UNIVERSITE DE STRASBOURG

École doctorale des Sciences de la Vie et de la Santé

Habilitation à diriger des recherches

Présentée par COLLONGUES Nicolas

Spécialité NEUROSCIENCES

De l'évaluation clinique et thérapeutique de la neuromyélite optique à la recherche translationnelle en neuroprotection

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Soutenue le 17 Mai 2018 devant la commission d'examen

Professeur	**Jérôme DE SEZE**	**Directeur de recherche**
Professeur	**Maurice LAVILLE**	**Rapporteur externe**
Professeur	**Jean Christophe CORVOL**	**Rapporteur externe**
Professeur	**Jacques KOPFERSCHMITT**	**Rapporteur interne**
Professeur	**David LAPLAUD**	**Examinateur**
Professeur	**Laurent MONASSIER**	**Examinateur**

REMERCIEMENTS

Tout d'abord un grand Merci à Monsieur le Professeur **Jérôme de Seze**, médecin coordonnateur du CIC INSERM 1434 et PU-PH de Neurologie, sans qui je n'aurai jamais eu l'occasion de m'intéresser d'aussi près à la neuromyélite optique ni même à la recherche clinique. En plus d'être un mentor, tu as su diffuser en moi ta bienveillance, me donner le gout d'entreprendre, et me montrer un chemin professionnel que j'aime à arpenter chaque jour. Merci également de me démontrer depuis maintenant 12 ans que les patients sont notre raison d'exercer et font la richesse de notre métier.

Merci à Monsieur le Professeur **Guy Mensah-Nyagan**, directeur de l'unité INSERM 1119, dont j'ai pu apprécier l'exigence, la plasticité intellectuelle et la motivation communicative qui sont autant de qualités fondatrices pour tout chercheur avide de découverte. Ce que je ne savais pas c'est qu'elles le sont également pour la gestion d'une équipe et la recherche de financements. Merci pour ton enseignement Guy et nos nombreuses discussions à huit clos où tu m'as fait partager les enjeux et les difficultés liées à ta fonction.

Merci au **Service de Neurologie de Strasbourg**, à ses acteurs actuels, passés et futurs. Tous ces échanges, ces moments intenses auprès des malades, ces doutes et ces petites victoires sont gravés dans ma mémoire. Je pense tout particulièrement à **Francine, Caroline, Speciose, Lucretia** et à Madame le **Docteur Marie Fleury** qui sont là depuis le début et qui m'ont vu grandir. Merci aussi aux **Professeurs Christine Tranchant** et **Edouard Hirsch** qui, chacun à

leur manière, ont cru en moi à des moments cruciaux dans ma carrière de clinicien.

Merci à l'équipe du **CIC de Strasbourg**, pour le dévouement dont chacune et chacun fait preuve au quotidien auprès de nos patients. Nous avons débuté ensemble en 2013 ce projet de CIC sur le site de Hautepierre, rendu possible grâce à votre dynamisme et votre enthousiasme. Plus précisément, Merci à Madame le **Docteur Catherine Mutter**, PH et médecin délégué au CIC INSERM 1434, avec qui j'ai la chance de travailler. Le CIC sans toi Catherine, c'est un peu comme un moulin sans eau ou une voiture sans moteur. Tu me montres au quotidien ta mobilisation pour la recherche et ton goût pour un travail de qualité.

Merci à Messieurs les **Professeurs Laville, Corvol, Laplaud et Kopferschmitt**, qui m'avez fait l'honneur d'être membre de mon jury. Vous portez chacun une expertise et un projet médical qui sont autant d'exemples à suivre. L'exercice de la Thérapeutique et la gestion d'un CIC s'apprennent principalement sur le terrain et rendent votre expérience précieuse à mes yeux. Merci à Monsieur le **Professeur Laurent Monassier**, PU-PH de Pharmacologie et également membre de mon jury, d'avoir su m'intéresser aux mécanismes d'action des antihypertenseurs, anticoagulants et autres molécules, loin de celles utilisées dans ma spécialité d'origine. Je te suis reconnaissant de m'avoir permis de m'impliquer autant auprès des étudiants dans l'exercice de la Pharmacologie appliquée.

Merci à mes parents pour l'éducation qu'ils m'ont transmise, leur amour et leur soutien ainsi qu'à mes frères Vincent et Antoine. Je n'oublie pas mon grand père Robert Collongues, ancien directeur de l'Ecole Nationale Supérieure de Chimie de Paris, qui de là-haut me regarde sans doute avec curiosité et admiration.

Merci à mes amis, tous ceux et celles qui comptent encore et toujours : Pierre-Yves, Guillaume, Vincent, Julien, Pierre, Alexandre, Lionel, Gildas, Caroline, Kasia, et Fanny.

Enfin, merci à mon épouse Marie, qui a toujours su me rappeler à mes ambitions quand cela était nécessaire. Tu as toujours été là pour m'aider à m'accomplir. Que de moments heureux passés ensemble à regarder dans la même direction, regards auxquels s'ajoutent aujourd'hui ceux de nos petites merveilles Louis et Amélie. Je vous dédie naturellement cette étape importante dans une vie d'universitaire.

A Marie, Louis et Amélie

TABLE DES MATIERES

Liste des abréviations

AAN : American Academy of Neurology

ADC : coefficient apparent de diffusion

ALLO : allopregnanolone

APNET : Association Pédagogique Nationale pour l'Enseignement de la Thérapeutique

AQP4 : aquaporine-4

BN : Brown Norway

BOC : bandes oligoclonales

CIC : centre d'investigation clinique

CMT1A : Charcot-Marie-Tooth de type 1A

DFNIE : densité de fibres nerveuses intraépidermiques

DRCI : direction de la recherche Clinique

EAE : encéphalomyélite autoimmune expérimentale

ECTRIMS : European Committee for Treatment and Research in Multiple Sclerosis

EDEN : European Devic Neuromyelitis Optica project

EDSS : Expanded Disability Status Scale

ELISA : enzyme-linked immunosorbent assay

EMC : encyclopédie médico-chirurgicale

FIEC : Formation des Investigateurs aux Essais Cliniques des médicaments

GFAP : glial fibrillary acidic protein

IF : impact factor

Ig : immunoglobuline

JNLF : Journées de Neurologie en Langue Française

LB : lymphocyte B

LCR : liquide céphalo-rachidien

LFSEP : Ligue Française contre la Sclérose en Plaques

LT : lymphocyte T

MAI : maladie auto-immune

MATLE : myélite aigue transverse longitudinalement étendue

MBP : myelin basic protein

MMF : mycophenolate mofetil

MOG : myelin oligodendrocyte glycoprotein

NMO : neuromyélite optique

NMOSD : maladies du spectre de la neuromyélite optique

NPF : neuropathie des petites fibres

OFSEP : Observatoire Français de la Sclérose en Plaques

PHRC : Projet Hospitalier de Recherche Clinique

PMP 22 : peripheral-myelin-protein 22

RT-PCR : reverse transcription polymerase chain reaction

RTX : rituximab

SEP : sclérose en plaques

SFPT : Société Française de Pharmacologie et de Thérapeutique

SFSEP : Société Francophone de la Sclérose en Plaques

SIGAPS : Système d'Interrogation, de Gestion et d'Analyse des Publications Scientifiques

SNC : système nerveux central

TAP : taux annualisé de poussée

Th : T helper

I. Présentation du candidat : Titres et Travaux

I.1. IDENTITE

Nicolas COLLONGUES

Né le 31/07/1977 à Paris 20ème

Marié, 2 enfants

N°ordre : 67/9751

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I.2. INTRODUCTION PERSONNELLE

J'exerce actuellement en tant que Maitre de Conférence des Universités - Praticien Hospitalier en Neurologie et au **Centre d'Investigation Clinique** (CIC) à Strasbourg. Mes activités d'enseignement sont en rapport avec cette double affiliation et concernent les domaines de la **thérapeutique** (UE10 et filières paramédicales), de la **méthodologie** des essais cliniques (UE1, 10 et 12) et de la **neurologie**. Mon activité de recherche s'effectue depuis 2007 au sein du service de neurologie du Pr. de Seze, en collaboration avec un réseau national appelé NOMADMUS, travaillant sur les maladies du spectre de la neuromyélite optique (NMOSD) mais aussi, depuis 2013, au sein de l'unité INSERM 1119 dirigée par le Pr. Mensah visant à développer de nouvelles stratégies thérapeutiques dans le domaine de la neuroprotection ainsi qu'au CIC INSERM 1434 en tant que médecin délégué adjoint. J'ai ainsi principalement contribué à une meilleure compréhension du diagnostic, du pronostic et de la réponse thérapeutique dans

la NMOSD. Dans le domaine de la neuroprotection, plusieurs travaux en cours m'ont permis de développer de nouveaux biomarqueurs diagnostiques et de proposer de nouvelles approches thérapeutiques. Mes nouveaux projets s'inscrivent dans ces deux champs d'expertise et sont fortement ancrés dans le domaine de la thérapeutique. Ils concernent l'évaluation de la réponse thérapeutique dans la NMOSD, la myasthénie ou la neuropathie des petites fibres ainsi que la mise en place d'études translationnelles évaluant l'aspect neuroprotecteur des stéroïdes dans la neuropathie de Charcot-Marie-Tooth de type 1A (CMT1A), les neuropathies chimio-induites ou la sclérose en plaques (SEP).

I.3. PARCOURS PROFESSIONNEL

2013-aujourd'hui :

Maitre de Conférences des Universités-Praticien Hospitalier. Echelon 1ère classe depuis 2017. Neurologie/CIC.

Médecin délégué adjoint au CIC adulte et pédiatrique du CHU de Strasbourg.

Affiliations double UMR 1119 INSERM/UDS : Biopathologie de la Myéline, Neuroprotection et Stratégies Thérapeutiques [Pr. Mensah-Nyagan] et UMR 1434 INSERM/UDS : CIC de recherche clinique polythématique [Pr. de Seze].

2012-2013 : Praticien attaché au CIC.

2008-2012 :

Chef de Clinique des Universités-Assistant Hospitalier de neurologie à Strasbourg.

Examinateur certifié sur Neurostatus (niveau C) pour les études au sein du CIC.

Affiliation à 2 unités de recherche UMR 7237 CNRS/UDS : Biopathologie de la myéline [Dr. Ghandour] et UMR 1002 INSERM/UDS : CIC de recherche clinique polythématique [Pr. de Seze].

2008-2011 : **Thèse de Neurosciences** à l'Institut de Physique Biologique. UMR 7191 CNRS/ULP, Strasbourg, France.

2007 : **Master de Neurosciences** à l'Institut de Physique Biologique. UMR 7191 CNRS/ULP, Strasbourg, France.

2003-2008 : **Interne des Hôpitaux Universitaires de Strasbourg en Neurologie** :

1. Service de Neurologie A. Pathologies inflammatoires du SNC. Pr. Confavreux, Lyon.
2. Service de Neurologie. Pathologies inflammatoires du SNC. Pr. de Seze, Hôpital Civil, Strasbourg.
3. Service de Neurologie. Mouvements Anormaux. Pr. Tranchant, Hôpital Civil, Strasbourg.
4. Service de Neurologie. Epilepsies. Pr. Hirsch, Hôpital Civil, Strasbourg.
5. Service de Neurologie. Centre Hospitalier de Mulhouse, Mulhouse (Dr. Cohen).
6. Service de Rhumatologie. Hôpital de Hautepierre, Strasbourg (Pr. Kuntz).
7. Service de Médecine Interne. Hôpital de Hautepierre (Pr. Schlienger), Strasbourg.
8. Service de Pneumologie. Hôpital Civil (Pr. Pauli), Strasbourg.

I.4. DIPLOMES OBTENUS

2013 : Diplôme interuniversitaire de pédagogie médicale.

2011 : **Thèse de biologie, spécialité Neurosciences.**

2011 : Diplôme interuniversitaire de formation des investigateurs aux essais cliniques des médicaments (responsable national Pr. Bergmann).

2010 : Diplôme universitaire de neuro-ophtalmologie (faculté Pitié-Salpêtrière, Pr. le Hoang).

2008 : **Thèse de médecine, spécialité Neurologie.**

2007 : Master 2 de neurosciences cellulaires et intégrées. Mention bien.

2003 : Maitrise de sciences biologiques et médicales.

1999 : Certificats de biologie du développement (faculté Saint-Antoine, Pr. Roux). et de biochimie générale humaine (faculté Pitié-Salpêtrière, Pr. Raisonnier).

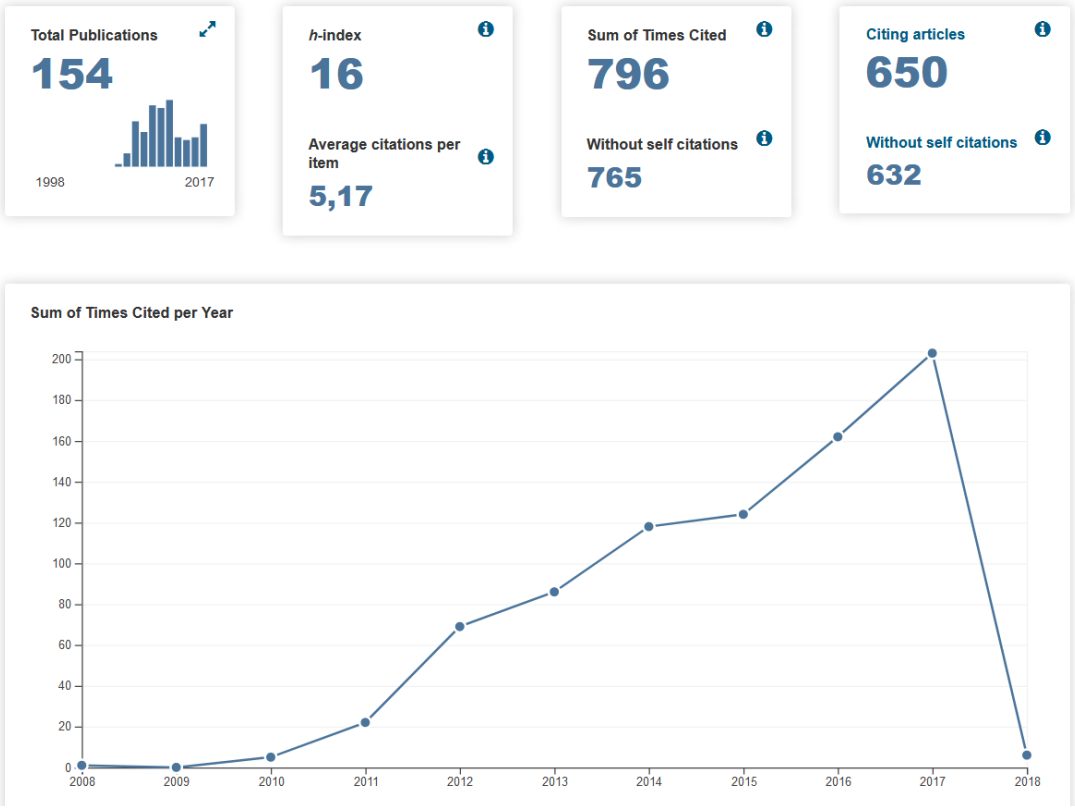
1995 : Faculté de médecine Saint-Antoine. Paris 6.

I.5. PUBLICATIONS

Au 1er février 2018 :

- 66 articles publiés dont 19 en premier auteur (dont 3 avec IF>5) et 5 en dernier auteur.
- Impact factor (IF) pondéré cumulé à 117.6 calculé selon les règles du CNU de Neurologie, soit :
    - Premier ou dernier auteur: IF x 1
    - 2ème auteur : IF x 0.8
    - 3ème auteur : IF x 0.5
    - >3ème auteur : IF x 0.2

- Score de publications sur le Système d'Interrogation, de Gestion et d'Analyse des Publications Scientifiques (SIGAPS) : 715
- H factor à 16 (web of knowledge)



I.6. PRIX ET RECOMPENSES

European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS) 2012 : 3ème prix du meilleur poster sur 1109 communications affichées.

ECTRIMS 2009 et 2010 : « travel grant » pour la qualité de la contribution scientifique au congrès.

American Academy of Neurology (AAN) 2008 : Poster sélectionné parmi les « highlight » : Devic's neuromyelitis optica MOG-induced in rat Brown Norway is associated with anti-AQP4 antibodies.

Journées Nationales des Internes de Neurologie 2008 : 2ème prix pour la présentation de l'étude de l'aquaporine-4 dans un modèle animal de neuromyélite optique.

I.7. FINANCEMENTS OBTENUS POUR LA RECHERCHE

2017 : PHRC Interrégional HUS N° (en attente). Budget : 400k€.

Rôle : conception de l'étude, écriture du projet et co-investigateur principal.

Titre : Traitement de la SEP récurrente-rémittente (SEP-RR) par la testostérone : effets potentiels sur la neuroprotection et la remyélinisation (TOTEM-RRMS).

2016 : PHRC Interrégional HUS N° 6638. Budget : 300k€.

Rôle : conception de l'étude et investigateur associé.

Titre : Etude de phase II, multicentrique, randomisée, en double aveugle, contrôlée versus placebo, pour évaluer l'efficacité et la sécurité de l'allopregnanolone dans la prévention des neuropathies périphériques induites par l'administration d'oxaliplatine chez des sujets atteints de cancer colorectal (ALLOXA).

2014 : PHRC Interrégional HUS N° 6100. Budget : 400k€.

Rôle : conception de l'étude, écriture du projet et co-investigateur principal.

Titre : Effet a long terme et tolérance de l'acétate d'ulipristal dans la maladie de Charcot-Marie-Tooth de type 1a (UPACOMT).

2011 : PHRC Interrégional HUS N° 4961. Budget : 300k€.

Rôle : conception de l'étude, écriture du projet et investigateur principal.

Titre : Etablissement d'une base normée de densité de fibres nerveuses à l'aide de la biopsie cutanée.

2010 : PHRC National. Budget : 460k€.

Rôle : Conception de l'étude, Investigateur associé et membre du conseil scientifique.

Titre : Création d'une cohorte française de Neuro-optico-myélite aiguë de Devic et des syndromes neurologiques apparentés (NOMADMUS).

I.8. TRAVAIL D'EXPERTISE SCIENTIFIQUE

1. Reviewer pour 28 journaux scientifiques nationaux et internationaux :
    - Annals of Neurology (IF : 9.89)
    - Autoimmunity (IF : 2.7)
    - BMJ Open (IF : 2.3)
    - Clinical Ophtalmology
    - CNS Neuroscience & Therapeutics (IF : 3.9)
    - European Journal of Neurology (IF : 4)
    - European Neurological Review (IF : 2.7)
    - European Neurology
    - Evidence based complementary and alternative medicine (IF : 1.9)
    - Expert Opinion On Therapeutic Patent (IF : 4.3)
    - Expert Review of Neurotherapeutics (IF : 2.8)
    - Expert Review of Ophtalmology
    - Eye and Brain
    - International Journal of Molecular Sciences (IF : 3)
    - Journal of Neurology (IF : 3.3)
    - International Journal of Neurosciences (IF : 1.5)

- Journal of Neuroinflammation (IF : 4.6)
- Journal of Neurology Neurosurgery and Psychiatry (IF : 6.8)
- Journal of the Neurological Sciences (IF : 2.5)
- Journal of Paediatric Neurology
- La Revue de Médecine Interne (IF : 1)
- Multiple Sclerosis International
- Multiple Sclerosis Journal (IF : 4.8)
- Muscle Nerve (IF : 2.3)
- Neurology (IF : 8.3)
- PLOsOne (IF : 3.2)
- Reproductive System and Sexual Disorders (IF : 1.1)
- Revue Neurologique (IF : 0.6)

2. Expertise pour les projets de recherche soumis à la direction de la recherche Clinique (DRCI) :
    a. des Hôpitaux Universitaires de Strasbourg : 2 projets en 2014 et 1 en 2015.
    b. des Hôpitaux Universitaires de Nîmes/Montpellier : 1 projet en 2017.
3. Expertise pour les projets de recherche soumis à l'Observatoire Français de la Sclérose en Plaques (OFSEP) et la Ligue Française contre la Sclérose en Plaques (LFSEP).
4. Membre du jury du master 2 de neurosciences de Strasbourg en 2015 et 2016.

I.9. COMMUNICATIONS EN CONGRES

1. Communications affichées en premier auteur :
    - AAN : 2008 : 1 ; 2009 : 2 ; 2010 : 2 ; 2011 : 2 ; 2012 : 1 ; 2013 : 1 ; 2014 : 1 ; 2015 : 1.
    - ECTRIMS : 2009 : 3 ; 2010 : 3 ; 2011 : 2 ; 2012 : 1 ; 2015 : 1 ; 2017: 1.
2. Communications affichées en dernier auteur :
    - AAN : 2016 : 1 ; 2017 : 2.
    - ECTRIMS : 2017 : 2.
3. Communications orales :

2018 : Journée nationale des internes de Neurologie : Controverse sur le traitements des formes progressives de sclérose en plaques.

2017 : Société Francophone de la Sclérose en Plaques (SFSEP) : Diagnostic et pronostic des maladies du spectre NMO.

2016 : 1ères Journées de Neurophysiologie Clinique : La biopsie cutanée dans la neuropathie des petites fibres. Modérateur de la session sur la neuropathie des petites fibres.

2016 : VIIIe Journée annuelle du réseau national des CIC : Effet a long terme et tolérance de l'acétate d'ulipristal dans la maladie de charcot-marie-tooth de type 1a (UPACOMT).

2015 : European Congress of Neurology : Rituximab in refractory neuromyelitis optica.

2015 : Rencontres de Neurologie : Les biothérapies en neurologie.

2012 : Journées de Neurologie en Langue Française (JNLF) : Comment traiter la forme oculaire de myasthénie auto-immune?

2011 : JNLF : Actualité épidémiologique et clinique dans la neuromyélite optique.

2008 : Journée annuelle extraordinaire de la Société Française de Neurologie : Étude de l'quaporine-4 dans un modèle animal de neuromyélite optique.

I.10. ENSEIGNEMENT

79h/an dont :

1. **43h de Thérapeutique/Pharmacologie et Méthodologie des essais cliniques sur les thématiques suivantes :**
   - Les biothérapies,
   - Facteurs de variabilité de la réponse aux médicaments et suivi thérapeutique,
   - Médicaments en neurologie,
   - Anti-inflammatoires stéroïdiens et non-stéroïdiens, interactions médicamenteuses,
   - « Evidence Based Medicine »,
   - Concept de qualité de vie et les différents outils de mesure,
   - Les essais de non infériorité,
   - Initiation à la lecture critique d'articles,
   - Les métiers de la recherche clinique.

Thérapeutique UE10 DFGSM3 et DFASM2 : 18h/an.

Lecture critique d'articles UE1, 10, 12 : 8h/an.

Master 2 de Neuro-immunologie : 3h/an.

Master 2 de Neurosciences : 8h/an.

DIU de Formation des Investigateurs aux Essais Cliniques des médicaments (FIEC) : 2h/an.

Infirmières anesthésistes : 2h/an.

Internes de Neurologie : 2h/an.

2. 36h de Neurologie sur les thématiques suivantes :

- Sclérose en plaques,
- Pathologies inflammatoires de la substance blanche,
- Myélopathies,
- Myasthénie,
- Voies visuelles et oculomotricité,
- Syndromes parkinsoniens.

TD DFASM1 et 2 : 8h/an.

Orthophonie : 8h/an.

Internes de Neurologie : 6h/an.

ECN DFASM1 : 4h/an.

Master 2 de Neurosciences : 4h/an.

DIU de Neuroradiologie : 2h/an.

Manipulateurs de radiologie : 2h/an.

Kinésithérapeutes : 2h/an.

I.11. PEDAGOGIE

Certifié sur le Système Informatique Distribué d'Evaluation en Santé (SIDES) niveau 2, membre de la commission d'évaluation des dossiers SIDES depuis 2015.

Membre fondateur et membre du comité scientifique de la réunion pédagogique BISEPs (Brainstorming Interactif autour de la Sclérose En Plaques) affiliée à la

SFSEP, dédiée au perfectionnement des jeunes praticiens (CCU-AH, PH) dans le domaine de la SEP depuis 2016.

Publications à visée didactique et pédagogique dans plusieurs journaux français de formation continue : Neurologies, Neurologie Pratique, La Lettre du Neurologue et Médecine Thérapeutique.

Livres :

2017 :

- Ecriture du chapitre « thérapies de 2ème ligne dans la SEP » dans la mini-collection thématique « La Sclérose en Plaques ». Elsevier Masson SAS.

2016 :

- Ecriture du chapitre « Les neuromyélites optiques » dans la mini-collection thématique « La Sclérose en Plaques ». Elsevier Masson SAS.
- Ecriture du chapitre « La neuromyélite optique » dans le précis de Neuro-ophtalmologie, 2e édition. Collection Atlas en ophtalmologie. Elsevier Masson SAS.
- Ecriture du chapitre de l'encyclopédie médico-chirurgicale (EMC) sur les "myélopathies aigues".

I.12. ENCADREMENT

Directeur de Thèse de médecine de Neurologie du Dr. Alves Do Rego Cécilia en juillet 2017.

Membre du jury de thèse de médecine en Neurologie des Dr. Benoilid en 2011 et Dr. Kremer en 2014.

Encadrement de Master 2 de Neurosciences des Dr. Benoilid en 2009, Dr. Bourre en 2010 et Dr. Chiara en 2012.

Encadrement des mémoires de stage des infirmières anesthésistes, des étudiants FIEC et de 2 internes en santé publique depuis 2013 au sein du CIC.

Responsable de l'UE de formation à l'investigation clinique spécialisée dans le cadre du Master 2 de physiopathologie : de la molécule à l'homme. Rôle de tuteur encadrant 4 étudiants/an dans cette UE depuis 2015.

Encadrement des stages intégrés des étudiants en médecine DFGSM1 et DFGSM2 de 2008 à 2012.

I.13. ACTIVITE DE SOIN

Visites de service et consultations dans le domaine des pathologies inflammatoires du système nerveux (service du Pr. de Seze).

Participation à une consultation mensuelle de neuro-ophtalmologie (Pr. Speeg).

Participation à une réunion mensuelle de neuro-médecine interne (Pr. Martin).

Participation à une réunion bi-mensuelle de neuro-rhumatologie (Pr. Gottenberg).

Médecin délégué adjoint du CIC de Hautepierre :

- Organisation du bon déroulement des protocoles de recherche,
- Aide à la conception des projets de recherche,
- Mise en relation du CIC avec les autres acteurs de la recherche,
- Développement de la communication autour de la recherche clinique.

I.14. SOCIETES SAVANTES ET GROUPES DE TRAVAIL

Membre associé de l'Association Pédagogique Nationale pour l'Enseignement de la Thérapeutique (APNET).

Membre de la Société Française de Pharmacologie et de Thérapeutique (SFPT).

Membre du comité éditorial du journal de la LFSEP.

Membre de la commission de biothérapie au CHU de Strasbourg depuis 2014.

Membre du réseau Alsacep de lutte contre la sclérose en plaques.

Membre du comité scientifique de l'OFSEP, parties « thérapeutique », "neuromyélite optique (NOMADMUS)" et "formes progressives".

Membre du comité scientifique de la SFSEP.

Membre du comité scientifique de la "Strasbourg Pain Initiative" regroupant 11 UMR.

I.15. COLLABORATIONS

Locales :

UMR 1119 INSERM/UDS : Biopathologie de la Myéline, Neuroprotection et Stratégies Thérapeutiques, équipe du Pr. Mensah-Nyagan.

UMR 1434 INSERM/UDS : Centre d'Investigation Clinique polythématique, équipe du Pr. de Seze.

Institut d'Histologie UDS : équipe du Pr. Boehm.

UMS 3489 CNRS/UDS : Centre d'Investigations Neurocognitives & Neurophysiologiques, équipe du Pr. Dufour.

UMR 7357 CNRS/UDS : Plateforme IMAGINES - ICube, équipe du Dr. Armspach.

Nationales :

UMR 1028 INSERM/UL : Centre de Recherche en Neurosciences de Lyon (CRNL), responsable Olivier Bertrand.

Observatoire Français de la Sclérose en Plaques (OFSEP).

Internationales :

Department of Biomedicine, University Hospital Basel : équipe des Pr. Derfuss et Pr. Kappos.

II. Introduction générale

La neuromyélite optique (NMO) est une pathologie rare appartenant au champ des maladies inflammatoires du système nerveux central (SNC). Cette pathologie touche avec prédilection les nerfs optiques et la moelle épinière mais aussi de nombreuses régions de l'encéphale et du tronc cérébral dont la découverte plus récente a nécessité de modifier les critères diagnostiques en 2015.[1] Par ailleurs, la NMO est la première pathologie inflammatoire du SNC pour laquelle un biomarqueur sérique a été découvert en 2004. Il s'agit de l'anticorps anti-aquaporine-4 (AQP4), qui cible les astrocytes dans le SNC.[2, 3]

Si les premiers cas ont été décrits au 19ème siècle, il s'agit donc d'une maladie redécouverte au 21ème siècle et qui est un sujet de préoccupation majeur pour les neurologues notamment du fait de la gravité des poussées susceptibles de confiner les patients à la cécité et la paraplégie.

Mes travaux dans ce domaine reposent sur l'expertise clinique locale du Pr. de Seze et sur une collaboration nationale appelée NOMADMUS, tissée suite à l'obtention en 2010 d'un PHRC national (Création d'une cohorte française de Neuro-optico-myélite aiguë de Devic et des syndromes neurologiques apparentés) par le Dr. Marignier neurologue à Lyon.

Cet axe de recherche sur la NMO sera traité en 2 parties :

1 : Critériologie et épidémiologie de la NMO

2 : Traitement de la NMO et de la myasthénie

III. Critériologie et épidémiologie de la neuromyélite optique

III.1. INTRODUCTION

La NMO est une maladie autoimmune qui touche préférentiellement les nerfs optiques et la moelle épinière. Elle a longtemps été confondue avec la SEP et il faudra attendre la découverte en 2004 d'un biomarqueur diagnostic qu'est l'anticorps anti-AQP4 pour identifier clairement ces deux pathologies ayant des mécanismes physiopathologiques différents.[2, 3]

Depuis les premières descriptions de cette pathologie au 19ème siècle caractérisées par des formes sévères et monophasiques, la critériologie n'a pas cessé d'évoluer proposant différents sets de critères depuis 1999 (tableau 1).[1, 4, 5] Ceux-ci intègrent la présence de l'anticorps anti-AQP4 en 2006.[5] Par la suite, la spécificité de cet anticorps étant importante,[6, 7] et sa cible à l'origine de la pathogénie étant l'astrocyte,[8, 9] le spectre des ces "aquaporinopathies" s'est étendu permettant la description de formes impliquant l'encéphale, le tronc cérébral, l'area postrema, ou le diencéphale et conduisant à un nouveau set de critères diagnostiques en 2015.[1] La sensibilité et la spécificité de ces critères ont été évalués pour ceux de 1999 et 2006. En revanche, il n'existe pas d'étude évaluant ces deux éléments pour les critères de 2015. Ce point est par ailleurs crucial car à la différence des critères de 1999 et 2006 qui ont étudié une cohorte de malades puis modélisé la performance de leurs critères diagnostiques, les critères de 2015 reposent sur une revue de la littérature et l'avis d'un groupe d'experts international.

En 2013, la découverte d'une sous-population de patients séronégatifs pour les anti-AQP4 mais positive pour les anti-myelin oligodendrocyte glycoprotein (MOG), une protéine mineure de la myéline, est retrouvée chez 20-40% des patients NMO séronégatifs pour les anti-AQP4. Actuellement, sur la base de ces biomarqueurs, il est défini trois sous-populations de malades suspectées d'appartenir à un même spectre : les aquaporinopathies AQP4+, les patients MOG+ et les patients double séronégatifs.

La plupart des études épidémiologiques ont été faites à partir des critères de 2006. La cohorte française que nous avons publiée en 2010 utilise donc ces critères.[10] Elle a ensuite servi à plusieurs études ancillaires qui ont permis de mieux caractériser cette population de malades.[11, 12] L'ensemble de nos travaux a contribué fortement à l'obtention d'un PHRC (NOMADMUS) destiné à développer une base de données nationale de patients atteints de NMO et validée par un groupe d'experts dans les pathologies inflammatoires du SNC. Plusieurs collaborations ont suivi et permis l'étude des formes bénignes (comparaison entre la cohorte française métropolitaine et la cohorte antillaise en 2013)[13] et des formes à début tardif (regroupant les universités d'Allemagne, de Turquie, du Royaume Uni, et de France, participants à un projet européen appelé European Devic Neuromyelitis Optica project [EDEN]).[14]

Enfin, nous avons publié avec le groupe d'experts "NOMADMUS" des recommandations pour le bilan diagnostique et étiologique des myélites aigues transverses longitudinalement étendues, qui sont une caractéristique des myélites retrouvées dans les maladies du spectre de la NMO.[15] Ces recommandations ont visé la communauté des neurologues français afin d'homogénéiser la prise en charge de ces patients et de clarifier les diagnostics

des patients susceptibles d'être intégrés dans la base "NOMADMUS". Nous avons également mené un travail plus large qui a conduit à l'écriture d'un chapitre de l'EMC portant sur les myélites aigues.

L'aspect plus fondamental de notre recherche s'est porté sur les NMO séronégatives pour les anticorps anti-AQP4. Le but de cette recherche a été de mieux comprendre les mécanismes physiopathologiques sous-tendus par la découverte des anticorps anti-AQP4 ou anti-MOG, Nous avons ainsi généré au laboratoire (UMR 7237 CNRS) un modèle animal induit par la MOG, et correspondant ainsi à un sous-groupe de patients atteints de NMO avec anticorps anti-MOG. Si ce modèle animal était bien à l'origine d'une inflammation et d'une démyélinisation optico-médullaire comme dans la NMO, cependant les lésions générées ne ressemblaient pas à celles créées dans les autres modèles d'immunisation avec l'anticorps anti-AQP4, suggérant un mécanisme physiopathologique distinct.

III.2. ARTICLES CLINIQUES ET FONDAMENTAUX

1. **Collongues N**, Kremer S, de Sèze J. Myélopathies aiguës. EMC - Neurologie 2016;0(0):1-14 [Article 17-071-A-10].

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3. **Collongues N**, Marignier R, Jacob A, Leite M, Siva A, et al. Characterization of neuromyelitis optica and neuromyelitis optica spectrum disorder patients with a late onset. Mult Scler 2014;20:1086-1094.

4. **Collongues N**, Chanson JB, Blanc F, Steibel J, Lam CD, et al. The Brown Norway opticospinal model of demyelination: does it mimic multiple sclerosis or neuromyelitis optica? Int J Dev Neurosci. 2012 Oct;30(6):487-97.

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7. **Collongues N**, Marignier R, Zéphir H, Papeix C, Fontaine B, et al. Long-term follow-up of neuromyelitis optica with a pediatric onset. Neurology. 2010 Sep 21;75(12):1084-8.

8. **Collongues N**, Marignier R, Zéphir H, Papeix C, Blanc F, et al. Neuromyelitis optica in France: a multicenter study of 125 patients. Neurology. 2010 Mar 2;74(9):736-42.

Myélopathies aiguës

N. Collongues, S. Kremer, J. de Sèze

Les myélopathies aiguës sont caractérisées par une atteinte médullaire dont le nadir est compris entre quatre heures et trois semaines après le début des symptômes. Partant de cette définition, les causes retrouvées sont infectieuses, auto-immunes, paranéoplasiques, toxiques ou idiopathiques. L'interrogatoire associé à un examen physique complet permet d'orienter rapidement l'enquête étiologique. L'imagerie par résonance magnétique (IRM) médullaire est l'examen clé permettant de confirmer le diagnostic. La localisation de l'atteinte, son étendue dans le plan axial et sagittal, la coexistence d'une atteinte méningée ou radiculaire, sont autant d'éléments qui, associés aux résultats de la ponction lombaire, permettent souvent de retrouver la cause. Le traitement des myélopathies aiguës est celui de la cause et nécessite dans tous les cas une prise en charge urgente pour éviter les séquelles.

Mots-clés : Myélopathie aiguë ; Myélite ; Myélite partielle ; Myélite transverse ; Myélite infectieuse ; Sclérose en plaques ; Neuromyélite optique

Plan

Introduction

Le terme « myélopathie » définit l'ensemble des atteintes de la moelle épinière, quelle que soit leur origine. La sémiologie d'une myélopathie se définit par un syndrome sous-lésionnel correspondant à une somatotopie médullaire, souvent caractérisée par une distribution bilatérale des signes pyramidaux ou sensitifs, un niveau sensitif franc, une atteinte sphinctérienne ou thermoalgique isolée, ou la coexistence de signes centraux et périphériques sur un même membre. Un syndrome lésionnel signe l'atteinte radiculaire. Celui-ci est principalement observé dans les causes compressives ou tumorales, et souvent absent dans les autres cas. Enfin, un syndrome rachidien caractérisé par une douleur à la mobilisation des épineuses et/ou une contraction des muscles paravertébraux est observé essentiellement dans les processus tumoraux. La myélopathie peut être visualisée en imagerie par résonance magnétique (IRM). Cet examen oriente le clinicien vers un mécanisme physiopathologique.

Le qualificatif « aigu » est ajouté lorsque le mode d'installation s'effectue sur quelques heures. La durée précise de l'installation des symptômes varie en fonction des auteurs : installation en plus de 48 heures mais moins de trois semaines [1], nadir entre quatre heures et trois semaines [2], ou aggravation maximale en 24 heures [3]. Quelle que soit la définition utilisée, ces intervalles de temps permettent en théorie de s'affranchir des myélopathies vasculaires et traumatiques d'apparition soudaine, et des myélopathies métaboliques, dégénératives ou postradiques d'apparition plus progressive et chronique. Par conséquent, ces aspects des affections médullaires ne seront pas détaillés ici mais feront l'objet de chapitres spécifiques de ce traité. Il faut noter que, néanmoins, un certain degré de chevauchement entre ces étiologies et leur mode d'installation peut exister en pratique, mettant ainsi en avant l'importance du contexte de survenue.

Ce chapitre a pour objectif de traiter les différents aspects et les étiologies des myélopathies aiguës, comprenant les affections infectieuses ou para-infectieuses, auto-immunes, toxiques, paranéoplasiques et idiopathiques.

Définitions de la myélopathie partielle, transverse et longitudinalement étendue

Définitions cliniques

Plusieurs définitions cliniques ont émergé ces dernières années, destinées à préciser l'étendue de l'atteinte médullaire.

En pratique courante, l'étendue limitée de la myélopathie est désignée sous le terme de « myélite partielle », également rapportée sous l'appellation « myélite aiguë partielle transverse », ajoutant encore plus à la confusion qui existe autour de ces différents termes. La myélopathie partielle peut être définie par un déficit unilatéral ou incomplet, moteur ou sensitif, ou sphinctérien. Cette définition est très proche de celle mentionnée par Scott de la myélite transverse partielle [4]. La confusion entre

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Tableau 1.
Définitions sémiologiques de la myélite transverse dans la littérature.

Myélite transverse idiopathique Consortium 2002	Myélite transverse complète Scott 2007	Myélite transverse partielle Scott 2007
Atteinte sensitive, motrice ou dysautonomique d'origine médullaire	Atteinte motrice modérée ou sévère symétrique et dysautonomie d'origine médullaire	Atteinte légère sensitive et/ou motrice, bilatérale ou unilatérale d'origine médullaire. Quand un déficit sévère est présent, une asymétrie marquée est observée
Symptômes bilatéraux (non nécessairement symétriques) définissant un niveau sensitif	Présence d'un niveau sensitif symétrique	Symptômes définissant un niveau sensitif (bilatéral ou unilatéral) ou présence d'une IRM typique de myélite
Nécessite l'exclusion d'autres pathologies démyélinisantes (sclérose en plaques, pathologie infectieuse, auto-immunes systémiques...)	Idem	Idem
Inflammation de la moelle épinière démontrée par une pléiocytose du LCR ou un index d'IgG élevé ou une prise de gadolinium en IRM	L'inflammation du LCR ou à l'IRM peut ne pas être présente	L'inflammation du LCR ou à l'IRM peut ne pas être présente
Nadir entre 4 heures et 21 jours	Idem	Idem

IRM : imagerie par résonance magnétique ; LCR : liquide céphalorachidien ; IgG : immunoglobuline G.

myélite partielle et transverse en termes de diagnostic et de pronostic a placé l'IRM médullaire en première ligne des examens complémentaires. Cet examen est ainsi devenu un élément incontournable de la réflexion stratégique clinique et thérapeutique et doit désormais être réalisé rapidement en cas de suspicion d'atteinte médullaire.

À cette forme particulièrement limitée de myélopathie aiguë s'oppose le cadre nosologique des myélopathies étendues dans le plan axial ou longitudinal. Ainsi, le terme « transverse » est habituellement employé pour désigner une certaine sévérité des manifestations cliniques. Dans la littérature, ce terme est utilisé de façon équivoque : « myélite transverse », « myélite transverse partielle », « myélite transverse complète », « myélite transverse extensive » ou « myélite longitudinale transverse extensive ». La multiplicité des termes employés a conduit en 2002 à la publication d'un article de revue dans lequel les auteurs ont défini le caractère transverse par la présence d'un niveau sensitif à l'examen [2]. Une définition complémentaire rapportant les spécificités cliniques de ce type de myélopathie en fonction de leur caractère complet ou partiel a été publiée en 2007 [4] et est rappelée dans le Tableau 1. Dans cette définition, la myélite transverse peut être plus ou moins complète, correspondant alors à un déficit sensitivo-moteur bilatéral (plus ou moins symétrique) et des troubles sphinctériens [2, 4].

On note que la myélopathie transverse a alors une définition clinique, aucune définition radiologique de ces termes ou même aucune corrélation clinicoradiologique n'ayant été rapportée à cette époque.

Depuis 2004, l'individualisation de la neuromyélite optique (NMO), après la découverte des anticorps antiaquaporine-4 (anti-AQP4), a révélé la valeur prédictive diagnostique et pronostique péjorative de l'extension longitudinale d'une myélite. Celle-ci est définie en IRM par un hypersignal T2 étendu sur au moins trois métamères dans le plan longitudinal [5]. Cette donnée, intégrée dans les critères diagnostiques de la NMO depuis 1999, prend plus d'importance encore en 2006 puis en 2008 [5–7]. Les termes anglo-saxons utilisés pour décrire la myélite de la NMO sont alors « myélite extensive », « myélite transverse extensive » ou encore « myélite longitudinale transverse extensive ». L'atteinte longitudinale ne peut pas être suspectée cliniquement de façon isolée et ne peut être précisée que par l'IRM. Elle est presque systématiquement associée à une atteinte étendue dans le plan transversal, caractérisée par un déficit bilatéral sévère et à tous les modes. Il est important de noter que le caractère longitudinalement étendu ne préjuge pas du type de syndrome médullaire observé sur le plan clinique.

Par conséquent, l'utilisation de plus en plus courante de l'IRM médullaire en coupes sagittales et axiales dans le domaine des pathologies inflammatoires du système nerveux central (SNC), ainsi que la valeur diagnostique et pronostique de la myélite étendue, a nécessité de reconsidérer le cadre nosologique des myélites aiguës transverses longitudinalement étendues (MATLE) afin de guider au mieux le clinicien dans sa démarche diagnostique.

Tableau 2.
Cadre nosologique de la myélite aiguë transverse longitudinalement étendue.

Myélite aiguë : nadir entre 4 heures et 3 semaines Transverse : syndrome médullaire bilatéral (symétrique ou non) moteur, sensitif et sphinctérien, ou extension de la lésion en IRM dans le plan transversal sur une surface ≥ 50 % Longitudinalement étendue : extension de la lésion en IRM dans le plan longitudinal ≥ 3 segments

IRM : imagerie par résonance magnétique.

Données en imagerie par résonance magnétique

L'IRM doit explorer l'ensemble de la moelle épinière pour déterminer l'étendue longitudinale de la lésion, mais également les racines de la queue de cheval afin de rechercher une atteinte radiculaire associée. Un plan de coupe sagittale est suivi d'un plan transversal centré sur la lésion. Ainsi sont réalisées des séquences pondérées en T2, complétées dans certains centres par une séquence STIR (*short tau inversion recovery*), puis des séquences pondérées en T1, suivies si nécessaire de séquences pondérées en T1 après injection de gadolinium. Encore à l'heure actuelle, l'acquisition en 1,5 Tesla reste préférable compte tenu des nombreux artefacts encore visibles sur les IRM 3 Tesla, malgré une nette amélioration de leur qualité récemment.

Un examen réalisé dans les premières heures après l'apparition des symptômes peut être normal. Il sera reconduit dans les 48 heures. L'IRM ne permet pas à elle seule d'affirmer un mécanisme purement inflammatoire, puisque l'œdème ou la prise de contraste ne sont pas spécifiques et s'observent dans d'autres situations cliniques comme l'ischémie médullaire ou la myélopathie postradiothérapie [8].

Ainsi, une première caractérisation de la myélopathie partielle apparaît en 2001, désignée alors par une lésion médullaire focale dans le plan transversal, souvent triangulaire à base externe (Fig. 1) [8]. Une donnée importante est le fait que le niveau clinique d'un syndrome sous-lésionnel ne correspond pas toujours au niveau de la lésion visible en IRM, celle-ci étant souvent située au-dessus, notamment dans les causes inflammatoires.

Concernant la myélite transverse, il n'existe actuellement pas de définition radiologique en IRM, cette dernière étant avant tout clinique. Il est tout de même admis de façon consensuelle que le terme transverse est associé radiologiquement à une lésion médullaire étendue sur au moins 50 % de la surface transversale de la moelle. Le caractère étendu dans le plan longitudinal est quant à lui bien défini, caractérisé par une lésion s'étendant de façon continue sur au moins trois corps vertébraux (Fig. 1). Un travail collaboratif français a ainsi permis de colliger les pratiques sur le territoire national et de proposer une définition de la MATLE, intégrant pour la première fois dans sa définition le rôle de l'IRM en coupe axiale et longitudinale (Tableau 2).

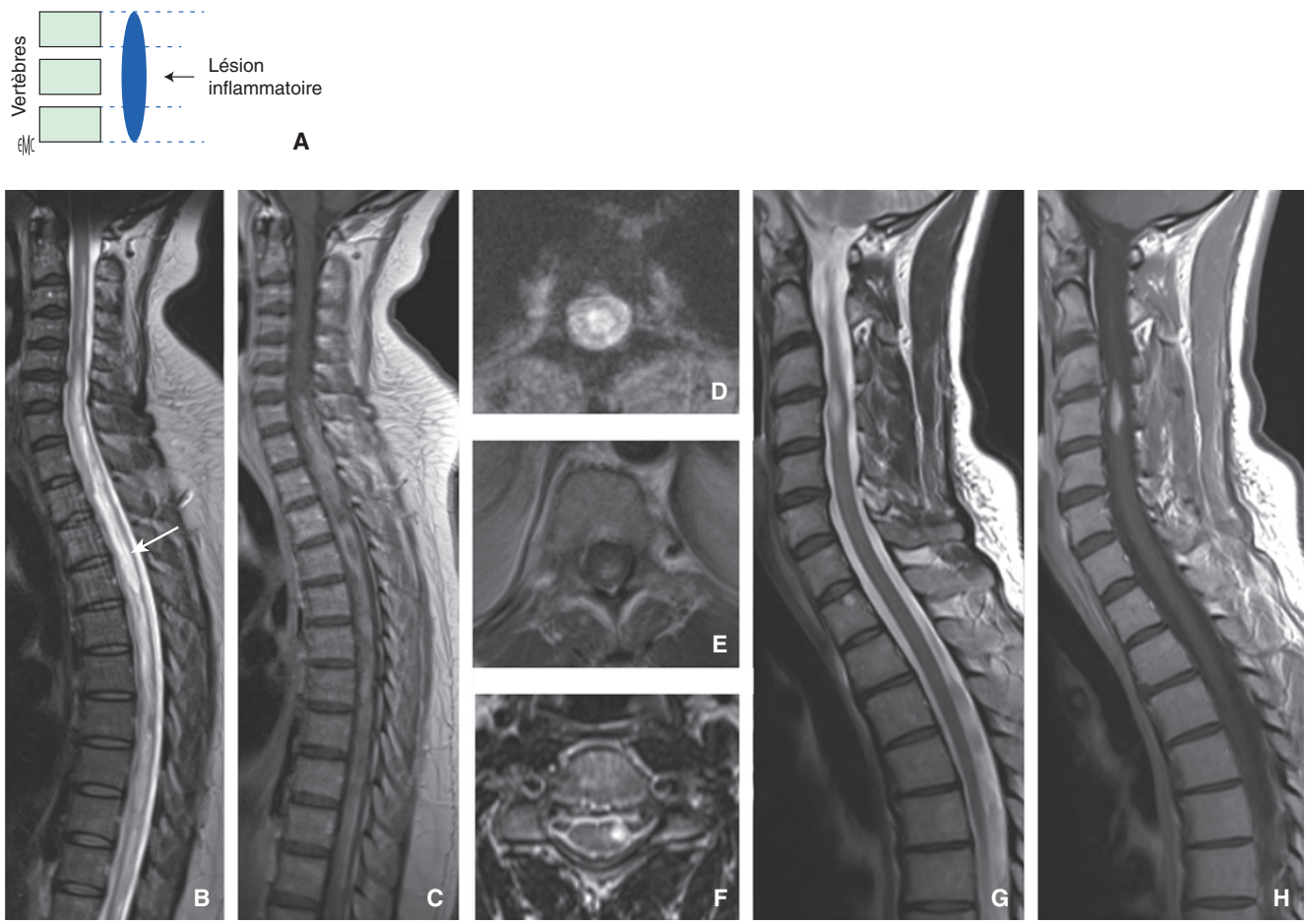

Figure 1. Aspect IRM des myélites étendues et des myélites partielles : la myélite longitudinalement étendue est définie en IRM par une extension de la lésion dans le plan sagittal au moins égale à 3 métamères (A). Myélite étendue de neuromyélite optique (NMO) en IRM dans le plan sagittal en séquence T2 (B) et T1 avec injection de gadolinium (C) mais aussi dans le plan axial en séquence T2 (D) et T1 avec injection de gadolinium (E). Myélite partielle de sclérose en plaques (SEP) en IRM sur la séquence T2 dans le plan axial (F) et sagittal (G), prenant le contraste après injection de gadolinium sur la séquence T1 (H). Noter un aspect en hypersignal T2 marqué de la myélite de NMO (flèche) et une prédominance de l'atteinte de la substance grise (D).

L'IRM médullaire peut être complétée par une IRM cérébrale pour rechercher d'autres lésions associées afin d'affiner le diagnostic étiologique.

■ Cadre nosologique de la myélopathie aiguë

Situations typiques

Depuis les premières descriptions des « myélites aiguës » en 1882 [9], le diagnostic de myélite repose sur des critères cliniques, le suffixe « -ite » désignant les affections médullaires d'origine inflammatoire, infectieuse ou paranéoplasique. Pour les affections médullaires de causes métaboliques, vasculaires, dégénératives ou traumatiques, le terme de myélopathie est préféré. Le terme « myélite » fait référence à un mécanisme inflammatoire, et est donc consacré a posteriori, après l'identification de la cause. Cependant, le diagnostic est orienté d'emblée par le mode d'installation. En effet, un mode d'installation aigu, classiquement défini par un nadir des symptômes entre quatre heures et trois semaines, permet de s'affranchir des causes vasculaires et traumatiques, le plus souvent d'apparition soudaine, ainsi que des causes métaboliques, dégénératives et postradiques généralement d'installation progressive. Partant de cette définition, le champ étiologique des myélopathies aiguës comprend les myélites infectieuses/parainfectieuses, auto-immunes et paranéoplasiques. Compte tenu de leur rareté, une place à part est faite aux myélopathies aiguës toxiques. Enfin, on traitera des myélopathies idiopathiques.

Situations atypiques

Alors que le mode d'installation et les contextes cliniques permettent d'orienter vers ces différentes étiologies, certaines situations cliniques atypiques sont à connaître. À titre d'exemple, un syndrome médullaire révélant un infarctus peut s'installer sur plus de quatre heures, parfois en plusieurs temps, ou être longitudinalement étendu [10, 11]. Des pathologies d'évolution classiquement progressive (fistule durale, cause métabolique ou postradique) peuvent se révéler de manière aiguë à l'occasion d'une décompensation rapide.

C'est pour ces raisons qu'il est important d'éliminer en premier lieu la présentation atypique d'une étiologie vasculaire ou métabolique fortement influencée par le contexte de survenue. Pour les myélopathies « ischémiques », ces contextes sont les suivants : patient masculin, âge supérieur à 50 ans, facteurs de risque cardiovasculaires. Pour les myélopathies « métaboliques », il s'agit des éléments suivants : perte de poids, intoxication éthylique, troubles du comportement alimentaire, malabsorption, chirurgie digestive, troubles cognitifs, troubles psychiatriques/hallucinations. Dans ce dernier cas, les dosages sériques des vitamines B_{12} (par le dosage de l'acide méthylmalonique) et B_9, de l'homocystéine, du cuivre et de la céruléoplasmine sont réalisés.

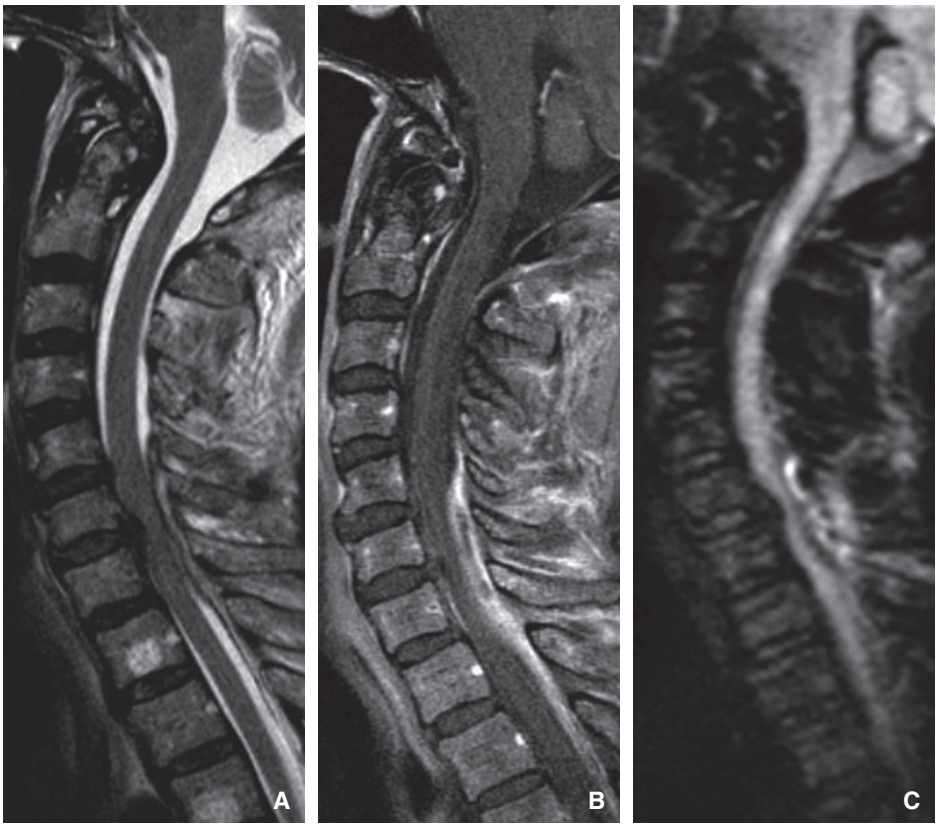

Figure 2. Abcès épidural cervical à staphylocoque avec compression médullaire : coupe sagittale T2 (A), coupe sagittale T1 gadolinium (B), coupe sagittale diffusion (C) : collection épidurale postérieure étendue de C5 à C7. Prise de contraste périphérique et zone centrale en hypersignal en diffusion évoquant un abcès à pyogène. Effet de masse sur la moelle avec probable hypersignal T2 intramédullaire évoquant une souffrance.

Enfin, la recherche étiologique n'inclut pas la sérologie du virus humain T-lymphotrope de type I (HTLV-1) pour plusieurs raisons :

- cliniquement, la myélite n'est jamais aiguë, la minorité des formes subaiguës s'associant à un canal rachidien étroit ;
- la myélite n'est qu'exceptionnellement transverse ;
- chez un sujet qui n'est pas originaire d'une zone d'endémie (Antilles, Afrique subsaharienne, Amérique du Sud, Japon), la sérologie HTLV-1 n'est qu'exceptionnellement positive [12]. Par ailleurs, même si ce contexte est présent, le caractère longitudinal reste une exception. En effet, dans la base de données antillaise d'au moins 100 MATLE, la positivité de la sérologie HTLV-1 n'a été observée qu'une seule fois. Là aussi, elle doit être interprétée en fonction de la prévalence de la maladie dans cette région [13].

■ Étiologies des myélopathies aiguës

Myélites infectieuses

Les myélites infectieuses représentent une part variable des myélopathies aiguës selon la définition utilisée. Si l'on se fonde sur la notion d'un syndrome infectieux précessif, cette proportion est estimée entre 20 et 45 % [1, 3, 14–17]. En revanche, si la mise en évidence d'un agent pathogène est nécessaire, cette proportion chute à 6 % [8]. Plusieurs situations diagnostiques différentes, correspondant à une sémiologie IRM spécifique, sont à distinguer : compression directe de la moelle épinière par un abcès le plus souvent épidural, lésion infectieuse directe, para-infectieuse ou postvaccinale.

Myélopathie liée à une compression médullaire

Cette situation clinique se voit principalement avec les agents infectieux bactériens comme le bacille de Koch (BK) ou le *Staphylococcus aureus*, ou parasitaires comme *Toxoplasma gondii*. D'autres germes peuvent être impliqués, souvent dans des contextes d'immunodépression ou d'anomalies anatomiques de la moelle ou de la colonne vertébrale. Les abcès compressifs ne sont qu'exceptionnellement de localisation intramédullaire ou sous-durale, et sont généralement des extensions épidurales de lésions osseuses.

Le tableau clinique se développe souvent sur quelques jours avec de la fièvre et une raideur méningée. En cas d'abcès épidural, dans la plupart des cas, une raideur rachidienne et des douleurs sont localisées au niveau de l'abcès. La myélopathie évolue le plus souvent de façon chronique sur un mode progressif, mais celle-ci peut aussi évoluer sur le mode aigu ou subaigu, nécessitant un bilan d'imagerie par IRM de façon urgente.

Infection à *Staphylococcus aureus*

Ce germe est retrouvé dans 67 % des cas d'abcès infectieux, 15 % d'entre eux étant une souche résistante à la méthicilline [18]. Il s'agit la plupart du temps d'infection survenant sur un terrain favorisant : diabète, toxicomanie avec drogues injectables, alcoolisme, insuffisance rénale chronique, infection cutanée ou des tissus mous ou endocardite, intervention chirurgicale ou immunodépression [18, 19]. Il est très rare que l'atteinte primitive soit épidurale, elle est le plus souvent secondaire à une spondylodiscite ou à une arthrite septique. L'aspect de l'abcès en IRM n'est pas spécifique du germe impliqué. Il apparaît en hypersignal T2 et hyposignal T1 avec une prise de contraste annulaire (Fig. 2). La réalisation d'une séquence de diffusion peut montrer un hypersignal avec une restriction de l'*apparent diffusion coefficient* (ADC), témoin des abcès à pyogènes [20]. Le diagnostic est fait par ponction de l'abcès et analyse mycologique, même si la rentabilité n'est pas très bonne [14]. La ponction lombaire (PL) n'est pas recommandée compte tenu du risque potentiel de diffusion de l'infection. Le traitement repose sur une antibiothérapie adaptée et une exérèse chirurgicale de l'abcès.

Tuberculose

Les atteintes de la tuberculose sont les plus fréquentes des atteintes médullaires bactériennes dans les pays en voie de

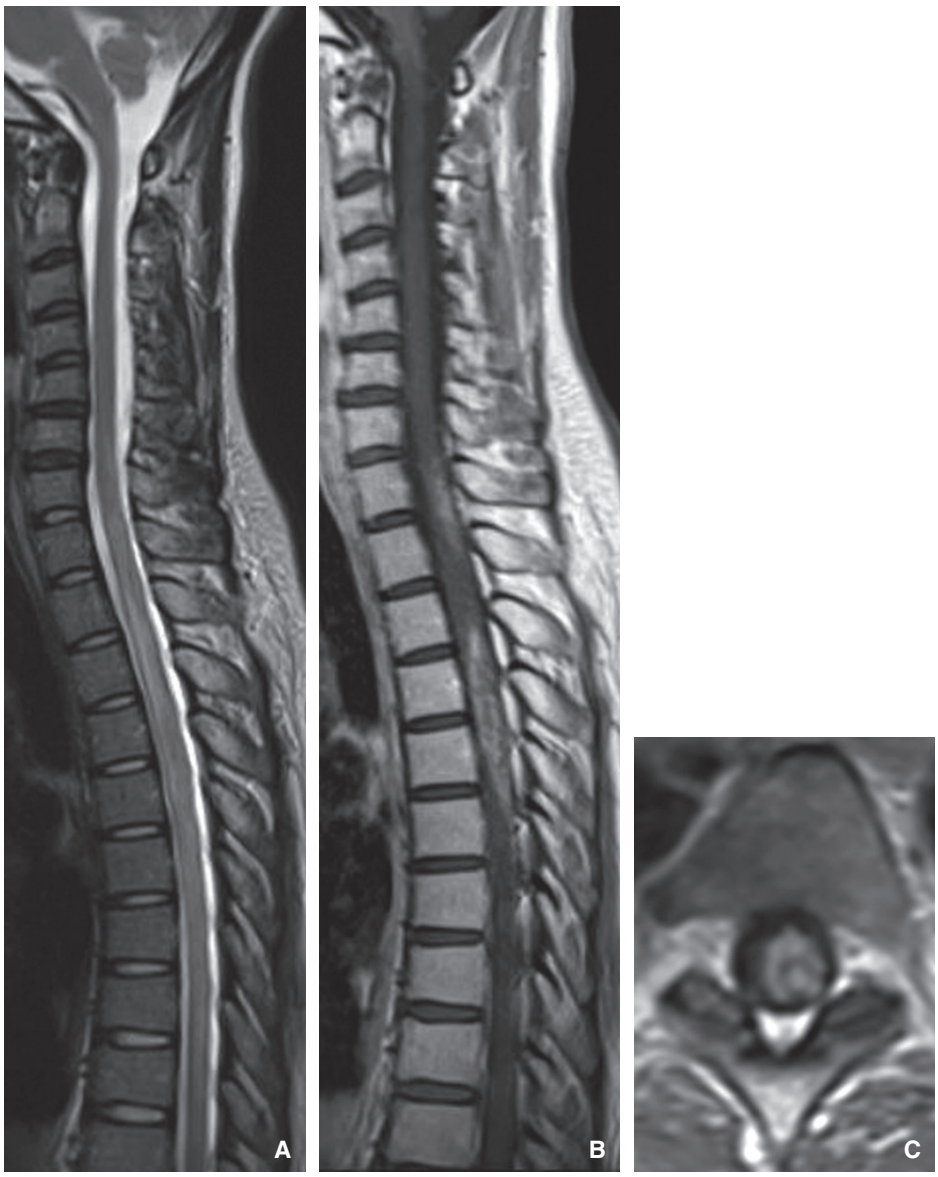

Figure 3. Abcès intramédullaires toxoplasmiques : coupe sagittale T2 (A), coupe sagittale T1 gadolinium (B), coupe axiale T1 gadolinium (C) : prises de contraste intramédullaires en forme de cible avec un nodule central excentré. Œdème périlésionnel.

développement. Ces atteintes sont surreprésentées chez les patients immunodéprimés, atteints par le virus de l'immunodéficience humaine (VIH) ou transplantés [21]. On distingue cinq types d'atteintes de mécanisme différent :

- tuberculose du corps vertébral et compression médullaire par contiguïté (abcès, granulomatose ou luxation) ;
- granulomatose épidurale compressive sans atteinte osseuse ;
- vascularite médullaire avec thrombose ;
- tuberculome intramédullaire ;
- arachnoïdite.

En cas de tuberculose, le liquide céphalorachidien (LCR) montre une hypercytose initialement panachée ou à prédominance de polynucléaires. Une hypoglycorachie peut être observée ainsi qu'une hyperprotéinorachie souvent importante correspondant à plusieurs grammes par litre. En cas d'arachnoïdite isolée, le LCR peut être normal ou montrer une hyperprotéinorachie isolée.

Dans tous les cas, le diagnostic de certitude repose sur l'isolement du germe dans le LCR ou après ponction de l'abcès par examen direct, culture ou *polymerase chain reaction* (PCR) [22].

Le traitement est fondé sur une antibiothérapie spécifique. Cependant, aucun consensus n'existe quant au nombre d'antituberculeux ni sur la durée du traitement ou l'utilisation concomitante d'une corticothérapie. Une chirurgie peut être parfois proposée, surtout en cas d'atteinte vertébrale avec compression médullaire.

Toxoplasmose

La toxoplasmose médullaire est rare et se voit essentiellement chez les patients immunodéprimés par le VIH [23]. Elle se manifeste par un abcès médullaire (Fig. 3) qui peut montrer un aspect tumoral avec effet de masse [24]. Le diagnostic repose sur la positivité des anticorps dans le LCR et, plus rarement, la biopsie médullaire quand la situation clinique ne permet pas d'éliminer un possible lymphome médullaire.

Myélite comme conséquence directe de l'infection

Dans ces myélites, l'agent infectieux entraîne une lésion directe de la moelle épinière. La symptomatologie associe une fièvre et des signes généraux non spécifiques. La fièvre peut être contemporaine des symptômes neurologiques ou se terminer quelques jours avant leur apparition dans un tiers des cas environ. La sémiologie médullaire est celle d'une myélite transverse d'aggravation progressive sur 2 à 3 jours. D'autres atteintes neurologiques notamment périphériques comme la méningoradiculite peuvent

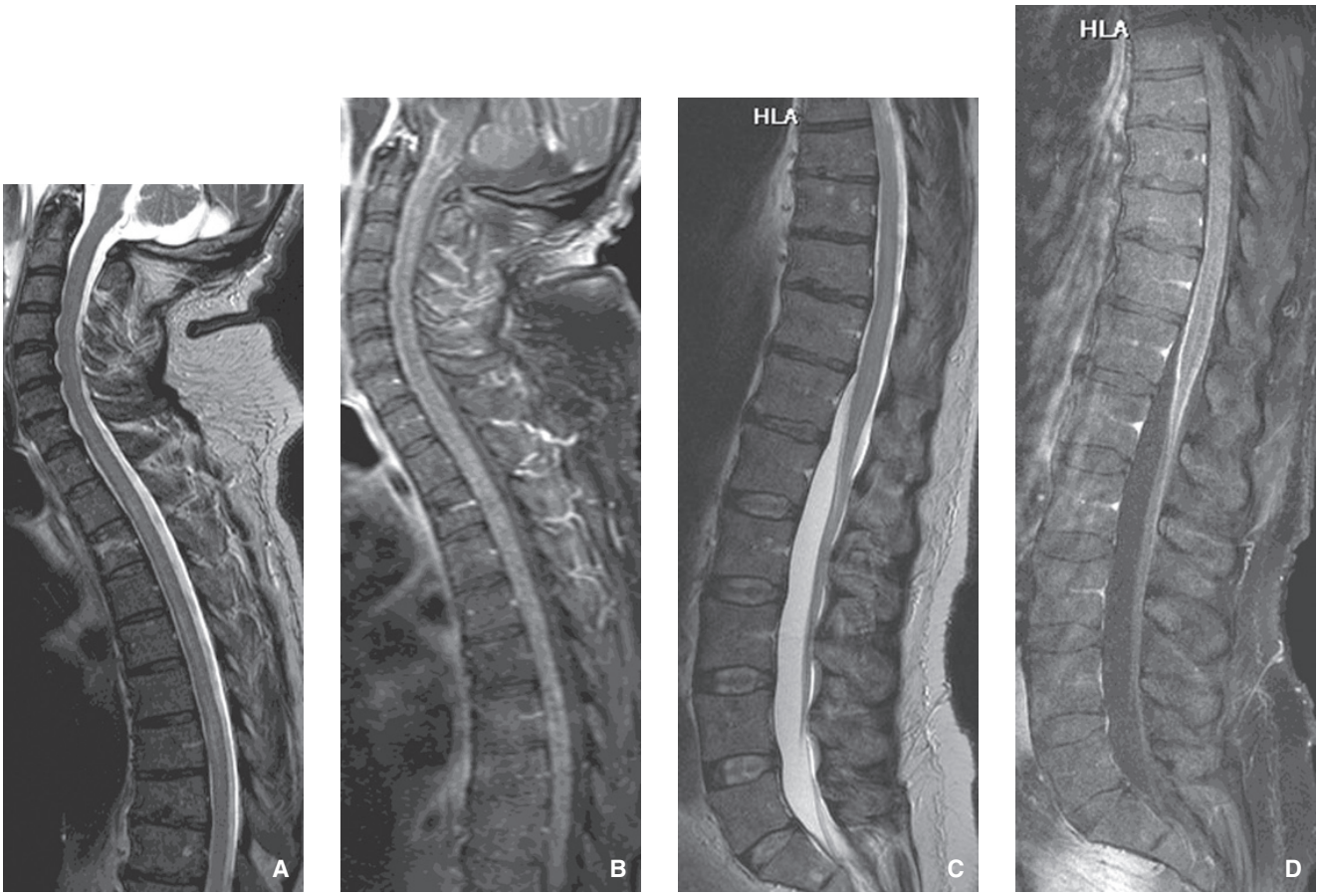

Figure 4. Myéloméningo radiculite à Epstein-Barr virus (EBV) : coupes sagittales T2 (A, C), coupes sagittales T1 gadolinium (B, D) : hypersignal T2 intramédullaire étendu associé à une prise de contraste leptoméningée et des racines de la queue de cheval.

Tableau 3.
Principaux virus responsables de myélopathie aiguë.

Groupe des virus herpes	Cytomégalovirus (CMV) Epstein-Barr virus (EBV) Virus herpes simplex 1 et 2 (HSV) Virus de la varicelle et du zona (VZV)
Groupe des entérovirus	Poliovirus 1, 2, 3 Coxsackievirus Entérovirus Échovirus
Virus des hépatites	Hépatite B (VHB) Hépatite C (VHC)

parfois être associées (Fig. 4), ce qui est un élément différenciant des myélites auto-immunes qui sont souvent de localisation centrale uniquement. La topographie lésionnelle est principalement thoracique ou lombaire, à l'origine de troubles sphinctériens précoces. Les germes à l'origine de ces myélites sont multiples et doivent être recherchés activement (Tableau 3). Des agents tels que le virus West Nile, le virus de l'encéphalite à tiques centro-européenne, le virus John-Cunningham ou encore le virus Zika peuvent aussi être responsables de myélites aiguës [25–28]. Certains ont un pronostic particulièrement mauvais compte tenu de leur toxicité vasculaire comme c'est le cas avec le virus herpes simplex (HSV), le cytomégalovirus (CMV) ou le virus varicelle-zona (VZV) [29–31]. L'interrogatoire permet d'orienter le diagnostic étiologique en fonction du terrain clinique et du type d'immunosuppression. Les myélites à CMV ou VZV surviennent essentiellement chez les immunodéprimés infectés ou non par le VIH [30, 32]. Le VIH en lui-même ne donne pas de myélopathie aiguë sévère mais plutôt une myélopathie vacuolaire d'aggravation progressive [33]. Cependant, de rares cas de myélopathie aiguë spontanément régressive ont été décrits pendant la phase de séroconversion ou durant l'évolution de la maladie. L'atteinte par le poliovirus à l'origine de la poliomyélite antérieure aiguë n'est pas traitée dans ce chapitre.

L'IRM retrouve principalement une MATLE qui n'est pas spécifique du mécanisme infectieux. Une des spécificités des atteintes médullaires infectieuses est la fréquente association IRM à une fine prise de contraste leptoméningée et à des prises de contraste radiculaires touchant en particulier des racines de la queue de cheval.

Compte tenu de leur particulière gravité, de leurs spécificités physiopathologiques et IRM, il est possible d'illustrer ce chapitre avec les agents infectieux suivants :

- pour les virus : HSV de type 1 et 2 (HSV1-2), CMV, VZV ;
- pour les bactéries : *Treponema pallidum*, *Borrelia burgdorferi* ;
- pour les parasites : schistosomiase.

Groupe des virus herpes simplex

Virus herpes simplex de type 1. La myélite à HSV1 est rare mais de mauvais pronostic, pouvant parfois conduire au décès [34]. Elle peut survenir chez le sujet immunodéprimé ou âgé immunocompétent. L'association à des lésions cutanées ou une encéphalite permet d'évoquer rapidement ce germe. Des myélites récurrentes ont rarement été décrites [35]. L'IRM est fréquemment anormale, les lésions pouvant correspondre à une MATLE ou à une myélite partielle. L'analyse du LCR retrouve une pléiocytose lymphocytaire et une hyperprotéinorachie modérée. Le diagnostic est documenté par l'augmentation du taux d'anticorps anti-HSV dans

le sang et surtout par la détection du virus par PCR dans la PL. Dès la suspicion du diagnostic, un traitement antiviral par aciclovir doit être mis en place et ajusté en fonction du résultat de la PCR dans le LCR [36].

Virus herpes simplex de type 2. Comme la myélite à HSV1, la myélite à HSV2 est d'évolution défavorable et laisse souvent d'importantes séquelles [37]. L'analyse histopathologique des lésions a révélé de larges zones de nécrose contenant des antigènes viraux et une vascularite médullaire [29, 38]. Cette myélite se voit essentiellement chez les enfants ou l'adulte immunodéprimé. La symptomatologie est celle d'une myélite transverse avec, au premier plan, des dysesthésies des membres inférieurs, des paresthésies génitales et des troubles sphinctériens. Une éruption cutanée peut être identifiée, touchant principalement les régions génitales. Cette myélite, en plus de son très mauvais pronostic, a la particularité de pouvoir récidiver de façon contemporaine aux éruptions cutanées [39]. L'IRM retrouve une MATLE. Le LCR peut retrouver une pléiocytose à prédominance de polynucléaires ou une hyperlymphocytose. Une hyperprotéinorachie peut être associée à une glycorachie normale ou parfois abaissée, ce qui ne doit pas orienter forcément vers une cause bactérienne. La stratégie diagnostique et thérapeutique est identique à celle effectuée en cas de myélite à HSV1.

Cytomégalovirus. La myélite à CMV survient essentiellement chez les patients atteints par le VIH au stade sida mais peut aussi survenir exceptionnellement chez les immunocompétents [40–42]. Elle est souvent associée à une polyradiculonévrite pouvant atteindre les nerfs crâniens [43]. La myélite s'aggrave sur plusieurs jours avec une symptomatologie de myélite transverse préservant souvent la proprioception [44]. Dans la majorité des cas, l'atteinte neurologique est associée à d'autres manifestations cliniques comme la rétinite, l'atteinte digestive ou pulmonaire qu'il faut systématiquement rechercher. L'IRM est normale dans 40 à 50 % des cas mais montre autrement soit une MATLE, soit une prise de contraste des racines correspondant à la polyradiculonévrite [43]. Noter que le CMV peut entraîner une myélopathie ischémique liée à une vascularite nécrosante de mauvais pronostic [30].

Le LCR est anormal dans 75 % des cas, avec une pléiocytose à polynucléaires neutrophiles ou une hyperlymphocytose, une hyperprotéinorachie modérée et une hypoglycorachie dans un tiers des cas. Le diagnostic de certitude est fait sur la détection d'une virémie, d'une virurie ou d'une réplication dans le LCR par PCR. Le traitement repose sur l'utilisation de ganciclovir [45].

Virus de la varicelle et du zona. La myélite peut se voir chez les immunodéprimés et notamment lors de traitements immunosuppresseurs comme le fingolimod, la corticothérapie ou les anti-*tumor necrosis factor* alpha (anti-TNFα) [46]. Elle peut également survenir chez les patients âgés immunocompétents. La myélite est contemporaine ou non du zona [47, 48]. Quand celui-ci survient, il est localisé le plus souvent au thorax et apparaît deux semaines avant le début de la myélite. Les symptômes débutent du côté de la lésion cutanée par un déficit moteur qui se complète le plus souvent en un tableau de myélite transverse en 1 à 3 semaines. Cependant, d'autres formes cliniques sont à connaître comme le syndrome de Brown-Séquard ou encore son association à une polyradiculonévrite [49, 50]. Plus rarement, la myélite évolue rapidement sur quelques heures, réalisant un tableau de vascularite nécrosante de pronostic catastrophique [31, 51]. L'IRM est souvent anormale, réalisant un tableau de MATLE ou retrouvant une prise de contraste des racines en cas de polyradiculonévrite associée. La myélite peut prédominer au niveau des cordons postérieurs [52].

La PL peut être normale, surtout au début de l'infection, ou montrer une méningite lymphocytaire avec hyperprotéinorachie. La glycorachie est normale. La détection du virus pas PCR dans le LCR permet de poser définitivement le diagnostic [51].

Le traitement repose sur l'aciclovir aux mêmes doses que pour l'encéphalite herpétique. La réponse thérapeutique n'est pas bonne et dépend de sa précocité ou de la survenue d'un ramollissement hémorragique [53]. La myélite du VZV fait partie des myélites mortelles pour laquelle un traitement antiviral doit être instauré en extrême urgence.

Syphilis

La manifestation médullaire aiguë de cette infection est caractéristique de la phase secondaire de la maladie, lors de la diffusion par voie hématogène du *T. pallidum*. Lors de cette phase méningovasculaire, une myélite à prédominance lombosacrée peut s'observer, associée ou non à une atteinte des artères spinales antérieures ou postérieures [21]. Cette complication est devenue exceptionnelle compte tenu de l'utilisation répandue de la pénicilline. Elle est néanmoins de mauvais pronostic en cas d'ischémie médullaire associée [54]. Une pachyméningite peut également orienter le diagnostic étiologique. L'analyse du LCR retrouve une hyperlymphocytose avec hyperprotéinorachie, présence de bandes oligoclonales et parfois une hypoglycorachie. Le diagnostic est avant tout sérologique (*venereal disease research laboratory* [VDRL], *Treponema pallidum hemagglutination assay* [TPHA], *fluorescent treponema-antibody absorption* [FTA-ABS]) dans le sang et le LCR. Le traitement repose sur l'utilisation de fortes doses de pénicilline [55].

Lyme

Les myélites aiguës sont rares et s'associent fréquemment à une atteinte radiculaire crânienne ou spinale. Celles-ci surviennent majoritairement à la phase aiguë, lors de l'apparition de l'érythème migrant [56]. L'atteinte est une MATLE souvent sévère cliniquement, confinant à la paraparésie dans près de 75 % des cas, mais pouvant aussi se limiter plus rarement à une atteinte sphinctérienne ou cordonale postérieure [57–59]. Parfois, la myélite n'est pas visualisée à l'IRM, une possible prise de contraste leptoméningée peut alors orienter le diagnostic, même si celle-ci n'est pas spécifique, et peut se retrouver dans toutes les infections. L'étude du LCR montre une méningite lymphocytaire et une hyperprotéinorachie. Une synthèse intrathécale est fréquente, surtout en cas de radiculite associée. L'infection à *B. burgdorferi* est évoquée lors de la mise en évidence d'anticorps spécifiques dans le sang par une technique *enzyme linked immunosorbent assay* (Elisa), souvent complétée par un western blot. Le neurolyme est quant à lui précisé lors de la détection dans le LCR d'un index de synthèse intrathécale d'immunoglobuline (Ig) anti-*B. burgdorferi* par Elisa ou par la mise en évidence du germe en PCR, même si cette dernière technique est spécifique mais peu sensible [60, 61]. Le traitement utilisé en priorité est la ceftriaxone, suivi de la doxycycline ou de l'amoxicilline.

Schistosomiase

La schistosomiase est la seconde endémie parasitaire dans le monde après le paludisme. L'atteinte médullaire est plus due à *Schistosoma mansoni* (Afrique, Amérique du Sud, Caraïbes) qu'à *S. haematobium* (Afrique) qui entraîne principalement des atteintes cérébrales [62]. Les myélites aiguës se voient lors de la primo-infection. Ces myélites sont la conséquence de granulomes contenant les œufs de ces vers hématophages. Ils sont alors extra- ou intrathécaux ou intramédullaires et siègent habituellement sous le niveau T9, essentiellement dans le cône terminal [63]. Parfois, la réaction inflammatoire permet de visualiser une myélite transverse à l'IRM, de niveau thoracique bas ou lombaire. D'exceptionnels cas de thrombose de l'artère spinale antérieure ont été décrits. L'atteinte clinique est souvent sévère, avec paraparésie plus ou moins flasque, abolition des réflexes ostéotendineux, troubles sensitifs et sphinctériens [64]. Le LCR contient 30 à 40 % d'éosinophiles avec une hyperlymphocytose, une hyperprotéinorachie avec ou sans bandes oligoclonales, et une glycorachie normale ou abaissée. Comme pour le neurolyme, un index de synthèse intrathécale peut être effectué, permettant de différencier les infections évolutives des cicatrices sérologiques [65]. Le diagnostic de certitude se fait sur la découverte des œufs dans les selles, les urines ou la biopsie rectale. Dès la suspicion diagnostique, un traitement urgent par praziquantel doit être initié, parfois associé à une corticothérapie. Une fois traitée efficacement, la myélite a une évolution favorable dans plus de 80 % des cas.

Myélite para-infectieuse et postvaccinale

Les myélites aiguës para-infectieuses, dont le mécanisme physiopathologique peut être rapproché des myélites postvaccinales,

sont fréquentes. Ces myélopathies sont liées à un dysfonctionnement transitoire du système immunitaire généré par l'infection, aussi appelé réaction immunoallergique postinfectieuse. Elles s'inscrivent principalement dans le cadre d'une encéphalomyélite aiguë disséminée (EMAD) mais peuvent parfois être confinées à une expression médullaire. En IRM, l'atteinte médullaire est une MATLE, focale ou mutifocale [66–68]. Le tableau clinique est souvent sévère, cependant une résolution spontanée est possible. Un traitement par bolus de corticoïdes intraveineux à forte dose est habituel et permet de faire régresser rapidement la quasi-totalité des symptômes.

Myélites auto-immunes

Les myélites auto-immunes comportent un grand nombre d'étiologies. La principale étiologie est la sclérose en plaques (SEP) qui constitue près de 50 % des myélopathies aiguës toutes présentations confondues [8]. La fréquence est augmentée si l'on ne prend en compte que les myélites partielles mais diminue si l'on considère seulement les myélites transverses (environ 10 à 15 %) [69]. Ainsi, la sémiologie clinique et surtout IRM est importante pour orienter le diagnostic (cf. supra). Dans ce paragraphe, les myélites associées à une SEP, une NMO, ou une sarcoïdose ne seront abordées que succinctement (ces pathologies faisant toutes partie d'un chapitre spécifique de l'*Encyclopédie médico-chirurgicale*).

Sclérose en plaques

Les myélopathies associées à une SEP sont le plus souvent de début subaigu, à prédominance sensitive et d'évolution favorable. Il s'agit de la présentation initiale de la SEP dans 20 à 30 % des cas selon les études portant sur les syndromes cliniques isolés [70, 71]. L'IRM montre habituellement une ou plusieurs lésions de petite taille couvrant moins de deux métamères dans le plan sagittal et moins d'une hémi-moelle dans le plan axial (Fig. 1). La fréquence de la survenue d'une SEP après un premier épisode de myélite partielle varie selon la durée de suivi et les données des autres examens complémentaires. Dans l'étude de Bourre et al., qui est la plus longue en termes de suivi, 63 % des patients développent une SEP après en moyenne huit ans de suivi [72]. Cette fréquence augmente à 92 % en cas d'anomalie du LCR (bandes oligoclonales) et de l'IRM cérébrale (au moins une lésion) mais est très basse (8 %) en cas de normalité de ces deux examens.

Les cas de myélopathie transverse sont plus rares mais non exceptionnels. Les myélopathies associées à une SEP représentent environ 10 à 20 % des myélopathies transverses [69]. Elles s'inscrivent parfois dans un tableau plus large de type EMAD. Dans ce cas, l'évolution est le plus souvent monophasique sans évolution vers une SEP, mais environ 30 % des patients évolueront tout de même vers une SEP, avec un risque plus élevé en cas de bandes oligoclonales à la PL et d'épargne de la substance grise (cortex et noyaux gris centraux) à l'IRM [73]. En ce qui concerne le pronostic de ces myélopathies inaugurales de SEP, il ne semble pas plus péjoratif qu'une atteinte optique ou encéphalique, notamment dans les études thérapeutiques portant sur les syndromes cliniques isolés.

Neuromyélite optique

Bien que faisant partie d'un chapitre séparé, il est difficile de ne pas évoquer la NMO dans les étiologies des myélopathies inflammatoires. En effet, il s'agit du principal diagnostic différentiel de la SEP devant la survenue d'une myélopathie aiguë. La découverte en 2004–2005 d'un anticorps spécifique de la maladie (anti-AQP4) [74, 75] a permis de réviser un certain nombre de diagnostics de SEP ou de myélopathies de causes indéterminées. La valeur prédictive de cet anticorps après un premier épisode de myélopathie aiguë transverse au regard du risque de rechute médullaire ou visuel est de plus de 50 % à un an, justifiant actuellement un traitement immunosuppresseur en cas de premier épisode de myélopathie aiguë avec anti-AQP4 [76]. Les critères de NMO ont d'ailleurs été récemment élargis dans ce cadre, permettant de poser les diagnostics de NMOSD (NMO *spectrum disorder* ou spectre NMO) plus précocement [77]. Plus récemment, un deuxième anticorps (*anti-myelin oligodendrocyte glycoprotein* [MOG]), dont la spécificité reste à évaluer, a été découvert [78, 79]. Les présentations sont assez souvent monophasiques et le pronostic un peu moins péjoratif que dans les formes avec anticorps anti-AQP4, mais la spécificité de cet anticorps reste à préciser, notamment chez l'enfant.

Dans la NMO, les myélopathies sont aiguës et souvent sévères. L'aspect en IRM est habituellement celui d'une MATLE avec un œdème important et une prise de contraste (Fig. 1). Parmi les MATLE, l'atteinte de la substance grise et l'aspect en hypersignal marqué en T2 semblent spécifiques de la NMO (Fig. 1) [80]. Beaucoup plus rarement, la myélite peut être partielle [81].

La possibilité de poser le diagnostic de NMO précocement a bien évidemment un impact thérapeutique. Compte tenu de la sévérité de ces tableaux de myélopathie, la mise en route d'un traitement immunosuppresseur dès le premier épisode en cas de positivité des anticorps s'impose. Plusieurs études ont montré l'impact largement supérieur des immunosuppresseurs sur les traitements immunomodulateurs habituellement utilisés dans la SEP [82], voire l'effet contraire de certaines de ces molécules comme les interférons, le natalizumab, l'alemtuzumab ou le fingolimod. La distinction entre SEP et NMO, si elle est le plus souvent facile à faire devant les différences de présentations cliniques et IRM (cérébrale et médullaire), reste un élément majeur de la discussion puisqu'actuellement, dans un cas comme dans l'autre, les patients sont le plus souvent traités dès le premier épisode neurologique.

Myélopathies aiguës et maladies systémiques

Les principales maladies systémiques associées aux myélopathies aiguës sont le syndrome de Gougerot-Sjögren (SGS) et le lupus. Ces myélopathies sont souvent associées à la NMO, ne modifiant pas la prise en charge thérapeutique de la NMO [83]. En revanche, il existe des cas non associés à la NMO, et la physiopathologie en est alors assez discutée. En effet, notamment dans le lupus, deux mécanismes physiopathologiques peuvent être observés [84]. Dans certains cas, le tableau clinique et radiologique ressemble à une ischémie médullaire (survenue brutale, atteinte sévère, atteinte IRM prédominant ou atteignant exclusivement la substance grise, etc.). Ces tableaux sont souvent associés à des anticorps antiphospholipides (syndrome des antiphospholipides [SAPL]). Il existe également des cas de myélopathies aiguës liées à un SAPL sans lupus avec souvent un caractère récidivant [85]. La présentation clinique des myélopathies aiguës liées au SGS est plutôt sur un mode inflammatoire, avec le plus souvent un hypersignal étendu, proche de ce qui est observé dans la NMO. Devant une myélopathie aiguë, un SGS doit être recherché attentivement, et la recherche doit être renouvelée à distance car les manifestations neurologiques du SGS peuvent précéder l'arrivée du syndrome sec et des anomalies biologiques et morphologiques [86].

De façon générale, toutes les maladies de système ont soit des hypersignaux médullaires ressemblant à ceux de la SEP, soit des atteintes médullaires étendues mais avec très peu ou pas de prise de contraste.

Sur le plan thérapeutique, malgré l'absence d'études randomisées, il existe un relatif consensus sur le traitement de la phase aiguë par bolus de corticoïdes intraveineux (3–5 g) et d'un relais assez rapide par cyclophosphamide pour limiter les séquelles [87]. L'ajout d'un antiagrégant, voire d'un anticoagulant, notamment en cas d'anticorps anticardiolipine, se discute au cas par cas mais a une certaine logique si l'on considère que certains de ces tableaux répondent au moins en partie à un mécanisme vasculaire.

Sarcoïdose

La sarcoïdose est associée de façon non exceptionnelle à des tableaux de myélopathies mais celle-ci s'exprime le plus souvent sur un mode subaigu voire chronique avec parfois des difficultés à différencier ces tableaux d'une pathologie tumorale [88]. Il s'y associe assez fréquemment une atteinte leptoméningée qui est très évocatrice de granulomatose. En IRM, l'atteinte est alors étendue avec une prise de contraste leptoméningée et radiculaire micronodulaire. Les manifestations neurologiques de la sarcoïdose peuvent être isolées, mais dans plus de 80 % des cas il existe une atteinte extraneurologique [89]. Il faut donc

s'attacher à les rechercher, permettant ainsi d'éviter une biopsie médullaire, de réalisation toujours délicate. La PL (lymphocytose, hyperprotéinorachie, rapport CD4/CD8 diminué), le TEP-scan, le dosage de l'enzyme de conversion, le scanner thoracique et la biopsie des glandes salivaires accessoires sont autant d'examens qui peuvent éventuellement apporter des arguments directs ou indirects en faveur de cette hypothèse. Le traitement d'une myélopathie sarcoïdosique est le même que pour les autres localisations systémiques et notamment neurologiques de la sarcoïdose : corticoïdes intraveineux à forte dose avec relais par voie orale à dose décroissante pendant une durée de plusieurs mois puis méthotrexate/cyclophosphamide/anti-TNFα en traitement de fond, en fonction de la sévérité et de la réponse au traitement initial [90].

Autres pathologies dysimmunitaires

Des cas exceptionnels ont été rapportés, notamment associés à une maladie de Wegener ou de Churg et Strauss [91]. Ces tableaux restent cependant rares et peuvent être pris en charge comme les autres myélopathies aiguës auto-immunes, notamment sur le plan thérapeutique avec, au premier plan, l'utilisation du cyclophosphamide.

Myélopathies toxiques

Chimique

Héroïne

Des myélopathies aiguës ont été décrites suite à la prise d'héroïne par voie intraveineuse ou intranasale, seule ou associée à d'autres substances [92–95]. Ces myélopathies surviennent généralement chez des héroïnomanes après une période d'abstinence [95] mais aussi chez des consommateurs chroniques [92]. Un cas après une première prise d'héroïne a également été publié [96]. Ces myélopathies sont souvent transverses avec une paraparésie sévère [97]. La toxicité médullaire pourrait avoir de multiples origines, souvent intriquées et biaisées par la prise concomitante d'autres substances. Parmi les mécanismes impliqués, il a été décrit une toxicité directe de l'héroïne, une vascularite, une hypotension ou une réaction d'hypersensibilité [98]. Dans cette dernière hypothèse, l'héroïne pourrait jouer le rôle d'haptène et se coupler à une protéine médullaire afin d'engendrer une réaction immunoallergique [93]. Un traitement par corticoïdes ou échanges plasmatiques sanguins peut alors être indiqué. Le pronostic de la myélopathie dépend du mécanisme impliqué, allant du décès dans les nécroses médullaires [92, 94, 99] à une récupération complète au bout d'un mois lors d'une réaction d'hypersensibilité [92, 97].

Médicaments cytotoxiques

Des myélopathies peuvent survenir de façon soudaine, aiguë ou retardée de plusieurs mois après l'injection intrathécale de méthotrexate, de cytosine arabinoside, de thiotépa ou de stéroïdes [100–102]. Leur fréquence est difficile à estimer mais elle est très rare avec le méthotrexate [100] et peut représenter jusqu'à 2,5 % des patients traités par cytosine arabinoside [101]. Le tableau clinique est varié en intensité et peut comprendre des douleurs des membres inférieurs, une paraplégie, une tétraplégie, des troubles sphinctériens ou un niveau sensitif. L'IRM médullaire est souvent initialement normale et peut montrer ensuite un aspect de myélite inflammatoire avec œdème ou parfois une atteinte localisée aux cordons postérieurs, latéraux, ou à la substance grise (Fig. 5) [103–105]. Le mécanisme physiopathologique reste hypothétique. Des taux élevés de *myelin basic protein* (MBP) ont été détectés chez des patients traités par méthotrexate intrathécal, plaidant en faveur d'une inflammation locale. Cependant, chez ces patients, l'aspect IRM de certaines myélopathies chroniques retardées ainsi que la réponse à un traitement substitutif par folate sont en faveur d'une carence folique [106]. Le problème à la phase aiguë est de distinguer une complication de la chimiothérapie d'une évolution de l'hémopathie. Dans ce contexte, le LCR peut montrer une pléiocytose modérée et une hyperprotéinorachie. L'évolution peut se faire vers la régression ou bien laisser des séquelles.

Anesthésie épidurale

La survenue d'une myélopathie aiguë au décours d'une anesthésie épidurale est un événement rare, estimé à un cas pour 11 000 anesthésies [107]. Les causes de ces accidents sont multiples : ischémie médullaire favorisée par la toxicité directe du produit anesthésique, hématome épidural, hypotension prolongée, position du patient lors de l'anesthésie. Celle-ci peut être due à une compression liée à la quantité de liquide anesthésique, ou à une toxicité plus importante de celui-ci dans certaines conditions anatomiques augmentant sa concentration locale (canal lombaire étroit).

Différents tableaux cliniques de pronostic variable ont été décrits. Habituellement, les patients développent quelques heures à quelques jours après l'intervention un tableau d'atteinte médullaire régressive.

Myélopathie après électrocution

Les signes neurologiques lors d'une électrocution peuvent être répartis en quatre groupes selon la classification de Cherington [108]. Le plus souvent, ils apparaissent immédiatement puis sont soit transitoires (groupe 1), soit permanents (groupe 2). Certaines manifestations sont retardées de 1 jour à 6 semaines (en moyenne 1 semaine), correspondent donc à des myélopathies aiguës et appartiennent alors au groupe 3 de la classification. Les signes neurologiques s'aggravent alors sur une période de 2 à 14 jours. Le tableau est celui d'une paraparésie ou d'une quadriparésie spastique associée à des troubles de la sensibilité profonde. Les troubles sphinctériens sont inhabituels. L'analyse du LCR peut montrer une hyperprotéinorachie sans pléiocytose. Les autopsies réalisées chez ces patients retrouvent des hémorragies pétéchiales, une dégénérescence rétrograde des faisceaux pyramidaux et des motoneurones, ou une démyélinisation sans perte axonale [109–111]. Le groupe 4 correspond à des hématomes sous-duraux, épiduraux ou sous-arachnoïdiens.

Myélites paranéoplasiques

Ces myélites sont rares et évoluent au cours d'un cancer. Leur évolution est plutôt chronique et progressive, mais des cas exceptionnels d'évolution aiguë monophasique ou à rechute ont été décrits [112]. Les femmes sont touchées dans 60 % des cas, l'âge médian de début est estimé à 62 ans, et la myélite précède généralement la découverte du cancer de plusieurs mois [113]. Le mécanisme impliqué est lié à la réponse immunitaire contre le cancer, qui génère une réaction auto-immune contre de nouveaux antigènes intracellulaires ou extracellulaires. Les antigènes intracellulaires entraînent une réponse lymphocytaire cytotoxique qui ne les rend pas directement pathogènes. À l'opposé, les antigènes extracellulaires entraînent une réponse immunitaire humorale où la toxicité de ces anticorps est directe. Les deux anticorps onconeuronaux les plus fréquemment retrouvés sont les anti-amphiphysine et anti-*collapsin response mediator protein 5* (anti-CRMP5) dans les cancers du sein et du poumon [112, 114].

La myélite est une MATLE symétrique, souvent limitée à une région anatomique définie : cordons postérieurs, faisceaux latéraux, substance grise centromédullaire [113]. Des phénotypes radiologiques semblables à ceux des maladies du spectre de la NMO ont été décrits [115]. Les principaux anticorps onconeuronaux retrouvés dans les myélites paranéoplasiques sont listés dans le Tableau 4.

Le traitement des myélites paranéoplasiques repose sur le traitement du cancer. Les manifestations neurologiques peuvent être traitées initialement par 3 à 5 cures de 1 g de méthylprednisolone (MP) suivies par des immunoglobulines intraveineuses ou des échanges plasmatiques en cas de non-réponse. Il n'y a pas de consensus sur la stratégie thérapeutique à adopter après la phase aiguë. Il est recommandé de favoriser l'action immunosuppressive sur les lymphocytes T en cas d'antigènes onconeuronaux intracellulaires (corticostéroïdes, mycophénolate mofétil, azathioprine, cyclophosphamide) et l'action sur le lymphocyte B (rituximab) en cas d'antigènes onconeuronaux extracellulaires [116].

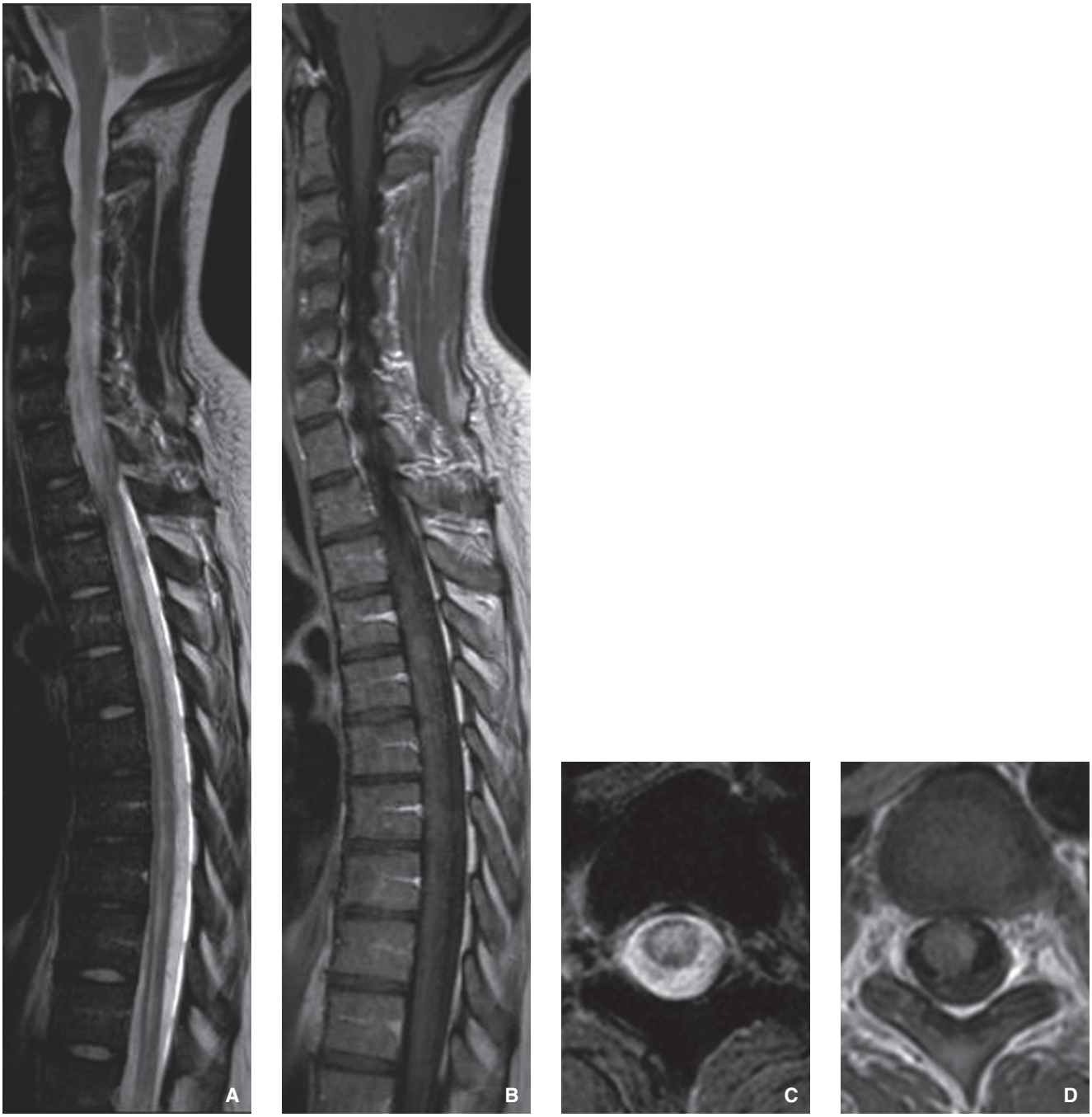

Figure 5. Myélopathie toxique au méthotrexate : coupe sagittale T2 (A), coupe sagittale T1 gadolinium (B), coupe axiale T2 (C), coupe axiale T1 gadolinium (D) : hypersignal T2 intramédullaire étendu prédominant au sein de la substance grise et s'accompagnant d'une prise de contraste discrète.

Myélites aiguës idiopathiques

Ces myélites sont celles pour lesquelles aucune étiologie n'a pu être retenue. Elles comportent un stigmate d'inflammation qui est soit une pléiocytose dans le LCR, soit la présence d'une synthèse intrathécale d'immunoglobulines, soit un rehaussement par le gadolinium [2]. En cas d'IRM ou de PL précoce ne retrouvant pas d'argument pour une inflammation, il est recommandé de refaire ces examens entre 2 et 7 jours après le début des symptômes. À l'heure actuelle, leur fréquence est difficile à déterminer car les données disponibles sur la question sont relativement anciennes, les premières descriptions de ces patients ayant été faites avant l'utilisation courante de l'IRM [1, 3, 15, 17]. Depuis lors, l'avènement de l'IRM cérébrale et médullaire pour les critères de SEP en 2010 ou de NMOSD en 2015, ainsi que l'arrivée de nouveaux biomarqueurs comme les anti-AQP4 et les anti-MOG pour la NMO ou encore les anticorps onconeuronaux pour les syndromes paranéoplasiques, ont permis d'améliorer la performance diagnostique et de diminuer le nombre de myélites aiguës idiopathiques. Certains cas restent cependant sans certitude diagnostique, notamment dans le contexte de myélite postinfectieuse, compte tenu de la normalité des examens biologiques et de la non-spécificité de l'IRM médullaire. Dans ces cas, le diagnostic est fortement suspecté sur la seule description d'un antécédent infectieux dans les semaines précédant la myélite.

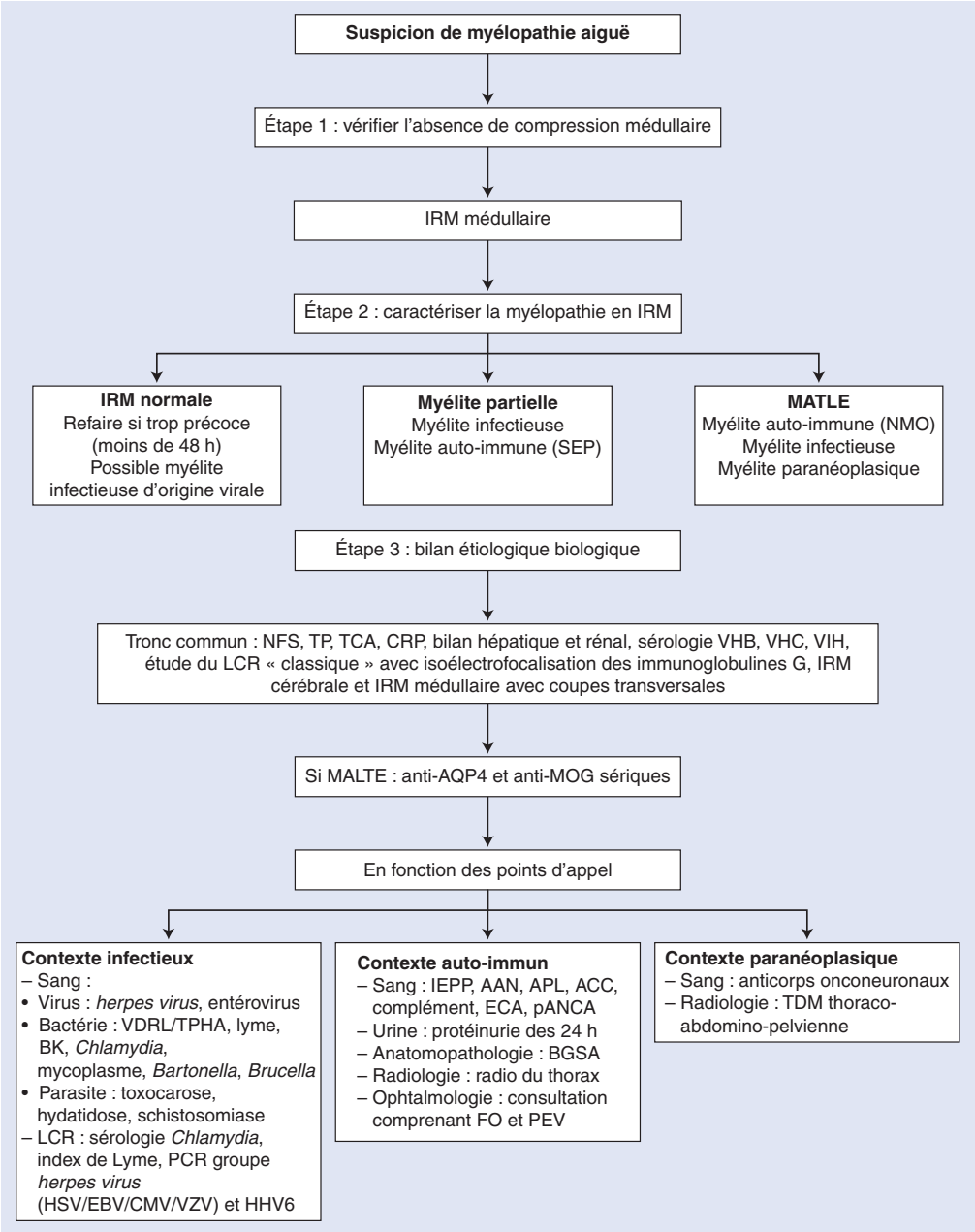

Figure 6. Conduite à tenir et bilan étiologique devant une suspicion de myélopathie aiguë. Après avoir éliminé une cause compressive et effectué un premier bilan correspondant au « tronc commun », trois situations cliniques principales sont à distinguer permettant d'orienter le bilan de seconde intention : contexte infectieux, contexte auto-immun, contexte paranéoplasique. Les causes toxiques ne nécessitent pas de bilan étiologique, le contexte et l'absence d'autres causes permettent de poser le diagnostic. NMO : neuromyélite optique ; MATLE : myélite aiguë transverse longitudinalement étendue ; SEP : sclérose en plaques ; BK : bacille de Koch ; NFS : numération formule sanguine ; TP : temps de prothrombine ; TCA : temps de céphaline activée ; CRP : C-réactive protéine ; VHB : virus de l'hépatite B ; VHC : virus de l'hépatite C ; VIH : virus de l'immunodéficience acquise humaine ; AQP4 : aquaporine-4 ; MOG : *myelin oligodendrocyte glycoprotein* ; LCR : liquide céphalorachidien ; IRM : imagerie par résonance magnétique ; VDRL/TPHA : *venereal disease research laboratory/Treponema pallidum hemagglutination assay* ; PCR : *polymerase chain reaction* ; HSV : virus herpes simplex ; EBV : virus d'Ebstein-Barr ; CMV : cytomégalovirus ; VZV : virus varicelle-zona ; HHV6 : *human herpes virus 6* ; IEPP : immunoélectrophorèse des protéines sériques ; AAN : anticorps antinucléaires (comprenant les antigènes solubles : ENA) ; APL : antiphospholipides ; ACC : anticoagulants circulants ; ECA : enzyme de conversion de l'angiotensine ; pANCA : anticorps anticytoplasme des polynucléaires neutrophiles à fixation périnucléaire ; BGSA : biopsie des glandes salivaires accessoires ; FO : fond d'œil ; PEV : potentiels évoqués visuels ; TDM : tomodensitométrie.

Un nouveau cadre nosologique semble se dégager, incluant des patients avec des myélites récurrentes idiopathiques isolées [117–120]. Cette entité pourrait être l'équivalent médullaire des névrites optiques récidivantes idiopathiques qui constituent un cadre nosologique à part [121, 122]. Cependant, là aussi, les études sont antérieures à la révision des critères de NMOSD en 2015, et souvent antérieures à l'avènement du test cellulaire pour les anti-AQP4 dont la sensibilité est très augmentée par rapport au test sur lame [123].

Tableau 4.
Anticorps onconeuronaux retrouvés dans les myélites paranéoplasiques.

Anticorps onconeuronal	Cancer les plus fréquemment retrouvés
Amphiphysine	CPPC, sein
Hu (ANNA-1)	CPPC
Ri (ANNA-2)	CPPC, carcinome mammaire
ANNA-3	CPPC
CV2/CRMP5	CPPC, thymome, sarcome utérin
Ma1	CPPC, sein, tube digestif, cellules germinales, lymphome non hodgkinien
Ma2	Tumeur germinale testiculaire
Yo (PCA-1)	Cancer de l'ovaire, sein, utérus
PCA-2	CPPC

CPPC : cancer pulmonaire à petites cellules.

Conduite à tenir devant une suspicion de myélopathie aiguë

Le diagnostic d'affection médullaire repose essentiellement sur la clinique. L'IRM, orientée par l'examen neurologique, permet d'en rechercher la cause et en particulier d'éliminer, en cas de myélopathie aiguë, une étiologie potentiellement chirurgicale comme une compression médullaire. Les autres techniques d'imagerie (radiographies standards, myélographie, scanner) n'ont plus leur place en première intention face à un syndrome médullaire non traumatique. Les données de l'IRM doivent être associées aux données cliniques, biologiques, sanguines, mais surtout du LCR, afin de guider le diagnostic étiologique.

Les myélopathies aiguës peuvent se traduire par un syndrome de myélopathie partielle (touchant une ou plusieurs voies de passage, en fonction de la localisation de la lésion) ou par une myélopathie transverse, caractérisée par un niveau lésionnel et une bilatéralité des symptômes. Cette distinction est importante, car elle peut guider le diagnostic étiologique, l'atteinte partielle étant par exemple plus évocatrice d'une SEP chez une femme jeune. L'ensemble de la conduite à tenir est schématisé sur la Figure 6.

Traitement

Le traitement est avant tout celui de la cause. Pour les myélites auto-immunes, liées à une EMAD ou idiopathiques, un consensus professionnel recommande de les traiter à la phase aiguë par de fortes doses de MP. L'absence d'études contrôlées ne permet pas de fixer une posologie précise. Cependant, de façon empirique, les patients sont traités à la dose de 1 g par jour pendant 3 à 5 jours. La place des corticoïdes per os n'est pas tranchée, mais s'ils sont utilisés, ils le sont le plus souvent en relais du MP. Une étude chez l'enfant a comparé l'évolution chez 12 patients traités par MP 1 g/1,73 m^2/j pendant 3 à 5 jours puis par une corticothérapie de relais, à une série historique de 17 enfants non traités ou traités par corticoïdes per os. À un mois, huit enfants sur 12 (66 %) avaient une marche autonome dans le groupe MP contre trois enfants sur 17 (17,6 %) dans le groupe contrôle ($p = 0,02$). À un an, plus d'enfants avaient totalement récupéré dans le groupe MP (75 % versus 23,5 %, $p = 0,006$) [124]. Les EP peuvent être envisagés chez les patients qui ne répondent pas aux corticostéroïdes à haute dose. Il est proposé aux patients 5 à 7 EP, au rythme d'une séance tous les deux jours sous contrôle de l'hémostase. Dans une étude randomisée en double aveugle, des patients ayant une myélite aiguë transverse ($n = 6$) ne répondant pas aux corticoïdes ont bénéficié d'EP réels ou simulés. Un changement de bras était effectué en cas de non-réponse au premier traitement par EP. Trois patients s'étaient nettement améliorés après EP réels, et aucun suite aux EP simulés [125].

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Recommandations

Cadre nosologique et stratégie diagnostique de la myélite aiguë transverse longitudinalement étendue

Nosology and etiologies of acute longitudinally extensive transverse myelitis

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RÉSUMÉ

La myélite aiguë transverse a connu de nombreuses appellations et définitions, reposant avant tout sur des critères cliniques. Le rôle de l'IRM dans l'exploration des myélites s'est précisé plus récemment après l'individualisation de la neuromyélite optique (NMO) en 2004. Cet examen a alors permis de préciser la valeur diagnostique et pronostique péjorative de la myélite aiguë transverse longitudinalement étendue (MATLE), définie par un hypersignal T2 étendu sur au moins trois métamères dans le plan longitudinal. Les limites de cette définition, la multiplicité des termes employés pour la caractériser ainsi que les nombreuses étiologies qui lui sont associées ont conduit notre groupe d'experts à préciser son cadre nosologique et ses étiologies. Nous avons mené une enquête nationale sur ce thème afin de

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longitudinalement étendue
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Sclérose en plaques

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proposer une nouvelle définition de la MATLE. Les examens complémentaires de première puis seconde intention ont été déterminés en fonction du contexte clinique. Des causes infectieuses/para-infectieuses, inflammatoires ou paranéoplasiques peuvent ainsi être identifiées. Il est indispensable que la cause d'une MATLE soit précisée dans un délai court, puisque la plupart de ses étiologies sont d'évolution sévère et nécessitent un traitement urgent.

ABSTRACT

Acute transverse myelitis had many names and definitions, based primarily on clinical criteria. The role of MRI in the exploration of myelitis has increased recently after the individualization of neuromyelitis optica (NMO) in 2004. This approach has enabled clarification of the diagnostic and prognostic value of acute longitudinally extensive transverse myelitis (LETM), defined by an extensive T2 lesion affecting three vertebral segments in the sagittal plane. The limitations of this definition, the multiplicity of terms used to characterize it as well as the large number of etiologies associated with it led our group of experts to clarify its etiology and nosology. We conducted a national survey on this subject in order to propose a new definition of LETM. Additional first- and second-intention examinations were determined according to the clinical context. Infectious/para-infectious, inflammatory or paraneoplastic causes can thus be identified. To determine within a short time the cause of LETM is essential, since most of its causes are severe and require urgent treatment.

1. Introduction

Depuis les premières descriptions des « myélites aiguës » en 1882 (Bastian, 1882), le diagnostic de myélite repose sur des critères cliniques, le suffixe « ite » désignant les affections médullaires d'origine inflammatoire, infectieuse ou paranéoplasique. Pour les affections médullaires de causes métaboliques, vasculaires, dégénératives ou traumatiques, le terme de myélopathie est préféré. Cette distinction lexicale reste d'usage encore aujourd'hui. Le qualificatif « aiguë » est ajouté lorsque le mode d'installation s'effectue sur quelques heures. La durée précise de l'installation des symptômes ou signes varie en fonction des auteurs : installation en plus de 48 heures mais moins de 3 semaines (Berman et al., 1981), nadir entre 4 heures et 3 semaines (Transverse Myelitis Consortium Working Group, 2002) ou aggravation maximale en 24 heures (Jeffery et al., 1993). Quelle que soit la définition utilisée, ces intervalles de temps permettent en théorie de s'affranchir des myélopathies vasculaires et traumatiques d'apparition soudaine, et des myélopathies métaboliques, dégénératives ou post-radiques d'apparition plus progressive et chronique. Néanmoins, un certains degré de chevauchement entre ces étiologies et leur mode d'installation peut exister en pratique, mettant ainsi en avant l'importance du contexte de survenue comme développé plus loin. Par la suite, le terme « transverse » a été ajouté et utilisé de façon équivoque par plusieurs équipes : « myélite transverse », « myélite transverse partielle », « myélite transverse complète », « myélite transverse extensive » ou « myélite longitudinale transverse extensive ». La multiplicité des termes employés prêtait à confusion et a conduit en 2002 à la publication d'un article de revue dans lequel les auteurs ont défini les myélites aiguës transverses idiopathiques par la présence d'un niveau sensitif à l'examen (Transverse Myelitis Consortium Working Group, 2002). Une définition complémentaire rapportant les spécificités cliniques de ce type de myélite en fonction de leur caractère complet ou partiel a été publiée en 2007 (Scott, 2007) et est rappelée dans le Tableau 1. L'ensemble de ces définitions concernant la myélite transverse est clinique, aucune définition radiologique de ces termes ou même aucune corrélation clinico-radiologique n'ayant été rapportée depuis lors. Cependant, l'individualisation récente de la neuromyélite optique (NMO) après la découverte des anticorps anti-aquaporine-4 (anti-AQP4) en 2004, a révélé la valeur prédictive diagnostique et pronostique péjorative de l'extension longitudinale d'une myélite, définie en IRM par un hypersignal T2 étendu sur au moins 3 métamères dans le plan longitudinal (Wingerchuk et al., 2006). Cette donnée, intégrée dans les critères diagnostiques de la NMO depuis 1999, prend plus d'importance encore en 2006 puis 2008 (Wingerchuk et al., 2006 ; Wingerchuk et al., 1999 ; Miller et al., 2008). Les termes anglo-saxons utilisés pour décrire la myélite de la NMO sont alors « myélite extensive », « myélite transverse extensive » ou encore « myélite longitudinale transverse extensive ».

À cette forme particulièrement étendue de myélite aiguë s'oppose le cadre nosologique des myélites partielles, caractérisées par une lésion médullaire focale dans le plan transversal, souvent triangulaire à base externe (de Seze et al., 2001). La première cause de ce type de myélite est la sclérose en plaques (SEP), une myélite étendue dans les plans transversaux ou longitudinaux n'étant observée dans cette pathologie que dans moins de 1 % des cas (Scott, 2007). Le terme consacré de « myélite aiguë partielle » est également rapporté sous l'appellation « myélite aiguë partielle transverse », ajoutant encore plus à la confusion qui existe autour de ces différents termes. Par la suite, la confrontation de ces deux entités en terme de diagnostic et de pronostic a

Tableau 1 – Définitions sémiologiques de la myélite transverse dans la littérature.

Myélite transverse idiopathique (Transverse Myelitis Consortium Working Group, 2002)	Myélite transverse complète (Scott, 2007)	Myélite transverse partielle (Scott, 2007)
Atteinte sensitive, motrice ou dysautonomique d'origine médullaire	Atteinte motrice modérée ou sévère symétrique et dysautonomie d'origine médullaire	Atteinte légère sensitive et/ou motrice, bilatérale ou unilatérale d'origine médullaire. Quant un déficit sévère est présent, une asymétrie marquée est observée
Symptômes bilatéraux (non nécessairement symétriques) définissant un niveau sensitif	Présence d'un niveau sensitif symétrique	Symptômes définissant un niveau sensitif (bilatéral ou unilatéral) ou présence d'une IRM typique de myélite

placé l'IRM médullaire en première ligne des examens complémentaires. Cet examen, devenu un élément incontournable de la réflexion stratégique clinique et thérapeutique, doit désormais être réalisé rapidement en cas de suspicion d'atteinte médullaire.

Par conséquent, l'utilisation de plus en plus courante de l'IRM médullaire en coupes sagittales et axiales ainsi que la valeur diagnostique et pronostique de la myélite étendue dans le domaine des pathologies inflammatoires du système nerveux central (SNC) nécessitent de reconsidérer le cadre nosologique des myélites aiguës transverses longitudinalement étendues (MATLE) afin de guider au mieux le clinicien dans sa démarche diagnostique.

2. Méthode

Un consortium d'experts dans le cadre du projet Nomadmus a réuni différents spécialistes de la NMO et des pathologies médullaires. La première étape a consisté à définir le cadre nosologique de la MATLE. Ensuite, il a été demandé à chaque expert représentant les villes de Lille, Lyon, Marseille, Nantes, Paris, Strasbourg et Toulouse de proposer une démarche diagnostique face à une MATLE. Les différentes propositions ont alors été discutées au sein du groupe. Dans un second temps un comité scientifique composé de spécialistes expérimentés dans le domaine des pathologies inflammatoires du SNC a validé les propositions faites par les experts. L'ensemble de la démarche est illustrée dans la Fig. 1. La dernière étape a été de proposer une stratégie diagnostique et étiologique en fonction des différents contextes rencontrés en pratique clinique.

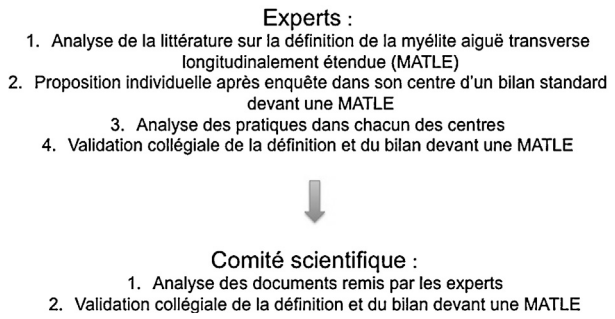

Fig. 1 – Procédure utilisée pour établir un consensus au sein du groupe Nomadmus.

3. Résultats

3.1. *Définitions cliniques*

La sémiologie d'une myélopathie se définit par un syndrome sous-lésionnel correspondant à une somatotopie médullaire, souvent caractérisée par une distribution bilatérale des signes pyramidaux ou sensitifs, un niveau sensitif franc ou une atteinte sphincterienne ou thermoalgique isolée. Un syndrome lésionnel signe l'atteinte radiculaire : principalement observé dans les causes compressives ou tumorales, il est souvent absent dans les autres cas. Enfin un syndrome rachidien caractérisé par une douleur à la mobilisation des épineuses et/ou une contraction des muscles paravertebraux est observé essentiellement dans les processus tumoraux. Une donnée importante est le fait que le niveau clinique d'un syndrome lésionnel ou sous-lésionnel ne correspond pas toujours au niveau de la lésion visible en IRM, celle-ci étant souvent située au-dessus notamment dans les causes inflammatoires.

Le terme « myélite » est un terme qui fait référence à un mécanisme inflammatoire, et sera donc consacré a posteriori, après l'identification de la cause. Cependant, le diagnostic est orienté d'emblée par le mode d'installation. En effet, un mode d'installation aigu, que nous avons défini par un nadir des symptômes entre 4 heures et 3 semaines, permet de s'affranchir des causes vasculaires et traumatiques, le plus souvent d'apparition soudaine, ainsi que des causes métaboliques, dégénératives et post-radiques le plus souvent d'installation progressive.

Concernant la myélite transverse, nous avons retenu la définition du consortium d'expert de 2002, elle-même reprise en grande partie dans la définition de Scott de la myélite transverse complète. Dans cette définition, la myélite transverse correspond cliniquement à un syndrome médullaire complet, i.e. associant un déficit sensitivo-moteur bilatéral (plus ou moins symetrique) et des troubles sphinctériens (Transverse Myelitis Consortium Working Group, 2002 ; Scott, 2007).

L'atteinte longitudinale ne peut pas être suspectée cliniquement de façon isolée et ne peut être précisée que par l'IRM. Elle est presque systématiquement associée à une atteinte étendue dans le plan transversal, caractérisée alors par un déficit bilatéral, sévère, et à tous les modes. Il est important de noter que le caractère longitudinalement étendu ne préjuge pas du type de syndrome médullaire observé sur le plan clinique. À l'opposé, dans notre expérience, la myélite partielle peut être définie par un déficit unilatéral ou incomplet, moteur

ou sensitif ou sphinctérien. Cette définition est très proche de celle mentionnée par Scott de la myélite transverse partielle (Scott, 2007).

3.2. *Définitions en IRM*

L'IRM doit explorer l'ensemble de la moelle épinière pour déterminer l'étendue longitudinale de la lésion. Un plan de coupe sagittal sera suivi d'un plan transversal centré sur la lésion. Ainsi seront réalisées des séquences pondérées en T2 complétées dans certains centres par une séquence *Short Tau Inversion Recovery* (STIR), puis des séquences pondérées en T1, suivies si nécessaires de séquences pondérées en T1 après injection de gadolinium. On pourra également évoquer l'utilisation dans certains centres de la séquence *Phase Sensitivie Inversion Recovery* (PSIR) qui est probablement la meilleure séquence actuellement pour différencier des lésions juxtaposées d'une lésion unique. Dans tous les cas, l'acquisition en 1.5 Teslas reste préférable compte tenu des nombreux artefacts visibles sur les IRM 3 Teslas.

Un examen réalisé dans les premières heures après l'apparition des symptômes, peut être normal et sera reconduit dans les 48 heures. L'IRM ne permet pas à elle seule d'affirmer un mécanisme purement inflammatoire, puisque l'œdème ou la prise de contraste ne sont pas spécifiques et s'observent dans d'autres situations cliniques comme l'ischémie médullaire ou la myélopathie post-radiothérapie (de Seze et al., 2001).

Il n'existe pas de définition radiologique en IRM de la myélite transverse, cette dernière étant avant tout clinique. Ce sujet est étayé par l'expérience acquise dans chaque centre. Pour certains il s'agit de myélite transverse complète radiologique si l'hypersignal T2 s'étend sur toute la largeur de la moelle, et transverse partielle radiologique si celui-ci fait plus de 50 % de la surface transversale sans concerner toute la surface médullaire. Pour d'autres la myélite transverse radiologique est définie par un hypersignal T2 étendu sur au moins 50 % de la surface en axial et la myélite partielle par un hypersignal étendu sur moins de 50 % de la surface en axial. Dans tous les cas il semble que le terme transverse soit associé radiologiquement à une lésion médullaire étendue sur au moins 50 % de la surface transversale de la moelle. C'est cette dernière définition qui a permis de dégager un consensus au sein du groupe d'expert.

Le caractère étendu dans le plan longitudinal est quant à lui bien défini, caractérisé par une lésion s'étendant de façon continue sur au moins 3 corps vertébraux (Fig. 2).

3.3. *Définition de la MATLE*

En s'appuyant sur les termes définis plus haut et sur l'expérience acquise dans les différents centres en France ayant participé à l'étude, nous avons défini la MATLE par l'association de données cliniques et IRM (Tableau 2). Le

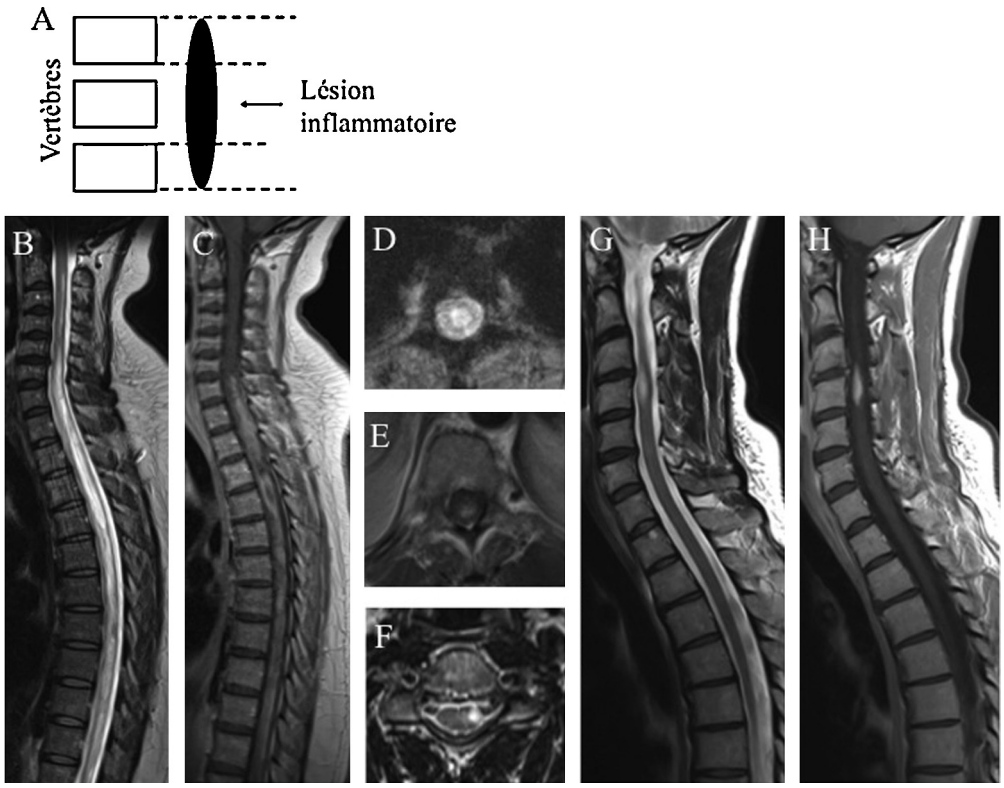

Fig. 2 – Aspect IRM des myélites étendues et des myélites partielles. La myélite longitudinalement étendue est définie en IRM par une extension de la lésion dans le plan sagittal au moins égale à 3 métamères (A). Myélite étendue en IRM dans le plan sagittal en séquence T2 (B) et T1 avec injection de gadolinium (C) mais aussi dans le plan axial en séquence T2 (D) et T1 avec injection de gadolinium (E). Myélite partielle en IRM sur la séquence T2 dans le plan axial (F) et sagittal (G), prenant le contraste après injection de gadolinium sur la séquence T1 (H).

tableau clinique de myélite transverse pouvant être parfois atypique, sans atteinte dysautonomique par exemple, on retiendra le terme « transverse » d'après sa présentation clinique ou en IRM, la présence d'une lésion étendue dans le plan transversal en IRM suffisant à poser ce diagnostic. Le caractère longitudinalement étendu reprend les données de l'IRM en coupe sagittale, montrant une myélite étendue sur plus de 3 métamères.

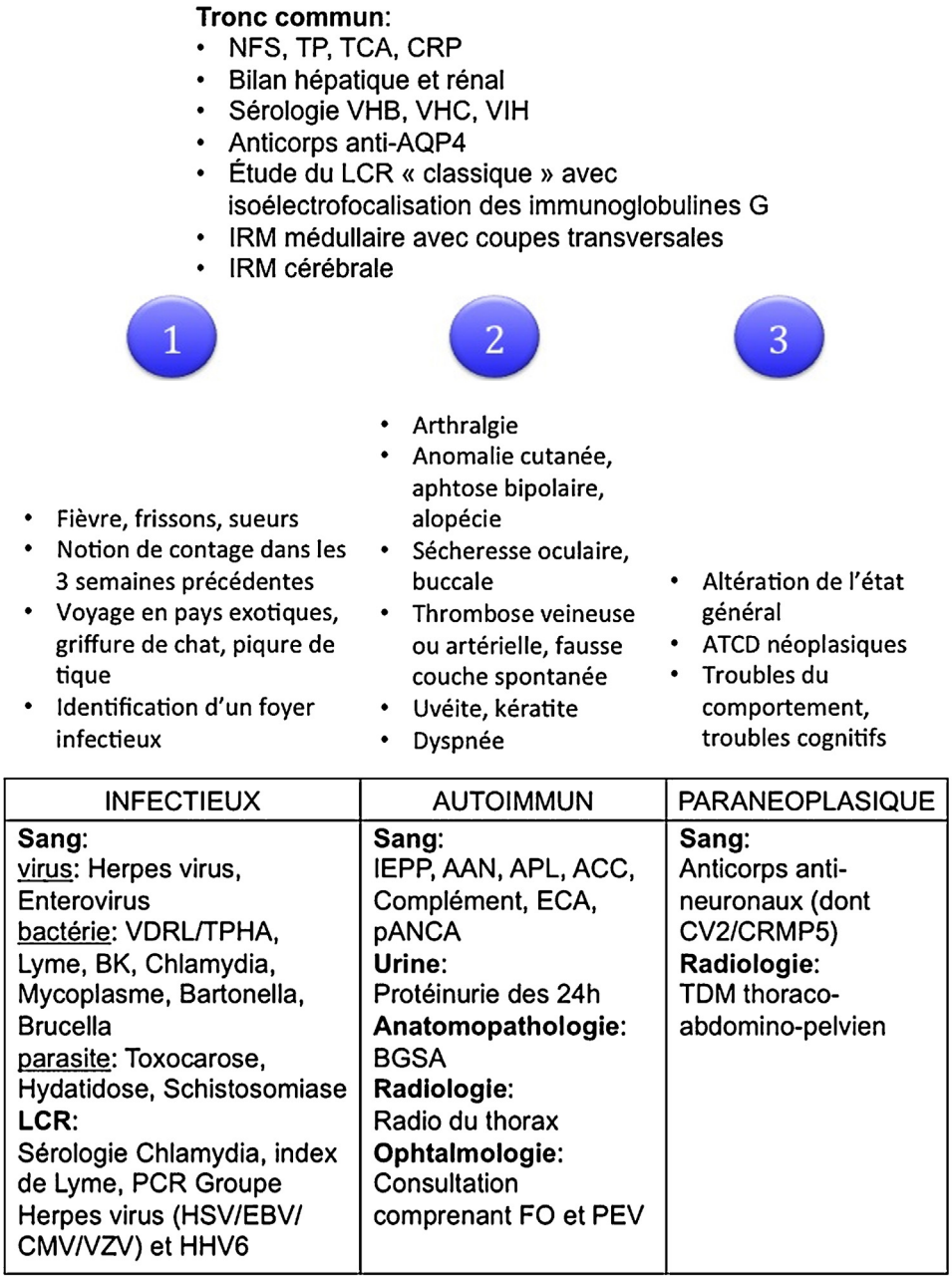

Fig. 3 – Bilan étiologique de première et seconde intention en cas de myélite aiguë transverse longitudinalement étendue. Après avoir éliminé une cause vasculaire et effectuer un premier bilan correspondant au « tronc commun », trois situations cliniques sont à distinguer permettant d'orienter le bilan de seconde intention : 1. Contexte infectieux ; 2. Contexte auto-immun ; 3. Contexte paranéoplasique. NFS : numération formule sanguine ; TP : temps de prothrombine ; TCA : temps de céphaline activée ; CRP : C-réactive protéine ; VHB : virus de l'hépatite B ; VHC : virus de l'hépatite C ; VIH : virus de l'immunodéficience acquise humaine ; AQP4 : aquaporine-4 ; LCR : liquide céphalo-rachidien ; IRM : imagerie par résonance magnétique ; VDRL/TPHA : *venereal disease research laboratory/Treponema Pallidum hemagglutination assay* ; PCR : *polymerase chain reaction* ; HSV : *herpes simplex virus* ; EBV : *Ebstein-Barr virus* ; CMV : *cytomegalovirus* ; VZV : *varicelle-zona virus* ; HHV6 : *human Herpes virus 6* ; IEPP : immunoélectrophorese des protéines sériques ; AAN : anticorps anti-nucléaires (comprenant les antigènes solubles : ENA) ; APL : anti-phospholipides ; ACC : anti-coagulants circulants ; ECA : enzyme de conversion de l'angiotensine ; pANCA : anticorps anti-cytoplasme des polynucléaires neutrophiles à fixation périnucléaire ; BGSA : biopsie des glandes salivaires accessoires ; FO : fond d'œil ; PEV : potentiels évoqués visuels ; TDM : tomodensitométrie.

Tableau 2 – Cadre nosologique de la myélite aiguë transverse longitudinalement étendue.

	Cadre nosologique
Myélite aiguë	Nadir entre 4 heures et 3 semaines
Transverse	Syndrome médullaire bilatéral (symétrique ou non) moteur, sensitif et sphinctérien, ou extension de la lésion en IRM dans le plan transversal sur une surface $\geq$ 50 %
Longitudinalement étendue	Extension de la lésion en IRM dans le plan sagittal $\geq$ 3 segments

3.4. *Les étiologies des MATLE*

Partant de la définition ci-dessus, la stratégie exploratoire a été déclinée par les différents centres en fonction des contextes cliniques. Trois situations sont proposées correspondant aux contextes infectieux/para-infectieux, auto-immuns et paranéoplasiques. La stratégie diagnostique est illustrée dans la Fig. 3. On notera que l'encéphalomyélite aiguë disséminée est considérée comme une réaction immunoallergique post-infectieuse pour laquelle l'atteinte médullaire est souvent étendue (Palace, 2011 ; Pohl and Tenembaum, 2012 ; Tenembaum et al., 2007).

Alors que le mode d'installation et les contextes cliniques permettent d'orienter vers ces différentes étiologies, certaines situations cliniques atypiques sont à connaître. À titre d'exemple, un syndrome médullaire révélant un infarctus peut s'installer sur plus de 4 heures, parfois en plusieurs temps, ou être longitudinalement étendu (Masson et al., 2004 ; Novy et al., 2006). Des pathologies d'évolution classiquement progressive (fistule durale, cause métabolique ou post-radiques) peuvent se révéler de manière aiguë à l'occasion d'une décompensation rapide.

C'est pour ces raisons qu'il est important d'éliminer en premier lieu la présentation atypique d'une étiologie vasculaire ou métabolique fortement influencée par le contexte de survenue. Pour les myélopathies « ischémiques », ces contextes sont les suivants : patient masculin ; âge $>$ 50 ans ; facteurs de risques cardio-vasculaires. Pour les myélopathies « métaboliques » il s'agit des éléments suivants : perte de poids ; intoxication éthylique ; troubles du comportement alimentaire ; malabsorption ; chirurgie digestive ; troubles cognitifs ; troubles psychiatriques/hallucinations. Dans ce dernier cas, les dosage sériques des vitamines B12 (par le dosage de l'acide methylmalonique) et B9, de l'homocystéine, du cuivre et de la céruléoplasmine seront réalisés.

Enfin, nous n'avons pas inclus dans l'arbre diagnostique la recherche de la sérologie HTLV-1 et cela pour plusieurs raisons :

- cliniquement, la myélite n'est jamais aiguë, la minorité des formes sub-aiguës s'associant à un canal rachidien étroit ;
- la myélite n'est qu'exceptionnellement transverse ;
- chez un sujet qui n'est pas originaire d'une zone d'endémie (Antilles, Afrique sub-saharienne, Amérique du Sud, Japon) la sérologie HTLV-1 n'est qu'exceptionnellement positive (De Castro-Costa et al., 2006).

Par ailleurs, même si ce contexte est présent, le caractère longitudinal reste exceptionnel. En effet, dans la base de données antillaise d'au moins 100 MATLE, la positivité de la sérologie HTLV-1 n'a été observée qu'une seule fois. Là aussi, elle doit être interprétée en fonction de la prévalence de la maladie dans cette région (Olindo et al., 2010).

4. Conclusion

L'individualisation récente de la NMO a permis de souligner la valeur diagnostique majeure de la MATLE. Elle n'est qu'exceptionellement révélatrice d'une SEP. Ces données soulignent l'importance de réaliser une IRM avec des plans sagittaux et axiaux. Les étiologies infectieuses/para-infectieuses, inflammatoires ou paranéoplasiques relèvent, elles, d'une prise en charge spécifique. Elles sont suspectées devant un contexte clinique évocateur et les résultats des examens complémentaires de première ligne. Les examens de deuxième intention, plus complets, permettent de préciser ces étiologies et conduisent à un diagnostic étiologique rapide. Un délai court pour établir la cause d'une MATLE est d'autant plus indispensable que la plupart de ses étiologies sont graves et nécessitent un traitement urgent.

Déclaration d'intérêts

Les auteurs B. A., D. L., R. M., F. C., et F. D. D. déclarent ne pas avoir de conflits d'intérêts en relation avec cet article.

N. C. déclare avoir reçu des honoraires comme consultant ou pour des présentations de Biogen Idec, Almirall, Novartis, Merck-Serono, Teva Pharma et fait parti du comité éditorial du journal de la ligue française contre la sclérose en plaques.

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Characterization of neuromyelitis optica and neuromyelitis optica spectrum disorder patients with a late onset

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Abstract

Background: Few data are available for patients with a late onset (≥ 50 years) of neuromyelitis optica (LONMO) or neuromyelitis optica spectrum disease (LONMOSD), defined by an optic neuritis/longitudinally extensive transverse myelitis with aquaporin-4 antibodies (AQP4-Ab).

Objective: To characterize LONMO and LONMOSD, and to analyze their predictive factors of disability and death.

Methods: We identified 430 patients from four cohorts of NMO/NMOSD in France, Germany, Turkey and UK. We extracted the late onset patients and analyzed them for predictive factors of disability and death, using the Cox proportional model.

Results: We followed up on 63 patients with LONMO and 45 with LONMOSD during a mean of 4.6 years. This LONMO/LONMOSD cohort was mainly of Caucasian origin (93%), women (80%), seropositive for AQP4-Ab (85%) and from 50 to 82.5 years of age at onset. No progressive course was noted. At last follow-up, the median Expanded Disability Status Scale (EDSS) scores were 5.5 and 6 in the LONMO and LONMOSD groups, respectively. Outcome was mainly characterized by motor disability and relatively good visual function. At last follow-up, 14 patients had died, including seven (50%) due to acute myelitis and six (43%) because of opportunistic infections. The EDSS 4 score was independently predicted by an older age at onset, as a continuous variable after 50 years of age. Death was predicted by two independent factors: an older age at onset and a high annualized relapse rate.

Conclusion: LONMO/LONMOSD is particularly severe, with a high rate of motor impairment and death.

Keywords

Aging, aquaporin-4, aquaporin antibody, late onset, neuromyelitis optica, morbidity, mortality, prognosis, spectrum disorders

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Introduction

Neuromyelitis optica (NMO) is a rare inflammatory disease affecting predominantly both the optic nerve and the spinal cord. Since the discovery of antibody against aquaporin-4 (AQP-4 Ab) in 50–80% of Caucasian NMO patients,[1–5] the clinical disorder spectrum is defined by isolated optic neuritis (ON) or longitudinally extensive transverse myelitis (LETM) with AQP-4 Ab.[6] These patients are highly likely to develop the definite bifocal form of NMO.

Since the availability of AQP4-Ab test,[7,8] epidemiological data show, in Caucasian NMO patients, that there is a female/male ratio of 3–6 females per male, and an average onset at 33–40 years of age.[1–3,9] The main important points associated with these epidemiological data are the high proportion of females among AQP-4 seropositive patients,[1,3,9] and specific clinical and paraclinical features in children, resulting in brain involvement and fast visual disability.[9–11] Importantly, contrary to multiple sclerosis (MS), the range of NMO onset age is larger, including 25% of patients with a pediatric onset or a late onset after 50 (LONMO).[2,3,9] Whereas data are now available for pediatric NMO patients,[10–13] nobody has just analyzed a homogeneous group of NMO/NMOSD patients with onset age $\geq$ 50, which represent a population of patients with a higher risk of death than young patients.[9,10,14]

In the literature, sparse data for LONMO are reported in 50–77-year-old patients.[9,15–17] An additional autopsy case reveals NMO onset at 81 years, but brain involvement in that patient raised the question of possible MS.[18] To date, observational studies show a predominance of myelitis during the first attack and a possible susceptibility to motor impairment or death in AQP-4 Ab-seropositive LONMO cases;[9,14] however, in these studies, interfering factors like AQP-4 Ab seropositivity or ethnicity may also play a role in the disability outcome in LONMO.

There are still questions about demographical and clinical data in elderly patients, as well as the course of the disease and response or tolerance to treatment, in patients with LONMO.

Here we report the demographic, clinical and paraclinical features, and disease outcome in a large population of LONMO/LONMOSD patients, mainly of Caucasian origin, sampled from four European and Turkish cohorts.

Patients and methods

We retrospectively studied LONMO/LONMOSD patients sampled from two national NMO and NMOSD cohorts, on behalf of French NMO group (NOMADMUS) and German NMO centers, but also the Turkish LONMO group and the UK cohorts (Liverpool and Oxford centers for the British NMO national service). These centers take part in the European Devic Neuromyelitis Optica (EDEN) project. In total, we identified 430 patients with NMO or NMOSD from January 2012 through September 2012, the study end point.

Among the late onset NMO/NMOSD patients, 63 NMO patients fulfilled the 2006 criteria,[19] and 45 had either isolated ON or isolated LETM and AQP-4 Abs (thus, NMOSD).[6] Data were collected from hospital files or using standardized assessment forms designed for LONMO and were entered in the European Database for MS, adapted to NMO specificities. Ethical approval, data confidentiality and security were ensured, in keeping with the recommendations of the appropriate ethics committee, and the study was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki.

For each patient, we recorded demographic data (gender, race, age at onset, follow-up), medical history of attacks, disability and treatments received (type and number). A monophasic course was defined by one single attack within days to 4 weeks. Residual disability was assessed at least 6 months after the last attack, in terms of Expanded Disability Status Scale (EDSS) score and severe residual visual loss (SRVL), defined as visual acuity (VA) $\leq$ 1/10 at least in one eye (corresponding to +1 on LogMAR or 20/200 on Snellen Visual Acuity charts). We also recorded laboratory (AQP-4 Ab and cerebrospinal fluid (CSF)) and magnetic resonance imaging (MRI) data (number and location of lesions). Most of the patients from France, UK and Germany were tested for AQP-4 Ab by cell-based assay, using the described immunofluorescence technique on AQP-4 M23-transfected cells.[7,8] Only two patients from Germany were tested with radioimmunoprecipitation assay.[20] Patients from Turkey were tested for AQP-4 IgG Abs by the initial indirect immunofluorescence assay.[5] Brain MRIs were classified as either not fulfilling or fulfilling MS criteria.[21]

We compared categorical data using the chi-squared or Fischer test and quantitative data using the Mann-Whitney test. We used the Kaplan-Meier technique to estimate time to assignment of EDSS scores. We compared survival curves using the log-rank test. We analyzed the predictive factors of disability using the Cox proportional model. The variables tested were: gender, age at onset, year at onset, disease duration, number of T2-hyperintense lesions on initial brain MRI, seropositivity for AQP-4 Ab, presence of oligoclonal bands (OCB) in CSF, number of white cells in CSF, initial topography of the disease, optico-spinal delay, time between first and second attack, mean annualized relapse rate (ARR), type of treatment (immunosuppressive treatment (oral or intravenous), immunomodulatory treatment, or a combination), and time between disease onset and first treatment. Age at onset was analyzed as a continuous variable in the population of LONMO/LONMOSD patients. Two-sided p values < 0.05 were considered significant. We performed all computations using Statistical Package for the Social Sciences (SPSS) for Windows, version 14.0.

Results

Demographic and disease-related characteristics

We followed up on 63 patients with LONMO and 45 with LONMOSD over a mean of 4.6 years. The LONMO/LONMOSD cohort was mainly of Caucasian origin (93%), female (81.5%) and seropositive for AQP-4 Ab in 85% (74% in NMO patients). The median age was 56 years and the eldest patient was 82.5 years old at disease onset. Myelitis was the first attack in 67% of cases, followed by ON in 29%. A relapsing form was observed in 82% of cases, a monophasic form in 18% and we noted no progressive form. The mean ARR was 1.4 with a mean first inter-attack interval of 17.5 ± 24 months. The mean number of lesions on initial brain T_2 MRI was 2.2 ± 3.2 and 9% of patients fulfilled Barkhof's criteria. We found that 90% of the patients experienced LETM during the disease course, with a preferential location for the cervical area, followed by the cervico-thoracic area. We observed CSF OCB in 26% of patients. Separate assessment of LONMO/LONMOSD and AQP-4 Ab+/AQP-4 Ab– patients are detailed in **Table 1**. Compared to LONMO, LONMOSD patients were characterized by a larger representation of initial myelitis ($p < 0.01$), a more monophasic disease course ($p = 0.015$) and fewer brain MRI lesions ($p = 0.04$). Furthermore, patients with NMO and AQP-4 Ab+ were most likely women ($p < 0.01$) than the NMO AQP-4 Ab seronegative patients.

Disability and death

At the end of the follow-up, the final median EDSS score was 5.5 in the LONMO and 6 in the LONMOSD groups, and 14 patients had died at the end of the study after a median of 5 attacks (**Table 2**). Their deaths were mainly due to extensive myelitis, but six patients died because of sepsis under immunosuppressive therapy. Among them, two patients died after the first attack: Patients number 3 and 14 died after extensive cervical myelitis (seen enhanced by gadolinium). Deaths related to NMO were secondary to extensive myelitis with respiratory failure ($n = 6$) and pulmonary embolism ($n = 1$).

In the whole cohort, the final median functional system scores were significantly higher for pyramidal functions (functional EDSS score of 3 corresponding to severe monoparesis, or mild/moderate paraparesis or hemiparesis) compared to bowel and bladder, sensory and visual functions (**Figure 1**). In LONMO/LONMOSD patients, the median time to EDSS 4 was 1.3 years (95% CI 0.6–2.1) and to death was 13.2 years (95% CI 12.9–13.5), as illustrated in **Figure 2**. The median time to immunosuppression was 1.2 years, ranging from 0–11.2 years. The analysis of subgroups showed no difference in median EDSS scores between the LONMO/LONMOSD ($p = 0.4$) and AQP-4 Ab+/AQP-4 Ab– ($p = 0.47$) patients.

In LONMO patients, the final median residual functional system score for visual function was 2 (worse eye VA between 0.67–0.34) and six patients experienced a SRVL in at least one eye. Only three patients with isolated ON were included in our study. In these patients, one had a VA < 0.1 in at least one eye at last follow-up, while the second had a VA between 0.2–0.1 and the third a VA between 0.67–0.34.

Using a Cox proportional model for univariate analysis, the predictive factors for the assignment of EDSS 4 was age (HR 1.055; 95%CI[1.018–1.093]; $p = 0.005$); and for death were age (HR 1.127; 95%CI [1.042–1.218]; $p = 0.003$), the historical year of onset (HR 1.331; 95%CI [1.052–1.683]; $p = 0.005$) and the mean ARR (HR 2.629; 95%CI [1.345–5.138]; $p < 0.001$). Using a Cox proportional model for multivariate analysis, the predictive factors of the assignment of EDSS 4 was age (HR 1.052; 95%CI [1.013–1.094]; $p = 0.02$); and for death were age (HR 1.128; 95%CI [1.025–1.241]; $p < 0.001$) and the mean ARR (HR 2.732; 95%CI [1.356–5.501]; $p < 0.001$). If only considering AQP-4 seropositive patients, multivariate analysis found only age (HR 1.108; 95%CI [1.021–1.201]; $p < 0.001$) as a predictive factor of death.

Discussion

We describe a large, mainly Caucasian population of NMO and NMOSD patients with a late onset, in a multinational cohort. For these two disease presentations, we encountered similar data except for an overrepresentation of monophasic myelitis in the NMOSD patients. Outcome was mainly characterized by motor disability, yet good visual function. We found a high proportion of deaths related to NMO, secondary to extensive myelitis or opportunistic infection due to immunosuppressive therapy. Death was predicted by two independent factors in these LONMO/LONMOSD patients: (a) an older age at onset; (b) a high ARR. This data argue for preferential spinal cord involvement in this population of patients, as was shown by the high proportion of myelitis at onset and also by the low rate of improvement following myelitis. As for death, the related EDSS 4 score was independently predicted by an older age at onset in the LONMO/LONMOSD patients.

The first results in our study attempted to characterize and therefore identify differences among subgroups, corresponding to two frequent clinical presentations according to a bifocal (NMO) or monofocal (NMOSD) disease course, and to AQP-4 Ab+ or AQP-4 Ab– serological status. In NMOSD, we observed a predominance of LETM, compared to ON. One explanation could be due to an overrepresentation of LETM in AQP-4 Ab+ patients, because AQP-4 seropositivity is quite low in unselected ON cohorts. Effectively, except in some atypical cases of bilateral simultaneous ON or severe visual residual impairment despite corticosteroid therapy, AQP-4 Abs are not tested systematically in ON. This is not the case in LETM, in which AQP-4

Table1. Demographic and disease-related characteristics of late-onset NMO/NMOSD patients.

	Whole cohort of late onset patients			*p*	Late onset NMO		*p*
	NMO + NMOSD	NMO	NMOSD		AQP4Ab+	AQP4Ab–	
Number of patients	108	63	45	NS	46	17	NS
Number of female (%)	88 (81.5)	50 (79)	38 (84.4)	NS	41 (89.1)	9 (52.9)	< 0.01
Median age,	56.6	55.7	58.9	NS	56	55	NS
[range] years	[50–82.5]	[50–82.5]	[50–76]		[50–82.5]	[50–75]	
Number of Caucasian (%)[a]	80 (93)	45 (96)	35 (94.6)	NS	32 (88.9)	13 (100)	NS
Mean follow-up ± SD years	4.6 ± 4	5 ± 4	4 ± 4	NS	5.1 ± 4.2	4.6 ± 3.4	NS
Initial event *n*, (%)[a]							
Myelitis	72 (67.3)	30 (48.4)	42 (93.3)	< 0.01	20 (44.4)	9 (53)	NS
Optic neuritis	31 (29)	28 (45.1)	3 (6.7)		22 (48.9)	6 (35.3)	
Both	4 (3.7)	4 (6.5)	-		3 (6.7)	2 (11.7)	
Mean first interattack interval ± SD months	17.5 ± 24	18 ± 27.7	16.6 ± 16.1	NS	20.2 ± 31.2	15.3 ± 17.5	NS
Mean opticospinal interval ± SD months	21.5 ± 29.5	21.5 ± 29.5	-	-	24.4 ± 32.7	12.5 ± 14.4	NS
Ratio relapsing:monophasic:progressive	89:19:0	57:6:0	32:13:0	0.015	42:4:0	15:2:0	NS
Total *n* of attacks	359	216	143	NS	182	34	NS
Mean ARR	1.4	1.6	1.2	NS	1.8	0.9	NS
AQP-4 Ab %	85	74	100	-	100	0	-
Mean *n* T_2 lesions on initial brain MRI	2.2 ± 3.2	2.5 ± 3.5	1.3 ± 2.8	0.04	2.9 ± 3.9	1.3 ± 1.2	NS
Patients with Barkhof criteria %	9	11.1	4.4	NS	15.2	0	NS
OCB in CSF %[a]	25.9	18	37.1	NS	20	20	NS
Preventive treatments used *n* (%)[a]							
Immunosuppressors	87 (89)	51 (88.3)	36 (90)	NS	35 (85.4)	16 (94)	NS
Immunomodulators (IFN, GA)	4 (4)	1 (1.7)	3 (7.5)		1 (2.4)	0	
Both	4 (4)	3 (5)	1 (2.5)		2 (4.9)	1 (6)	
None	3 (3)	3 (5)	0		3 (7.3)	0	
Mean time to first treatment ± SD, years	1.9 ± 2.4	2 ± 2.5	1.7 ± 2.2	NS	2.4 ± 2.7	1.2 ± 1.5	NS
Death *n*, (%)	14 (13)	12 (19)	2 (4.4)	NS	11 (23.9)	1 (6)	NS

[a]Missing data are not included in the calculation. Data were available for OCB in 85/108 patients.
Ab: Antibody; AQP-4: aquaporin 4; CSF: cerebrospinal fluid; GA: glatiramer acetate; IFN: interferon; MRI: magnetic resonance imaging; NMO: neuromyelitis optica; NMOSD: NMO spectrum disease; NS:; OCB: oligoclonal bands.

Table 2. Characteristics of deceased patients in the late onset NMO/NMOSD cohort of patients.

Patient number	Gender	Race	Age at onset	Yr of onset	Duration of the disease (yrs)	Disease Course	AQP4 assay	Number of attacks	ARR	Topography of the first attack	First interattack interval (mo)	Optico-spinal interval time (mo)	Cause of death
1	F	C	50.50	1998	13.25	R NMO	+	4	0.30	ON	64.8	64.8	Sepsis secondary to pneumonia
2	F	C	53.25	2003	5.75	R NMO	+	5	0.87	SC	2	9	Sepsis secondary to pneumonia
3	F	C	53.92	2009	0	Mo NMOSD	+	1	NA	SC	NA	NA	Extensive myelitis
4	F	C	54.08	2001	4.67	R NMO	+	2	0.43	ON + SC	53	0	Extensive myelitis
5	F	NC	56.67	2001	5.92	R NMO	+	6	1.01	ON	39	39	Sepsis secondary to pneumonia
6	F	C	58.83	2008	0.50	R NMO	+	5	10	SC	3	5	Extensive myelitis
7	F	C	60.50	1997	13.17	R NMO	+	9	0.68	ON	24	100	Sepsis secondary to pneumonia
8	F	C	60.50	2005	5.42	R NMOSD	+	3	0.55	SC	16	NA	Lung cancer
9	M	C	61.25	2003	0.75	R NMO	-	2	2.67	SC	7	7	Extensive myelitis
10	F	C	61.33	1997	13.08	R NMO	+	8	0.61	ON	14	14	Sepsis secondary to urinary infection
11	M	C	67.67	1999	8.83	R NMO	+	4	0.45	ON	3,0	43.1	Extensive myelitis
12	M	C	69.25	1997	10.08	R NMO	+	6	0.60	ON	9	9	Extensive myelitis
13	F	C	75.50	2008	3.75	R NMO	+	5	1.33	SC	14.9	36.3	Sepsis secondary to urinary infection
14	M	C	82.67	2010	0	Mo NMO	+	1	NA	ON + SC	NA	0	Extensive myelitis

ARR: Annualized relapse rate; AQP4: aquaporin-4; C: Caucasian; F: female; M: male; Mo: monophasic; NA: not available; NC: non-Caucasian; NMO: neuromyelitis optica; NMOSD: NMO spectrum disease; ON: Optic neuritis; R: relapsing; SC: Spinal cord.

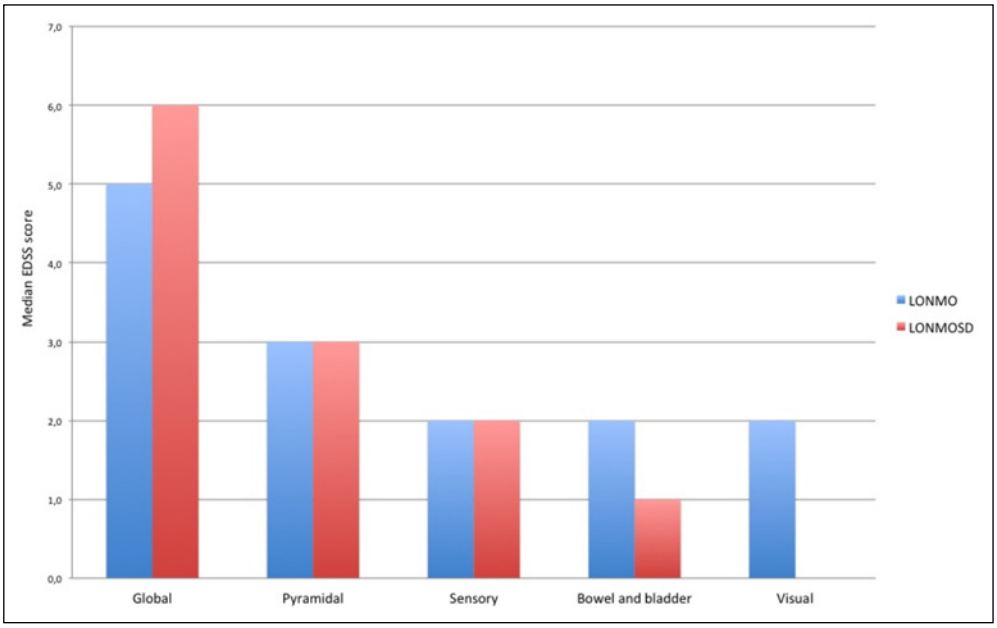

Figure 1. Median EDSS scores in late onset NMO (LONMO) and NMOSD (LONMOSD) patients.
In the whole cohort, the final median EDSS scores were significantly higher for pyramidal functions compared to bowel and bladder, sensory and visual functions ($p < 0.05$).
EDSS: Expanded Disability Status Scale; LONMO: late onset NMO; NMO: neuromyelitis optica; NMOSD: NMO spectrum disease.

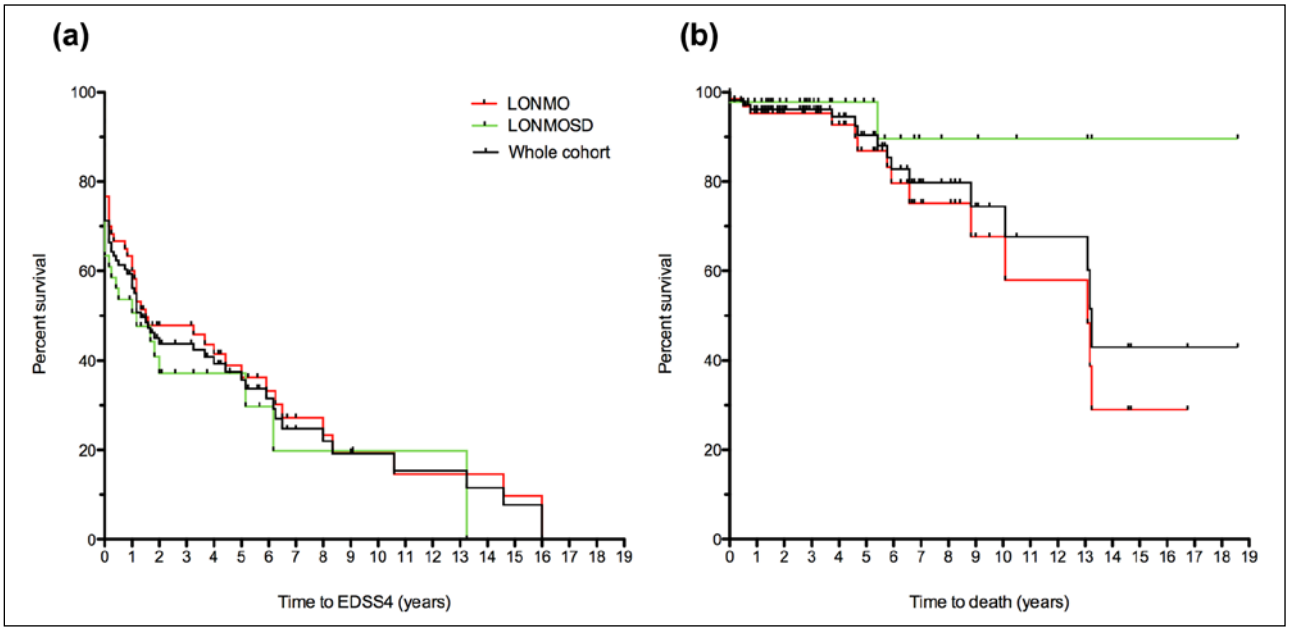

Figure 2. Time to the assignment of (a) EDSS 4 and (b) death in the whole cohort, and in each LONMO and LONMOSD subgroup.
In the whole cohort, the median time to EDSS 4 was 1.3 years (95% CI 0.6–2.1) and to death was 13.2 years (95% CI 12.9–13.5). No difference was noted between the different subgroups of patients for EDSS 4 ($p = 0.7$) and death ($p = 0.5$).
EDSS: Expanded Disability Status Scale; LONMO: late onset NMO; LONMOSD: late onset NMO spectrum disease; NMO: neuromyelitis optica.

Ab testing has become an indispensable part of the diagnostic work-up. In LONMOSD we observed a correlation between the low ARR and the low level of deaths. This data is in line with our predictive approach, showing that the ARR is a key determinant independent factor to explain the high rate of deaths in our study. The low level of brain MRI lesions could be explained by the low number of patients with ON in this population. Effectively, a correlation could exist between brain involvement, as seen on brain MRI, and the optic nerve impairment in NMO.[2]

No difference was observed between AQP-4 seropositive and seronegative patients, except an overrepresentation of women in the AQP-4 Ab+ patients; however, this result is compromised by the low proportion of seronegative cases in our cohort.

The second part of this study sought to identify predictive factors of disability in LONMO/LONMOSD patients. The age at onset played a major role in the assignment of EDSS 4 or death. It is likely that older patients are more susceptible to disability, because of a decrease in repair mechanisms or less immune tolerance, leading to a deficient anti-inflammatory process.[22,23] It is also possible that LONMO/LONMOSD patients had a less good response to immunosuppressive therapy and experienced more side effects, as was shown by the six patients (43%) patients whom died because of severe opportunistic infections. Also, ARR played a prognostic role in disability. The extreme rarity of a progressive course,[24] despite a focused analysis of old patients in our cohort, argued in favor of a correlation between the number of attacks and disability, as is shown in other studies.[25,26]

In our study, LONMO/LONMOSD was particularly severe, with a high rate of death and motor disability in the remaining patients after a mean of 4.6 years of follow-up. These results corroborated data from others large NMO/NMOSD cohorts with a special focus on late onset patients. In a recent cohort of 106 AQP-4 Ab seropositive patients from the UK and Japan, one-third of patients developed permanent motor disability and 9% of them died.[9] Within this study, the age at disease onset appears to be determinant according to the disability type, with a higher risk of motor impairment, wheelchair dependence and death with increasing age. This effect was particularly observed when patients had a disease onset at 50 years of age or later (LONMO/LONMOSD, $n = 29$) as is shown by the high prevalence of LETM (66%) compared to ON (28%). In another Japanese cohort including 583 AQP-4 Ab seropositive patients, myelitis was also the most common initial symptom during the later age onset.[14] Of note in these studies, all patients were AQP-4 Ab seropositive and ethnicity was not homogenous. Our study, which focused on late onset patients from mainly Caucasian origin, enabled us to analyze the role of AQP-4 Ab in the functional outcome.

The role of age in disability outcome has been evaluated in a large cohort, including 87% Caucasian patients and AQP-4 Ab seronegative patients.[2] LONMO was observed in 20 cases and an old age at onset was predictive of the assignment of an EDSS score of 4, 6, or 7; but only in an univariate analysis. This result was not confirmed in a multivariate analysis, mainly due to confounding factors like type and duration of the treatments received.

As was shown in our study, the age at onset could be a determining demographic aspect of NMO-related disability and death. Effectively, the clinical presentation and disability of NMO patients with an onset at an age ≥ 50 years contrast with what is observed in a pediatric-onset group described in the literature.[10–13] Pediatric-onset NMO is especially characterized by frequent brain involvement at presentation and it seems to have a better prognosis than adult-NMO, in terms of disability. The time from onset to EDSS scores 4 and 6 was longer in patients with pediatric-onset NMO, largely explained by the severity of the first myelitis in the adult-onset NMO group. Furthermore, few deaths are observed in pediatric patients. Against the relevance of the age at onset in NMO disability, it could be proposed that the effect on disability is due to an overrepresentation of AQP-4 IgG Ab seropositivity in late onset patients, especially in women after 50 years of age.[27] We don't think that this repartition of AQP-4 IgG Ab seropositivity may influence our results, because we demonstrated that AQP-4 IgG Ab could not be an independent factor of disability and death in our population of LONMO/LONMOSD patients. And lastly, no difference in terms of age at onset is found between the seropositive and seronegative patients in several recently published cohort studies.[28,29]

Because of the retrospective design of this study, some limitations are notable. First, we were limited in our analysis by the missing data in the field of EDSS functional scores. A complete EDSS score was not ruled systematically during the follow-up; and therefore, the repartitions of these scores were disseminated in time and not homogenous. A second point is about treatment. We were not able to extract data to monitor the efficacy and tolerance of each therapy, in each patient. This task would be really challenging in such a study cohort, because of the large number of patients included and because of the huge number of treatments tried during the long period of follow-up. A future study with a specific endpoint to analyze the therapeutic management and socioeconomic consequences of the disease related to age would surely be of interest. In contrast to the French and German cohorts, the UK and Turkish cohorts were not population based, so we cannot rule out different referral and follow-up biases, nor the influence in the speed of diagnosis and treatment between the NMO centers. Despite this limitation, we should note that each NMO center in the UK and Turkey has a referral role in a large regional area that could catch a representative local population of patients. The most critical point was about NMOSD patients and the overrepresentation of LETM, compared to ON: We think that a part of this observation is due to the absence of a consensus approach to diagnose NMOSD, particularly isolated ON, as compared to NMO, which is difficult to diagnose. Despite these limitations, we are unable to postulate a systematic bias to explain our findings.

In conclusion, we have described the demographic, clinical and disease-related characteristics of 108 LONMO/LONMOSD patients. We also compared the features of LONMO/LONMOSD and AQP-4 Ab positive/negative

subgroups of patients. Despite the rarity of the disease, the large set of data in this study population was a unique occasion for us to perform a multivariate analysis with a Cox proportional model, to isolate the independent factors related to disability and death.

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Conflict of interest

Dr. Collongues serves on scientific advisory boards for and has received honoraria from Biogen Idec, Merck Serono, sanofi-aventis, and Bayer Schering Pharma.

Dr. de Seze serves on scientific advisory boards for and has received honoraria from Biogen Idec, LFB, Merck Serono, sanofi-aventis, and Bayer Schering Pharma; and serves on the editorial board of *Revue Neurologique*.

Dr. Paul has received speaker honoraria, travel reimbursement and research support from Biogen Idec, Merck Serono, Teva, Bayer Schering, Novartis and Sanofi Genzyme, and has received travel reimbursement from the Guthy Jackson Charitable Foundation. Dr. Paul is member of the steering committee of the OCTIMS study (Novartis).

Dr Kitley is supported by the NHS National Specialised Commissioning Group for Neuromyelitis Optica and has received travel grants from Biogen Idec, Novartis and Teva and speaker honoraria from Novartis.

Dr Altintas, Marignier, Kuscu have nothing to disclose

Dr. Palace serves on the scientific advisory board for Charcot Foundation, and has performed advisory work for Biogen Idec, Merck Serono Ltd, Bayer Schering Pharma, Novartis Pharmaceuticals UK Ltd, Teva Pharmaceutical Industries Ltd, Gilenya, Ono Pharmaceutical Co Ltd, Primary i-research, Chugai Pharma Europe and CI Consulting. She receives research support from the MS Society, QIDIS, Merck Serono Ltd and Bayer Schering Pharma, plus conference expenses from Novartis and Merck Serono Ltd

Dr. Confavreux reports receiving for the last three years Consulting fees from Biogen-Idec, Gemacbio, Genzyme, Novartis, Sanofi-Aventis, Teva, UCB; lecture fees from Bayer-Schering, Biogen-Idec, Genzyme, Merck-Serono, Novartis, Octopharma, Sanofi-Aventis, Teva; research support from Bayer-Schering, Biogen-Idec, Merck-Serono, Novartis, Sanofi-Aventis, Teva.

Dr. Elsone has received travel grants to attend scientific meetings from Novartis and Teva.

Dr. Mutch received honoraria for speaking on NMO from Biogen Idec.

Dr. Jacob has received honoraria from Biogen Idec and Chugai pharmaceuticals for giving talks and participating in clinical trial advisory boards.

Dr. Wildemann has served on a scientific advisory board for Novartisand Biogen Idec, has received funding for travel and speaker honoraria from Biogen Idec, Bayer Schering Pharma, Merck Serono, Teva Pharmaceutical Industries Ltd., and Genzyme-A Sanofi Company; and has received researchsupport from Bayer Schering Pharma, Merck Serono, Biotest PharmaceuticalsCorporation, Teva Pharmaceutical Industries Ltd., and the Bundesministeriumfür Bildung und Forschung (BMBF).

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Dr. Zéphir served on scientific advisory board for biogen idec, genzyme, teva, served as consultant for biogen idec, bayer, merck, novartis, sanofi, teva, received research support from teva, received payments for lectures for biogen idec, bayer, novartis.

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The Brown Norway opticospinal model of demyelination: Does it mimic multiple sclerosis or neuromyelitis optica?

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ABSTRACT

Opticospinal demyelinating diseases in humans are mostly characterized by the opticospinal form of multiple sclerosis (MS) and neuromyelitis optica (NMO). Increasing attention has recently focused on astrocyte markers, aquaporin-4 (AQP4) and glial fibrillary acidic protein (GFAP) in these diseases. We induced opticospinal demyelination in Brown Norway rats with soluble recombinant rat myelin oligodendrocyte glycoprotein (1–116) and incomplete Freund's adjuvant. Clinical, MRI, neuropathological and immunological evaluations were performed, with a focus on AQP4 and GFAP. We confirmed the opticospinal phenotype, including extensive myelitis, but also showed the MRI-characterized involvement of the periventricular area. Expression levels of myelin, AQP4 and GFAP showed the early involvement of astrocytes before demyelination in the optic nerve. The overexpression of AQP4 was particularly pronounced in the spinal cord and was concomitant with demyelination and astrocyte apoptosis. The disability scores were correlated with demyelination and inflammation but not with AQP4/GFAP expression. No antibodies against the linear and conformational epitopes of AQP4 were detected. Whereas a NMO-like phenotype was observed in this model, the AQP4/GFAP expression during the disease process was more closely related to opticospinal MS than NMO. However, this model raises the question of a continuum between opticospinal MS and the seronegative NMO subtype.

1. Introduction

Opticospinal inflammatory/demyelinating diseases in humans are mostly characterized by two distinct entities, the opticospinal form of multiple sclerosis (MS) and neuromyelitis optica (NMO). NMO is usually a more severe pathology than MS, characterized by disabling optic neuritis and longitudinally extensive transverse myelitis. Autopsies have shown that NMO was characterized by extensive demyelination of the optic nerves and spinal cord, owing to a massive myelin protein loss that occurs in both the white and gray matter. Contrary to MS, brain MRI is often normal at disease onset in NMO, but brain lesions appear in about 50% of patients during the disease, with the involvement of periependymal regions such as the hypothalamus, corpus callosum, periaqueductal brainstem and periventricular areas (Pittock et al., 2006).

Recently, the involvement of astrocyte aquaporin-4 (AQP4), the dominant central nervous system (CNS) water channel protein, has been demonstrated in NMO. A specific and pathogenic serum autoantibody, called NMO-IgG, recognizing AQP4, has been reported in 50–70% of NMO patients but only in 10% of MS patients (Kira, 2011; Lennon et al., 2004; Mata and Lolli, 2011). Neuropathological studies of glial fibrillary acidic protein (GFAP), a specific marker of astrocytes, and AQP4, particularly abundant in the optic nerves, spinal cord and periependymal regions (Nagelhus et al., 1998; Pittock et al., 2006; Vitellaro-Zuccarello et al., 2005), have demonstrated a decreased expression of AQP4 and GFAP in the optic

Abbreviations: AQP4, aquaporin-4; BN, Brown Norway; CSF, cerebrospinal fluid; EAE, experimental autoimmune encephalomyelitis; ELISA, enzyme-linked immunosorbent assay; GFAP, glial fibrillary acidic protein; HE, haematoxylin/eosin; IFA, incomplete Freund's adjuvant; LFB/c, luxol fast blue/cresyl violet; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; NMO, neuromyelitis optica; OD, optical density; ON, optic nerve.

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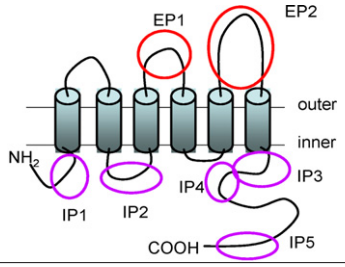
Table 1
Synthetic peptides of AQP4 used in this study.

1. IP1 = p.2–21	SDGAAARRWGKSGHSSSRQS
2. IP2 = p.96–115	INPAVTVAMVCTRKISIAKS
3. EP1 = p.137–156	TPPSVVGGLGVTMVHGNLTA
4. EP 2 = p.207–231	YTGASMNPARSFGPAVIMGNWENHW
5. IP 3 = p.251–269	VFCPDVEFKRRFKEAFSKA
6. IP 4 = p.267–285	SKAAQQTKGSYMEVEDNRS
7. IP 5 = p.301–319	VIDVDRGEEKKGKDQSGEV



IP 1–5: intracellular peptides 1 to 5. EP 1 and 2: extracellular peptides 1 and 2.

nerve and spinal cord of NMO patients before demyelination (Misu et al., 2007; Roemer et al., 2007; Sinclair et al., 2007). This pattern of immunoreactivity seems different from that encountered in MS where loss of AQP4 has been found mainly in strongly demyelinated regions in intensive and acute MS lesions, whereas overexpression of AQP4 has mostly been observed in other chronic demyelinated tissue due to astrogliosis.

Animal models of experimental autoimmune encephalomyelitis (EAE) suggest that myelin oligodendrocyte glycoprotein (MOG) plays an important role in opticospinal demyelination (Sakuma et al., 2004; Stefferl et al., 1999; Storch et al., 1998). A model in a Brown Norway (BN) rat strain was induced by immunization with rat MOG (1–125) (rMOG) in incomplete Freund's adjuvant (IFA) (Storch et al., 1998). This animal model, characterized by major antibody-mediated inflammation, results in myelitis and optic neuritis between 30 and 70 days post immunization (PI). Furthermore, as in NMO, a perivascular deposition of immunoglobulin and complement C9 antigen occurs in this model. Another model, involving Lewis rats immunized with rMOG in complete Freund's adjuvant (CFA), also results in myelitis and optic neuritis (Sakuma et al., 2004), but with a lower anti-MOG antibody titer than the BN model (Stefferl et al., 1999). More recently, a double-transgenic mouse strain TCR^{MOG} and IgH^{MOG} was engineered, which spontaneously develops a form of EAE similar to NMO (Bettelli et al., 2006; Krishnamoorthy et al., 2006). Besides the role of MOG antigen in inducing opticospinal demyelination, the BN animal model differs from the others by its particular immunological process. It has been demonstrated that activation of B lymphocytes was less pronounced in Lewis rats than in BN rats (Stefferl et al., 1999) and that MOG antigen was more abundant in the optic nerves and spinal cord of BN rats than of Lewis rats (Pagany et al., 2003). Unlike the Lewis or Dark Agouti rat models, the BN rat model with its capacity to trigger an antibody response to MOG, induces NMO-like lesions. However, none of the above-mentioned animal models has been characterized by MRI or with respect to AQP4/GFAP expression during pathogenesis.

In view of the reported similarities between the BN MOG-induced model and opticospinal disease in humans, we used it to explore the opticospinal phenotype, to report neuropathological measurements of myelin, AQP4 and GFAP expression and to look for correlations with disability. We also explored the hypothesis of MOG immunization-induced epitope spreading in reporting immunization against linear and conformational AQP4 epitopes.

2. Experimental procedures

2.1. Animals

Immunization with MOG: experiments were performed on 8- to 10-week-old female BN rats (130–150 g) obtained from Janvier (France). A total of 32 rats were analyzed in the present study. During the observation period the rats were kept under environmentally controlled conditions. The experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications no. 80–23, revised 1996); the Institutional Animal Care and Use Committee of Strasbourg University approved all the experiments.

2.2. Reagents

Recombinant rat MOG protein corresponding to the N-terminal sequence of rat MOG (1–116) (rMOG) was prepared as previously described (Delarasse et al., 2003). IFA was purchased from Sigma–Aldrich (France). Recombinant rMOG and AQP4 peptides were used for the enzyme-linked immunoassay (ELISA). Seven AQP4 peptide sequences were selected (Table 1): intracellular peptides (IP1 to IP5) and two extracellular peptides (EP1 and EP2). The peptides were synthesized by the proteomic platform of Neuroscience IFR 37 (Strasbourg, France) and purified by RP-HPLC. The purity of the peptides was above 95% and their identities were confirmed by mass spectrometry.

2.3. Induction and evaluation of EAE

Twenty-five rats were immunized at the base of the tail with 100 μl of rMOG emulsified with the same volume of IFA in a total volume of 200 μl, corresponding to 100 μg of rMOG per rat. Anesthesia for immunization and blood collection was induced by intra-peritoneal injection of 37 mg/kg of ketamine (Ketalar, Parke-Davis) and 5.5 mg/kg of xylazine (Rompun, Bayer). Seven rats were used as controls and were immunized with IFA alone.

The animals were weighed and examined daily for EAE clinical signs and scored on the following scale: 0 = no weakness; 1 = weakness of the tail only; 2 = walking disability without any motor dysfunction; 3 = walking disability with motor dysfunction; 4 = paraplegia; 5 = tetraplegia; 6 = death. Relapse and remission were defined respectively as an increase or a decrease in clinical score of at least one point for at least two days. Consecutive relapses or remissions were considered as a single event.

2.4. MRI

The brains, optic nerves and spinal cords were assessed *in vivo* by MRI at different time intervals. Three time-points were chosen on the basis of clinical disability as described in the literature (Storch et al., 1998), corresponding to the early stage (days 0 to 29), the disease stage when clinical signs were visible (days 30 to 70) and the late stage (after 70 days), when no further clinical signs were noted. Four MOG-immunized rats were examined by MRI and then sacrificed during the early stage of the disease at day 10 and day 25 and during the disease stage at day 45. Longitudinal follow-up by

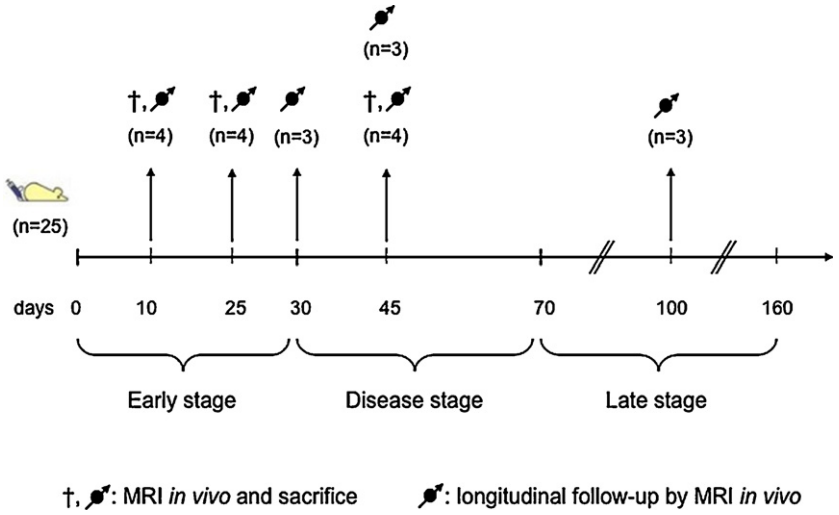


Fig. 1. A work plan summarizing the MRI experimental procedure for the groups of rats immunized with MOG. Two IFA-injected rats were used as controls at each stage of the disease course.

MRI was performed for three rats from the disease stage, at day 30 and day 45 until the late stage, at day 100 (Fig. 1). Two IFA-injected rats were used as controls for each MRI time-point. The rats were anaesthetized with a mixture of isoflurane and oxygen at 5% for induction and 1.5% for maintenance. Imaging was performed using a 4.7 T MR magnet, equipped with self-shield gradient coils from Magnex Sci. Ltd. (Oxford, United Kingdom) and an MR spectrometer from S.M.I.S. Ltd. (now M.R.R.S., Guildford, United Kingdom). The animals were placed in a stereotaxic device to immobilize the head, with integrated heating facility to maintain the body temperature at 37 °C. An anatomically shaped ^{1}H surface coil for small animals (Rapid Biomedical GmbH, Würzburg, Germany) was placed over the head to serve as a receiver for the magnetic resonance signal. The system was then placed into a ^{1}H resonator (Rapid Biomedical GmbH) for rats and mice, which was slid into the magnet. Multiparametric T2-weighted MRI was performed with a repetition time of 3800 ms and an echo time of 30 ms in the control and MOG-immunized rats. The slice thickness was 1 mm and the field of view (FOV) ranged from 25 to 50 mm depending on the anatomic areas explored. For an FOV 25 mm × 25 mm with a data matrix 256 × 256 (zero filled to 512 × 512), the in-plane resolution was 50 μm.

2.5. Histopathology and immunohistochemistry

After MOG injection, rats were sacrificed at different time-points: eight at the early stage, eight at the disease stage and nine at the late stage. Seven control rats were terminated as follows: two at the early and disease stages, respectively, and three at the late stage. For tissue fixation, the rats were perfused via the heart with 4% paraformaldehyde. Brains, optic nerves and spinal cords were removed and postfixed in the same fixative. Paraffin-embedded tissues were then processed for conventional light microscopy. Eight-micrometer-thick sections were used for demyelination evaluation stained with luxol fast blue/cresyl violet (LFB/c) and inflammation was evaluated with hematoxylin/eosin (HE).

In adjacent serial sections, immunohistochemistry was performed on paraffin-embedded sections using indirect immunofluorescence or a peroxidase-biotinylated antibody for the amplification technique (Vector VIP SK-4600, Vector Laboratories). The primary antibodies used are listed in Table 2. The anti-MOG monoclonal antibody was a gift from Dr. Linnington (Linnington et al., 1984). Control sections were incubated without primary antibody or with non-immune rabbit serum.

LFB/c and antibodies against Myelin basic protein (MBP) were used to quantify demyelination, definite as extensive when a transverse spinal cord or bilateral optic nerve demyelination was observed. HE and ED1 were used to characterize the extent of inflammation. A marked inflammation was noted when the number of infiltrating cells was ≥20 cells in a 100 μm × 100 μm area, determined by using the NIS. Elements D3.2 imaging software (Nikon Inc., New York, USA). AQP4 and GFAP expression was evaluated using the following scores: −2, marked decreased expression; −1, mild decreased expression; 0, similar to control; +1, mild increased expression; +2, marked increased expression.

Antibodies against caspase3 were used to determine apoptotic cells. The number of caspase3 positive cells was counted in a 100 μm × 100 μm area, determined by using the NIS. Elements D3.2 imaging software (Nikon Inc., New York, USA). The mean number of apoptotic cells was determined for each animal on an average of 10 complete cross sections of spinal cord. Histological scores were established compared to controls IFA-injected rats. For each rat, three sections/areas (including the optic nerves, the spinal cord and the periventricular areas) were used. This scale was calculated from results obtained from blind tests of tissue sections by two independent observers.

2.6. Enzyme-linked immunoassay

Blood was collected either from the tail vein or by cardiac puncture immediately before perfusion with the fixative. After clotting at 4 °C the sera were collected by centrifugation and stored at −20 °C. Antigens were coated on 96-well plates. Antigens were diluted at 20 μg/ml in a coating buffer solution and incubated for 1 h at 37 °C. Antigen-coated plates were incubated with 1:10–1:1000 serial dilutions of sera from normal and immunized animals for 1 h at 37 °C. After washing, an appropriate dilution of peroxidase-conjugated anti-rat IgG (A9037, Sigma) was applied at 1:10000 for 1 h. The reaction product was then visualized after incubation of the substrate ortho-phenylene-diamine diluted at 1.5 mg/ml, for 30 min. The optical density (OD) was measured at 450 nm. The results are given as a mean of triplicate values. Cut-off was determined as the mean OD in the IFA group with three standard deviations. Separate assays were carried-out for each individual

Table 2
Antibodies used for immunohistochemistry.

Antigen	Ab type	Target	Dilution	Ab anti-rat/revelation	Source
AQP4	Polyclonal	Astrocytes/endothelium/ependymocytes	1:20	Rabbit IgG/DAB	Sigma
Caspase3	Polyclonal	Apoptotic cells	1:200	Rabbit IgG/DAB	USbiological
ED1	Monoclonal	Macrophages/activated microglia	1:200	Mouse IgG/DAB	Serotec
GFAP	Polyclonal	Astrocytes	1:200	Rabbit IgG/VIP	DAKO
MBP	Monoclonal	Myelin	1:50	Mouse IgG/fluo.	Home made
MOG	Monoclonal	Myelin/oligodendrocytes	1:10	Mouse IgG/DAB	(Linnington et al., 1984)

AQP4: aquaporin-4, GFAP: glial fibrillary acidic protein, MBP: myelin basic protein, MOG: myelin oligodendrocyte glycoprotein.

AQP4 peptide noted in Table 1. Control wells were incubated without primary antibodies.

2.7. AQP4-Ig assay

All serum samples were examined for the presence of NMO-IgG using the described immunofluorescence technique on AQP4 transfected cells, developed by INSERM U842 laboratory (Lyon, France) (Marignier et al., 2010) with a sensitivity of 60% and specificity of 100%. Briefly, a full-length human AQP4-M1 cDNA (NM_001650, Origene, Rockville, USA) was amplified by PCR and inserted directionally into the Bgl2 and Kpn1 restriction sites of the pEGFP-C1 vector (Clontech, France). The identity of AQP4 was confirmed by protein sequencing. The purified plasmid was used to transfect human embryonic kidney cells (HEK 293) using the calcium phosphate method in compliance with the manufacturer's protocol (Invitrogen, France). Twenty-four hours post transfection, the cells were fixed with 4% paraformaldehyde, washed in PBS and blocked with PBS containing 2% BSA and 0.1% Triton X-100. Rat sera were incubated overnight at a dilution of 1:50. Alexa fluor 546 anti-human IgG (Invitrogen, France) was used as secondary antibody. Positive controls were performed using a rabbit anti-rat AQP4 polyclonal antibody (Millipore, France) and a human serum clearly positive for NMO-IgG.

2.8. Statistical analysis

A Mann–Whitney *U* test was used to analyze MOG immunoreactivity in serum samples of rat immunized with MOG, as a function of postimmunization time. Spearman's rank correlation test and coefficients (r) were used to test a sequence of pairs of values including the clinical score, the OD, the presence of demyelination, inflammation and the histological AQP4 and GFAP scores in the optic nerves, spinal cord and periventricular areas for all the rats at each stage of the disease. Bonferroni correction was made because of multiple comparisons performed for statistical correlation. Two-sided p values <0.005 were considered statistically significant. Statistical analysis was performed using SPSS 14.0 for Windows (SPSS Inc., Chicago, USA).

3. Results

3.1. Clinical course of MOG-induced EAE

All animals were evaluated daily using the clinical scale described in Section 2.3. In the control groups, no disability was detected. Clinical data for MOG-immunized rats at different time points (early, disease and late stages) are summarized in Table 3. After immunization with MOG, the mean time to the first clinical

Table 3
Clinical and histological evaluations of the MOG-injected rats.

Rats	Days post-immunization			Scores		Optic nerves				Spinal cords				Periventricular areas			
	CS ≥ 1	Max. CS	Sac.	Max.	Sac.	DM	INF	AQP4[a]	GFAP[a]	DM	INF	AQP4[a]	GFAP[a]	DM	INF	AQP4[a]	GFAP[a]
1.	–	–	10	0	0	–	–	0	0	–	+	0	0	–	–	+1	0
2.	–	–	10	0	0	–	–	0	0	–	–	0	0	–	–	+1	+1
3.	–	–	10	0	0	–	–	−1	−1	–	–	0	0	–	–	0	0
4.	–	–	10	0	0	–	–	0	0	–	–	0	0	–	–	0	0
5.	–	–	25	0	0	–	–	−1	−1	–	–	+1	+1	–	–	0	0
6.	–	–	25	0	0	–	–	0	−1	–	–	+1	+1	–	–	0	0
7.	–	–	25	0	0	–	–	−1	0	–	–	+1	+1	–	–	0	0
8.	–	–	25	0	0	–	–	0	0	–	–	0	0	–	–	0	0
9.	19	21	35	5	5	+, ext.	+, marked	−1	0	+, ext.	+, marked	+2	+1	–	–	0	0
10.	45	45	45	1	1	+	+, marked	−1	+1	+	+	+1	+2	–	–	+1	+1
11.	32	34	45	4	4	+	+, marked	−1	+1	+	+	+1	+2	–	–	1	0
12.	45	45	45	4	4	+	+	0	+1	+	+	+1	+2	–	–	0	+1
13.	–	–	45	0	0	–	+	−1	+1	+	+	+1	+1	–	–	+1	+1
14.	22	37	50	2	1	+, ext.	+	−2	−1	+, ext.	+, marked	+1	+2	–	–	+1	0
15.	45	58	60	3	3	+	–	−2	−2	+, ext.	+, marked	+1	0	–	–	+1	+1
16.	58	58	60	1	1	+	–	−1	+1	–	–	0	0	–	–	0	0
17.	64	64	100	1	0	+	+	−1	−2	+	–	+2	+1	–	–	0	0
18.	49	79	100	1	0	+	–	−2	−1	–	–	+2	+2	–	–	0	+1
19.	64	64	100	1	1	+	–	−2	−2	+	–	+1	+1	–	–	+1	0
20.	30	31	160	1	0	–	–	0	+1	–	–	0	+1	–	–	0	0
21.	–	–	160	0	0	+	–	+1	+2	–	–	0	+1	–	–	0	+1
22.	30	33	160	4	0	+, ext.	–	+1	+2	–	–	0	+1	–	–	+1	+1
23.	32	32	160	1	0	+, ext.	–	+1	+2	–	–	+1	+1	–	–	0	+1
24.	–	–	160	0	0	–	–	0	+2	–	–	+1	+1	–	–	0	0
25.	37	37	160	4	0	–	–	0	0	–	–	0	0	–	–	0	0

Three time–points were chosen for sacrifice on the basis of clinical disability as described in the literature, corresponding to the early stage (days 0–29), the disease stage when clinical signs were visible (days 30–70) and the late stage (after 70 days), when no further clinical signs were noted.

[a] For AQP4/GFAP, histological scores were assessed as follows: 1, −2 graduated decreased expression, 0 no modification compared to IFA-injected rats, +1, +2 graduated increased expression (for details see Experimental procedures). CS: clinical score; DM: demyelination; INF: inflammation. Max.: maximal; Sac.: sacrifice; Ext.: extensive.

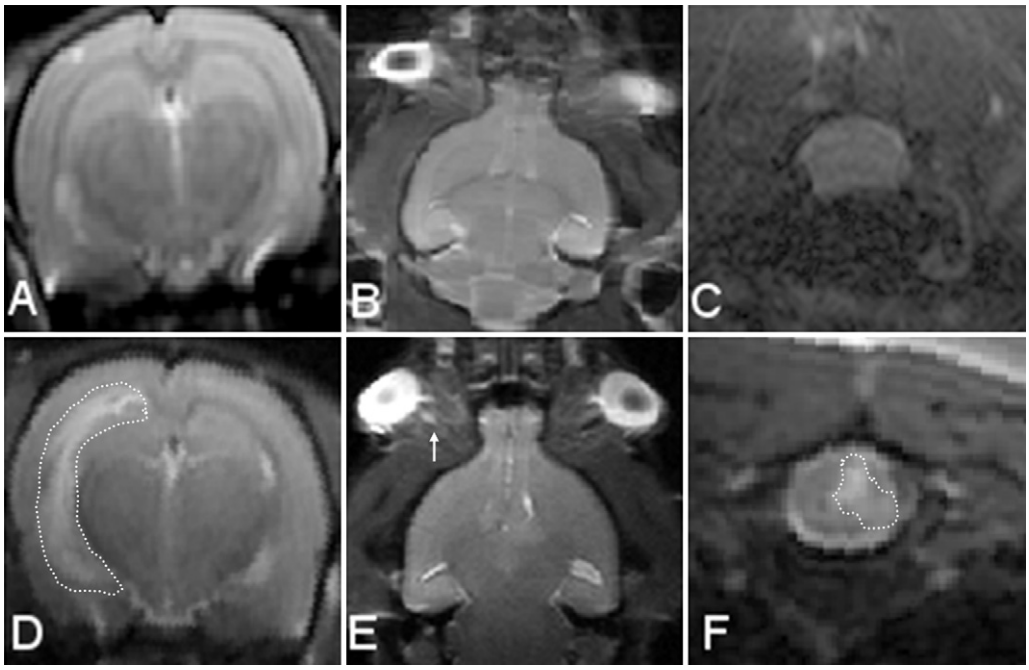


Fig. 2. Brain, optic nerve and spinal cord MRI in control and MOG-immunized rats. The figure shows brain, optic nerve and spinal cord in control rats (A–C) and MOG-immunized rats (D–F). During the disease stage at day 30 in the three rats examined by MRI, T2-hypersignals were detected in the periventricular area and optic nerves of two rats (D and E) and the spinal cord in one rat (F). The dotted lines in D and F delineate the T2-hypersignal in the periventricular area and spinal cord, respectively. The arrow in E shows T2-hypersignal in the optic nerve.

score $\geq$1 was 41 $\pm$ 14 days and 46 $\pm$ 16 days to the maximal clinical score. As expected, only two MOG-immunized rats developed clinical signs during the early stage of the disease. MOG-induced EAE was observed in 82% of rats at the two subsequent stages of the disease. A single clinical event was observed in 93% of rats, followed by an onset of recovery. Only one rat experienced a relapsing course, with two distinct clinical events five days apart. No progressive course was noted. No rats died during the clinical course.

3.2. Lesion topography

3.2.1. Topography of the lesions by MRI

No abnormality was observed in control rats at the different stages of the experimental procedure. In MOG-injected rats, during the early stage, the animals were healthy and the MRI scans were similar to controls (Fig. 2A–C). Out of the three rats examined in the disease stage at day 30, T2-hypersignal abnormalities were observed in the periventricular zone (Fig. 2D) and optic nerve (Fig. 2E) in two rats and one rat showed abnormalities in the spinal cord (Fig. 2F). MRI follow-up of these rats showed a persisting T2-hypersignal in the spinal cord whereas other abnormalities disappeared after day 45, when the clinical disability score was dramatically reduced. MRI examination of four additional rats at day 45 showed T2-hypersignal in the spinal cord of two rats only. After day 70, no abnormal T2-hypersignal was identified in these regions of interest.

3.2.2. Topography of demyelination and inflammation

No histopathological signs of EAE were observed in the control group. Chronological evaluation of demyelination and inflammation in the rats immunized with MOG is reported in Table 3. In these rats, the brain was always spared whereas signs of demyelination were found in the spinal cord, optic nerves/tracts and optic chiasms at the disease and late disease stages. None of the rats sacrificed at the early stage presented demyelination or inflammation in the optic nerve. During the disease stage, demyelination was noted in more than 80% of the optic nerves (Fig. 3A and B) and spinal cords (Fig. 3G–I). Most of them were strongly infiltrated by inflammatory ED1 positive cells in demyelinated areas of the optic nerve (Fig. 3C–F) and spinal cord (Fig. 3J–M). Out of seven animals with optic nerve involvement, demyelination was bilateral in two animals. In the spinal cord, three rats experienced extensive demyelination with transverse myelitis affecting the cervical, thoracic and lumbosacral regions. At the late stage, we mainly observed isolated demyelination without inflammatory cells and bilateral optic nerve demyelination was noted in two rats whereas no extensive myelitis was observed.

3.3. Expression of AQP4 and GFAP

The analysis of the intensity of immunostaining was performed using antibodies against AQP4 to examine AQP4 expression during the development of the disease, whereas antibodies against GFAP were used to evaluate astrocyte expression. Scores were calculated on the basis of AQP4 and GFAP immunostaining in IFA rats at the different stages of experimentation (see Experimental procedures). AQP4 and GFAP histological scores are presented in Table 3. In the optic nerve, immunoreactivity to AQP4 and GFAP was mildly decreased at the early stage, before the onset of visible demyelination and inflammation. During the disease stage, AQP4 levels continued to decrease while GFAP levels increased significantly and remained high until the late stage of the disease. AQP4 and GFAP levels in the spinal cord and periventricular area were increased constantly throughout all three stages but the level in the periventricular area showed lower scores when compared with the spinal cord.

Optic nerve transverse sections of MOG-immunized rats, at the early stage, showed reduced AQP4 and GFAP immunostaining in 3/8 rats examined (Fig. 4B and F). At the disease stage, a decrease

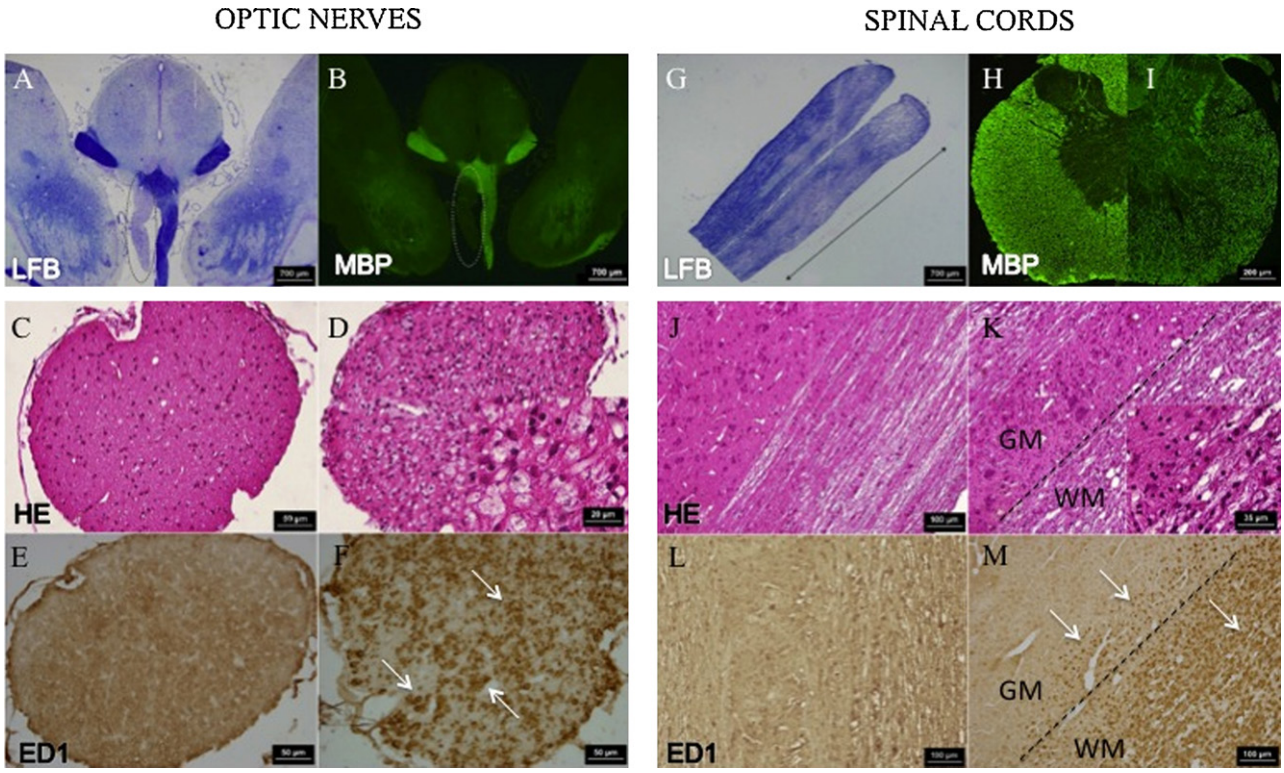


Fig. 3. Optic neuritis and myelitis at the disease stage in MOG-immunized rats. The longitudinal diencephalic section reveals a defect in blue staining with LFB/c in the left optic nerve (A, dotted line). The same section of the same rat confirms a loss of myelin, as shown by a loss of MBP immunostaining, corresponding to a whole unilateral demyelination of the optic nerve (B, dotted line). Compared to control rats (C), transverse sections of a demyelinated optic nerve (D) stained with HE shows marked mononuclear cell infiltration with phagocytic activity in the whole section, as shown at higher magnification in box D and attested by the ED1 immunostaining. Compared to control (E), infiltrating cells (arrows) were revealed by ED1 immunostaining in (F). The longitudinal section of thoracic spinal cord in a rat at the disease stage shows a defect in blue staining with LFB/c, corresponding to extensive myelitis, over 6 mm (G, double arrow). The transverse section of the same rat confirms the loss of myelin through reduced MBP immunostaining and shows transverse demyelination (I, right side) compared to control rats (H, left side). Compared to control rats (J), coloration with HE in a demyelinated spinal cord area shows cell infiltration in the gray and white matter (K). Higher magnification in this box shows marked cells infiltration, ED1 positive (M, arrows) compared to control (L).

in the expression of AQP4 was observed in 7/8 rats (Fig. 4C). In comparison, GFAP expression increased in 5/8 and 5/9 animals at the disease (Fig. 4G) and late stages (Fig. 4H) respectively. AQP4 immunostaining intensity remained lower for a longer time than GFAP in some animals, with 3/9 rats showing decreased levels and 3/9 showing increased levels at the late stage (Fig. 4D).

In spinal cord of affected rats, an increased AQP4 immunostaining level was accompanied by an increase in GFAP

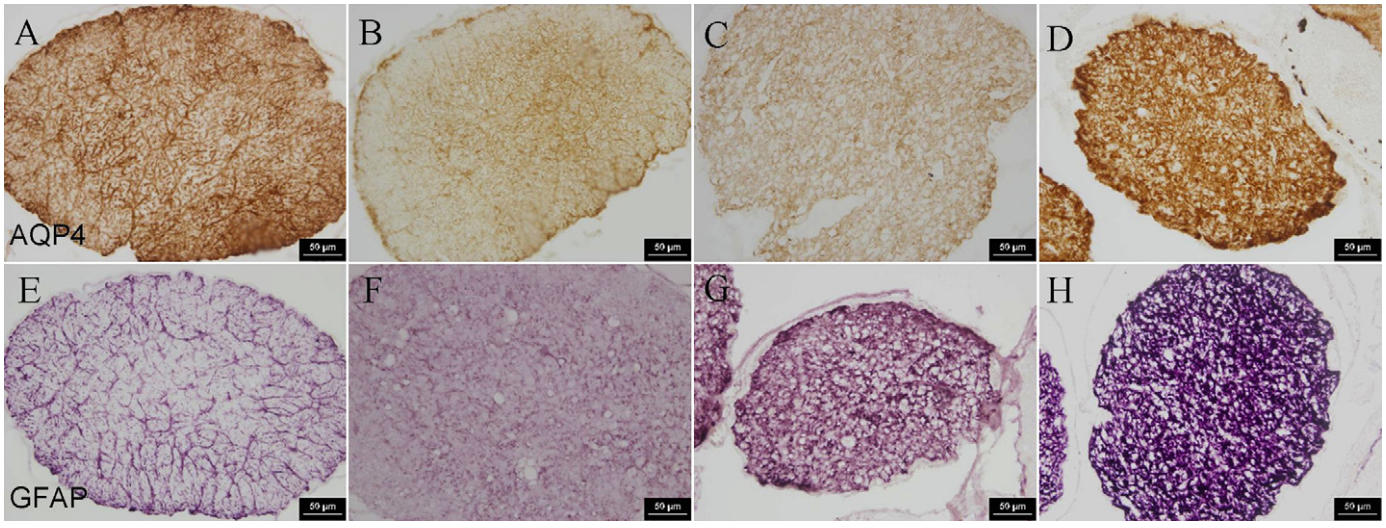


Fig. 4. AQP4 and GFAP immunostaining in the optic nerve of control and MOG-immunized rats with histological scores (HS) (for detail of the histological score, see Experimental procedures) during the disease process. The figure illustrates AQP4 and GFAP immunostaining in the optic nerve of control (A and E) and MOG-immunized rats (B–D, F–H). In the optic nerve, decreased expression of AQP4 (−1) was noted at the early stage (B), with a maximal decrease at the disease stage (−2) (C). AQP4 overexpression was delayed until the late stage of the disease (+2) (D). A decreased expression of GFAP (−1) was observed at the early stage (F). A progressive overexpression of GFAP was noted from the disease stage (+1) (G) to the late stage of disease (+2) (H).

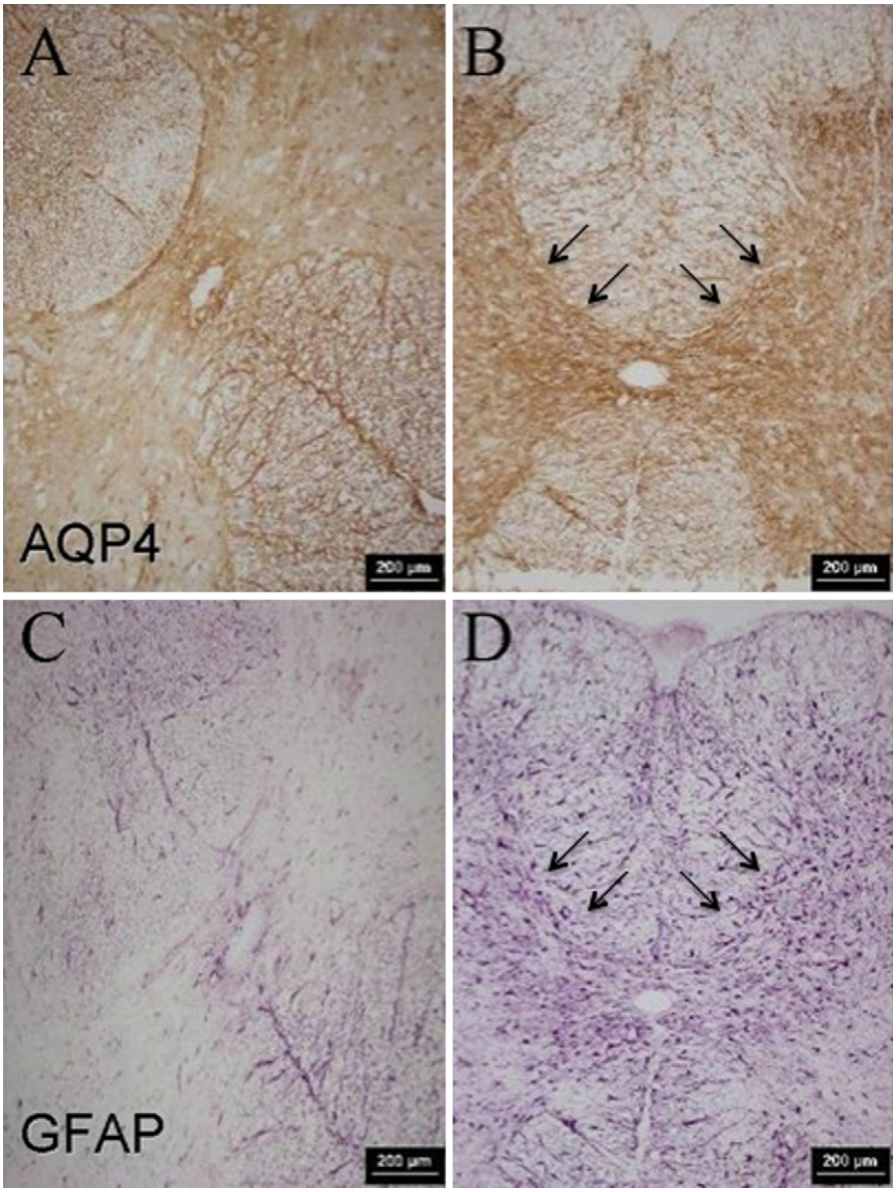


Fig. 5. Disease stage: AQP4 and GFAP immunostaining in the spinal cord of control and MOG-immunized rats with histological scores (HS) (for detail of the histological score, see Experimental procedures). The figure illustrates AQP4 and GFAP immunostaining in the spinal cord of control (A and C) and MOG-immunized rats at the disease stage (B and D) at the same spinal cord level. In the spinal cord, a continuous increase in expression of AQP4 (+2, arrows in B) and GFAP (+2, arrows in D) associated with gliosis and astrocytic hypertrophy was observed during demyelination.

immunoreactivity at the early stage in 3/8 rats. At the disease stage, both AQP4 and GFAP immunostaining intensities were increased in 7/8 rats and 6/8 rats, and was observed in both the gray and white matter of the spinal cord (Fig. 5B and D). The immunoreactivity was particularly strong at the disease stage and accompanied by astrocytic hypertrophy (Fig. 5D) when compared to controls (Fig. 5C). At the late stage, both AQP4 and GFAP immunostaining intensities were increased in 5/9 rats and 8/9 rats. Brain periventricular areas showed a slight increase in AQP4 and GFAP immunostaining at the early stage. The level of both markers continued to increase during the disease stage (Fig. 6B and D) and remained high until the late stage.

3.4. Expression of caspase3

The analysis of apoptosis was performed using antibodies against caspase3, a protein which plays a central role in the execution-phase of cell apoptosis. Whereas no significant apoptosis was observed in the optic nerves during the disease process (data not shown), a statistically significant increase of apoptotic cells was noted in the spinal cord at the disease stage compared to controls and rats at early stage (Fig. 7A–C). At the disease stage, the majority of caspase3 positive cells are GFAP positive (Fig. 7D–E). At the late stage, the cell death of astrocyte/GFAP positive is observed at higher rate.

3.5. Immunological evaluation

3.5.1. ELISA detection of immunoreactivity against MOG and AQP4

ELISA experiments confirmed that immunization with rMOG induced an antibody response increasing from day 0 to day 46, when the maximal OD was noted (Fig. 8). No significant

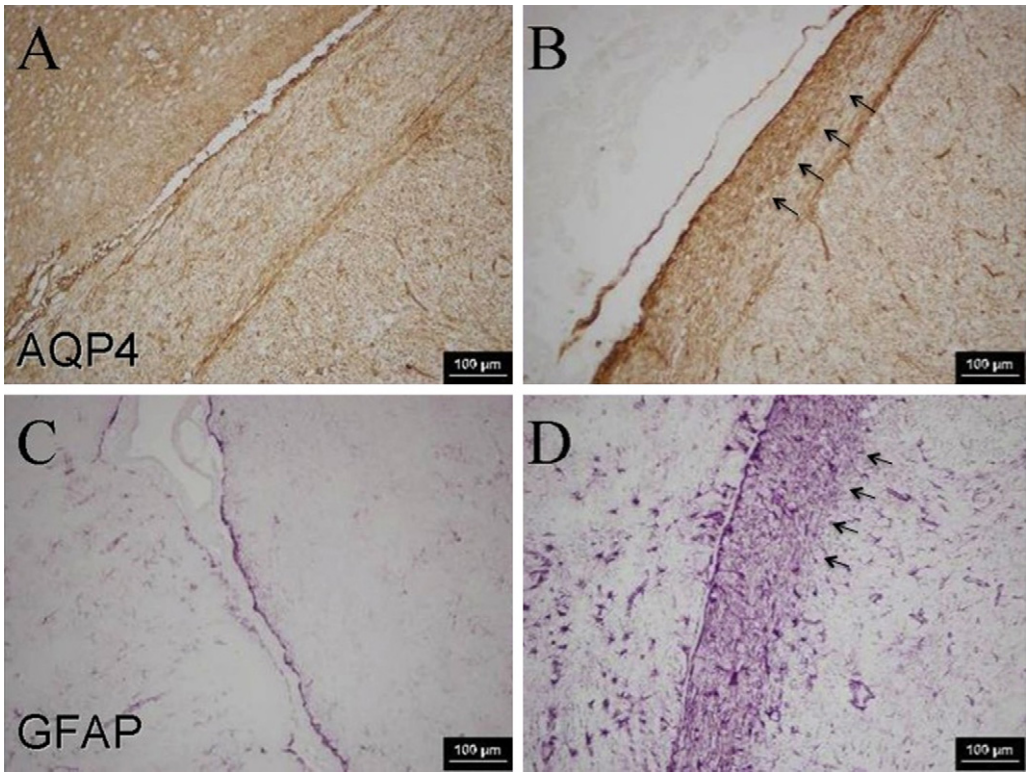


Fig. 6. Disease stage: AQP4 and GFAP immunostaining in periventricular areas of control and MOG-immunized rats with histological scores (HS) (for detail of the histological score, see Experimental procedures). The figure illustrates AQP4 and GFAP immunostaining in periventricular areas of control (A and C) and MOG-immunized rats at the disease stage (B and D). In the brain, a moderate, continuous increase in expression of AQP4 (+1, arrows in B) and GFAP (+1, arrows in D), accompanied by gliosis and astrocytic hypertrophy was observed without demyelination.

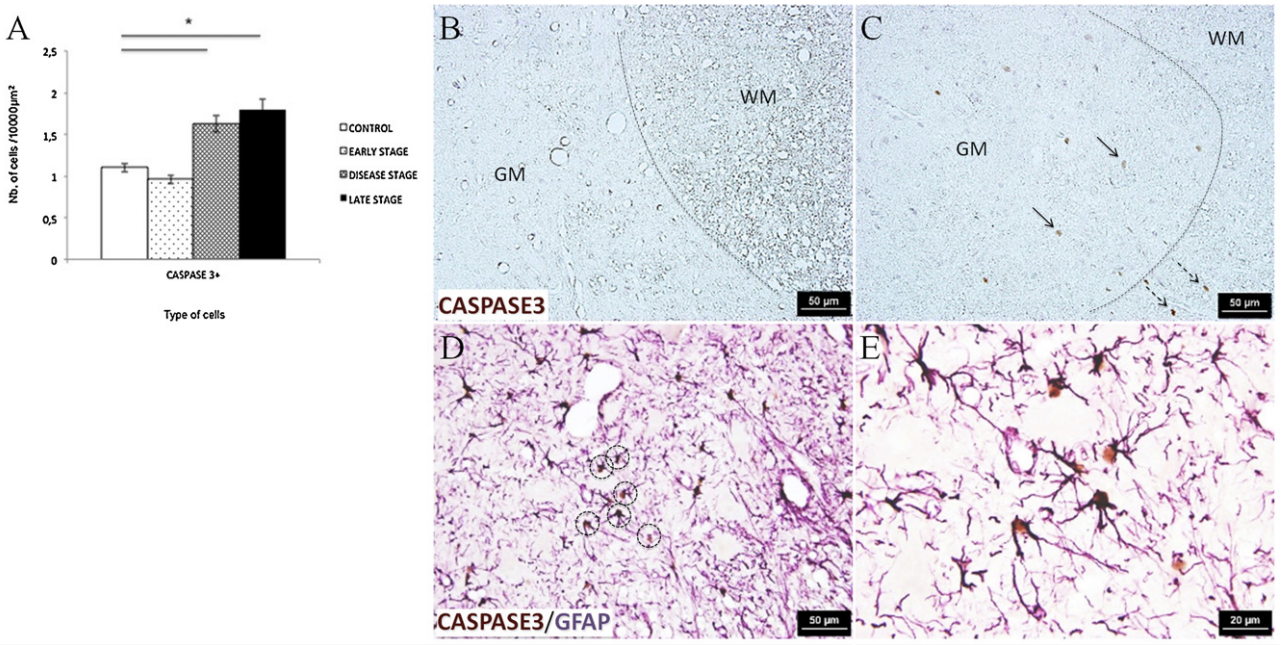


Fig. 7. Evaluation of GFAP and caspase3 immunostaining in spinal cords of control and MOG-immunized rats. Mean number of caspase3 positive cells is increased in the rats at the disease and late stage compared to controls (A). Compared to control (B), caspase3 marked cells in MOG-immunized rat at the disease stage were localized in gray (solid arrows) and white matter (dotted arrows) of the spinal cord (C). Double immunostaining with GFAP and caspase3 revealed a pronounced astrocytic apoptosis at the disease stage (D). Cells delineated with dotted circles in D are presented at higher magnification in E. * $p < 0.001$; GM = gray matter; WM = white matter.

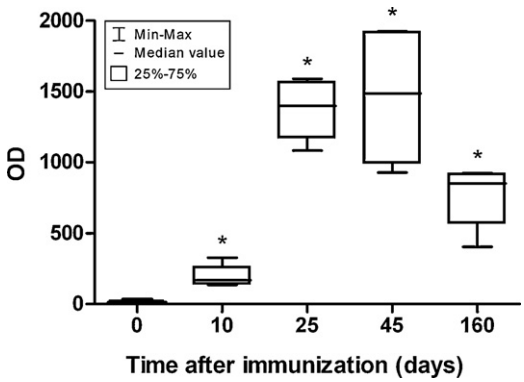


Fig. 8. Boxplot of MOG immunoreactivity (ELISA) in serum samples of MOG-immunized rat, as a function of post-immunization time. Before immunization (day 0), the mean optical density (OD) was (mean ± standard deviation) 14 ± 8 (n = 7). After immunization, the mean OD was 200 ± 89 at day 10 (n = 4) and 1368 ± 242 at day 25 (n = 4) for the early stage of the disease and 1456 ± 539 at day 45 (n = 4) at the disease stage. At the late stage, the mean OD was 779 ± 196 at day 160 (n = 6). * Statistical difference compared to day 0.

immunoreactivity against individual AQP4 peptides described in Table 1 was found by ELISA.

3.5.2. AQP4-Ig assay detection of immunoreactivity against AQP4

Rat sera tested against conformational AQP4 using the immunofluorescence and immunocytochemical techniques with human AQP4 transfected cell line were also negative (data not shown).

3.6. Correlations between the clinical and histological scores and MOG antibody level

Using a nonparametric test, we observed a correlation between the high clinical score at sacrifice and the increase in histological scores for demyelination and inflammation in the optic nerve (r = 0.6; p < 0.001) and spinal cord (r = 0.8; p < 0.001). The modification of histological scores for AQP4 was not correlated with clinical scores in the spinal cord (r = 0.3; p = 0.02), optic nerve (r = −0.5; p = 0.02) or periventricular area (r = 0.3; p = 0.1). The modification of histological scores for GFAP was not correlated with clinical scores in the spinal cord (r = 0.3; p = 0.51), optic nerve (r = −0.1; p = 0.74) or periventricular area (r = 0.03; p = 0.84). No correlation was found between the clinical scores and the level of MOG antibodies in immunized rat sera (r = 0.4; p = 0.1).

4. Discussion

In this study, we characterized by MRI and histology the opticospinal phenotype of the BN MOG-induced model of demyelination. We added to its previous description the involvement of the periventricular area and we recorded the expression of myelin, AQP4 and GFAP in the optic nerves, spinal cords and periventricular areas. The modification of AQP4 level and astrocyte apoptosis occurred despite the absence of antibodies tested against linear and conformational epitopes of AQP4. Furthermore, the elevation of disability scores in rats was only synchronized with the severity of demyelination/inflammation in the optic nerve and spinal cord but not with the modification of AQP4 or GFAP expression.

In the present study, we added the involvement of the periventricular area to the opticospinal phenotype in the BN MOG-induced animal model. This area was free of demyelination during the disease process but showed T2-hypersignal on MRI at the disease stage. This observation indicated a water flux abnormality in this area and was supported by a local modification of AQP4 expression, playing a crucial role in water transport across the blood–brain barrier. With the optic nerve and spinal cord, the periventricular area underlines the implication of regions containing high levels of AQP4 in this model (Nielsen et al., 1997; Venero et al., 1999).

AQP4 immunostaining showed different patterns of expression in the three regions of interest. During the disease process in the optic nerve, we observed a transiently low level of AQP4, which starts before demyelination, and can be attributed to vasogenic edema, blood–brain barrier impairment and/or inflammation that may result in astrocyte degeneration (Ke et al., 2001; Sattler et al., 2008). In view of the excess fluid seen in the white matter and the regressive T2-hypersignal observed at the disease stage in the optic nerve, vasogenic edema seems to be the main actor in the AQP4 physiopathological process. During the later stage, a healing process may explain the extensive gliosis and increased AQP4 expression. In contrast, astrocytes in the spinal cord and the periventricular areas of the brain became hypertrophic early on in the gray and white matter and expressed higher immunoreactivity to AQP4 throughout the disease course. This finding may have resulted from cytotoxic edema fluid, characterized by astrocyte hypertrophy, which is known to accumulate in both the gray and white matter (Papadopoulos et al., 2004). This hypothesis is also supported by the persistent T2-hypersignal MRI in the spinal cord at the late stage, whereas the course of the vasogenic edema is usually regressive after the inflammatory process. Further experimentation with diffusion and gadolinium MRI is in progress in our laboratory to try to resolve this question.

Analysis of apoptotic cells showed a pronounced astrocytic death in spinal cord at the disease stage. Oppositely to classical EAE in which massive oligodendrocyte cell death was observed (Hisahara et al., 2003), the cell death in this model involves mainly astrocytes. In human, this data argues for an antibody-independent AQP4 astrocytopathy in the MOG-induced model of demyelination, as observed in heterogenous demyelinating conditions including Balo's disease, NMO and MS (Kira, 2011).

As expected, disability was correlated with the presence of demyelinating and inflammatory lesions. On the opposite, disability was not correlated with the AQP4 response as attested by the increasing immunostaining level of AQP4 in the spinal cord and the decreasing immunostaining level in the optic nerve. No correlation was found between disability and the level of GFAP expression in the spinal cord because astroglisosis at the late stage of the disease may represent the glial scar in a monophasic disease without sequellae at the late stage. Nevertheless, the expected differences between the type of edema in the spinal cord and optic nerve suggest that there may also be differences in the physiological functions of the blood–brain barrier and the blood–spinal cord barrier (Saadoun and Papadopoulos, 2010).

In EAE animal models, few studies reported an increase in AQP4 expression in the spinal cord and brain using RT-PCR (Miyamoto et al., 2009) or a strong astrocyte reactivity in the spinal cord by immunohistochemistry (Pham et al., 2009). No data were available about modifications in the expression of both AQP4 and GFAP in the reported EAE models in the optic nerve or the location of acute AQP4 overexpression in the brain. A recent study in a MOG-induced EAE model showed the attenuation of disease progression in AQP4 knockout mice (Li et al., 2009). Other experimental data were obtained with a MOG-induced EAE model in NOD/Lt and C57BL/6 mice, known to develop a diffuse inflammatory process not limited to the opticospinal location. Furthermore, CFA and pertussis toxin were largely used, which are known to alter the blood–brain barrier permeability (Lu et al., 2008; Namer et al., 1994).

Comparison with opticospinal demyelinating diseases in humans suggests that whereas an NMO-like phenotype is observed in this model, the course of AQP4/GFAP expression during the disease process is closer to opticospinal MS than NMO. Effectively, the

overexpression of AQP4 in the spinal cord has been observed in MS but not in NMO. Furthermore, no antibody against linear and conformational epitopes of AQP4 was found. On the other hand, 50% of patients with NMO experienced brain involvement (including the periventricular region) during the disease course, and 30–50% of patients test negative to NMO antibodies. Lastly, the decrease of AQP4 expression before demyelination in the optic nerve fits what is observed in NMO. Altogether, our experimental data raise the question of a continuum between opticospinal MS and the seronegative NMO subtype, reported in several countries (Cabre et al., 2009; Collongues et al., 2010; Misu et al., 2002).

5. Conclusion

At this point, several authors have attempted to reproduce NMO lesions in an animal model. In the animal models involving the injection of anti-AQP4 antibody (Bradl et al., 2009; Kinoshita et al., 2009), the pathogenicity of such antibodies has been clearly demonstrated but no animal has concomitantly developed opticospinal demyelination. The animals of our BNMOG-induced model develop an NMO-phenotype, an early implication of AQP4 in the optic nerves due to vasogenic edema and a pronounced astrocytic apoptosis in spinal cord during the disease process. Disability was correlated with the intensity of the inflammatory process in the demyelinated spinal cord and optic nerve. Despite these similarities with NMO in humans, the cytotoxic edema with AQP4 overexpression in the spinal cord during the disease process, the involvement of periventricular area and the absence of anti-AQP4 antibodies are closer to what is observed in MS. We think that this model raises the question of a continuum between opticospinal MS and the seronegative NMO subtype.

Acknowledgments

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ORIGINAL CONTRIBUTION

A Benign Form of Neuromyelitis Optica

Does It Exist?

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Background: Few data exist on a possible benign form of neuromyelitis optica (NMO).

Objectives: To identify NMO with a good outcome (go-NMO) among a large population of patients and to describe demographic and clinical variables associated with go-NMO vs standard NMO and benign multiple sclerosis.

Design: Observational retrospective multicenter study.

Setting: Twenty-five medical centers in metropolitan France (MF) and 3 medical centers in the French West Indies (FWI).

Patients: A total of 175 patients with NMO were retrospectively analyzed from 2 cohorts: 125 in MF and 50 patients of nonwhite race/ethnicity in the FWI. Patients in MF fulfilled the 2006 NMO criteria, whereas patients in the FWI fulfilled the 1999 or 2006 NMO criteria. Neuromyelitis optica and multiple sclerosis databases were reviewed, and patients with a score of 3 or lower on the Expanded Disability Status Scale after a 10-year follow-up period were considered to have go-NMO.

Main Outcome Measures: Clinical, laboratory, and magnetic resonance imaging data and course of disability.

Results: In MF, go-NMO was observed in 11 patients, including 3 untreated patients. In the FWI, NMO was severe because of disability related to optic neuritis. Compared with standard NMO, go-NMO was associated with a lower annualized relapse rate (0.3 vs 1.0, $P < .01$), and 8 of 11 patients with go-NMO showed complete regression of myelitis on magnetic resonance imaging during the disease course. Three patients experienced a disabling attack of NMO after 15 years of follow-up. A good outcome occurred less frequently among patients with NMO than among patients with multiple sclerosis (12.0% vs 22.4%, $P = .03$).

Conclusions: Among patients in MF, go-NMO occurs rarely. However, because a disabling attack may occur after a long follow-up period, a benign form of NMO cannot be defined.

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NEUROMYELITIS OPTICA (NMO) is a relapsing and disabling autoimmune disease of the central nervous system, characterized by severe optic neuritis and extensive myelitis. A direct consequence of NMO is disability, including complete vision loss or paraplegia, which may be lethal in 2.9% to 25% of patients, mainly through brainstem involvement.[1-5] Optic neuritis is initially followed by severe residual vision loss (visual acuity [VA], ≤0.1 minutes of arc [corresponding to 20/200 on Snellen visual acuity charts]) in 30% of patients descended from nonwhite populations and in 22% of patients descended from the white population.[6] After a first episode of myelitis, ambulation is limited to 500 m, corresponding to a residual Expanded Disability Status Scale (EDSS) score of 4 or higher, in 37.3% of patients.[2] These data argue for development of severe disabling inflammatory disease in the absence of immunosuppressive therapies among patients with NMO.

Despite the putative severity of NMO, there are rare reports of a benign course in patients with NMO.[7-9] Such patients developed NMO secondary to celiac disease[7] or dengue infection.[9] In a third case report, NMO was observed in a patient without evidence of systemic autoimmune or infectious disease.[8] These reported cases fulfilled the 2006 diagnostic criteria for NMO.[10] Cohort investigations that included more than 30 patients with NMO have not estimated the proportion of patients with good outcome NMO (go-NMO) (ie, those with a

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Group Information: The NOMADMUS and CF-SEP Study Group members are listed on page 923.

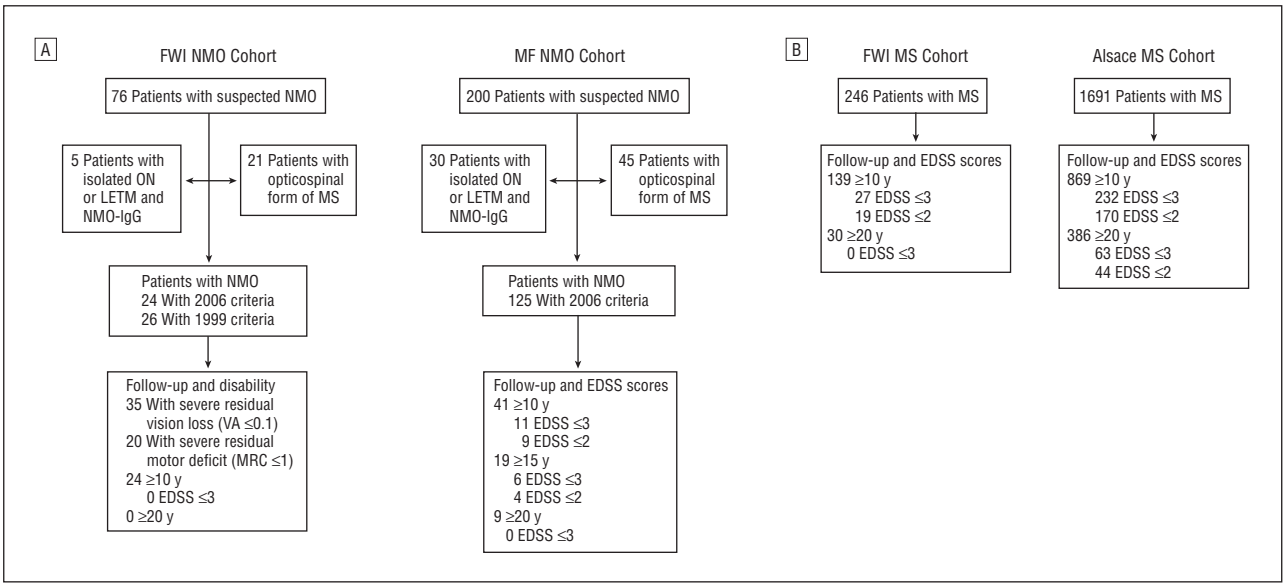

Figure 1. Selection of patients with neuromyelitis optica (NMO) (A) and with multiple sclerosis (MS) (B) in the French West Indies (FWI) and in metropolitan France (MF). A, Patients with NMO were analyzed for a good outcome based on the follow-up period and the level of disability (Expanded Disability Status Scale [EDSS] score, visual acuity [VA], and Medical Research Council [MRC] score). B, Patients with MS were selected for a benign form based on the follow-up period and the level of disability (EDSS score). In MF, MS data were obtained from the database for Alsace, a representative French region. ON indicates optic neuritis; LETM, longitudinal extensive transverse myelitis.

score of ≤3 on the EDSS after a 10-year follow-up period). Evaluation of disability in these cohorts suggests that the disease may be less severe among patients of white[3,5,11] vs nonwhite[1,4] race/ethnicity.

We analyzed 2 NMO cohorts, one in metropolitan France (MF) with 125 patients and the other in the French West Indies (FWI) with 50 patients of nonwhite race/ethnicity, to identify patients with go-NMO. We further describe demographic and clinical variables associated with go-NMO vs standard NMO and benign multiple sclerosis (MS) in these geographic areas to develop hypotheses about benign inflammatory disease of the central nervous system.

METHODS

We performed an observational retrospective multicenter study of NMO in France, including MF and the FWI. Data were collected from September 1, 2007, through September 1, 2010, corresponding to the end point of the study. In MF, 25 tertiary hospital centers recruited 200 patients with a suggested diagnosis of NMO; 125 of these fulfilled the 2006 NMO criteria and were included in the study. Data derived from this cohort have been published elsewhere.[2] In the FWI, 3 tertiary hospital centers recruited 76 patients with a suggested diagnosis of NMO; 50 of these were included in the study (26 fulfilled the 1999 NMO criteria and 24 fulfilled the 2006 NMO criteria). Selection of patients with NMO for the study is shown in **Figure 1** A. Selection of patients with benign MS for the study is shown in Figure 1B. Data were derived from hospital medical records and from a clinical information questionnaire specifically designed for NMO. If needed, additional data were obtained from the participating centers by e-mail or by telephone. All data were entered in the European Database for Multiple Sclerosis, recently modified to include NMO.[12] In MF, MS data were obtained from the database for Alsace, a representative French region. In the FWI, MS data were obtained from the geographic Caribbean database. Data confidentiality and security were ensured consistent with recommendations of the French data protection authority (Commission Nationale de l'Informatique et des Libertés), which also approved the study.

Assessed for each patient with NMO were demographic data, medical history, treatment, laboratory test results, neuroimaging data, and key episodes in the course of NMO (relapses and successive disability score dates). In patients with go-NMO, initial spinal cord magnetic resonance (MR) imaging was performed a mean (SD) of 7.8 (6.5) years after disease onset, 4.8 (4.1) years after the first episode of myelitis. Imaging was performed immediately after the first episode of myelitis in patients 1, 2, 3, and 5 (**Table 1**). Initial brain MR imaging was performed a mean (SD) of 7.3 (6.2) years after disease onset and was classified as normal in the presence of criteria by Paty et al[13] or by Barkhof et al[14] or as abnormal in the absence of those criteria. Serum samples from 111 patients in MF and from 24 patients in the FWI were tested for NMO-IgG with an indirect immunofluorescence assay on a substrate of adult rat cerebellum and midbrain using a previously described technique.[15,16] Among the MF cohort of patients with go-NMO in whom the immunofluorescence assay was negative (patients 1, 2, 3, and 11), anti–aquaporin 4 antibody detection was performed using a routinely used cell-based assay with aquaporin 4–transfected cells.[17] For the other patients, the NMO-IgG test was unavailable at NMO onset and was not necessary to diagnose NMO according to the 1999 or 2006 criteria. Residual disability was assessed during at least 6 months using EDSS score and VA. An EDSS score of 3 corresponded to benign MS, and an EDSS score of 2 reflected better vision. Visual acuity was included in the vision variable of the EDSS score.[18] An EDSS score of 3 was compatible with a maximum VA of 0.1 or less (20/200 Snellen) in the worse eye, and an EDSS score of 2 indicated a large scotoma or a maximum VA of 0.33 to 0.2 (20/60 to 20/100 Snellen) in the worse eye.

Comparisons of categorical data were performed using χ^2 test. Comparisons of quantitative data were conducted using Mann-Whitney test. Kaplan-Meier technique was used to estimate time to the second episode and time to initial treatment. Survival curves were compared using log-rank test. Two-

Table 1. Demographic and Clinical Characteristics of Patients Having Neuromyelitis Optica (NMO) With a Good Outcome

Patient No./Sex/ Race/Ethnicity/ Age at Onset, y/ Follow-up, y	Topography of First Attack	Opticospinal Interval, mo	First Interattack Interval, mo	Annualized Relapse Rate, Mean	NMO-IgG	Cerebrospinal Fluid, OCB/WBC Count, Cells/mm^3	Abnormal Spinal MR Imaging, No. of Lesions/ Location of LETM	First Brain MR Imaging, No. of Lesions[a]	Treatment
1/F/W/17.0/15.2	ON	22	22	0.7	+	+/0	1/CT	0	Interferon
2/M/W/51.2/14.3	SC	4	4	0.4	–	–/29	1/CT	1	Azathioprine
3/M/W/27.4/16.4	SC	108	108	0.2	–	+/0	2/C	0	. . .
4/M/W/37.8/19.2	ON	48	36	0.4	–	–/5	1/C	0	Interferon, glatiramer acetate
5/F/W/20.2/15.3	ON	96	24	0.3	–	–/3	3/C, T	1	. . .
6/F/W/24.8/25.4	ON	120	60	0.3	+	–/0	2/C, T	0	Interferon, cyclophosphamide
7/M/A/14.7/18.2	ON	75	75	0.3	+	–/128	1/CT	0	Cyclophosphamide
8/F/W/29.8/11.4	SC	119	119	0.2	+	+/16	1/. . .	0	. . .
9/F/B/35.5/14.3	ON	48	12	0.5	+	+/4	1/C	0	Interferon, mycophenolate mofetil
10/F/W/52.3/13.7	ON	3	3	0.2	–	–/5	1/C	0	Azathioprine
11/F/W/36.7/16.3	ON	48	48	0.4	+	–/12	1/T	0	Cyclophosphamide

Abbreviations: A, Asian; C, cervical; CT, cervicothoracic; ellipsis, not applicable; LETM, longitudinal extensive transverse myelitis; MR, magnetic resonance; OCB, oligoclonal bands; ON, optic nerve; SC, spinal cord; T, thoracic; WBC, white blood cell; +, positive; –, negative.

SI conversion factor: To convert white blood cell count to $\times 10^9$/L, multiply by 0.001.

[a] For criteria by Paty et al[13]/by Barkhof et al,[14] all results were negative/negative.

sided $P < .05$ was considered statistically significant. All analyses were performed using commercially available statistical software (SPSS for Windows, version 14.0; SPSS Inc, Chicago, Illinois).

RESULTS

PATIENTS WITH go-NMO

Good-outcome NMO was observed in 11 patients, all of whom were in the MF cohort (Figure 1A). When 33 patients with an unknown long-term clinical course (ie, <10 years' follow-up and EDSS score of ≤3) were excluded, 11 of 92 patients with go-NMO represented 12.0% of the NMO cohort. Table 1 gives individual demographic and clinical characteristics of 11 patients with go-NMO. The first episode of myelitis was characterized by an isolated sensitive symptom in 5 patients and by a sensorimotor deficit in 6 patients (5 with sphincter impairment). After intravenous corticotherapy, recovery from myelitis was complete in 5 patients, including 3 patients with myelitis at NMO onset. The initial episode of clinical optic neuritis was bilateral in 1 patient. Visual acuity was 0.1 or worse (≤20/200 Snellen) in 6 patients, and the mean VA was 0.2 (20/100 Snellen) in the disabled eye. After intravenous corticotherapy, recovery from optic neuritis was complete in 9 patients. After a mean (SD) follow-up period of 16.4 (3.9) years, the course of NMO was relapsing-remitting in 10 patients and monophasic in patient 8, who was lost to follow-up because of suicide 11.7 years after disease onset. Using an EDSS cutoff score of 2, patients 10 and 11 were not considered to have go-NMO. At the end of the follow-up period, patient 3 had an EDSS score of 7 because of severe myelitis, and patients 6 and 7 had EDSS scores of 4.5 and 5, respectively, because of combined severe VA loss and myelitis. Residual VA of 0.1 or worse (≤20/200 Snellen) in 1 eye was observed in patients 7 and 8, but the mean VAs among the entire go-NMO cohort at the end of the follow-up period were 0.8 (20/25 Snellen) in both eyes.

Longitudinal extensive transverse myelitis was observed a mean (SD) of 9.7 (5.8) years after NMO onset and a mean (SD) of 5.8 (4.3) years after the first episode of myelitis and was observed in 7 patients on initial spinal cord MR imaging. Patient 8 was free of extensive myelitis at the end of the follow-up period. Apparent complete regression of myelitis (**Figure 2**) was observed in 8 of 11 patients with go-NMO.

In the FWI cohort, no go-NMO was observed. Most patients had severe optic neuritis with residual VA of 0.1 or worse (≤20.200 Snellen), despite immunosuppressive therapy (Figure 1A).

DEMOGRAPHIC AND CLINICAL VARIABLES ASSOCIATED WITH NMO VS BENIGN MS

Demographic and clinical data associated with NMO vs benign MS are summarized in **Table 2**. Excluding demographic data (higher ratios of female to male patients and nonwhite to white races/ethnicities in the FWI), disease-related characteristics of standard NMO were similar in the FWI and MF cohorts and allow a comparison between go-NMO and standard NMO. Compared with standard NMO, go-NMO was associated with a lower annualized relapse rate and demonstrated a trend to delayed time from onset to a second episode. Whereas patients with standard NMO were often treated with immunosuppression, 3 patients with go-NMO required no treatment, and 2 patients with go-NMO needed only immunomodulatory therapy.

A lower percentage of patients having NMO experienced a good outcome of the disease compared with patients having benign MS in Alsace (12.0% vs 22.4%, $P = .03$). Except for a predominance of nonwhite patients in the FWI cohort, demographic and clinical characteristics of patients with benign MS were identical in Alsace and in the FWI. Similar results were noted when the cutoff for go-NMO was an EDSS score of 2 or lower.

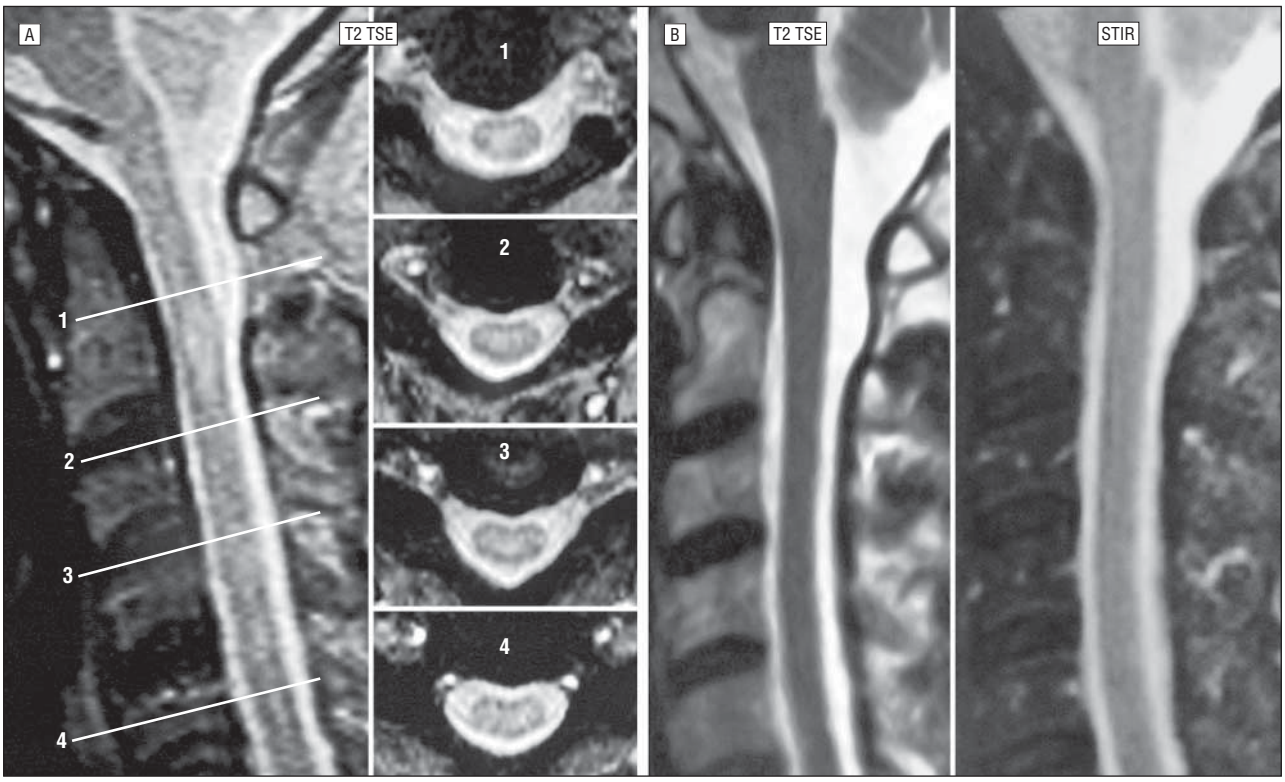

Figure 2. Apparent regression of spinal cord magnetic resonance imaging hypersignal during the follow-up period in patient 4 (Table 1) having neuromyelitis optica with a good outcome. A, Longitudinal extensive transverse myelitis was diagnosed on a longitudinal section of a 0.5-T T2-weighted turbo spin-echo sequence (TSE/M) in 1999 (left). Transverse sections of the lesions at different levels confirmed extensive transverse myelitis, with a length of 3 or more vertebral segments (right). B, Longitudinal section on a 1.5-T T2-weighted TSE/M (left) and a short tau inversion recovery (STIR) sequence (right) in 2004 showed complete regression of myelitis.

COMMENT

Our study found go-NMO in 12.0% of patients among the MF NMO cohort. No patients in the FWI NMO cohort had a good outcome because of severe optic neuritis among this population. Compared with patients having standard NMO, patients having go-NMO had a lower annualized relapse rate, and complete regression of myelitis on spinal cord MR imaging was observed in 8 of 11 patients with go-NMO.

Based on our results, it is difficult to conclude that a benign form of NMO exists. First, the term *benign* is based on an assessment of the natural history of the disease, whereas only 3 patients in our cohort with go-NMO were untreated. Second, the expression "benign form" implies a permanent condition, whereas patients with NMO may have a good outcome for many years and then have a disabling attack, as observed in 3 patients after 15 years of follow-up.

To date, the so-called benign form of NMO has been described only in case reports. Patients with opticospinal demyelination secondary to celiac disease or dengue infection dramatically improved after causative treatment and a short course of corticotherapy.[7,9] Primary benign NMO has been described in a patient with opticospinal demyelination, longitudinal extensive transverse myelitis, and normal brain MR imaging.[8] The disease was characterized by a 6-year interval between the first 2 attacks and by marked swelling of a cervical lesion, which was initially suggestive of a low-grade tumor. The patient responded to corticotherapy and experienced progressive improvement after a few months. Six years after this event, spinal cord MR imaging showed complete regression of the initial lesion. This is in accord with our description of go-NMO, including spontaneous improvement, regressive myelitis, and a low annualized relapse rate. In contrast, clinical predictors of death among the FWI cohort with NMO included a high frequency of episodes during the first year of disease, blindness or sphincter signs at onset, and lack of recovery after the first attack.[1] Among the MF cohort, independent risk factors for EDSS scores of 4, 6, and 7 were not identified, but a large number of lesions on brain MR imaging during the disease course of NMO may predict a VA outcome of 0.1 or worse (≤20/200 Snellen).[2]

Results of this study suggest some possible explanations for low disability among patients with NMO. The most important of these relate to the low annualized relapse rate and the complete regression of myelitis on MR imaging in 8 of 11 patients with go-NMO. These observations indicate that disability may be exclusively linked to relapses in NMO, without progressive aggravation of disability between relapses. The findings underline a major difference in the pathophysiological processes between NMO and MS, in which a progressive course is common.[2,19] Regression of myelitis on MR imaging in inflammatory demyelinating diseases of the central nervous system has been described in acute disseminated

Table 2. Characteristics of Patients Having Neuromyelitis Optica (NMO) in Metropolitan France (MF) and in the French West Indies (FWI) and of Patients Having Benign Multiple Sclerosis (MS) in Alsace and in the FWI[a]

Characteristic	NMO in FWI (n=50)	Standard NMO in MF (n=81)	go-NMO in MF (n=11)	*P* Value[b]	Benign MS in Alsace (n=232)	*P* Value[c]	Benign MS in FWI (n=27)	*P* Value[d]
Female-male ratio	9:1	2.5:1	1.75:1	.85	2.8:1	.70	8:1	.13
White-nonwhite ratio	0:50	9:1	4.5:1	.79	28:1	.10	0:27	<.01
Age at onset, mean (SD), y	31.8 (13.0)	35.2 (14.0)	31.6 (12.6)	.36	29.4 (8.7)	.71	26.3 (8.9)	.13
Disease course monophasic:remitting ratio	0:50	1:4	1:10	.66	0:232	.03	0:27	<.01
Time from onset to second attack, mean (SD), mo	23.3 (45.0)	31.2 (44.6)	46.5 (40.2)	.08	59.5 (32.7)	.17	58.9 (64.2)	.17
Annualized relapse rate, mean (SD), mo	0.9 (0.7)	1.0 (1.5)	0.3 (0.1)	<.01	0.4 (0.2)	.24	0.4 (0.2)	.19
Patients receiving treatments, No. (%)	39 (78.0)	76 (93.8)	8 (72.7)	.08	181 (78.0)	.97	20 (74.0)	.82
No. of treatments received, mean	...	...	...	.06	...	<.01	...	.31
IM	1	3	2	...	148	...	19	...
IS	30	48	4	...	7	...	0	...
IM+IS	8	25	2	...	26	...	1	...
Time to first treatment, median (95% CI), y	4.5 (2.3-6.7)	2.1 (1.4-2.7)	12.0 (8.3-15.7)	.03	11.0 (5.0-17.0)	.65	11.0 (9.6-12.4)	.50
NMO-IgG, No. (%)	15 (62.5) (n=24)	34 (50.0) (n=68)	6 (54.5)	.96	...	...	...	...

Abbreviations: CI, confidence interval; ellipsis, not applicable; go-NMO, NMO with a good outcome; IM, immunomodulatory treatment; IS, immunosuppressive treatment.

[a] Patients with an Expanded Disability Status Scale score lower than 3 and with less than 10 years' follow-up are excluded.

[b] Comparison between NMO subgroups in MF.

[c] Comparison between go-NMO and benign MS in Alsace.

[d] Comparison between benign MS in Alsace and in the FWI.

encephalomyelitis and in the pseudotumoral form of MS.[20] Such observations are uncommon in classic MS, whatever the course of the disease. In NMO, this point is poorly documented, but reported cases demonstrate that marked swelling in myelitis can shrink after high-dose corticotherapy.[8,21] In a study by Cassinotto et al[21] that included 17 patients with NMO, complete regression was observed in only 2 patients after a 26-month follow-up period. In most cases, these spinal cord lesions progress to atrophy and necrosis, leading to syrinxlike cavities on T1-weighted images. From a pathological point of view, it would be relevant to correlate transient myelitis in NMO with the presence of NMO-IgG, which can appear and disappear during the course of the disease.[22] Involvement of immunosuppressive therapies in this transitory detection was not demonstrated in a large prospective follow-up study,[2] but our data show that the phenomenon can occur without any treatment.

Good-outcome NMO was observed in the MF cohort but not in the FWI cohort. Differences in genetic backgrounds may be a key factor in accounting for these results, as suggested by the low proportion of nonwhite patients with go-NMO in the MF cohort. Despite immunosuppressive therapy, the median time in the FWI cohort from NMO onset to an EDSS score of 3 was 1 year, and death occurred in 25% of patients.[1] In other NMO cohorts of primarily white race/ethnicity, percentages of patients with NMO-related death were as follows: 2.9% in a Mexican cohort,[4] 3.2% in an MF cohort,[2] 13% in an Italian cohort,[3] and 22.5% in a North American cohort.[5] The apparent severity of NMO in the North American cohort may be related to the following: (1) the high proportion of untreated patients in 1999, when the new NMO spectrum was first described; (2) a selection bias for severe hospitalized cases; and (3) the unspecified proportion of nonwhite patients in this population. The severe disability in our FWI cohort was mainly because of early and severe vision impairment, characterized by a permanent loss of vision after 2 attacks in a given eye and by a median time of 2 years from onset to monocular vision loss.[6] Nevertheless, some patients in the FWI cohort experienced complete regression of their myelitis and remained fully ambulatory. These data raise questions about the validity of the vision scale used to calculate the EDSS score when applied to patients with NMO.

Several biases may limit interpretation of our study results. First, we may have inadvertently selected patients with good prognosis in the MF cohort, as demonstrated by the low proportion of deaths compared with that in the FWI cohort. This could have occurred because of selection bias, as all patients in the MF cohort were being followed up at the start of the study and the 2006 NMO criteria were used in this population. In contrast to the 1999 NMO criteria used in the FWI cohort, the 2006 NMO criteria do not include the severity of motor disability and vision impairment in the diagnosis and may select less disabled patients.[23] Second, whereas the collected data among this NMO cohort were among the most complete in the literature, the retrospective study design induced a lack of power in the search for predictive factors of disability. For example, good recovery after the first clinical event reported among patients with go-NMO cannot be compared with that among patients of the entire MF and FWI cohorts because of the wide range of treatments used after the first event, as well as the difficulty of assessing this in patients with a 10-year follow-up period. Third, the definition used for go-NMO was derived from that used for MS. Visual acuity was included in the EDSS score, and a converted vision score of 3 was compatible with a maximum VA of 0.1 or worse (≤20/200 Snellen) in the worse eye, which raises questions about whether symptoms were benign. That is why we also considered an EDSS score of 2 compatible with a large scotoma or a maximum VA of 0.33 to

0.2 (20/60 to 20/100 Snellen) in the worse eye. However, such considerations do not alter the results of the study.

Because a disabling attack may occur after a long follow-up period, a benign form of NMO cannot be defined. However, results of this study show that go-NMO exists. This finding is applicable to a small percentage of patients with NMO descended from white populations with a low annualized relapse rate of NMO. The result is independent of NMO-IgG seropositivity, which does not affect the course of NMO.[1,2,16,22] As in MS, a long first interattack interval could be indicative of a less disabling course of NMO.[24] Furthermore, the resolution of myelitis without sequelae on MR imaging (spontaneously or after a short course of oral corticotherapy) may suggest a potentially good outcome of NMO. When NMO is suspected, complete regression of symptoms and of signal abnormalities on MR imaging may also be indicative of a good outcome. These considerations are valid only in the absence of the predictors of severity mentioned herein.

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High-risk syndrome for neuromyelitis optica: a descriptive and comparative study

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Abstract

Background: Neuromyelitis optica (NMO) frequently begins with a monofocal episode of optic neuritis or myelitis. A concept named high-risk syndrome (HRS) for NMO has been proposed for patients with monofocal episodes and NMO-IgG antibodies.

Objective: To describe HRS patients and compare them with NMO patients.

Methods: We identified 30 patients with HRS: 18 with extensive myelitis (HRM) and 12 with optic neuritis (HRON), in a database pooling patients from 25 centres in France. Clinical, laboratory/magnetic resonance imaging (MRI) data and outcome were analysed and compared with a national cohort of 125 NMO patients extracted from the same database.

Results: Mean follow-up was 4.8 years. Mean age at onset was 42.8 years (range: 12.4–70) with a female:male ratio of 0.9. Asymptomatic lesions were report on visual evoked potentials in 4/8 tested HRM patients and on spinal cord MRI in 2/7 HRON patients. Three patients died, two owing to a cervical lesion. HRS and NMO patients had similar clinical/paraclinical data, except for a predominance of men in the HRS group and a later mean age at onset in the HRM subgroup.

Conclusion: The description of HRS patients is compatible with a monofocal form of NMO. Asymptomatic lesions could be included in a new set of NMO diagnostic criteria.

Keywords

Cohort study, Devic's syndrome, high-risk syndrome, NMO-IgG, optic neuritis, transverse myelitis

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Introduction

The bifocal location in neuromyelitis optica (NMO) is a necessary condition according to all diagnostic criteria.[1,2] A specific serum autoantibody, NMO-IgG,[3] which binds to the dominant central nervous system water channel protein aquaporin-4 (AQP4), has recently been identified in 50–70% of patients with NMO,[3,4] but also in 5–10% of patients with multiple sclerosis (MS).[5–7]

As suggested in small cohorts, a concept named high-risk syndrome (HRS) has been proposed for patients with a monofocal episode of longitudinal

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extensive transverse myelitis (LETM) (high-risk myelitis: HRM) or optic neuritis (ON) (high-risk optic neuritis: HRON) and testing positive for NMO-IgG antibodies.[8–10] These patients are considered at high risk to develop NMO.

The aim of this study was to complete these data by (1) providing detailed clinical and paraclinical data of a large group of HRS patients, and (2) comparing HRM with HRON patients and HRS with NMO patients in the same cohort.

Patients and methods

We performed an observational and retrospective study of NMO in 25 tertiary medical centres in France from September 2007 to September 2008. Two hundred patients with a suspected diagnosis of NMO or HRS were identified. Of these, 125 patients fulfilled the 2006 NMO criteria[2] and 30 patients presented LETM ($n = 18$) or ON ($n = 12$) and positive NMO-IgG serology,[4] as indicated in Figure 1. LETM was defined by spinal cord magnetic resonance imaging (MRI) signal abnormalities extending over more than two vertebral segments, and ON was defined by clinical visual impairment and altered visual evoked potentials (VEP). Data were collected from hospital files or using standardized assessment forms designed for NMO and have been entered in the European Database for MS, adapted to NMO specificities.[11] The study was approved by the local ethics committee and data confidentiality and security were ensured in keeping with the recommendations of the French data protection authority, which approved the study.

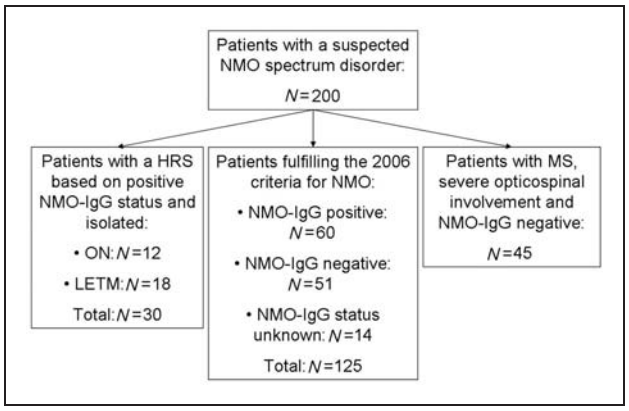

Figure 1. Selection of patients with a high risk syndrome (HRS) for neuromyelitis optica (NMO). Patients in 25 centres in France with a suspected NMO spectrum disorder were divided into the following groups: 1. HRS patients; 2. NMO patients fulfilling the 2006 NMO criteria; 3. MS patients with severe opticospinal involvement and negative NMO-IgG status. ON, optic neuritis; LETM, longitudinal extensive transverse myelitis; MS, multiple sclerosis.

For each patient, we recorded demographic data (gender, race/ethnicity, age at onset, follow-up), medical history of attacks and disability, laboratory data (NMO-IgG, VEP, oligoclonal bands (OCB) in cerebrospinal fluid (CSF)), MRI data and treatments. We excluded other known causes of myelitis and ON. Brain MRI was performed in all patients, spinal cord MRI in 25 patients and VEP in 20 patients. Serum samples were tested for NMO-IgG by an indirect immunofluorescence (IIF) assay on a substrate of adult rat cerebellum and midbrain following the original technical procedures.[3,5] The Expanded Disability Status Scale (EDSS) score was recorded to determine the extent of the neurological disability.

Categorical data were compared using the chi-squared test, and quantitative data using the Mann–Whitney test. The Kaplan–Meier technique was used to estimate time to assignment of the second attack and the EDSS scores. Survival curves were compared using the log-rank test. Two-sided p values <0.05 were considered statistically significant. All computations were performed using SPSS for Windows, version 14.0.

Results

The clinical characteristics of the HRM and HRON subgroups are summarized in Table 1. One patient had a primary Sjögren's syndrome and one patient had cryoglobulinaemia type III. OCB in CSF were found in 7/18 (38.9%) patients in the HRM subgroup and 5/12 (41.7%) patients in the HRON subgroup. In the HRM subgroup, VEP were altered in four of the eight patients tested. These four were tested 1, 3, 25 and 78 months after disease onset, respectively. In the HRON subgroup, a single, asymptomatic, short central or posterior cervical T2-hyperintense lesion on spinal cord MRI was observed in two patients, 7 and 12 years after the disease onset, respectively. Initial brain MRI can show T2-hyperintense lesions but was normal in nine patients (50%) with HRM and in eight patients (66.7%) with HRON (Table 1). Two patients (10%), both in the HRM subgroup, experienced both OCB and brain MRI lesions.

Eight patients with HRS experienced only one attack and 22 patients had a relapsing–remitting disease course. Monophasic and relapsing patients were similar in terms of demographic, clinical and paraclinical data, but mean follow-up was shorter in monophasic than in relapsing patients (3.9 vs. 5.1 years, $p = 0.06$). Six patients with HRM and four patients with HRON were followed up for more than 5 years and had a mean annualized relapse rate of 0.46 ± 0.55. No progressive course was observed. In the HRS group, EDSS score 4 was reached by 15 patients (50%), score 6 by nine patients (30%) and score 7 by seven

Table 1. Characteristics of the 30 patients with high-risk myelitis (HRM) and optic neuritis (HRON)

	HRM ($N = 18$)	HRON ($N = 12$)	p
Demography, *n*			
Female : male ratio	8 : 10	6 : 6	0.76*
White : non-white ratio	16 : 2	10 : 2	0.76*
Age at onset (years)			
Mean ± SD	46.4 ± 17.5	37.4 ± 17.4	0.16#
Median [range]	48 [12.4–70]	32.4 [15.1–67.7]	
Distribution, *n* (%)			
0–18	1 (5.6)	1 (8.3)	0.23*
19–29	3 (16.7)	6 (49.8)	
30–49	6 (33.3)	2 (16.6)	
≥50 years	8 (44.4)	3 (24.9)	
Associated AID, *n* (%)	1 (5.6)	1 (8.3)	0.65*
Lesions on first brain MRI, *n*			
0/1–2/3–8/≥9	9/3/3/3	8/3/0/1	0.48*
Follow-up (years)			
Mean ± SD	3.8 ± 2.9	6.3 ± 5.5	0.44#
Median [range]	3.8 [0–11.7]	5 [1.5–18.7]	
Course of the disease			
Relapsing–remitting : Monophasic	11 : 6	10 : 2	0.4*
Mean number of attacks after onset			
0–1 year	1.7	1.9	0.37#
0–2 years	1.8	2.1	0.32#
Mean annualized rate of attacks	0.75	0.97	0.58#
First inter-attack interval (months)			
Median [95% CI]	27 [16.2–37.8]	6 [0–16.2]	0.09$
Median time from onset to EDSS 4/6/7 (years)	2.3/3.7/10	7.8/NA/NA	0.06$°
Death, *n* (%)	2 (11.1)	1 (8.3)	0.8*

AID, autoimmune disease; EDSS, Expanded Disability Status Scale; MRI, magnetic resonance imaging; SD, standard deviation; NA, not available. For statistical analysis: *chi-squared test, #Mann–Whitney test, $log-rank test. °log-rank was used for EDSS 4 comparison only.

patients (23.3%), though at different interval times (see Table 1). The first myelitis attack was immediately followed by an EDSS score of 4 in 7.7%, 6 in 11.1% and 7 in 33.3% of cases. The high number of censored patients in the HRON subgroup for EDSS scores 6 and 7 is because the interval time to disability was not evaluated. Three patients died, two owing to a diffuse cervical lesion (a 63-year-old woman after the first attack and a 52-year-old woman after a follow-up of 3.5 years) and a 76-year-old man who died from extra-neurological diseases after a mean follow-up of 8.3 years. Among the 30 HRS patients, 25 were treated with immunosuppressive therapies, including the three patients who died. According to the severity of the disease, 14 patients with HRM and eight with HRON were treated with immunosuppressive therapies after the first attack, including five monophasic HRM patients. Three patients with HRM and two with HRON remained free of treatment after a mean follow-up of 6.7 years, including one patient with monophasic HRM and two patients with monophasic HRON.

Compared with NMO patients, HRS patients had similar demographic and clinical data, except on two points: first, there was a significant predominance of men in the HRS group, especially when compared with the NMO-IgG-positive subgroup of patients with NMO; second, there was a higher mean age at onset, but only in the HRM subgroup (Tables 1 and 2), whatever the NMO-IgG status of the NMO patients. No difference was found when female and male seropositive NMO-IgG patients were compared in the NMO and HRS cohorts. The other points of comparison are mentioned in Table 2.

Table 2. Comparison between NMO and high-risk syndrome (HRS) patients

	NMO ($N = 125$)	HRS ($N = 30$)	p
Demography, *n*			
Female : male ratio	94 : 31	14 : 16	0.002*
White : non-white ratio	74 : 11	26 : 4	0.36*
Age at onset (years)			
Mean ± SD	34.5 ± 13.2	42.8 ± 17.7	0.02#
Median [range]	34.7 [4–66]	44.5 [12.4–70]	
Topography of the first attack, *n* (%)			
Spinal	57 (45.6)	18 (60)	0.8*
Optic	46 (36.8)	12 (40)	
OCB in CSF, *n* (%)	25 (23.4)	12 (40)	0.27*
Lesions on first brain MRI, *n*			
Mean ± SD	0.9 ± 2.4	1.6 ± 3.3	0.52#
Follow-up (years)			
Mean ± SD	10 ± 7.8	4.8 ± 4.2	<0.001#
Median [range]	8.7 [0.1–39.5]	3.5 [0–18.8]	
Mean number of attacks			
0–1 year	1.8	1.8	0.5#
0–2 years	2.3	1.9	0.26#
Annualized relapse rate	0.99	0.8	0.66#
First inter-attack interval (months)			
All patients, median [95% CI]	12 [9–15]	22 [8.4–35.6]	0.62$
Patients with relapse, median [95% CI]	12 [9.2–14.8]	10 [1.7–18.3]	0.1$
Time from onset to disability (years)			
EDSS 4, median [95% CI]	7.3 [4.7–9.9]	7.4 [0.1–14.7]	0.25$
EDSS 6, median [95% CI]	10 [7.4–12.6]	8.3 [0.1–16.5]	0.98$
EDSS 7, median [95% CI]	21.4 [7.7–35.1]	10 [7.5–12.5]	0.27$
Censored patients for EDSS scores of 4/6/7	33/50/80	15/21/23	
Death, *n* (%)	4 (3.2)	3 (10)	0.11#

CSF, cerebrospinal fluid; EDSS, Expanded Disability Status Scale; MRI, magnetic resonance imaging; OCB, oligoclonal bands; SD, standard deviation; CI, confidence interval. For statistical analysis: *chi-squared test, #Mann-Whitney test, $log-rank test.

Discussion

HRS patients seemed to be similar to definite NMO patients in terms of clinical and paraclinical data, except for a monofocal clinical expression, a predominance of men and, but only in the HRM subgroup, an older mean age at onset. These similarities are supported by the discovery of asymptomatic lesion in HRS patients, which could shorten the time for the diagnosis of NMO according to the bifocal condition of the disease. Importantly, HRS patients also seemed to have a poor prognosis for disability taking into account the two patients who died owing to a diffuse cervical lesion.

Our demographic data show a predominance of men and a late mean age at onset (43 years) in the HRS group. The sex and age profiles of HRS patients are difficult to interpret because of the small size of the HRS cohort. However, it should be noted that these data argue against a first attack of MS, in which the first demyelinating event predominantly occurs in women around 30 years old. It might appear that a late onset of the disease in men could represent a specific subgroup in which central nervous system inflammation is limited to a monofocal form. However, the following data argue against this hypothesis: first, these demographic data have either not been reported in previous HRS studies[8–10] or were not reported to be a good prognostic factor for disability;[4,12] second, no difference was observed between male and female seropositive NMO-IgG patients in the NMO and HRS groups. Therefore, we cannot exclude the possibility that these

particularities were the result of a selection bias due to the size of the HRS cohort.

Considering the high specificity of NMO-IgG and the similarity between the HRS and NMO groups, HRS can be categorized as an NMO spectrum disorder in which: (1) most patients do not present both ON and myelitis within a short time period; and (2) some patients may have a clinical course characterized by recurrent ON or relapsing myelitis only. Furthermore, we found that the HRS group likely corresponded to NMO spectrum patients with a subclinical bifocal disease, as suggested by the altered asymptomatic VEP in HRM patients or by T2-hyperintense spinal cord lesion in HRON patient. In view of these new findings, we propose that asymptomatic lesions could be included in a new set of NMO diagnostic criteria.

To date, only four clinical studies, each including fewer than 15 HRS patients, have been published. Despite the small size of their HRS cohorts, these studies appear to show that HRS patients are likely to develop NMO or recurrent attacks of myelitis/ON. One study found that after a 1-year follow-up of nine patients with HRM, a new transverse myelitis attack had occurred in four patients and ON in one patient[10]. More rarely, recurrent episodes of LETM have been described in a patient with acute partial transverse myelitis, defined as short asymmetric lesions ($\leq$2 vertebral segments).[13] Two retrospective studies reported that after a mean follow-up of 5 and 6 years, 50% of the patients with recurrent HRON, compared with fewer than 20% of seronegative patients, developed NMO and that visual acuity was more severe in the seropositive group.[8,9] These clinical approaches are supported by the neuropathological findings in a patient with HRM, including a pattern of loss of AQP4 expression in demyelinated areas identical to that of NMO patients.[14]

Our results confirm, in a large cohort of patients, that HRS corresponds to an NMO spectrum disease and should therefore be an indication for early immunosuppressive therapy. In our study, this conclusion is corroborated by the poor prognosis for disability in HRS patients. To shorten the time for the diagnosis of NMO, we propose that asymptomatic lesions in HRS patients could be included in a new set of NMO diagnostic criteria.

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Conflict of interest statement

The authors declare that they have no conflicts of interest.

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Long-term follow-up of neuromyelitis optica with a pediatric onset

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ABSTRACT

Background: Neuromyelitis optica (NMO) is a rare inflammatory disease. Average age at onset is 35 years. Few data exist on patients with pediatric-onset NMO (p-NMO), with disease onset before age 18 years. We report the clinical and paraclinical features and long-term outcome of patients with p-NMO and compare them with a large adult-onset NMO (a-NMO) cohort.

Methods: We performed a retrospective, multicenter study of patients with p-NMO in pediatric and adult medical centers. We identified 125 patients with NMO (12 p-NMO; 113 a-NMO) fulfilling the 2006 criteria. Data were collected using hospital files and standardized assessment forms for NMO.

Results: Patients with p-NMO were followed up during a mean 19.3 years. Median age at onset was 14.5 years (4.1–17.9) with a female:male ratio of 3:1. Three patients (25%) fulfilled Paty criteria for multiple sclerosis on first brain MRI, including one patient with acute disseminated encephalomyelitis. Median interval between onset and residual Expanded Disability Status Scale (EDSS) score 4 was 20.7 years, score 6 was 26 years, and score 7 was 28.7 years. Median interval between onset and residual visual loss ≤1/10 was 1.3 years. Compared with a-NMO, p-NMO showed a longer time to EDSS scores 4 and 6, largely explained by the severity of the first myelitis in the a-NMO group. Time to first treatment was longer in the p-NMO group (13.1 vs 3.4 years).

Conclusion: Patients with p-NMO can present a diffuse inflammatory process on first brain MRI and have a longer time to disability than patients with a-NMO. *Neurology*® 2010;75:1084–1088

GLOSSARY

a-NMO = adult-onset neuromyelitis optica; **EDSS** = Expanded Disability Status Scale; **IgG** = immunoglobulin G; **LETM** = longitudinal extensive transverse myelitis; **MS** = multiple sclerosis; **NMO** = neuromyelitis optica; **p-NMO** = pediatric-onset neuromyelitis optica; **SRVL** = severe residual visual loss.

Neuromyelitis optica (NMO) is a rare inflammatory disease occurring at an average age of 35 years (range 4–66).[1-3] Apart from case reports,[4,5] only 2 recent retrospective studies have contributed to epidemiologic and laboratory data on children with NMO: one on 17 children fulfilling the 1999 NMO criteria followed up for an average of 3 years and the other on 9 children fulfilling the 2006 NMO criteria followed up for an average of 4 years.[1,6]

Pediatric-onset NMO (p-NMO) is especially characterized by frequent brain involvement at presentation, expressed clinically by encephalopathy or seizure associated with multiple sclerosis (MS)–like lesions on brain MRI.[1,6] Laboratory findings revealed a high prevalence of NMO-immunoglobulin G (IgG) positivity (78%) in the relapsing population but NMO-IgG positivity was estimated at 12.5% in the population with a monophasic disease course.[1]

However, there are currently no data on time to disability in patients with p-NMO during a long follow-up. Furthermore, no study has compared p-NMO with adult-onset NMO (a-NMO), analyzed with the same design and using the same inclusion criteria.

We report the clinical and paraclinical features and long-term outcome of patients with p-NMO and compare them with a large a-NMO cohort.

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Table 1 Demographic, clinical, and paraclinical characteristics of patients with pediatric-onset NMO

Patient no.	Sex	Age at onset, y	Race	Follow-up, y	Topography of the first attack	Opticospinal interval, mo	First interattack interval, mo	ARR	NMO-IgG	CSF OCB	CSF WBC/mm^3	First spinal MRI No. of lesions	First spinal MRI Location of LETM	First brain MRI No. of lesions	First brain MRI MS criteria (Paty/Barkhof)	Treatments used
1	F	4.1	Asian	3.7	ON	12	12	0.8	+	–	28	2	C + T	1	–/–	—
2	F	7.8	White	32.7	ON	264	154	0.3	+	–	5	1	–	0	–/–	INF, AZA
3	F	8	Black	9.3	SC	7	7	0.9	+	–	12	1	–	2	–/–	INF, CYC, MMF
4	F	10.3	White	28.7	SC	12	12	0.5	+	–	11	1	C	4	+/–	AZA
5	M	11.9	White	15.2	ON	18	8	1.6	–	–	3	1	C	0	–/–	MITO, AZA, MMF
6	F	14.5	American Indian	28.7	OS	0	48	0.1	+	–	4	1	T	0	–/–	CYC
7	M	14.7	Asian	23.7	ON	75	75	0.2	+	–	128	1	CT	0	–/–	CYC, RTX
8	F	15.3	White	24.7	SC	137	137	0.6	–	–	110	2	C	6	+/–	INF, MMF, CYC
9	F	16.1	White	31.3	ON	15	15	0.4	+	+	4	2	C	10	+/–	AZA
10	F	17	White	13.7	ON	22	22	0.8	–	+	0	1	–	0	–/–	INF
11	M	17.2	White	15.7	SC	12	12	0.2	–	ND	ND	2	T	0	–/–	CYC, MMF
12	F	17.9	White	4.3	SC	70	17	1.2	+	+	3	1	C	1	–/–	GA, INF

Abbreviations: ARR = annualized relapse rate of attacks; AZA = azathioprine; C = cervical; CT = cervicothoracic; CYC = cyclophosphamide; GA = glatiramer acetate; IgG = immunoglobulin G; INF = interferon; LETM = longitudinal extensive transverse myelitis; MITO = mitoxantrone; MMF = mycophenolate mofetil; MS = multiple sclerosis; ND = not done; NMO = neuromyelitis optica; OCB = oligoclonal bands; ON = optic nerve; OS = opticospinal; RTX = rituximab; SC = spinal cord; T = thoracic; WBC = white blood cells.

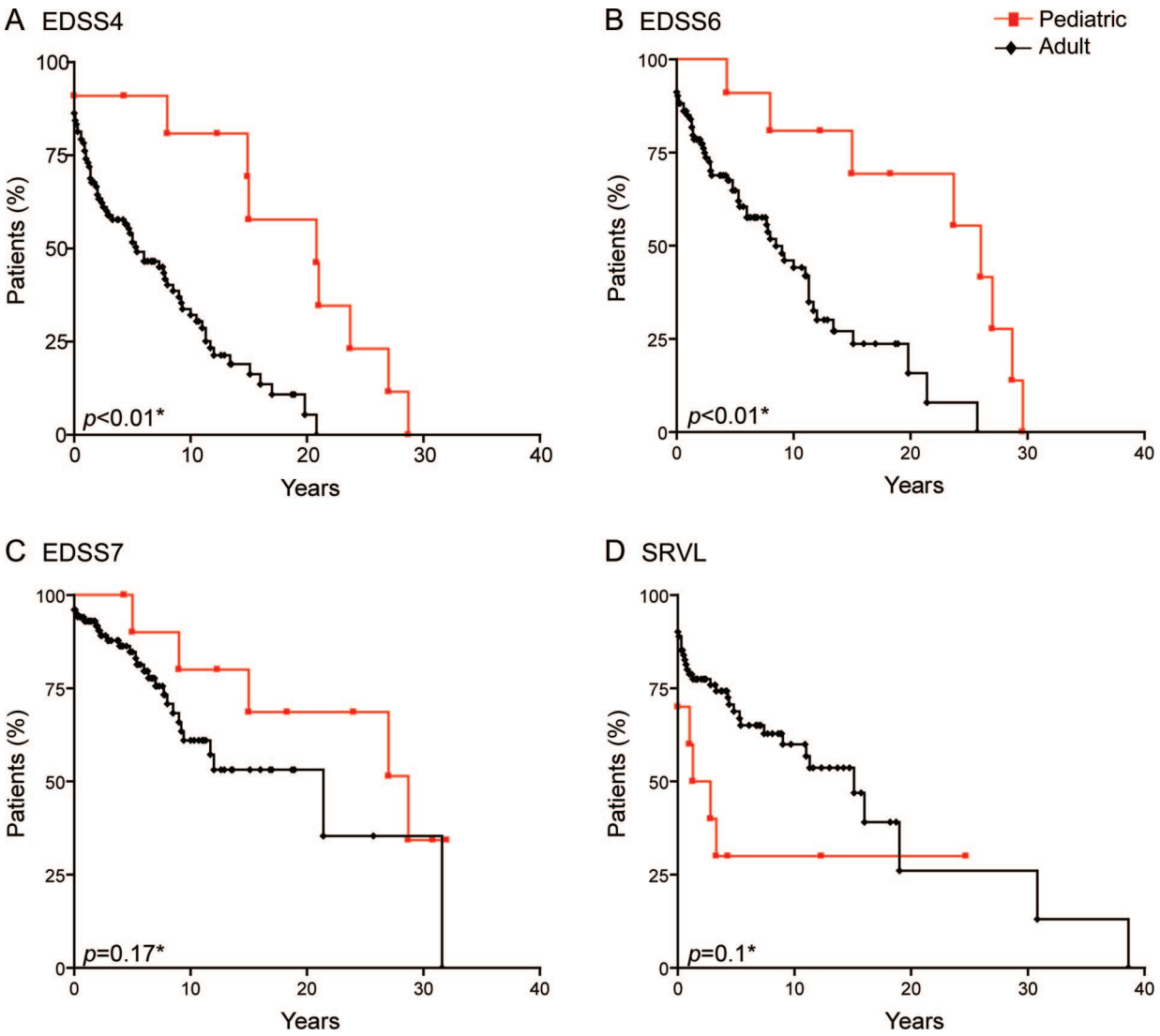
METHODS We retrospectively studied patients with p-NMO in 25 pediatric and adult medical centers in France. Through the Club Francophone de la Sclérose en Plaques, a French network of MS centers, we identified 200 patients with suspected NMO from September 2007 through August 2008, the study endpoint. Seventy-five experienced isolated optic neuritis/longitudinal extensive transverse myelitis (LETM) or MS. The remaining 125 fulfilled the 2006 criteria for NMO, namely optic neuritis, myelitis, and at least 2 of the following 3 items: LETM, NMO-IgG seropositivity, and brain MRI not meeting Paty criteria for MS.[7] In this cohort, we identified 12 patients with p-NMO (onset before 18 years of age) and 113 with a-NMO. Data were collected from hospital files or using standardized assessment forms designed for NMO and have been entered in the European Database for MS, adapted to NMO specificities.[8] Ethical approval, data confidentiality, and security were ensured in keeping with the recommendations of the French data protection authority, which approved the study.

For each patient, we recorded demographic data (gender, race, age at onset, follow-up), medical history of attacks and disability, laboratory data (NMO-IgG, CSF), MRI data (number and location of lesions), and treatments (type and number). Brain MRIs were classified as either not fulfilling or fulfilling MS criteria (Barkhof or Paty) and were performed a mean 84 ± 139 months after the first clinical event. Spinal cord MRIs were performed a mean 26 ± 13 months after the first clinical event. Sera of 111 patients were tested for NMO-IgG in Lyon, France, using an indirect immunofluorescence assay, as previously described.[9] Residual disability was assessed during at least 6 months in terms of Expanded Disability Status Scale (EDSS) score and severe residual visual loss (SRVL), defined as a visual acuity ≤1/10 (corresponding to +1 on logMAR or 20/200 on Snellen visual acuity charts).

Categorical data were compared using the χ^2 test and quantitative data using the Mann-Whitney test. The Kaplan-Meier technique was used to estimate time to assignment of EDSS scores. Survival curves were compared using the log-rank test. Two-sided p values <0.05 were considered significant. All computations were performed using SPSS for Windows, version 14.0.

RESULTS **Characteristics and long-term outcome of patients with p-NMO.** Twelve children were followed up during a mean 19.3 years. Table 1 shows their clinical and paraclinical characteristics. Three patients (25%) fulfilled Paty criteria for MS on first brain MRI, including one patient with acute disseminated encephalomyelitis. During the course of the disease, patient 10 developed LETM and patient 8 experienced a regression of the brain MRI lesions, and both were also considered as NMO. The course of NMO was relapsing-remitting in all p-NMO cases. The median interval between onset and disability was 20.7 years for EDSS score 4, 26 years for EDSS score 6, and 28.7 years for EDSS score 7, and was 1.3 years for SRVL (figure). The median interval between the first myelitis and the assignment of EDSS scores 4, 6, and 7 was as follows: 15 years for score 4, 23.7 years for score 6, and 27 years for score 7. No patient reached an EDSS score ≥4 immediately after the first myelitis. After the first optic neuritis, median time to SRVL was 1.3 years.

Figure Actuarial survival analysis of patients with neuromyelitis optica (NMO), according to age at onset



Kaplan-Meier estimates of time from NMO onset to the assignment of Expanded Disability Status Scale (EDSS) scores 4 (A), 6 (B), and 7 (C) and to severe residual visual loss (SRVL) (D) in patients with NMO with pediatric (p-NMO) or adult onset (a-NMO). Compared to patients with a-NMO (n = 101), patients with p-NMO (n = 12) had a longer median time to EDSS 4 (20.7 vs 5.3 years; $p < 0.01$) and EDSS 6 (26 vs 8.5 years; $p < 0.01$), whereas no difference was found for median time to either EDSS 7 (28.7 vs 21.4; $p = 0.17$) or SRVL (1.3 vs 15.1; $p = 0.1$). *Log-rank test was used to compare survival curves.

Comparison between p-NMO and a-NMO cohorts. Table 2 compares the p-NMO and a-NMO cohorts. Brain MRI fulfilled Paty criteria in 3 patients (25%) in the p-NMO group and 8 patients (7.1%) in the a-NMO group, but the difference did not reach significance. No differences were found in either the number or aspect of spinal cord lesions on MRI.

Compared to patients with a-NMO, patients with p-NMO had a longer interval before EDSS scores of 4 and 6 but not 7 (figure). The interval between the first myelitis and EDSS score assignment was longer in the p-NMO than in the a-NMO cohort for EDSS scores 4 (15 vs 4.4 years; $p = 0.04$) and 6 (23.7 vs 7.7 years; $p < 0.01$), but not 7 (27 vs 21.5 years; $p = 0.22$). The first myelitis attack was immediately followed by EDSS scores of 4 in 21.6%, 6 in 13.7%, and 7 in 5.9% of a-NMO cases. After the first optic neuritis, time to SRVL was 1.3 years in the p-NMO and 11.3 years in the a-NMO cohort ($p = 0.06$).

DISCUSSION Our study underlines 2 points: the first brain MRI in p-NMO can show a diffuse inflammatory process, such as MS lesions (2 patients) or acute disseminated encephalomyelitis (1 patient); similarly to pediatric MS, p-NMO seems to have a better prognosis than a-NMO in terms of disability: time from onset to EDSS scores 4 and 6 was longer in patients with p-NMO, largely explained by the severity of the first myelitis in the a-NMO group.

To date, there is no explanation for the high level of inflammation in the brain of children with

Table 2 Demographic, disease-related, and paraclinical characteristics of the 125 patients with NMO, as a function of age at onset

	Pediatric (n = 12)	Adult (n = 113)	p
Demographic data			
Female:male ratio	9:3	85:28	0.74[a]
White:nonwhite ratio[b]	8:4	66:7	0.07[a]
Age at onset, y, median (range)	14.5 (4.1–17.9)	33.8 (19.1–66.3)	
Duration of follow-up, y, median (range)	19.3 (3.7–32.7)	8.8 (0.1–39.5)	<0.01*[c]
Clinical history			
Patients with spinal attack as first event, n (%)	5 (41.7)	52 (46)	0.51[a]
Patients with ON attack as first event, n (%)	6 (50)	40 (35.4)	
Patients with opticospinal attack as first event, n (%)	1 (8.3)	21 (18.6)	
Opticospinal interval, mo, median (range)	18 (0–264)	15 (0–204)	0.29[c]
First interattack interval, mo, median (range)	17 (7–154)	12 (1–204)	0.08[c]
Mean annualized rate of attacks	0.6	1	0.28[c]
Treatments			
Patients treated first with IM, n (%)	1 (8.3)	6 (5.3)	0.75[a]
Patients treated first with IS, n (%)	8 (66.7)	71 (62.8)	
Patients treated first with IM and IS, n (%)	2 (16.6)	29 (25.7)	
Time from onset to first treatment, y, mean ± SD	13.1 ± 9.3	3.4 ± 4.8	<0.01*[c]
No. of treatments used, mean ± SD	1.7 ± 1.2	2.2 ± 1.7	0.23[c]
Laboratory findings			
Patients with NMO-IgG,[d] n (%)	8 (66.7)	52 (52.5)	0.43[a]
Patients with OCB,[e] n (%)	3 (27.3)	21 (26.2)	0.96[a]
No. of WBC in CSF,[f] mean ± SD	28 ± 45.8	33.6 ± 65.2	0.59[c]
First brain MRI			
No. of lesions, mean ± SD	2 ± 3.2	0.7 ± 2.2	0.06[c]

Abbreviations: IgG = immunoglobulin G; IM = immunomodulatory; IS = immunosuppressive; NMO = neuromyelitis optica; OCB = oligoclonal bands; ON = optic neuritis; WBC = white blood cells.
* Significant.
[a] χ^2 test.
[b] Data were available for 85 patients, including all pediatric NMO cases.
[c] Mann-Whitney test.
[d] NMO-IgG was tested for 111 patients, including all pediatric NMO cases.
[e] OCB was tested for 91 patients, including 11 pediatric NMO cases.
[f] WBC in CSF was available for 76 patients, including 11 pediatric NMO cases.

NMO.[1,3,6] The short time to SRVL in the patients with p-NMO is in accordance with the predictive role of the number of lesions on brain MRI for SRVL in NMO and a possible susceptibility of the optic nerve to inflammation.[2]

The good prognosis of patients with p-NMO is in line with a retrospective chart review, in which 9 patients with p-NMO experienced good visual and motor recovery after corticotherapy, with the absence of sequelae during an average 5-year follow-up.[10] However, response to treatment is unlikely to be the sole explanation for the good prognosis, given the long interval before the first treatment in the p-NMO group.

Due to the retrospective design of this study, selection bias may limit the interpretation of the results. We cannot exclude the possibility that patients with severe p-NMO had died or were otherwise lost to follow-up at the time of the study. The predominant recruitment of patients in adult centers may have led to benign forms of p-NMO being underrepresented. No difference was found in the number of brain MRI lesions between the a-NMO and p-NMO groups. This may be due to a lack of statistical power due to the small number of p-NMO cases and the long delay in some cases between the first clinical event and the first brain MRI. Finally, the outcome of treatment is difficult to assess given the many different treatments used in the patients with p-NMO.

Prospective studies are needed to determine the disease course in children and assess the impact of immunosuppressive therapies in p-NMO.

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Neuromyelitis optica in France

A multicenter study of 125 patients

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ABSTRACT

Background: There have been few epidemiologic studies on neuromyelitis optica (NMO) and none used the recent 2006 diagnostic criteria. Here we describe the clinical, laboratory, MRI, and disability course of NMO in a French cohort of 125 patients.

Methods: We performed an observational, retrospective, multicenter study. Data were collected from September 2007 through August 2008, corresponding to the endpoint of the study. We identified 125 patients fulfilling the 2006 NMO criteria. Selection was made using hospital files and a specific clinical questionnaire for NMO.

Results: Mean age at onset was 34.5 years (range 4–66) with a mean disease duration of 10 ± 7.8 years at the endpoint. The patients were mainly (87%) Caucasian, with a female:male ratio of 3:1. In 90% of cases, the association of optic neuritis, longitudinal extensive myelitis, and a Paty-negative initial brain MRI was sufficient to fulfill the supportive criteria. Eighty-eight percent of patients were treated with immunosuppressive therapies. Median delay from onset to Expanded Disability Status Scale (EDSS) score 4 was 7 years; score 6, 10 years; and score 7, 21 years. The first episode of myelitis was immediately followed by an EDSS score ≥4 in 37.3% of cases, and a severe residual visual loss was observed in 22% of patients after the first episode of optic neuritis. Multivariate analysis did not reveal any predictors of a poor evolution other than a high number of MRI brain lesions at diagnosis, which were predictive of a residual visual acuity ≤1/10.

Conclusions: Our demographic data provide new data on disability in patients with neuromyelitis optica, most of whom were receiving treatment. *Neurology*® 2010;74:736–742

GLOSSARY

CI = confidence interval; **EDSS** = Expanded Disability Status Scale; **LETM** = longitudinal extensive transverse myelitis; **MS** = multiple sclerosis; **NMO** = neuromyelitis optica; **OCB** = oligoclonal bands; **ON** = optic neuritis; **SRVL** = severe residual visual loss.

Neuromyelitis optica (NMO) is a rare inflammatory and demyelinating disease of the CNS characterized by optic neuritis (ON) and myelitis, mostly extensive longitudinally and transversally (longitudinal extensive transverse myelitis [LETM]). In addition to these clinical data, the finding in 2004 of a specific antibody, called NMO-IgG,[1] was a determining factor in the development of new diagnostic criteria for NMO in 2006.[2] Previous epidemiologic studies[3-10] revealed a female:male ratio of 4:1, a median age at onset of around 37 years, and an association with autoimmune disease in approximately 20% of cases. A relapsing-remitting clinical course was observed in more than 80% of NMO cases. In contrast, primary or secondary progression was noted in only 2% of cases.[11] The remainder were patients with a monophasic form, defined by the absence of further relapses after the index events of ON and myelitis. Only one study,[6] with 46 patients, evaluated disability outcome: the median time to reach Expanded Disability Status Scale (EDSS)[12] score 3 was 0.5 years and EDSS score 6 was 7 years. Severe visual loss in at least one eye was observed in half of the patients within 5 years of disease onset.[10] According to the above epidemiologic studies, the clinical manifestations of NMO are severe, with death occurring in 13% to 25% of patients during the follow-up.[6,8,10]

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Table 1 Epidemiologic data derived from the 3 principal cohorts of patients with neuromyelitis optica

	American cohort[10]	Italian cohort[6]	Mexican cohort[9]
Diagnostic criteria	1999	1999	1999
Patients, n	80	46	34
Mean time of follow-up, y	7.6	8.8	5.8
Median age at onset, y	38	35	35
Ratio F/M	2.5/1	4.1/1	2.4/1
Associated autoimmune disease	25	22	0
Initial topography, %			
Spinal	NA	39.1	35.3
Optic	NA	56.6	23.5
Optic and spinal	12.5	4.3	41.2
Optico-spinal interval, mo (mean/median)	NA	NA	12.4/3.5
Interval between first and second attack, mo (mean/median)	NA/NA	17/NA	NA
Median time from onset to the assignment of disability score, y			
EDSS score 3	NA	0.5	NA
EDSS score 6	NA	7	NA
EDSS score 8	NA	NA	NA
Death events, n (%)	18 (22.5)	6 (13)	1 (2.9)
Annual rate of relapse (mean)	NA	1.3	NA

Abbreviations: EDSS = Expanded Disability Status Scale; NA = not available.

Although the above epidemiologic studies provide useful data, they have 2 serious limitations: only 3 studies included more than 30 patients[6,9,10] (table 1) and none of them used the 2006 NMO criteria, and thus did not take into account the new enlarged spectrum of NMO.

We describe a cohort of 125 patients with NMO with a mean disease course of 10 ± 7.8 years.

METHODS We performed an observational and retrospective study of NMO in 25 tertiary medical centers in France. Data were collected from September 2007 through August 2008, corresponding to the endpoint of the study. Through the "Club Francophone de la Sclérose en Plaques" (French network of multiple sclerosis [MS] centers), we identified 200 patients with a suspected diagnosis of NMO. Of these, 125 patients fulfilled the 2006 NMO criteria and were included in the study. Data were obtained from hospital files and a clinical information questionnaire specifically designed for NMO. Any additional data required were obtained from the participating centers by e-mail or telephone. All data used in the study have been entered in the European Database for Multiple Sclerosis (EDMUS), recently adapted to NMO specificities.[13] Data confidentiality and security are ensured in keeping with the recommendations of the French data protection authority (Commission Nationale de l'Informatique et des Libertés [CNIL]), which also provides approval. Every effort was made to exclude patients with MS: hospital files of patients with oligoclonal bands (OCB), Barkhof criteria[14] on brain MRI, or nonextensive myelitis were reviewed by an NMO study committee of 7 neurologists.

Epidemiologic data were assessed for each patient including demographic data, medical history, key episodes in the NMO course (relapses, onset of the progressive phase, and dates of assignment of the successive scores of disability), biologic results, neuroimaging data, and treatment. Brain MRIs were classified as normal and abnormal with or without Paty's[15] or Barkhof's criteria. Serum samples from 111 patients were tested for NMO-IgG with an indirect immunofluorescence assay on a substrate of adult rat cerebellum and midbrain, using a previously described technique very similar to the original procedure.[1,16] The EDSS score was recorded to determine the level of neurologic disability. Severe residual visual loss (SRVL) was defined as a visual acuity ≤1/10 during at least 6 months. We focused on EDSS scores 4, 6, and 7 and the SRVL, since these could be easily ascertained, even by retrospective interview. Visual acuity is included in the visual parameter of the EDSS.

Comparisons of categorical data were made using the χ^2 test. Student t test was used for the comparison of quantitative data. The Kaplan-Meier technique was used to estimate the time to the assignment of EDSS scores 4, 6, and 7. Survival curves were compared using the log-rank test. Predictive factors of disability were analyzed using the Cox proportional model. The variables tested were gender, age at onset, disease duration, number of T2 hypersignals on brain MRI at diagnosis, seropositivity for NMO-IgG, presence of OCB and number of white cells in CSF, initial topography of the disease, optico-spinal delay, time between first and second attack, number of attacks during first year after onset, first type of treatment and global treatment received (immunosuppressive treatment [oral or IV], immunomodulatory treatment, or a combination), time between disease onset and first treatment, and number of treatments received. Two-sided p values <0.05 were considered significant. All computations were performed using SPSS for Windows, version 14.0, and curves were realized using GraphPad Prism 4.

RESULTS **Clinical and biologic.** Clinical and demographic data of patients with NMO are summarized in table 2. The NMO population was mainly Caucasian (87%) with a high proportion of women (female:male ratio, 3:1). Non-Caucasian patients originated from Sub-Saharan Africa (45%), Asia (37%), and Latin America (18%). Mean age at onset was 34.5 ± 13.2 years and in 25.6% of patients onset was either before 18 years (9.6%) or after 50 years (16%). In 90% of patients, the association of optic neuritis, LETM on spinal cord MRI, and Paty-negative initial brain MRI as supportive criteria was sufficient for the diagnosis of NMO according to the 2006 criteria. Thus, NMO-IgG was mandatory for the diagnosis in only 10% of patients. The disease course was relapsing-remitting in 73% and monophasic in 26% of patients, without any difference in terms of gender ($p = 0.33$) or median age at onset (33 years vs 38 years; $p = 0.07$), but a difference was observed in the median time of follow-up

Table 2 Demographic and disease-related characteristics of 125 patients with neuromyelitis optica (NMO)

	NMO
Gender, n (%)	
Female	94 (75.2)
Male	31 (24.8)
Ethnicity, n (%)	
White	74 (87)
Nonwhite	11 (13)
Initial topography, n (%)	
Spinal	57 (45.6)
Optic	46 (36.8)
Optic and spinal	22 (17.6)
Age at onset, y	
Mean ± SD	34.5 ± 13.2
Median	34.7
Range	4–66
Distribution, n (%)	
0–18	12 (9.6)
19–29	37 (29.6)
30–49	56 (44.8)
>50 y	20 (16)
Opticospinal interval, mo	
Mean ± SD	35.6 ± 48
Median	15
Range	0–264
A1–A2 interval, mo	
Mean ± SD	30.8 ± 43.1
Median	12
Range	1–204
Duration of follow-up, y	
Mean ± SD	10 ± 7.8
Median	8.7
Range	0.1–39.5
Mean number of attacks after onset	
0–1 y	1.8
0–2 y	2.3
Mean annualized rate of attacks	0.99

Abbreviations: A1 = first attack; A2 = second attack.

Table 3 Characteristics of the first spinal and brain MRI in patients with neuromyelitis optica

	Values
Spinal cord	
No. of lesions, mean ± SD	
Total	1.2 ± 0.6
Typology of the lesions, n (%)	
Cervical T2	86/103 (83.5)
Cervical T1 gado+	27/45 (60)
Cervical extensive[a]	58/120 (48.3)
Dorsal T2	59/81 (72.8)
Dorsal T1 gado+	21/49 (42.8)
Dorsal extensive[a]	38/120 (31.7)
Brain	
No. of lesions, mean ± SD	
Total	0.9 ± 2.4
Periventricular	0.1 ± 0.6
Juxtacortical	0 ± 0.1
>9 Lesions	0.1 ± 0.2
Typology of the lesions, n (%)	
Supratentorial	19/100 (19)
Infratentorial	12/91 (13.2)
Barkhof criteria	2/125 (1.6)
Paty criteria	9/125 (7.2)

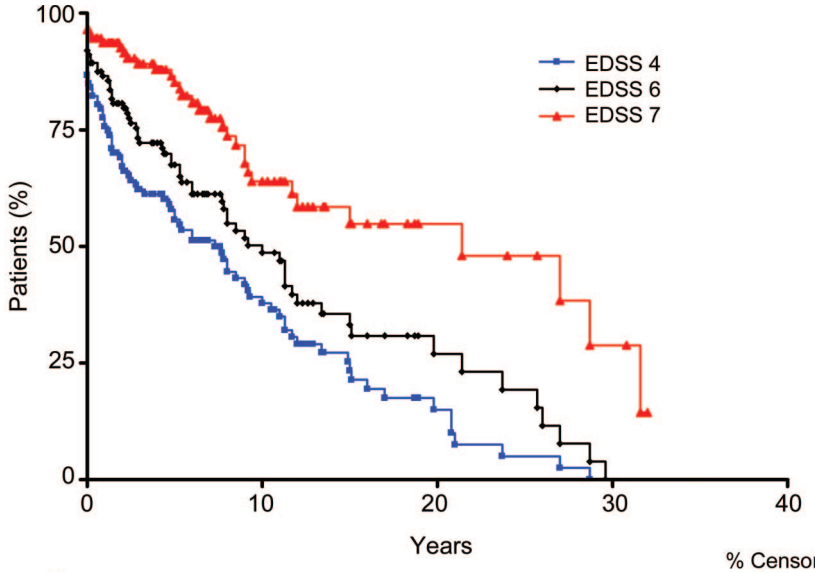
[a]More than 2 vertebral segments.

(10.7 years vs 7.5 years; $p = 0.04$). Among patients with a monophasic form, 8 (6.4%) experienced only one bifocal attack. A progressive course was observed in 2 patients (1.6%). Of the 111 patients tested, 54% were NMO-IgG-positive. CSF results showed a mean number of lymphocyte cells/mm^3 of 23 ± 7.4, a mean number of polynuclear cells/mm^3 of 9 ± 14.7, a mean protein level of 0.54 ± 0.34 g/L, and OCB in 23.8% of cases. An autoimmune disease was associated in 10.4% of cases, including 4 patients with lupus erythematosus, 3 with primary Sjögren syndrome, 3 with autoimmune thyroiditis, and 3 with unspecified autoimmune disease.

MRI. Details of the first brain and spinal cord MRI are summarized in table 3. Among the 8 patients with lesions at first brain MRI, without Barkhof or Paty's criteria, aspecific lesions were noted in 2, lesions suggesting vasculitis in 2, and acute disseminated encephalomyelitis with gadolinium enhancement in 4. During the course of the disease, brain MRI remained normal in 93 patients (74.4%). In the remaining 25.6% of patients, we found a mean 3.66 ± 3.22 lesions; among these patients, only 3 (2.4%) met Barkhof's criteria. During the follow-up, 122 patients (97%) presented LETM on at least one spinal cord MRI. Of these 122 patients, 16 were negative for LETM on the first spinal cord MRI.

Course of NMO and disability. During the follow-up, we observed a decrease in the mean number of attacks after 2 years ($p < 0.01$). The mean interval between the 2 first attacks was shorter if the initial topography was bifocal (11.1 months), compared to the spinal form (33.1 months, $p = 0.04$) or the optic

Figure Actuarial survival analysis of patients with neuromyelitis optica (NMO)



Number at risk					% Censored subjects*
EDSS 4	113	29	7	1	29%
EDSS 6	113	32	8	1	44%
EDSS 7	113	33	9	4	70%

Kaplan-Meier estimates of the age of the patients with NMO at the time of assignment of the scores of Expanded Disability Status Scale (EDSS) 4, 6, and 7, according to the initial course of NMO. Among 113 patients, the median time from the onset of NMO to the assignment of EDSS scores 4, 6, and 7 was as follows: score 4, 7 years; score 6, 10 years; score 7, 21 years. *Data on patients who had not reached an endpoint were censored at the time of the last clinical evaluation.

form (34 months, $p = 0.01$). In the whole population of patients with NMO, 80 (70.8%) reached EDSS score 4, 63 (55.8%) reached EDSS score 6, and 33 (29.2%) reached EDSS score 7. Four patients died, at 28, 37, 43, and 44 years of age, during the follow-up, 2 owing to a diffuse brainstem lesion, including 1 after the first attack, and 2 owing to extraneurologic diseases, after a mean follow-up of 9 years. The median time from onset to EDSS 4, 6, and 7 was 7.3 (95% confidence interval [CI] 4.7–9.9), 10 (95% CI 7.4–12.6), and 21.4 (95% CI 8.3–34.5) years, respectively (figure). No correlation was observed between the topography of the first attack and the subsequent EDSS score. After the first episode of myelitis, the median time to an EDSS score of 4, 6, and 7 was 5 (95% CI 1.6–8.3), 8 (95% CI 4.9–11.1), and 21.4 (95% CI 7.7–35.1) years, respectively. The first myelitis attack was immediately followed by an EDSS score of 4 in 19.5% of cases, an EDSS score of 6 in 12.5% of cases, and an EDSS score of 7 in 5.3% of cases.

Visual acuity assessments were collected in 92 patients. Twenty-nine patients (31.5%) experienced a unilateral and 12 (13%) a bilateral SRVL. At the endpoint, mean visual acuity was 5.9/10 in the left eye and 6.3/10 in the right eye. Median time to SRVL was 11.3 years (95% CI 5.8–16.8). According to the topography of the first attack, statistical differences in survival curves were observed in terms of SRVL, with a more severe visual disability after an opticospinal event (median time of 5.2 years [95% CI 0–12.1]) than after optic neuritis (median time of 10 years [95% CI 0–20.2]) or after myelitis (median time of 15 years [95% CI 4.8–23.3]) (log rank = 0.017). After the first episode of optic neuritis, the median time to SRVL was 11 years (95% CI 3.6–18.4). Among the 41 patients with SRVL, 20 (48.8%) experienced unilateral or bilateral SRVL after the first episode of optic neuritis.

During the follow-up, patients received a mean 1.9 ± 1.4 lines of treatment, including immunosuppressive or immunomodulatory treatments: cyclophosphamide (26%), mycophenolate mofetil (20%), interferon beta (16%), azathioprine (14%), mitoxantrone (12%), glatiramer acetate (3%), rituximab (2.5%), methotrexate (2%), natalizumab (1.5%), and other treatments (3%). The mean time from onset to first treatment was 4.6 ± 5.9 years, divided into 3 treatment classes: immunomodulatory treatment in 20 patients (19%), oral immunosuppressive treatment in 34 (32%), and IV treatment in 52 (49%). In 8 patients, no treatment had been initiated by the end of follow-up (4.9 ± 3.9 years after onset).

Predictive factors of disability. In the univariate survival analysis of EDSS, a late onset of the disease, a short interval between the first 2 attacks, a high number of attacks during the first year of the disease, and the type of treatments received were significantly predictive of the assignment of an EDSS score of 4, 6, or 7. Using the Cox proportional model, we found that the clinical variables had no influence on the time from the onset of NMO to the assignment of an EDSS score of 4, 6, or 7. Because of the presence of confounding factors, due especially to the treatments received, predictive factors isolated with univariate analysis were not positive with multivariate analysis. Patients who were not treated were assigned an EDSS score comparatively later than treated patients and were more likely to be patients with a less severe form of NMO.

In the univariate analysis of SRVL, data of specific interest were age at onset, initial topography of the disease, and number of lesions on brain MRI. Using the Cox proportional model, we found that a high number of lesions on brain MRI was associated with a shorter time from the onset of NMO to the diagnosis of SRVL ($p < 0.01$).

An analysis of both the relapsing-remitting and monophasic groups of patients with NMO did not reveal any differences compared to the whole cohort.

DISCUSSION Our demographic data are in accordance with those of previous studies, especially as regards NMO in a predominantly Caucasian population: female:male ratio 3:1; median age at onset 34.7 years; a predominantly relapsing-remitting course with extremely rare progressive forms (1.6%). Our study is the first to have evaluated the importance of NMO-IgG for the diagnosis of NMO. In 90% of cases, the association of optic neuritis, Paty-negative initial brain MRI, and LETM criteria was sufficient to diagnose NMO. NMO-IgG was necessary to confirm the diagnosis in only 12 cases (10%). In 26% of patients with NMO, the disease began either before 18 years or after 50 years, which is therefore higher than the corresponding figure of 15% reported in patients with MS.[17] Other important information arising from our study includes the median time between the first 2 attacks and the 2 index events, the median survival time in treated patients, and the search for predictive factors of disability. The median delay before a bifocal location in NMO was approximately 1 year. Considering the severity of the pathology, this delay is particularly long and emphasizes the importance of identifying early predictive factors for developing NMO. To this end, NMO-IgG may be of interest as a means of predicting evolution to definite NMO, as shown in previous studies.[18-20] Concerning disease severity, multivariate analysis did not allow us to identify predictors of a poor evolution, other than a high number of MRI brain lesions, which was predictive of a residual visual acuity $\leq 1/10$.

In our cohort, there was a relatively low proportion of cases with a monophasic course compared to other series.[9,21] It should be noted, however, that in those studies, the longer the follow-up, the lower the proportion of monophasic cases. Another potential source of bias is the severity of the optic and spinal attacks in NMO. The early massive destruction of these areas may lead to further, asymptomatic attacks, despite an ongoing inflammatory process. In a study on a population of Afro-Caribbean patients with NMO, the median time to unilateral SRVL was 2 years, compared to 11 years in our study.[22] The apparent good prognosis of optic neuritis in our Caucasian patients may well be linked to the lower rate of optic neuritis attack and the efficacy of the immunosuppressive treatment, since the patients in the non-Caucasian study were not treated. It is noteworthy that the proportion of patients who experienced blindness in at least one eye after the first optic neuritis was similar: 30% in the non-Caucasian NMO population and 22% in our Caucasian NMO population.

Using multivariate analysis, we were unable to identify any risk factors that could predict the EDSS in NMO. To date, only one study has used this statistical approach in order to identify predictive factors for the development of a progressive course.[11] The only available data concerning predictive factors for disability were published by the Italian Devic's Study Group,[6] but were obtained using univariate analysis without consideration of confounding factors. Another approach would be to evaluate predictive factors for treatment efficacy, according to the type of immunosuppressive therapy. In the only published study with data on the median time to EDSS scores 3, 6, and 8, no information was given about the treatments received, thus making it difficult to analyze the results.[6] A retrospective evaluation of the efficacy of treatment is clearly difficult, with many potential biases. In our retrospective study, the main bias is that treatment was given according to the severity of the NMO. Untreated patients therefore had a less severe disease than treated patients.

We found that the number of lesions on MRI during the disease may be indicative of the outcome of an SRVL. This observation could be a reflection of the intensity of the inflammatory process, as observed in children presenting diffuse cerebral abnormalities on MRI and a high level of autoimmune activity.[23,24] Another physiopathologic explanation is

the extension of the inflammation behind the optic nerves, involving the optic chiasma or tracts. This finding indicates that the brain tropism of NMO may be a source of visual disability and suggests that particular attention should be given to these patients, who may have been diagnosed with MS.

In 2008, new NMO criteria were proposed.[25] In contrast to the 2006 criteria, the 2008 criteria consider LETM and the absence of other autoimmune disease to be strictly required for the diagnosis. Out of our cohort of 125 patients, 16 would not have fulfilled these new criteria: 3 patients without LETM and 13 patients with other autoimmune disease. However, among the 3 patients without LETM, only one had a progressive course. For these 3 patients, the median follow-up was 1.6 years and the rate of relapse was 1.7 attacks/year/patient. The possibility of them subsequently developing more severe attacks with LETM cannot therefore be ruled out. This suggests that the 2008 criteria may lack sensitivity, especially at the beginning of NMO. Furthermore, the importance of NMO-IgG for NMO diagnosis should not be overestimated; in our study the test was positive in only 54% of the 111 patients tested and was necessary in order to confirm the diagnosis in only 10% of the whole cohort of patients.

In addition to being retrospective, our study has other limitations. First, we included only patients who were hospitalized at least once during the follow-up. Second, the low proportion of deaths compared to other studies may have been due to a selection bias, since all of our patients were being followed up at the start of the study. Third, the delayed survival time observed for an EDSS score of 7 may have been overestimated because of a bias. Disease severity in the high number of censored patients 10 years after NMO onset is unknown and may have influenced the survival curves after this point.

However, our study contains the largest body of data published to date on patients with NMO. Our demographic data confirm the results of previous studies and provide new data on disability in a treated population. Our results argue in favor of early intensive treatment, especially in patients with frequent relapses at the beginning of the disease. The number of brain lesions on MRI during the follow-up should be seen as an indication of the risk of severe visual loss. A prospective study is now needed to define the course of the disease and provide an accurate assessment of the impact of immunosuppressive therapies in NMO.

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Dr. Collongues, Dr. Marignier, Dr. Zéphir, Dr. Papeix, Dr. Blanc, Dr. Ritleng, Dr. Tchikviladze, and Dr. Outteryck report no disclosures. Dr. Vukusic has received travel expenses and/or honoraria for lectures or educational activities not funded by industry; has received funding for travel from Biogen Idec and Merck Serono; and has received honoraria from the Serono Symposia MS Foundation. Dr. Fleury reports no disclosures. Dr. Fontaine serves on the editorial boards of *Gene and Immunity*, the *Journal of Neurology, Neurosurgery and Psychiatry*, *Revue Neurologique*, and the *Journal of Neurology*; receives research support from Biogen Idec, sanofi-aventis, Merck Serono, INSERM (National Research Agency), the University Pierre and Marie Curie, Association Française contre les myopathies, and Association pour la Recherche sur la Sclérose en Plaques (ARSEP). Dr. Brassat receives research support from the French Ministry of Health, the French Multiple Sclerosis Society 2005, and the European Union. Dr. Clanet serves on an independent data monitoring committee for Genmab A/S; serves as Clinical Editor of the *International MS journal* and on the editorial board of *Revue Neurologique*; and has received honoraria from Bayer Schering Pharma. Dr. Milh reports no disclosures. Dr. Pelletier serves on scientific advisory boards for Biogen Idec and Novartis and has received research support from ARSEP. Dr. Audoin and Dr. Ruet report no disclosures. Dr. Lebrun-Frenay has received travel expenses and/or honoraria for lectures or educational activities not funded by industry; serves on the editorial board of *Revue Neurologique*; has received speaker honoraria from Biogen Idec, Bayer Schering Pharma, Merck Serono, sanofi-aventis, and Teva Pharmaceutical Industries Ltd.; and serves as a consultant to Institut National de Recherche en Informatique et en Automatique (INRIA). Dr. Thouvenot receives research support from ARSEP. Dr. Camu has received consulting fees from sanofi-aventis and Merck Serono. Dr. Debouverie has served on a scientific advisory board for Bayer Schering Pharma; has received travel expenses and/or honoraria for lectures or educational activities not funded by industry; and has received honoraria for speaking engagements or educational activities for Biogen Idec, Merck Serono, Bayer Schering Pharma, Teva Pharmaceutical Industries Ltd., and sanofi-aventis. Dr Créange serves on a scientific advisory board for Bayer Schering Pharma; serves on the editorial advisory board of *Revue Neurologique* and has received honoraria for speaking engagements or educational activities for Biogen Idec, LFB, Merck Serono, Bayer Schering Pharma, Teva Pharmaceutical Industries Ltd., and sanofi-aventis. Dr. Moreau has served on scientific advisory boards for Bayer Schering Pharma and sanofi-aventis; has received travel expenses and/or honoraria for lectures or educational activities not funded

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III.3. COMMENTAIRES

III.3.1. Nouvelles données épidémiologiques et cliniques en France

Le recensement effectué en 2010 des patients NMO à l'échelle nationale n'a pas été exhaustif mais a mobilisé 25 hôpitaux répartis sur l'ensemble du territoire français.

Ce recensement a permis d'estimer, pour la première fois en France, la prévalence de la pathologie à environ 0.3/100 000 habitants (données démographiques INSEE 2010), ce qui correspond à une ratio NMO:SEP de 1:300, soit 10 fois plus faible qu'à Cuba et 40 fois plus faible qu'aux Antilles françaises.

Ces études ont permis de mieux documenter le profil clinique de ces patients. Elles ont notamment permis de préciser quelques points importants non recensés jusqu'alors :

1. L'âge de début peut varier de 4 à 82 ans et 28% de patients débutent avant 18 ans ou après 50 ans. Il s'agit là d'une différence majeure avec la SEP où seulement 15% des patients débutent avant 18 ans ou après 50 ans.
2. le délai médian au diagnostic (séparant l'atteinte optique de l'atteinte médullaire) est évalué autour de un an et correspond aussi au taux annualisé moyen de poussée (TAP) dans cette pathologie.
3. Après une durée de suivi de plus de 10 ans en moyenne, l'évolution de cette pathologie se fait essentiellement par poussée, les formes d'allure progressive intéressant moins de 2% des patients. La rareté des formes dégénératives dans cette pathologie en font une maladie essentiellement

inflammatoire et oriente prioritairement la recherche thérapeutique vers le développement de médicaments immunosuppresseurs.

4. L'étude de la sévérité de la première poussée a permis de constater une forte altération de la fonction visuelle, 22% des patients ayant une acuité visuelle résiduelle (persistante à 6 mois) ≤ 0.1. Cette donnée est retrouvée dans la cohorte antillaise et atteint même 30% des patients après la première névrite optique.[16] La première myélite était également suivie d'un handicap sévère, suivie d'un EDSS ≥ 4 dans 37% des cas et représentait le facteur de handicap le plus important dans la NMO à l'âge adulte.
5. Il n'existe pas de formes dites "bénignes" comme cela a pu être décrit dans la SEP. L'étude de ces formes supposées de bon pronostic a révélé la possibilité de poussées très handicapantes pouvant survenir après 15 ans d'évolution favorable.
6. Le handicap a une expression différente selon l'âge de début de la NMO (**figure 1**).

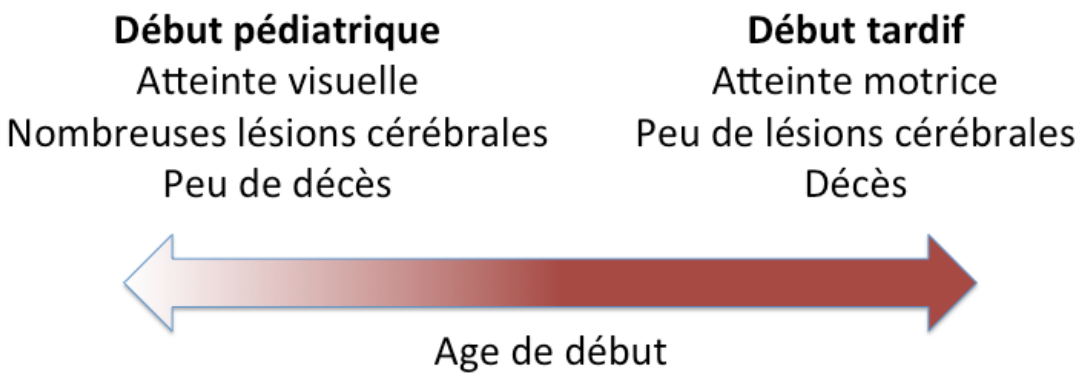

Figure 1. Types d'atteintes en fonction de l'âge de début. Nos études ont retrouvé une atteinte sévère de l'acuité visuelle et aucun décès dans la NMO pédiatrique après 20 ans de suivi. A contrario, les NMO/NMOSD à début tardif (≥50 ans) sont particulièrement sévères avec un taux de décès élevé comparé aux patients débutant avant 50 ans.

7. le handicap dans la NMO est lié aux poussées et les traitements immunosuppresseurs sont efficaces pour lutter contre les poussées et le handicap à long terme. En effet, d'une part le TAP des formes supposées de bon pronostic est particulièrement bas et d'autre part il diminue de façon significative après traitement immunosuppresseur dans les formes standard, associé alors à un long délai au handicap comme le montre l'évaluation des scores EDSS chez ces patients traités.
8. Il existe une différence dans l'expression de la NMO chez les patients d'ascendance africaine comparée aux patients d'ascendance caucasienne. (**tableau 1**).

Caractéristiques des patients inclus	Cohorte Antillaise/Cubaine 2009	Cohorte Française 2010
Critères diagnostiques	1999/2006	2006
Patients, *n*	96	125
Suivi, *an (moy)*	9.5	10.4
Age au début, *an (méd)*	29.5	34.5
Ratio Femmes/Hommes	11/1	3/1
MAI, *%*	8	10
Topo. initiale prédo.	Optique	Médullaire
Délai P1-P2, *m (moy/méd)*	27.6/11.5	30.8/12
Délai OM,[#] *m (moy/méd)*	34.8/24	35.6/15
EDSS 6, *an*	8	10
Décès, *n (%)*	24 (25)	4 (3.2)
TAP, *(méd)*	0.7	0.99
NMO-IgG, *%*	32	54
BOC dans le LCR, *%*	-	23.8

Tableau 1. Comparaison des cohortes françaises Antillaises et Métropolitaine de patientes NMO. La comparaison de ces deux cohortes ethniquement différentes retrouve une sévérité plus importante chez les patients d'ascendance africaine en terme de décès et de délai d'atteinte de l'EDSS 6. *Moy :*

moyenne; Méd : médiane; MAI : maladie auto-immune; P1 : première poussée; P2 : deuxième poussée; # : délai séparant la première poussée optique et médullaire, quel qu'en soit l'ordre; TAP : taux annualisé de poussée; BOC : bandes oligoclonales; LCR : liquide céphalo-rachidien.

9. Les patients avec une myélite aiguë transverse longitudinalement étendue et des anticorps anti-AQP4 ont des myélites sévères.

III.3.2. Critériologie et approche translationnelle

La description du modèle animal a permis de compléter les données manquantes, nécessaires à la comparaison du modèle avec la NMO humaine, mentionnées dans le **tableau 2.**

Caractéristiques	**Souris 2D2xTH**	**EAE du rat BN induite par la MOG**	**Patients NMO**
Évolution clinique	Aiguë rémittente	Aiguë rémittente	Aiguë rémittente
Pathologie de la moelle épinière	Inflammation sous-piale, démyélinisation, éosinophiles	Atteinte vasculaire: Inflammation, nécrose, éosinophiles. Destruction de la substance grise et blanche	Atteinte vasculaire: Inflammation, nécrose, éosinophiles, hyalinisation des petits vaisseaux. Destruction de la substance grise et

			blanche
Extension de la myélite	Un métamère	Au moins deux métamères	Au moins deux métamères
Atteinte encéphalique	Non	Dans 3% des cas touchant le septum et les fimbria des hippocampes	Dans 50% des cas au cours de l'évolution touchant le tronc cérébral, l'hypothalamus, les régions périventriculaires ou le corps calleux
Pathogénie humorale	Pas de dépôt d'Ig dans les lésions, pas de corrélation avec la sévérité de la pathologie.	Dépôt d'Ig et de complément (C9) dans les lésions.	Dépôt d'Ig et de complément (C9) dans les lésions. Efficacité des échanges plasmatiques.
Anti-AQP4	Absence	Non recherché	Présence

Tableau 2. Données cliniques, anatomopathologiques et immunologiques des principaux modèles de démyélinisation optico-médullaire comparées avec la NMO humaine.

Souris 2D2xTH : souris transgéniques ayant un récepteur aux LT particulièrement affin pour la MOG et générant une production massive d'IgM anti-MOG ; EAE : encéphalomyélite autoimmune expérimentale ; BN : Brown Norway ; Ig :

immunoglobuline.

Ainsi, nous avons pu montrer que :

1. ce modèle remplit les critères diagnostiques chez l'humain,
2. les lésions encéphaliques sont limitées à un oedème périventriculaire transitoire,
3. les modifications du niveau d'expression de l'AQP4 sont secondaires à l'oedème vasogénique (diminution de son expression), à l'oedème cytotoxique ou à la gliose cicatricielle (augmentation de son expression dans les deux cas),
4. ce modèle ne produit pas d'anticorps anti-AQP4.

Par ailleurs, nous avons observé que le handicap est lié aux lésions démyélinisantes actives, pouvant générer un handicap sévère allant jusqu'à la tétraplégie. Ce handicap n'est pas lié aux modifications d'expression des molécules astrocytaires testées, semblant pouvoir écarter l'hypothèse d'une astrocytopathie inaugurale dans ce modèle. Cette donnée est cohérente avec le protocole d'immunisation utilisé, basé sur la genèse d'une réaction immunitaire dirigée contre la MOG qui est un antigène de la myéline.

Cette étude ouvre la porte à une discussion sur la critériologie en cours actuellement sur la NMO. Il est important de noter que dans les nouveaux critères de NMOSD, à coté du rôle majeur de l'anti-AQP4, l'atteinte optico-médullaire garde une place importante. Dans cette population de patients séronégatifs pour l'anti-AQP4, les études actuelles distinguent un sous-groupe de patients MOG+ et d'authentiques double séronégatifs. Cependant le mécanisme physiopathologique de ces maladies semble très différent et pose la question de

la nosologie actuelle. La comparaison des données issues de notre modèle primitivement démyélinisant à tropisme optico-médullaire pourrait correspondre aux NMO avec anticorps anti-MOG, même si la pathogénicité de cet anticorps chez l'humain reste à démontrer. L'analyse des cohortes de patients MOG+ converge vers l'idée qu'il s'agit d'une pathologie à part, de meilleur pronostique que les patients AQP4+ mais pour laquelle la frontière avec certaines encéphalomyélites aiguës disséminées ou SEP à tropisme optico-médullaire est encore imprécise.

III.4. LIMITES DES ETUDES

III.4.1. Chez l'humain

La principale limite de notre étude de cohorte est certainement son caractère rétrospectif. Par conséquent nous avons perdu un certain nombre de données concernant les IRM ou les scores de handicap (Expanded Disability Status Scale [EDSS]), tels que le signifient les 113 EDSS exploitables sur 125.

D'autre part, l'absence d'exhaustivité du recrutement nous a certainement confronté à un effet centre et à un biais de sélection drainant ainsi les cas les plus sévères référés à un centre expert. Cette limite a conduit à l'élaboration et l'obtention d'un PHRC national (NOMADMUS) permettant de colliger tous les patients sur le territoire français. Cette étude permet à chaque neurologue d'inclure son patient dans la base nationale de NMO "NOMADMUS" en remplissant une fiche standardisée et en l'envoyant au CHU de Lyon. En pratique, il est aussi possible de retrouver les patients séropositifs pour les anti-AQP4 ou anti-MOG à partir du résultat des tests sérologiques effectués aussi au CHU de

Lyon. Chaque patient est ensuite discuté, IRM et bilan biologique à l'appui, lors d'une réunion du comité scientifique (dont je fais partie) tous les 6 mois environ. Nous espérons ainsi obtenir une base de données la plus exhaustive possible tant sur le recrutement que sur les données. Malgré tous les efforts déployés pour implémenter cette base, le niveau de complexité de certaines études ancillaires nécessite néanmoins de retourner aux dossiers cliniques et relègue l'exploitation de cette base à un rôle consultatif basé sur l'identification de malades potentiellement incluables. Cet aspect est particulièrement flagrant pour les études visant à évaluer l'efficacité des traitements qui nécessitent de connaitre précisément les doses reçues et leurs dates d'utilisation.

III.4.2. Chez l'animal

A l'exception de la recherche d'anticorps conformationnels anti-AQP4 qui a été faite à Lyon, l'ensemble des procédures expérimentales pour caractériser ce modèle a été réalisé au sein du laboratoire de biopathologie de la myéline à Strasbourg. Cette approche a donc nécessité des compétences en imagerie de la myéline par résonance magnétique, en immunologie liées à la réalisation des immunomarquages et des tests par dosage d'immunoabsorption par enzyme liée (ELISA), ainsi qu'en histologie pour l'analyse des lames chez les rats autopsiés. L'avantage crucial d'une telle démarche centralisée a été de permettre une vision globale du sujet, d'obtenir rapidement des résultats exploitables et donc d'accroitre la réactivité face à une problématique nouvelle. A coté de cela, de nombreuses manipulations expérimentales ont dû être répétées plusieurs fois

avant de devenir performantes et certaines techniques dont nous ne disposions pas n'ont pas été utilisées.

Une première limite réside dans l'absence de quantification des immunomarquages de l'AQP4 et de la glial fibrillary acidic protein (GFAP). Celle-ci aurait pu être effectuée par reverse transcription polymerase chain reaction (RT-PCR)[17] sur le cerveau, la moelle et les nerfs optiques ou par microfluorimétrie[18] adaptée aux grandes surfaces tissulaires. Ces techniques connaissent néanmoins des limitations dans leur utilisation. Pour la RT-PCR, un volume conséquent de tissu doit être prélevé chez l'animal pour ensuite être amplifié. Il n'est donc pas possible d'établir chez un même animal une corrélation entre la quantité d'ADN exprimé en un site donné et le niveau d'intensité des marquages immunohistologiques au même niveau. La microfluorométrie était initialement utilisée pour quantifier l'intensité lumineuse émise par des cellules en culture marquées avec un anticorps fluorescent. Une extension de son utilisation a permis par la suite de l'appliquer aux tissus fixés et d'étendre la surface analysée. Les problèmes qui se posent dans l'exploitation de cette technique résident d'une part dans l'acquisition du matériel de comptage spécifique et d'autre part dans la variabilité intra-individuelle de l'expression de la fluorescence. En effet, l'intensité de la fluorescence n'est pas constante et diminue avec le temps et selon les conditions de conservation. Enfin, l'absence de marquage aurait pu être détaillée par une analyse en Western Blot afin de vérifier s'il s'agissait d'une baisse de l'expression des molécules ou une perte complète d'antigène liée à une destruction cellulaire par exemple. Cette technique n'était pas faite en routine dans notre laboratoire.

Nous avons réalisé deux types de suivi en IRM chez les rats injectés avec de la MOG : un suivi longitudinal sur trois rats et un suivi IRM précédant une histologie chez 12 rats. Ces données ont été contrôlées à chaque stade du processus pathologique par deux rats contrôles injectés avec de l'adjuvant incomplet de Freund seul. De nombreuses difficultés se sont posées pour obtenir des images de bonne qualité *in vivo*. Alors que l'IRM est habituellement réalisée sur l'encéphale des rats, les acquisitions de nerfs optiques ou de moelle épinière *in vivo* restent cependant très difficiles à obtenir dans les modèles d'EAE et expliquent le peu de données disponibles dans la littérature à ce sujet [19, 20] nécessitant parfois une approche *ex vivo.*[21] Dans notre modèle, même si le coté régressif des images et leur tropisme pour la substance blanche évoque un oedème vasogénique plus que cytotoxique dans les nerfs optiques et les régions périventriculaires, d'autres séquences pondérées en diffusion ou calculant le coefficient apparent de diffusion (ADC) auraient permis de mieux individualiser ces deux mécanismes. L'injection d'un produit de contraste comme le gadolinium aurait également permis d'authentifier une rupture de la barrière hémato-encéphalique, argument en faveur d'un oedème vasogénique.

Enfin, on pourrait regretter l'absence de recherche de corrélation entre les modifications de signal en IRM et les données histologiques. Le protocole n'avait pas été conçu pour cela, avec seulement quatre rats passés en IRM et sacrifiés pendant la période des signes cliniques.

L'étude de la réponse inflammatoire dans notre modèle aurait pu être un élément supplémentaire pour distinguer une SEP à tropisme optico-médullaire d'une NMO. En effet, la réaction inflammatoire dans la SEP et l'EAE comprend une activation des lymphocytes T helper (Th) 1 incluant la présence

d'interleukine-2, d'interferon-γ, de tumor necrosis factor-α et de nombreuses chemokines. En plus des lymphocyte T (LT) 4 ainsi activés, des LT8 et les macrophages connaissent une expansion clonale au sein des lésions, à l'origine d'une transsection axonale.[22] Enfin, une activation de type Th2 participe également au processus auto-immun, générant l'activation de lymphocytes B (LB) et la production d'anticorps contre la MOG et la myelin basic protein (MBP) par exemple dans certaines plaques.[23, 24] Cette réponse humorale est prédominante dans la NMO, s'exprimant par un aspect de vascularite nécrosante, associant un dépôt d'anticorps et de complément activé périvasculaires.[25] Cette analyse a été réalisée dans le même modèle que le nôtre pour les macrophages/microglie activée (marquage ED1), les LT (marquage W3/13), le complément C9[26] et les anticorps (marquage IgG).[27] Elle montre un dépôt d'IgG et de C9 le long des gaines de myéline en marge des plaques. Dans les plaques sont retrouvés de nombreux macrophages périvasculaires contenant des débris cytoplasmiques, ainsi que des éosinophiles, des IgG et du C9. La présence d'éosinophiles est également un élément supplémentaire indiquant l'activation des LB par le biais de la production de chemokine CCR3.[28] Dans notre étude ainsi que dans celle de Storch et collaborateurs, cette infiltration éosinophile a été retrouvée de façon abondante dans les lésions inflammatoires grâce à la coloration hématoxyline-éosine. Chez le rat Brown Norway, Dark Agouti et Lewis, l'analyse de l'infiltration par les LT8 à l'aide du marqueur CD8 est difficile car cet antigène est également porté par les cellules ED1+.[29] Enfin, les séquences utilisées en IRM : T1, T2, transfert de magnétisation, pondération en diffusion, ADC, volume sanguin régional, injection de gadolinium, ne permettent pas de

distinguer les différents mécanismes inflammatoires Th1 ou Th2 se déroulant dans l'encéphale.[30]

III.5. VERS UNE MODIFICATION DE LA NOSOLOGIE DES MYELITES ETENDUES

Chez l'humain, la description du spectre des maladies appartenant à la NMO a donné une place centrale à la myélite aigue transverse longitudinalement étendue (MATLE), appelée aussi "longitudinally extensive transverse myelitis". La diversité des diagnostics qui lui sont rattachés a nécessité de redéfinir son bilan diagnostique et étiologique. Cette réflexion nous a conduit à écrire un chapitre de l'EMC sur les myélites aiguës et des recommandations concernant la nosologie de la MATLE sous l'égide du groupe "NOMADMUS" qui ont permis de modifier nos pratiques pour une meilleure prise en charge des malades. Ces recommandations ont permis également de mieux documenter les patients avec atteinte médullaire inclus dans la base NOMADMUS et d'augmenter le niveau de confiance des décisions prises lors des réunions du groupe expert. En effet, en cas de bilan insuffisant, le patient est signalé auprès du centre référent pour compléter cet aspect de la prise en charge.

IV. Traitement de la neuromyélite optique et de la myasthénie

IV.1. INTRODUCTION

Il est important de garder à l'esprit que tous les traitements utilisés dans la NMO sont issus de l'expérience des centres experts dans le traitement des pathologies inflammatoires du SNC. Aucun d'entre eux n'a été pour l'instant validé par le biais d'essais cliniques, conférant ainsi à leur utilisation un niveau de preuve d'efficacité relativement faible. Afin de remédier à cela, deux essais cliniques de phase 3 utilisant de nouvelles cibles thérapeutiques sont actuellement en cours. D'autre part, la recherche clinique évaluant la réponse aux thérapies existantes a pris une place importante dans notre pratique. Ainsi, nous avons pu décrire l'efficacité et la tolérance du rituximab (RTX), un anticorps monoclonal anti-CD20 permettant de cibler le LB, dans une série de cas locaux de myasthénie ainsi que dans une cohorte nationale de NMO dans le cadre du projet "NOMADMUS". L'étude de cette cohorte a également permis d'évaluer la réponse thérapeutique au mycophenolate mofetil (MMF), un immunosuppresseur non sélectif. Pour ces deux traitements immunosuppresseurs, leur efficacité ainsi que leur bonne tolérance observées dans nos études ont contribués à en faire des produits de référence dans le traitement de la NMO.

La connaissance thérapeutique que chaque expert a pu partager au sein du projet "NOMADMUS" a conduit à l'écriture de recommandations nationales pour encadrer la prescription de RTX. J'ai ainsi pu encadrer ce travail qui permet d'homogénéiser les pratiques sur le territoire national et de réaliser une analyse moins biaisée des patients traités. En dehors de la NMO, l'analyse de la réponse

thérapeutique au RTX dans la myasthénie ainsi que l'écriture d'une revue de la littérature sur le mode d'action et l'efficacité du RTX en particulier et des biothérapies en général m'a permis de développer une expertise dans ce domaine qui a conduit à la publication d'une revue de la littérature sur l'utilisation des biothérapies dans les pathologies inflammatoires du SNC.

IV.2. ARTICLES THERAPEUTIQUES

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Guidelines for the use of Rituximab in neuromyelitis optica spectrum disorders

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Abstract

There is growing evidence of a preventive effect of Rituximab (RTX) in neuromyelitis optica spectrum disorders (NMO-SD). This monoclonal antibody against CD20 is becoming the most widely used preventive therapy in NMO-SD, as a first line therapy or as a rescue therapy. Nevertheless, considerable heterogeneity still exists concerning the pre-treatment work-up, the vaccinations required before and under treatment, the number and dosage of infusions, prevention of the risk of infusion-related reactions, prevention of infections under treatment, and frequency of therapeutic cycles.
Thanks to a collaborative work among NMO-SD experts belonging to the NOMADMUS project, we provide here recommendations for all these topics concerning RTX use in NMO-SD.

Introduction

The term neuromyelitis optica spectrum disorders (NMO-SD) refers to an autoimmune inflammatory demyelinating disease of the central nervous system, which is distinct from multiple sclerosis (MS). NMO-SD is potentially a life-threatening condition where disability is driven by acute attacks. Thus, the aim of maintenance therapy in NMO-SD is to avoid any further attack. The International Panel for NMO Diagnosis (IPND) has recently revised the diagnostic criteria for NMO-SD, allowing the diagnosis to be made earlier and a suitable preventive therapeutic strategy to be implemented.[1] NMO-SD is a rare disorder for which no prospective randomized trials have been achieved yet. However, there is a large body of evidence from retrospective studies suggesting that immunoactive drugs targeting B cell lineage are efficient in NMO-SD.

Among these drugs, rituximab (RTX) is a widely used preventive therapy in NMO-SD. RTX is a chimeric monoclonal antibody against CD20, a human B-lymphocyte antigen. CD20 is expressed at the membrane of B-lymphocytes from the early stages of development (pre-B cells) to mature B cells, but is no longer expressed at the membrane of plasma cells, which are the next stage of B cells and which produce antibodies.

There is growing evidence of a preventive effect of RTX in NMO-SD.[2] Some practitioners use RTX as a first line therapy in order to prevent relapses of NMO-SD,[3] whereas others use it as a rescue therapy after unsuccessful first line therapy.[4]

Although RTX is increasingly employed in NMO-SD, considerable heterogeneity still exists concerning the following: pre-treatment work up, vaccinations required before and under treatment, number and dosage of infusions, prevention of the risk of infusion-related reactions, prevention of infections under treatment, and frequency of therapeutic cycles.

In order to provide guidelines about these topics, a consortium of NMO-SD experts belonging to the NOMADMUS study group met regularly and set up a working group. First of all, the working group established a spreadsheet for the above questions concerning the use of RTX, in order to collect the experience of each expert. Answers were discussed collegially, considering the experience of all the experts and the literature data. For each point, a proposition was made according to evidence-based data, national or international recommendations, or if a consensus emerged among the experts (majority of experts agreeing to a proposition). Finally, the working group drafted propositions of guidelines regarding the management of RTX in NMO-SD and submitted them to the members of the French societies for MS and NMO-SD (*Société Francophone de la Sclérose en Plaques* and *Observatoire Français de la Sclérose en Plaques*) for final revision. The use of RTX in paediatric patients suffering from NMO-SD is out of the scope of these guidelines.

Figure 1 summarizes recommendations for RTX use in NMO-SD.

Question 1: work up before starting rituximab

We discussed whether the following laboratory tests were essential before starting rituximab: complete blood count, lymphocyte subset counts (CD19, CD4, CD8), serum protein electrophoresis, immunoglobulin subset amounts (IgG, IgM, IgA, +/- IgG subclasses), serology tests for hepatitis C, hepatitis B, HIV and VZV, tuberculin skin test and/or quantiFERON®-TB Gold, and β-HCG. We also discussed whether chest X-ray or other radiological examinations (for example dental panoramic, sinus X-ray…) were useful.

We all recommended complete blood count and lymphocyte subset counts, given that serum CD19+ lymphocytes reflect the circulating B-cell population quite well (although this antigen is not expressed at the membrane of all plasma cells). There was also a consensus for performing serum protein electrophoresis, immunoglobulin subset amounts, HIV, hepatitis B, hepatitis C and VZV serology tests.

One expert usually performs JC virus serology as some cases of progressive multifocal leukoencephalopathy (PML) have been described under RTX therapy, but almost exclusively in non-neurological indications. Nevertheless, the experts did not recommend a systematic analysis of JCV serology since cases of PML remain exceptional and since the risk of PML under RTX appears to be significantly lower in autoimmune disorders such as NMO-SD (or rheumatoid arthritis, lupus, vasculitis) than in lymphomas. In fact, the increased risk observed in non-neurological indications may be explained either by the disease itself (lymphoma and other haematological diseases lead to increased risk of PML), or by a combination of immunosuppressant and/or chemotherapy, which is not the rule as far as NMO-SD are concerned.

The experts did not recommend biological tests for tuberculosis since the risk of tuberculosis under RTX seems to be very low. Actually, data about the risk of this infection under RTX in neurological indications are very scarce. In rheumatological (rheumatoid arthritis) and onco-haematological (lymphoma) indications, RTX does not increase the risk of tuberculosis and a past history of tuberculosis is not a contraindication for RTX use.[5–7] Of course, a past history of untreated or insufficiently treated tuberculosis should be sought, since tuberculosis chemoprophylaxis would be necessary for such situations.

We all recommended measurement of β-HCG for women of childbearing age (see section below about pregnancy).
We all recommended chest X-ray but none of us recommended any other systematic radiographic examination. Nevertheless, a detailed clinical examination was strongly recommended in order to detect any infection; consequently, some appropriate radiographic examination could be added if necessary.

We recommend the following work up before RTX initiation: complete blood count, lymphocyte subset counts (including CD19+, CD3+, CD4+ and CD8+ cells), serum protein electrophoresis, immunoglobulin subset amounts (IgG, IgA, IgM, +/- IgG subclasses), HIV, hepatitis B, hepatitis C and VZV serology tests, β-HCG (for women of childbearing age) and chest X-ray (expert opinion).

Question 2: vaccinations before starting rituximab and under treatment

Pneumococcal vaccine, influenza vaccine, varicella-zoster virus (VZV) vaccine and all vaccines included in the vaccination schedule were discussed.
The "Haut Conseil de Santé Publique" (HCSP), a French body of independent experts involved in public health, recommends a similar preventive strategy for all patients involved in immunosuppressive therapy: ensure that the vaccination schedule is up to date before starting treatment, perform pneumococcal vaccination before starting treatment, keep the vaccination schedule up to date under treatment, and administer the influenza vaccine under treatment every year.[8]
The measles mumps rubella vaccination is recommended during childhood. If it has not been given and then if measles serology is negative, it is recommended that this vaccination be performed before starting RTX since it involves a live attenuated vaccine. This is all the more important as there is currently an increase in measles in France and several other European countries.
For adults and children over 5 years old, the pneumococcal vaccine strategy is based on a first injection with 13-valent vaccine (Prevenar-13®) followed, at least 2 months later, by a second injection with 23-valent vaccine (Pneumo-23®,

which is being replaced by Pneumovax®). Recently, the HCSP has proposed to repeat vaccination with 23-valent vaccine 5 years after the latest injection with this vaccine (but not before this period of 5 years), but it is not clearly said if this should be repeated every 5 years over the long term.[9]

The necessity for two injections for pneumococcal vaccination, with at least 2 months between the first and the second dose, delays the beginning of the treatment. Considering the potential severity of NMO-SD relapses, delaying the start of the disease modifying therapy may lead to a substantial risk of disability worsening if another relapse occurs before the preventive treatment takes effect. Therefore, we proposed starting RTX after the first vaccine injection. The main limitation of this strategy is the uncertainty of the degree of efficacy of the second vaccine injection, which would be performed under immunosuppressive therapy.

The zoster vaccine is recommended by the vaccination schedule for people between 65 and 74 years of age, even those who have already experienced zoster. As a live attenuated vaccine, it has to be done before starting RTX. Since some severe cases of infections by the herpes group (including VZV) have been reported under RTX, we also recommended the VZV vaccine for people under the age of 65 if they have not been immunized against this virus (those who do not have IgG against VZV).[10] As a live attenuated vaccine, it also has to be done before starting RTX.

Another question concerns the optimal period for administering a vaccine (influenza, diphtheria-tetanus-polio, etc.) under RTX. As for other immunosuppressants, all live attenuated vaccines are contraindicated. Non-live vaccines are allowed but the vaccine efficacy may be decreased by the immunosuppression conferred by RTX. In fact, those vaccines can be given at any time under this treatment; it might appear better to perform vaccination a few days before repeating RTX infusion since the vaccine immunogenicity might increase with time since the latest infusion. Nevertheless, we must admit that no consistent data really support this strategy, especially as a continuous effect of the treatment is expected over time with the maintenance regimen detailed below.

Therefore, we advised performing recommended vaccinations under RTX without any preference concerning the optimal period.

We recommend the following: a) update the vaccination schedule, in particular vaccination against measles, and perform pneumococcal vaccination before starting RTX (official recommendation); b) concerning pneumococcal vaccination, start RTX after the first vaccine injection (expert opinion); c) under 65 years old, perform VZV vaccination before starting RTX if the patient is not immunized against this virus (expert opinion); d) between 65 and 74 years old, perform zoster vaccination before starting RTX, even if VZV serology is positive (official recommendation); e) keep the vaccination schedule up to date and perform influenza vaccination every year under RTX (official recommendation); f) perform recommended vaccinations under RTX without any preference concerning the optimal period for doing so (expert opinion).

Question 3: timing of rituximab start

We discussed whether or not an interval was necessary between the end of the attack treatment (by high doses of steroids and/or plasma exchanges) and the start of RTX. There was a consensus among us for recommending no therapeutic window between the end of the attack treatment and the initiation of RTX.

We also discussed the optimal interval between discontinuing a previous disease modifying therapy and starting RTX for patients whose NMO-SD was already treated by another immunosuppressant. There is no evidence-based data available that would enable an easy decision to be taken on such concerns, considering that a decision to switch treatment often occurs in the setting of breakthrough disease. We recommended a case by case analysis for switching from a previous immunosuppressant to RTX. The decision should assess the risk to benefit ratio. Notably, the risk of cumulative effects of two immunosuppressants has to be balanced against the need for rapid disease control.

In the case of a switch from a previous immunosuppressant to RTX, we recommended no delay after azathioprine or mycophenolate mofetil. After

cyclophosphamide or mitoxantrone infusion, we recommended taking the nadir of neutropenia into account: neutrophil count has to be > 1500/mm3 to start RTX.

We do not recommend any therapeutic window between the end of the relapse treatment and the initiation of RTX (expert opinion).
In the case of a switch from a previous immunosuppressant to RTX, we recommend no delay after azathioprine or mycophenolate mofetil (expert opinion). After cyclophosphamide or mitoxantrone infusion, we recommend taking the nadir of neutropenia into account: neutrophil count has to be > 1500/mm3 to start RTX (expert opinion).

Question 4: modality of induction treatment by rituximab

There is no standardized RTX protocol in NMO-SD. In neurologists' clinical practice, its application is informed by the experience of each physician.
In general, the first therapeutic cycle, commonly referred to as "induction treatment", consists of either 1 g infused twice over a 2-week interval (1 g at day 1 and 1 g at day 15), or 375 mg/m2 once weekly for 4 weeks. The "1 g twice over a 2-week interval" protocol has already been adopted in the treatment of neurological inflammatory diseases. For example, it was the therapeutic strategy chosen in the OLYMPUS phase II/III randomized double-blind placebo-controlled trial that concerned RTX in primary progressive MS.[11] This therapeutic strategy has also been widely applied in NMO-SD.[3,12–15] A consensus on recommending the 1 g two weeks apart protocol emerged in our expert group as, in our experience, neither lower efficacy nor increased side effects have been observed with this regimen. This is of importance considering the medico-economic cost, but an evaluation involving this parameter has not yet been conducted. In addition, a rapid onset of treatment effect is desirable in NMO-SD, and it might be expected (at least theoretically) that this induction regimen allows a more rapid depletion of B cell subsets than the 4 weeks induction regimen.

The most common adverse event reported under RTX is infusion-related reaction. The relative frequency of such reactions has been estimated at 20-30% of cases after the first infusion, decreasing with further infusions.[16,17] Most of these reactions are benign (headache, asthenia, fever, hypo/hypertension, nausea, bronchospasm, rash). Life-threatening adverse events are extremely rare (0.5%) and have been observed mainly during the first infusion.

In NMO-SD, adverse events have not been systematically documented across the studies, so estimating the prevalence of such side effects is difficult. Moreover, prevention therapy is generally not mentioned. In a recently published meta-analysis of RTX in NMO-SD, infusion related adverse events were reported in 45/438 (10.3%) of cases.[18] In a Korean cohort, for which we have long-term follow-up, such events were related in 26% of cases during the first infusion but the incidence declined with subsequent infusions.[15] Thus, there is no clear evidence supporting the idea that the type and frequency of infusion-related events occurring in NMO-SD patients differ from those found in other similar conditions (rheumatoid arthritis).

IV methylprednisolone at 100 mg, 1 hour prior to RTX infusion, has been demonstrated to mitigate the risk of infusion-related side effects.[19] A combination of steroids with IV antihistamine and paracetamol is recommended, but without evidence. Slow titration of RTX is also recommended in order to decrease the risk of such reactions, mainly at the first infusion.

We recommend the "1 g two weeks apart" protocol for the treatment induction (expert opinion).

We recommend mitigating RTX infusion-related events by: a) the use of IV methylprednisolone (100 mg) 1 hour prior to each RTX infusion (evidence-based), in combination with an anti-histaminic drug and paracetamol (expert opinion); b) a slow titration of RTX infusion, especially at the first infusion (official recommendation).

Question 5: infectious risk under rituximab and preventive measures

We discussed whether some medications were useful in preventing opportunistic infections.

None of the experts proposed systematic pneumocystis prevention based on either cotrimoxazole (sulfamethoxazole + trimethoprim), or pentamidine. As for other situations where RTX is used (lymphomas, rheumatoid arthritis, vasculitis), this prevention might be suggested in case of T4 lymphopenia < 200/mm3 (or, for some practitioners, in case of global lymphopenia < 500/mm3).

None of us proposed systematic zoster or herpes prevention. As mentioned above, we recommended before starting RTX systematic zoster vaccination for patients between 65 and 74 years of age, and VZV vaccination for patients under 65 years of age if not immunized against VZV. For those under 65 years who are already immunized, we assumed that the risk of recurrence (zoster) was not increased enough to lead to a decision in favour of systematic treatment by valaciclovir (or aciclovir). Nevertheless, such preventive treatment might be discussed for patients having had a zoster or recurrence of genital herpes under RTX.

We recommended a systematic determination of immunoglobulin subset amounts (IgG, IgM, IgA, +/- IgG subclasses) just before repeating infusions of RTX. We did not recommend systematic supplementation by intravenous (or subcutaneous) immunoglobulins but this might be discussed in case of recurrent infections and low dosage of IgG (< 5 g/l) under RTX. In case of such preventive treatment, immunoglobulins should be infused according to a substitution regimen similar to that proposed in common variable immunodeficiency, for example 1 day every 3 or 4 weeks at 0.4 g/kg if intravenous administration.

Some cases of delayed neutropenia have been described under RTX, requiring closer clinical and biological follow-up but not specific medication. Therefore, we recommended a complete blood count at month 3 and month 6 following the latest RTX infusion.

We do not recommend systematic pneumocystis prevention, but this prevention should be considered if T4 cells are below 200/mm3, as for HIV+ patients (official recommendation). Similarly, we do not recommend systematic zoster or herpes

prevention by antiviral drug, but it should be considered in cases of zoster or recurrence of genital herpes under RTX (expert opinion). We recommend a systematic determination of immunoglobulin subset (IgG, IgA, IgM, +/- IgG subclasses) amounts just before repeating RTX infusions (expert opinion). Supplementation by intravenous or subcutaneous immunoglobulins (according to a substitution regimen) should be considered only in cases of recurrent infections coupled with low amounts of IgG (< 5 g/l) (expert opinion). We also recommend a complete blood count at months 3 and 6 following the latest RTX infusion (expert opinion).

Question 6: monitoring of RTX re-infusion: tools, rate and posology

We discussed strategies for the long-term management of RTX. The first strategy consists of repeating RTX infusions every 6 months without biological monitoring of its effects. The second strategy is based on biological monitoring of RTX effects by a count of circulating B-cells. CD19+ cells are a good reflection of the global circulating B-cells. Some authors have suggested repeating RTX infusions when CD19+ cells become detectable (which had been defined as over 0.01 x 10^9/L)[20] or when they reach more than 0.1% of total lymphocytes.[21] CD27+ cells are a subset of CD19+ cells (therefore also called CD19+CD27+ cells) corresponding to memory B-cells. Kim et al. have suggested that monitoring CD27+ cells in peripheral blood could optimize the maintenance regimen of RTX, by repeating treatment only when CD27+ cells were above 0.05% of peripheral blood mononuclear cells (PBMC).[13] This strategy is supposedly more precise than the monitoring of CD19+ cells, since the risk of reactivation of the disease appears to be correlated with the re-emergence of memory B-cells, but not with the re-emergence of the whole B-cell population.[14] This difference in sensitivity in detecting reactivation of an inflammatory disease under RTX has already been suggested in myasthenia gravis, where total CD19+ cells were not associated with relapse of clinical symptoms in all patients, whereas recovery of the CD19+CD27+ subset was predictive of relapse risk.[22] Nevertheless, CD27+ cell monitoring is not an absolute guarantee against the risk of relapse, as demonstrated by Kim et al.: among 100 NMO-SD patients, 9 patients had relapses

(accounting for 11 relapses) although memory B-cells were below the therapeutic target.[15] This might be partly explained by a higher threshold of memory B-cells being required for repeating treatment (> 0.1% of PBMC) after 2 years of treatment than during the first 2 years (> 0.05% of PBMC) in this study.

Recently, Cohen et al. underlined the interest of CD27+ cell monitoring in order to detect short responder patients who needed their RTX infusions to be repeated more frequently than every 6 months in NMO-SD.[23] Before CD27+ cell monitoring, those patients were considered as having treatment failure; now they are considered as potential responders who need a more frequent therapeutic regimen to ensure therapeutic success. The same team has demonstrated encouraging results with the strategy involving repeating RTX infusions only on the basis of the CD27+ cell count, including beyond 6 months following the latest RTX infusion.[23] Nevertheless, this last approach seems to have insufficient validity to become a widespread practice at the moment.

The third strategy is based on monitoring AQP4-Ab titres. This is a logical approach, based on the effect of treatment on the deleterious antibody itself. A first report underlined potential interest of such monitoring, demonstrating a correlation between treatment response and antibody titre, especially after RTX treatment.[24] However, this result has not been confirmed.[25–27] Thus, we did not recommend monitoring RTX infusion by AQP4-Ab titre in AQP4-Ab positive NMO-SD. The question is under investigation in MOG-Ab associated NMO-SD as a link between disease activity and MOG-Ab titre has recently been reported.[28]

Others strategies, where monitoring is carried out through evaluation of the RTX level in the blood and/or presence of human anti-chimeric antibody against RTX (HACA) have not been evaluated in the field of NMO-SD. In rheumatoid arthritis, the frequency of such HACA has been evaluated at 10% but their impact on efficacy and tolerability is not yet demonstrated.[29]

Another question centres on the optimal number and dosage of RTX infusions during the maintenance regimen. Classical maintenance therapy in our experience was either two infusions of 1 g 2 weeks apart, or only one infusion of 1 g or, more rarely, only one infusion of 375 mg/m2. Some studies have shown that it is possible to reduce the dose of RTX without increasing the risk of disease reactivation.[30,31] Similarly, in our experience, the risk of relapse does not

increase when the number of RTX infusions is reduced from 2 to 1 every 6 months at a dose of 1 g.

The last question is whether a maximal cumulative dose exists or not. At present, there are no data suggesting a dose-dependent increase of any severe side effects, but the duration of follow-up is quite limited in neurological indications of RTX. Nevertheless, we can look toward the experience of physicians in non-neurological indications of RTX, particularly rheumatologists, who have been using RTX in rheumatoid arthritis for more than 15 years, without having established a maximal cumulative dose.

We recommend repeating RTX infusions every 6 months, except for patients who need a shorter retreatment interval because of an early CD27+ cell re-emergence (expert opinion). Thus, we do not recommend CD27+ cell monitoring beyond 6 months after the latest RTX infusion (expert opinion).

We recommend a CD27+ cell (and total CD19+ cells) count at any time in case of relapse, in order to distinguish short responders from patients for whom the RTX treatment has genuinely failed (evidence-based).

We propose to assess CD27+ cells at month 3 after the latest RTX infusion, and then once a month until month 6 after the latest RTX infusion, in the aim of detecting short responder patients (evidence-based). If it is not possible to perform this monthly analysis, we recommend a count of CD27+ cells at least at months 3 and 6 after the latest RTX infusion (expert opinion). Total CD19+ cells may be counted on the same dates, even though this will not determine whether or not to repeat RTX infusion (expert opinion). For practitioners who do not have easy access to a count of CD27+ cells, counting only the total CD19+ cells is an acceptable compromise (expert opinion).

As a maintenance regimen, we recommend only one infusion of 1 g, the main alternative being two infusions of 1 g at day 1 and day 15 (expert opinion).

Figure 2 summarizes biological monitoring recommended related to RTX pharmacodynamy.

Question 7: therapeutic combination

None of the experts are used to combining RTX with another immunosuppressant in NMO-SD, except for steroids, which are used by some experts - mainly transiently while waiting for the therapeutic coverage of RTX (given its onset of full action estimated at 2 months) or, more rarely, in the long term as an add-on therapy. It should be noted that steroids remain immunosuppressive beyond 10 mg per day for more than 2 weeks. Actually, literature data and our experience suggest that RTX seems to be very effective in NMO-SD without the need for therapeutic intensification, even a combination with steroids[2,18]. In addition, RTX alone is well tolerated in general but a combination with another immunosuppressant would most certainly increase the risk of side effects, mainly an infectious risk (opportunistic infections such as PML, and community acquired infections). Therefore, we recommended using RTX only as a monotherapy in NMO-SD.

The question seems to be different in other neurological diseases, such as myasthenia gravis, where some other therapies may be combined, such as cholinesterase inhibitors. However, most of the time, no other immunosuppressant drug is co-prescribed, apart from steroids, which are sometimes combined with RTX or other immunosuppressive drugs.

We recommend using RTX only as a monotherapy in NMO-SD (evidence-based).

Question 8: pregnancy, breastfeeding

Since NMO-SD is a rare disease, data addressing NMO evolution during pregnancy are scarce. Following the first original paper published by our group in 2012, five cohorts have been described.[32–37] Unlike MS patients, for whom the relapse rate decreases throughout the pregnancy before flaring in the post-partum period, NMO-SD women are more prompt to show acute exacerbation during the pregnancy and also a significantly higher risk of relapse in the first 3 months post-partum. In a Japanese cohort, 46.8% of NMO-SD patients manifested a pregnancy-related attack.[35] Treating our patients of childbearing age is therefore a complex issue.

Before pregnancy and pregnancy exposed to rituximab

Based on the European Medicines Agency labelling, RTX is contraindicated during pregnancy and breastfeeding, and women of childbearing potential should use effective contraceptive methods during and for 12 months following treatment with RTX, due to the long retention time of RTX in B cell depleted patients.

Actually, the biological effects of RTX generally persist in the body for 6 to 9 months after the end of treatment.[38] After 2 infusions of RTX 1000 mg 2 weeks apart, the mean half-life is 19.7 days (12.3-34.6 days). Considering 5 half-lives (one maximal half-life: 35 days), we should consider that nearly all RTX is eliminated in 61-175 days, i.e. between 2 and 6 months. Pregnancy could be started 1 menstrual cycle after complete elimination of RTX e.g. 6.7 months after the last infusion.

IgG immunoglobulins are known to cross the placental barrier, but B cell levels in human neonates following maternal exposure to RTX have not been studied in clinical trials.

The RTX global drug safety database identified 153 pregnancies with known outcomes associated with maternal exposure to RTX.[39] Ninety pregnancies resulted in live births (59%), including 22 children born prematurely. There were also 33 spontaneous miscarriages, 28 elective terminations (including 1 therapeutic abortion because of trisomy 13), 1 still birth and 1 maternal death due to an underlying condition. Only 3 congenital malformations were identified (clubfoot, oesophageal atresia and cardiac abnormality). Moreover, details of concomitant medication were not always available. Eleven new-borns had haematological abnormalities, without any infection reported. B cell status was reported only for 11 children and was low for 6 patients. Of the 6 children with preconception or first trimester exposure, only one had low B cell levels. All the 5 children with second or third trimester exposure had low or absent B cell levels. All recovered spontaneously in a few months.

Recently, the British RTX registry reported 32 pregnancies in 23 women, with 21 live births (66%), without any major malformation.[40] For 10 women, pregnancy

occurred less than 6 months after last RTX infusion, with 6 live births (60%), 1 miscarriage (methotrexate was associated), 1 stillbirth and 1 termination. No data on the B-cell status of the new-borns was available.

One case report described a NMO-SD woman exposed to RTX during pregnancy, where the outcome was favorable for the mother and the child, without infectious events despite B cell depletion.[41] Another case report from the same group described a NMO-SD woman who became pregnant a few months after RTX exposure, with again a favorable outcome for the mother and the child.[42]

However, those data are not enough to validate RTX use during pregnancy.

In practice, pregnancy should not be forbidden in women with NMO-SD. Considering the potential severity of any attack, the reported higher risk of relapse during the postpartum period, and the potential risk of AQP4-IgG induced miscarriage, maintenance of an immunoactive treatment should be discussed, preferentially with azathioprine and/or low dose oral steroids.[43]

We recommend NMO-SD women with childbearing potential should use effective contraceptive methods during treatment with RTX (official recommendation). Pregnancy must be planned after discussion with the neurologist (official recommendation).

Registry data are reassuring if conception occurs beyond 6 months after the last RTX infusion (evidence-based). Therefore, we recommend effective contraceptive methods during and for only 6 months following treatment with RTX, although EMA labelling advises such contraception for 12 months following treatment with RTX (expert opinion).

Breastfeeding

Because maternal IgG are excreted in human milk, and since rituximab was detectable in milk from lactating monkeys, EMA labelling recommends women should not breastfeed while treated with RTX and for 12 months following RTX treatment.

Bragnes et al. reported a woman treated by RTX and pregnant 5 months after her last RTX infusion.[44] The healthy new-born was breastfed. Three months after

delivery, 1 g of RTX was administered, and samples of the mother's serum and milk were analysed, but not the child's serum. RTX concentration in human milk was very low (240 times less than the amount present in the maternal serum), a result which is quite similar to those on the concentration of other monoclonal antibodies.

Since RTX is supposed to be stopped before conception, RTX should not be present in the maternal serum 9 months later. Therefore, we do not consider that breastfeeding is contraindicated if RTX was stopped before conception (expert opinion). Introduction of RTX after delivery precludes breastfeeding for NMO-SD patients, because of the lack of data concerning RTX excretion in the mother's milk (official recommendation). So, we recommend that the decision for the mother to restart RTX treatment early or to breastfeed should be discussed case by case with the neurologist and the patient, depending on the activity of the disease (expert opinion).

Paternal exposure

Due to the long retention time of rituximab in B cell depleted patients, males should use effective contraceptive methods during and for 12 months following treatment with RTX, according to EMA labelling.
Data on paternal exposure are limited. The RTX global drug safety database reported 9 cases of men exposed to RTX at the time of conception: outcomes included 7 healthy term infants and 2 spontaneous miscarriages.[39]

Given the half-life of RTX and for the same reasons as those detailed for women, we recommend that conception be allowed beyond 6 months after the last RTX infusion (expert opinion).

Conclusion

RTX is a standard treatment of NMO-SD but, so far, strong heterogeneity exists concerning its use. Here, some guidelines are provided in the intention of helping

to make standard care with RTX more homogeneous. They have been drawn up according to national or international recommendations, evidence-based data and expert opinion.

In the future, further advances in the knowledge of RTX use can be expected, enabling more safety and more personalized treatment of NMO-SD patients with RTX. Repeating RTX only on the basis of CD27+ cell count (including beyond 6 months after the latest infusion), reducing doses of RTX below 1 g (administered by only one infusion) for the maintenance regimen, and repeating pneumococcal vaccination every 5 years are some of the potential future directions for forthcoming recommendations. Other immunoactive drugs targeting B cell lineage (anti-CD20 drugs like ocrelizumab, anti-CD19 drugs) are being developed in NMO-SD. Guidelines for RTX use should probably apply, at least partially, to these emerging treatments.

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Conflict of interest

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Biotherapy in Inflammatory Diseases of the CNS: Current Knowledge and Applications

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Opinion statement

Biotherapy represents an innovative therapeutic approach that includes immunotherapy (vaccines, apheresis, and antibodies); gene therapy; and stem cell transplants. Their development helps to cross the bridge from bench to bedside and brings new hope of a cure for severe diseases in different fields of medicine. In neurology, a growing range of applications is being developed for these medications. Valuable results are now available in the field of autoimmunity, neuro-oncology, paraneoplastic manifestations, and neurodegenerative disorders. In this review, we examine the current and future applications of biotherapy in the field of inflammation of the central nervous system. We demonstrate its contribution in clinical practice, where it has enabled a significant level of effectiveness to be achieved. Indeed, the efficacy of these new biodrugs provides a solution for patients refractory to standard therapies, such as intravenous immunoglobulins in limbic encephalitis, plasma exchanges in neuromyelitis optica and anti-CD20 monoclonal antibodies in multiple sclerosis. They also mark the first steps towards individualized medicine.

Introduction

Biotherapies use drugs derived from biotechnology. These treatments are therefore performed with substances from living organisms. Biotherapy development is a growing part of translational research in the therapeutic field. It has largely contributed to the production of promising new medicines with a wide range of applications, as illustrated by the time line in Fig. 1. Biotherapies have already been developed in various fields of medicine, and most commonly in oncology, cardiology, infectiology, rheumatology,

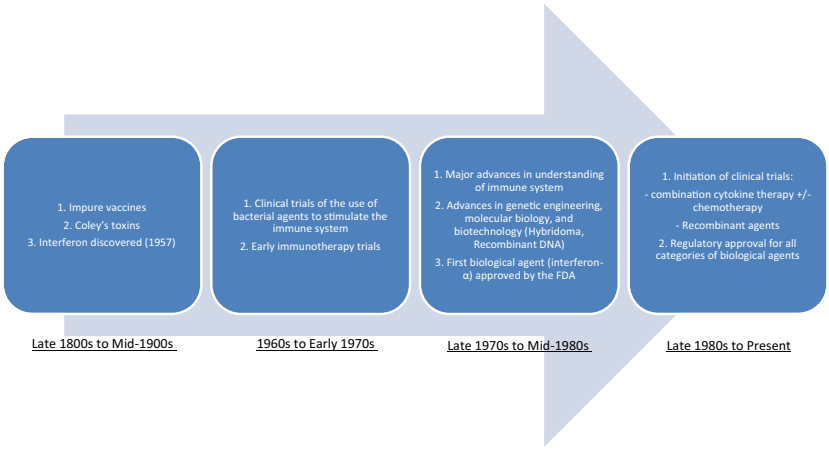


Fig. 1. Time line of key events in the development of biotherapy.

and immunology [1–5]. As reported in a national cohort, this therapeutic domain is mainly represented by vaccines (35%), followed by monoclonal antibodies (17%), growth factors (9%), hormones (9%), and enzymes (8%) [6]. It also includes more confidential applications in the field of gene therapy, stem cell therapy, and tissue engineering [7, 8]. In the case of neurology, numerous applications of these medications are gradually being developed. They rely upon four approaches. These are, in decreasing order of frequency of usage:

A. Immunoglobulins and plasma exchanges;
B. Monoclonal antibodies;
C. Stem cell transplants;
D. Gene therapies.

As illustrated in Fig. 2, many of these applications occur in the field of autoimmune diseases and especially in inflammatory disorders of the CNS. To date, biodrugs are the most efficient option for treating CNS inflammation and are a growing part of our therapeutic arsenal in this field. This review will cover the spectrum of this innovative approach with a special focus on inflammatory disorders of the CNS.

A. Immunoglobulins and plasma exchange

Intravenous immunoglobulins

Polyclonal immunoglobulins G (IgG) are usually used in replacement therapy in patients with immunoglobulin deficiencies. However, at high doses, these immunoglobulins may have an anti-inflammatory and immunomodulatory effect in the treatment of several autoimmune diseases. Intravenous immunoglobulins (IVIG) are polyclonal IgG coming from several thousand healthy donors. They contain antibodies directed against not only a wide range of pathogens but also some autoantigens. Their functions are complex and are not yet fully understood. The immunoglobulin protein consists of several functional areas: [1] the antigen-specific Fab domain, [2] the constant Fc part that mediates the effector functions (complement activation or cross-linking of the immune complexes). Both portions are important for the effectiveness of IVIG [9].

The various mechanisms include the blocking of immune cell receptors, cytokines, complement and autoantibody neutralization and blocking and modulation of the activation of Fc receptors. As a consequence, these actions

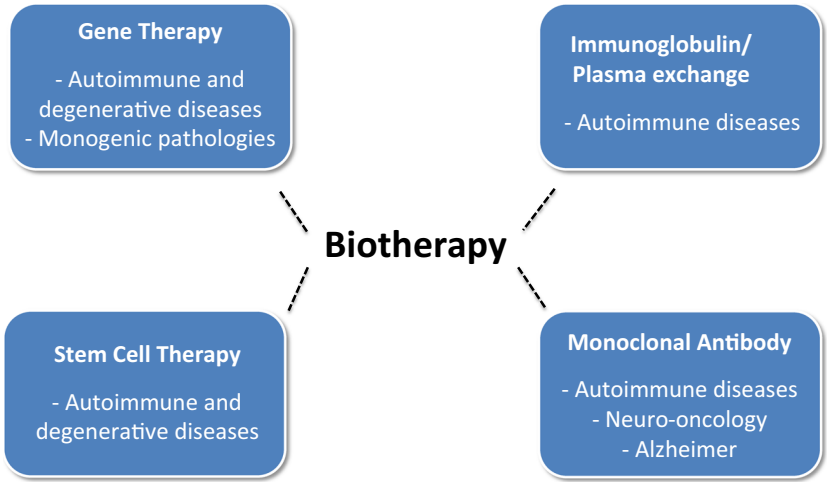


Fig. 2. Main applications of biotherapy in the field of neurology.

are mainly immunomodulatory and anti-inflammatory and make this therapeutic very useful in inflammatory diseases of the CNS. The range of neurological indications is increasing, including a wide marketing authorization and some temporary treatment recommendations. Their use in inflammatory diseases of the CNS are restricted to off-group indications for Rasmussen's encephalitis (RE), paraneoplastic syndromes such as limbic encephalitis (LE) but also neuromyelitis optica (NMO) or acute demyelinated encephalomyelitis (ADEM) in children [10].

For these indications, studies are scarce and limited to case series and open-label trials. Intravenous immunoglobulins have also been studied in multiple sclerosis (MS), especially in progressive forms, but the results were not convincing with only a trend of efficacy in primary progressive MS but not in secondary progressive or relapsing MS [11, 12]. This therapeutic has also been tested for the prevention of postpartum relapses but the level of evidence was low with only retrospective studies [13–15]. Therefore, level of proofs for IVIG in inflammatory diseases of the CNS is restricted to expert therapeutics guidelines [16–19]. In children, this therapeutic approach benefits from a very good safety and tolerance.

Side effects are classified as minor or major. Minor side effects observed during the first minutes of perfusion in 10% of patients include headache, flu-like symptoms, and chest or back pain. In such cases, the infusion should be stopped for 30 min and then resumed at a slower speed. Within 24 h after the infusion, fatigue, fever, and nausea can be observed. After 48 h, 5% of patients present skin lesions such as urticaria, lichenoid lesions, petechiae at the extremities, palmar itching, or eczema, which can be relieved with symptomatic treatments. Major side effects must be identified and diagnosed rapidly and include anaphylactic shock due to the presence of IgA and arterial or venous thrombosis. The latter is favored by atherosclerosis, age, thromboembolic history, immobilization, excessive dosage, or rapid administration of IVIG [20]. Within 10 days, acute renal failure may occur with acute tubular necrosis, mostly due to hyperosmolar sucrose. This is partially reversible, but in 50% of cases requires hemodialysis [21]. Risk factors are poor hydration, pre-existing

renal disease, age >65 years, diabetes, hypertension, hyperviscosity, or concomitant use of nephrotoxic drugs [20].

Plasma exchange and immunoabsorption

Plasma exchange (PLEX) involves extracting extracorporeally patient plasma and separating the proteins of the plasma from the blood cells. The most commonly used technique is centrifugation. Compensation for protein loss can be achieved by using macromolecules or albumin. Filter pore diameters measure up to 0.2 μM, resulting in filtration of substances up to a molecular weight of approximately 3.10^6 Da, such as autoantibodies, circulating immune complexes, and monoclonal antibodies directed at components of the central and peripheral nervous system. Plasmapheresis has been shown to reduce IgG, IgM, and total complement levels by 63.4, 68.9, and 57.1%, respectively, after one exchange and 80.1, 79.5, and 59.7%, respectively, after five [22].

The neurological indications are poor therapeutic responses to IVIG or corticosteroids including severe relapse of MS, NMO, ADEM, Sydenham's chorea, and RE [23]. As for IVIG, no class I or II studies are available in those pathologies, mainly because of their rarity, except for MS. Very limited data mentioned their use as a long-term management in NMO and RE [24, 25]. Because of its ability to filter some monoclonal antibodies, PLEX is used in MS if progressive multifocal leukoencephalopathy (PML) occurs during natalizumab treatment. Several teams use PLEX as a rescue therapy for severe or corticoids non-responsive relapses in MS. However, there is no randomized published study on this topic. A placebo-controlled randomized study called "PLASMASEP" is currently in progress in order to test this hypothesis (NCT01442233).

The major advantage of this therapeutic approach is its safety; the only listed side effects are related to vascular access, the bleeding risk by filtration of coagulation factors and hypotension.

Another technique used immunoabsorption (IA) that leads only to a distraction of pathologic antibodies sparing other plasma proteins [26]. Therefore, it may avoid potential allergic side effects resulting from albumin substitution and unselective elimination of other plasma proteins in patients treated with PLEX. Additionally, IA is less challenging for the circulation and there is no risk related to the transmission of human material. To date, IA is not licensed in some countries such as the USA but widely used in Germany. In NMO, a large retrospective study of treatment used during the relapses has shown that IA or PLEX may increase the recovery after acute myelitis compared to a classical treatment with intravenous steroids [27].

B. Monoclonal antibodies

The history of monoclonal antibodies dates back to 1975, when the hybridoma technique was developed (immortalized mouse cells producing monoclonal antibodies). Kohler and Milstein received the Nobel Prize for Medicine and Physiology for this discovery that opened the way for production of therapeutic antibodies [28•]. These monoclonal antibodies, identified by the suffix "mab" (for monoclonal antibody) are IgG and recognize only one epitope on a given antigen. They cannot be used orally. Their tissue diffusion is low and their

molecular weight does not allow them to cross the blood–brain barrier (BBB) unless it is inflamed and more leaky [29].

The prefixes used allow us to determine the composition of the mab (Figure 3). Research is currently moving towards the development of new humanized mab, which are less immunogenic and have an increased half-life as well as a higher efficacy compared to their original chimeric predecessors [30]. Table 1 lists the different indications for these antibodies in neurology. We present below the antibodies most frequently used in treating neuroinflammation of the CNS. Multiple sclerosis represents the main field of application, because of the frequency of the disease in which phase II/III trials have recently shown very good results in terms of efficacy and manageable side effects.

Natalizumab

Natalizumab (NTZ) is a humanized monoclonal antibody directed against the α chain of the integrin α4β1 (VLA-4) expressed on the surface of leukocytes. The α4β1 integrin is expressed on the surface of lymphocytes and monocytes. Its binding to its ligand VCAM-1 expressed on the surface of endothelial cells of the BBB is necessary for the migration of leukocytes within the brain parenchyma. This product has been only developed to treat MS and is one of its most specific therapeutics according to its mode of action. This molecule has demonstrated clinical and radiological efficacy in relapsing-remitting MS (RRMS) and has received marketing authorization as second-line or first-line therapy for patients with an aggressive form [31, 32]. The effect on inflammatory process is very

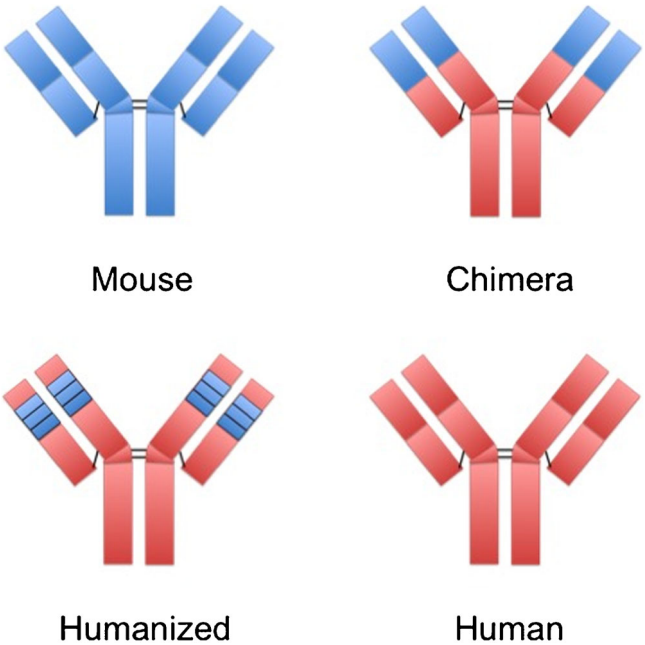


Fig. 3. Illustration of different types of monoclonal antibodies used in therapy. *Blue* murine part (mouse); *red* human part. Their denominations are classified as follows: • ... o = murine mab, • ... xi = chimeric mab, • ... zumab = humanized, and •... (m) u = human mab

Table 1. Indications of monoclonal antibodies in neurology

Systemic diseases: - Neurosarcoidosis - Neuro-Sjoegren - Neurolupus	Rituximab (anti-CD20) Infliximab (anti-TNFα)
Multiple sclerosis	*Natalizumab* (*anti-VLA4*) *Alemtuzumab* (*anti-CD52*) Daclizumab (anti-CD25) Rituximab Ocrelizumab (anti-CD20) Ofatumumab (anti-CD20)
Neuromyelitis optica	Rituximab Tocilizumab (anti-RIL6) Eculizumab (anti-C5)
Anti-MAG neuropathy	Rituximab
Myasthenia	Rituximab
Inflammatory muscle diseases	Rituximab
Neurooncology	Rituximab *Bevacizumab* (*anti-VEGF*)*

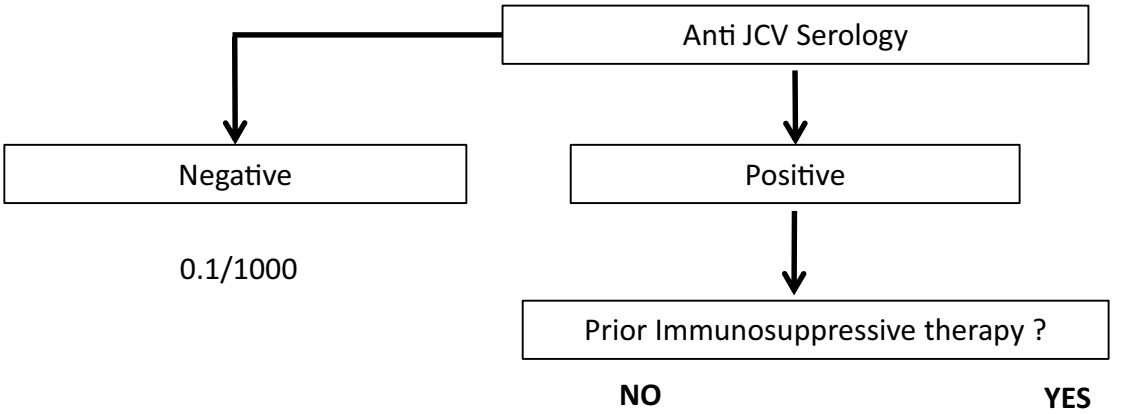
In italic characters, the used molecules that have the marketing authorization
*Relapsing metastatic cancers. Glioblastoma only in USA

high, achieving lack of any disease activity in more than one third of the patients after 2 years [33].

However, blocking the VLA-4/VCAM-1 interaction inhibits the migration of leukocytes into the CNS and causes an increase of the white blood cell count in the peripheral blood and a decrease in the cerebrospinal fluid (CSF) [34, 35]. Because of this CNS "immunosuppression," NTZ is associated with a risk of serious infections, especially PML. This infection is caused by the reactivation of John Cunningham virus (JCV) within the CNS (638 cases in March 2016, extracted from Biogen database, not published). In terms of prognosis, historically, 20–25% of patients who developed PML died, and around 80% of those that survived presented mild to severe residual disability [36]. With time, this mortality and level of morbidity are lowering with increased awareness and surveillance. This risk can now be stratified using a combination of predictive factors. If MS patients have been exposed to previous immunosuppressive therapy, the presence of JCV antibodies (with index) and the duration of treatment with NTZ [37, 38•] play a decisive role in determining the risk of developing PML (Table 2).

A biomarker is currently being considered that could help to refine the evaluation of the risk of PML among patients treated with NTZ: the CD62L expression by $CD4^+$ T cells [39].

Table 2. Estimation of the risk of progressive multifocal leukoencephalopathy under natalizumab (NTZ) according to JCV serology, prior exposure of immunosuppressive therapy and index of JCV synthesis. The PML Risk is estimated by the number of patients with PML per 1000 anti-JCV antibody-positive patients



Duration of NTZ treatment (months)	Index ≤ 0.9	0.9< Index ≤1.5	Index >1.5	
1-12	0.1	0.1	0.2	0.3
13-24	0.1	0.3	0.9	0.4
25-36	0.2	0.8	3	4
37-48	0.4	2	7	8
49-60	0.5	2	8	8
61-72	0.6	3	10	6

Alemtuzumab

Alemtuzumab is a humanized monoclonal antibody, initially developed for the treatment of B chronic lymphocytic leukemia, which has proven clinical and radiological efficacy in RRMS [40, 41]. This antibody is directed against CD52, a glycoprotein expressed on the surface of different leukocyte populations, including T lymphocytes (T cells), B lymphocytes (B cells), and natural killer cells (NK cells). Binding of this antibody with CD52 on the surface of leukocytes causes their apoptosis through complement-mediated cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC). Lymphocyte reconstitution

varies and requires on average 6 months for B cells and more than a year for T cells [42]. Efficacy in RRMS has been demonstrated in two studies showing a higher efficacy of alemtuzumab compared with high-dose/frequency interferon Beta 1A given subcutaneously twice a week. One of the major side effects associated with alemtuzumab is the risk of autoimmunity, estimated at 30% for thyroid autoimmunity (mainly Greaves disease) and 3% for idiopathic thrombocytopenic purpura after 5 years [41]. The development of these autoimmune diseases is probably related to an abnormal B- and T-lymphocyte immune reconstitution as observed in hematopoietic stem cells transplantation [43].

Anti-CD20

Rituximab

In 2014, rituximab (RTX) was the third most sold monoclonal antibody in the world after adalimumab and infliximab. RTX is a chimeric IgG1 isotype binding to CD20 proteins specifically expressed by B cells (except plasmablasts and plasmocytes). Apoptosis of B cells occurs through ADCC and CDC after 1 month and can last between 6 and 12 months depending on the initial dose [44]. However, plasma cells that do not express CD20 are not affected and antibody secretion remains unchanged. RTX does not have marketing authorization for any neurological disease but is commonly used in NMO as a first- or a second-line therapy [45] and in some systemic pathology with neurological expression. RTX has been tested in relapsing-remitting and primary progressive MS with promising results [46, 47] but ocrelizumab (OCR) and ofatunumab rather than RTX are currently developed in these indications. RTX has also entered clinical practice in SLE. Despite failing to meet the primary end points in two phase III clinical trials (EXPLORER and LUNAR) of renal and non-renal lupus [48, 49], RTX remains part of the therapeutic strategy in refractory lupus due to its apparent efficacy in earlier phase and post marketing studies [50].

The major difficulty in its use lies in the dose. An induction dose is recommended (1 g every 15 days for 1 month or 375 mg m^2 weekly for 1 month) followed by a maintenance therapy. The efficacy of RTX can be monitored using the count of $CD19^+$ B cells or that of $CD19^+CD27^+$ B cells (memory B cells) [51]. The main side effects are related to the infusion (headache, nausea, fatigue, or pruritus) and infections of the upper respiratory or urinary tracts. Severe opportunistic infections were reported in patients with lymphoma, lupus, or rheumatoid arthritis treated with RTX. Rare cases of PML (over 150 cases) have been described under RTX but only in immunocompromised patients receiving multiple immunosuppressive agents [52]. However, in MS and NMO patients, in whom RTX is used as a monotherapy, no cases of PML have been reported to date.

Ocrelizumab and ofatumumab

Ocrelizumab (OCR) is a humanized monoclonal antibody that has the same therapeutic target as RTX. The epitope is overlapping with the binding site of RTX and depletes B cells by ADCC, whereas RTX acts more in a CDC manner, which is due to differences in the Fc portion of the antibodies. OCR has demonstrated a dramatic effect on inflammatory process

in RRMS with a higher efficacy compared first with placebo in a phase II trial [53] then with high-dose/frequency interferon Beta 1A given subcutaneously twice a week in two phase III studies [54]. This drug was also tested in primary progressive MS and allows a reduction of disease progression compared with placebo of 25% [55].

OCR was also tested for the treatment of SLE in two phases III clinical trials: the BEGIN study (trial number: NCT00539838) was ended prematurely because of absence of benefit in the patients. In the other phase III study (BELONG; trial number: NCT00626197), some patients included in the OCR group presented serious opportunistic infections that led to the interruption of the trial [56].

The fully human anti-CD20 antibody is called ofatumumab (OFA). It binds to an epitope distinct from that of OCR or RTX, located in both the small and large loops of the CD20 molecule. OFA increased complement activation potential, particularly in the presence of low CD20 expression levels [57]. The cytotoxicity in vitro is superior to RTX, since OFA was able to deplete RTX-resistant B cell lines [58–60]. One phase II study has been published in MS and confirms the efficacy on brain MRI during the 24 weeks of follow-up [61].

Daclizumab

Daclizumab is a humanized monoclonal antibody directed against the α chain of interleukine (IL)-2 receptor, a molecule upregulated on activated T cells. This drug has been used for more than 10 years in the prevention of kidney transplant rejection and is now approved for use subcutaneously in RRMS.

Studies showed that CD25 antagonism was responsible of an expansion of $CD56^{bright}$ NK regulatory cells, resulting in decreased T cell activation [62]. The SELECT trial studied a 52-week treatment by monthly daclizumab 150 or 300 mg compared to placebo in 600 patients with active RRMS [63]. Treatment with daclizumab resulted in a 50 to 54% reduction of annualized relapse rate (ARR) compared to placebo. DECIDE, the other phase III trial, compared monthly injection of daclizumab with interferon Beta 1A and showed a 45% reduction of ARR [64].

The most common adverse events reported were infections, cutaneous adverse events, and elevation of liver aminotransferase levels.

Belimumab

B cell activating factor (BAFF) is a cytokine of the TNF ligand superfamily produced and secreted by monocytes, dendritic cells, and activated neutrophils, which promote B cell activation and survival.

Belimumab, a fully IgG1 monoclonal antibody that targets BAFF, was specifically approved for use in lupus in the USA and Europe in 2011. In fact, two phases III (BLISS 52 and BLISS 76) included patients with active SLE (but no nephritis or CNS lupus) met their primary endpoint without safety issues [65, 66].

Some trials including more severe lupus (nephritis and CNS) are now underway with a clinical trial in lupus nephritis due to be completed in 2019 (trial number: NCT01639339).

Anti-type I IFN

Evidence of the activation of the innate immune system in SLE, such as upregulated IFNα signaling and increased expression of IFN-regulated genes, led to the development of new biological agents targeting specifically this cytokine.

Several anti-type I IFN agents have been evaluated in clinical trials like two anti-IFNα monoclonal antibodies (sifalimumab and rontalizumab) and one anti-IFNα receptor (anifrolumab). The results of the phase II clinical trials are promising with reduction of clinical disease activity measures associated to an acceptable safety profile [67–69]. To date, anifrolumab has progressed to a phase III trial (trial number: NCT02446912).

Others

Secukinumab is a fully human IgG1K monoclonal antibody that neutralizes human IL17-A. A randomized proof of concept study performed in 73 RRMS patients provided the first evidence that blocking IL17-A with an antibody may reduce MRI lesion activity in MS [70].

In NMO, interleukin-6 that is implicated in B cell differentiation is significantly elevated in serum and CSF. Tocilizumab, a humanized antibody targeting the interleukin-6 receptor, has been reported as effective in some refractory NMO patients [71].

Other therapeutical strategies are emerging in the NMO field as the specific inhibition of the complement cascade with a C5 humanized monoclonal antibody (eculizumab) with very promising results on relapses [72].

C. Stem cell transplants

Stem cells are defined by their capacity for self-renewal and differentiation in at least two types of specialized cells. There are different types of stem cells, including hematopoietic, mesenchymal, neural, and embryonic stem cells. Embryonic stem cells have the unique ability to differentiate into all cell types of the organism. To compensate for the ethically problematic use of these cells, an alternative has been developed from transformed skin fibroblasts, namely induced pluripotent cells (IPS) [73••]. John Gordon and Shinya Yamanaka received the Nobel Prize in 2012 for this discovery. Currently, none of these therapies has marketing authorization for neurological diseases but clinical trials in MS are ongoing.

The allogeneic transplant of hematopoietic stem cells has already been the subject of numerous publications on patients with RRMS. The initial goal of treatment is first to eradicate autoreactive cells with an immunosuppressive process and then to restore the immune system with the transfusion of hematopoietic stem cells. Only phase I/II trials are currently available. However, this treatment appears to be very effective in some selected cases of MS patients with

relapses and/or active MRI lesions, under 40 years old and with a short disease duration [74, 75]. Regarding tolerance, the mortality rate was estimated at around 1.3% within the 2001–2007 time frame [76], and deaths were mainly due to systemic infections and preferentially affected more severe disabled patients treated with a high regimen intensity of immunosuppression [77]. Furthermore, about 10% of transplanted patients were affected by autoimmune diseases within the first 2 years [78]. Hematopoietic stem cells can be considered as an alternative therapy in patients with aggressive RRMS [75].

Mesenchymal stromal cells (MSC) have been the intense focus of not only in vitro studies but also animal models of autoimmune diseases [79]. Many mechanisms for the mode of actions have been proposed. In MS, six small studies with less than ten patients have been published suggesting some clinical responses [80–85] including only one small randomized, double blind, placebo-controlled, crossover phase II study [85]. Long-term and powered randomized controlled clinical trials are still needed to evaluate the potential benefit of this therapeutic strategy.

D. Gene therapies

Gene therapy is defined as the use of nucleic acids (DNA or RNA) to cure or prevent diseases. Depending on the disease, this can be achieved by delivering to the cells a functional gene that replaces the defective gene (transgene), a therapeutically active gene or RNA capable of regulating or partially blocking the expression of an altered gene. These nucleic acids are often transported into cells via a viral vector and can be done via in vivo or ex vivo methods. There are a number of viruses being used as vectors, and these viruses differ in tropism, host genome interaction, packaging capacity, immune response in target cells, relative viral titer, and transduction efficiency and therefore affect strength of transgene expression and distribution throughout the brain. The most widely used one currently seems to be the adeno-associated virus (AAV). Other virus like herpes simplex (HSV) and lentivirus, can be used if a neurotropic action is needed. Adenovirus could be used to target directly the astrocytes. The risk of this approach is mainly due to the possible induction of tumor if the inserted DNA is incorrectly placed and trigger toxic, immune, or inflammatory reactions through the virus itself. Some newer vectors have features that mediate site-specific DNA integration in the genome, which will not cause tumorigenic problem. For example, exogenous nanoparticles (liposomes, dendrimers, carbon nanotubes, magnetic nanoparticles, and cationic polymers); endogenous nanoparticles (extracellular vesicles like microparticles, microvesicles, exosomes, and apoptotic bodies); or hybrid system (hybrid bacteriophage vector [AAV/P], which contains genetic elements of AAV packaged within a bacteriophage capsid) are under development [86•].

This therapeutic approach was put into practice for the first time in 1990 following the injection of stem cells and modified lymphocytes in a patient with severe immunodeficiency [87]. Currently, more than 1800 clinical trials are underway, mainly in phase I or II. However, only 1–2% of these tests are related to neurology in Parkinson's disease, Alzheimer's disease, leukodystrophy, or MS. In inflammatory diseases of the CNS, development of such approach concern preclinical studies. For example, a plasmid construct coding for rat IL-10 was used in the experimental autoimmune encephalomyelitis (EAE)

model of MS. In two studies, it has shown a reduction of motor disability and an improvement of EAE-induced sensitivity to touch and allodynia when used intrathecally [88, 89]. Another model of myelin oligodendrocyte glycoprotein (MOG)-induced EAE was treated with hBM-MSCs for secreting IFN-β (MSCs-IFN-β) via adenoviral transduction [90]. Results have shown a decreasing inflammatory cell influx and a suppression of demyelination associated with an immunomodulatory effects on the Th1/Th2 balance.

Another approach results in DNA vaccination. The most advanced therapeutic expertise concerns vaccination against myelin proteins and was achieved with the immunization with a plasmid encoding an encephalitogenic T cell epitope, the guinea pig myelin basic protein 68–85 (MBP68–85) that has suppressed clinical and histopathological signs of EAE and reduced the IFN-γ production [91]. Therefore, a plasmid vector (BHT-3009) was constructed by cloning the 18.5 kD isoform of the full-length human MBP complementary DNA (cDNA) into a modified pVAX1 plasmid, in which the immunostimulatory CpG motifs were removed and immunoinhibitory GpG motifs were included. Phase I and II trials of BHT-3009, demonstrated its safety, tolerability, and effectiveness in induction of immune tolerance to the autoantigen. Moreover, BHT-3009 showed efficacy in reducing brain lesion activity as well as clinical relapses in patients that were immunologically active at baseline [92, 93].

Conclusion

Biodrugs are a modern and innovative therapeutic approach. Their development in the field of inflammatory diseases of the CNS has made a real contribution to clinical practice and enabled a significant degree of effectiveness to be achieved. The efficacy of biodrugs provides a solution for patients who are refractory to standard therapies, such as IVIG in LE, PLEX in NMO, and anti-CD20 in MS.

Biodrugs are the first steps towards personalized medicine, which aims at:

- Better therapeutic targeting with monoclonal antibodies and gene or cell therapies;
- The ability to specifically change the patient's cells using gene or cell therapies;
- Potentially opening the way for tissue repair and neuroregeneration using gene or cell therapies.

In a near future, extraordinary therapeutic perspectives are going to open up in connection with recent discoveries in biology. Among them, CRISPR-Cas9 RNA-guided DNA endonuclease that can induce site-specific modifications in the genomes of cells, bi-specific monoclonal antibodies that can enhance the immune response or T cell vaccination with attenuated autoreactive T cells capable of inducing T cell-dependent inhibition of autoimmune responses could lead to applications in all branches of biotechnology, as well as strategies for human therapeutics.

With all these major innovations, it is time to consider the financial aspects of these expensive treatments. Their cost would appear to be largely justified by

their development and the advanced technologies required for their production.

Finally, as the indications for these treatments increase, it is our duty as doctors to assess the efficacy and the risk of these biotherapeutics. Many collaborative projects are currently underway in the various fields of neuroscience to learn how better to use these products.

Compliance with Ethical Standard

Conflict of Interest

Nicolas Collongues has received board membership fees, honoraria, and paid travel accommodations from Biogen, Merck, Novartis, and Sanofi. Laure Michel has received honoraria payments from Genzyme and Roche and paid travel accommodations from Novartis. Jérôme de Seze has received board membership fees from Biogen, Genzyme, Teva, Merck, Novartis, and Roche.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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MULTIPLE SCLEROSIS JOURNAL | MSJ

Original Research Paper

Effectiveness of mycophenolate mofetil as first-line therapy in AQP4-IgG, MOG-IgG, and seronegative neuromyelitis optica spectrum disorders

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1–8

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Abstract

Objective: To evaluate the effectiveness and tolerance of mycophenolate mofetil (MMF) as a first-line treatment in neuromyelitis optica spectrum disorder (NMOSD).

Methods: In all, 67 NMOSD patients treated by MMF as first-line therapy, from the NOMADMUS cohort were included. A total of 65 fulfilled 2015 NMOSD criteria, and 5 were myelin oligodendrocyte glycoprotein (MOG)-immunoglobulin G (IgG) positive. Effectiveness was evaluated on percentage of patients continuing MMF, percentage of patients free of relapse, pre- and post-treatment change in the annualized relapse rate (ARR), and Expanded Disability Status Scale (EDSS).

Results: Among 67 patients, 40 (59.7%) continued treatment till last follow-up. A total of 33 (49.3%) were relapse-free. The median ARR decreased from one pre-treatment to zero post-treatment. Of 53 patients with complete EDSS data, the score improved or stabilized in 44 (83%; $p < 0.05$). Effectiveness was observed in aquaporin-4 (AQP4)-IgG (57.8% continued treatment, 46.7% relapse-free), MOG-IgG (3/5 continued treatment, 4/5 relapse-free), and seronegative NMOSD (64.7% continued treatment, 61.3% relapse-free). In 16 patients with associated steroids, 13 (81.2%) continued MMF till last follow-up versus 15 of 28 (53.6%) in the non-steroid group. Nine patients discontinued treatment for tolerability purpose.

Conclusion: MMF showed effectiveness and good tolerability as a first-line therapy in NMOSD, whatever the AQP4-IgG status. Concomitant use of oral steroids at start could limit the risk of treatment failure.

Keywords: Aquaporin-4, neuromyelitis optica, treatment response, myelin oligodendrocyte glycoprotein

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Introduction

Devic's neuromyelitis optica (NMO) is a severe relapsing autoimmune inflammatory disease of the central nervous system. Around 70% of NMO cases are associated with aquaporin-4 (AQP4)-immunoglobulin G (IgG),[1,2] which play a key role in NMO pathogenesis and have allowed enlarging the clinical phenotype of the disease to NMO spectrum disorders (NMOSDs).[3–6] AQP4-IgG-negative NMO patients display specific demographic and clinical features and are likely to encompass a number of different diseases.[7] Recently, auto-antibodies directed against the myelin oligodendrocyte glycoprotein (MOG)-IgG have been reported in 20%–25% of AQP4-IgG-seronegative NMO patients.[8,9]

Relapse prevention in NMO is based on early and prolonged immunosuppressive treatments including azathioprine (AZA), mycophenolate mofetil (MMF), methotrexate, mitoxantrone, and rituximab (RTX).[10] A better understanding of AQP4-IgG-mediated NMO pathophysiology recently led to the proposal of more

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targeted therapies including eculizumab, tocilizumab, and aquaporumab.[11–13] It is not clear whether typical NMO patients tested negative for AQP4-IgG should be treated differently from the seropositive ones. However, as the course and the pathophysiology are possibly different, long-term therapy with these treatments, aiming at reducing antibody production and/or complement activation, might not be appropriate in all AQP4-IgG-negative NMO.

MMF is an immunosuppressant drug with proven efficacy and good tolerance in organ transplantation and an increasing number of immune-mediated disorders.[14] MMF is an inhibitor of inosine monophosphate dehydrogenase, which inhibits de novo guanosine nucleotide synthesis, targeting proliferation of T and B lymphocytes. The use of MMF in NMO and NMOSD has been investigated only in a few retrospective studies, including mainly AQP4-IgG-positive cases and mixing the use of MMF as first-line or rescue therapy. All of them suggested some benefits in reducing the relapse rate and disability.[15–17]

The purpose of our work was to evaluate the effectiveness and tolerance of MMF as first-line therapy in a large French multicentre cohort of NMOSD patients, whatever their AQP4-IgG status.

Methods

Patients

Patients were selected from the French NOMADMUS cohort of NMO and related disorders that was constituted in 2011 and included 310 cases at the date of the study (www.edmus.org). The cohort involves the main referral centers for neuro-inflammatory disorders in France organized in a network within the "Observatoire Français de la Sclérose en Plaques" (OFSEP) and also patients spontaneously reported by general practitioners, neurologists, or pediatricians. Data are recorded and anonymized in a specific database using the EDMUS standardized language, that is, the EDEN software dedicated to NMO (http://www.edmus.org/en/soft/index.html). Among the NOMADMUS cohort, we have included the following:

Patients fulfilling the 2015 criteria for NMOSD;[5]

or MOG-IgG-positive patients;[18]

and patients naive of treatment before MMF.

All the cases included in NOMADMUS are assessed by an expert committee.[19]

Of a total of 310 cases, we identified 67 patients who received MMF as first-line therapy for at least 12 months.

Effectiveness was evaluated on several parameters: percentage of patients continuing MMF, percentage of patients free of relapse, pre- and post-treatment change in the annualized relapse rate (ARR), and Expanded Disability Status Scale (EDSS).

Standard protocol approvals, registrations, and patient consents

The French data protection authority approved the study. All patients gave their informed consent to participate in the study.

Auto-antibodies' detection

All samples were tested for AQP4-IgG using a cell-based assay method previously described.[6] When available, AQP4-IgG-negative samples were further tested for MOG-IgG using a cell-based assay.[19]

Statistical analyses

The ARR and EDSS score, before and after treatment, were compared using the Wilcoxon signed-rank test. Percentage of treatment continuation and relapse-free patients between different populations was compared using Fisher's exact test. Statistical significance cut-off was reached at $p < 0.05$. We performed computations using SAS software version 9.1 (SAS Institute, Inc., Cary, NC, USA).

Results

Patient characteristics

In all, 67 patients were included, 50 females and 17 males. The median age at disease onset was 37.9 years (range, 6–67 years), and the median time interval from disease onset to treatment with MMF was 21 months (range, 0–454 months). The dose of MMF used was 2000 mg/day. Totally, 65 fulfilled the 2015 NMOSD criteria,[5] including 40 patients who fulfilled 2006 criteria and 24 tested positive for AQP4-IgG but without involvement of both the optic nerve and the spinal cord. Among the 22 patients with AQP4-IgG-negative status, 11 were tested for MOG-IgG and 5 of 11 were positive (3 NMO and 2 longitudinally extensive transverse myelitis (LETM)). Clinical and demographic characteristics of the patients are outlined in Table 1.

Table 1. Patient characteristics.

Characteristics	Value
Patients, no.	67
Female (sex ratio), no.	50 (2.9/1)
Age at disease onset, mean (range), years	37.9 (6–67)
Relapse before MMF treatment, mean (range), no.	3.1 (1–13)
AQP4-IgG-positive patients, no.	45
AQP4-IgG-negative patients	22
Seronegative NMOSD, no.	17
MOG-IgG-positive patients, no. (%)	5/11 (45.4)

MMF: mycophenolate mofetil; AQP4: aquaporin-4; IgG: immunoglobulin G; NMOSD: neuromyelitis optica spectrum disorder; MOG: myelin oligodendrocyte glycoprotein.

Treatment effectiveness

In the whole group. Among the 67 patients, the median follow-up was 24 months (range, 1–156 months; Table 2). In all, 40 (59.7%) patients continued treatment till last follow-up (mean follow-up 43 months). Totally, 33 (49.3%) of the 67 patients were relapse-free. Among the 55 patients with a follow-up of 1 year or more, the median ARR decreased from 1 (0.1–3.2) pre-treatment to 0 (0–3) post-treatment ($p < 0.05$; Figure 1). The median post-treatment EDSS score was improved compared with the median pre-treatment (4 (0–8.5) to 3.75 (0–10); $p < 0.05$). Of 53 patients with complete data about EDSS, the score was improved or stabilized in 44 patients (83%).

If we consider only the 46 patients with at least 1 year of pre-treatment periods, the median ARR decrease from 0.88 (0.1–3.2) pre-treatment to 0 (0–3) post-treatment ($p < 0.05$).

According to AQP4-IgG status. Of 45 patients positive for AQP4-IgG with a median follow-up of 24 months (range, 3–156 months), 26 (57.8%) continued their treatment till last follow-up versus 11 of 17 (64.7%) in the AQP4-IgG-seronegative NMOSD group with a median follow-up of 21 months (range, 1–59 months). Among patients with AQP4-IgG-positive status, 21 of 45 (46.7%) were relapse-free in comparison to 8 of 17 (61.3%) in the AQP4-IgG-negative NMOSD. The evolution of the ARR was roughly similar for the two groups with a median post-treatment ARR of 0.21 (0–1.12; $p < 0.05$) in the AQP4-IgG-seropositive group and 0 (0–0.8; $p < 0.05$) in the NMOSD AQP4-IgG-seronegative group.

According to MOG-IgG status. Of five patients positive for MOG-IgG with a median follow-up of 43 months (range, 7–67 months), three (60%) were continuing MMF till the end of follow-up and four (80%) were relapse-free. Three of the five patients had a monophasic course during the follow-up (under treatment). The median ARR post-treatment was 0. The results of evolution of ARR were not significant ($p = 0.07$).

According to concomitant steroid use. Comprehensive information regarding steroid use was available for 47 patients. In all, 28 did not receive concomitant steroids, 16 received steroids with a regimen based on high starting dose of $\geqslant 1$ mg/kg during 3 months, followed by a slow tapering for minimum 3 additional months (Table 3), and 3 patients received steroids at lower dose (these 3 patients are not considered in the results). Of the 16 patients with associated steroids with a median follow-up of 25 months (range, 12–71 months), 13 (81.2%) continued MMF till the end of follow-up versus 15 of 28 (53.6%) in the non-steroid group ($p = 0.1$) with a median follow-up of 29 months (range, 1–156 months). Among patients with concomitant steroids, 10 of 16 patients (62.5%) were relapse-free compared to 17 of 28 (60.7%) in the non-steroid group ($p = 1$). The evolution of the ARR was similar for the two subpopulations with a median post-treatment ARR of 0 ($p < 0.05$). However, the median post-treatment EDSS score was improved compared with the median pre-treatment EDSS score in the concomitant steroids group (EDSS median, 4.5 (0–8) to 4 (0–8.5); $p < 0.05$) which was not the case in the non-steroid group (EDSS median, 4 (0–8) to 4 (0–8.5); $p = 0.4$). The median delay of occurrence of relapse under treatment was 11.1 months (range, 3–28.4 months) in the 6 patients of the concomitant steroid group (with 1 patient who underwent relapse before 6 months of treatment) compared to 6.5 months (range 1.1–54 months) in the 11 patients of the non-steroid group (with 5 patients who underwent relapse before 6 months of treatment; $p < 0.05$).

Treatment discontinuation

In all, 32 patients discontinued treatment permanently or transiently during follow-up. Causes for discontinuation were divided into several categories: lack of efficacy (15 patients), intolerance with digestive predominant symptoms (6 patients), infection (3 patients), pregnancy or pregnancy expectation (5 patients), and other causes (3 patients) including personal choice and switch of treatment for associated pathological condition. One patient died during the follow-up. Death was secondary to complication of

Table 2. Results of subpopulations.

	Continuation of treatment (% of patients)	Free of relapse (% of patients)	ARR (median and range)	EDSS score (median and range)
Whole group ($n=67$ and median follow-up of 24 months)				
Before MMF treatment	59.7	49.3	1 (0.1–3.2)	4 (0–8.5)
After MMF treatment			0 (0–3)	3.75 (0–10)
Patients with adequate steroids ($n=16$ and median follow-up of 25 months)				
Before MMF treatment	81.2	62.5	1 (0.11–3)	4.5 (0–8)
After MMF treatment			0 (0–0.77)	4 (0–8.5)
Patients without steroids ($n=28$ and median follow-up of 29 months)				
Before MMF treatment	53.6	60.7	0.95 (0.1–3)	4 (0–8.5)
After MMF treatment			0 (0–1.12)	4 (0–10)
Patients with AQP4-IgG-positive ($n=45$ and median follow-up of 24 months)				
Before MMF treatment	57.8	46.7	1 (0.17–3)	4 (0–8)
After MMF treatment			0.21 (0–3)	4 (0–8.5)
Patients with MOG-IgG-positive ($n=5$ and median follow-up of 43 months)				
Before MMF treatment	60	80	1 (1–1)	4 (2–6)
After MMF treatment			0 (0–0)	3.5 (0–6)
Patients with AQP4-IgG- and MOG-IgG-negative ($n=17$ and median follow-up of 21 months)				
Before MMF treatment	64.7	47.1	0.91 (0.1–3.2)	3.5 (0–8.5)
After MMF treatment			0 (0–0.8)	4 (0–10)

ARR: annualized relapse rate; EDSS: Expanded Disability Status Scale; MMF: mycophenolate mofetil; AQP4: aquaporin 4; IgG: immunoglobulin G; MOG: myelin oligodendrocyte glycoprotein.

immobilization, as EDSS was already 8.5 at treatment introduction. Among the 32 patients, 6 stopped only transiently. The median duration of MMF at treatment discontinuation was 16 months.

Among patients with AQP4-IgG-positive status, 10 of 45 (22.2%) discontinued treatment because of lack of efficacy in comparison to 4 of 17 (23.5%) in the AQP4-IgG-negative NMOSD group and 1 of 5 (20%) in the MOG-IgG-positive group. Of the 16 patients with associated steroids, 2 (12.5%) stopped MMF because of infection versus 1 of 28 (3.6%) in the non-steroid group.

Discussion

Our work confirms the efficacy of MMF as first-line therapy in NMO and related disorders in a large cohort of Caucasian patients. We reported efficacy on the rate of patients continuing treatment till last follow-up, the percentage of patients free of relapse, and the decrease in ARR and EDSS pre- and post-treatment. Interestingly, we found a positive outcome in AQP4-IgG-positive, -seronegative NMOSD, and MOG-IgG-positive patients. The strength of our study is to be large sized and multicentric with patients from more than 15 centers described in a computerized standardized manner. Our study reflects the whole spectrum of the disease with an important proportion of AQP4-IgG-negative NMOSD patients and some MOG-IgG-positive ones for whom data are lacking. Last but not least, we deliberately selected only patients receiving MMF as first-line therapy to minimize the risk of confusing biases, including the remaining effect of the preceding treatments and selection of a specific population. In our work, 50.7% of patients had relapse under MMF. However, 59.7% continued treatment and 83% had stabilization or improvement of EDSS till the end of the follow-up. Thus, relapse under treatment should not be the only parameter for assessing treatment efficacy in NMOSD. All relapses do not have same consequences, and our results suggest that relapse under treatment could be less severe in terms of disability, as recently reported.[20]

In 2009, Jacob et al.[15] published a cohort of 24 North-American patients treated with MMF, 7 of them as first-line treatment. They suggested the efficacy of MMF with a decrease in the ARR for 19 of 24 patients (79%) and a stabilization or decrease in disability in 22 of 24 patients (91%). Recently, Huh et al.[16] published a retrospective study about efficacy of MMF in 58 NMOSD patients from three expert centers in South Korea, half of them were naive of treatment and 90% were AQP4-IgG-positive. All patients were

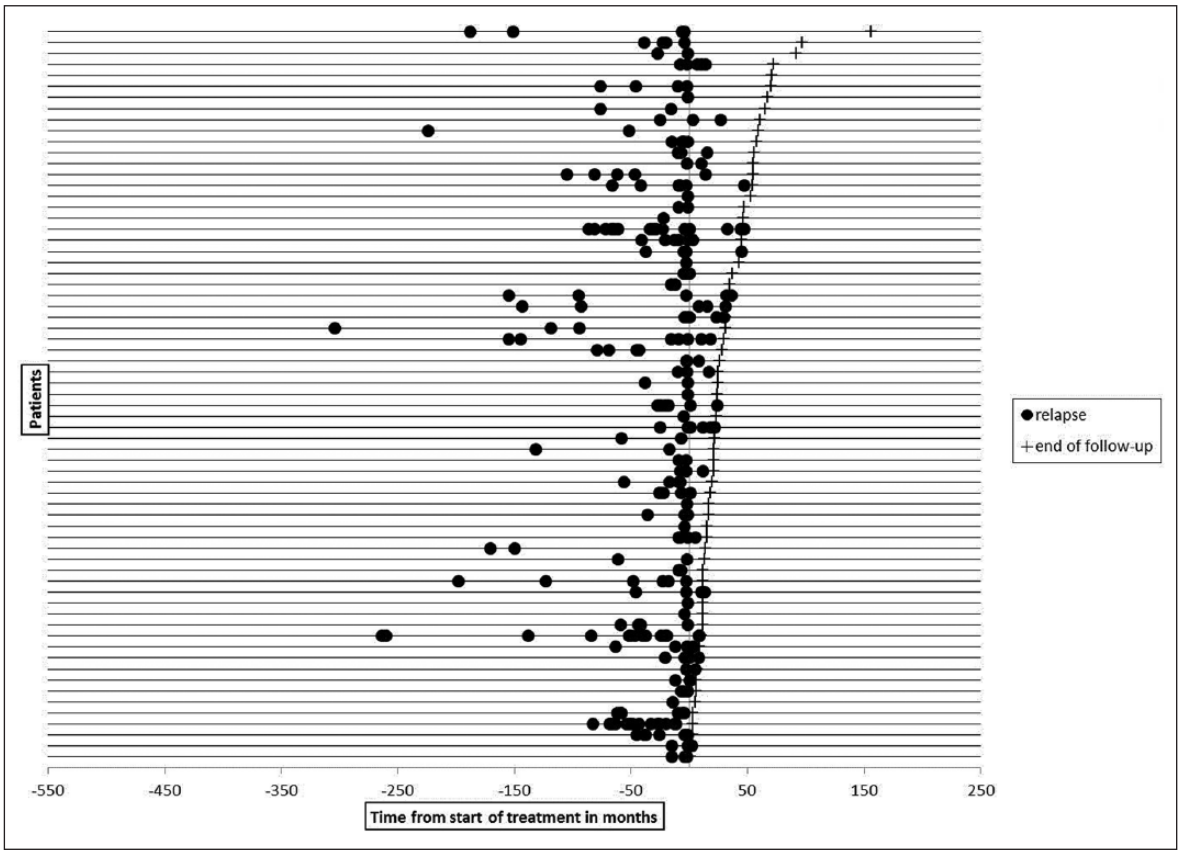

Figure 1. Relapses before and after start of mycophenolate mofetil.

Table 3. Patients characteristics of steroid and non-steroid group.

Characteristics	Steroid group	Non-steroid group
Patients, no.	16	28
Female (sex ratio), no.	13 (4.3/1)	18 (1.8/1)
Age at disease onset, mean (range), years	40.9 (6–67)	38.5 (14–67)
ARR before treatment, median (range)	1 (0.1–2.5)	0.95 (0.1–3)
Duration of follow-up, mean (range)	32 (12–71)	37 (1–156)
AQP4-IgG-positive patients, no.	12	17
AQP4-IgG-negative patients	4	11
Seronegative NMO, no.	3	10
Seronegative LETM/ON, no.	1	1
MOG-IgG-positive, no. (%)	2	2

ARR: annual relapse rate; AQP4: aquaporin; IgG: immunoglobulin G; NMO: neuromyelitis optica; LETM: longitudinally extensive transverse myelitis; ON: optic neuritis; MOG: myelin oligodendrocyte glycoprotein.

treated with a combined oral steroid for at least 2 months. After a median follow-up of 20.4 months, 60% of patients were free of relapse with a stabilization or improvement in EDSS score in 91%. Our work confirms these positive results on a large cohort of Caucasian patients. However, in all of these studies, a proportion of patient is non-responder to MMF. We were unable to identify any demographic or clinical characteristics that could predict this lack of efficacy. Recently, several single-nucleotide polymorphisms (SNPs) in the drug's target enzyme, inosine monophosphate dehydrogenase (IMPDH), have been implicated in the renal transplant literature as affecting response to MMF therapy.[21,22] Thus, in the near

future, identifying such a specific genetic profile could help to predict MMF efficacy in NMO patients.

The two other main treatments currently used in NMO are RTX[23,24] and AZA.[25,26] A comparison between these different treatments and MMF cannot be done reliably due to the lack of randomization, because the treatment was chosen at the discretion of the clinician depending on the context and due to differences in populations.[27,28] However, Mealy et al.[17] in a cohort of 90 patients evaluating the efficacy of MMF, AZA, and RTX concluded of no major difference between MMF and RTX and found an important proportion of treatment failure in AZA (53%). Jeong et al.,[29] in a cohort of 138 NMOSD patients treated with AZA, MMF, or RTX, concluded a better efficacy of RTX than MMF and MMF than AZA, when used first line.

As it is the case for AZA, the clinical effects of MMF are usually not seen before 3–6 months after initiation of therapy emphasizing the need of additional oral steroids during this period. We found a higher percentage of patients continuing treatment till last follow-up in the concomitant steroid group (81.2% against 53.6% without steroids; $p < 0.05$). This was associated with a lower ARR till last follow-up and a higher percentage of patients free of relapse compared to the non-steroid group. This underlines that side effects associated with steroids were minor or at least did not lead the clinician to stop treatment more frequently compared to the non-steroid group. The mean time to first relapse was longer in steroid group (11.1 months) than in non-steroid group (6.5 months), even if decision of concomitant use of steroids was the choice of neurologists with some differences in the two groups (Table 3).

In our study, MMF seems to be effective in all subpopulations, possibly even more among AQP4-IgG-seronegative. However, we must take into account that AQP4-IgG-negative NMO, including MOG-IgG NMOSD, expressed specific demographic and disease-related features, characterized by over-representation of monophasic course, a lower relapse rate, and a better motor and visual acuity outcome compared to seropositive NMO.[6,30,31] Some MOG-IgG-positive patients might have been relapse-free without MMF therapy. However, in our study, MMF therapy was started after onset events without the knowledge of MOG-IgG status, as MOG-IgG testing was done retrospectively. This good response from MOG-IgG patients is, however, of importance as there is currently no recommendation for the management of MOG-IgG patients. Of course, further studies are needed to determine how to treat these patients efficiently. In any case, these good results on all NMOSD populations may be due to the broad immunosuppressive action of MMF targeting B and T cells. In the near future, it will be important to monitor the efficacy of new treatments on seronegative patients. Lack of knowledge of the pathophysiology of AQP4-IgG-negative NMO and the possibility of a different pathophysiological mechanism could lead to a difference in efficacy of the new treatments between AQP4-IgG-positive and AQP4-IgG-negative NMOSD.

Conclusion

Our work supports that MMF provides consistent and sustained efficacy with good tolerability in some patients with NMOSD, whatever is the AQP4-IgG status or MOG-IgG status. The use of concomitant steroids at initiation of treatment is associated with a better efficacy. However, as illustrated in our study, a number of patients still experienced relapse or discontinued MMF. Thus, new treatments are needed in NMOSD. Ideally, the next step would be to assess the exact benefit of MMF in comparison to other active drugs and/or placebo, in randomized clinical trial.

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Short Report

Efficacy of rituximab in refractory neuromyelitis optica

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Abstract

Background: Despite a growing use of rituximab (RTX) in neuromyelitis optica (NMO), data are lacking in patients with refractory NMO (RNMO), defined as cases with at least one relapse during immunosuppressive therapy.

Objective: The purpose of this study was to assess RTX as a maintenance therapy in RNMO.

Methods: Out of a total of 305 NMO cases from a population-based cohort, 21 RNMO patients received RTX during a mean follow-up period of 31 months.

Results: After RTX, 11 patients (52.3%) were relapse free, meaning that 47.7% were refractory to RTX. The mean annualized relapse rate decreased from 1.3 to 0.4 ($p<0.001$) and median EDSS from 5 to 3 ($p=0.02$). Body mass index (BMI) was predictive of EDSS worsening.

Conclusions: RTX is an effective and well-tolerated treatment in RNMO. BMI could be a predictive factor for efficacy.

Keywords: Neuromyelitis optica, rituximab, refractory, disability, body mass index

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Introduction

Neuromyelitis optica (NMO) is a rare but severe disease. Patients that do not respond to at least one immunosuppressor (IS) prescribed at the appropriate dose and duration are considered as having refractory NMO (RNMO) and urgently need a new line of treatment.

Accumulated evidence suggests a robust effect of rituximab (RTX), an anti-CD20 therapy, in NMO. In these retrospective studies, a decrease in annualized relapse rate (ARR) or disability was observed, though with a wide range of response due to the inclusion of heterogeneous groups of patients and different dosage strategies.[1–5] Indeed, the term 'refractory' was not defined and therefore no clear data were provided on the dose and duration of each IS before RTX therapy.

In view of the questions that remain about RTX efficacy in RNMO patients, we performed a nationwide study of RNMO patients treated with RTX.

Methods

Patient

Out of a total of 305 NMO cases from a population-based cohort (NOMADMUS; www.edmus.org), 21 RNMO patients received RTX as a maintenance therapy. Data were collected from September 2013 through July 2014, corresponding to the end point of the study. All of the patients included in this study fulfilled the following inclusion criteria: 1. NMO diagnosis based on the Wingerchuck 2006 criteria;[6] 2. Maintenance therapy with RTX for at least 6 months; 3. Expanded Disability Scale Score (EDSS)$\leqslant$8; 4. At least one relapse under previous well-conducted IS therapy. RTX was given as an induction protocol followed by a maintenance strategy, which was determined differently in each center according to the habits of the treating physician.

A range of immunosuppressive therapies was used before RTX, such as azathioprine (AZA),

mycophenolate mofetil (MMF), cyclophosphamide (CP), methotrexate (MTX), mitoxantrone (MT), corticosteroid (CS) or hydroxychloroquine sulfate (HS). Each immunosuppressive agent was given for a period of time longer than the time to clinical effect established for each drug: at least 6 months for AZA (2 mg/kg/d), MMF (2 g fixed dose), MTX (0.2 mg/kg/d) and HS (7 mg/kg/d) and 3 months for CP (700 mg/m^2), MT (12 mg/m^2) and CS (1 mg/kg/d). Corticosteroids were used with AZA and MMF for patient No. 9. As soon as a relapse occurred under immunosuppressive therapy, the patient was switched to RTX treatment. Except for patients Nos 3 and 9, no concomitant IS was given during RTX therapy.

Data collection

Data confidentiality and security were ensured in keeping with the recommendations of the French data protection authority, which also provided approval.

Epidemiologic data were assessed for each patient with NMO, including demographic data, medical history, key episodes in the NMO course (relapses and dates of relapses and assignment of the successive scores of disability), biological results (AQP4 antibodies), neuroimaging, treatments before RTX, time to RTX, dosage regimen used, and the date and number of infusions. Clinical disability was assessed using the EDSS.

Statistical analysis

The Mann-Whitney-Wilcoxon matched-pairs signed-rank test was used for the comparison of ARR and EDSS before and under RTX. A random subject effect and a fixed time effect were used to assess predictive factors of clinical activity. Two-sided p values <0.05 were considered statistically significant. All computations were performed using SPSS for Windows, version 14.0 (SPSS, Chicago, IL).

Results

Twenty-one RNMO patients were included in the study (Table 1). Mean age at onset was 37.7 years (female/male ratio: 19/2). Antibodies against AQP4 were found in 19/21 (90.5%) patients, longitudinally extensive transverse myelitis (LETM) in 18/21 (85.7%), and Paty-negative brain MRI in 16/21 (76.2%). Before the introduction of RTX therapy, a mean of 1.7 different immunosuppressive therapies were used during a meantime of 22 months. RTX was used during a mean time of 31±18 months. All patients remained under RTX at the end of the study. Rituximab was used concomitantly with CS (per os for 6 months) and MMF (for 12 months) for patients No. 3 and No. 9, respectively. No serious side effects were reported in any of the patients.

Considering the two periods of time including the two years before RTX and the years during RTX therapy, the RNMO patients experienced a decrease in mean ARR from 1.3 ± 0.5 to 0.4 ± 0.5 (p<0.001) (Figure A). Eleven patients (52.3%) were relapse free under RTX. Median time to the first relapse was 17 months, including 2 patients with a relapse in the first month after RTX induction. The median EDSS score decreased from 5.0 [1.5-8] to 3.0 [0-7.5] (p=0.02) (Figure B). The mean annualized infusion rate was 2.3±1.6 and the mean annualized dose was 1664±1002 mg.

No predictive impact was found for time to RTX, number and type of treatment used before RTX, and the annualized dose used. Whereas demographic and AQP4 serological status had no influence on RTX response, body mass index (BMI) was significantly predictive of disability, as assessed on the EDSS (p=0.04) (Table 2). When the BMI increased by 1 point, the mean EDSS score increased by 0.12. Importantly, none of these data were predictive of the disease course before RTX treatment.

Discussion

In this population-based study, we report the efficacy of RTX in an IS-unresponsive population of patients. Efficacy was not associated with a specific demographic profile; neither was it associated with pre-RTX clinical activity or disability. Our search for predictive factors of RTX response showed a putative role of BMI in disability outcome.

Because RNMO patients represent a disabled population treated in tertiary and referral centers involved in the NOMADMUS project, we consider it likely that we included all the RNMO patients treated with RTX as a maintenance therapy in France. The dosage used for maintenance therapy was closer to 1 g every 6 months than 2 g, resulting in a lower occurrence of side effects and a lower cost. The absence of dosage strategy-related predictive factors could be due in part to the narrow range of dosages used and the low number of patients. Nevertheless, BMI could play a role in RTX unresponsiveness, likely due to the increase in dilution volume and its impact on the effective dose in patients.

Our data on efficacy are in line with previously published studies. Mainly of these studies, which included 5 to 30 patients, showed a decrease in the ARR and the EDSS score.[1–5, 7] A marked effect on

Table 1. Characteristics of patients with refractory NMO treated with rituximab.

Patient No.	Gender	Racial background	Age at onset (years)	Body mass index	AQP4-antibodies	LETM	Paty-negative brain MRI at onset	Chronology of previous treatments and their duration (months)	Time to IS treatment from disease onset (months)	Time to RTX from disease onset (months)
1.	F	West Indian	60	28.8	+	+	+	AZA (3) MT (4)	6	19
2.	F	Asian	46	22	+	+	+	CP (6) AZA (10)	1	20
3.	F	African	37	32.4	+	+	–	CP (6) MMF (36) CS (6)	1	44
4.	F	Hispanic	38	22.6	+	+	+	INF (66) MMF (47)	18	134
5.	F	West Indian	21	25	–	+	+	AZA (1) MMF (1) MTX (13)	13	28
6.	F	Caucasian	16	19.5	+	–	+	AZA (8)	2	10
7.	F	Caucasian	25	18.2	+	+	+	MMF (24)	10	36
8.	F	Caucasian	69	20.5	+	+	–	MMF (6) AZA (6)	62	93
9.	F	Caucasian	51	19.2	+	+	+	CS (18) AZA (3) MMF (24)	0	18
10.	F	West Indian	54	27.3	+	+	+	MT (3) MMF (23)	4	34
11.	F	Caucasian	53	18.4	+	+	+	MT (12)	10	38
12.	F	West Indian	14	23.5	+	+	+	INF (41) AZA (90)	84	226
13.	F	Caucasian	56	22.8	+	+	+	INF (24) CP (4)	2	32
14.	M	Caucasian	37	31.3	+	+	–	CP (3)	17	21
15.	F	Caucasian	34	18.7	+	+	+	CP (11) MMF (7)	0	18
16.	F	Caucasian	35	19.8	–	+	+	AZA (18)	2	20
17.	M	Caucasian	39	27.3	+	–	+	MMF (29)	3	35
18.	F	Caucasian	40	42.6	+	+	–	AZA (6)	28	56
19.	F	Caucasian	18	19.1	+	+	+	CP (5)	1	8
20.	F	African	28	23.9	+	–	+	MMF (10)	5	15
21.	F	Caucasian	22	42.7	+	+	–	CS (4) HS (20)	54	80

F: female; M: male; AZA: azathioprine; AQP4: aquaporin-4; INF: interferon; IS: immunosuppressant; LETM: longitudinally extensive transverse myelitis; MMF: mycophenolate mofetil; CP: cyclophosphamide, CS: corticosteroids (per os); MT: mitoxantrone; MTX: methotrexate; RTX: rituximab: HS: hydroxychloroquine sulfate.

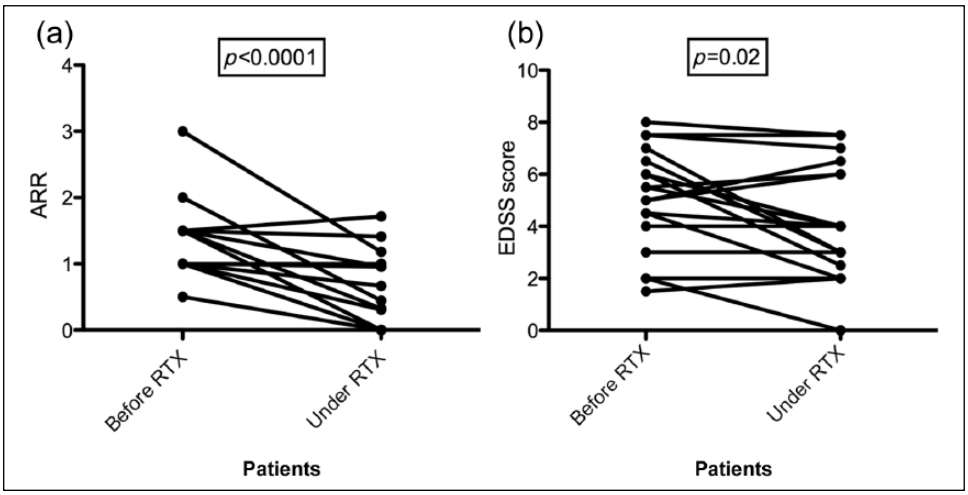

Figure. Efficacy of RTX on relapses and EDSS.
(a) Annualized relapse rate (ARR) before and at the end of follow-up under RTX showed a decrease for 17/21 RNMO patients.
(b) EDSS score before and at the end of follow-up under RTX showed a decrease for 11/21 and was stable for 6/21 RNMO patients.

Table 2. Level of significance in a multivariate analysis looking for predictive factors of annualized relapse rate (ARR) and EDSS under rituximab.

Non continuous data	Number	ARR	EDSS
Gender (F/M)	19/2	*p=0.83*	*p=0.81*
Racial background (C/NC)	13/8	*p=0.52*	*p=0.41*
LETM	18	*p=0.12*	*p=0.4*
Initial brain MRI	16	*p=0.59*	*p=0.06*
Anti-AQP4 antibodies	19	*p=0.88*	*p=0.38*
Continuous data	**Mean ± SD**		
Body mass index	25 ± 7.2	*p=0.33*	*p=0.04*
Age at disease onset	37.7 ± 15.5	*p=0.53*	*p=0.66*
Previous treatments	1.7 ± 0.8	*p=0.15*	*p=0.58*

F: female; M: male; C: Caucasian; NC: non Caucasian; LETM: longitudinally extensive transverse myelitis; SD: standard deviation.

EDSS was also encountered in our study, whereas the relapse rate was less decreased, possibly due to the inclusion of naive patients at the onset of the disease in the other studies. Additionally, our study has some specificities that have not been systematically reported in previous studies: 1. A maintenance therapy with RTX was performed for all patients; 2. A majority of patients were seropositive for AQP4-Ab; 3. Evaluation of patients was done in a population-based study; 4. Predictive factors of RTX response were looked for.

Six out of 21 patients have received CP alone or in combination, which have been reported to improve but also to exacerbate NMO in one study.[8] In this study, the lack of timing between the onset of CP and the first relapse could explain a part of the results, as CP needs several months to be effective. Furthermore, 1 g every 2 months was used whereas the dose of 1 g monthly seems preferable.

The maintenance regimen is a matter of debate as it is not mentioned in most of the studies.[3, 5, 9, 10] In our study we suggest that BMI could drive a dose-effect response linked to the volume of RTX dilution. This is consistent with a previous study that showed that low doses of RTX were associated with a high rate of early B-cell repopulation.[2]

The main limitation of our study is its retrospective design. In particular, CD19+/CD27+ memory B cells could be of interest in monitoring RTX pharmacodynamics.[11] To our knowledge, only Kim et al. have used this approach and further studies are needed to confirm their findings.

To conclude, we showed a strong efficacy of RTX as a maintenance and rescue therapy. We suggest that calculating RTX dose by BMI or weight could help to ensure optimal efficacy of the maintenance therapy in NMO patients.

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Conflict of interest

Dr. Collongues serves on scientific advisory boards for and has received honoraria from Biogen Idec, Merck Serono, sanofi-genzyme, Novartis, Bayer Schering Pharma and Alexion Pharmaceutical. Dr. Brochet serves on scientific advisory boards for and has received honoraria or research support from Biogen Idec, Merck Serono, sanofi-genzyme, Bayer Schering Pharma, teva. Dr. De Seze serves on scientific advisory boards for and has received honoraria from Biogen Idec, Merck Serono, sanofi-genzyme, Bayer Schering Pharma, Chugai and Alexion Pharmaceutical. Dr JC Ouallet has received consultancy fees, speaker fees, research grants (non-personal), and honoraria from Novartis, Biogen-Idec, Merck-Serono, Bayer Schering, Roche, Almirall, Teva and Genzyme. Dr. Marignier serves on scientific advisory board for MedImmune and has received honoraria from Biogen Idec, Merck Serono, Novartis, Sanofi-Genzyme. Drs. Brassat, Vukusic, Maillart, Labauge, Carra Dalliere, Moreau, Papeix, Bourre and Audoin report no disclosures.

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An update on the evidence for the efficacy and safety of rituximab in the management of neuromyelitis optica

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***Abstract*:** Neuromyelitis optica spectrum disorders (NMOSDs) is a new concept which includes classical neuromyelitis optica (NMO) and partial forms of NMO such as recurrent optic neuritis with positive aquaporin-4 antibodies (AQP4) or brainstem symptoms (intractable hiccups or vomiting). This disease is clearly distinguished from multiple sclerosis (MS) and the therapeutic approach is clearly different. Rituximab is actually considered to be one of the most efficient treatments of NMOSD, even if class I studies are clearly lacking. In the present review, we describe the state of the art about rituximab treatment in NMOSD, including adults and children, plus its efficacy and tolerance and we also underline the questions that should be addressed in the near future.

Keywords: Rituximab, CD20, B lymphocyte, neuromyelitis optica, monoclonal antibody

Introduction

Neuromyelitis optica (NMO) is a rare inflammatory and demyelinating disease of the central nervous system. The therapeutic strategy to prevent relapses is based on the use of immunosuppressants (ISs). When NMO is particularly severe or when patients do not respond to a first line therapy, a new IS infused intravenously is usually prescribed. In these patients, data suggest an effect of cyclophosphamide, mitoxantrone and rituximab (RTX), each used at a dosage similar to that used in multiple sclerosis (MS) [Collongues *et al.* 2011]. To date, there is an increasing amount of evidence for a strong effect of RTX in NMO leading to a growing number of publications in recent years. We propose to review the data on the efficacy and tolerability of RTX in NMO.

Pharmacology of rituximab

RTX is a chimeric monoclonal antibody (mAb) against human B-lymphocyte antigen, CD20, initially approved for the treatment of non-Hodgkin B-cell lymphomas. CD20 is expressed at the membrane of the B lymphocyte from the stage of pre-B cells to mature B lymphocytes. In an exceptional manner, CD20 is also expressed in less than 5% of T lymphocytes [Hultin *et al.* 1993]. This cluster is not present on stem cells and plasmocytes that permit maintenance of a constant level of immunoglobulin, and therefore confers a relative protection against opportunistic infection.

RTX consists of a variable light chain of murine anti-CD20 and a constant heavy chain (Fc) of human IgG-1 associated with a light chain Kappa. Its major mechanism of action results in a destruction of B cells via CD20 linkage, caused by phagocytosis by macrophage and neutrophils, complement-dependent cytotoxicity (CDD) or antibody-dependent cellular cytotoxicity (ADCC) involving natural killer (NK) cells. These mechanisms depend on the Fc portion of the antibody binding to the Fc gamma receptors (FcγRs) on immune cells. Other mechanisms are aggregation of targeted cells or direct cell death through CD20 signaling [Golay *et al.* 2013].

Studies on pharmacokinetics (PK) show that RTX infused intravenously has a terminal half-life of about 120 hours and can persist in the body for up to 6–9 months after treatment stops [Boye *et al.* 2003]. A weak diffusion in the CNS has been observed because RTX may not traverse the blood–brain barrier. After intravenous (IV) administration, maximal RTX levels in cerebrospinal

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fluid (CSF) are generally less than 1% of serum levels [Harjunpaa *et al.* 2001; Lampson, 2011]. RTX depletes B cells from the circulation 1 month after administration. Depth of B-cell depletion is variable among patients but restoration of the B-cell repertoire generally takes 9–12 months from the last perfusion of RTX [Dass *et al.* 2008]. In the setting of a disrupted blood–brain barrier, depletion occurs not only in the periphery but also in the perivascular area in the brain parenchyma [Batchelor *et al.* 2011].

Efficacy of rituximab in adults with neuromyelitis optica

Open-labeled studies

To date, no randomized controlled trials have been performed to explore the effect of RTX in NMO. Available studies are open labeled and have provided consistent data in favor of a positive effect of RTX in NMO [Cree *et al.* 2005; Jacob *et al.* 2008; Bedi *et al.* 2011; Kim *et al.* 2011, 2013a, 2015; Pellkofer *et al.* 2011; Lindsey *et al.* 2012; Ip *et al.* 2013; Yang *et al.* 2013; Collongues *et al.* 2015; Radaelli *et al.* 2015; Zephir *et al.* 2015]. The main results of studies, all including at least five NMO patients, are summarized in Table 1. Except for two of the studies [Cree *et al.* 2005; Jacob *et al.* 2008], all patients meet the 2006 NMO criteria [Wingerchuk *et al.* 2006]. The studies show a strong reduction in the annualized relapse rate (ARR) in a wide range of follow up, from 12 to 60 months. In four studies, the mean ARR was null, and patients were free from relapse in a mean 60% of cases. Disability, evaluated by the Expanded Disability Status Scale (EDSS), was improved in most of the studies, except for two. In the study by Lindsey and colleagues, two patients experienced a major impairment from EDSS, from 3.5 to 8.3 and 0 to 8.0, respectively, that raises the question of the time period between relapse and EDSS evaluation, because these data are not provided in the study. [Lindsey *et al.* 2012]. For example, in the study by Bedi and colleagues, EDSS data were used only when the assessments were made at least 1 month before, or after, an exacerbation [Bedi *et al.* 2011]. Therefore, these data do not support classifying the disability as residual. In the study by Pellkofer and colleagues, one patient died due to cardiovascular failure that could impact the overall results in this cohort of 10 patients [Pellkofer *et al.* 2011].

In addition, timing of relapse after RTX treatment needs to be considered, as for Lindsey and colleagues, three patients had relapse within the first month after RTX [Lindsey et al. 2012], and for Pellkofer and colleagues, one patient died during the first month after RTX induction [Pellkofer *et al.* 2011].

Another caveat is that most of these studies have included patients who received interferon (IFN) before RTX that could artificially worsen the course of NMO before RTX and therefore inflate the efficacy of RTX. The absence of precision concerning the time between relapse and EDSS pre-RTX could also drive the same conclusion.

Rituximab in the area of predictive factors of disability

A retrospective study has defined the effect of IS treatment in NMO and NMOSD, that is, longitudinally extensive transverse myelitis or optic neuritis with AQP4 antibodies, on ARR [Mealy *et al.* 2014]. Modalities of prescription were made with respect of at least 6 months of treatment for azathioprine (AZA; $n = 32$) or mycophenolate mofetil (MMF; $n = 28$), and 1 month for RTX ($n = 30$). After a mean follow up of 2 years, RTX reduced the ARR of 88.2% and a complete remission was observed in 66%. The MMF reduced the ARR of 87.4% and AZA of 72.1%. The comparative analysis of the efficacy related to RTX; MMF and AZA show a similar efficacy to MMF and RTX but a lower efficacy of AZA alone. It was noted that refractory patients could be responders to RTX, despite a nonresponse of MMF or AZA prescribed as a first-line therapy.

Another study has found that the time to next attack in 58 patients with NMO or NMOSD was independently increased by 1.31 times (95% confidence interval (CI) 1.02–1.67, $p = 0.035$) with each additional cumulative attack experienced, by 5.34 times (95% CI 1.57–18.13, $p = 0.007$) with combined AZA treatment and continued oral prednisolone, and by 4.26 times (95% CI 1.09–16.61, $p = 0.037$) with RTX treatment [Kim *et al.* 2013b. Interestingly, the multivariate analysis did not find any association with AZA alone, mitoxantrone, MMF, IFNβ, cyclophosphamide or methotrexate.

Concern on the use of rituximab in neuromyelitis optica

An important point is that subsequent studies reported patients who experienced a severe relapse

Table 1. Main open-label studies on rituximab in neuromyelitis optica.

	Number of Patients	Patients with anti-aquaporin-4 antibodies	Patients naive of any treatment before RTX[1]	Mean or median time of follow up	Mean or median ARR pre-RTX	Mean or median ARR post-RTX	Patients relapse free (%)	Median EDSS score pre-RTX	Median EDSS score post-RTX	Add-on therapy
Cree *et al.* [2005]	8	NA	4	12 (m)	2.6 (med.)	0	6 (75)	7.5	5.5	No
Jacob *et al.* [2008]	25	14	7	19 (med.)	1.7 (med.)	0	13 (52)	7	5	Yes
Pellkofer *et al.* [2011]	10	10	2	29 (m)	1.7 (m)	0.93 (m)	4 (40)	6.5	7	Yes
Bedi *et al.* [2011]	23	15	3	32.5 (med.)	1.87 (med.)	0	17 (74)	7	5.5	No
Kim *et al.* [2011]	30	21	20	24 (m)	2.4 (m)	0.3 (m)	21 (70)	4	3	No
Lindsey *et al.* [2012]	9	6	0	16.5 (med.)	2 (med.)	2 (med.)	3 (33)	3.5	4.5	Yes
Ip *et al.* [2013]	7	4	5	24 (med.)	1.38 (med.)	0.69 (med.)	5 (71)	8	7	No
Yang *et al.* [2013]	5	4	0	12 (m)	1.16 (m)	0	5 (100)	4.5	4	No
Kim *et al.* [2013]	30	23	20	60 (med.)	2.4 (m)	0.3 (m)	18 (60)	4	3	No
Kim *et al.* [2015]	100[2]	94	NA	67 (med.)	2.4 (m)	0.1 (m)	70 (70)	4	3	Yes[3]
Zephir *et al.* [2015]	32[2]	28	32	28.7 (m)	3.8 (m)	0.1 (m)	25 (78)	6	3.5	No
Radaelli *et al.* [2015]	21[2]	17	4	48 (m)	2	0.16	12 (57)	5.5	4	No
Collongues *et al.* [2015]	21	19	0	31 (m)	1.3 (m)	0.4 (m)	11 (52)	5	3	Yes[4]

RTX, rituximab; ARR, annualized relapse rate; EDSS, Expanded Disability Status Scale; NA, not available; m, mean; med., median.
[1]Naïve patients include patients without any treatments or patients treated with immunomodulators (interferon β, glatiramer acetate or immunoglobulin) before RTX.
[2]It included definite 2006 neuromyelitis optica (NMO) patients but also limited forms of NMO with anti-aquarporin-4 antibodies.
[3]Three patients received mitoxantrone and one received cyclosporine and corticosteroids during RTX therapy.
[4]Two patients received corticosteroids or mycophenolate mofetil during RTX therapy.

3 months after the last RTX infusion [Capobianco *et al.* 2007; Nasir *et al.* 2009], or posterior reversible encephalopathy syndrome 24 hours after the first infusion [Sanchez-Carteyron *et al.* 2010; Berger *et al.* 2014]. These observations lead to the hypothesis that B cells could have an anti-inflammatory effect whereas the relapses were T-cell mediated. Another possibility is that RTX leads to anti-AQP4 release, and transiently enhances the pool of these pathogenic antibodies [Nakashima *et al.* 2011]. These data are offset by those of another study that showed a decrease in anti-AQP4 antibodies in three out of four patients treated with RTX; the fourth patient experienced a relapse 27 and 99 days after RTX and elevated anti-AQP4 antibodies [Jarius *et al.* 2008].

Monitoring

In the literature, a single-induction protocol is insufficient to suppress disease activity as shown by the high number of patients who experience relapses early after the first course of RTX. For example, in the Bedi study, among four patients who had induction with 4-weekly doses of RTX, two patients relapsed just short of their planned retreatment at 12 months. These two patients who relapsed short of 1 year have not been retreated at 6 months [Bedi *et al.* 2011]. In contrast, administration of RTX doses biweekly every 6 months has resulted in an impressive absence of relapses, and disease stability.

Optimizing maintenance therapy with rituximab

There is an absence of a standardized RTX protocol in NMO. In clinical practice, its use is driven by the experience acquired in each center by each physician. There is a general agreement that the induction phase should be based on the infusion of about 2 g during 1 month, consisting of either 1 g, 2 weeks apart or 375 mg/m^2 every week for 4 consecutive weeks. The maintenance regimen is a matter of debate, as it is not mentioned in most of the studies. Protocols differ from one another: reinfusion of RTX (375 mg/m^2) could be used when the CD27+ memory B-cell frequency was at least 0.05% in peripheral blood mononuclear cells [Kim *et al.* 2011, 2013b], or 2 g RTX divided into two biweekly infusions every 6–9 months or when the CD19 population was greater than 0.1% [Pellkofer *et al.* 2011; Mealy *et al.* 2014], or every 6–9 months based on clinical status and the patient's preference [Ip *et al.* 2013], or a 100 mg infusion once a week for 3 consecutive weeks depending on circulating B-cell repopulation [Yang *et al.* 2013]. In this last study, including 30 patients with MS or NMO, the mean number of days after a 100 mg dose of RTX until the CD19 population was greater than 2% was 99 ± 36 days (range 43–172), compared with 184 ± 72 days (range 106–288) after a 1000 mg dose of RTX. One study shows that the effect of altered body composition on drug disposition and therapeutic outcome could be associated with an increase in body mass index [Collongues *et al.* 2015]. These data are consistent with a previous study that showed that low doses of RTX were associated with a high rate of early B-cell repopulation [Greenberg *et al.* 2012]. As suggested by Kim and coworkers, repopulation of CD19+ B cells could not be a determining factor to ensure RTX efficacy [Kim *et al.* 2013a]. Nevertheless, CD19+/CD27+ memory B cells could be of interest in monitoring RTX pharmacodynamics. This approach is only used in Korea and further studies are needed to confirm these findings [Kim *et al.* 2011, 2013a] whereas in a large study including 100 NMOSD patients, 11 relapses in nine patients occurred during periods where memory B-cells were below the therapeutic target [Kim *et al.* 2015]. The same team has showed that the FcγR3A-158F allele, coding for FcγR present on immune cells, was associated with a risk of insufficient memory B-cell depletion and a short retreatment interval during the initial 2 years [Kim *et al.* 2015].

Interestingly, some studies have reported that nonresponse to RTX in patients with rheumatoid arthritis was correlated with higher circulating preplasma cell numbers at baseline and incomplete B-cell depletion [Dass *et al.* 2008; Vital *et al.* 2010]. At last, a recent study has shown that CD19+/CD24high/CD38high B cells' (regulatory B cells) quantities and functions were impaired during relapses in NMO [Quan *et al.* 2015]. In these patients, RTX led to the repopulation of B cells, which was characterized by the predominance of regulatory B cells. Therefore, RTX restored the numerical balance between regulatory and memory B cells in favor of regulatory B cells. This mechanism could be a way to research and closely monitor the efficacy of RTX in NMO.

Expert opinion

At this level of our knowledge and according to the pharmacodynamics of RTX, we could advise

to start with an induction phase consisting either of 1 g, 2 weeks apart, or 375 mg/m^2 every week for 4 consecutive weeks, which could have a rapid and profound effect on B-cell depletion.

Despite the absence of consensus, it seems reasonable to perform a count of CD19+ B cells every 3 months and to reinfuse the patients as soon as CD19+ B cells become detectable. The advantage of this approach is related to its feasibility in centers, contrary to the threshold of CD19+/CD27+ memory B cells corresponding to 0.05% of peripheral blood mononuclear cells (PBMCs), that requires being able to detect very few cells in the serum and needs technique standardization of flow cytometry. Furthermore, the count of CD19+ B cells includes the CD19+/CD27+ ones. The posology of the infusion for the maintenance therapy is a matter of debate. We propose to consider the posology of 1 g as a dose effect that has been suggested in many studies and seems to be a good compromise in preventing underdosing therapy. In the near future, new biomarkers like the FcγR3A-158F allele could impact the therapeutic strategy with RTX.

Safety

The tolerability of RTX is well established in several autoimmune diseases, especially rheumatoid arthritis. The main side effects are reaction to infusion, opportunistic and nonopportunistic infection. Infusion reactions are very common but can usually be managed by pretreatment with IV steroids, antihistamine and slow titration of RTX. A large number of infections has been reported, mostly herpetic rashes and tuberculosis, but also progressive multifocal leukoencephalopathy (PML). The risk of PML in rheumatoid arthritis is calculated to be 1/25,000 [Clifford *et al.* 2011]. To date, the only case of NMOSD reported was on AZA [Flanagan and Weinshenker, 2014]. Data concerning tolerability of RTX specifically in NMOSD are scarce. Overall, the adverse events profile of RTX in NMO appears to be consistent with the known safety profile of the drug. Only two studies recorded fatal outcomes in RTX-treated NMO patients: one patient died from septicemia [Jacob *et al.* 2008], and another to presumed cardiovascular failure that occurred 3 days after an RTX infusion [Pellkofer *et al.* 2011]. However, it is difficult to attribute this last side effect to RTX.

Efficacy and safety in children with neuromyelitis optica

There are few data concerning only NMO and RTX. However, several other diseases are frequently treated by RTX in children, especially juvenile arthritis and nephrotic syndrome [Basu *et al.* 2015; Sakamoto *et al.* 2015]. Tolerance is good in these different populations. Although there are different dose regimens, the recommended dose in children is 375 mg/m^2 weekly for 4 weeks, with additional infusions depending on the CD 19+B-cell count to maintain immunosuppression.

In the few studies focused on NMOSD, children treated with RTX demonstrated significant reductions in the relapse rate, with 60–70% of patients remaining relapse free, and stabilization or improvement of disability [Mahmood *et al.* 2011; Kimbrough *et al.* 2012; Kavcic *et al.* 2013; Longoni *et al.* 2014]. The main question remains what to use for maintenance therapy, as for adults. There is no clear identified strategy on NMO, but in nephrotic syndrome, Basu and colleagues recently recommended a treatment with MMF following induction with RTX. Such strategies could be proposed in NMOSD [Basu *et al.* 2015].

Questions unresolved and futures directions of research

Gender effect

Several studies on lymphoma have shown a gender-dependent difference in RTX PK [Jager *et al.* 2012; Muller *et al.* 2012]. It is characterized in females by a higher minimal concentration and area under the curve than in males, both in the induction and maintenance phases. Modification of PK was also followed by a better quality of response. Interestingly, these effects were observed only in premenopausal and not postmenopausal women and occur independently of weight [Gisselbrecht *et al.* 2012]. Further studies are needed to confirm these data on PK and efficacy.

Subcutaneous route

IV administration is related to a prolonged infusion time and a reduced autonomy for patients. A more convenient administration would be the oral route, but is limited by the degradation of RTX in the gastrointestinal tract and its inefficient diffusion through the intestinal epithelium.

The subcutaneous (SC) administration fulfills criteria to improve acceptability in patients compared with the IV route, including a shorter infusion time (~5 min *versus* 150 min or more) and the possibility to treat at home, facilitating a better autonomy for patients. The SC doses for RTX are fixed doses ranging from 1400 to 1600 mg, to compensate for the portion lost during the absorption phase (~40%). This formulation has been tested in patients with follicular lymphoma and chronic lymphocytic leukemia with comparable PK and tolerability with the IV route [Davies *et al.* 2014; Salar *et al.* 2014; Assouline *et al.* 2015].

New monoclonal antibodies targeting B cells

New generations of anti-CD20 antibodies that have enhanced immune-mediated activities are now under development in clinical trials for hematologic neoplasm or relapsing–remitting MS. Ocrelizumab is a humanized mAb, which binds to the large loop of the CD20 molecule. The epitope is overlapping with the binding site of RTX and depletes B cells by ADCC, whereas RTX acts more in a CDC manner, which is due to differences in the Fc portion of the antibodies. Positive results in a phase II trial in MS [Kappos *et al.* 2011] have allowed procedure of two phases III studies that are in progress [ClinicalTrials.gov identifiers: NCT01247324 and NCT01194570]. The fully human anti-CD20 antibody is called ofatumumab. It binds to an epitope distinct from that of ocrelizumab or RTX, located in both the small and large loops of the CD20 molecule. Ofatumumab increased complement activation potential, particularly in the presence of low CD20 expression levels [Teeling *et al.* 2006]. The cytotoxicity *in vitro* is superior to RTX, since ofatumumab was able to deplete RTX-resistant B-cell lines [Wierda *et al.* 2011; Bologna *et al.* 2013; Barth *et al.* 2015]. Two phase II studies are ongoing to establish the relation between the dose and efficacy after IV or SC administration in the field of MS [ClinicalTrials.gov identifiers: NCT00640328 and NCT01457924]. At last, obinutuzumab (GA101), a humanized and glyco-engineered mAb, shows increased binding to FcγR3A, enhanced NK-mediated ADCC and increased direct cell-death induction [Mossner *et al.* 2010]. This drug is tested in a phase II study in the maintenance treatment of patients with a central nervous system lymphoma [ClinicalTrials.gov identifier: NCT02498951].

Another innovative drug called MEDI-551 is a humanized mAb that binds to the B-cell-specific antigen CD19. Contrary to RTX, it results in depletion from pre-B cells to plasmablasts, these last being responsible for the production of autoimmune antibodies involved in NMOSD. Furthermore, the affinity-optimization and α-fucosylation of CD19 enhanced the ADCC resulting in a lower effective dose than RTX [Ward *et al.* 2011]. This product is in entered in a phase IIb in NMOSD and phase I in the relapsing form of MS [ClinicalTrials.gov identifiers: NCT02200770 and NCT01585766].

Conclusion

This review underlines the efficacy and tolerance of RTX in NMOSD. This treatment is widely recognized as the best second-line therapy in this rare disease despite lack of class A evidence from therapeutic trials. Due to the severity of the disease, placebo-controlled trials appear unethical. However, several questions remain open, including the use of this treatment as a first-line therapy, especially after the first relapse in patients with AQP4-positive antibodies. We have also to better understand the maintenance therapy program and the surveillance dosage in order to detect and prevent a possible new relapse. Finally, new anti-CD20 drugs such as ocrelizumab or ofatumumab should be tested, as fewer side effects, especially infusion reactions, have been described with these humanized monoclonal antibodies.

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RITUXIMAB IN REFRACTORY AND NON-REFRACTORY MYASTHENIA: A RETROSPECTIVE MULTICENTER STUDY

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ABSTRACT: *Introduction:* Few data are available about the effect of rituximab (RTX) on refractory (RM) and non-refractory (NRM) myasthenia. *Methods:* This retrospective multicenter study involved 13 RM and 7 NRM patients treated with sequential RTX infusions over 2 years, on average. RTX was used as a substitute for corticosteroids in NRM patients. Disability was assessed using the annualized relapse rate (ARR) and Myasthenia Gravis Foundation of America (MGFA) scores. *Results:* RTX induction decreased the ARR from 2.1 to 0.3 ($P < 0.001$), and lowered MGFA scores from 5–3b to 4b–0 in RM patients, and from 1.9 to 0.1 ($P < 0.001$) and 4b–2b to 3b–0 in NRM patients. No side effects were reported in either group, except for 1 case of spondylodiscitis 1 year after the last RTX infusion. Within a year after RTX induction, complete corticosteroid withdrawal was obtained in 7 RM and 4 NRM patients. *Conclusions:* RTX is efficacious and well-tolerated. Its use allows for dose reduction or withdrawal of corticosteroids.

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Acquired myasthenia gravis (MG) is an autoimmune antibody-mediated disorder of the neuromuscular junction, which results in a cholinergic transmission defect. Patients are treated in a symptomatic manner with oral cholinesterase inhibitors, which increase the amount of acetylcholine in the neuromuscular junction. Despite the absence of randomized, controlled clinical trials, thymectomy is often performed early in the disease course and may result in a positive effect.[1] Patients with mild generalized myasthenia and oropharyngeal/respiratory weakness receive oral corticosteroids as a first-line treatment, and second-line therapy may include sequential immunoglobulin infusions or plasma exchanges, or 1 of several immunosuppressive drugs such as azathioprine, cyclophosphamide, mycophenolate mofetil, cyclosporine, or tacrolimus.[2]

Despite the lack of a conventional definition for refractory myasthenia (RM), when patients with MG fail to respond to thymectomy and at least 1 or 2 successive immunosuppressive drugs, with or without associated oral corticosteroids, they are usually considered to have RM. This situation urgently calls for a new line of treatment.[3] Recent data suggest that rituximab (RTX), an anti-CD20 therapy that specifically targets B lymphocytes, has a beneficial effect in RM. RTX is currently used in cases of rheumatoid arthritis resistant to first-line therapy, including antitumor necrosis factor, and in the treatment of lymphoproliferative disorders, but it may also be prescribed "off-label" in many other autoimmune diseases, such as anti-neurophil cytoplasmic antibody-associated vasculitis, systemic lupus erythematosus, immune thrombocytopenic purpura, and pemphigus vulgaris.[4] Because these diseases are rare, except for rheumatoid arthritis, patients have been mostly evaluated through retrospective studies.

Of some 70 reported cases of MG treated with RTX, most have had medical benefit. Eight patients have been described whose condition did not improve, and the disease actually worsened in 1 patient.[5–8] However, the retrospective design, low population numbers, and short follow-up period have been limitations in all those studies. Furthermore, no specific data were made available about non-refractory (NRM) patients—that is, patients who improved after 1 or 2 successive immunosuppressive drugs or thymectomy. We report a large, national, multicenter experiment in treating myasthenia with RTX. Two populations were considered: 1 with RM and the other with NRM. In the latter, RTX was used as a non-conventional treatment for milder disease in an attempt to reduce or

Abbreviations: AChR, acetylcholine receptor; ARR, annualized relapse rate; IVIg, intravenous immunoglobulin; LEMS, Lambert–Eaton myasthenia syndrome; MG, myasthenia gravis; MGFA, Myasthenia Gravis Foundation of America; MuSK, muscle-specific receptor tyrosine kinase; NRM, non-refractory myasthenia; PE, plasma exchange; RM, refractory myasthenia; RTX, rituximab

Key words: myasthenia, neuroimmunology, non-refractory form, refractory form, rituximab

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withdraw corticosteroids and prevent their side effects.

METHODS

We performed this observational study in France, which included 4 tertiary-care medical centers. The patients were included retrospectively, and they were followed in centers that specialize in the treatment of MG. The data were collected from April 2010 to September 2011, when the clinical trial was terminated.

The patients were considered to have fulfilled the diagnostic criteria for MG on the basis of clinical history, neurological examination, and electrophysiological evidence of a neuromuscular transmission defect. Patients with Lambert–Eaton myasthenic syndrome (LEMS) were excluded. Prior immunosuppressive treatments included prednisone as the first-line drug and, when the response was not adequate or the requisite doses were too high, second-line immunosuppressors were introduced. Each immunosuppressive agent had been given for a minimum period of 6 months and, when MG had worsened, intravenous infusions of immunoglobulin (IVIg) or plasma exchange (PE) were used. The patients who had failed to respond to thymectomy (performed prior to their enrollment in the study) and at least 2 immunosuppressive drugs, including corticosteroids, were considered to have RM. The other patients were considered to have NRM. Before the first infusion of RTX, immunosuppressive treatments were stopped, except for corticosteroids, which were tapered progressively. Rituximab was given according to 2 different protocols: (i) patients 1–8, 10, 11, 13, 16, 17, and 20 received 375 mg/m^2 weekly for 4 consecutive weeks (induction stage), and subsequently 375 mg/m^2 every 3 months; and (ii) patients 9, 12, 14, 15, 18, and 19 received 2 infusions of 1 g each, 2 weeks apart (induction stage), and subsequently 1 g, as required if symptoms worsened. The treatment was followed up for 2 years, on average. During that period, if a relapse occurred, patients were treated promptly with IVIg or PE.

All data were obtained from hospital files and from a clinical information questionnaire specifically designed for this study, which included demographic data, clinical course, and biological results [acetylcholine receptor (AChR) and muscle-specific receptor tyrosine kinase (MuSK) antibodies]. The CD19$^+$ lymphocyte percentage in the total lymphocyte population was calculated using quantitative flow cytometry. Disability was assessed on the basis of 2 criteria: (1) the number of attacks, corresponding to disease aggravation requiring hospitalization and treatment with IVIg or PE; and (2) the Myasthenia Gravis Foundation of America (MGFA) clinical score.[9] Data confidentiality and security were enforced as per the recommendations of the French data protection authority, which also provided its approval. This study was approved by the appropriate ethics committees and was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki. All participants gave informed consent prior to enrollment in the study. Details have been omitted as necessary from the following case descriptions to ensure anonymity.

A Mann–Whitney *U*-test was used to compare the quantitative data. Two-sided $P < 0.05$ was considered statistically significant. All computations were performed using SPSS for Windows (version 14.0).

RESULTS

Characteristics of the Cohort. Individual characteristics of the 13 RM and 7 NRM patients enrolled are listed in Table 1.

The mean age at onset was 43.6 and 58.3 years for RM and NRM patients, respectively. Seventeen of 20 patients (85%) tested positive for antibodies against AChR or MuSK. Patient 1 had antibodies against AChR (32 nmol/L) and MuSK (0.2 nmol/L). All patients (except 2 in the NRM group) had undergone thymectomy, on average, 14.6 months after disease onset in the RM group, and 13 months after disease onset in the NRM group. Before institution of RTX, on average, 3.2 and 1.4 different long-term therapies had been prescribed in the RM and NRM groups, respectively. Despite such therapeutic management, the disability level remained high in the RM patients, whose MGFA score ranged from 3b to 5. In the NRM group, that score ranged from 2b to 4b.

Follow-Up during Treatment with Rituximab. The mean follow-up period was 26 ± 13 months for RM patients and 25 ± 13 months for NRM patients. In comparing a 2-year interval before and after institution of RTX, we found that, in RM patients, the annualized relapse rate (ARR) decreased from 2.1 ± 0.3 to 0.3 ± 0.1 ($P < 0.001$). In the NRM group, the ARR decreased from 1.9 ± 0.3 to 0.1 ± 0.1 ($P < 0.001$). Such decreases were associated with improved MGFA scores, as indicated in Table 2.

Among the 10 patients who were followed up for >2 years, only 1 (patient 1) had a relapse, which occurred 28 months after RTX induction. No side effects were reported in the RM and NRM groups except for 1 patient (patient 10), who was admitted to an intensive care unit because of spondylodiscitis 1 year after the last RTX infusion. For most patients, prednisone withdrawal was gradually

Table 1. Characteristics of patients with a refractory or non-refractory form of myasthenia.

Patient no.	Gender	Age at onset (years)	Antibodies	Time to thymectomy (months), histology	Previous treatments	Time to RTX (months)	
						From disease onset	From IS* withdrawal
Refractory form							
1	F	50	AChR+MuSK	12, hyperplasia	C, CYC, MMF	150	1
2	F	74	AChR	12, hyperplasia	C, AZA	38	1
3	M	49	AChR	12, hyperplasia	C, CYC, AZA, MMF	75	1
4	M	16	MuSK	24, hyperplasia	C, AZA, CYC, MMF	87	1
5	F	39	—	12, hyperplasia	C, AZA, CYC, MMF	51	1
6	F	50	MuSK	24, hyperplasia	C, AZA, CYC, MMF	39	1
7	M	69	AChR	12, hyperplasia	C, AZA	89	1
8	F	26	AChR	1, hyperplasia	C, AZA, MMF	155	35
9	M	65	AChR	12, thymoma	C, MMF	39	3
10	F	30	AChR	14, hyperplasia	C, AZA, CYC, MMF	302	4
11	M	57	AChR	21, normal	C, AZA	33	1
12	F	13	—	12, normal	C, MMF	531	19
13	F	29	MuSK	22, normal	C, AZA, CYC, CS, MMF	217	3
Non-refractory form							
14	M	65	MuSK	12, hyperplasia	C	18	—
15	M	74	AChR	2, normal	C	6	—
16	F	46	AChR	1, thymoma	C	8	—
17	M	81	AChR	0, hyperplasia	C	27	—
18	F	39	AChR	—	C, AZA	22	11
19	M	55	AChR	—	C, MMF	20	4
20	F	48	—	51, normal	C, MMF	70	7

F, female; M, male; AChR, acetylcholine receptor; MuSK, muscle-specific receptor thyrosine kinase; C, corticosteroids; AZA, azathioprine; MMF, mycophenolate mofetil; CS, cyclosporine; CYC, cyclophosphamide, RTX, rituximab; IS, immunosuppressants
**In this column, IS do not include corticosteroids, IV immunoglobulin, or plasma exchange.*

initiated within the first month after institution of RTX, except for patients 11 (6 months), 13 (3 months), 19 (3 months), and 20 (3 months). Six months after institution of RTX, corticosteroids were withdrawn in 6 RM and 2 NRM patients. After 1 year, the mean dose of prednisone was decreased from 38.5 ± 6.6 mg/day to 8.7 ± 3.7 mg/day ($P = 0.002$) in RM patients and from 42.8 ± 8.4 mg/day to 6.4 ± 3.5 mg/day ($P = 0.003$) in NRM patients, with complete corticosteroid withdrawal in 7 RM and 4 NRM patients. After 18 months, corticosteroids were terminated in all NRM patients, whereas RM patients still received a mean dose of 6 mg/day (Figure 1).

Despite the difference in therapeutic strategy, no $CD19^+$ cells were detectable in blood analyses of each patient during follow-up.

DISCUSSION

Our study demonstrates the strong efficacy of RTX in MG on the basis of the decreased ARR and MGFA disability scores. These results were obtained in a large patient sample, which included RM and NRM patients, who may be representative of the patient populations usually referred to tertiary-care medical centers. Except for patient 11, who had particularly severe disease, the use of RTX allowed rapid withdrawal of corticosteroids.

These findings argue for wider use of RTX as a treatment for MG by demonstrating efficacy within the first year of treatment. No such effect has been reported with other immunosuppressants.[2] In NRM patients, the rapid dose decrease and often complete corticosteroid withdrawal argue for early use of RTX, despite the previous use of steroid-sparing agents in 4 patients.

The second point is tolerability. In the 14 patients we followed for at least 2 years, no side effects were detected, apart from spondylodiscitis in 1 patient. However, the implication of RTX in this complication is not clear because of the long time period between the last RTX infusion and the appearance of infection. In the literature, a major concern is the risk of progressive, multifocal leukoencephalopathy, which has been reported in 56 patients treated with RTX for lymphoproliferative disorders and 5 for other autoimmune diseases.[10–12] Although those patients were treated with RTX in combination with other immunosuppressants, this issue should not be ignored, because RM patients are also likely to have been heavily pretreated with other immunosuppressants.

To date, only a few studies have been published on the use of RTX in MG, but all reported good efficacy in RM patients. Three studies included >10 patients; 1 study involved 10 patients with MG and 2 with LEMS, followed for over 18 months,

Table 2. Course of myasthenia before and during treatment with rituximab (RTX) as a function of the number of attacks and the Myasthenia Gravis Foundation of America (MGFA) disability score.

		Number of attacks				MGFA scores		
		Before RTX		With RTX		Before RTX	With RTX	
Patient no.	Follow-up (months)	2-1 y /ICU	1-0 y /ICU	0-1 y /ICU	1-2 y /ICU	At the onset of RTX	Over 2 years	At end of follow-up
Refractory form								
1	43	1/1	2/2	0/0	1/0	4b	2b	2b
2	24	0/0	2/0	0/0	0/0	4b	3a	3a
3	34	0/0	3/2	0/0	0/0	3b	2b	2b
4	48	1/0	3/1	0/0	0/0	4b	2a	2a
5	48	0/0	3/1	0/0	0/0	3b	2b	2b
6	48	0/0	3/2	0/0	0/0	3b	0	0
7	24	2/0	1/0	0/0	0/0	3b	2a	2a
8	12	1/0	1/0	0/0	NA	3b	NA	2a
9	12	5/0	4/0	0/0	NA	4b	NA	2a
10	32	3/0	5/1	1/1	1/1	4b	2b	2b
11	24	5/0	2/1	2/0	1/0	5	4b	4b
12	12	0/0	3/0	0/0	NA	4b	NA	3a
13	35	0/0	4/0	1/0	1/0	3b	2a	2a
Non-refractory form								
14	24	0/0	2/0	0/0	0/0	3b	2a	2a
15	12	NA	2/1	0/0	NA	3b	NA	2b
16	25	NA	2/1	0/0	1/0	4b	3b	3b
17	48	0/0	3/0	0/0	0/0	3b	2b	2b
18	29	3/0	1/0	0/0	0/0	2b	1	1
19	12	1/1	3/0	0/0	NA	4b	NA	3a
20	12	3/0	3/0	0/0	NA	2b	NA	0

and the 2 others involved 14 patients with MG who were followed for 14 and 17 months, on average.[6,8,13] The results reported in those studies corroborate our own data, showing decreased disability in 58–92% of patients, without any major side effects. However, most patients in the published studies were considered RM patients, despite the lack of a consensus definition of this condition. As such, they may not have undergone sustained immunosuppressive therapy, or thymectomy.[6–8,13–15]

One may argue that, because our study was retrospective, a notable limitation arises from the different RTX protocols used. This can be explained by the absence of consensus in the RTX dosing schedule in MG. Yet, despite this limitation, evidence for a pharmacological effect of RTX in these protocols is shown by the sustained depletion of $CD19^+$ blood cells during follow-up. Patients were treated again with RTX after the first month of induction. Because MG is a chronic condition, re-treating patients with RTX appears to be logical when necessary.

In conclusion, RTX is efficacious and well-tolerated. It use permits reduction or withdrawal of corticosteroids and should be considered more widely for the treatment of MG. It is now clear that the time has come to evaluate RTX in MG in a randomized, controlled trial. It is worth noting, however, that, because of previous failures of

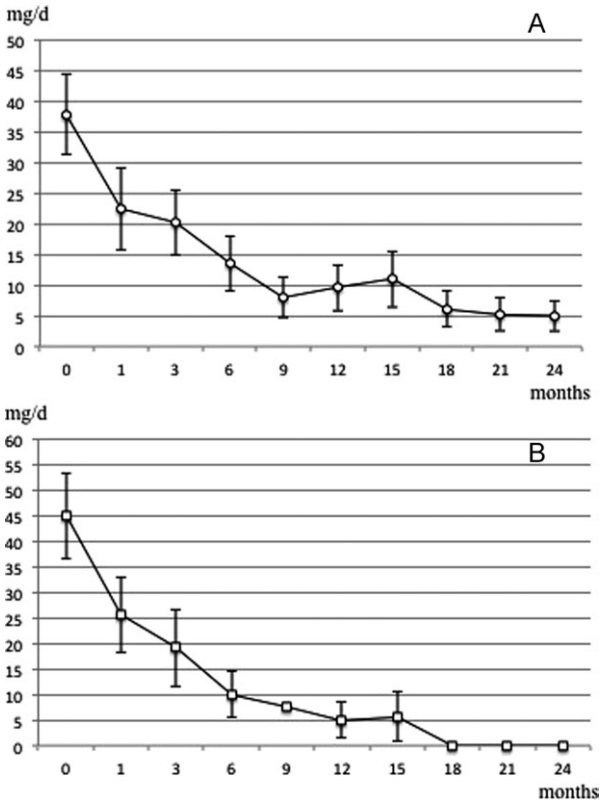

FIGURE 1. Oral corticosteroid withdrawal in RM (**A**) and NRM (**B**) MG patients during a 2-year follow-up after the first infusion of rituximab.

several immunosuppressive treatments in RM patients, the choice of control therapy will be challenging. In RM patients, as they were defined in our study, a point of interest could be to test RTX against sequential immunotherapy such as IVIg or PE. Our study also argues for the primary use of RTX in NRM patients, which may constitute the first step to determine the optimal dose and efficacy range of this drug. At this point, our results should help investigators to design future therapeutic trials to assess the potential of RTX as an alternative treatment for MG.

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IV.3. COMMENTAIRES

IV.3.1. Efficacité du mycophénolate mofetil en première intention dans la neuromyélite optique

Nous avons rapporté les résultats d'efficacité thérapeutique dans une des plus larges cohortes de patients NMO traités par MMF.

En dehors de l'efficacité du MMF évalué selon les critères cliniques habituels, l'action conjointe de la corticothérapie est un point important du manuscrit. En effet, comme dans la myasthénie, les corticoïdes ont un effet majeur dont l'utilisation est limitée par la survenue d'effets secondaires. Ce papier montre que les patients sous MMF qui ont bénéficié d'une corticothérapie orale pendant les trois premiers mois de sa prescription ont un risque moindre de faire des poussées sous MMF. Plusieurs arguments convergent vers l'idée que les corticoïdes permettraient de combler un manque d'efficacité du MMF dans les trois à six premiers mois, ce qui correspond au délai nécessaire pour observer son action pharmacodynamique sur les lymphocytes. Par ailleurs, plusieurs autres équipes appliquent déjà cette stratégie thérapeutique mais sans avoir publié sur la question.

IV.3.2. Efficacité du rituximab dans les cas réfractaires de neuromyélite optique et de myasthénie

Nous avons reporté l'efficacité du RTX dans deux pathologies neurologiques médiées par des anticorps que sont la NMO et la myasthénie.

Nous avons analysé l'évolution d'une vingtaine de patients dans chaque étude sur une durée supérieure à 2 ans et retrouvé une efficacité majeure du RTX sur

plusieurs déterminants cliniques reconnus pour évaluer l'activité de la maladie que sont TAP et le handicap résiduel. Dans chaque étude nous avons analysé une population de patients très active, ce qui fait du RTX un produit particulièrement intéressant en cas d'échec des thérapeutiques de première ligne. Notre étude sur le RTX s'ajoute à d'autres études rétrospectives que nous avons recensées dans une revue de la littérature et qui retrouvent le même type d'effet thérapeutique. A côté de ces résultats, la tolérance au long cours du RTX est apparue excellente et les résultats d'efficacité dans la population de patients non réfractaires de myasthénie ainsi que l'étude du RTX en première ligne dans la NMO publiée sous l'égide du groupe "NOMADMUS" en font également un traitement intéressant en première intention.[31]

IV.4. LIMITES DES ETUDES

Ces études souffrent des mêmes limitations que pour la cohorte initiale publiée en 2010, liées à leur caractère rétrospectif et la difficulté de produire des analyses en sous-groupes compte tenu de la rareté de la pathologie. De fait, ces études se sont faites dans le cadre d'une collaboration nationale afin d'obtenir un nombre de patients convenables pour effectuer des analyses statistiques. Une autre difficulté a été la multiplicité des schémas thérapeutiques parmi les centres compte tenu de l'absence de standardisation des pratiques autour du RTX notamment.

IV.5. VERS UNE MODIFICATION DE LA STRATEGIE THERAPEUTIQUE

L'ensemble de ces travaux a contribué à mieux prescrire le MMF et le RTX dans le contexte des maladies neurologiques à anticorps comme la NMO ou la myasthénie. Ces deux pathologies partagent un mécanisme d'action impliquant au premier plan le LB, conférant ainsi au RTX une place majeure dans l'arsenal thérapeutique. Concernant le RTX, nous avons montré qu'il permettait de traiter les cas les plus réfractaires de NMO et de myasthénie.

Fort de ces travaux, nous avons pu proposer des recommandations nationales sur l'utilisation du RTX, sur le modèle de ce qui existe déjà en rhumatologie pour les biothérapies et le RTX en particulier. Ces recommandations permettent entre autres d'optimiser la posologie de RTX à utiliser et de mieux encadrer le risque infectieux lié à son utilisation. Ces recommandations se basent sur la médecine fondée sur les preuves pour guider l'attitude du neurologue face à de nombreuses situations pratiques comme la survenue d'anomalies du bilan sanguin lors du suivi, la conduite à tenir face à une corticothérapie associée ou la prévision d'une grossesse ou d'un allaitement.

V. Perspectives

V.1. NEUROMYELITE OPTIQUE ET MALADIES APPARENTEES

Ce domaine d'expertise a été l'objet de nombreuses publications et m'a permis d'intégrer le groupe de réflexion national sur la NMO appelé "NOMADMUS". Par ce biais, de nombreux autres projets se dessinent répartis sur des axes variés comme la critériologie ou la thérapeutique.

Deux travaux en cours ont fait l'objet de présentations lors du congrès international sur la sclérose en plaques (ECTRIMS) en octobre 2017.

Le premier a contribué à déterminer la sensibilité et la spécificité des critères 2015 de NMOSD par l'analyse de la base de donnée nationale de NMO. J'ai ainsi eu l'occasion d'encadrer ce travail qui a nécessité la relecture et la classification de 235 patients à partir de données cliniques et IRM entre 2012 et 2014. Pour ce travail il a fallu mobiliser les centres de Strasbourg (Pr. de Seze et Dr. Kremer), de Paris Pitié-Salpêtrière (Dr. Papeix et Dr. Maillart) et de Lyon (Dr. Marignier et Dr. Durand-Dubief). Nous avons organisé une relecture des données des patients par des réunions communes qui ont eu lieu dans chacun des centres. L'exhaustivité des nombreuses données requises et le nombre important de malades a nécessité 2 ans de travail et l'implication de plusieurs attachés de recherche notamment dans les centres de Strasbourg et Lyon. Les résultats ont montré une grande sensibilité des critères de 2015, notamment comparativement aux critères plus anciens de 1999 et 2006, mais aussi une spécificité imparfaite. Certains patients ne remplissent que les critères de 1999, d'autres ont de véritables formes frontières remplissant les critères de 1999 et de SEP. La notion de SEP optico-médullaire avancée depuis le début de l'histoire

de la critériologie dans la NMO trouve donc ici un sens nouveau et définit une faible proportion de malades qui sera nécessaire d'étudier plus spécifiquement dans les années à venir. Ce travail est en cours de rédaction pour publication.

Le deuxième travail est dans la continuité des précédents et concerne l'évaluation des thérapeutiques dans la NMO. Alors que de nombreux auteurs se sont intéressés au traitement de fond dans cette pathologie, il n'existe que peu de travaux évaluant le traitement des poussées. J'ai ainsi entrepris en 2015 d'essayer d'identifier des facteurs de réponse thérapeutique dans le traitement des poussées de NMOSD. L'enjeu est à mon sens important puisque la NMOSD est une pathologie auto-immune démyélinisante dans laquelle le handicap est lié à la sévérité des poussées. Par conséquent, la réponse thérapeutique des poussées est un élément fondamental à considérer dans la prise en charge de ces patients car c'est elle qui définit le handicap résiduel. Un premier travail a consisté à sonder la base de données "NOMADMUS" et nous sommes arrivés à la conclusion que nous n'avions pas les moyens de répondre à la question posée par manque de précision des données disponibles. Par la suite, les centres participant au projet "NOMADUS" ont été contactés pour colliger les données pertinentes et un premier travail a été présenté à l'ECTRIMS en 2017. Si cette étude révèle que certains facteurs semblent pronostiques de la récupération des poussées comme le statut pour l'anticorps anti-AQP4 ou le délai séparant les deux lignes habituelles de traitement anti-inflammatoire (corticoïdes puis échanges plasmatiques), le manque de puissance n'a pas permis de sortir des résultats significatifs en analyse multivariée. Nous avons ainsi fait le choix de confier la suite du travail à un interne de neurologie à Rouen (Dr. Guillaume) qui fera le tour des centres experts dans la NMO pour recueillir les données directement

dans les dossiers. Nous espérons ainsi doubler le nombre de patients et de poussées analysées et obtenir la puissance nécessaire pour notre analyse des facteurs pronostiques. Ce travail fera par ailleurs l'objet de la thèse de médecine du Dr. Guillaume que j'aurai l'honneur de présider.

Un troisième travail est en cours et fait suite à notre publication sur les recommandations nationales sur l'utilisation du rituximab dans la NMOSD. Ce projet consiste à développer nos recommandations pour les faire évoluer au niveau européen.

V.2. RECHERCHE TRANSLATIONNELLE EN NEUROPROTECTION

A côté de la thématique sur la NMO, mon intégration dans l'équipe du Pr. Mensah en 2013 m'a permis d'ouvrir d'autres horizons de recherche dans le domaine de la thérapeutique et plus spécifiquement de la neuroprotection. D'autre part, mon rôle au CIC et les nombreuses études auxquelles j'ai pu directement ou indirectement participer ont rendu possible la concrétisation de ces projets en m'apportant un éclairage tout particulier sur la façon de réaliser une recherche translationnelle consistant à transférer chez l'humain les données issues de l'expérimentation préclinique.

V.2.1. La neuropathie des petites fibres

Notre travail s'est orienté vers la recherche d'outils diagnostiques dans le domaine des neuropathies des petites fibres (NPF; PHRC HUS 4961). Les NPF appartiennent au cadre nosologique des douleurs neuropathiques chroniques et s'expriment souvent par des acrodysesthésies à type de brulure ou remplissant

les autres critères du DN4. L'absence de lésion bien identifiée expliquant la symptomatologie rend le diagnostic de NPF particulièrement difficile et a nécessité la recherche de biomarqueur comprenant la densité de fibres nerveuses intraépidermiques (DFNIE) recueillie à l'aide de la biopsie cutanée.

Notre étude s'est déroulée au CIC de Strasbourg et a permis de mettre au point une base normée de DFNIE qui pourra être comparée aux résultats chez les patients et aider ainsi à poser le diagnostic de NPF. Dans le même temps, nous avons réalisé au CIC un fichier hébergeant les coordonnées de ces sujets sains susceptible d'être utilisé par les investigateurs travaillant avec le CIC.

Notre étude a confirmé les seuils quantitatifs de DFNIE retrouvés dans 2 autres études mais nous avons en plus proposé, à l'aide d'une modélisation statistique, un calcul plus précis de ce seuil en fonction de l'âge selon la formule suivante :

DFNIE : 7.6156 - 0.0769 x âge (années) + 1.5506 x sexe (femme = 1 ; homme = 0)

Nous avons également effectué une analyse qualitative des fibres qui suggère que la présence de dilatations de gros diamètre ou de ramifications dans l'épiderme était rare chez les sujets sains et pouvait être utilisée pour le diagnostic de NPF chez les patients.[32]

Ainsi, cette étude est une première étape pour permettre de classer nos malades suspects de NPF et colligés depuis 2013 en collaboration avec le laboratoire d'histologie du CHU de Strasbourg. En parallèle, nous avons alimenté une base de données de 200 patients suspects de NPF. Celle-ci a été implémentée par Dr. Cecilia Alves Do Rego sous ma supervision. Cette base est actuellement la plus

grosse cohorte existant sur cette pathologie. L'analyse de cette cohorte est en cours et fera prochainement l'objet d'une publication.

V.2.2. Antagoniste des récepteurs à la progestérone et maladie de Charcot-Marie-Tooth de type 1A

L'expertise du laboratoire du Pr. Mensah dans le rôle neuroprotecteur des stéroïdes et celle exercée au CIC dans le domaine de la thérapeutique ont conduit à l'écriture et la réalisation d'un PHRC sur l'effet de l'ulipristal acétate dans la maladie de Charcot-Marie-Tooth de type 1A (CMT1A; PHRC HUS 6100).

Le CMT1A est la plus fréquente des neuropathies périphériques héréditaires, pour laquelle aucun traitement n'a fait la preuve de son efficacité. Elle est de transmission autosomique dominante, liée à une duplication de la région chromosomique 17p11.2 qui conduit à une surexpression du gène puis de la protéine peripheral-myelin-protein 22 (PMP22), un composant majeur de la myéline périphérique.

Chez l'animal et chez l'humain, les taux d'ARNm *PMP22*, de *Glutathione S-transferase theta 2* et *Cathepsin A* (marqueurs du stress oxydant), détectés dans une biopsie de peau sont des marqueurs pouvant jouer un rôle pronostique dans l'évolution de la pathologie. Par ailleurs, plusieurs études ont montré que l'administration de progestérone augmentait l'expression du gène *PMP22* (mesurable dans une biopsie de peau) et aggravait les symptômes. En revanche, les anti-progestérones permettent de réduire la synthèse de *PMP22* et améliorent les symptômes chez le rat CMT1A.

La tolérance à long terme des anti-progestérones a été évaluée pour la mifepristone (RU486) et l'ulipristal acetate (EllaOne®). Peu d'effets secondaires ont été rapportés comprenant quelques cas d'hyperplasie endométriale réversible à l'arrêt du traitement. Avec le RU486, de rares cas d'insuffisance surrénalienne et androgénique ont été observés. En revanche, EllaOne® a une faible action antagoniste sur les récepteurs des glucocorticoïdes et aucune action sur les récepteurs des androgènes. Nous pensons par conséquent qu'il sera bien toléré chez l'homme et permettra de diminuer la synthèse de PMP22, ainsi que l'action du stress oxydant en améliorant le handicap des malades.

L'objectif principal de notre étude est donc de tester l'efficacité et la tolérance d'un antagoniste des récepteurs à la progestérone sous forme de comprimé, dont l'action devrait diminuer la synthèse de *PMP22* et ainsi améliorer le handicap des patients atteints de CMT1A. L'étude est randomisée en double aveugle et permettra d'inclure 45 patients répartis en 3 groupes correspondant à 2 doses différentes de produit et un groupe placebo. Les patients bénéficient d'une biopsie de peau initiale de 3mm au niveau d'une phalange qui est répétée à la fin de l'étude qui dure 1 an. Le critère de jugement principal est le taux de *PMP22* testé par RT-PCR dans une biopsie de peau entre le début et la fin de l'étude.

L'intérêt de cette approche est double puisqu'elle permettra de confirmer chez l'humain l'effet potentiel des antagonistes de la progestérone sur la synthèse de *PMP22* obtenu chez l'animal, mais aussi d'espérer obtenir des données intéressantes sur l'efficacité et la tolérance au long cours chez l'homme. Cette étude est en cours de recrutement avec 30 malades inclus pour un objectif de 45.

V.2.3. Allopregnanolone et neuropathies chimio-induites

L'allopregnanolone (ALLO) est un neurostéroïde endogène dérivé de la progestérone qui a fait l'objet de nombreuses études et publications au laboratoire INSERM U1119. Ces travaux expérimentaux ont démontré ses propriétés neurorégénératives et neuroprotectrices. L'ALLO a fait l'objet de quelques études en clinique humaine et deux études (phase 1 dans la maladie d'Alzheimer, et phase 2 dans les traumatismes cérébraux) sont actuellement référencées, mais aucun travail ne fait état de recherche avec cette molécule dans les neuropathies périphériques chez l'humain. Les neuropathies chimio-induites par l'oxaliplatine sont en revanche l'objet de nombreuses études pour l'évaluation de thérapeutiques (incluant divers composés neuroprotecteurs dont thiols, antioxydants, facteurs neurotrophiques, anticonvulsivants…) ou stratégies préventives (réduction ou espacement des doses), témoignant de l'absence actuelle de traitement efficace prophylactique ou curatif et du besoin crucial d'identifier de nouvelles molécules. Ainsi j'ai pu participer à l'élaboration du PHRC HUS 6638 ALLOXA dont l'**objectif principal** est d'évaluer si l'administration d'ALLO lors de chaque cycle de chimiothérapie à base d'oxaliplatine permet de réduire la fréquence de survenue des neuropathies périphériques chimio-induites chez des patients atteints de cancer colorectal. Ce projet a reçu un financement en 2016 mais n'a pas encore débuté.

V.2.4. Testostérone et sclérose en plaques

La SEP est une maladie neurologique chronique auto-immune dont les manifestations cliniques principales sont associées à une inflammation et une

démyélinisation du SNC. Elle représente la 1ère cause non traumatique de handicap sévère acquis chez le sujet jeune. Les traitements actuels sont limités à des agents anti-inflammatoires et il existe un besoin urgent de thérapies innovantes capables de promouvoir la neuroprotection et la réparation de la myéline. De nombreuses études épidémiologiques et cliniques ont mis en évidence des différences de pronostic entre la femme et l'homme atteints de SEP. Ces observations ont stimulé l'intérêt pour les effets protecteurs potentiels des hormones sexuelles, et en particulier de la **testostérone** chez les patients atteints de SEP.

Les données actuelles montrent que la testostérone agit selon trois effets distincts :

1. Un effet **anti-inflammatoire** mis en évidence chez l'humain et dans les modèles expérimentaux de SEP induits chez l'animal.
2. Un effet **neuroprotecteur** capable de protéger les neurones de la moelle épinière en culture contre de la toxicité induite par le glutamate, ainsi que des lignées cellulaires neuronales contre le stress oxydant. La testostérone est également capable de préserver la transmission synaptique excitatrice dans l'hippocampe au cours de l'EAE.
3. Un effet **remyélinisant.** Dans un modèle murin de démyélinisation chronique induit par la cuprizone, il a été démontré que le traitement par la testostérone stimulait efficacement la formation de myéline ainsi que sa régénération, en mettant en évidence le rôle spécifique du récepteur neural des androgènes**.** Chez l'humain, cet effet remyélinisant n'a pas été clairement démontré. Cependant une étude de phase 2 chez dix patients

traités par testostérone laisse supposer qu'il existe un rôle neuroprotecteur ou remyélinisant comme le montre son effet sur le ralentissement de l'atrophie cérébrale et sur l'atrophie de la substance grise.

Ainsi, nous projetons d'étudier l'effet de la testostérone dans le traitement de la démyélinisation induite lors de la SEP. Nous proposons une étude clinique pilote de phase II (multicentrique, randomisée, en double aveugle, en groupes parallèles, contre placebo) qui mettra l'accent sur les effets bénéfiques d'un traitement par la testostérone chez des hommes atteints de SEP rémittente récurrente ayant des taux faibles de testostérone (inférieurs à 15 nmol/L) et traités de manière stable par le natalizumab (Tysabri®) depuis au moins un an.
L'objectif principal de l'étude sera de déterminer par IRM conventionnelle les bénéfices du traitement sur l'évolution de l'atrophie cérébrale, marqueur indirect de la remyélinisation et secondairement d'évaluer l'efficacité du traitement sur des paramètres d'IRM non-conventionnels dont l'analyse approfondie pourrait permettre d'estimer au mieux la neuroprotection et la rémyélinisation cérébrale. La tolérance au traitement ainsi que son efficacité seront également évaluées en utilisant diverses échelles cliniques. Ce projet a été soumis à l'appel d'offre pour les PHRC interrégionaux 2017 et a été retenu pour financement.

V.2.5. Biomarqueurs de réponse thérapeutique chez les patients atteints de forme progressive de sclérose en plaques et traités par biotine à haute dose (MD1003)

En France, des hautes doses de biotine (MD1003) sont utilisées *per os* pour traiter les patients atteints de sclérose en plaques progressive. Malgré une étude de phase 2 et un essai thérapeutique de phase 3 montrant des résultats positifs sur l'évolution du handicap, le mécanisme d'action du MD1003 reste incertain. Pour différencier un effet symptomatique d'un effet neuroprotecteur, nous avons étudié prospectivement au CHU de Strasbourg (Pr. de Seze) et en collaboration avec l'université de Bâle (Pr. Derfuss et Dr. Kuhle), une cohorte de 30 patients nouvellement traités par MD1003. Ce travail a été l'objet d'une mobilité de 6 mois que j'ai pu effectuer dans le département de biomédecine de l'université de Bâle. L'évolution des taux sériques de neurofilament, qui constitue un marqueur robuste de neuroprotection, a été effectuée à J0, 6 mois et 12 mois. Ces données ont été corrélées aux données d'atrophie cérébrale et médullaire, estimées à l'aide d'un nouveau logiciel développé à Bâle. Les données cliniques correspondent au "no evidence for disease progression" et comportent le score EDSS, le 9HPT et le T25FW. Ce travail est en cours et les derniers patients seront analysés courant été 2018.

VI. Conclusion

Les travaux de recherche décrits dans ce document montrent la double expertise que j'exerce actuellement. D'une part il existe un champ de recherche dans le domaine des pathologies inflammatoires du SNC telles que la NMOSD et la SEP, et d'autre part un vaste programme de recherche translationnelle, rendu possible grâce à ma fonction au CIC, portant sur le développement de nouvelles approches diagnostiques et thérapeutiques chez l'humain dans différents domaines de la neurologie et plus particulièrement dans le domaine de la neuroprotection.

Ces projets ont pu se développer grâce à ma forte implication dans plusieurs groupes de travail nationaux (OFSEP, NOMADMUS) qui ont d'ores et déjà permis d'élaborer des recommandations nationales diagnostiques (myélites aigues, MATLE) et thérapeutiques (utilisation du rituximab dans la NMOSD). Au niveau international, une collaboration avec le département de biomédecine de Bâle (projet sur la biotine) et un groupe de travail européen sur la NMO (EDEN) m'ont permis de développer plusieurs travaux de recherche clinique (formes tardives de NMOSD) et thérapeutique (recommandations européennes sur l'utilisation du rituximab, biotine et SEP).

La pérennité de ces axes de recherche est assurée de par la fiabilité des structures sur lesquelles elle s'appuie (OFSEP, INSERM 1119, CIC INSERM 1434) et la mobilisation des chercheurs dans les groupes de travail qui lui sont dédiés. De plus, mon rôle au CIC et mon investissement dans l'enseignement et la formation des étudiants à la thérapeutique m'ont permis d'acquérir une meilleure compréhension de la recherche clinique et de concevoir plusieurs

études translationnelles ayant bénéficié d'un financement de type PHRC (ALLOXA, UPACOMT, TOTEM-RRMS). Plusieurs internes en médecine ont déjà bénéficié des ces projets de recherche pour leurs travaux de thèse à Strasbourg (Dr. Alves Do Rego) ainsi que dans d'autres CHU en France (Dr. Guillaume à Rouen).

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