variables, the mean, median, and standard deviation were calculated. For qualitative variables, frequencies were determined. Comparisons were carried out using the Mann–Whitney test for non-parametric variables and the Khi^2 test for categorical data.
A retrospective study was conducted in the Immunology Department of the University Hospital Center of Beni Messous over a 10-year period (2015–2025), including 162 patients.

The mean age of the study population was 54.75 ± 16.8 years. Most patients were women (113/162, 69.75%), while men accounted for 30.24% (49/162), with a sex ratio (M/F) of 0.43.

MM was present in 43 out of 162 patients (26.54%, group 1), including 27 women (62.79%) and 16 men (37.20%), with a sex ratio (M/F) of 0.59. The mean age was 53.51 ± 16.95 years.

MGUS was found in 119 cases (73.45%, group 2), including 86 women (72.26%) and 33 men (27.73%), with a sex ratio (M/F) of 0.38. The mean age was 55.26 ± 16.8 years.

These patients underwent the following serum and urinary immunological tests:

- **Serum Protein Electrophoresis (SPEP):**

SPEP is an essential laboratory method for the qualitative and quantitative analysis of major serum protein fractions. It highlights a monoclonal component in the gamma, beta, or, more rarely, alpha region. It appears as a narrow, dense, and symmetrical band in one of these zones. The test was performed using either capillary techniques (Capillarys Octa 3® – Sebia, V8® – Helena) or agarose gel electrophoresis (SAS3®, SAS4® – Helena, Hydrasys Focusing® – Sebia).

- **Serum Protein Immunofixation (IFE):**

IFE confirms the presence of a monoclonal component and identifies precisely the immunoglobulin isotype (IgG, IgA, IgM, etc.) and the type of light chain (kappa or lambda). It was performed using SAS3®, SAS4® Helena Biosciences, or Hydrasys Focusing Scan® Sebia.

- **Serum Beta-2-Microglobulin Assay:**

This assay was performed by laser nephelometry using BNproSpec®, and carried out in only 11 patients from group 1.

- **Serum Free Light Chains (sFLC) Assay and FLC Ratio Calculation:**

Performed by turbidimetry on SPA Plus® or laser nephelometry on BNproSpec®, or on SPA Plus Binding Site. It was done in 5 patients from group 1 and 6 patients from group 2.

- **Urine Protein Electrophoresis (UPEP):**

Conducted on agarose gel (SAS3®, SAS4® Helena®, or Hydrasys Focusing Scan®, Sebia®), performed in 8 patients from group 1 and 3 patients from group 2.

- **Urine Protein Immunofixation:**

Performed using SAS3 IFE-9 kits (SAS3®, SAS4®, Helena®) or Bonce Jonce kits (Hydrasys Focusing Scan®).

Some of our patients also underwent an **autoimmunity workup**, including the detection of antinuclear antibodies (ANA) by indirect immunofluorescence (IIF) on HEp-2 cells or by chemiluminescence (CLIA) (MAGLUMI X3). Identification of specific autoantibodies was performed using Immunodot (dot-blot), ELISA (enzyme-linked immunosorbent assay), or CLIA. All techniques were carried out in our immunology laboratory in accordance with manufacturer recommendations, with both internal and external quality control.

Results:

1. Clinical Data:

Group 1 (MM): Characterized by CRAB features, notably anemia in 34.88% (15/43), renal insufficiency in 20.93% (9/43), and bone lesions in 27.90% (12/43). Clinical manifestations related to autoimmune diseases were also observed: joint involvement (arthritis and arthralgia) in 34.88% (15/43), sicca syndrome (ocular–oral dryness) in 16.27% (7/43), and pleuropulmonary manifestations in 13.95% (6/43). Other manifestations, such as nephrotic syndrome, proteinuria, hepatic, and cutaneous involvement, were noted at much lower frequencies.

Group 2 (MGUS): Clinical manifestations were more frequent, dominated by joint involvement in 49.57% (59/119), followed by sicca syndrome in 21.84% (26/119), pleuropulmonary manifestations in 13.44% (16/119), and cutaneous manifestations in 6.72% (8/119). Other signs, including nephrotic syndrome, proteinuria, hematuria, digestive, and hepatic involvement, were reported but with much lower frequencies.

Table 1. Demographic and clinical data

Parameters	Group 1 (MM, n=43)	Group 2 (MGUS, n=119)	Total (n=162)	Comments
Epidemiological data				
Mean age ± SD	53.51 ± 16.95 yrs	55.26 ± 16.8 yrs	54.75 ± 16.8 yrs	Younger than literature (65–70 yrs, Kyle 2006)
Sex ratio (M/F)	0.59	0.38	0.43	Opposite to classical sex ratio (M/F ≈ 1.7)
Clinical data				
Anemia	15 (34.9 %)	/	/	Typical of MM
Renal insufficiency	9 (20.9 %)	/	/	Consistent with literature
Bone lesions	12 (27.9 %)	/	–	Classical in MM
Joint involvement	15 (34.9 %)	59 (49.6%)	74 (45.7%)	More frequent in MGUS
ocular and salivary gland dryness	7 (16.3 %)	26 (21.8%)	33 (20.4%)	Consistent with SS (20–44%)
pleuropulmonary manifestations	6 (13.9 %)	16 (13.4%)	22 (13.6%)	Similar frequencies
Nephrotic syndrome, proteinuria, hepatic, and cutaneous involvement	Rare	17 (14.28 %)	24 (14.81 %)	More frequent in MGUS

2. Frequencies of Autoimmune Diseases (AID):

Group 1 (MM): The most common AID was rheumatoid arthritis (RA) in 18.60% (8/43), followed by pernicious anemia and Sjögren's syndrome, each in 16.27% (7/43), and celiac disease in 9.30% (4/43). Unclassified autoimmune diseases accounted for 16.27% (7/43). Systemic lupus erythematosus (SLE), ANCA-associated vasculitis, and membranous glomerulonephritis were each found in 4.65% (2/43). Systemic sclerosis and inflammatory bowel disease (IBD) were noted in one patient each (2.32%).

Group 2 (MGUS): RA was also the most frequent AID, identified in 35.29% (42/119), followed by unclassified AID in 15.96% (19/119), Sjögren's syndrome in 14.28% (17/119), SLE in 8.40% (10/119), and systemic sclerosis in 5.88% (7/119). Autoimmune liver diseases (autoimmune hepatitis and primary biliary cholangitis) were found in 4.2% (5/119). Autoimmune thyroiditis, polymyositis, and ankylosing spondylitis were found in 2.52% each (3/119). ANCA vasculitis, celiac disease, pernicious anemia, and IBD were each observed in 1.68% (2/119). A single case was reported for antiphospholipid syndrome and autoimmune pancreatitis (0.84% each).

Overall cohort (n=162): RA was the most frequent AID, present in 32.09% (52/162), followed by unclassified AID in 21.84% (26/162), Sjögren's syndrome in 14.19% (23/162), SLE in 7.40% (12/162), pernicious anemia in 5.55% (9/162), systemic sclerosis in 4.93% (8/162), and celiac disease in 3.7% (6/162). Autoimmune liver diseases were present in 3.08% (5/162). Less frequent AIDs comprised ANCA vasculitis (2.46%), polymyositis, inflammatory bowel disease, ankylosing spondylitis, and autoimmune thyroiditis (1.85% each), membranous glomerulonephritis (1.23%), and more rarely antiphospholipid syndrome and autoimmune pancreatitis (0.61% each).

Table 2. Frequency of autoimmune diseases

Parameters	Group 1 (MM, n=43)	Group 2 (MGUS, n=119)	Total (n=162)	Comments
Frequency of autoimmune diseases (AID)				
Rheumatoid arthritis	8 (18.6%)	42 (35.3%)	52 (32.1%)	Most frequent AID (10–40% in literature)
Sjögren's syndrome	7 (16.3%)	17 (14.3%)	23 (14.2%)	Consistent with literature (20–44%)
Systemic lupus erythematosus (SLE)	2 (4.6%)	10 (8.4%)	12 (7.4%)	Lower than literature (10–15%)
Pernicious anemia	7 (16.3%)	2 (1.7%)	9 (5.6%)	More frequent in MM
Systemic sclerosis	1 (2.3%)	7 (5.9%)	8 (4.9%)	Rare but described
Celiac disease	4 (9.3%)	2 (1.7%)	6 (3.7%)	More frequent in MM
Unclassified AID	7 (16.3%)	19 (16.0%)	26 (16.1%)	Similar proportions
Rare AID (ANCA vasculitis, MG, IBD, APS, etc.)	Isolated cases	Isolated cases	<5%	Rare but consistent with literature

3. Immunological Data:

Group 1 (MM): Monoclonal MM (single M-protein) was predominant (79.06%, 34/43), followed by biclonal MM (16.27%, 7/43) and triclonal MM (4.65%, 2/43). The most frequent isotype was IgG Lambda (34.88%, 15/43), followed by IgG Kappa (27.90%, 12/43), IgA Kappa (11.62%, 5/43), and light-chain Kappa (2.32%, 1/43). Other rare combinations included IgA Lambda (2.32%), IgG Lambda + IgM Kappa (6.97%), IgA Lambda + IgG Kappa (2.32%), double IgG Lambda/Kappa (2.32%), IgG + double IgM (2.32%), and IgG + double IgA (2.32%). The mean M-protein concentration was 4.75 ± 1.06 g/L. Electrophoretic migration was mostly in the gamma region (83.72%, 36/43), less often in the beta region (9.30%, 4/43), and in both beta and gamma regions in biclonal/triclonal cases (6.97%, 3/43). Hypogammaglobulinemia was found in 32.55% (14/43). Bence Jones proteinuria (BJP) was tested in 8 patients (18.60%) and was positive. Beta-2 microglobulin was measured in 12 patients (27.90%), with a mean level of 4.07 ± 3.67 g/L. ISS staging (based on available data) classified 58.33% (7/12) in stage I, 8.33% (1/12) in stage II, and 25% (3/12) in stage III. Free light chain assay was performed in 5 patients (11.62%), with abnormal results in 2 and normal in 3.

Group 2 (MGUS): Monoclonal MGUS was predominant (77.31%, 92/119), followed by biclonal MGUS (19.32%, 23/119) and triclonal MGUS (3.36%, 4/119). The most frequent isotype was IgG Kappa (42.85%, 51/119), followed by IgG Lambda (26.89%, 32/119) and IgA Lambda (5.88%, 7/119). Other rare isotypes included IgM Lambda (1.68%) and combinations such as IgG Lambda + IgM Kappa (4.20%), double IgG Lambda (0.84%), IgG + IgA + Lambda light chain (1.68%), etc. The mean M-protein concentration was 4.13 ± 4.16 g/L. Migration was mostly in the gamma region (81.51%, 97/119), less often in the beta region (14.28%, 17/119). Hypogammaglobulinemia was present in 21% (25/119). BJP was tested in 3 patients (2.5%) and was positive. Free light chain assay was performed in 7 patients. Prognostic evaluation (based on isotype and M-protein concentration) showed that 67.7% (77/119) had no risk factors, 35.29% (42/119) had one risk factor (mostly non-IgG isotypes), and 2 patients had abnormal FLC ratio. Overall, most patients had a favorable prognosis.

Overall cohort (n=162): Monoclonal gammopathy (single M-protein) was predominant (77.77%, 126/162), followed by biclonal (18.51%, 30/162) and triclonal (3.70%, 6/162). The mean M-protein concentration was 4.12 ± 4.34 g/L. Migration was mostly in the gamma region (82.09%, 133/162), less often in the beta region (12.96%, 21/162). Biclonal and triclonal cases showed beta/gamma and beta/gamma/gamma migration patterns (3.08% and 0.61%, respectively). The most frequent isotype was IgG Kappa (38.88%, 63/162), followed by IgG Lambda (29.01%, 47/162), IgA Kappa (5.55%, 9/162), and IgA Lambda (4.93%, 8/162). Several combinations were also noted: IgG + IgM (4.93%), double IgG (6.17%), IgA + IgG (3.08%), etc. Hypogammaglobulinemia was present in 24.07% (39/162). BJP was positive in 6.79% (11/162).

Table 3. Immunological data.

Parameters	Group 1 (MM, n=43)	Group 2 (MGUS, n=119)	Total (n=162)	Comments
Immunological data				
Mean M-protein conc. ± SD	4.75 ± 1.06 g/L	4.13 ± 4.16 g/L	4.12 ± 4.34 g/L	Moderate means, overall concordant with literature
Biclonal GM	7 (16.3%)	23 (19.3%)	30 (18.5%)	Higher than general series (<10%), may reflect chronic antigenic stimulation due to AID
Triclonal GM	2 (4.7%)	4 (3.4%)	6 (3.7%)	Rare
Predominant isotype	IgG Lambda (34.9%)	IgG Kappa (42.9%)	IgG Kappa (38.9%)	Consistent with literature (IgG majority)
Predominant isotype	IgG Lambda (34.9%)	IgG Kappa (42.9%)	IgG Kappa (38.9%)	Consistent with literature (IgG majority)
Positive BJP	8 (18.6%)	3 (2.5%)	11 (6.8%)	Typical of MM
Migration site				
Gamma	36 (83.72%)	97 (81.51%)	133 (82.09%)	In line with published series (>80%)
Beta	4 (9.30%)	17 (14.28%)	21 (12.96%)	Beta position rarer, often associated with IgA
Beta/Gamma	1 (2.32%)	3 (2.52%)	4 (2.46%)	Rare

Beta/Gamma/Gamma	2 (4.65%)	2 (1.68 %)	4 (2.46 %)	Rare
Prognostic factors				
ISS (MM)	Stage I: 58.3%; Stage III: 25%	/	/	Mostly good prognosis but small sample
Mayo Clinic (MGUS)	/	67.7 % no risk; 35.3 % one risk	/	Overall low-risk cohort

Discussion:

Within the immunology department of our university hospital, 5,020 cases of monoclonal gammopathies were recorded between 2015 and 2025. In comparison, our current study cohort represents 3.22% of all cases. Although modest, this proportion brings together patients with a dual characteristic: the presence of a monoclonal gammopathy (MGUS or MM) associated with an autoimmune disease. This subgroup therefore deserves particular attention because of its potentially distinct epidemiological, semiological, and prognostic value.

In our study, MGUS represented the most frequently observed monoclonal gammopathy, affecting 119 out of 162 patients, i.e., 73.45% of the study population. This predominance is consistent with literature data, which identify MGUS as an entity far more frequent than multiple myeloma (MM) or other malignant lymphoid hemopathies (13). The prevalence of MGUS increases with age, affecting around 3% of individuals over 50, and up to 5% after 70 years, according to large epidemiological series such as Kyle et al. (2006), who conducted a large-scale cohort study on more than 21,000 patients from Minnesota (13).

Unlike the classical data described by Kyle et al. (2006), which reported a male predominance in monoclonal gammopathies (male-to-female ratio ~1.7), our study shows a clear female predominance (69.75%). This divergence could be explained by an immunological selection bias, since autoimmune diseases are more frequent in women, suggesting a distinct pathophysiological profile in our cohort (13).

The mean age at diagnosis was 54.75 ± 16.8 years, significantly younger than that observed in the general GM population of our department, estimated at 63–65 years (internal CHU Beni Messous data, 2014–2025). Literature data report that monoclonal gammopathies generally occur at an advanced age, with a median age at diagnosis between 65 and 75 years, depending on the GM type. MGUS is often detected around 70 years, while multiple myeloma is usually diagnosed between 69 and 71 years (14,15). Several autoimmune diseases, particularly systemic lupus erythematosus (SLE), preferentially affect young adults, with a peak incidence around 30 years. This distribution could explain the earlier age observed in our cohort.

In Group 1, we observed that rheumatoid arthritis (RA) was the most frequent autoimmune disease (23.25%), followed by Biermer's disease (16.27%) and Sjögren's syndrome (SS) (13.95%). Rarer AIDs such as celiac disease or ANCA-associated vasculitis were also present. In Group 2, RA was also the most frequent AID (35.29%), followed by Sjögren's syndrome (14.28%) and systemic lupus erythematosus (8.40%). A variety of rarer pathologies were also observed: systemic sclerosis, autoimmune thyroiditis, autoimmune hepatopathies, etc. Moreover, rheumatoid arthritis (RA) was present in 52 patients (32.09%) of our cohort, with a clear predominance in the MGUS group. The prevalence of monoclonal components (MC) in RA patients is estimated at around 10–19%, according to Kyle et al. (2006) (13). A Swedish study by Baecklund (2021) demonstrated that RA increased the risk of developing lymphoid malignancies, including MGUS and MM, by 2- to 3-fold (17). The retrospective Polish study by Wojciechowska et al. (2020) also found RA to be the most frequent AID in patients with MG, with a frequency of 40%, comparable to that observed in our MGUS group (18).

Sjögren's syndrome (SS) was present in 23 patients (14.19%) of our cohort, the majority in the MGUS group. SS is one of the autoimmune diseases most strongly associated with monoclonal gammopathies. The prevalence of MC in SS patients varies between 20% and 45%, reaching 43.9% in some studies. This strong association is explained by chronic B-cell stimulation, favoring clonal proliferation (19).

Systemic lupus erythematosus (SLE) was present in 12 patients (7.4%) of our cohort. The frequency of detecting

monoclonal components in SLE patients has been estimated at around 14–15%. Recent studies (Zucchi et al., 2025) confirm this prevalence and emphasize that the presence of such components increases the risk of progression to lymphoproliferative disorders, including lymphomas and multiple myeloma (20). In our cohort, Biermer's disease was found in 9 (5.55%) patients with monoclonal components, whereas the cited study reported a 13.6% incidence of cobalamin deficiency among patients with IgA myeloma and MGUS. Furthermore, a case-control study showed a 1.47-fold increased risk of developing myeloma among patients with Biermer's disease, suggesting a potential epidemiological link between these two entities (21).

Other AIDs such as systemic sclerosis, celiac disease, ANCA-associated vasculitis, or Hashimoto's thyroiditis were observed at frequencies ranging from 1% to 5%. These frequencies are broadly in line with the literature, which describes them as less frequently associated with monoclonal gammopathies, yet not negligible. Overall, the frequencies observed in our cohort are somewhat higher than expected. These data support the hypothesis that autoimmune diseases, by promoting prolonged B-cell activation, increase the risk of monoclonal clone emergence, thus providing a favorable ground for the development of MGUS and even MM.

The table below illustrates a comparison of the frequencies of autoimmune diseases associated with monoclonal gammapathies in our cohort and in the literature:

Table 4. Distribution of autoimmune diseases in patients with monoclonal gammopathies – our single-center study and literature comparison

Autoimmune disease	Frequency in our cohort (%)	Frequency in literature (%)	References
Rheumatoid arthritis (RA)	32.09%	10–20–40%	Baecklund 2021; Filik 2016; Wojciechowska et al. 2020
Sjögren's syndrome (SS)	14.19%	20–44%	Ramos-Casals 2019; Voulgarelis 1997
Systemic lupus erythematosus (SLE)	7.4%	10–15%	Muchtar 2018
Biermer's disease	5.55%	2–5%	Azzabi 2017
Systemic sclerosis	4.93%	~3–5%	Various studies
Celiac disease	3.7%	<3%	Limited data
ANCA-associated vasculitis	2.46%	<2%	Limited data
Autoimmune thyroiditis / Hashimoto	1.85%	<2%	Limited data
IBD / APS / Polymyositis	1.23% or less	<2%	Limited data

Immunological Findings:

In our cohort, the IgG Kappa isotype was the most frequently observed, affecting 38.88% of patients, followed by IgG Lambda (29.01%), and then IgA isotypes (Kappa or Lambda) at lower frequencies. This distribution is consistent with classical literature data, where IgG accounts for more than 60% of isotypes in MGUS and remains predominant in MM. The presence of rare isotypes or biclonal/triclonal forms (22.21% in our series) is noteworthy and slightly higher than in general series (often <10%), which could reflect an immunologically unstable background or prolonged antigenic stimulation due to autoimmune diseases.

The mean MC concentration was 4.12 ± 4.34 g/L for the whole cohort, with a mean of 4.75 g/L in Group 1 and 4.13 g/L in Group 2. These values remain generally moderate, but some patients presented concentrations ≥ 15 g/L, which represents an important risk factor for progression in MGUS according to the Mayo Clinic model (1).

Prognostic assessment revealed that in Group 1, 63.63% of patients were classified as stage I (good prognosis) and 27.27% as stage III (poor prognosis). In Group 2, 64.70% had no risk factors for progression (low risk), 35.29% had a single risk factor (intermediate risk), and no patient presented all three major risk factors (high risk). This suggests that most patients in our cohort remain at low risk with a favorable prognosis, despite the autoimmune context. Nevertheless, it should be noted that most patients did not undergo β2-microglobulin or free light chain (FLC) testing, which makes the prognostic evaluation incomplete.

According to some observational studies, the presence of MGUS could in some cases modulate the immune response, maintaining a transient immunological balance in patients with autoimmune diseases. However, no large-cohort study has conclusively confirmed a protective effect, and further longitudinal research is needed to assess the true prognostic impact of this interaction. Long-term follow-up and expansion of the cohort are required to confirm the actual prognostic value, identify potential evolutionary factors, and better understand the interplay between monoclonal gammopathy and autoimmune disease.

The literature highlights that the monoclonal peak observed in MGUS can be either transient or persistent, with notable prognostic implications. According to Buadi et al. (Blood Cancer Journal, 2021), the absence of clonal bone marrow plasmacytosis is frequently associated with transient peaks, particularly in light IgG forms, often incidentally discovered (23). Furthermore, Kyle and Rajkumar (Immunological Reviews, 2003) report that such peaks may disappear or fluctuate in the context of concomitant inflammatory, infectious, or thrombotic diseases, making their diagnostic interpretation sometimes challenging (24). Studies such as Cesana et al. (2002) also report that the absence of clonal bone marrow plasmacytosis is often linked to transient monoclonal peaks, especially in IgG forms. These peaks may disappear or fluctuate depending on the evolution of concomitant inflammatory or thrombotic diseases, further complicating diagnostic interpretation (4).

Study limitations:

The retrospective nature of our study and the limited number of specialized tests (e.g., free light chain assay, β2-microglobulin measurement, Bence Jones protein detection) represent methodological limitations. A broader longitudinal follow-up and cytogenetic analyses (e.g., translocations involving the 14q32 region [IgH heavy chain gene], deletions of the short arm of chromosome 17 [17p], the locus of the TP53 gene) would help clarify the evolutionary mechanisms toward malignant forms.

Perspectives:

It would be of interest to monitor the evolution of MGUS patients with autoimmune diseases to determine whether they present a higher transformation rate to MM. Furthermore, analysis of cytokine profiles (IL-6, TNF-α) and gene expression signatures of autoimmune-associated plasma cells could open new perspectives for the development of targeted or preventive therapeutic strategies.

Conclusion:

Our retrospective study conducted on a cohort of 162 patients with monoclonal gammopathies associated with autoimmune diseases (AID) highlights several major findings. On one hand, monoclonal gammopathy of undetermined significance (MGUS) remains the most frequent form, occurring at an earlier age than reported in classical series of isolated gammopathies. On the other hand, rheumatoid arthritis, Sjögren's syndrome, and systemic lupus erythematosus emerge as the most frequently associated autoimmune diseases.

From an immunological perspective, IgG isotypes predominate, and the monoclonal component (MC) concentration remains moderate in most cases, conferring an overall favorable prognostic profile. This good prognosis may be attributed to close immunological monitoring enabling earlier diagnosis, but it may also be overestimated due to the limited sample size.

Finally, some recent data suggest that the presence of MGUS could, in certain cases, favorably modulate the course of some autoimmune diseases—a compelling hypothesis that nevertheless requires confirmation through larger-scale prospective studies.

Regular, long-term clinical and immunological follow-up is essential for these patients, in order to better define evolutionary trajectories and refine prognostic stratification.

Conflict of interest:

The autour declare that the research was conducted without any conflict of interest.

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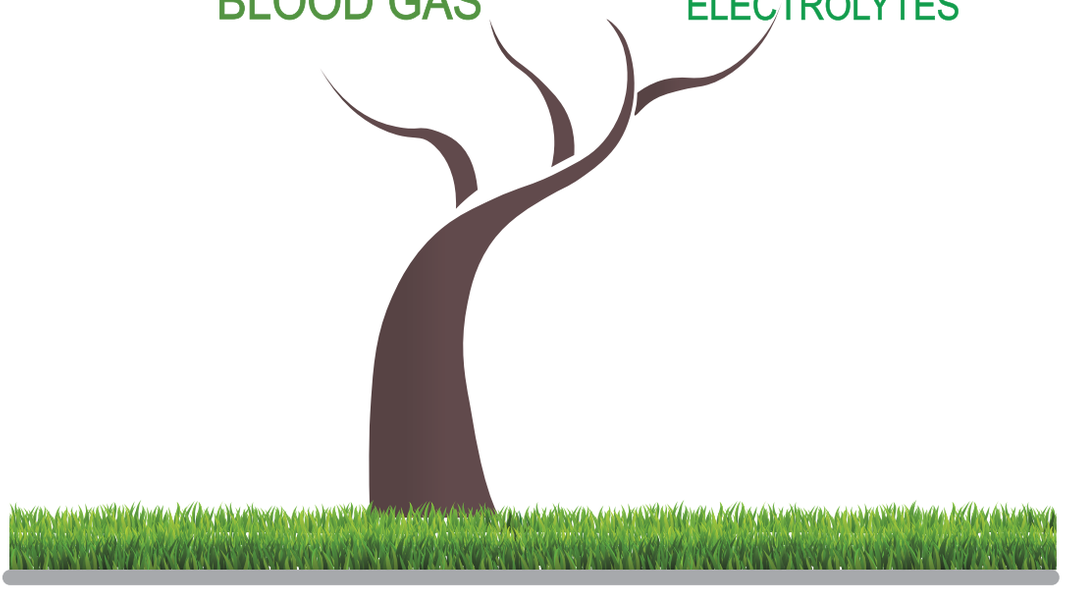

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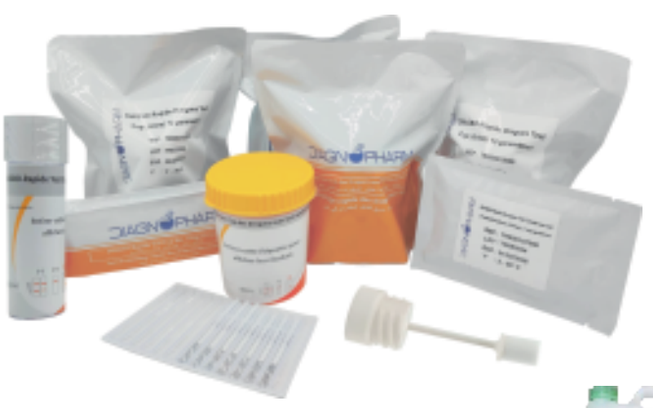

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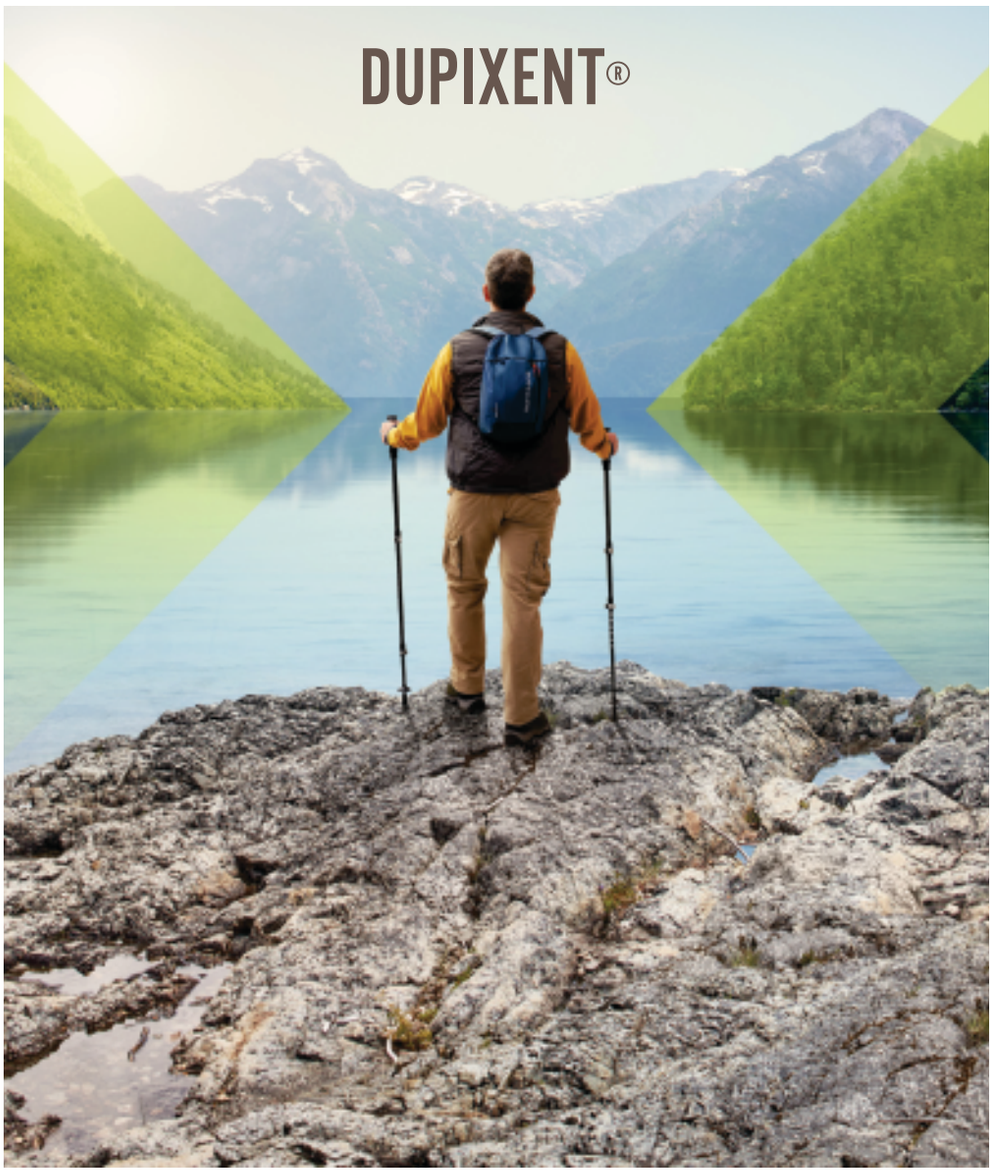

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