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THESE POUR LE DIPLOME D'ETAT
DE DOCTEUR EN MEDECINE

Diagnostic, caractéristiques et devenir des déficits sélectifs en anticorps anti-polysaccharides : à propos d'une cohorte de 55 patients adultes.

Mise au point d'un test de détection des anticorps anti-polysaccharides par électrochimiluminescence.

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Par Nicolas PERRARD

JURY

Président :

Monsieur le Professeur David LAUNAY

Assesseurs :

Monsieur le Professeur Eric HACHULLA

Madame le Docteur Sarah STABLER

Monsieur le Docteur Sébastien HUMBERT

Directeur de thèse :

Monsieur le Docteur Guillaume LEFEVRE

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Sigles

CEREDIH	CEntre de REfrérence Déficits Immunitaires Hérititaires
CID	Déficits immunitaire combinés
CPP	Comité de Protection des Personnes
DETECT	Detection of specific polysaccharide antibody deficiency in adult patients with unexplained recurrent severe bacterial infections
DICV	Déficit Immunitaire Commun Variable
DIP	Déficit Immunitaire Primitif
ECL	ÉlectroChimiLuminescence
ELISA	Enzyme-Linked Immunosorbent Assay
ESID	European Society for ImmunoDeficiencies
GREPI	Groupe de Recherche et Enseignement en Pneumo-Infectiologie
HAS	Haute Autorité de Santé
IUIS	International Union of Immunological Societies
MaRIH	Maladies Rares Immuno-Hématologiques
NK	Natural Killer
OMS	Organisation Mondiale de la Santé
OPA	Opsonophagocytosis Assay
PNDS	Protocole National de Diagnostic et de Soins
SPAD	Déficit Sélectif en Anticorps anti-Polysaccharides

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Introduction

Généralités sur les déficits immunitaires

1. Le système immunitaire

Les êtres vivants sont confrontés à de nombreux organismes pathogènes et non pathogènes pouvant menacer l'homéostasie normale de chaque individu. On distingue des agents infectieux pathogènes strictes, et des organismes commensaux ayant un comportement bénéfique pour l'hôte, que ce dernier doit tolérer et contrôler afin de pouvoir bénéficier du comportement symbiotique. *A contrario*, les organismes pathogènes possèdent divers mécanismes grâce auxquels ils se répliquent, se propagent et menacent les fonctions normales de l'hôte [1].

Le système immunitaire représente donc un ensemble complexe de mécanismes permettant l'élimination des agents pathogènes et des protéines toxiques ou allergènes, tout en évitant les réponses impliquant des dommages excessifs aux fonctions vitales de l'organisme ou risquant d'éliminer les organismes commensaux bénéfiques. Une telle discrimination hôte-pathogène ou hôte-toxine est essentielle pour permettre à l'hôte d'endiguer les menaces sans endommager ses propres fonctions [2].

Les réponses immunitaires peuvent être divisées en 2 systèmes interconnectés :

- Système immunitaire inné, primitif mais très efficace, regroupant des réponses programmées reconnaissant des motifs partagés à la fois par de nombreux microbes et toxines qui ne sont pas présents chez l'hôte. On retrouve parmi les principaux acteurs les polynucléaires, les monocytes/macrophages, les cellules dendritiques, les cellules Natural Killer (NK) et des acteurs protéiques tels que le système du complément et les médiateurs lipidiques [3] ;

- Système immunitaire adaptatif, évolué, regroupant des réponses spécifiques d'antigène codées par des éléments génétiques qui se réorganisent pour assembler des molécules uniques de réponse à un antigène donné. Cette adaptabilité moléculaire caractérise les lymphocytes T et B principalement. Les lymphocytes T exercent le rôle de « chef d'orchestre » du système immunitaire, tandis que les lymphocytes B produisent les immunoglobulines [4].

Le système immunitaire doit réguler sa propre réponse contre les antigènes du soi tout en assurant également une fonction anti-tumorale vis-à-vis d'antigènes modifiés ou nouveaux, et permet de lutter contre l'apparition de cancers notamment [5].

2. Les déficits immunitaires et leurs réseaux de soin

Les déficits immunitaires primitifs (DIP) sont des maladies rares, dont l'incidence est estimée à environ 1 sur 4000 naissances. Il existe à ce jour plus de 500 DIP, dont la majorité ont une cause monogénique et de transmission récessive [6,7]. Néanmoins, les DIP les plus fréquents sont souvent sans cause génétique connue.

De manière générale, les dysfonctions du système immunitaire, qu'elles soient génétiques ou acquises au cours de la vie, se manifestent par une susceptibilité accrue aux infections, par l'apparition de cancers, et par une réponse inappropriée contre l'hôte par défaut de régulation responsable d'auto-immunité, d'auto-inflammation ou de syndrome d'activation macrophagique [8,9].

La mission de l'European Society for ImmunoDeficiencies, (ESID) et du CEntre de REfrérence Déficits Immunitaires Héritaires (CEREDIH) est « d'améliorer la sensibilisation, le diagnostic, le traitement et la compréhension des déficits immunitaires primitifs par l'éducation, la documentation, l'uniformisation des pratiques, la recherche et le suivi des patients » [10,11].

Dans cette optique, un registre européen des DIP a été créé en 2004, rapidement suivi au niveau national français par le registre du CEREDIH en 2005. La prévalence en France est estimée à 9,35 PID /100 000 habitants, deuxième plus haute prévalence en Europe probablement en raison de la haute qualité du référencement Français (les patients français représentent 28% des patients du registre européen) [12].

En janvier 2024, le registre du CEREDIH référençait 8924 patients porteurs d'un DIP, dont 55% étaient des hommes (plus forte prévalence de maladies génétiques liées à l'X) et 59% des adultes. Les déficits immunitaires référencés concernaient à part égale l'immunité T (38%) et l'immunité B humorale (43%), suivi ensuite par les anomalies de l'immunité innée (18%) et les anomalies du système du complément (4%). A noter qu'une anomalie génétique imputable n'est retrouvée que chez la moitié des patients du registre. Parmi les manifestations les plus fréquentes, 88% des patients présentaient au moins une infection, inaugurale de la maladie dans 65% des cas, 39% de l'auto-immunité, 27% des manifestations allergiques sévères (i.e. nécessitant le recours à une consultation spécialisée et/ou l'introduction d'un traitement spécifique) et 11% l'apparition d'une néoplasie [12].

Sur le plan physiopathologique, l'étude des bases génétiques des DIP permet de comprendre le fonctionnement des différents acteurs du système immunitaire et leur implication dans la défense contre les infections, l'immunité antitumorale ou le maintien de l'homéostasie.

3. Classification

En 2019, le comité d'experts des « Inborn Errors of Immunity » de l'International Union of Immunological Societies (IUIS) ont proposé une mise à jour de la classification des DIP en fonction du phénotype clinico-biologique, permettant une application en pratique clinique que la classification génétique ne permettait pas, avec [13] :

- I. Déficits immunitaire combinés (CID), touchant l'immunité cellulaire (lymphocyte T) et humorale (lymphocyte B) ;
- II. CID avec caractéristiques syndromiques associées (malformations, dysmorphies) ;
- III. Déficits de l'immunité humorale isolés ;
- IV. Défauts de l'homéostasie du système immunitaire ;
- V. Déficits des cellules phagocytaires en nombre et/ou en fonction (granulocytes, cellules dendritiques, monocytes et macrophages) ;
- VI. Déficits de l'immunité innée ;
- VII. Maladies auto-inflammatoires ;
- VIII. Déficits du complément (opsonisation) ;
- IX. Aplasies médullaire primitives ;
- X. Phénocopies de PID (modification non héréditaire du phénotype, induite par l'environnement)

Chaque composant du système immunitaire, qu'il soit cellulaire ou protéique, comporte une fonction précise et, par extension, chaque déficit d'un composant précis va se manifester par un spectre précis d'infections et d'autres symptômes associés. Cette classification permet donc d'associer un spectre infectieux et clinique à un défaut d'une fonction biologique, et donc d'orienter les hypothèses diagnostiques et les explorations génétiques.

4. Spectres infectieux

Au cours de l'évolution, certaines composantes du système immunitaire se sont spécialisées dans la lutte contre certains facteurs de virulence spécifiques des pathogènes, souvent conservés pendant l'évolution car efficaces et déterminants pour la survie du pathogène [14].

Par exemple, de manière schématique, le système humoral (immunoglobulines) assuré par les lymphocytes B et les plasmocytes est particulièrement efficaces pour lutter contre les

pathogènes comportant une capsule. Cette capsule composée de lipopolysaccharides et d'un glycocalix protège les bactéries de la phagocytose, permet d'augmenter l'adhérence aux muqueuses et la colonisation de l'hôte, ou encore réduit l'interaction avec la composante protéique du système immunitaire (complément, récepteurs solubles) ou d'autres composants toxiques tels que les antibiotiques, se comportant donc comme un authentique facteur de virulence. Les immunoglobulines permettent de contourner ces fonctions et de lutter contre de telles bactéries en se fixant à la capsule, l'empêchant d'exercer ses fonctions d'adhérences et de colonisation. Elles permettent également de rétablir le rôle cytolitique du complément et de recruter les acteurs cellulaires de la phagocytose et de la cytotoxicité. Ainsi, un déficit immunitaire du compartiment humoral prédisposera à des infections à organismes encapsulés [15].

Ces spectres infectieux sont illustrés dans le Tableau 1.

Tableau 1 : Spectre infectieux en fonction du type de déficit immunitaire. Adapté d'après Notarangelo LD. Primary immunodeficiencies. JACI. 2010. [14]

Organisme	Déficit humoral	Déficit cellulaire	Déficit en phagocytose	Déficit en complément
Virus	Entérovirus	Tous, particulièrement : CMV, VRS, EBV, Parainfluenza de type 3	Non	Non
Bactéries	<i>Streptococcus pneumoniae</i> , <i>Haemophilus influenzae</i> , <i>Moraxella catarrhalis</i> , <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , <i>Nisseria meningitidis</i> , <i>Mycoplasma pneumoniae</i>	Idem que les déficits humoraux, avec : <i>Salmonella typhi</i> , <i>Listeria monocytogenes</i> , Entérobactéries	<i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , <i>Nocardia asteroides</i> , <i>Salmonella typhi</i>	Idem que les déficits humoraux Voie terminale uniquement : <i>Nisseria meningitidis</i> ,
Mycobactérie	Non	Mycobactéries atypiques, BCG	Mycobactéries atypiques, BCG	Non
Champignon	Non	<i>Candida</i> spp <i>Aspergillus</i> spp <i>Cryptococcus neoformans</i> <i>Histoplasmosis capsulatum</i>	<i>Candida</i> spp <i>Aspergillus</i> spp	Non
Protozoaire	<i>Giardia lamblia</i>	<i>Pneumocystis jiroveci</i> <i>Toxoplasma gondii</i> <i>Cryptosporidium parvum</i>	Non	Non

CMV : Cytomégalo virus, VRS : Virus Respiratoire Syncytial, EBV : Epstein-Barr Virus, BCG : Bacille de Calmette et Guérin.

Les DIP de l'adulte

1. De l'enfance à l'âge adulte

Si la plupart des DIP de l'enfant sont d'origine génétique et se manifestent dans les premières années de vie par des déficits graves de l'immunité cellulaire T et du système immunitaire inné, les DIP débutants à l'âge adulte concernent *a contrario* l'immunité humorale principalement, avec des manifestation souvent plus tardives et moins sévères. Rarement, certains DIP classiquement diagnostiqués dans l'enfance souffrent d'un retard diagnostique majeur en raison d'un phénotype moins sévère, d'un défaut d'accès aux soins ou d'une méconnaissance des praticiens par exemple, et le diagnostic est porté tardivement à l'âge adulte [16].

Classiquement, la prise en charge diffère également entre ces deux populations. La prise en charge des DIP de l'enfant, de manifestation souvent précoce et grave, nécessite des thérapeutiques curatives parfois lourdes, dangereuses et/ou coûteuses telle que l'allogreffe de cellules souches hématopoïétique ou la thérapie génique. Ces traitements permettent une guérison du patient [17]. *A contrario*, la prise en charge des DIP de l'adulte, de manifestation souvent plus tardive et moins sévère, relève de traitements « suspensifs » moins lourds et moins coûteux comme une antibiothérapie préventive ou une supplémentation en immunoglobulines polyvalentes permettant de remplacer le contingent humoral défaillant par exemple. Ces traitements ne permettent pas une guérison, et doivent être poursuivis tout au long de la vie, ce qui nécessite un recours constant aux systèmes de soins et impacte la qualité de vie du patient [18,19].

2. Manifestations et points d'appel des DIP humoraux

Le spectre infectieux des DIP humoraux comporte principalement des infections à bactéries encapsulées (pneumopathies, otites, rhino-sinusites, méningites ou autres sites atypiques), plus rarement des infections digestives bactériennes (*Salmonella spp.*, *Campylobacter spp.*) ou

parasitaires (*Giardia intestinalis*). Les infections sont volontiers atypiques, plus fréquentes, plus graves et plus difficiles à traiter, et ces caractéristiques doivent faire soupçonner la présence d'un déficit immunitaire chez ces patients supposés immunocompétents. L'association de ce spectre infectieux spécifique avec des manifestations auto-immunitaires, allergiques et/ou une ou plusieurs néoplasies confortent cette suspicion de DIP [8,16]. En collaboration avec le CEREDIH, l'association de patients déficits immunitaires primitifs IRIS a édité en 2019 une brochure de sensibilisation du grand public aux signes d'alerte devant faire suspecter un déficit immunitaire chez l'adulte (primitif ou secondaire), et nécessitant une consultation auprès d'un médecin spécialiste (Figure annexe - [20])

Plus récemment en décembre 2022, le CEREDIH a publié la première édition du Protocole National de Diagnostic et de Soins (PNDS) « Déficits Immunitaire Héréditaires », un référentiel de bonne pratique prévue par le deuxième plan national maladies rares 2011-2014, et confiée aux centres de référence et de compétence maladies rares à l'aide d'une méthode proposée par la Haute Autorité de Santé (HAS). L'objectif d'un PNDS est d'explicitier aux professionnels concernés la prise en charge diagnostique et thérapeutique optimale et le parcours de soins d'un patient atteint d'une maladie rare donnée. Les PNDS comportent une partie « synthèse destinée au médecin traitant », ayant pour objectif la sensibilisation du corps médical entourant le patient et l'amélioration de la prise en charge globale. Ce PNDS reprend les signes d'alerte devant alerter les professionnels de santé de la possible présence d'un déficit immunitaire, et détaille le bilan diagnostique nécessaire permettant de (i) éliminer un déficit immunitaire secondaires, et (ii) déterminer le type de déficit immunitaire primitif [21].

Parmi les principaux DIP humoraux pouvant se manifester à l'âge adulte, on retrouve principalement :

- Les déficits complets en IgA [22] ;
- Les déficits immunitaires communs variables (DICV) [23] ;

- Les déficits en sous-classes (IgG2 et IgG3) [24] ;
- Les déficits sélectifs en anticorps anti-polysaccharides (SPAD).

Les trois premiers DIP sont des déficits humoraux quantitatifs pouvant être suspectés et/ou diagnostiqués par des examens simples réalisés en routine de détermination de la quantité d'immunoglobulines circulantes, tandis que le dernier est un déficit qualitatif, concernant la qualité de la réponse humorale contre les antigènes polysaccharidiques.

La suite de cette introduction sera consacrée spécifiquement à ce DIP.

3. Le déficit sélectif en anticorps anti-polysaccharides

Le déficit sélectif en anticorps anti-polysaccharides (SPAD) est un DIP se caractérisant par des explorations quantitatives normales du système immunitaire (taux normaux d'immunoglobulines y compris des sous-classes d'IgG, dosages des protéines du complément normaux et immunophénotypage lymphocytaire normal), et par une réponse immunitaire aux polysaccharides altérée. Il a été décrit pour la première fois par Ambrosino *et al.* en 1987 dans le *New England Journal of Medicine* à travers le cas d'un homme de 30 ans ayant présenté au cours des 15 années précédentes 11 épisodes de pneumopathies documentées à *Streptococcus pneumoniae* et *Haemophilus influenzae*. L'examen clinique de retrouvait pas d'arguments pour un déficit immunitaire par ailleurs, les examens pulmonaires (fibroscopie, explorations fonctionnelles respiratoires, radiologie) ne montraient pas d'anomalies en dehors d'un asthme confirmé par un test à la métacholine, dans un contexte de tabagisme actif estimé à 20 paquets/années. Les explorations quantitatives du système immunitaire étaient normales, mais il était observé un défaut complet de réponse aux antigènes polysaccharidique, soit l'absence d'immunisation après vaccination contre *Streptococcus pneumoniae*, *Nisseria meningitidis* et *Haemophilus influenzae*. Ce type de défaut de réponse vaccinale avait déjà été décrit associée

à d'autres DIP tels que le DICV, l'ataxie-télangiectasie ou le syndrome de Wiskott-Aldrich par exemple, mais aucun autre DIP n'avait été découvert chez ce patient [25].

Cette description princeps est une bonne illustration de l'errance diagnostique dont souffrent encore actuellement les patients porteurs d'un SPAD.

a. Le diagnostic précoce du SPAD comme enjeu pronostic majeur

L'errance diagnostique et l'absence de prise en charge spécifique du SPAD a un impact direct sur la qualité de vie des patients et augmente le risque de séquelles post-infectieuses définitives à long terme (bronchiectasies, séquelles neurologiques, conséquence des hospitalisations à répétition ...) [26].

Récemment, une étude prospective multicentrique a été menée dans les Hauts-de-France avec pour objectif l'étude de la prévalence des DIP au sein d'une cohorte de patients présentant des infections évocatrices d'un déficit de l'immunité humorale (*i.e.* infections invasives et/ou graves et/ou récidivantes à bactéries encapsulées, ou infections récidivantes bénignes ORL et/ou pulmonaires bronchiques). Parmi les 118 patients explorés, 38 patients présentaient un SPAD, soit une prévalence de 31% [27].

La difficulté à poser le diagnostic de SPAD et la méconnaissance de ce DIP de l'adulte sont responsables de ce retard diagnostique.

b. Le diagnostic du SPAD reste difficile et peu codifié

L'étude des réponses vaccinales est difficile, requière des techniques de laboratoire de pointe par méthode ELISA permettant l'étude des sérologies sérotype par sérotype après vaccination par le vaccin anti-pneumococcique non conjugué [28].

De plus, l'interprétation des taux pré- et post-vaccinaux nécessite une expertise particulière car la définition d'une réponse vaccinale normale fait l'objet que de peu d'études, repose

essentiellement sur un consensus d'experts et dépend notamment de l'âge du patient. Brièvement, chez l'adulte, une bonne réponse vaccinale pourra être retenue si, en comparant les taux d'anticorps sérotype par sérotype avant, et 4 à 8 semaines après vaccination par un vaccin polysaccharidique anti-pneumococcique non conjugué (PNEUMOVAX®) [29,30] :

- Les taux sont supérieurs à 1,3 µg/mL par sérotype,
- ET multiplication des taux concernés par 4 si le pré-vaccinal était inférieur à 1,3 µg/mL (par 2 si le pré-vaccinal était supérieur à 1,3 µg/mL),
- ET pour au moins 70 % des sérotypes testés (50 % pour les patients pédiatriques)

Enfin, il est possible d'observer un défaut de réponse anti-polysaccharidique chez les enfants de moins de 2 ans de manière physiologique (en témoigne le schéma vaccinal national par vaccin conjugué chez l'enfant de moins de 2 ans), chez les patients vaccinés depuis moins de 2 ans (hyporéponse post-vaccinale) ainsi que chez certains adultes sains ne présentant pas d'infections (jusqu'à 10-15% de la population selon certains auteurs) [31,32].

c. Il existe une méconnaissance du SPAD et des points d'appel infectieux

L'errance diagnostique est également à attribuer une apparente bénignité des infections que présentent les patients (rhino-sinusites, bronchites), c'est alors la fréquence élevée qui justifie une évaluation du système immunitaire [27,33].

La forte prévalence du SPAD décrite ci-dessus au sein d'une population sélectionnée nécessite donc une information et une éducation des professionnels de santé et des associations de patients pour accélérer la prise en charge diagnostique et thérapeutique de ces patients.

Objectifs de la thèse

Ce travail de thèse comportait 2 objectifs :

1. la description de caractéristiques non infectieuses au diagnostic et l'évolution des complications infectieuses après le diagnostic de SPAD chez l'adulte, grâce à une cohorte multicentrique de 55 patients adultes suivi dans les Hauts-de-France.
2. la description d'un test multiplex de dosage des anticorps anti-pneumococciques développé au CHU de Lille dans cette indication pour pallier aux limites d'accès de la méthode ELISA de référence approuvée par l'Organisation Mondiale de la Santé (OMS).

Je présenterai enfin l'étude prospective nationale multicentrique DETECT ayant pour objectif de sensibiliser les médecins des réseaux de soin du CEREDIH et du Groupe de Recherche et Enseignement en Pneumo-Infectiologie (GREPI) au diagnostic et à la prise en charge des patients porteurs d'un SPAD.

Diagnosis, characteristics, and outcome of selective anti-polysaccharide antibody deficiencies: a cohort of 55 adult patients

Authors

Nicolas Perrard ^(1, 2, 3, 12) ; Sarah Stabler ^(3, 4, 5, 12) ; Sébastien Sanges ^(1, 2, 12) ; Louis Terriou ^(1, 2, 12) ; Catherine Lamblin ^(6, 12) Sacha Gaillard ^(7, 12) ; Fanny Vuotto ^(4, 5, 12) ; Cécile Chenivresse ^(8, 12) ; Geoffrey Mortuaire ^(9, 12) ; Frédéric Batteux ^(10, 11) ; Aurore Collet ^(2, 3, 12) ; Sylvain Dubucquoi ^(2, 3, 12) ; Myriam Labalette ^(2, 3, 12) ; Eric Hachulla ^(1, 2, 12) ; David Launay ^(1, 2, 12) ; Guillaume Lefèvre ^(1, 2, 3, 12)

(1) Department of internal medicine and clinical immunology, CHU Lille, Lille, France;

(2) Univ. Lille, Inserm, CHU Lille, U1286 - INFINITE - Institut for Translational Recherche in Inflammation, F-59000 Lille, France;

(3) Institute of immunology, CHU Lille, Lille, France;

(4) Department of infectious and tropical diseases, CHU Lille, Lille, France;

(5) Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 - UMR 9017 - CIIL - Center for Infection and Immunity of Lille, Lille, France;

(6) Department of pneumology, Private Hospital La Louvière, Lille, France;

(7) Department of pneumology, Clinique Tessier, Valenciennes, France;

(8) Department of pneumology, CHU Lille, Lille, France;

(9) Department of oto-rhino-laryngology, CHU Lille, Lille, France;

(10) Cochin hospital, Department of biological immunology, 75014 Paris France;

(11) Cochin Institute, INSERM U1016, Univ. Paris, 75014 Paris, France;

(12) French National Reference Center for Primary Immune Deficiency (CEREDIH), Necker Enfants Malades University Hospital, Assistance Publique-Hôpitaux de Paris, Paris, France.

ABSTRACT (world count: 255/250)

Background:

Selective anti-polysaccharide antibody deficiency (SPAD) is a rare immunodeficiency predisposing individuals to encapsulated bacterial infections. The diagnosis is challenging, and literature reports are scarce in adult patients.

Objective:

To describe the demographic, infectious complications, immunological characteristics, therapeutic strategies, and outcome after SPAD diagnosis in adults.

Methods:

We conducted a multicenter retrospective study involving 55 adult patients in a tertiary clinical immunology department.

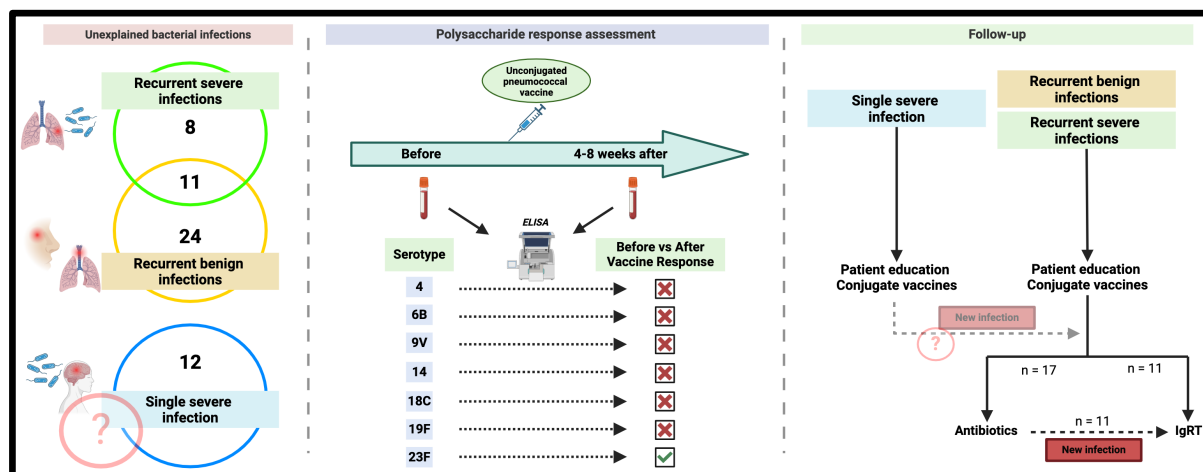
Results:

The median [interquartile range, IQR] age was 45 [36-60] years at diagnosis, and 74.5% were female patients. Twenty-one patients (38,2%) had a history of allergic and/or inflammatory disease, mainly asthma (n=10), rheumatic diseases (n=6) or autoimmune thyroiditis (n=4). Twelve patients (21,8%) were diagnosed after a first severe infection and 43 (78,2%) in a context of recurrent mild and/or severe infections. In the latter, the median [IQR] time from first infections to diagnosis was 74.5 [33-167] months. Diagnosis delay was significantly higher in patients presenting with bronchiectasis (24 months [14.5-74.5] versus 122 months [33-219.5], $p=0,0042$). Twenty-two patients received Ig replacement therapy (IgRT), the mean (min-max) frequency of antibiotic courses decreased from 7.9 (2-18) to 0.7 (0-2) courses per year after IgRT ($p < 0.001$) with a median follow-up period of 46 [27-73] months. SPAD patients diagnosed after a single severe infection did not have any relapse with a median follow-up of 85 [80.5-104.5] months after diagnosis.

Conclusion:

Adult SPAD patients have allergic or inflammatory disorders which could contribute to the diagnosis delay. IgRT is effective in preventing recurrent infections. Further studies are warranted to confirm if SPAD must be considered after a first unexplained severe bacterial infection.

Visual summary



Highlights box

What is already known about this topic?

- Specific anti-polysaccharide antibody deficiency (SPAD) predisposes to encapsulated bacterial infections despite normal antibody levels. Diagnosis is rare in adults: their characteristics, associated diseases and long-term outcome after diagnosis are not known.

What does this article add to our knowledge?

- Reporting a large cohort of adult patients, the present study explores new insights in allergic and inflammatory disorders which could have contribute to the diagnosis delay and highlights the potential risk of bronchiectasis.

How does this study impact current management guidelines?

- SPAD diagnosis must be considered even in patients with asthma and/or inflammatory disorders. IgRT displays long-term benefit for preventing recurrent infections in selected patients.

Key words.

Specific antibody deficiency, Selective anti-polysaccharide antibody deficiency, Primary immunodeficiency, Encapsulated bacterial infections, Preventive antibiotherapy, Immunoglobulin replacement therapy, Asthma

Abbreviations

AAAAI	American Academy of Allergy, Asthma & Immunology
COPD	Chronic Obstructive Pulmonary Disease
CRS	Chronical Rhinosinusitis
CVID	Common Variable Immunodeficiency
HibCV	<i>Haemophilus influenzae</i> serotype B conjugate Vaccin
IgRT	Immunoglobulin Replacement Therapy
IQR	Interquartile Range
OVA	Overall anti-pneumococcal Response Assay
PCV13	Pneumococcal Conjugate Vaccin 13-valent
PID	Primary Immunodeficiency
PnPS	Pneumococcal Capsular Polysaccharide
PPV23	Pneumococcal Polysacharide Vaccine 23-valent
PS	Polysaccharide
RA	Rheumatoid Arthritis
SITRAM	Systemic Inflammatory Trunk Recurrent Acute Macular Eruption
SPAD	Specific anti-polysaccharide antibody deficiency
U/LRTI	Upper/Lower Respiratory Tract Infection
WHO-ELISA	World Health Organization-Enzyme Linked Immunosorbent Assay

Introduction

Primary immunodeficiencies (PIDs) encompass rare disorders predisposing individuals to various infections, autoimmune diseases, and neoplasia [1]. Antibody deficiencies typically manifest through bacterial upper and/or lower respiratory tract infections (U/LRTIs), with pathogens such as *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Haemophilus influenzae* [2,3]. Among antibody deficiencies, the first full characterized case of specific anti-polysaccharide antibody deficiency (SPAD) was reported in 1987: Ambrosino and colleagues described an adult patient with a history of recurrent U/LRTIs, demonstrating impaired antibody responses to polysaccharide (PS) antigens but normal responses to protein antigens [4]. SPAD is now characterized by an impaired response to PS antigens, despite normal response to protein antigens, and normal levels of immunoglobulins (Ig), IgG subclasses, T cells, and B cells [5,6]. This assessment of anti-PS response is made by evaluating antibodies against pneumococcal capsular polysaccharides (PnPS), using a standardized, serotype-by-serotype approach before and 4 to 8 weeks after immunization with the non-conjugate 23-valent polysaccharide pneumococcal vaccine (PPV23, PNEUMOVAX®) [7].

In a recent multicenter prospective study, we demonstrated that specific SPAD may represent a common etiology for recurrent and/or severe bacterial infections when local or systemic predisposing conditions are excluded [8]. However, several considerations remain in the diagnosis and management of SPAD in adult patients. First, clinical practice shows that some SPAD patients could have common chronic respiratory diseases, tobacco use, or inflammatory disorders, despite it could be considered as predisposing conditions to infections [9]. Secondly, benefit of anti-PS response assessment need to be confirmed in patients who had a single

unexplained severe infection [8]. Thirdly, the long-term benefit of SPAD diagnosis and the efficacy of IgRT required further evaluation.

In this study, we aim to characterize a large cohort of SPAD patients managed in an expert center, detailing infectious complications, non-infectious associated conditions, and their outcome with prophylactic measures.

Materials and methods

Population

We conducted a retrospective analysis of patients diagnosed with SPAD at our center between March 2015 and November 2022. Thirty-six patients (65%) were identified through our prospective multicenter study, all of whom lacked any local or systemic predisposing conditions to infections (e.g., tobacco use, asplenia, neoplasia, chronic obstructive pulmonary disease) [8]. The remaining 19 patients (35%) were diagnosed at our tertiary clinical immunology clinic, either with or without concurrent chronic diseases and/or predisposing conditions which were not deemed responsible for their recurrent infections.

SPAD diagnosis

Briefly, SPAD diagnosis was made if a patient had normal levels Ig and IgG subclasses, as well as normal B cell counts; and an impaired anti-polysaccharide (PS) response 4 to 8 weeks after vaccination with PPV23. Poor anti-PnPS response was established in case of impaired responses to 3 or more PnPS among serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F using the third-generation World Health Organization enzyme-linked immunosorbent assay (WHO-ELISA) [2,7,10], or if the anti-PNPs antibodies remained below 110 mg/L using the overall anti-pneumococcal response assay (OVA), with a positive predictive value of 100% indicating poor anti-PnPS response as per the serotype specific assay criteria [11]. CD27+IgD-IgM- switched

memory B cells were considered low if their count was below 6.5% of CD19+ B-cells, and CD27+IgD+IgM+ marginal zone-like B cells if below 7.4% of total CD19+ B cells [12].

Data collection

Demographic characteristics and personal medical histories encompassing autoimmune or inflammatory disorders, allergies, neoplasia, and the presence of bronchiectasis were documented from medical records. Biological parameters including T cell, B cell (with naive/memory subsets), NK cell counts, and levels of IgG, IgA, and IgM were recorded at the time of diagnosis and throughout follow-up when available.

Bacterial infections evocative of SPAD were categorized as follows: (i) recurrent non severe infections treated as outpatients (occurring at least twice per year for a minimum of two years) and/or (ii) single or recurrent severe infections necessitating hospitalization and/or intravenous antibiotics.

When available, antibody responses to conjugate polysaccharide vaccines (pneumococcal conjugate vaccine PCV13, PREVENAR 13[®]), and conjugate anti-*Haemophilus influenzae* serotype B HibCV, ACT-HIB[®]) were collected only if at least one assessment was available within six months following immunization. Additional data were gathered in patients undergoing IgRT, including the frequency of infections and courses of antibiotics per year before and after IgRT initiation (at the latest follow-up visit).

Statistical analysis

Quantitative variables are expressed as median (interquartile range, IQR) regarding the non-Gaussian distribution of immune parameters. Normality distribution was assessed graphically and using the Shapiro-Wilk test.

Statistical tests were performed at the two-tailed alpha level of 0.05. Comparisons of baseline characteristics between subgroups were done using Student's t-test. Comparisons of infections frequency after IgRT versus baseline and diagnosis delay in presence versus absence of

bronchiectasis were done using the Wilcoxon' signed rank test. Data analyses and graphs were performed using the GraphPad Prism software version 9.1.2 (GraphPad Software, La Jolla, CA, USA).

Ethical Approval

All patients were aged 18 or older, and non-opposition on data use was collected ("Protection des Données – GHT Lille Métropole Flandre intérieure", number of agreement DEC23-109). Data from the 37 patients included from the SPIDAC study were collected after written consent from each patient, with approval of the Institutional Review Board of our institution and by an independent ethics committee according to French legislation ("Comité de Protection des Personnes Nord Ouest IV", number of agreement 2014-100739-38).

Results

Demographic and non-infectious characteristics at diagnosis

Fifty-five patients were included in the study, with 41 being female (74.5%), and the median (IQR) age at diagnosis was 45 years (36-60 years). Allergic and/or inflammatory disorders were present in 21 patients (38.2%), comprising asthma (n=12), rheumatic diseases (n=6; including rheumatoid arthritis (RA) [n=2], ankylosing spondylitis [n=2], polymyalgia rheumatica, and unclassified chronic inflammatory rheumatism [n=1] each), autoimmune thyroiditis (n=4), Sjogren's syndrome (n=2), cutaneous psoriasis (n=2), and a recently described auto-inflammatory disorder known as systemic inflammatory trunk recurrent acute macular eruption (SITRAME syndrome, n=1) [13]. Notably, these patients were not undergoing treatment with steroids or immunosuppressants at the time of anti-PS response assessment. In the two cases of RA, the use of steroids and immunosuppressants was contraindicated due to recurrent benign and/or severe infections, resulting in progressive joint destruction and a decline in quality of life prior to the diagnosis of SPAD. Additionally, four patients exhibited asymptomatic

immunological abnormalities, including low-level type III cryoglobulinemia (n=2), positive rheumatoid factor, and positive antinuclear antibodies (>1/1280) (n=1 each) (Table 1, Supp. Appendix Table 1). No history of lymphadenopathy, splenomegaly, or neoplasia was documented in our cohort.

Infectious characteristics at diagnosis

Twelve patients (21.8%) presented with a single severe infection (meningitis n=8, acute respiratory distress syndrome due to pneumonia n=2, pneumococcal endophthalmitis n=1, pneumococcal spondylodiscitis n=1), 24 patients (43.6%) presented with recurrent benign infections, and 8 patients (14.5%) presented with recurrent severe infections. Eleven patients (20%) had both recurrent benign and a history of a severe infection (meningitis n=2 and pneumonia n=3) or recurrent severe infections (*Streptococcus pneumoniae* meningitis n=1, pneumonia n=5) (Figure 1a). Thirteen patients (23%) presented with bronchiectasis (diffuse n=7, localized n=6). Patients with recurrent infections were diagnosed after a median delay of 74,5 (33-167) months. Diagnostic delay was significantly higher in patients presenting with bronchiectasis at diagnosis (24 months [14.5-74.5] versus 122 months [33-219.5], p = 0,0042) (Figure 2).

Twenty-nine bacterial infections were documented in 24 patients with identification of *S. pneumoniae* (n=12), *N. meningitidis* (n=8), *H. influenzae* (n=7) and *S. pyogenes* (n=2). Among patients who presented with a single severe infection, documentation was available for all patients (*S. pneumoniae* n=6 and *N. meningitidis* n=6) (Table 1, Supp. Appendix Table 2).

T cell, B cell and NK cell counts were available in 48 patients: T and B counts were normal in all patients, but a decreased NK cell count below 100/mm³ was present in 12 cases (25%). Naive/memory B cell counts were available in 30 patients and 7 had decreased relative counts of CD27+IgD-IgM- switched memory B cells, associated with decreased CD27+IgD+IgM+ marginal zone-like B cells in 5 (Supp. Appendix Figure 1).

Follow-up

The median duration of follow-up was 67.8 months (48-84 months). No death occurred during the study period.

All patients received conjugate vaccines (excepted some patients who received IgRT immediately after diagnosis) and were educated about warning signs necessitating prompt consultation with their general practitioner. Assessment of the post-vaccination response to conjugate vaccines was available in 17 patients. Pre- and post-vaccination paired serological assessments were only available for 5 patients receiving PCV13 and 5 patients receiving HibCV. Serum anti-*Hib* antibody level was below the protection threshold (1 mg/L) after HibCV in 2 of 12 tested patients, despite multiple vaccination attempts (Supp. Appendix Figure 4).

Seventeen patients underwent treatment with low-dose azithromycin as “bronchial anti-inflammatory therapy” (250 or 500 mg three times a week). Before azithromycin introduction, 10 experienced benign recurrent infections, 5 experienced severe recurrent infections, and 2 experienced both. The efficacy of this treatment was deemed partial in 5 cases and complete in 2 cases (7 out of 15 evaluated; 47%; 2 losses to follow-up prior to evaluation). Of the 13 patients with partial response or failure, 11 subsequently received IgRT, including one patient whom bronchiectasis progressed despite azithromycin (Figure 1b, Supp. Appendix, Figure 1). At a whole, 22 patients were treated with IgRT at an initial dose of 0.1g/kg/week (subcutaneous route) or 0.4 g/kg/month (intravenous route), including 8 with bronchiectasis. The mean frequency of antibiotic courses decreased significantly from 7.9 (2-18) courses per year to 0.7 (0-2) courses per year following IgRT ($p < 0.001$), with a median follow-up period of 46 months (27-73,3 months) (Figure 3). One patient with severe chronic obstructive pulmonary disease (COPD) and bronchiectasis, requiring supplemental oxygen therapy and non-invasive ventilation, had poor response to both PCV13 and PPV23. He received azithromycin and then,

IgRT was added: bacterial infections decreased from 1-2 per month prior diagnosis to 3-4 per year, and lung transplantation which was contraindicated was reconsidered.

Among the 47 patients with available follow-up data on IgG, IgA, and IgM levels, there was no significant decrease in IgA, IgM levels, or IgG levels in the 26 patients not receiving IgRT. IgRT was discontinued by 4 patients; however, recurrent infections recurred in all cases, and IgRT was started again. In 2 pregnant women who previously experienced a dose reduction of IgRT, new bacterial infections occurred, and the dose was increased subsequently with a recovered efficacy in preventing infections (Figure 4). Among 12 patients who presented exclusively with a single severe infection, no recurrence of relevant bacterial infection was observed during a median follow-up period of 85 months (80.5-104.5 months) after diagnosis. One patient with Sjögren's syndrome later developed systemic lupus erythematosus and thrombotic thrombocytopenic purpura. Despite various immunosuppressant therapies (including corticoids and rituximab), IgRT was maintained, and no infections occurred. Patients with rheumatoid arthritis who received IgRT for their specific anti-polysaccharide antibody deficiency were also optimally managed for their inflammatory disorder with agents such as rituximab, abatacept, tocilizumab, and certolizumab, without experiencing new infections (Supp. Appendix, Figure 3).

Discussion

We present a comprehensive case series of 55 patients with SPAD, detailing their characteristics at diagnosis and their outcomes after a median follow-up period exceeding 5 years.

We observed a notable prevalence of allergic and inflammatory diseases among SPAD patients. Twenty-two percent (n=12/55) were diagnosed with asthma, occasionally accompanied by severe and recurrent exacerbations linked to bacterial infections, with 10 patients experiencing recurrent bacterial sinusitis. U/LRTI can precipitate asthma exacerbations [14]. Consequently,

patients with frequent asthma exacerbations attributed to infections should be thoroughly investigated for underlying factors, such as bronchial dilatation [15,16], but also humoral deficiency [17].

Lee and colleagues reported a prevalence of antibody deficiencies 5.5% among 2866 asthmatic patients and concluded that they were associated to a significant risk factor for asthma exacerbation, and that IgRT should be considered in the management in adult asthmatics [18].

Early screening for PID in such patients and the implementation of targeted treatments have been demonstrated to reduce the frequency of infections, asthma exacerbations, and hospitalizations [19,20]. Similar observations have been reported in a cohort of chronic rhinosinusitis (CRS) patients, with the prevalence of SPAD up to 40.2% associated with a high prevalence of asthma in 23.4% to 58.6% of patients [21–23]. In a meta-analysis, Schwitzguébel and colleagues showed increased prevalence of IgG subclass deficiency (5% to 50%) and specific antibody deficiency (8% to 34%) in patients with CRS (31% to 72%) [24].

We report on a severe COPD patient with monthly bacterial infections. The prevalence of PID in COPD is not well known, an old study observed poor response to PPV23 in these patients but there was no interpretation of post-vaccinal threshold, and some patients were vaccinated multiple times before [25]. More recently, among 42 patients with very frequent COPD exacerbations of infectious origin, 29 were diagnosed with PID (8 common variable immunodeficiency [CVID], 20 SPAD, and 1 IgA deficiency). Twenty-two of these patients benefited from IgRT, resulting in a reduction in exacerbations and the use of corticosteroid therapy and antibiotics [26].

Our findings and those of other studies confirm that assessing the anti-PS response in these patients is crucial, and the diagnosis of SPAD may represent a significant turning point in asthma and COPD management when IgRT can prevent frequent recurrent infections.

Thirteen patients in our cohort presented with autoimmune and/or inflammatory disorders. In a previous work, impaired vaccine response to polysaccharides has been observed in rheumatoid arthritis (RA) patients, both with (3 out of 20 explored) and without bronchiectasis (2 out of 20 explored), suggesting a possible underestimation of SPAD diagnosis due to the assessment of only the OVA [27]. According to Broyde and colleagues, the presence of inflammatory rheumatism does not impact the anti-polysaccharide response in patients with and without specific biologics, and antibodies remain detectable even 10 years after vaccination. However, this observation was also based solely on the assessment of the OVA [28]. Further studies using the single serotype assay are warranted to assess the long-term efficacy of pneumococcal vaccines in these patients.

Among our patients with autoimmune/inflammatory disorders, 9 would have required oral steroids and/or immunosuppressants for conditions such as rheumatic disease, cutaneous psoriasis, or connective tissue disease. However, these patients either were not receiving treatment or received suboptimal treatment due to their recurrent infections. Following the diagnosis of SPAD, IgRT prevented recurrent severe respiratory tract infections and facilitated the introduction of specific therapeutics such as immunosuppressants.

In our study, we identified 3 infectious phenotypes: i) a single unexplained severe infection, ii) recurrent severe infections, and iii) recurrent benign infections. Some patients presented with both benign and severe infections. Bacteriological documentation was available for only 43.6% of patients; however, clinicians should endeavor to document infections as thoroughly as possible, to support long-term of low dose azithromycin or IgRT. Indeed, a defective response may be observed in healthy individuals depending on the choice of specific antibody levels, of pre/post immunization fold-increases, of the number of tested serotypes, and of the methodology employed [11,29]. Therefore, SPAD diagnosis must only been made in the

context of bacterial infections, and the latter should be considered as suspicious by their high frequency and/or severity in view of the patient's context [30].

The criteria defining a good or poor anti-PS response were established based on expert consensus in 2012 and grounded in various pediatric and adult studies [2,7]. We adhered to the proposed criteria and utilized the reference method, which relies on a complex and non-automated single-serotype assay [10]. However, the thresholds for specific antibody levels and the number of serotypes required to define a satisfactory immunization status remain subject to debate [31]. Several alternative tests to assess the anti-pneumococcal response were considered, such as allo-haemoagglutinins or the anti-*Salmonella typhi* response; however, these were not consistent with the anti-pneumococcal response [32–34]. We also proposed that, notwithstanding its limitations, the OVA could be utilized when a post-vaccination antibody titer below 110 mg/L was recorded, as this consistently correlated with a poor PPV23 response using the single-serotype assay [11]. Six patients in our cohort were diagnosed using this method, and five of them received IgRT, which effectively prevented recurrent infections.

T and B immunophenotyping was normal for all our patients but switched memory B cell counts were decreased in seven patients. A reduction in switched memory B lymphocytes has been previously documented in patients with SPAD, and it may be associated with an elevated risk of infectious complications and bronchiectasis in both adult and pediatric populations [35,36]. In the 12 patients who presented with a severe single infection, none experienced a recurrence of bacterial infections during a median follow-up of 85 months (80.5-104.5 months) after diagnosis. This observation could potentially be attributed to the administration of conjugate vaccines following the diagnosis of SPAD. However, this raises questions regarding the utility of investigating SPAD after a single severe infection, particularly considering the prevalence of low anti-PS responses in healthy individuals [23]. Serological evaluations following administration of PCV13 and HibCV were also conducted, revealing poor responses to

conjugate vaccines in some patients. However, the interpretation of these post-vaccination serologies lacks standardization, and further studies are needed to determine the efficacy of conjugate vaccines in patients with SPAD using the single serotype assay [37].

Patients experiencing recurrent infections often face delayed diagnosis and may develop bronchiectasis. Our observations suggest that antibiotic prophylaxis and IgRT are effective in preventing bacterial infections, even in the presence of bronchiectasis. Early diagnosis is crucial for averting life-threatening infections and lung disease, and the high incidence of bronchiectasis in our case series underscores the consequences of delayed diagnosis. In a study conducted by Van Kessel and colleagues in 2005, 26 patients with bronchiectasis of unknown etiology were examined, revealing that 15 of them were non-responders to PPV23. These non-responder patients exhibited a higher frequency of infections and antibiotic usage and had more severe bronchiectasis [38].

Finally, therapeutic management remains non-standardized and largely relies on expert recommendations and clinician preferences. This management typically includes administration of conjugate vaccines, patient education regarding the risks of bacterial infections, and, in cases of persistent recurrent infections, initiation of preventive antibiotic therapy or IgRT [39,40]. In our cohort, all patients received preventive vaccination with conjugate vaccines and were educated on symptoms and situations warranting prompt medical attention and early initiation of antibiotic therapy. A letter was also sent to their primary care physicians for the same purpose. We believe these measures are crucial for the effective management of these patients, given their safety profile and in alignment with recommendations for other PID. During the follow-up of our patients, we observed a partial and transient efficacy of low-dose azithromycin (but not other antibiotic prophylaxis such as cotrimoxazole [n=1] or V penicillin [n=1] for example). Among the 17 patients in our cohort

initially treated with azithromycin, 11 eventually required a switch to IgRT due to the absence, partial, and/or transient effectiveness of azithromycin.

In a prospective, double-blind, placebo-controlled, randomized study, 89 patients with antibody PID were assigned randomly to receive either azithromycin 250 mg administered once daily three times a week for two years (n = 44) or placebo (n = 45). Azithromycin was found to be effective in reducing the incidence of infections (3.6 vs. 5.2 per patient-year), decreasing the risk of hospitalization, and enhancing quality of life in patients with CVID and X-linked agammaglobulinemia [41]. Similar to its efficacy in other conditions such as asthma or COPD, low-dose macrolide therapy exhibits antimicrobial and anti-inflammatory properties, particularly in reducing LRTI and exacerbations, with effects on neutrophil chemotaxis and flagellin-induced inflammation [42].

IgRT demonstrated efficacy in 100% of cases, whether employed as first or second-line therapy. This observed efficacy surpassed that reported in previous case series [21–23,43]. This difference can be attributed to the systematic exclusion of all potential differential diagnoses prior to SPAD diagnosis in our patient cohort, as well as the deliberate selection of SPAD patients with a high incidence of bacterial infections before initiating IgRT.

Several retrospective studies have explored the efficacy and safety of antibiotic prophylaxis versus IgRT [44–46], but Smits and colleagues conducted the first prospective randomized controlled trial aimed at establishing the efficacy of IgRT compared to antibiotic prophylaxis in 64 patients (55 adults and 9 children) with infections and carriers of a humoral deficit in humoral immunity, including only 4 cases of SPAD [47]. At the conclusion of the study, no significant difference in efficacy was observed between prophylactic antibiotics and IgRT in terms of reducing the frequency of infections and courses of curative antibiotic therapy. However, patients experiencing persistent infections during antibiotic prophylaxis demonstrated fewer infections after transitioning to IgRT.

Monitoring of immunoglobulin levels in our patients receiving IgRT did not indicate a secondary reduction in IgA or IgM levels, suggesting no progression from a qualitative deficit to a quantitative deficit such as CVID [48]. After a median follow-up of 67.8 months of these adult patients, SPAD did not appear to be a transient state evolving toward another quantitative immunodeficiency, but a longer follow-up will have to confirm this point.

Finally, our retrospective cohort reports recurrence of infections in 4 patients who unexpectedly discontinued IgRT and 2 patients whose Ig doses were reduced, requiring the resumption of IgRT at the same dose. The efficacy of IgRT and the recurrence of infections upon discontinuation of IgRT confirm SPAD as a primary antibody deficiency.

Conclusion

Our study addresses the diagnostic challenges and management strategies associated with SPAD in a sizable case series of 55 adult patients. In clinical practice, the consideration of SPAD diagnosis becomes paramount in the presence of severe and/or recurrent infections attributable to encapsulated bacteria, alongside normal quantitative assessment of humoral immunity. This diagnosis may also be warranted in patients exhibiting autoimmune manifestations alongside such infectious complications, or in those with severe chronic lung disease experiencing difficult-to-treat exacerbations and/or unexplained bronchiectasis.

References

1. Seidel MG, Kindle G, Gathmann B, Quinti I, Buckland M, van Montfrans J, et al. The European Society for Immunodeficiencies (ESID) Registry Working Definitions for the Clinical Diagnosis of Inborn Errors of Immunity. *The Journal of Allergy and Clinical Immunology: In Practice*. 2019;7:1763–70.
2. Bonilla FA, Khan DA, Ballas ZK, Chinen J, Frank MM, Hsu JT, et al. Practice parameter for the diagnosis and management of primary immunodeficiency. *Journal of Allergy and Clinical Immunology*. 2015;136:1186-1205.e78.
3. Oksenhendler E, Gérard L, Fieschi C, Malphettes M, Mouillot G, Jaussaud R, et al. Infections in 252 Patients with Common Variable Immunodeficiency. *CLIN INFECT DIS*. 2008;46:1547–54.
4. Ambrosino DM, Siber GR, Chilmonczyk BA, Jernberg JB, Finberg RW. An Immunodeficiency Characterized by Impaired Antibody Responses to Polysaccharides. *N Engl J Med*. 1987;316:790–3.
5. Perez E, Bonilla FA, Orange JS, Ballow M. Specific Antibody Deficiency: Controversies in Diagnosis and Management. *Front Immunol*. 2017;8:586.
6. Perez EE, Ballow M. Diagnosis and management of Specific Antibody Deficiency. *Immunology and Allergy Clinics of North America*. 2020;40:499–510.
7. Orange JS, Ballow M, Stiehm ER, Ballas ZK, Chinen J, De La Morena M, et al. Use and interpretation of diagnostic vaccination in primary immunodeficiency: A working group report of the Basic and Clinical Immunology Interest Section of the American Academy of Allergy, Asthma & Immunology. *Journal of Allergy and Clinical Immunology*. 2012;130:S1–24.
8. Stabler S, Lamblin C, Gaillard S, Just N, Mihăilescu M, Viget N, et al. High Frequency of Specific Polysaccharide Antibody Deficiency (SPAD) in Adults With Unexplained, Recurrent and/or Severe Infections With Encapsulated Bacteria. *Clinical Infectious Diseases*. 2022;ciac842.
9. Berger M, Geng B, Cameron DW, Murphy LM, Schulman ES. Primary immune deficiency diseases as unrecognized causes of chronic respiratory disease. *Respir Med*. 2017;132:181–8.
10. Wernette CM, Frasch CE, Madore D, Carlone G, Goldblatt D, Plikaytis B, et al. Enzyme-Linked Immunosorbent Assay for Quantitation of Human Antibodies to Pneumococcal Polysaccharides. *Clin Vaccine Immunol*. 2003;10:514–9.
11. Lopez B, Bahuaud M, Fieschi C, Mehlal S, Jeljeli M, Rogeau S, et al. Value of the Overall Pneumococcal Polysaccharide Response in the Diagnosis of Primary Humoral Immunodeficiencies. *Front Immunol*. 2017;8:1862.
12. Warnatz K, Schlesier M. Flowcytometric phenotyping of common variable immunodeficiency. *Cytometry Part B Clinical*. 2008;74B:261–71.
13. Soria A, Amsler E, Garel B, Moguellet P, Tieulié N, Cordoliani F, et al. Systemic inflammatory trunk recurrent acute macular eruption (SITRAME): A new auto-inflammatory syndrome in adult? *J Eur Acad Dermatol Venereol*. 2023;37:e538–42.
14. Guilbert TW, Denlinger LC. Role of infection in the development and exacerbation of asthma. *Expert Rev Respir Med*. 2010;4:71–83.
15. Coman I, Pola-Bibián B, Barranco P, Vila-Nadal G, Dominguez-Ortega J, Romero D, et al. Bronchiectasis in severe asthma. *Annals of Allergy, Asthma & Immunology*. 2018;120:409–13.
16. García-Clemente M, Enríquez-Rodríguez AI, Iscar-Urrutia M, Escobar-Mallada B, Arias-Guillén M, López-González FJ, et al. Severe asthma and bronchiectasis. *J Asthma*. 2020;57:505–9.
17. Darveaux JJ, Lemanske RF. Infection-related asthma. *J Allergy Clin Immunol Pract*. 2014;2:658–63.
18. Lee S-H, Ban G-Y, Kim S-C, Chung C-G, Lee H-Y, Lee J-H, et al. Association between primary immunodeficiency and asthma exacerbation in adult asthmatics. *Korean J Intern Med*. 2020;35:449–56.
19. Tiotiu A, Salvator H, Jaussaud R, Jankowski R, Couderc L-J, Catherinot E, et al. Efficacy of immunoglobulin replacement therapy and azithromycin in severe asthma with antibody deficiency. *Allergology International*. 2020;69:215–22.
20. Vivarelli E, Matucci A, Parronchi P, Liotta F, Cosmi L, Rossi O, et al. Primary antibody deficiencies represent an underestimated comorbidity in asthma patients: efficacy of immunoglobulin replacement therapy in asthma control. *Journal of Asthma*. 2023;60:1227–36.
21. Carr TF, Koterba AP, Chandra R, Grammer LC, Conley DB, Harris KE, et al. Characterization of Specific Antibody Deficiency in Adults with Medically Refractory Chronic Rhinosinusitis. *Am J Rhinol Allergy*. 2011;25:241–4.
22. Kashani S, Carr TF, Grammer LC, Schleimer RP, Hulse KE, Kato A, et al. Clinical Characteristics of Adults With Chronic Rhinosinusitis and Specific Antibody Deficiency. *The Journal of Allergy and Clinical Immunology: In Practice*. 2015;3:236–42.
23. Keswani A, Dunn NM, Manzur A, Kashani S, Bossuyt X, Grammer LC, et al. The Clinical Significance of Specific Antibody Deficiency (SAD) Severity in Chronic Rhinosinusitis (CRS). *The Journal of Allergy and Clinical Immunology: In Practice*. 2017;5:1105–11.
24. Schwitzguébel AJ-P, Jandus P, Lacroix J-S, Seebach JD, Harr T. Immunoglobulin deficiency in patients with chronic rhinosinusitis: Systematic review of the literature and meta-analysis. *J Allergy Clin Immunol*. 2015;136:1523–31.
25. Dransfield MT, Nahm MH, Han MK, Harnden S, Criner GJ, Martinez FJ, et al. Superior immune response to protein-conjugate versus free pneumococcal polysaccharide vaccine in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2009;180:499–505.
26. McCullagh BN, Comellas AP, Ballas ZK, Newell JD, Zimmerman MB, Azar AE. Antibody deficiency in patients with frequent exacerbations of Chronic Obstructive Pulmonary Disease (COPD). *PLoS One*. 2017;12:e0172437.
27. Snowden N, Moran A, Booth J, Haeney MR, Swinson DR. Defective antibody production in patients with rheumatoid arthritis and bronchiectasis. *Clin Rheumatol*. 1999;18:132–5.
28. Broyde A, Arad U, Madar-Balakirski N, Paran D, Kaufman I, Levartovsky D, et al. Longterm Efficacy of an Antipneumococcal Polysaccharide Vaccine among Patients with Autoimmune Inflammatory Rheumatic Diseases. *J Rheumatol*. 2016;43:267–72.
29. Sorensen RU, Leiva LE, Javier FC, Sacerdote DM, Bradford N, Butler B, et al. Influence of age on the response to Streptococcus pneumoniae vaccine in patients with recurrent infections and normal immunoglobulin concentrations. *Journal of Allergy and Clinical Immunology*. 1998;102:215–21.
30. Lawrence MG, Borish L. Specific antibody deficiency: pearls and pitfalls for diagnosis. *Ann Allergy Asthma Immunol*. 2022;129:572–8.
31. Sorensen RU. A Critical View of Specific Antibody Deficiencies. *Front Immunol*. 2019;10:986.
32. Bucciol G, Schaballie H, Schrijvers R, Bosch B, Proesmans M, De Boeck K, et al. Defining Polysaccharide Antibody Deficiency: Measurement of Anti-Pneumococcal Antibodies and Anti-Salmonella typhi Antibodies in a Cohort of Patients with Recurrent Infections. *J Clin Immunol*. 2020;40:105–13.
33. Schaballie H, Bosch B, Schrijvers R, Proesmans M, De Boeck K, Boon MN, et al. Fifth Percentile Cutoff Values for Antipneumococcal Polysaccharide and Anti-Salmonella typhi Vi IgG Describe a Normal Polysaccharide Response. *Front Immunol*. 2017;8:546.

34. Schaballie H, Vermeulen F, Verbinnen B, Frans G, Vermeulen E, Proesmans M, et al. Value of allohaemagglutinins in the diagnosis of a polysaccharide antibody deficiency: Allohaemagglutinins for polysaccharide antibody deficiency diagnosis. *Clin Exp Immunol*. 2015;180:271–9.
35. Alachkar H, Taubenheim N, Haeney MR, Durandy A, Arkwright PD. Memory switched B cell percentage and not serum immunoglobulin concentration is associated with clinical complications in children and adults with specific antibody deficiency and common variable immunodeficiency. *Clinical Immunology*. 2006;120:310–8.
36. Leiva LE, Monjure H, Sorensen RU. Recurrent Respiratory Infections, Specific Antibody Deficiencies, and Memory B Cells. *J Clin Immunol*. 2013;33:57–61.
37. Milito C, Soccodato V, Collalti G, Lanciarotta A, Bertozzi I, Rattazzi M, et al. Vaccination in PADs. *Vaccines (Basel)*. 2021;9:626.
38. van Kessel DA. Impaired pneumococcal antibody response in bronchiectasis of unknown aetiology. *European Respiratory Journal*. 2005;25:482–9.
39. United Kingdom Primary Immunodeficiency Network (UKPIN) Immunoglobulin Decision to Treat Study Group, Edgar JDM, Richter AG, Huissoon AP, Kumararatne DS, Baxendale HE, et al. Prescribing Immunoglobulin Replacement Therapy for Patients with Non-classical and Secondary Antibody Deficiency: an Analysis of the Practice of Clinical Immunologists in the UK and Republic of Ireland. *J Clin Immunol*. 2018;38:204–13.
40. Joud Hajjar, Nguyen AL, Constantine G, Kutac C, Syed MN, Orange JS, et al. Prophylactic Antibiotics Versus Immunoglobulin Replacement in Specific Antibody Deficiency. *J Clin Immunol*. 2020;40:158–64.
41. Milito C, Pulvirenti F, Cinetto F, Lougaris V, Soresina A, Pecoraro A, et al. Double-blind, placebo-controlled, randomized trial on low-dose azithromycin prophylaxis in patients with primary antibody deficiencies. *J Allergy Clin Immunol*. 2019;144:584-593.e7.
42. Starner TD, Shrout JD, Parsek MR, Appelbaum PC, Kim G. Subinhibitory concentrations of azithromycin decrease nontypeable *Haemophilus influenzae* biofilm formation and diminish established biofilms. *Antimicrob Agents Chemother*. 2008;52:137–45.
43. Cheng YK, Decker PA, O'Byrne MM, Weiler CR. Clinical and laboratory characteristics of 75 patients with specific polysaccharide antibody deficiency syndrome. *Annals of Allergy, Asthma & Immunology*. 2006;97:306–11.
44. Barlan IB, Geha RS, Schneider LC. Therapy for patients with recurrent infections and low serum IgG3 levels. *Journal of Allergy and Clinical Immunology*. 1993;92:353–5.
45. Gandhi A, Brodsky L, Ballow M. Benefits of Antibiotic Prophylaxis in Children with Chronic Sinusitis: Assessment of Outcome Predictors. *allergy asthma proc*. 1993;14:37–43.
46. van Kessel DA, Hoffman TW, van Velzen-Blad H, Zanen P, Grutters JC, Rijkers GT. Long-term Clinical Outcome of Antibody Replacement Therapy in Humoral Immunodeficient Adults With Respiratory Tract Infections. *EBioMedicine*. 2017;18:254–60.
47. Smits BM, Kleine Budde I, de Vries E, ten Berge IJM, Bredius RGM, van Deuren M, et al. Immunoglobulin Replacement Therapy Versus Antibiotic Prophylaxis as Treatment for Incomplete Primary Antibody Deficiency. *J Clin Immunol [Internet]*. 2020 [cited 2020 Dec 22]; Available from: <http://link.springer.com/10.1007/s10875-020-00841-3>
48. Wall LA, Dimitriades VR, Sorensen RU. Specific Antibody Deficiencies. *Immunology and Allergy Clinics of North America*. 2015;35:659–70.

Tables

Table 1 – Patients' characteristics

	Total (n=55)
Female gender, n	41 (74.5%)
Age at diagnosis, years (median (IQR))	45 (36-60)
Infectious manifestations	
Single severe infection, n (%)	12 (21.8)
Recurrent benign infections, n (%)	24 (43.6)
Recurrent benign and severe infections, n (%)	11 (20)
Recurrent severe infections, n (%)	8 (14.5)
Bacteriological documentation, n (%)	24 (43.6%)
<i>Streptococcus pneumonia</i> , n	12
<i>Nisseria meningitidis</i> , n	8
<i>Haemophilus influenzae</i> , n	7
<i>Streptococcus pyogenes</i> , n	2
No documentation, n	31
Allergic/Inflammatory diseases, n (%)	21 (38.2%)
Asthma, n	12
Inflammatory rheumatism*, n	6
Autoimmune thyroiditis, n	4
Sjogren's syndrome, n	2
Psoriasis, n	2
Auto-inflammatory disease, n	1
Inflammatory bowel disease, n	1
Biological only autoimmunity**, n	4

* Rheumatoid arthritis (n = 2), ankylosing spondylitis (n = 2), polymyalgia rheumatica (n = 1), chronic inflammatory rheumatism (n = 1).

** Type III cryoglobulinemia (n=2), positive rheumatoid factor (n = 1), positive antinuclear antibodies > 1/1280 (n = 1)

Figures

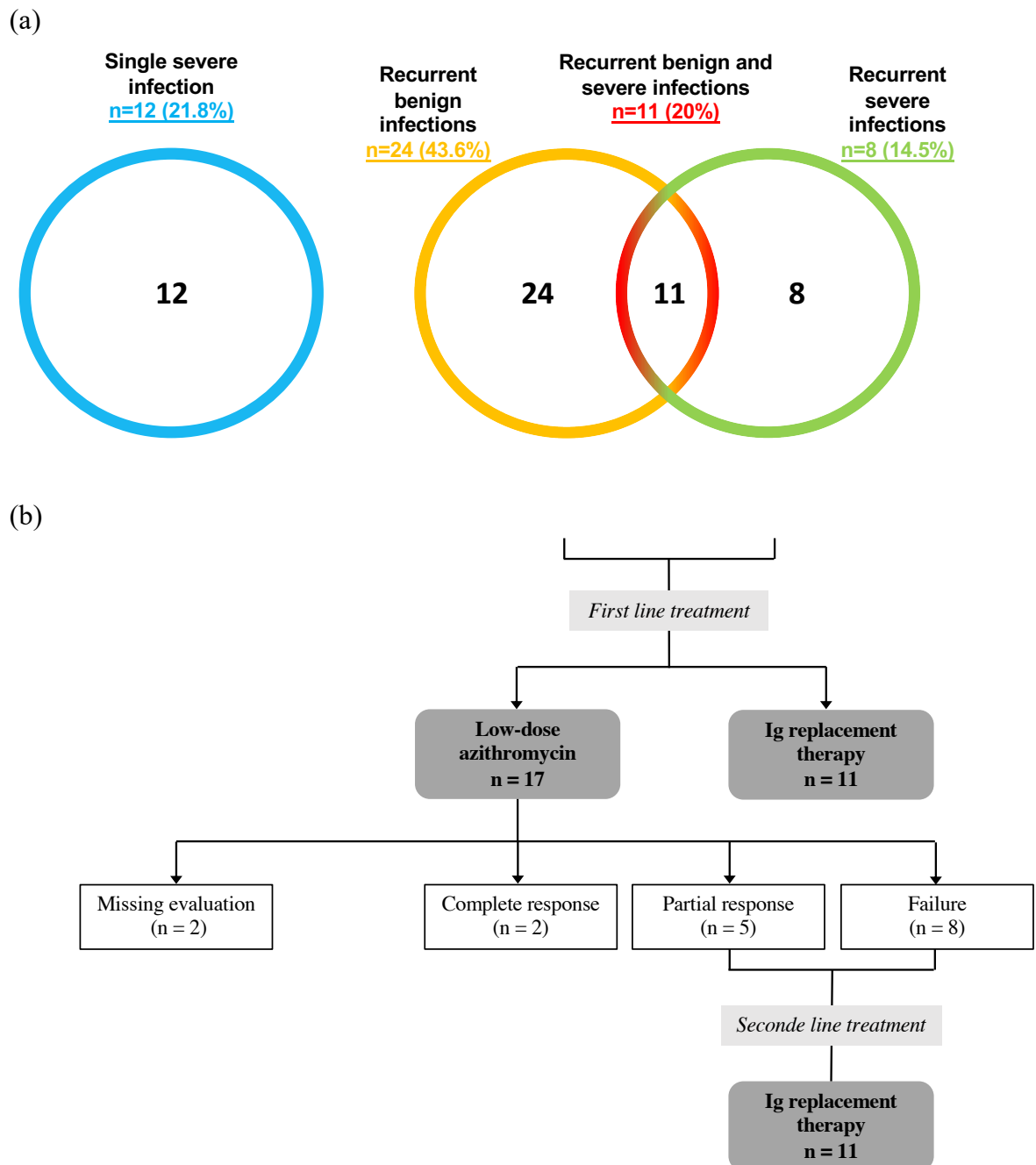


Figure 1 – Infectious phenotypes of SPAD and management. (a) Repartition of SPAD phenotype among 3 groups: a single severe infection, recurrent benign infections, and recurrent severe infections. (b) Therapeutic management of SPAD patients.

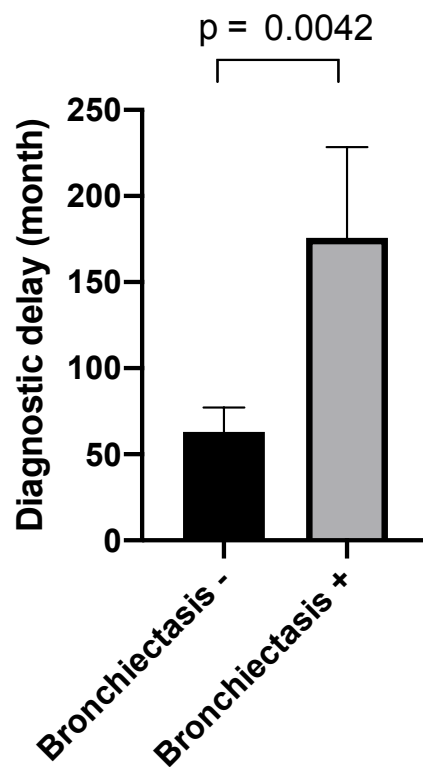


Figure 2: Diagnostic delay according to presence or absence of bronchiectasis.

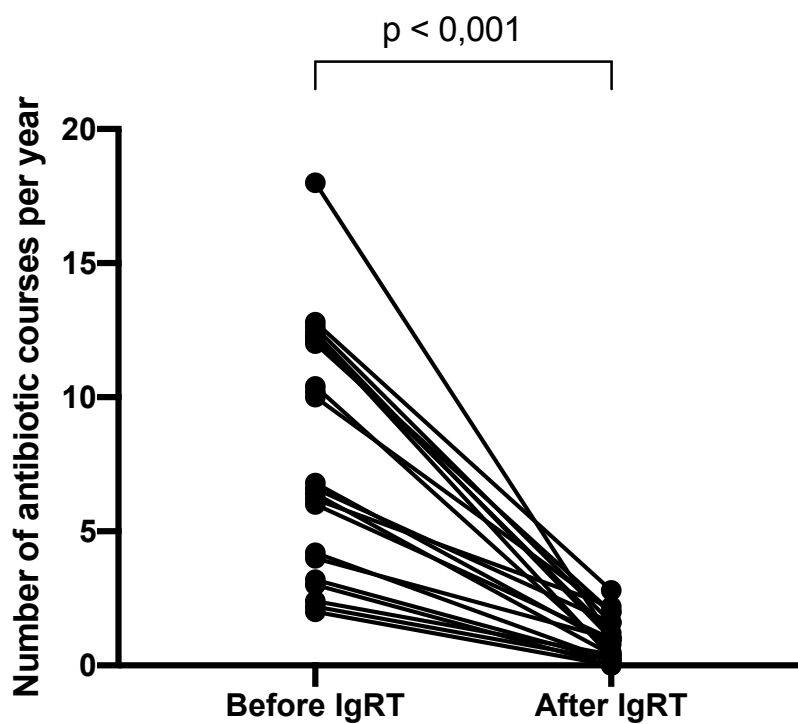


Figure 3 - Evolution of the number of antibiotic courses per year before and after introducing IgRT.

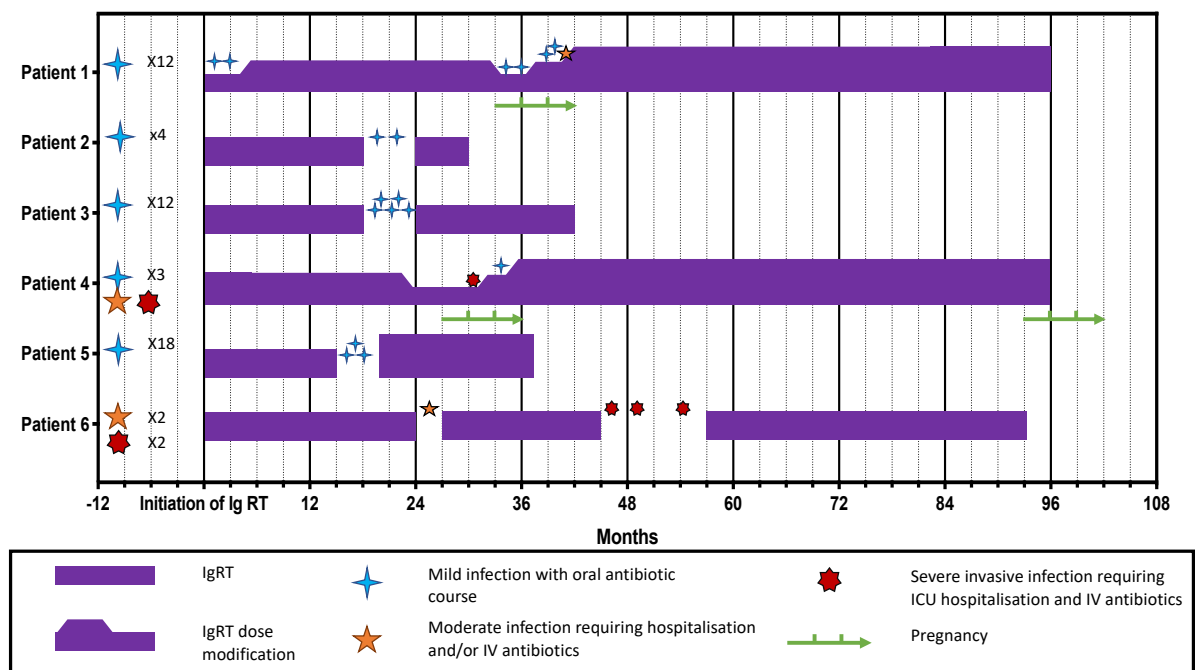


Figure 4 - Infectious events in 6 patients with reduction or discontinuation of IgRT.

Supplementary appendix

Supplementary Table 1: Repartition of patients among two groups

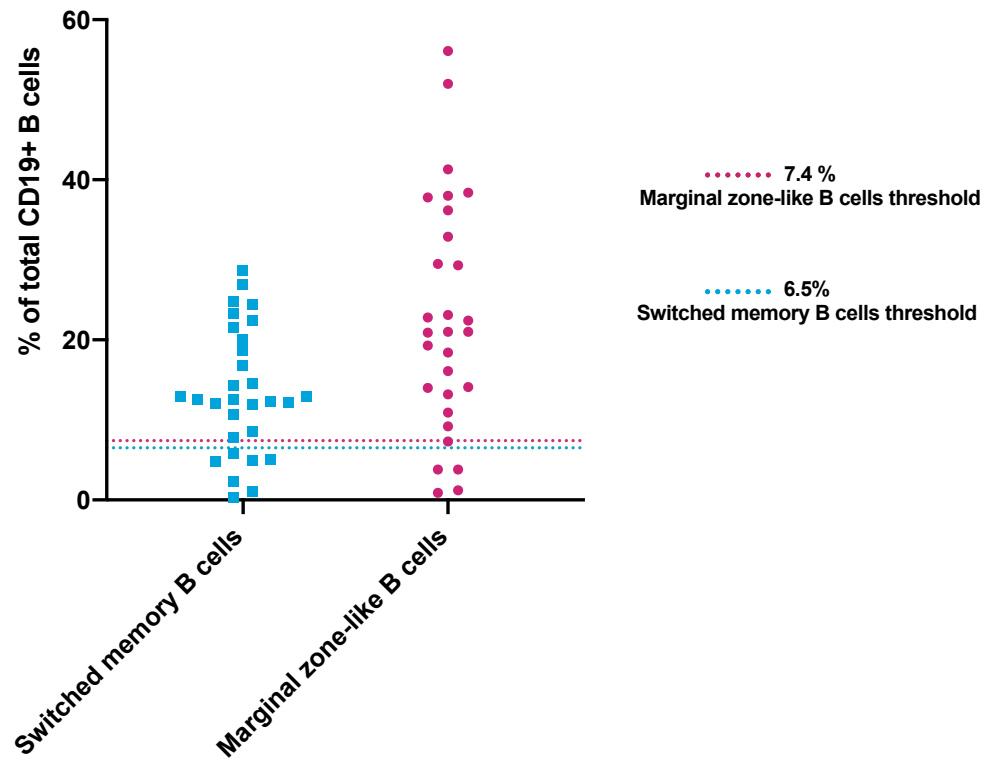
	Prospective cohort (n=36)	Local center group (n=19)
Female gender, n	26 (72%)	15 (78%)
Age at diagnosis, years (median (IQR))	40 (31.5-53.5)	37 (29-51)
Infectious manifestations		
Single severe infection, n (%)	8 (22.2%)	4 (21%)
Recurrent benign infections, n (%)	18 (50%)	6 (31.6%)
Recurrent benign and severe infections, n (%)	8 (22.2%)	3 (15.8%)
Recurrent severe infections, n (%)	2 (5.6%)	6 (31.6%)
Bacteriological documentation, n (%)	15 (41.7%)	9 (47.4%)
<i>Streptococcus pneumoniae</i> , n	7	5
<i>Nisseria meningitidis</i> , n	5	3
<i>Haemophilus influenzae</i> , n	3	4
<i>Streptococcus pyogenes</i> , n	1	1
No documentation, n	21	10
Allergic/Inflammatory diseases, n (%)	10 (27.8%)	11 (57.9%)
Asthma, n	8	4
Inflammatory rheumatism*, n	0	6
Autoimmune thyroiditis, n	1	3
Sjogren's syndrome, n	0	2
Psoriasis, n	2	0
Auto-inflammatory disease, n	0	1
Inflammatory bowel disease, n	1	0
Biological only autoimmunity**, n	0	4

* Rheumatoid arthritis (n = 2), ankylosing spondylitis (n = 2), polymyalgia rheumatica (n = 1), chronic inflammatory rheumatism (n = 1).

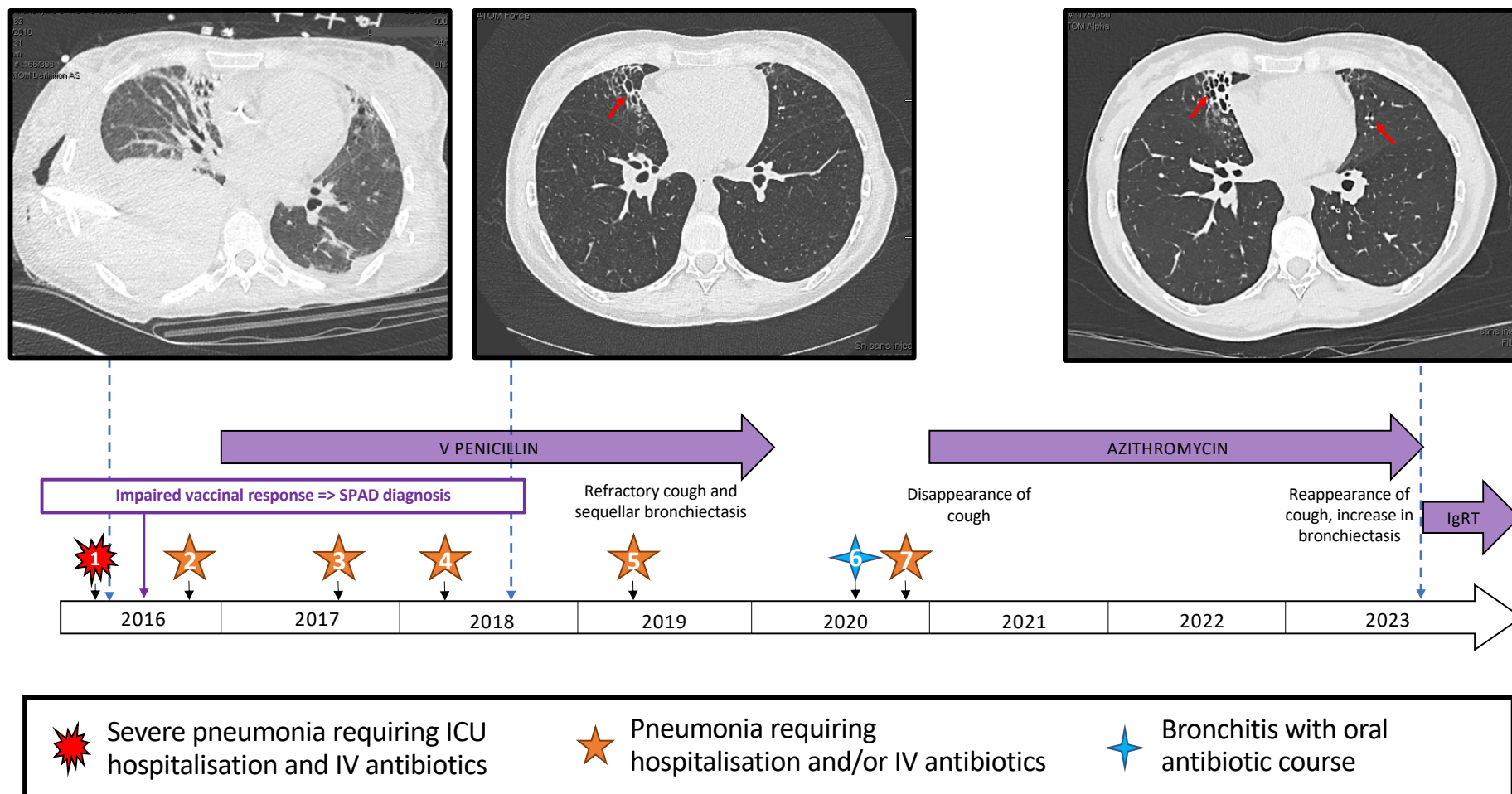
** Type III cryoglobulinemia (n=2), positive rheumatoid factor (n = 1), positive antinuclear antibodies > 1/1280 (n = 1)

Supplementary Table 2: Repartition of bacteriological documentation among infectious phenotypes. Some patients were documented for multiple bacteria.

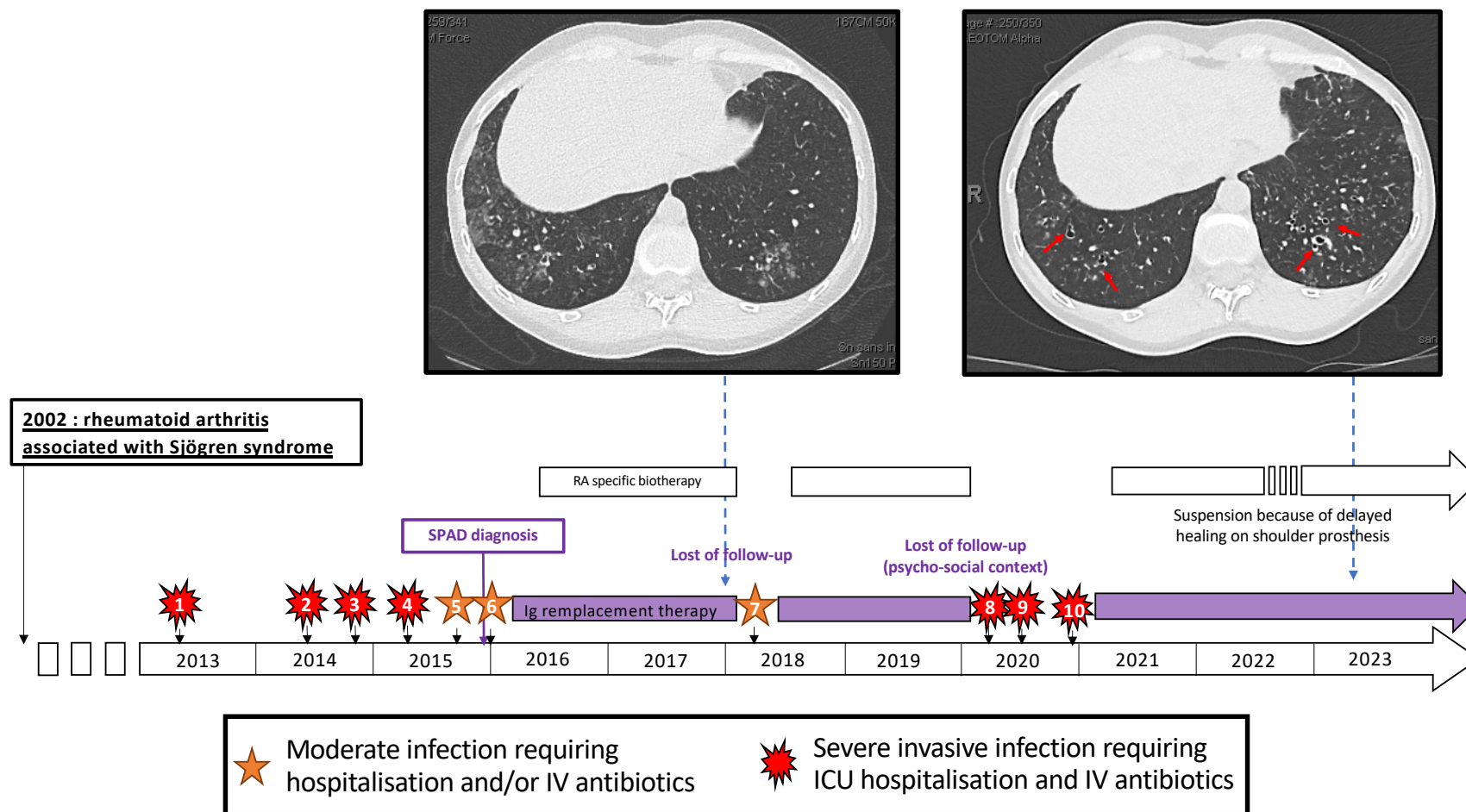
	Single severe infection (n=12)	Recurrent benign infections (n=24)	Recurrent benign and severe infections (n=11)	Recurrent severe infections (n=8)
<i>Streptococcus pneumoniae</i> , n (%)	6 (50%)	1 (4%)	3 (27%)	2 (25%)
<i>Nisseria meningitidis</i> , n (%)	6 (50%)	-	2 (18%)	-
<i>Haemophilus influenzae</i> , n (%)	-	3 (12%)	2 (18%)	2 (25%)
<i>Streptococcus pyogenes</i> , n (%)	-	1 (4%)	-	1 (12%)
No documentation, n (%)	0 (0%)	21 (87%)	5 (45%)	5 (62%)



Supplementary Figure 1 : Decreased relative counts of switched memory B cells (CD27+IgD-IgM-, n=7) and marginal zone-like B cells (CD27+IgD+IgM+, n=5) in SPAD patients.

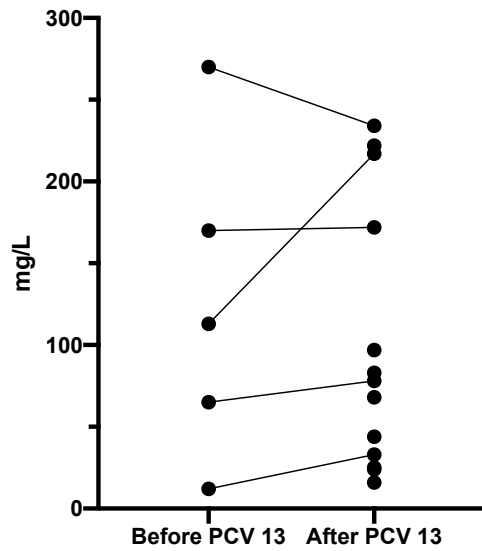


Supplementary Figure 2 – A case of SPAD patients with progressive worsening of bronchiectasis. 1: On-flu secondary infection by *Streptococcus pyogenes*, with refractory ARDS requiring v-vECLS and bacteriemia, multiple thrombosis including spleen ischemia, necrosis of the extremities requiring amputation of 4 limbs, 2,4,5,7: Pneumonia (*Haemophilus influenzae*), 3: Pneumonia (No documentation), 6: Bronchitis (*Moraxella catarrhalis*). ARDS: Acute respiratory distress syndrome, v-vECLS: Veno-venous extracorporeal life support, ICU: Intensive care unit, IV: Intravenous, IgRT: Immunoglobulin replacement therapy

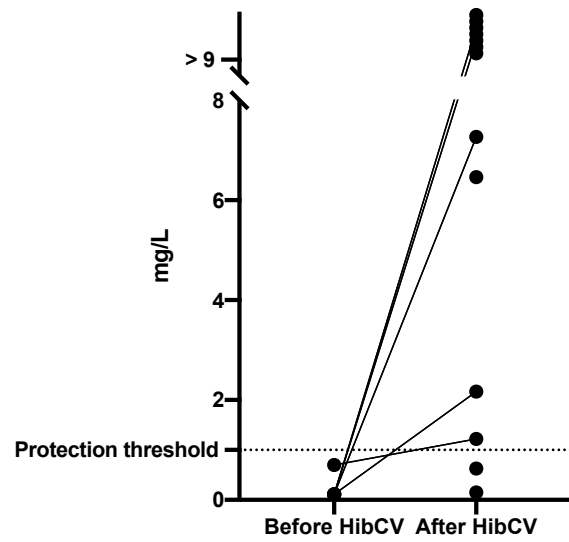


Supplementary Figure 3. A case of SPAD patient with rheumatoid arthritis. 1: *Pneumonia with empyema (Streptococcus pneumoniae)*, 2, 3, 4, 8 : *Pneumonia - ICU with septic shock (Klebsiella pneumoniae ESBL)*, 5 : *Bilateral maxillary sinusitis (Klebsiella pneumoniae ESBL)*, 6, 7 : *Pneumonia (No documentation)*, 9,10 : *Bilateral pneumonia - ICU with respiratory failure (No documentation)*. ICU: Intensive care unit, ESBL: Extended spectrum beta-lactamase, RA: Rheumatoid arthritis, IV: Intra-venous

(a)



(b)



Supplementary Figure 4 – Assessment of response to conjugate vaccines in SPAD patients. (a) Overall response assay of anti-pneumococcal antibodies before and/or after vaccination with PCV13. (b) Assessment of anti-*Haemophilus influenzae* serotype B antibodies before and/or after vaccination with HibCV;

Comparison of a multiplex electrochemiluminescence assay and the WHO reference ELISA for detection of anti-pneumococcal polysaccharide antibodies in the diagnosis of selective polysaccharide antibody deficiency

Authors

Nicolas Perrard ^(1,2), Aurore Collet ^(2,3), Sarah Stabler ^(2,4), Sandrine POIZOT ⁽³⁾, Myriam Labalette ^(2,3), Lucie Chevrier ⁽⁵⁾, Gatien Durand ⁽⁵⁾, Frédéric Batteux ⁽⁵⁾, Sylvain Dubucquoi ^(2,3), Guillaume Lefèvre ^(1, 2, 3)

(1) CHU Lille, Service de médecine interne et d'immunologie clinique, Centre de Référence des Déficits Immunitaire Hérititaires (CEREDIH), CHU Lille, F-59000 Lille, France ;

(2) Univ. Lille, INFINITE – Institute for Translational Research in Inflammation, Inserm U1286, Université de Lille, Lille.

(3) CHU Lille, Institut d'Immunologie, Lille, France; INFINITE - Institute for Translational Research in Inflammation, Inserm U1286, Université de Lille, F-59000 Lille, France ;

(4) CHU Lille, Service des Maladies Infectieuses, CHU Lille, F-59000 Lille, France ;

(5) Service d'Immunologie Biologique, AP-HP, Centre Hospitalier Universitaire (CHU) Cochin, Paris, F-75014, France ; Université Paris Cité, Institut Cochin, INSERM, CNRS, F-75014, Paris, France.

ABSTRACT (world count: 249/250)

Background:

Streptococcus pneumoniae can be responsible for severe infections, especially in patients with primary antibody deficiencies like selective anti-polysaccharide antibodies deficiency (SPAD). The reference method for assessment of anti-pneumococcal capsular polysaccharides (PnPS) IgG antibodies is a standardized serotype-specific ELISA (SSA), but this manual method is time-consuming and limit the number of evaluated PnPS.

Objective:

We aim to evaluate the performance values of a new multiplex assay based on electrochemiluminescence (ECL-plex).

Methods:

A panel of 164 sera from 82 patients sampled before and 4-8 weeks after immunization by the 23-valent pneumococcal polysaccharide vaccine (PPV23) were assessed by the reference WHO-SSA (7 to 13 serotypes) and by a 18-plex ECL assay (18 serotypes). All patients had normal serum Ig and IgG subclasses levels, and were classified as good (n=43) or poor responders (n=39, i.e SPAD patients) according to the reference SSA and according to the American Academy of Asthma, Allergy and Immunology's (AAAAI) current guidelines.

Results:

We observed excellent correlations between the two methods for 7 serotypes ($r = 0.88$ [95% CI: 0.87-0.90], n=124 sera) and 13 serotypes ($r = 0.87$ [0.87-0.89], n=40 sera). Using the AAAAI's guidelines for interpretation, the test performance of the 18-plex ECL assay for SPAD diagnosis showed a sensitivity of 95% and specificity of 84%, positive and negative predictive values of 84% and 95%, respectively. The percentage of agreement was 89% between the SSA and the 18-plex ECL assay.

Conclusion:

The 18-plex ECL assay is a reliable, rapid, and simple method for evaluating anti-PS response and screening patients with SPAD.

Key words.

Specific antibody deficiency, Selective anti-polysaccharide antibody deficiency, Primary immunodeficiency, pneumococcal vaccine, Electrochemiluminescence, ELISA, Multiplex

Abbreviations

4PL	4-parameter logistic curve
AAAAI	American Academy of Allergy, Asthma & Immunology
BSA	Bovine Serum Albumin
CV	Coefficient of Variation
CWPS	Cell Wall Polysaccharides
ECL-assay	Electrochemiluminescence Detection Assay
IQR	Interquartile Range
MSD	MesoScale Discovery[®]
N/PPV	Negative/Positive Predictive Value
OPA	Opsonophagocytosis Assay
PBS	Phosphate-buffered Saline
PnPS	Pneumococcal Capsular Polysaccharide
PPV23	Pneumococcal Polysaccharide Vaccine 23-valent
PS	Polysaccharide
SPAD	Specific anti-polysaccharide antibody deficiency
U/LLOQ	Upper/Lower Limit Of Detection
WHO-SSA	World Health Organization-Single Serotype Assay

Introduction

Streptococcus pneumoniae is an encapsulated anaerobic diplococcus found in the respiratory tract, responsible for various infections such as otitis, pneumonia, septicemia, or meningitis [1,2]. Unexplained, frequent and/or severe infections (e.g., endocarditis, meningitis, osteomyelitis) suggest an impaired immune response, mainly complement deficiencies and antibody deficiencies [3]. The bacterial capsule, composed of polysaccharides, constitutes a significant virulence factor for these bacteria due to its involvement in cell adhesion, resistance to phagocytosis, and complement activation [4]. The study of capsular polysaccharides allows the identification of pneumococcal serogroups and serotypes. Currently, there are more than

100 serotypes grouped into 48 serogroups described in the Lund Danish classification [5]. These capsular polysaccharides are the main targets of specific antibodies, enabling opsonization and degradation of the bacteria, while other capsular antigens are immunogenic but do not promote the destruction and clearance of the bacteria [6,7].

Specific anti-polysaccharide antibody deficiency (SPAD) is characterized by an impaired response to polysaccharides (PS), leading to recurrent and severe bacterial infections, including pneumococcal infections. This immune deficiency can be present despite normal responses to protein antigens and normal levels of immunoglobulins, T cells, and B cells [8,9]. The anti-pneumococcal capsular polysaccharide (PnPS) response is assessed after immunization with the unconjugated 23-valent pneumococcal vaccine (PPV23, PNEUMOVAX®). The exploration must be conducted serotype by serotype using a standardized method, both before and 4 to 8 weeks after immunization [10]. Interpretation criteria are based on expert consensus regarding specific antibody levels, fold-increases in antibody levels pre/post-immunization, the number of tested serotypes, and the method used. It's worth noting that, according to these criteria, a defective response can be found in healthy subjects [11,12].

In 2000, a consortium comprising representatives from various academies, governments, and industries, under the auspices of the World Health Organization (WHO), selected a single serotype enzyme-linked immunosorbent assay protocol for quantifying human IgG antibodies specific to *Streptococcus pneumoniae* capsular polysaccharides (PnPS) as the reference method [13]. This single-serotype assay (WHO-SSA) is relevant for analyzing serotype-specific antibody levels but is time-consuming and costly in terms of reagents and sera. The titers of specific anti-PnPS antibodies determined by this method correlate with the results of the opsonophagocytosis assay (OPA), which assesses the ability of these antibodies to destroy the pathogen through complement activation and phagocytic functions. Recommendations have

also been issued to guide the development of ELISA or other techniques in laboratories, ensuring correlation with WHO-SSA [14].

In 2007, Pickering et al. developed an electrochemiluminescence detection assay (ECL-assay) for the multiplex quantification of anti-PnPS antibodies using MesoScale Discovery® technology, with method validation and bridging with WHO-SSA, demonstrating good interchangeability between the two techniques [15]. This technique is faster and less costly in terms of reagents and sera, making it an interesting method for quantifying anti-PnPS antibodies.

Here, we present the development of this ECL technique in our laboratory, the results of the bridging conducted towards WHO-SSA, the value of this test in the diagnosis of SPAD.

Materials and methods

Polysaccharides (PS)

PnPS. All PnPS powders for serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A 19F, 23F were courtesy provided by Pfizer®, and transmitted to MesoScale Discovery® Manufacturing Division, West Point, PA for coating of ECL plates.

Cell Wall Polysaccharides (CWPS) and 22F. CWPS is a purified pneumococcal cell wall polysaccharide obtained from the Statens Serum Institut, Copenhagen, Denmark (Ref 3459). PnPS 22F was obtained from the Statens Serum Institut, Copenhagen, Denmark (Ref 76966). CWPS and 22F were reconstituted in type I reagent grade water, with a final concentration following reconstitution at 10 mg/ml. These PS are used for serum preadsorption to improve assays specificity [16].

Sera

Reference serum. The International Standard for Human Anti-pneumococcal capsule Reference Serum, lot 007SP, was prepared from 287 healthy volunteers following vaccination with 23-

valent pneumococcal polysaccharide vaccine (PPV23 - PNEUMOVAX II®). Calibration for 24 serotype specific IgG concentrations was made in 2011 during an International Collaborative Study involving 5 laboratories. These values were derived following double adsorption of 007sp with cell wall polysaccharide (CWPS) and Ps 22F. This reference serum is distributed by the U.S. Food and Drug Administration, Bethesda, MD.

Adult serum samples. The concordance between the 18-plex ECL assay and the WHO-SSA was made using 124 adult samples documented in IgG for 7 serotypes (serotypes 4, 6B, 9V, 14, 18C, 19F and 23F) and 40 documented in IgG for 13 serotypes (7 serotypes plus serotypes 1, 3, 5, 6A, 7F and 19A) before and after vaccination with PPV23. All specific IgG for each PnPS were assessed using the WHO-SSA in the immune-monitoring facility at Cochin University Medical Center (Paris, France). These sera were collected from March 2015 to February 2020.

External quality controls. Four samples provided by an external quality assessment service (UK NEQAS®) were tested with the 18-plex ECL. We used the mean results provided after the final analysis of all participating centers (n=?), for each serotype, for comparison with the 18-plex ECL: as it merged results obtained with LUMINEX technology assay and WHO-SSA, this comparison with 18-plex ECL was made independently.

We also use pharmacological-industry pooled human polyvalent immunoglobulins CLAIRYG® (Ig 50 mg/mL, IgG >95% (IgG1 55-67%, IgG2 29-37%, IgG3 1-4% and IgG4 1-3%), IgA < 0,1% and IgM 0%) to assess reproducibility of the assay, and possible use as internal quality controls in IgG.

18-plex ECL assay

The MesoScale Discovery® (MSD) technology is based on ECL detection buffer as documented previously [17]. Briefly, MSD-multispot carbon microplates were incubated with samples or reference serum, then incubated with detection secondary SULFO-TAG antibodies. MSD-reader Quick-Plex SQ 120 applied electrical current on MSD-multispot carbon microplates and,

in presence of tripropylamine read buffer, a series of electrically induced reactions leads to oxydation of the ruthenium tris(bipyridine) complexes on SULFO-TAG antibodies and produces a luminescent signal.

The multispot configuration used was two 96-well plates with 10 spots/well format. Each well was coated with 100 ng per spot of PnPs serotypes. First plate ('plate A') was coated with PnPS 1, 3, 4, 5, 6A, 6B, 7F, 8 and 9V, and second plate ('plate B') was coated with PnPS 10A, 11A, 12F, 14, 15B, 18C, 19A 19F and 23F. Each well also contained one 200 ng CWPS spot, which was used to assess and the effectiveness of adsorption with CWPS and 22F and the background reactivity of the assay (i.e., the response associated with serum and a labeled secondary antibody).

007SP was use at 8 different 3-fold dilutions (from 1:150 to 1:328,050) in replicates to create a reference standard curve. Sera were tested at a fixed dilution of 1:2000.

The reference serum 007SP, quality controls, and sera were diluted at appropriate dilutions in phosphate-buffered saline containing 0.05% Tween 20 (PBS-T), 1% Bovine Serum Albumin (BSA), 20 µg/ml CWPS, 20 µg/ml PnPS 22F, and were incubated overnight at 4°C on a stirring plate. Each antigen-coated plate was incubated at ambient temperature for 1 h on an orbital shaker at 700 rpm with 5% BSA and PBS-T. Plates were washed 3 times with PBS-T, and 50 µL per well of the preadsorbed and diluted test sera/reference serum/quality control was added and incubated for 2 hours at ambient temperature on an orbital shaker. Plates were washed 3 times with PBS-T; and 50µL MSD Sulfo-tag-labeled-goat anti-human IgG secondary antibody diluted 1:1000 in 1% BSA PBS-T was added to each well and incubated for 1 hour at ambient temperature on an orbital shaker. Plates were washed for 3 times with PBS-T, and 150 µl of MSD ECL read buffer was added to each well. The plates were read using MSD-reader Quick-Plex SQ 120[®], the correct operation of the machine was tested before each reading using an electronical plate. The concentrations of antibodies were determined using MSD Discovery

Workbench[®] software, by referencing their ECL responses against a standard 4-parameter logistic (4PL) model curve generated from the serially diluted 007SP reference serum.

WHO ELISA method.

The WHO ELISA method used at Cochin University Medical Center has been described previously and can be accessed online [13]. Briefly, medium-binding 96-well microtiter plates were coated with various concentrations of PnPs 1, 3, 5, 4, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. Human sera and international reference standard 007SP were absorbed with 5 µg/ml CWPS and 5 µg/ml serotype 22F capsular polysaccharide 30 min at room temperature to neutralize antibody binding to CWPS and other contaminants found in the pneumococcal polysaccharide coating antigens.

Serial dilutions of absorbed sera were added to Ps-coated plates. Serotype-specific antibodies present in sera were detected using an alkaline phosphatase-labeled goat anti-human secondary antibody, followed by addition of the substrate, p-nitrophenyl phosphate. The optical density of each well was measured at 405 nm and 620 nm (reference) using an ELISA plate reader. The optical density of each sample well was compared to the one of the standard in order to determine the concentration of antibodies in the human serum using a 4PL model.

Validation of the concordance with WHO-SSA in SPAD diagnosis

Interpretation of anti-PnPS response and SPAD diagnosis were defined in accordance with the recommendations of the American Academy of Allergy, Asthma & Immunology (AAAAI) [10,18], i.e. (i) normal Ig and IgG quantitation, normal B cell count and (ii) impaired anti-PS response after vaccination with PPV23. The anti-PS response is considered as normal for a single serotype if the post-immunization antibody titer was upper 1.3 µg/mL (considered to be protective) and achieved a 4-fold increase relative to the pre-immunization value or achieved a 2-fold increase if the pre- immunization value was already greater than 1.3 µg/mL. A normal

response to five serotypes among the seven tested (70%) was interpreted as a good immunization.

The objective of the concordance experimentation was to assess the concordance of the ECL-assay results with those generated by the international WHO-SSA for a set of 164 pre and post-vaccination samples from 82 adult patients. As some of these patients were diagnosed SPAD after the WHO-SSA exploration of anti-PnPS response, we applied our results to all patient to assess the diagnostic performance of the ECL-assay in this indication.

All these criteria are only validated for IgG vaccinal response.

Statistical analysis

Correlation between ECL-assay and WHO-SSA was assessed using Spearman correlation test for all 7 and 13 serotypes, and for each serotype individually. Concordance between 18-plex ECL and WHO-SSA was assessed using Bland-Altman method comparison for all 7 and 13 serotypes (difference between each method values versus average). ROC curves were used to determine the performance of 1.3 µg/mL threshold in ECL-assay compared to WHO-SSA.

Aberrant values (more than ten times difference between the two methods) were excluded from per-protocol bridging analyses, representing between 1 and 3% of results depending on serotypes, but were kept for intension-to-tread SPAD performances of the assay.

The level of imbalance in the discordance between 18-plex ECL and WHO-SSA was statistically assessed using a McNemar's exact test. Cohen's κ -coefficient was also calculated, providing an estimate of the agreement between assays beyond which might exist due to chance alone. The value of the κ -coefficient ranges from -1.0 to 1.0. Agreement consistent with chance alone yields a κ -coefficient near 0, whereas agreement far exceeding chance alone yields a κ -coefficient approaching 1.0 (from 0.61 to 0.80 been seen as substantial agreement).

Data analyses and graphs were performed using the GraphPad Prism software version 9.1.2 (GraphPad Software, La Jolla, CA, USA) and online tests provided by developers at <https://www.graphpad.com/quickcalcs>.

Ethical disclosures

Local sera were collected during a prospective multicentered study (NCT02972281) aiming to evaluate a systematic research strategy of primary immunodeficiencies in adults presenting unexplained bacterial infections, including exploration of pre and post-vaccinal response to PPV23, with approval of the Institutional Review Board of our institution and by an independent ethics committee according to French legislation (“Comité de Protection des Personnes Nord Ouest IV,” number of agreement 2014-100739-38). Non-opposition was collected for assessment of patient immunodeficiency status (“Protection des Données – GHT Lille Métropole Flandre intérieure”, number of agreement DEC23-109).

Results

18-plex ECL set up

The range and dilutions of calibrant 007SP were visually determined to achieve an optimal 4PL curve at both low and high antibody concentrations for each serotype. The optimal measurement range for 007SP started at an initial dilution of 1:150 with seven 1:3 dilutions (final dilution at 1:328,050). This dilution allowed for the use of minimal serum (10µL/sample), achieving the optimal Lower Limit of Quantification (LLOQ) and Upper Limit of Quantification (ULOQ) for pre- and post-vaccination levels to support interpretation of threshold of 1.3 mg/L and a 2- to 4-fold-change in vaccine response. The LLOQ and ULOQ for each polysaccharide at a 1:2000 dilution are presented in Table 1. The highest LLOQ was obtained for the the serotype 14 due to its high concentration of anti-PnPS antibodies in 007SP (Figure 1).

Twelve patient samples were initially tested at two serial dilutions of 1:500 and 1:2000: the 1:2000 dilution had the best signal linearity and was chosen for the inter assay comparison.

Adsorption of non-specific antibodies by 22F and CWPS, was compared between 10 and 20 µg/mL each in the adsorption solution: 20 µg/mL was optimal to negate the CWPS spot signal at a dilution of 1:2000.

Measurement variability

To observe inter-plate (4 sets of 2 plates) and inter-operator (2 operators) reproducibility in IgG, concentrations of anti-PnPS antibodies were determined for the industrial sample of polyvalent immunoglobulins (CLAIRYG[®]) and two sera from one patient (pre and post-vaccination) on 3 plates. We found an average coefficient of variation (CV) of 21.77% (CVmin at 9.5% for serotype 6B, CVmax at 44.7% for serotype 19F), regardless of the serotype or magnitude of the determined concentration. This totaled 162 measurements across 18 serotypes and 3 samples (one patient pre and post-vaccination and CLAIRYG[®]).

Interassay comparison

The overall correlation between the 18-plex ECL and WHO-SSA, as determined by Spearman's correlation test, showed excellent correlation across 7 serotypes (1089 measurements, $r = 0.8850$ [95% CI: 0.8710-0.8975]) and 13 serotypes (1307 measurements, $r = 0.8735$ [0.8696-0.8852]). Bland-Altman tests revealed a light tendency for underestimation with the ECL assay for both 7 and 13 serotypes, with a negative bias of -1.038 and -1.044, and 95% limits of agreement ranging from -18.31 to 16.21 and from -17.09 to 15.00, respectively (Figure 2a). The correlation, detailed serotype by serotype, is illustrated in Figure 2b, with correlations ranging from $r = 0.6693$ (0.4241-0.8230) for serotype 6A to $r = 0.9626$ (0.9215-0.9824) for serotype 19A. A mild but systematic underestimation was observed for serotypes 5 and 7F.

There were also excellent correlations with the mean results from the samples collected in an external quality assessment (18 serotypes) with Spearman's correlation coefficients of $r =$

0.9590 (0.9342-0.9746) (Figure 3). Notably, there was a systematic underestimation of serotype 5, as well as for our sera (Figure 2 and Figure 3).

Using the threshold defined by expert consensus in the diagnosis of SPAD at 1.3 µg/mL, ROC curves found an AUC of 0.9327 (95% CI: 0.9185-0.9468) across 7 serotypes and 0.9194 (0.9050-0.9338) across 13 serotypes (Figure 4).

18-plex ECL diagnostic performance for SPAD

Using the results of the ECL-assay, serotype-by-serotype response was assessed across 18 serotypes for 43 controls and 39 patients with SPAD (Figure 5).

Based on ROC curves, the expected percentage of serotypes with a good response required for the diagnosis of SPAD with the 18-plex ECL assay was maintained at 70%. We observed good diagnostic performances compared to the WHO-SSA, with a sensitivity of 94.9% and specificity of 83.7% (PPV = 84.1%, NPV = 94.7%) in the 82 evaluated patients. The tests did not significantly differ according to McNemar's exact test ($p = 0.1824$), with a substantial agreement rate (Cohen's κ -coefficient at 0.781 [95% CI: 0.647 to 0.915], percentage of agreement at 89.02%). Among them, 20 patients were diagnosed using a 13-serotype evaluation and 62 with a 7-serotype evaluation. When focusing on the 20 patients diagnosed using a 13-serotype evaluation, the diagnostic performance increased to a sensitivity of 100% and specificity of 85.7% (PPV = 92.9%, NPV = 100%) (Table 2).

Discussion

We introduce a new method for assessment of anti-PnPS antibody response to PPV23 in the diagnosis of primary antibody deficiencies. Our results suggest that the 18-plex ECL assay can be considered to diagnose SPAD and compensate for the disadvantages of WHO-SSA.

Our results demonstrate excellent correlation with the WHO ELISA reference method, as well as adequate inter-operator and inter-plate reproducibility, despite a tendency to underestimate

antibody concentrations. This strong correlation has been previously demonstrated by several research teams [15,17,19–21].

The ECL-assay performs well in screening for SPAD with excellent sensitivity and NPV. Furthermore, it appears that the assay is particularly concordant when evaluating 13 serotypes than only 7 serotypes. Potentially, diagnosing SPAD requires more than 7 serotypes to be reliable in patients, which may partly explain the discrepancies observed with the ECL-assay on 13 and 18 polysaccharides. By comparing the response rate to each polysaccharide and the previous work from our team [22], a consistent difference is observed in most serotypes between SPAD patients and controls, suggesting that each explored polysaccharide could potentially improve the performance of SPAD diagnosis.

However, it appears that specific serotypes may be underestimated by the 18-plex ECL assay (for example serotype 5 or 7F) or exhibit weaker correlation with the WHO-SSA compared to others. This last is particularly notable for serotypes 6A and 6B ($r = 0.66$ and 0.80 respectively), which could potentially be affected by cross-reactivity within the same serogroup [5,23]. Subsequent advancements are imperative to ascertain the optimal coating concentration of polysaccharides within the well, or their distribution across plates, to mitigate methodological interference and cross-reactivity between serogroups.

More in-depth explorations of the pathogenesis of SAPD seem necessary in order to ultimately redefine the diagnostic criteria for this rare immune deficiency [24].

Conclusion

The ECL is a promising method for exploring anti-PnPS immunity and diagnosing SPAD. We demonstrate here excellent correlation and diagnostic performance compared to the WHO-SSA reference method.

References

- Henriques-Normark B, Tuomanen EI. The pneumococcus: epidemiology, microbiology, and pathogenesis. *Cold Spring Harb Perspect Med*. 2013;3:a010215.
- Batah J, Varon E. . Rapport d'activité 2017. 2017;
- Sadarangani M. Protection Against Invasive Infections in Children Caused by Encapsulated Bacteria. *Front Immunol*. 2018;9:2674.
- Diard M, Hardt W-D. Evolution of bacterial virulence. *FEMS Microbiol Rev*. 2017;41:679–97.
- Geno KA, Gilbert GL, Song JY, Skovsted IC, Klugman KP, Jones C, et al. Pneumococcal Capsules and Their Types: Past, Present, and Future. *Clin Microbiol Rev*. 2015;28:871–99.
- Nielsen SV, Sørensen UB, Henrichsen J. Antibodies against pneumococcal C-polysaccharide are not protective. *Microb Pathog*. 1993;14:299–305.
- Lee C, Chun HJ, Park M, Kim RK, Whang YH, Choi SK, et al. Quality Improvement of Capsular Polysaccharide in *Streptococcus pneumoniae* by Purification Process Optimization. *Front Bioeng Biotechnol*. 2020;8:39.
- Perez E, Bonilla FA, Orange JS, Ballow M. Specific Antibody Deficiency: Controversies in Diagnosis and Management. *Front Immunol*. 2017;8:586.
- Perez EE, Ballow M. Diagnosis and management of Specific Antibody Deficiency. *Immunology and Allergy Clinics of North America*. 2020;40:499–510.
- Orange JS, Ballow M, Stiehm ER, Ballas ZK, Chinen J, De La Morena M, et al. Use and interpretation of diagnostic vaccination in primary immunodeficiency: A working group report of the Basic and Clinical Immunology Interest Section of the American Academy of Allergy, Asthma & Immunology. *Journal of Allergy and Clinical Immunology*. 2012;130:S1–24.
- Lopez B, Bahuau M, Fieschi C, Mehlal S, Jeljel M, Rogeau S, et al. Value of the Overall Pneumococcal Polysaccharide Response in the Diagnosis of Primary Humoral Immunodeficiencies. *Front Immunol*. 2017;8:1862.
- Sorensen RU, Leiva LE, Javier FC, Sacerdote DM, Bradford N, Butler B, et al. Influence of age on the response to *Streptococcus pneumoniae* vaccine in patients with recurrent infections and normal immunoglobulin concentrations. *Journal of Allergy and Clinical Immunology*. 1998;102:215–21.
- World Health Organization Pneumococcal Serology Reference Laboratories at the Institute of Child Health, University College London, London, England and the Department of Pathology at the University of Alabama at Birmingham, Birmingham Alabama, USA. Training manual for Enzyme linked immunosorbent assay for the quantitation of *Streptococcus pneumoniae* serotype specific IgG (Pn PS ELISA). (007sp Version) [Internet]. Available from: [\[https://www.vaccine.uab.edu/uploads/mdocs/ELISAProtocol\(007sp\).pdf\]](https://www.vaccine.uab.edu/uploads/mdocs/ELISAProtocol(007sp).pdf)([https://www.vaccine.uab.edu/uploads/mdocs/ELISAProtocol\(007sp\).pdf](https://www.vaccine.uab.edu/uploads/mdocs/ELISAProtocol(007sp).pdf))
- WHO. Recommendations to assure the quality, safety and efficacy of pneumococcal conjugate vaccines, Annex 3, TRS No 977 [Internet]. Available from: <https://www.who.int/publications/m/item/pneumococcal-conjugate-vaccines-annex3-trs-977>
- Pickering JW, Hoopes JD, Groll MC, Romero HK, Wall D, Sant H, et al. A 22-plex Chemiluminescent Microarray for Pneumococcal Antibodies. *Am J Clin Pathol*. 2007;128:23–31.
- Poolman JT, Frasch CE, Käyhty H, Lestrade P, Madhi SA, Henckaerts I. Evaluation of Pneumococcal Polysaccharide Immunoassays Using a 22F Adsorption Step with Serum Samples from Infants Vaccinated with Conjugate Vaccines. *Clin Vaccine Immunol*. 2010;17:134–42.
- Marchese RD, Puchalski D, Miller P, Antonello J, Hammond O, Green T, et al. Optimization and Validation of a Multiplex, Electrochemiluminescence-Based Detection Assay for the Quantitation of Immunoglobulin G Serotype-Specific Antipneumococcal Antibodies in Human Serum. *Clin Vaccine Immunol*. 2009;16:387–96.
- Bonilla FA, Khan DA, Ballas ZK, Chinen J, Frank MM, Hsu JT, et al. Practice parameter for the diagnosis and management of primary immunodeficiency. *Journal of Allergy and Clinical Immunology*. 2015;136:1186-1205.e78.
- Goldblatt D, Ashton L, Zhang Y, Antonello J, Marchese RD. Comparison of a New Multiplex Binding Assay versus the Enzyme-Linked Immunosorbent Assay for Measurement of Serotype-Specific Pneumococcal Capsular Polysaccharide IgG. *Clin Vaccine Immunol*. 2011;18:1744–51.
- Feyssaguet M, Bellanger A, Nozay F, Friel D, Merck E, Verlant V, et al. Comparison between a new multiplex electrochemiluminescence assay and the WHO reference enzyme-linked immunosorbent assay to measure serum antibodies against pneumococcal serotype-specific polysaccharides. *Vaccine*. 2019;37:2208–15.
- Nolan KM, Zhang Y, Antonello JM, Howlett AH, Bonhomme CJ, Greway R, et al. Enhanced antipneumococcal antibody electrochemiluminescence assay: validation and bridging to the WHO reference ELISA. *Bioanalysis*. 2020;12:1363–75.
- Stabler S, Lamblin C, Gaillard S, Just N, Mihailescu M, Viget N, et al. High Frequency of Specific Polysaccharide Antibody Deficiency (SPAD) in Adults With Unexplained, Recurrent and/or Severe Infections With Encapsulated Bacteria. *Clinical Infectious Diseases*. 2022;ciac842.
- Eddy BE. Cross Reactions between the Several Pneumococcal Types and Their Significance in the Preparation of Polyvalent Antiserum. *Public Health Reports (1896-1970)*. 1944;59:485.
- Sorensen RU. A Critical View of Specific Antibody Deficiencies. *Front Immunol*. 2019;10:986.

Figures

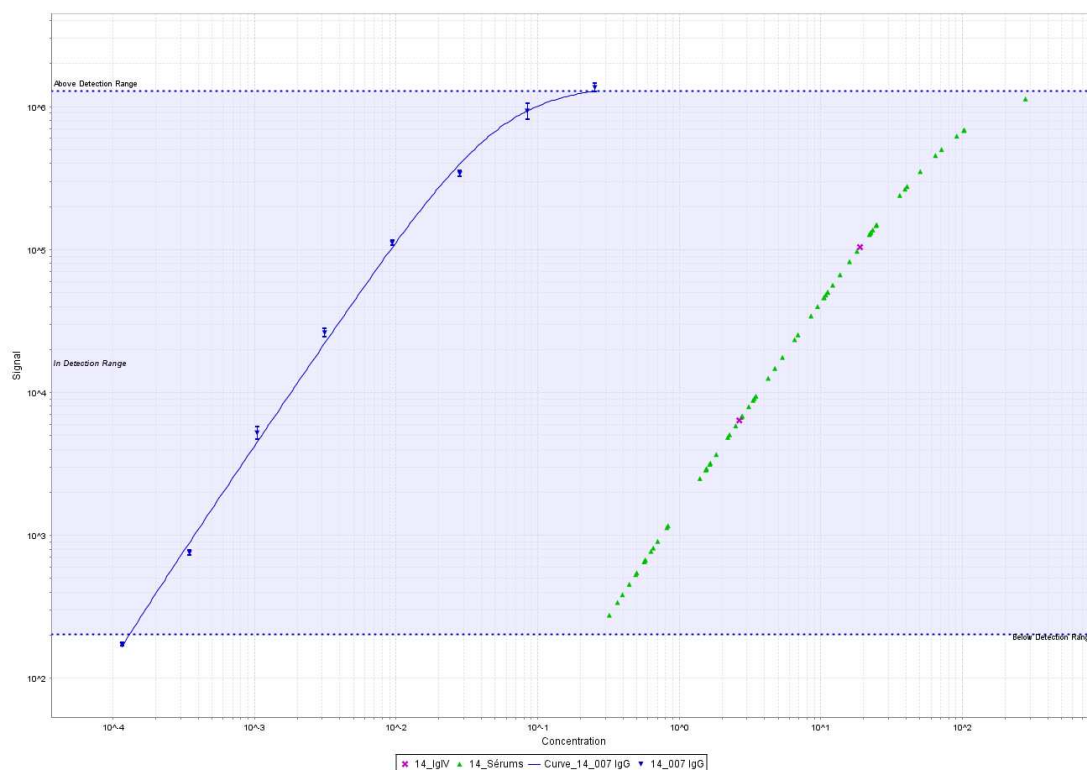
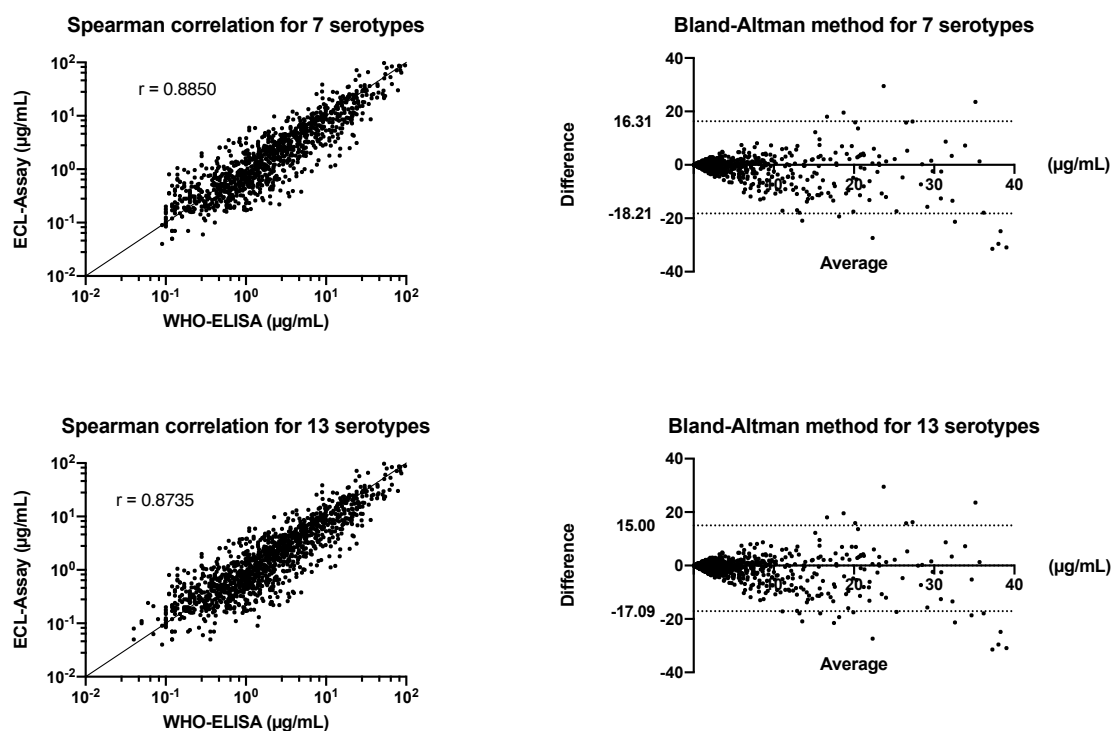


Figure 1: Extraction of serotype 14 4-PL curve from MSD Discovery Workbench[®] in a concentration($\mu\text{g/mL}$)/signal graphic (blue triangles and curve), with representation of samples determination of anti-PnPS serotype 14 antibody (green triangles) and 2 dilutions of industrial human polyvalent immunoglobulins CLAIRYG (purple crosses).

(a)



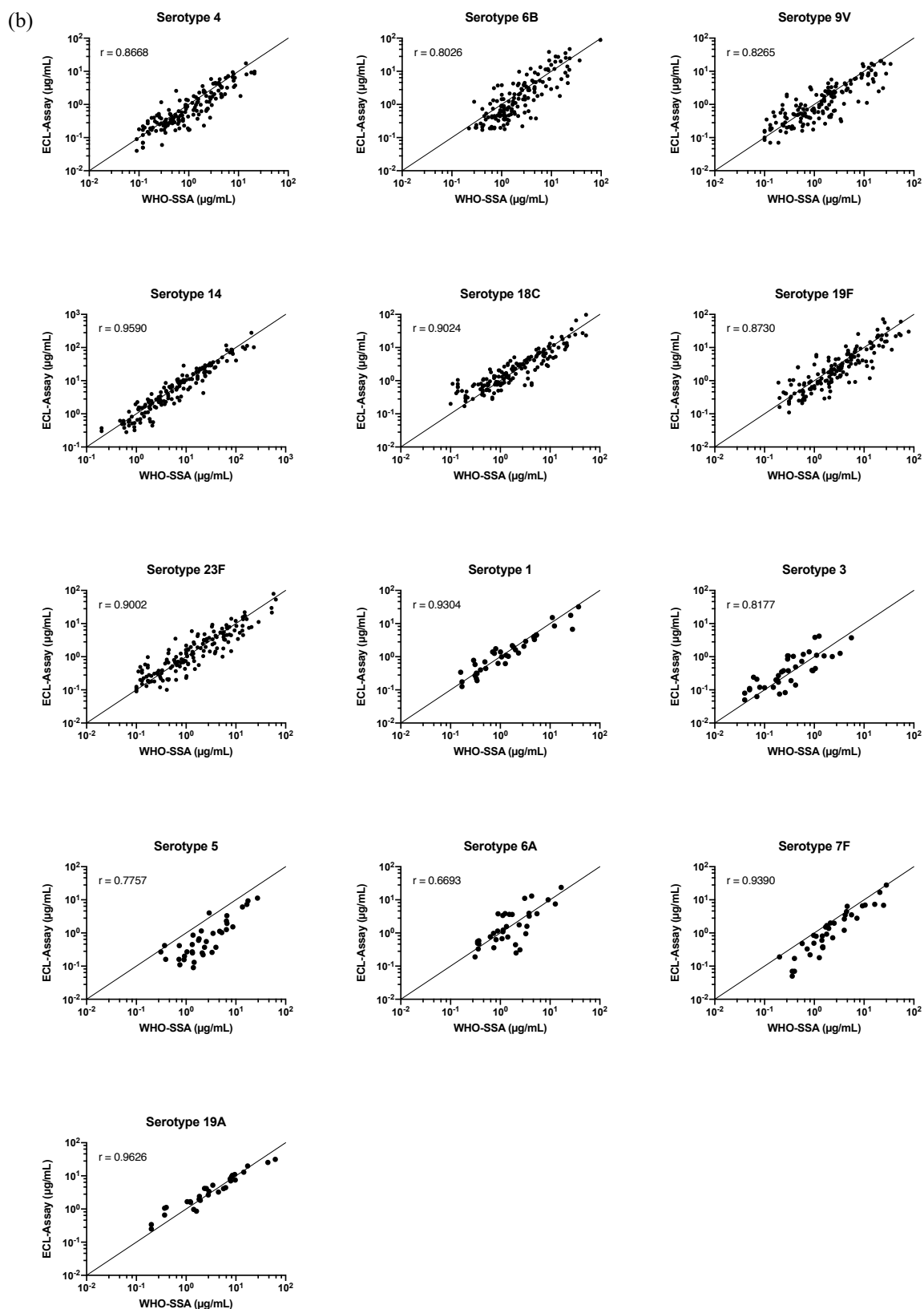


Figure 2: Spearman correlation and Bland-Altman tests between WHO-SSA and 18-plex ECL assay for pooled 7 serotypes $n = 1089$, and 13 serotypes $n = 1307$ (a), and detailed correlation tests for serotypes 4, 6B, 9V, 14, 18C, 19F and 23F, 7 serotypes $n = 154$ to 158 per serotype, and for serotypes 1, 3, 5, 6A, 7F and 19A, 13 serotypes $n = 35$ to 39 per serotype (b). Plotted function represents ideal test $x = y$.

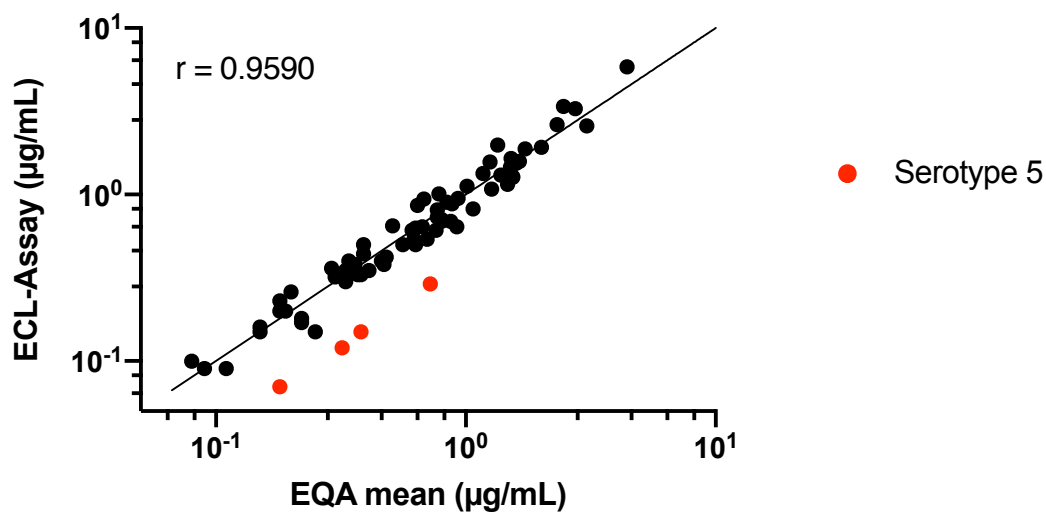


Figure 3: Comparison between 18-plex ECL assay and mean results obtained from 4 samples provided in an external quality assessment (WHO-SSA and Luminex-Based Multiplex Immunoassays) (n=4 for 18 serotypes) On the PT sera graph, red dots represented underestimated anti-serotype 5 antibodies. Plotted function represents ideal test $x = y$. EQA: External Quality Assessment

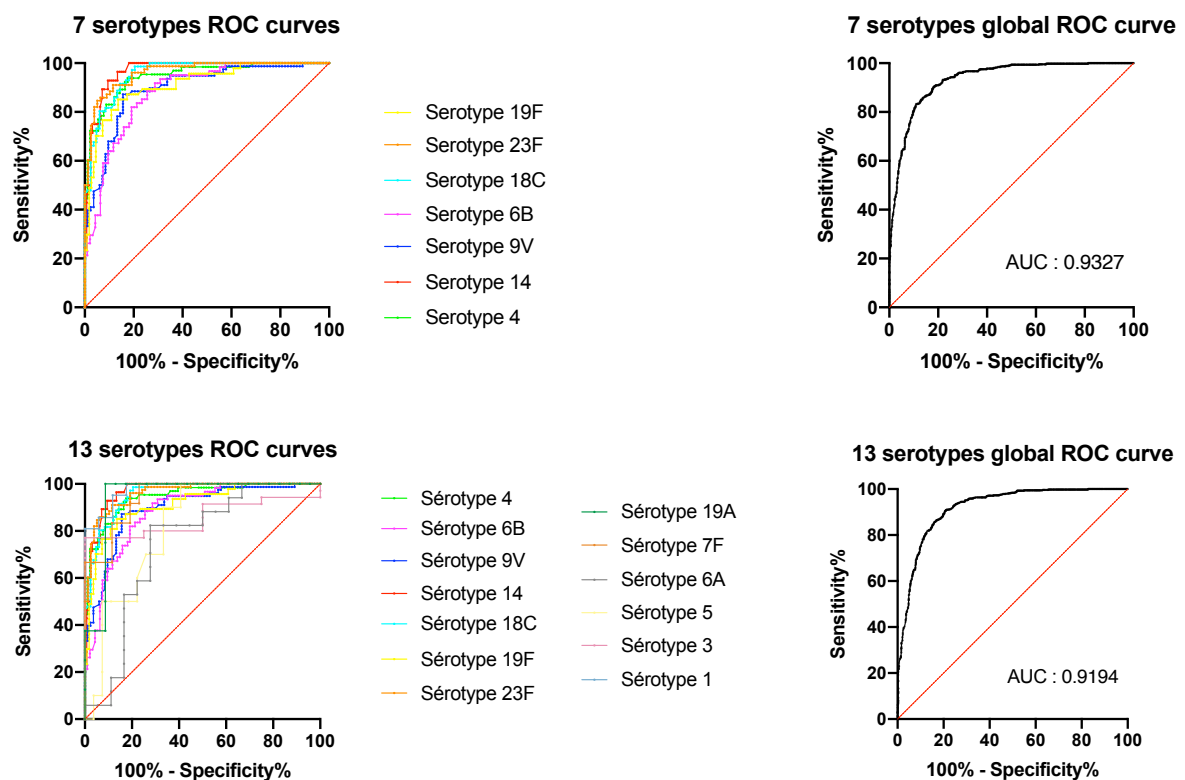


Figure 4: ROC curves for 1.3 $\mu\text{g/mL}$ threshold reliability of the ECL-assay for 7 serotypes (serotypes 4, 6B, 9V, 14, 18C, 19F and 23F) and 13 serotypes (serotypes 1, 3, 5, 6A, 7F and 19A).

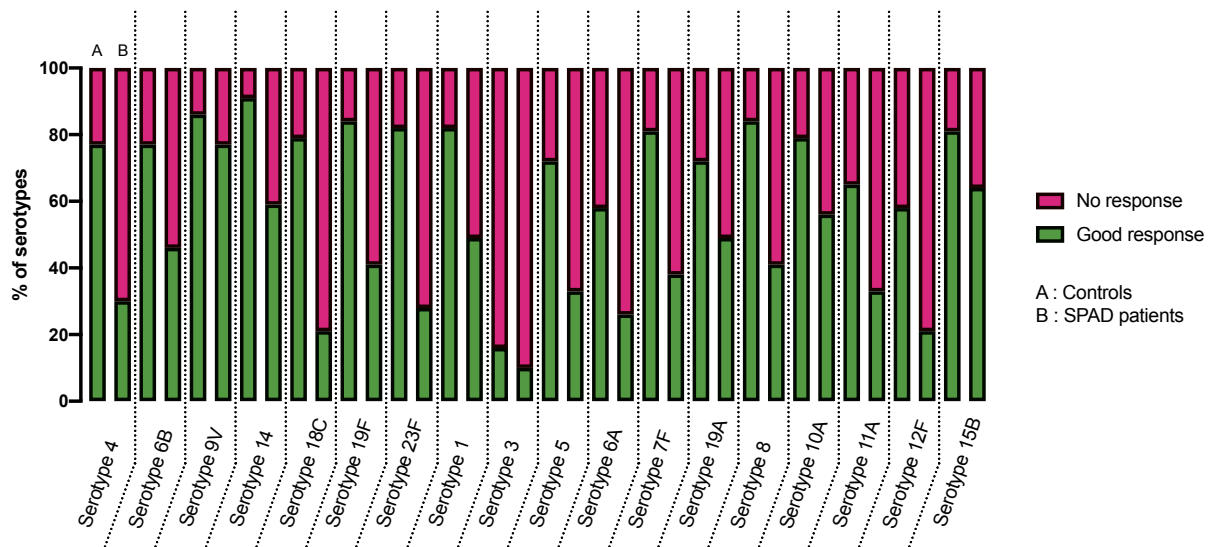


Figure 5: Assessment of specific responses to PnPS in the 18-plex ECLA assay in SPAD patients and controls. For each serotype, a good response was considered according to the AAAAI's composite criteria taking account of pre- and post-PPV23 antibody levels. A "good response" was defined if i) the post-immunization anti-body titer was upper 1.3 $\mu\text{g/mL}$ and ii) it achieved a 4-fold increase relative to the pre-immunization value or achieved a 2-fold increase if the pre- immunization value was already greater than 1.3 $\mu\text{g/mL}$. If one of the 2 criteria was absent, it was considered as a "poor response".

Tables

Table 1: Detection range for each 18 polysaccharides for 1:2000 dilution samples in IgG

IgG (1:2000 dilution)			IgG (1:2000 dilution)		
Serotype	LLOQ (µg/mL)	ULOQ (µg/mL)	Serotype	LLOQ (µg/mL)	ULOQ (µg/mL)
1	0,097	113,4	10A	0,1134	173
3	0,0208	19,34	11A	0,097	67,8
4	0,0574	44,4	12F	0,0344	29,4
5	0,0608	100,2	14	0,264	506
6A	0,1422	52,4	15B	0,1766	226
6B	0,1918	126,6	18C	0,0624	97,4
7F	0,0824	110,6	19A	0,1186	185
8	0,1448	189,8	19F	0,1234	194,8
9V	0,0716	85,8	23F	0,0772	79,4

LLOQ: Low Lower Limit of Quantification, ULOQ: Upper Limit of Quantification

Table 2: SPAD diagnostic performance of 18-plex ECL-assay compared with WHO-SSA: global performance

N = 82 (controls n = 43, patients with SPAD n = 39)

<i>18-plex ECL-assay</i> <i>N=82</i>	<i>WHO-SSA</i> <i>Global performance</i>	
	<i>SPAD +</i>	<i>SPAD -</i>
<i>Test +</i>	37	7
<i>Test -</i>	2	36
	<i>Se: 94.9%</i>	<i>Sp: 83.7%</i>
	<i>Cohen's κ-coefficient:</i>	
	0.781	
	% of agreement:	
	89.02%	
	<i>McNemar's exact p-value:</i>	
	0.1824	

N/PPV: Negative/Positive Predictive Value, Se: Sensibility, Sp: Specificity, SSA: Single Serotype Assay

Table 3: SPAD diagnostic performance of 18-plex ECL-assay compared with WHO-SSA : 7 and 13 serotype performance

<i>ECL-assay</i> <i>N=62</i>	<i>WHO-SSA</i> <i>7 serotype</i>		
	<i>SPAD +</i>	<i>SPAD -</i>	
<i>Test +</i>	24	6	<i>PPV: 80.0%</i>
<i>Test -</i>	2	30	<i>NPV: 93.8%</i>
	<i>Se: 92.3%</i>	<i>Sp: 83.3%</i>	
<i>ECL-assay</i> <i>N=20</i>	<i>WHO-SSA</i> <i>13 serotype</i>		
	<i>SPAD +</i>	<i>SPAD -</i>	
<i>Test +</i>	13	1	<i>PPV: 92.9%</i>
<i>Test -</i>	0	6	<i>NPV: 100%</i>
	<i>Se: 100%</i>	<i>Sp: 85.7%</i>	

N/PPV: Negative/Positive Predictive Value, Se: Sensibility, Sp: Specificity, SSA: Single Serotype Assay

Discussion

Ce travail de Thèse portant sur le diagnostic et la prise en charge des déficits sélectifs de réponses aux antigènes polysaccharidiques, ou SPAD, a été mené sur 2 volets : la description d'une cohorte de patients et le développement d'un test diagnostique alternatif à la méthode de référence ELISA, dont l'accès est limité.

Description de la cohorte de 55 patients porteurs d'un SPAD

La description de cette cohorte de 55 patients porteurs d'un SPAD apporte des éléments nouveaux sur cette maladie rare :

- Il existe une fréquence élevée de comorbidités inflammatoires et/ou auto-immunes associées compliquant la prise en charge globale des patients en cas de nécessité de traitement immunosuppresseur ;
- Nous n'avons pas constaté de récurrence d'infections chez les patients qui avaient été diagnostiqués après une infection grave unique. Même si on peut supposer le bénéfice des mesures préventives (vaccination, éducation), ceci pose la question du l'intérêt du diagnostic dès le premier épisode d'infection à bactérie encapsulée, bien que grave et inexpliquée ;
- En revanche, chez les patients présentant des infections répétées, nous observons un retard diagnostique plus important dans le sous-groupe de patients porteurs d'une dilatation des bronches, suggérant fortement l'importance du diagnostic précoce chez ces malades, à l'instar des autres déficits en anticorps ;
- Nous confirmons avec un suivi plus long, nos données préliminaires qui suggéraient une excellente efficacité de l'administration d'immunoglobulines polyvalentes dans la

réduction du nombre d'infections et de prises d'antibiotiques chez les patients sélectionnés sur une fréquence élevée des infections.

De manière intéressante, les caractéristiques de nos patients se superposent parfaitement à la première description de patient SPAD dans la littérature en 1987: des patients jeunes, souvent porteurs d'un asthme, présentant de multiples infections graves, et en errance diagnostique depuis de nombreuses années [25]. Cette errance diagnostique persiste encore 4 décennies après la description princeps de la maladie, probablement pour de multiples raisons : le caractère parfois « banal » des symptômes rapportés (rhino-sinusite chronique, bronchites), la méconnaissance de cette pathologie ou encore la difficulté d'accès et d'interprétation du test ELISA de référence [34].

Sur la base de ce constat, nous avons débuté en 2023 l'étude prospective nationale multicentrique DETECT (Detection of specific polysaccharide antibody deficiency in adult patients with unexplained recurrent severe bacterial infections) au sein des services hospitaliers membres du CEREDIH et du GREPI à l'échelle nationale (CPP n°2022-A00574-39) [35]. Cette étude bénéficie également du soutien de la filière des Maladies Rares Immuno-Hématologiques (MaRIH) *via* une bourse de financement de projet recherche.

Elle a pour objectif principal de déterminer la fréquence des SPAD chez les patients ayant des infections bactériennes respiratoires récurrentes inexplicables, c'est-à-dire sans cause locale et générale, et incluant un bilan immunologique par ailleurs normal. Les objectifs secondaires sont i) l'évaluation des paramètres non infectieux chez les patients diagnostiqués (i. e. manifestations allergiques/dysimmunes) et ii) l'évaluation de l'impact de la prise en charge 12 mois après le diagnostic sur la fréquence mensuelle des cures d'antibiotiques, sur l'absentéisme scolaire/retentissement professionnel et sur la qualité de vie (questionnaire SF-36).

Cette étude permettra de partager à l'échelle nationale l'expertise acquise au sein du CEREDIH-Lille dans le diagnostic et la prise en charge des SPAD, ainsi que d'approfondir les connaissances et la prise en charge de ce DIP sur un effectif plus important.

Mise au point de la méthode ECL 18-plex

La deuxième partie de ce travail de thèse visait à pallier la difficulté d'accès au test de référence par la mise au point à l'Institut d'Immunologie du CHU de Lille d'une méthode de détection des anticorps anti-polysaccharides pneumococciques par électrochimiluminescence. Notre travail montre que :

- La corrélation entre la méthode ECL et la méthode de référence par ELISA recommandée par l'OMS est excellente ;
- La performance diagnostique de ce nouveau test est similaire à la méthode de référence pour le diagnostic de SPAD, et ce d'autant plus que le diagnostic était porté sur l'analyse de 13 polysaccharides.

Les résultats sur la méthode ECL 18-plex en IgG sont concordants avec les précédentes mises au point de cette méthode ECL décrites dans la littérature [36–38]. Elle a pour avantage d'être plus simple, plus rapide et moins couteuse en sérum que la méthode de référence ELISA. Nous notons cependant une corrélation diminuée pour certains polysaccharides (sérotypes 5, 6A et 6B par exemple) avec parfois tendance à la sous-estimation du taux, pouvant être expliqué par certaines spécificités de la méthode ECL dans la lecture des plaques, ou par des réactions croisées entre les polysaccharides de mêmes sérogroupes au sein d'un même puit altérant la détermination en ECL (dans le cas des sérotypes 6A et 6B par exemple) [39]. De plus amples explorations sont donc nécessaires pour isoler ces sérotypes sur différentes plaques et améliorer la corrélation avec la méthode ELISA pour ces sérotypes en particulier. A terme, cette méthode

ECL pourrait supplanter la méthode ELISA dans l'exploration de l'immunité anti-pneumococcique et le diagnostic de SPAD.

Plusieurs études récentes n'ont pas réussi à démontrer de corrélation après administration d'un vaccin pneumococcique conjugué entre la détermination des IgG anti-pneumococciques par méthode ECL et le résultat des tests d'opsonophagocytose (OPA), méthode historique de référence d'évaluation de la réponse vaccinale anti-pneumococcique par vaccin conjugué [40,41]. Néanmoins, l'OPA explore les trois isotypes d'immunoglobulines anti-pneumococciques, et cette absence de corrélation pourrait s'expliquer par la détermination seule des IgG par la méthode ECL. Néanmoins, un des avantages de la méthode ECL est la possibilité de détermination des IgA et IgM anti-polysaccharides pneumococciques par simple changement d'anticorps secondaire de détection.

Données préliminaires : exploration de la réponse IgA anti-pneumococcique

Grâce à la méthode ECL, nous disposons également de données sur la réponse IgA anti-pneumocoque dans le SPAD (données non détaillées dans le manuscrit). Nous avons constaté que :

- Il existe une bonne corrélation entre les réponses IgG et IgA spécifiques ($r = 0.5722$ (95% (intervalle de confiance 95% : 0.5170 - 0.6227), $p < 0.001$, Figure 1 ci-dessous)
- La réponse globale tous sérotypes confondus en IgA est moins élevée chez les patients SPAD comme observé avec les IgG, à la fois en quantitatif (taux médian d'IgA post-vaccinal (écart interquartile) à 0.6590 $\mu\text{g/mL}$ (0.2640-1.377) vs. 0.8740 $\mu\text{g/mL}$ (0.3960-1.701) respectivement, $p = 0.0037$, Figure 2a) et en intensité de réponse (multiplication médiane des taux entre pré et post-vaccinal à 2.732 (1.581 - 7.471) vs. 6.628 (3.123 - 14.14) respectivement, $p < 0.0001$ Figure 2b).

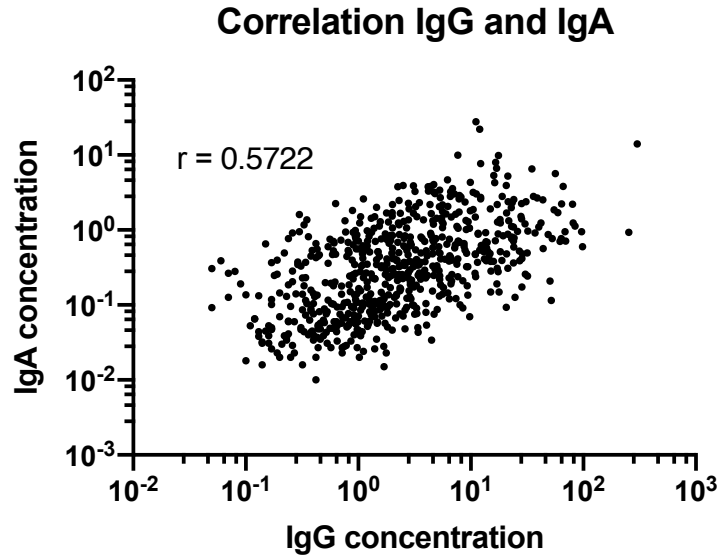


Figure 1 : Corrélation de Spearman entre les IgG et IgA du test ECL pour 12 sérotypes n=662.

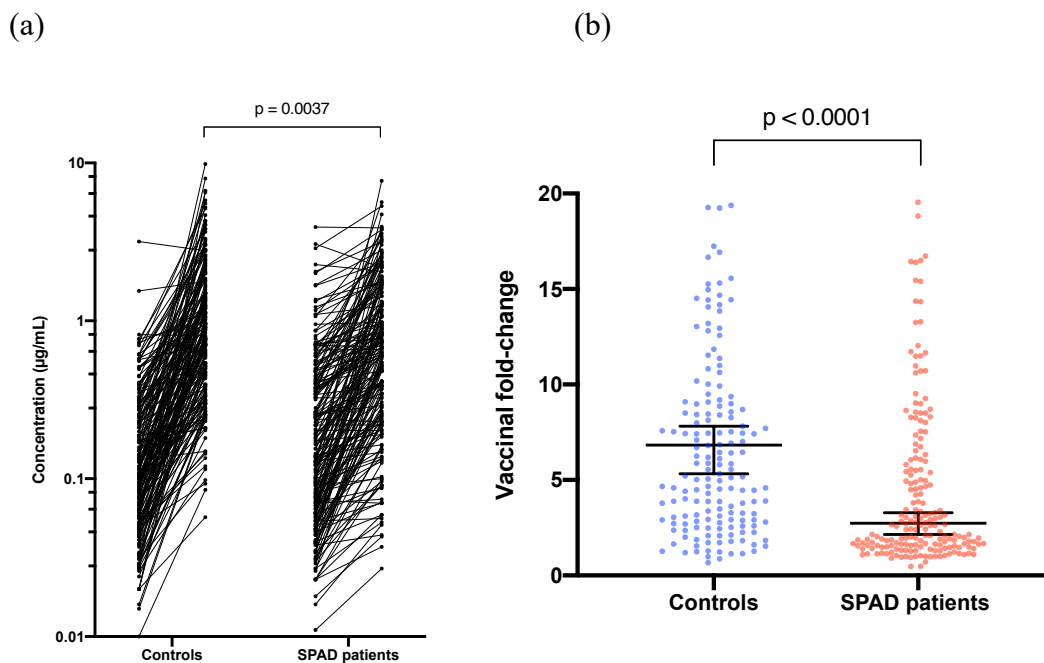


Figure 2 : Réponse vaccinale IgA anti-polysaccharides pour 12 sérotypes après vaccination chez 16 contrôles et 17 patients atteints de SPAD en concentration pré- et post-vaccinale (a) et multiplication du taux de base (b)

L'identification d'une réponse IgA spécifique anti-polysaccharides post-vaccinale apporte également de nouveaux éléments de compréhension de la physiopathologie du SPAD, et de plus amples explorations et analyses, en IgA mais également en IgM, permettront à terme de

préciser les mécanismes physiopathologiques du défaut de production des anticorps anti-polysaccharidiques et peut-être également de comprendre les phénotypes infectieux différents non expliqués par la réponse IgG seule, mais par exemple selon les réponses IgA et IgM.

Conclusion et perspectives

Les SPAD sont des maladies aux phénotypes hétérogènes, de diagnostic difficile, et dont la prise en charge reste peu codifiée. Néanmoins, le diagnostic est essentiel pour la prise en charge des patients atteints, avec des complications en cas de retard diagnostique, et des thérapeutiques drastiquement efficaces sur la prévention de la survenue d'infections.

De nombreuses avancées dans la compréhension, le diagnostic et la prise en charge de cette pathologie sont attendues à court et moyen terme avec le développement de nouvelles méthodes diagnostiques telle que la méthode ECL, ou encore grâce aux données à venir de l'étude nationale DETECT.

Références

- [1] Chaplin DD. Overview of the immune response. *J Allergy Clin Immunol* 2010;125:S3-23. <https://doi.org/10.1016/j.jaci.2009.12.980>.
- [2] Parkin J, Cohen B. An overview of the immune system. *Lancet Lond Engl* 2001;357:1777-89. [https://doi.org/10.1016/S0140-6736\(00\)04904-7](https://doi.org/10.1016/S0140-6736(00)04904-7).
- [3] Kaur BP, Secord E. Innate Immunity. *Immunol Allergy Clin North Am* 2021;41:535-41. <https://doi.org/10.1016/j.iac.2021.07.003>.
- [4] Bonilla FA, Oettgen HC. Adaptive immunity. *J Allergy Clin Immunol* 2010;125:S33-40. <https://doi.org/10.1016/j.jaci.2009.09.017>.
- [5] Abbott M, Ustoyev Y. Cancer and the Immune System: The History and Background of Immunotherapy. *Semin Oncol Nurs* 2019;35:150923. <https://doi.org/10.1016/j.soncn.2019.08.002>.
- [6] Kathleen E Sullivan, MD, PhD, Soma C Jyonouchi, MD. Inborn errors of immunity (primary immunodeficiencies): Classification 2024.
- [7] Collège des enseignants d'immunologie, editor. *Immunologie fondamentale et immunopathologie: enseignements thématique et intégré tissu lymphoïde et sanguin, immunopathologie et immuno-intervention*. 2e éd. Issy-les-Moulineaux: Elsevier Masson; 2018.
- [8] Turvey SE, Bonilla FA, Junker AK. Primary immunodeficiency diseases: a practical guide for clinicians. *Postgrad Med J* 2009;85:660-6. <https://doi.org/10.1136/pgmj.2009.080630>.
- [9] Amaya-Urbe L, Rojas M, Azizi G, Anaya J-M, Gershwin ME. Primary immunodeficiency and autoimmunity: A comprehensive review. *J Autoimmun* 2019;99:52-72. <https://doi.org/10.1016/j.jaut.2019.01.011>.
- [10] ESID Vision & Mission n.d.
- [11] CEREDIH - Le Centre de Référence Déficits Immunitaires Héritaires n.d.
- [12] Mahlaoui N. Le registre du CEREDIH 2024.
- [13] Bousfiha A, Moundir A, Tangye SG, Picard C, Jeddane L, Al-Herz W, et al. The 2022 Update of IUIS Phenotypical Classification for Human Inborn Errors of Immunity. *J Clin Immunol* 2022;42:1508-20. <https://doi.org/10.1007/s10875-022-01352-z>.
- [14] Diard M, Hardt W-D. Evolution of bacterial virulence. *FEMS Microbiol Rev* 2017;41:679-97. <https://doi.org/10.1093/femsre/fux023>.
- [15] Sadarangani M. Protection Against Invasive Infections in Children Caused by Encapsulated Bacteria. *Front Immunol* 2018;9:2674. <https://doi.org/10.3389/fimmu.2018.02674>.
- [16] McCusker C, Upton J, Warrington R. Primary immunodeficiency. *Allergy Asthma Clin Immunol Off J Can Soc Allergy Clin Immunol* 2018;14:61. <https://doi.org/10.1186/s13223-018-0290-5>.
- [17] Kohn LA, Kohn DB. Gene Therapies for Primary Immune Deficiencies. *Front Immunol* 2021;12:648951. <https://doi.org/10.3389/fimmu.2021.648951>.
- [18] Miloto C, Pulvirenti F, Cinetto F, Lougaris V, Soresina A, Pecoraro A, et al. Double-blind, placebo-controlled, randomized trial on low-dose azithromycin prophylaxis in patients with primary antibody deficiencies. *J Allergy Clin Immunol* 2019;144:584-593.e7. <https://doi.org/10.1016/j.jaci.2019.01.051>.
- [19] ANSM. Hiérarchisation des indications des immunoglobulines humaines polyvalentes n.d.
- [20] IRIS - Association de patients déficits immunitaires primitifs n.d.
- [21] LES DEFICITS IMMUNITAIRES HEREDITAIRES - PROTOCOLE NATIONAL DE DIAGNOSTIC ET DE SOINS n.d.
- [22] Yazdani R, Azizi G, Abolhassani H, Aghamohammadi A. Selective IgA Deficiency: Epidemiology, Pathogenesis, Clinical Phenotype, Diagnosis, Prognosis and Management. *Scand J Immunol* 2017;85:3-12. <https://doi.org/10.1111/sji.12499>.
- [23] Yazdani R, Habibi S, Sharifi L, Azizi G, Abolhassani H, Olbrich P, et al. Common Variable Immunodeficiency: Epidemiology, Pathogenesis, Clinical Manifestations, Diagnosis, Classification, and Management. *J Investig Allergol Clin Immunol* 2020;30:14-34. <https://doi.org/10.18176/jiaci.0388>.
- [24] Preud'homme JL, Hanson LA. IgG subclass deficiency. *Immunodef Rev* 1990;2:129-49.
- [25] Ambrosino DM, Siber GR, Chilmonczyk BA, Jernberg JB, Finberg RW. An Immunodeficiency Characterized by Impaired Antibody Responses to Polysaccharides. *N Engl J Med* 1987;316:790-3. <https://doi.org/10.1056/NEJM198703263161306>.
- [26] Sorensen RU, Edgar D. Specific Antibody Deficiencies in Clinical Practice. *J Allergy Clin Immunol Pract* 2019;7:801-8. <https://doi.org/10.1016/j.jaip.2019.01.024>.

- [27] Stabler S, Lamblin C, Gaillard S, Just N, Mihailescu M, Viget N, et al. High Frequency of Specific Polysaccharide Antibody Deficiency (SPAD) in Adults With Unexplained, Recurrent and/or Severe Infections With Encapsulated Bacteria. *Clin Infect Dis* 2022;ciac842. <https://doi.org/10.1093/cid/ciac842>.
- [28] Wernette CM, Frasci CE, Madore D, Carlone G, Goldblatt D, Plikaytis B, et al. Enzyme-Linked Immunosorbent Assay for Quantitation of Human Antibodies to Pneumococcal Polysaccharides. *Clin Vaccine Immunol* 2003;10:514–9. <https://doi.org/10.1128/CDLI.10.4.514-519.2003>.
- [29] Wall LA, Dimitriades VR, Sorensen RU. Specific Antibody Deficiencies. *Immunol Allergy Clin North Am* 2015;35:659–70. <https://doi.org/10.1016/j.iac.2015.07.003>.
- [30] Sorensen RU. A Critical View of Specific Antibody Deficiencies. *Front Immunol* 2019;10:986. <https://doi.org/10.3389/fimmu.2019.00986>.
- [31] Gilchrist JJ, Arnold DF, Pretorius PM, Milford CA, Misbah SA. Adult-Onset Recurrent Bacterial Meningitis: Immunological Red Herrings. *Clin Infect Dis* 2018;66:635–6. <https://doi.org/10.1093/cid/cix862>.
- [32] Sorensen RU, Leiva LE, Javier FC, Sacerdote DM, Bradford N, Butler B, et al. Influence of age on the response to Streptococcus pneumoniae vaccine in patients with recurrent infections and normal immunoglobulin concentrations. *J Allergy Clin Immunol* 1998;102:215–21. [https://doi.org/10.1016/S0091-6749\(98\)70089-2](https://doi.org/10.1016/S0091-6749(98)70089-2).
- [33] Cheminant M, Suarez F. [When should we think to primary immune deficiency in adult patients?]. *Rev Prat* 2019;69:659–65.
- [34] Lawrence MG, Borish L. Specific antibody deficiency: pearls and pitfalls for diagnosis. *Ann Allergy Asthma Immunol Off Publ Am Coll Allergy Asthma Immunol* 2022;129:572–8. <https://doi.org/10.1016/j.anai.2022.05.028>.
- [35] SPAD in Adult Patients (DETECT). University Hospital, Lille: n.d.
- [36] Goldblatt D, Ashton L, Zhang Y, Antonello J, Marchese RD. Comparison of a New Multiplex Binding Assay versus the Enzyme-Linked Immunosorbent Assay for Measurement of Serotype-Specific Pneumococcal Capsular Polysaccharide IgG. *Clin Vaccine Immunol* 2011;18:1744–51. <https://doi.org/10.1128/CDLI.05158-11>.
- [37] Feyssaguet M, Bellanger A, Nozay F, Friel D, Merck E, Verlant V, et al. Comparison between a new multiplex electrochemiluminescence assay and the WHO reference enzyme-linked immunosorbent assay to measure serum antibodies against pneumococcal serotype-specific polysaccharides. *Vaccine* 2019;37:2208–15. <https://doi.org/10.1016/j.vaccine.2019.03.011>.
- [38] Nolan KM, Zhang Y, Antonello JM, Howlett AH, Bonhomme CJ, Greway R, et al. Enhanced antipneumococcal antibody electrochemiluminescence assay: validation and bridging to the WHO reference ELISA. *Bioanalysis* 2020;12:1363–75. <https://doi.org/10.4155/bio-2020-0023>.
- [39] Geno KA, Gilbert GL, Song JY, Skovsted IC, Klugman KP, Jones C, et al. Pneumococcal Capsules and Their Types: Past, Present, and Future. *Clin Microbiol Rev* 2015;28:871–99. <https://doi.org/10.1128/CMR.00024-15>.
- [40] Platt HL, Cardona JF, Haranaka M, Schwartz HI, Narejos Perez S, Dowell A, et al. A phase 3 trial of safety, tolerability, and immunogenicity of V114, 15-valent pneumococcal conjugate vaccine, compared with 13-valent pneumococcal conjugate vaccine in adults 50 years of age and older (PNEU-AGE). *Vaccine* 2022;40:162–72. <https://doi.org/10.1016/j.vaccine.2021.08.049>.
- [41] Lupinacci R, Rupp R, Wittawatmongkol O, Jones J, Quinones J, Ulukol B, et al. A phase 3, multicenter, randomized, double-blind, active-comparator-controlled study to evaluate the safety, tolerability, and immunogenicity of a 4-dose regimen of V114, a 15-valent pneumococcal conjugate vaccine, in healthy infants (PNEU-PED). *Vaccine* 2023;41:1142–52. <https://doi.org/10.1016/j.vaccine.2022.12.054>.

Annexes

DÉFICITS IMMUNITAIRES PRIMITIFS – DIP



10 SIGNES CLINIQUES D'ALERTE D'UN DIP

CHEZ L'ADULTE POUR UN DIAGNOSTIC PRÉCOCE !

En France, plus de 6 000 personnes sont concernées.

En 2018, il existe près de 350 déficits immunitaires primitifs différents. Ce sont des maladies génétiques, aujourd'hui sous-diagnostiquées.

- Les plus sévères se manifestent dès la naissance, certaines mettent en jeu le pronostic vital. Les autres peuvent se déclarer plus tard, même à l'âge adulte.
- Un diagnostic précoce permet au patient de recevoir des traitements appropriés qui autorisent, dans de nombreux cas, un vie quasi normale. En revanche, un patient non soigné à un fort risque de présenter une dégradation de son état de santé et parfois de façon irréversible.

C'est pourquoi le **diagnostic précoce** de ces maladies encore trop méconnues est **si important !**



1

Infections bactériennes ORL répétées et/ou graves, inexpliquées (otites, sinusites aiguës, sinusite chronique, ...).



2

Infections bactériennes broncho-pulmonaires répétées et/ou graves, inexpliquées.



3

Dilatations des bronches sans cause reconnue.



4

Diarrhée chronique avec perte de poids.



5

Infections invasives à bactéries encapsulées (pneumocoque, méningocoque, ...).



6

Mycose cutané-muqueuse persistante.



7

Syndrôme lymphoprolifératif (adénopathie, splénomégalie, lymphome) et/ou granulomatose.



8

Cas d'immunodéficience connu dans la famille.



9

Cytopenie auto-immune (anémie, thrombopénie, neutropénie).



10

Infections virales répétées ou chroniques (herpès, zona, verrues, condylomes, infections génitales chez la femme).

Attention :
la présence de l'un ou l'autre de ces signes cliniques peut laisser penser à un déficit immunitaire primitif, sans toutefois qu'il en soit systématiquement ainsi.

Ce document a été conçu par l'association IRIS avec les conseils du centre de référence pour les déficits immunitaires héréditaires (CEREDIH), d'après un concept de la Jeffrey Modell Fondation et réalisé avec le soutien du laboratoire Shire.



Figure annexe 1 – 10 signes cliniques d'alerte d'un DIP (Association IRIS)

AUTEUR : Nom : PERRARD

Prénom : Nicolas

Date de Soutenance : 17/05/2024

Titre de la Thèse : Diagnostic, caractéristiques et devenir des déficits sélectifs en anticorps anti-polysaccharides : à propos d'une cohorte de 55 patients adultes.

Mise au point d'un test de détection des anticorps anti-polysaccharides par électrochimiluminescence.

Thèse - Médecine - Lille 2024

Cadre de classement : Médecine

DES + FST ou option : Médecine Interne et Immunologie Clinique

Mots-clés : Déficit immunitaire primitif, Déficit sélectif en anticorps anti-polysaccharides, Immunoglobulines polyvalentes, Electrochimiluminescence, Multiplex

Résumé :

Contexte : Le déficit sélectif en anticorps anti-polysaccharides (SPAD) est un déficit immunitaire rare prédisposant aux infections à bactéries encapsulées. Il s'agit d'un déficit immunitaire méconnu, peu décrit et dont le diagnostic est difficile car nécessitant une exploration fonctionnelle post-vaccinale du système immunitaire humoral par méthode ELISA.

Objectifs et méthode : Nous décrivons une cohorte de 55 patients porteurs d'un SPAD diagnostiqués et suivis dans les Hauts-de-France.

Nous détaillons également la mise au point d'un nouveau test diagnostique du SPAD reposant sur la détection d'anticorps anti-polysaccharides par électrochimiluminescence (ECL).

Résultats : La médiane d'âge était de 45 ans au diagnostic, 74,5 % étaient des femmes et 38,2 % présentaient des antécédents de maladies allergiques/inflammatoires. Le délai diagnostique était plus long chez les patients présentant des bronchiectasies. Parmi 22 patients supplémentés en immunoglobulines, le nombre moyen de cures antibiotiques passait de 7,9 à 0,7 cures par an. Nous avons observé d'excellentes corrélations entre les deux méthodes ECL et ELISA pour 7 sérotypes ($r = 0,88$) et 13 sérotypes ($r = 0,87$). Les performances du test multiplex par ECL pour le diagnostic du SPAD montrent une sensibilité de 95 % et une spécificité de 84 %, des valeurs prédictives positives et négatives de 84 % et 95 %, respectivement.

Conclusion : A travers cette thèse, nous confirmons les caractéristiques cliniques et l'excellente réponse au traitement par immunoglobulines substitutives des patients porteurs d'un SPAD.

Nous présentons également un nouveau test de détection des anticorps anti-polysaccharidiques par ECL prometteur dans le diagnostic de SPAD.

Composition du Jury :

Président : Monsieur le Professeur David LAUNAY

Assesseurs : Monsieur le Professeur Eric HACHULLA
Madame le Docteur Sarah STABLER

Monsieur le Docteur Sébastien HUMBERT
Directeur : Monsieur le Docteur Guillaume LEFEVRE