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West Nile Virus Neuroinvasive Disease: why it should be considered in the Iberian Peninsula

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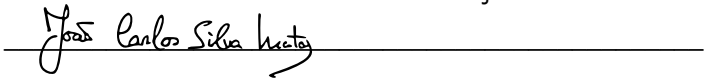

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DESIGNAÇÃO DA ÁREA DO PROJECTO

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West Nile Virus Neuroinvasive Disease: why it should be considered in the Iberian Peninsula

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Dedicatória

Aos meus pais pelo o apoio e paciência durante este todo percurso.

West Nile Virus Neuroinvasive Disease:

why it should be considered in the Iberian Peninsula

Abstract

Introduction: West Nile virus has lately caught the attention of the medical community given the spike of European cases in the season of 2018. The number of infected humans exceeded the total cases of the past seven years and the virus expanded to area previous disease-free, causing significant morbimortality along its way.

Objectives: to highlight from a clinical standpoint West Nile virus as a possible aetiology in neuroinvasive disease on humans in the Iberian Peninsula.

Development: West Nile virus can be transmitted by mosquitoes' bites, blood transfusion, and organ transplant. Although most infections are asymptomatic, <1% of patients develop neuroinvasive disease presenting as meningitis, encephalitis or acute flaccid paralysis. West Nile virus should be considered as a differential diagnosis in the face of neurological symptoms of unknown aetiology in the appropriate epidemiological circumstances. Diagnosis in the clinical setting is based in serological analysis of serum or cerebrospinal fluid. As available treatment is only supportive, preventive measures are key to diminish this virus' impact. Future course of West Nile virus is difficult to predict, even though ongoing global changes could be factors contributing to the virus geographic expansion.

Conclusion: West Nile virus is either relatively stable responsible for only small outbreaks or it can be the causative agent of large epidemics, depicting the unpredictability around it, hence, justifying the need of further surveillance and information in the Iberian Peninsula.

Key-words: West Nile virus, meningitis, encephalitis, acute flaccid paralysis, Iberian Peninsula

Introduction

Last year was unprecedented as the number of human cases of West Nile virus (WNV), 2083 autochthonous cases reported (figure 1), surpassed the last seven years all together (2010-2017), affecting new areas, and being a factor of severe morbimortality (181 deaths reported in EU and neighbouring countries) [1].

WNV is considered the most relevant agent of viral encephalitis and a good example of how an apparently stable zoonosis can emerge with unpredictable consequences. Most infected individuals are asymptomatic, however, it is important to be recalled as a differential diagnosis, especially in the case of severe presentations such as WNV's neuroinvasive manifestations, to prevent iatrogenic complications from misdiagnoses and subsequent therapies [2, 3].

The virus has been known to cause occasional outbreaks especially in countries of Central and Southern Europe in a much smaller scale. Hence, there is a growing concern over the future of WNV in whether global changes will increase the human and animal impact of this disease. In European countries which are affected every year (such as Italy, Greece, and Romania), WNV raises more awareness than in Iberian peninsula, even though human cases have been described and the virus is known to be in circulation [4, 5].

The WNV is a neurotropic arthropod-borne flavivirus genetically related to the Japanese encephalitis virus. It has an enzootic transmission cycle which comprises several species of wild migratory and/or resident birds (reservoir host) and mainly the *Culex* mosquitoes species (vector); humans and mammals like horses may be infected by these mosquitoes' bites, however they do not play a part on further transmission because their short-timed low level viremia does not allow it, being described as "dead-end hosts", composing the epizootic cycle [6].

WNV was first isolated from a human patient in 1937 in the West Nile district of Uganda, and from that point onwards it has been detected in several regions of the World even in the American continent with New York as the gateway in 1999 [2, 7]. In the past, the virus was mostly spread via migratory birds that overwinter in Africa which contributed to the usual WNV season of activity in Europe, ranging from mid-June to mid-November which matches

with the birds' return and also with the vectors peak of activity [8-10]. Nowadays, the virus is endemic to the Old Continent [6] - figure 2.

This manuscript intends to highlight from a clinical standpoint WNV infection as a differential diagnosis in the case of meningitis, encephalitis or acute flaccid paralysis, given the recent spike of cases in Europe, that might propel a greater spread of the virus in the Iberian Peninsula, considering the disseminated vector presence in the region.

Methods

To fulfil this objective, data of WNV epidemics and current recommendations were obtained on the websites of the Centres for Disease Control and Prevention and the European Centre for Disease Prevention and Control (ECDC) and it was conducted a review of the literature in PubMed electronic database using the terms: *West Nile virus, West Nile fever, neuroinvasive disease, meningitis, encephalitis and acute flaccid paralysis*. Articles written in English, Spanish and Portuguese were analysed with publication date till February 2019. There were no restrictions on the type of study. Additional studies found in the references of the selected ones and pertinent book chapters were also included.

Epidemiology Matters

Anamnesis is key to consider WNV as a differential diagnosis. Location of patient's residence and his travel history is of utmost importance in order to verify his presence in an area with active transmission - this factor may make or break the diagnostic hypothesis [11].

In order to identify a WNV infection it is important to recognize and comprehend its clinical presentations, however, discriminating each one of them is difficult and not as distinguishable as one may think since overlap is common [12] – figure 3.

The Virus and Transmission Routes

The virus is generally introduced in humans by a mosquito bite (usually a *Culex*) and the infection can be characterized in three phases: early phase – it infects skin cells in the point of entry; visceral dissemination phase – the virus spreads to the nearest lymphatic node from which it cause viremia followed by organs invasion such as the central nervous system (CNS), in which it presents as neuroinvasive disease – the CNS phase [13, 14]. This last phase is the most important because of its clinical repercussions.

While mosquito bites are the most common route of infection, WNV can be spread by transfusions of diverse products obtained from blood of infected donors [15]. Countering this exposure risk, a European directive (2004/33/EC) states that travellers who return from an area with ongoing transmission of WNV should be deferred from donating blood for 28 days. Bearing in mind a blood donations shortage, nucleic acid amplification techniques (such as polymerase chain reaction – PCR) to detect WNV RNA can be implemented in WNV affected countries and in countries without active transmission but with a considerable number of travellers returning from those areas, as the WNV peak of activity coincides with the high travel season in Europe.

Two other ways of transmission, although rare, should be kept in mind. One is through solid organ transplant from an infected individual, which could be particularly dangerous as patients are deeply immunocompromised [16]. Infection has been reported through organs from a donor with no traceable WNV RNA but detectable immunoglobulin (Ig) M/IgG anti-WNV which highlight the complexity involving transplantation and WNV control, as the virus

may reside hidden in organs after viremia was cleared or by long-term persistence of WNV characterized by a sporadic viremia [17, 18]. Therefore, implementing a screening test to donors is difficult and probably not cost-effective [19]. The other is vertical transmission (intrauterine and breastfeeding), an even rarer possibility, requiring more reports and investigation [20, 21].

Clinical Manifestations

Symptoms and signs of the patient are only detectable after an incubation period which can vary from 2 to 14 days but it can go up to 21 days in immunocompromised patients [15, 22]. Most infected patients will remain asymptomatic (around 80%) so it is only possible to verify its infection by a blood screening mostly performed in situations concerning blood donations [12].

The most common presentation is West Nile fever (WNF) in about 20% of patients infected, characterized as a flu-like syndrome with a sudden onset of fever (which may be of low grade), fatigue, headache, and myalgia [23]. Other symptoms include nausea, vomiting, and a morbilliform or maculopapular rash that affects 25 to 50% of patients [24, 25]. The rash usually appears in defervescence lasting less than a week and it tends to be non-pruritic, more prevalent in the head, neck, torso and extremities sparing palms and soles [24]. WNF ranges from a mild condition lasting only a few days to an incapacitating state persisting weeks or even months [26]. Although the majority of patients have a full recovery, age and comorbidities seem to be factors determining the clinical outcome: WNF in the elderly with several underlying diseases may prompt severe results, even death through respiratory failure or cardiovascular causes [27].

WNV is able to penetrate the blood-brain barrier affecting the meninges and causing neural parenchymal damage. This is more common in older and immunocompromised individuals [28, 29]. WNV neuroinvasive disease may present as meningitis, encephalitis, and acute flaccid paralysis (AFP) or as a combination of these different presentations – figure 3. Despite affecting 1% or less of infected patients, these conditions are the more damaging clinical outcomes of a WNV infection [26]. Symptoms/signs described for WNF are commonly present in the development of neuroinvasive disease.

Meningitis caused by WNV is similar to a typical viral meningitis, being impossible to distinguish by clinical manifestations. It is characterized by fever and retroorbital or frontal headache, meningeal signs - nuchal rigidity, Kernig's and/or Brudzinski's signs - photophobia, and phonophobia [30, 31]. The characteristic rash is less observed comparing to WNF [24]. Constitutional signs may also be present such as abdominal pain, anorexia, myalgia, nausea, and vomits [30, 32]. Other neurological signs and symptoms are usually not present [31].

In WNV encephalitis, mental status changes are very common and can range from mild confusional state to severe encephalopathy, coma, and death [12]. Behavioural differences are noted by some patients such as disorientation, confusion and irritability [31]. Diffuse and focal neurological manifestations are more common in the case of WNV encephalitis, such as extrapyramidal symptoms which with the appropriate epidemiologic context is a very characteristic feature [12, 31]. Chronologically, changes in the consciousness level precede initial symptoms, however, they are usually followed by movement disorders [12, 28]. Bilateral tremor and myoclonus may be present, both more common in the upper extremities and the latter also in facial muscles [26, 31]. Patients may develop cerebellar ataxia with gait imbalance and parkinsonism characteristics such as bradykinesia, rigidity, and postural instability which can lead to falls [31]. These symptoms arise given the neurotropism of WNV for extrapyramidal structures [33]. Seizures, increased intracranial pressure or cerebral oedema are not frequently observed [12].

Muscle weakness in the form of paresis or paralysis can be observed as an outcome of WNV infection and represents damage dealt to the lower motor neurons of the spinal cord [34-36]. On physical examination, this AFP syndrome is typically characterized by hyporeflexia or areflexia of the affected muscles [31, 34]. Muscle weakness related to AFP develops in the acute phase of infection, 24 to 48 hours after symptoms onset. It is frequently asymmetric affecting only one limb - monoplegia; nevertheless, as it depends on the extension of the spinal cord's lesions, AFP might present as quadriplegia associated with higher mortality and morbidity [26]. In some particular cases, bilateral cranial muscle weakness may be observed [36]. Persistent loss of muscular strength leads to muscular atrophy in late stages [33]. However, the most dangerous situation is when respiratory muscles are affected as it propels respiratory failure with need of further medical care [34, 35, 37]. In fact, invasive mechanical ventilation might be required for prolonged periods or

even permanently as some patients are unable to sustain ventilatory function without support, also increasing mortality and morbidity [35]. It is important to access patients with risk of developing respiratory failure, especially those that concomitantly have dysarthria, dysphagia or loss of gag reflex [29]. Bladder and bowel disfunction are common; pain is reported by some patients and it might become persistent; also, modification in sensory sensations are absent for most cases [29, 31, 34].

Differential Diagnosis

Summing up, in this stage with the details from patient's history and the presentation of neuroinvasive disease, doctors can formulate their hypothetical diagnoses. At this moment, more attention must be drawn towards WNV.

However, affirming the diagnosis of WNV infection based solely on the clinical manifestations is very challenging: apart from symptoms' lack of specificity, in areas with co-circulation of other flavivirus (dengue, yellow fever, Zika, Japanese encephalitis, St. Louis encephalitis viruses), due to similar clinical outcomes, the difficulty rises as it is hard to distinguish between them [6, 33]. Many other infectious (such as herpes simplex 1, enterovirus, varicella zoster and human immunodeficiency viruses or bacteria as meningococcus or pneumococcus) and not infectious conditions present as meningitis or encephalitis [38, 39]. AFP might also be described as a poliomyelitis-like syndrome for its resemblance with clinical manifestations of poliovirus infection [30]. Patients infected with WNV presenting with AFP can be misdiagnosed with Guillain-Barré syndrome (GBS) especially its acute motor axonal neuropathy subtype since it is a common cause of the same symptomology [33]. However, sensory impairment is another feature of GBS which is uncommon in AFP, which can be confirmed with physical examination or nerve conduction studies. Myopathies and neuromuscular junction disorders might too be listed as differential diagnoses. This clinical presentation can also be observed with other infectious diseases such as Lyme disease, syphilis, and botulism [40].

Diagnostic Considerations

Taking into account the clinical information gathered, the suspicion of WNV infection is confirmed by detection of IgM antibodies anti-WNV in serum or cerebrospinal fluid (CSF) by

an IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) which is sufficient in the majority of cases [33, 41]. IgM is highly suggestive of acute infection, rising as early as 4 days to 10 days after symptoms onset; consequently, it is possible to have a negative MAC-ELISA result if the test is performed before the rise of IgM level; in this situation, the test should be repeated days later (approximately 10 days) [23, 33]. IgM titres usually decline from the 21st day onwards being undetectable 1 to 2 months after clinical resolution, however IgM in some patients might persist for more than a year [33].

An important factor to consider is the possibility of cross-reactivity in MAC-ELISA test with other flaviviruses, depicting the importance of patient's travel history or his residence area can be an endemic to certain flavivirus and/or in the event of recent vaccination for Japanese encephalitis/yellow fever virus [23, 33, 41]. Therefore, a confirmatory test should be requested in order to verify if MAC-ELISA is falsely positive for WNV. Plaque reduction neutralization test (PRNT) indicates the highest dilution of serum able to neutralize WNV among other flaviviruses using cell cultures: the higher the titre, the higher the antibody' serum concentration and the test is considered positive and the diagnosis is confirmed when is documented a difference 4 times greater for WNV than for other flaviviruses tested [33, 41]. The problem of PRNT lies with its complexity, demanding a biosafety level 3 laboratory to be performed, limiting the utility of this test [41, 42].

Ultimately, the viremia level can be detected by PCR to prove an acute infection, but humans develop a frail viremia with low concentration of WNV genome material for a short amount of time, so trying to identify it is considered unpractical in a clinical setting, although being a very specific test [41, 42]. Exception could be made when testing an immunocompromised patient, since the immune response is impaired, the development of antibodies is delayed or absent and viremia is sustained for longer periods [43].

Peripheral blood analysis is rather unspecific in the case of WNV infection [26].

Lumbar punctures can be performed to conduct an analysis of the CSF, unless contraindicated: testing for IgM anti-WNV is important, since IgM is unable to cross the blood-brain barrier, it's detection in the CSF is a hallmark sign of WNV neuroinvasive disease; CSF is typically characterized by an increase of white blood cells (generally less than 500 cells/mm^3, but some patients have normal cell count, especially those with an

immunocompromised status that are unable to mount a significant inflammatory response against WNV infection) – and increased protein levels, the latter being of greater magnitude in the case of WNV encephalitis; glucose levels are normal most of the time [12, 30, 33, 44].

Neuroimaging studies should be performed, not because they are essential to diagnose WNV neuroinvasive disease, but in order to help exclude differential diagnosis. In fact, imaging examinations (in particular magnetic resonance imaging – MRI) do not correlate exactly with active severe infection as they can remain without relevant findings during several weeks after onset of the disease [12]. Meningitis may be represented as an increased signal intensity in MRI [45]. Concerning WNV encephalitis, the most characteristic MRI finding is bilateral signal abnormalities in the basal ganglia and the thalami on T2-, fluid-attenuated inversion recovery (FLAIR) and diffusion-weighted image sequences [45]. In an AFP setting, it is also possible to request a spinal MRI. Signal abnormalities are usually found especially in the anterior spinal cord, representing damage in the anterior corticospinal tract which is usually coherent with the segments through the motor neurons for the affected muscles travel [29].

Electroencephalography in a clinical setting of WNV encephalitis may present nonspecific abnormalities as generalized irregular slow waves (frequently at the frontal and temporal regions), triphasic sharp waves, or it can even document seizures [12, 31].

When muscle weakness is observed and AFP is suspected, electrodiagnostic studies of the peripheral nervous system such as an electromyography or a nerve conduction study can be requested. Typically, motor axonopathy is shown, normally without any demyelinating abnormalities, with intact sensory function [28, 31].

Treatment

There is no specific treatment for WNV and usually only supportive measures are deployed to assist patients [33]. Pain and emesis management are the most commonly used, however, as the case' severity increases, more aggressive attitudes should be implemented as ventilatory support in respiratory failure, prevention of superinfection and pressure sores or even seizures and intracranial pressure control in an encephalitis presentation [40, 43]. Some agents as interferon alpha-2b, ribavirin, and intravenous immunoglobulin have been

advanced as potential therapies but no studies showed a clear benefit in humans and most evidence for their proposed advantage comes from animal models or in vitro results [23, 46-48].

Prognosis

The general prognosis of a WNV infection is excellent taking into account the very low percentage of infected individuals that develop neuroinvasive disease, which is far more debilitating and life-threatening than other presentations [23, 33, 48]. Older age seems to be the most relevant prognostic factor [49, 50]. Some medical conditions were showed to have an association with the development of WNV neuroinvasive disease as hypertension, diabetes, history of alcohol abuse, history of cancer, and chronic renal disease [51, 52].

Follow-up of patients with neuroinvasive disease show that the majority of patients become functionally independent within several months to years, however a significant number might require long-term rehabilitation due to difficulties presented in performing activities of daily living [31, 53]. Mental status might remain persistently changed [31]. Neuroimaging studies might predict evolution of patients as a normal MRI test result have a better prognosis, with shorter hospitalizations and faster recovery time [45].

WNV meningitis is mostly associated with a positive outcome with a smaller chance of developing long-lasting neurological symptoms [31]. WNV encephalitis is a more dangerous condition when compared to WNV meningitis. In fact, its fatality rate is around 10% which can go up to 30% in older and/or immunocompromised patients [46, 52]. Persistent physical complains such as muscle weakness, fatigue, and myalgias are the most common findings [32, 53]. Parkinsonism features and myoclonus are also reported [31, 48]. Depression, apathy, and anxiety can be seen in the process of recovery from WNV encephalitis [32, 53]. Cognitive deficits are also described, such as loss of memory and of thought with increased difficulty in concentration [31, 32, 53]. The severity of the initial presentation is a debatable indicator of final outcome as patients with severe WNV encephalitis can recover without significant long-term functional losses [29, 48].

In the beginning of AFP’s convalescence phase quality of the life could be compromised as the majority of patients still report muscle weakness which may require therapy to improve

their physical impairment [29, 53]. In the months post-infection, usually after 6 to 8 months, most patients recover from muscle weakness, varying with the extension of the disease [29]. Severe situations such as respiratory muscles involvement (responsible of 50% of deaths related with AFP by WNV) or quadriplegia are more difficult to overcome, in fact, previous health state before the WNV infection may never be reached [29, 48, 53]. However, some patients recover successfully from dare clinical presentations [29]. The severity of the initial setting of APF is not the most reliable prognostic factor likewise WNV encephalitis, whereas electrodiagnostic studies might be more worthwhile, revelling the evolution of motor denervation when compared to initial tests [29, 54].

Prevention

Prevention is key to reduce the impact of WNV. Adopting individual precautions should follow the four D's rule: N,N-diethyl-m-toluamide or its acronym DEET – to use an insect repellent with DEET, especially when in outdoors activities; dress – to wear clothes with long sleeves and long pants; drain – to drain standing water near one's residence including tires, plant-pots, and rain gutters; dusk to dawn – to avoid being outdoors during this period of time [55, 56]. Community-based mosquitoes control policies take an important part in reducing the WNV's vector population by the means of insecticide spraying [55, 56].

Although being a demanding process, surveillance is always crucial to an effective and rapid response to an outbreak as it helps to predict an epidemic's magnitude improving the overall health system's preparedness [6]. A recommend approach is an integration of different systems, consisting in a collaboration of several public institutions regarding human, animal and environmental health which may improve the cost-effectiveness of WNV's infection control and prevention as the One Health approach [57, 58]. Blood screening protocols significantly helped reducing the risk of transmission through transfusion [58]. Animal surveillance is also recommended: horses can be studied to determine the existence of WNV in a certain area – however, with the availability of an equine vaccine against WNV, the utility of this parameter is limited; searching for WNV infection in birds is useful in order to early warn public health services of WNV circulation, but due to logistical and economic costs this type of study is reserved to countries which experience large epidemics [58].

All these measures should always be interpreted in a case to case basis, being influenced by the epidemy' scale and the ability of the country's health system to respond to such threat.

Another frontline of WNV infection prevention is related to the development of a vaccine for human beings and some studies have been conducted [59]. However, the cost of designing a vaccine, the wide geographic area in which WNV is endemic, and the benign course in the large majority of the infected individuals are disadvantages towards such goal, because the only objective would be of controlling an infection that does not need humans to strive in the wild [59].

Future Perspectives

WNV is subject to the constant changes the World has been facing the past decades, successfully adapting to the challenges presented by an ever-mutating society. Its geographic expansion is proof of the previous statement. What does the future hold for WNV, especially in the Iberian Peninsula? It is a difficult question to answer, however some predictions can be made regarding the current course of human development and its impact on the environment.

An overall human population increase is taken for granted in the upcoming decades with all of its consequences, regarding WNV in particular [60]. Higher density urban populations enable zoonotic diseases to flourish because transmission to humans becomes more frequent and mosquitoes have better breeding conditions specially in less sanitary conditions [2, 61]. The population boom in certain parts of the globe will push for a greater land usage which if not planned and regulated will put in further contact humans and vectors [62]. Another consequence will be the increased movement of people for work purposes, tourism, or migration and the accompanying international traffic of goods which may play a part in expanding WNV and/or its vector to new areas [2, 61]. Earth biosphere will be even more connected, and the smallest disturbance of balance might have a great impact on the general environment.

Present day concern about climate change can be approached when discussing the future of WNV, with the increase in median temperatures and modifications to precipitation patterns across the globe will facilitate the spread of mosquitoes as they would be able to

survive and thrive in areas that previously could not support their existence and will also alter birds' migration routes [2, 62, 63]. These factors would work together to globally extend the WNV period of activity and its geographic distribution.

It is also important to remember that WNV is capable of intrinsic change as well, by the means of genetic mutation. In this field an uncountable number of paths can be drawn. A new strain might develop sustained viremia in humans ending the status of "dead-end host" or the percentage of patients with neuroinvasive disease might increase if a more aggressive strain appears, just to exemplify a couple of mutation results in an endless set of scenarios [2].

Conclusion

WNV was discovered 82 years ago and what it has taught the scientific community that it should be prepared for the unexpected, besides the significant evolution of the knowledge about this virus in these past two decades. Additionally, it proved the public that tropical diseases are getting closer to their doorstep. In the presence of neurological symptoms and epidemiological links, WNV should be considered as a differential diagnosis, also in the Iberian Peninsula, as the vector *Culex* is widely distributed in the temperate regions. A serological analysis can make a difference between misdiagnosing and overtreating a patient. In conclusion, the dynamics of WNV have to be better understood in order to more effective measures can be implemented, whether in development of a vaccine, finding appropriate specific treatment or investing further on prevention. Each season of WNV is unique and provides a chance to gather more information, and one can only wonder how the future of WNV might play out.

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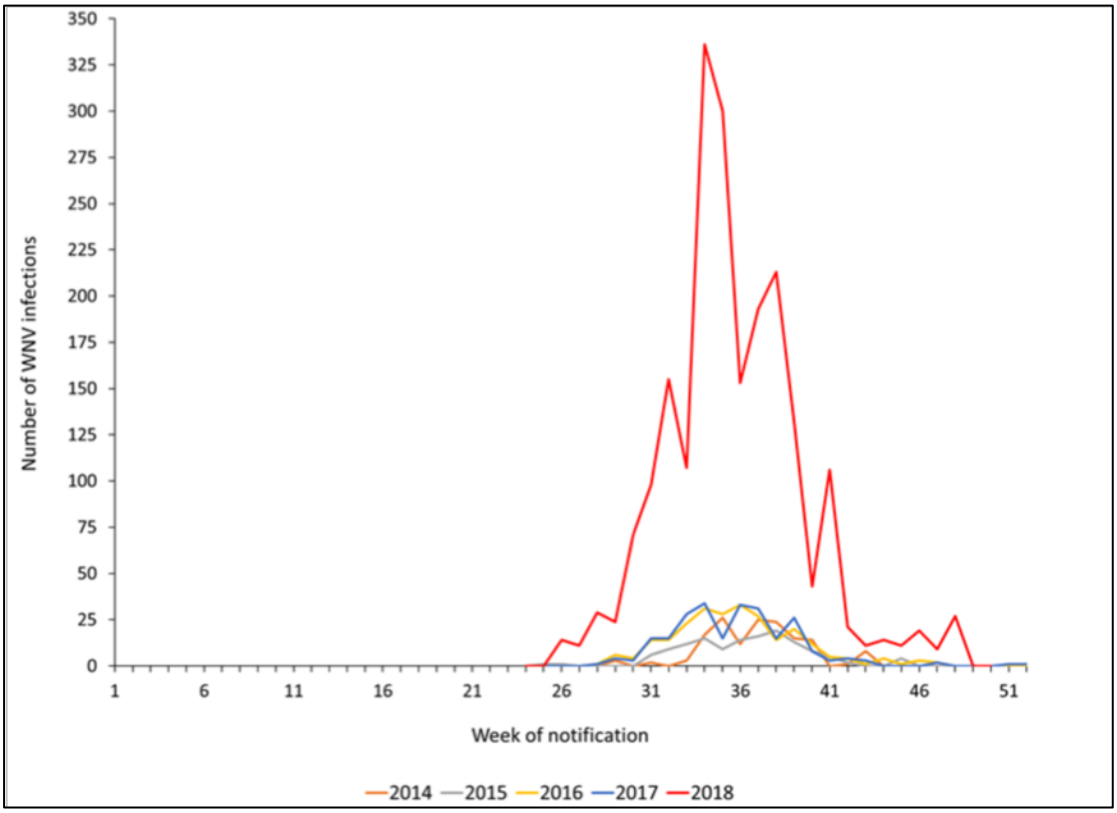

Figure 1. Number of WNV infections in European Union/ European Economic Area Member States and European Union neighbouring countries by epidemiological week of notification (to national authorities or if missing, week of notification to European Centre for Disease Prevention and Control - ECDC), 2014-2018. Graphic and data from the ECDC. WNV- West Nile virus

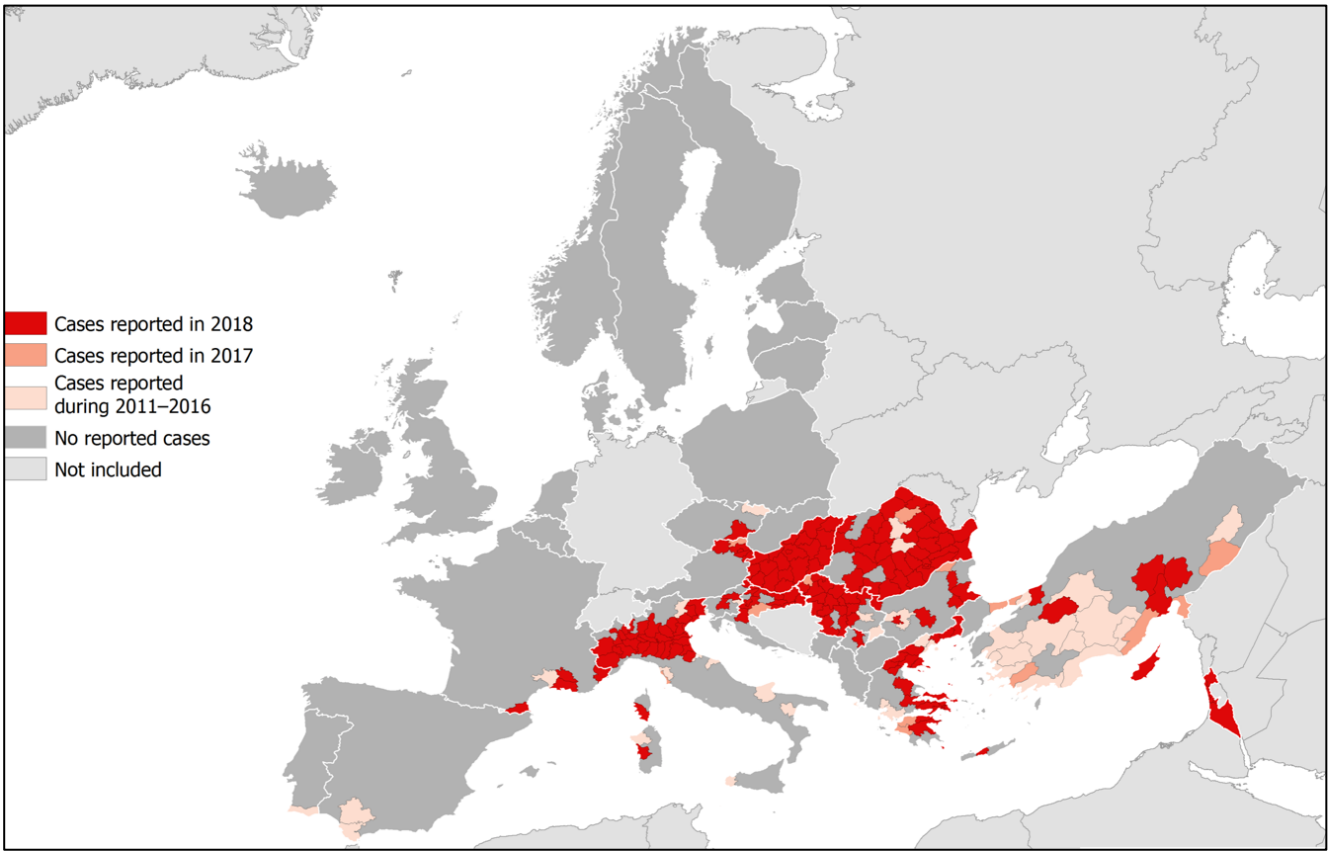

Figure 2. Distribution of West Nile virus infections in humans by affected areas in the European Union/ European Economic Area Member States and European Union neighbouring countries in the transmission season of 2018 and previous ones (with overlap); latest data update 13 December 2018. Map and data from the European Centre for Disease Prevention and Control.

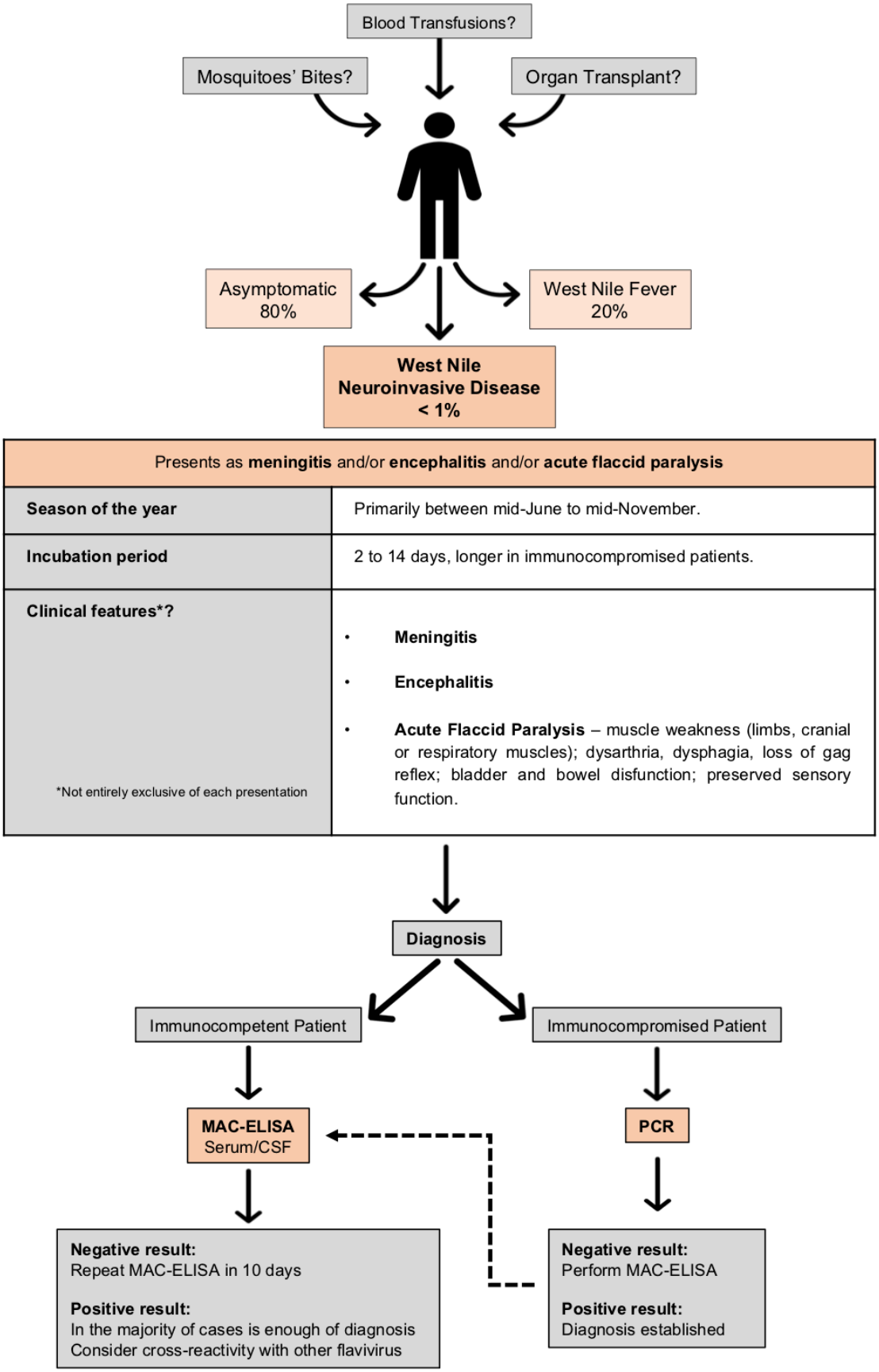

Figure 3. Resume of the characteristics of WNV infection, focusing on neuroinvasive disease and a short scheme of diagnostic work-up.

Agradecimentos

À minha orientadora, Doutora Cândida Abreu, pela sua orientação, compreensão e disponibilidade na elaboração deste projecto.

Aos meus amigos e colegas de curso, pela sua paciência, companhia, conselhos.

Anexos

NORMAS PARA LA PUBLICACIÓN DE ARTÍCULOS EN REVISTA DE NEUROLOGÍA

Revista de Neurología es una publicación de periodicidad quincenal (24 números al año), de ámbito y distribución mundial, destinada a la publicación de manuscritos sobre las neurociencias, tanto clínicas como experimentales. *Revista de Neurología* está indexada en MEDLINE/Index Medicus, SciSearch/Science Citation Index Expanded, EMBASE/Excerpta Medica, Research Alert, IME/Índice Médico Español e IBECS/Índice Bibliográfico Español en Ciencias de la Salud, entre otros, y ofrece una **versión electrónica *on-line* con contenidos completos de entrada libre en: http://www.neurologia.com**

CONTENIDO

Revista de Neurología consta de las siguientes secciones básicas: Editorial, Originales, Notas Clínicas, Revisiones, Revisión en Neurociencia, Historia y Humanidades, Neurología de la Conducta, Neuroimagen (descripción y comentario de imágenes de gran interés clínico), Correspondencia (trabajos de investigación o presentación de casos clínicos de menor extensión), Cartas al Director (comentarios a artículos publicados y las posibles réplicas a éstos), Crítica de Libros (comentario de obras literarias destacables por aspectos diversos relacionados con la Neurología).

REMISIÓN DE MANUSCRITOS

Los manuscritos destinados a su publicación se remitirán por correo electrónico a: secretaria@viguera.com.

PRESENTACIÓN DE LOS MANUSCRITOS

1. Los trabajos deben ser inéditos, no haberse enviado simultáneamente a otras revistas ni estar aceptados para su publicación. En el caso de que se hayan publicado de forma parcial, por ejemplo como resumen, deberá indicarse en el manuscrito.

2. La extensión y número de figuras y tablas máximos permitidos se detallan en el anexo I.

3. Para la redacción de los trabajos, los autores pueden utilizar como guía los *Uniform Requirements for Manuscripts Submitted to Biomedical Journals, updated Feb 2006* (http://www.icmje.org) elaborados por el Grupo de Vancouver (Rev Neurol 1997; 25: 795-803). El envío de artículos deberá incluir un archivo en Microsoft Word con el texto y tablas del artículo, y los distintos archivos con las figuras (un archivo para cada figura, indicando el programa utilizado para su elaboración).

4. Los trabajos sometidos a *Revista de Neurología* para su publicación podrán remitirse en español o inglés. En los trabajos enviados en inglés, todo el proceso de revisión se realizará en este idioma; posteriormente, se traducirán al español para su publicación en los números regulares, reservándose *Revista de Neurología* la posibilidad de publicarlos también en el idioma original. En todo caso, los autores de habla española deberán facilitar finalmente una versión en español para su publicación.

5. Los autores pueden sugerir la sección que consideren más apropiada para valorar su publicación, aunque el Comité Editorial no asume el compromiso de seguir dicha sugerencia.

6. Los trabajos se acompañarán de una carta de presentación dirigida al Director de *Revista de Neurología*, donde se hará constar la conformidad de todos los autores con los contenidos del manuscrito y los posibles conflictos de interés con todos ellos.

7. El manuscrito constará básicamente de tres partes: la primera empezará con el título del trabajo, nombre y apellidos de cada autor, nombre del departamento/s e institución/es donde se ha realizado el trabajo, nombre y dirección del autor responsable de la correspondencia (incluyendo fax y correo electrónico de contacto, aunque dicho autor podrá solicitar que estos últimos datos no se publiquen), agradecimientos, ayudas o fuentes de financiación total o parcial, conflictos de interés (o su inexistencia), resumen estructurado, palabras clave. y palabras de cabecera (opcional).

8. La segunda parte contendrá el cuerpo del artículo, que se dividirán en apartados, de acuerdo con el siguiente esquema:

Originales: Introducción, Objetivo, Pacientes (o Sujetos o Materiales) y métodos, Resultados, Discusión.

Notas clínicas: Introducción, Caso/s clínico/s, Discusión.

Revisiones, Revisión en Neurociencia, Neurología de la Conducta, Historia y Humanidades: Introducción, Objetivo, Desarrollo (con los subtítulos que el autor crea conveniente) y Conclusiones.

La segunda parte finalizará con la Bibliografía, el resumen estructurado en inglés (si el autor quiere facilitarlo) y los pies de las figuras.

9. En la tercera y última parte del manuscrito se incluirán las tablas, cada una de ellas en una hoja aparte o separada por un salto de página. Cada tabla irá encabezada por su título y finalizará con su pie correspondiente (si existe).

10. Las figuras siempre se presentarán aparte, cada una en un archivo.

11. Los autores pueden facilitar una versión en inglés de su artículo (sin compromiso de aceptación por parte del Comité Editorial), que se incluiría en la versión *on-line* (www.neurologia.com) para su difusión electrónica. Con el mismo fin también puede acompañarse un vídeo por artículo, en formato Quicktime (.mov) o Windows (.mpg), con un tamaño máximo, una vez comprimido, de 5 Mb.

ESTRUCTURA DEL TEXTO

1. **Resumen.** Debe tener una extensión comprendida entre 200 y 250 palabras, y estructurarse de acuerdo con el siguiente esquema:

Originales: Introducción, Objetivo, Pacientes (o Sujetos o Materiales) y métodos, Resultados, Conclusiones.

Notas clínicas: Introducción, Caso/s clínico/s, Conclusiones.

Revisiones, Revisión en Neurociencia, Neurología de la Conducta, Historia y Humanidades: Introducción, Objetivo, Desarrollo, Conclusiones.

Aunque no es imprescindible, los autores podrán proponer una versión inglesa de su resumen en los manuscritos remitidos en español.

2. **Palabras clave**. Se incluirán por lo menos 6 palabras clave, ordenadas alfabéticamente y separadas por un punto, que deben permitir la clasificación e identificación de los contenidos del manuscrito. Se utilizarán preferentemente los términos incluidos en la lista del *Medical Subject Headline* de Index Medicus (http://www.nlm.nih.gov/mesh/MBrowser.html).

3. **Palabras de cabecera**. Corresponde a la frase breve (dos o tres palabras) que aparece en el margen superior derecho del artículo impreso.

4. **Desarrollo del manuscrito**:

a) Introducción/objetivo. Debe exponer claramente los antecedentes y el objetivo del trabajo, así como resumir las razones que han motivado su realización.

b). Pacientes (o Sujetos o Materiales) y métodos. Debe describir claramente los criterios de selección del material del estudio, pacientes y diseño del mismo. Cuando el manuscrito haga referencia a seres humanos el apartado se titulará necesariamente Pacientes y métodos, o bien Sujetos y métodos (cuando se incluya un grupo control de sujetos sanos).

c) Casos clínicos. Deberán describirse detalladamente. Las referencias a fármacos deben realizarse a través de nombres genéricos. Las unidades de parámetros paraclínicos y de laboratorio deben ajustarse a las normas internacionales. Deben señalarse claramente los métodos de evaluación estadística, así como el poder y los grados de significación.

d) Resultados. Deben describirse únicamente los datos más relevantes y no repetirlos en el texto si ya se han mostrado mediante tablas o figuras.

e). Discusión. No deben aparecer datos que no se hayan descrito en los resultados. Se considera de especial interés la discusión de estudios previos publicados en español. Las conclusiones deben incluirse en este apartado, salvo en las Revisiones, Revisión en Neurociencia, Neurología de la Conducta e Historia y Humanidades.

f) *Bibliografía*. Debe estar actualizada y se recomienda la citación de trabajos publicados en español (sobre todo de los dos últimos años), en especial de *Revista de Neurología*, que sean considerados relevantes por el autor. Las referencias se identificarán en el texto mediante números arábigos entre corchetes, alineados con la escritura (p. ej. [1-3,6]. Se enumerarán correlativamente por orden de aparición en el texto y se describirán en la hoja correspondiente según el formato de referencia adoptado por el *Uniform Requirements for Manuscripts Submitted to Biomedical Journals* (http://www.icmje.org). El año de publicación se situará inmediatamente después del título abreviado de la revista. Si una referencia se halla pendiente de publicación deberá describirse como [in press], siendo responsabilidad de los autores la veracidad de ésta. Los títulos de las revistas se abreviarán según las recomendaciones de la *List of Journals Indexed in Index Medicus* (http://www.ncbi.nlm.nih.gov/PubMed). No es admisible la referencia de comunicaciones personales. Ejemplos de referencias bibliográficas se ofrecen en el anexo II, y el número máximo de citas bibliográficas por tipo de artículo, en el anexo I.

g) *Tablas.* Se presentarán en formato de texto, nunca como una figura incrustada en el documento. Cada tabla se presentará separada por un salto de página, indicando claramente su numeración (en números romanos), correlativa según la aparición en el texto. El pie de tabla detallará el significado de las abreviaturas que aparezcan en ella, así como las llamadas, señaladas correlativamente con una letra en superíndice (p. ej. [a], [b]). Si el autor propone una tabla obtenida de otra publicación debe tener el correspondiente permiso y acompañarlo. Las secciones de Neuroimagen, Correspondencia, Cartas al Director y Crítica de Libros no contendrán tablas, salvo con permiso expreso del Comité Editorial.

h) *Figuras.* Para las fotografías (digitalizadas) el mejor formato es TIFF, con resolución de 300 ppp para un tamaño de imagen de 8,5 cm de ancho (y proporciones inversas equivalentes, es decir, 150 ppp para una anchura de imagen de 17 cm, etc.). Independientemente del programa con que se hayan elaborado, las figuras (especialmente gráficos) deben permitir su posterior tratamiento y manipulación informática por parte de la editorial, por lo que nunca deberán incrustarse en un documento sin el vínculo con el programa que las ha creado. Las reproducciones en color se publican de forma excepcional y siempre contando con la participación económica del autor. Las letras, números y símbolos que aparezcan en las figuras deben ser claros y uniformes, y de tamaño suficiente para que la reducción de la figura no conlleve su ilegibilidad. Las figuras se enumerarán correlativamente en cifras arábigas según la aparición en el texto. Si el autor propone una figura obtenida de otra publicación debe tener el correspondiente permiso y acompañarlo. Los pies de figura se incluirán con el artículo, nunca formando parte de la misma figura. Las secciones de Correspondencia y Cartas al Director nunca contendrán figuras, salvo con permiso expreso del Comité Editorial.

i) *Abreviaturas*. Deben usarse solamente abreviaturas estándares, ya que el uso de abreviaturas no estándares puede resultar extremadamente confuso para el lector. Debe evitarse el uso de abreviaturas en el título del trabajo y minimizar al máximo su aparición en el resumen. Las abreviaturas utilizadas por el autor deben definirse y describirse en el texto la primera vez que se mencionen.

PROCESO DEL MANUSCRITO

Los manuscritos se registrarán con un número de referencia, a partir del cual los autores podrán obtener información sobre el estado del proceso de revisión, que sigue las siguientes fases:

1. **Revisión editorial**. Se remite el trabajo al Comité Editorial, quien lo revisa y decide si se somete a revisión externa.

2. **Revisión externa o por pares *(peer review)***. Se remiten a revisión externa todos los manuscritos no rechazados en primera instancia por el Comité Editorial. Los manuscritos se remiten al menos a dos revisores considerados como expertos por *Revista de Neurología*. La elección de los revisores para cada trabajo se realiza atendiendo al contenido del manuscrito, aunque la política editorial de *Revista de Neurología* tiende a que, en la medida de lo posible, haya por lo menos un revisor de Europa y otro de América. Dependiendo de los contenidos del manuscrito podrán solicitarse evaluaciones técnicas, estadísticas y farmacológicas, cuando los trabajos se refieran a ensayos clínicos y utilización de fármacos.

3. **Aceptación o rechazo del manuscrito**. A través de los informes realizados por los revisores, el Comité Editorial establece la decisión de publicar o no el trabajo, pudiendo solicitar a los autores la aclaración de algunos puntos o la modificación de diferentes aspectos del manuscrito. En este caso, el autor cuenta con un plazo máximo de dos meses para remitir una nueva versión con los cambios propuestos; pasado dicho término, si no se ha recibido una nueva versión, la editorial considerará retirado el artículo. Asimismo, el Comité Editorial puede proponer la

aceptación del trabajo en un apartado distinto al propuesto por los autores.

4. **Revisión editorial.** La editorial revisa los aspectos formales del trabajo, descritos en estas normas. Un manuscrito puede ser devuelto a sus autores por incumplimiento de las normas de presentación. La exclusión o rechazo de un trabajo no implica forzosamente que no presente suficiente calidad, sino que quizá su temática no se ajusta al ámbito de la publicación.

5. **Revisión tras aceptación del trabajo.** Una vez aceptados los trabajos, los manuscritos se someten a una corrección morfolingüística y de estilo. Los autores podrán comprobar los cambios realizados por el corrector al recibir las galeradas, aprobar dichos cambios o sugerir modificaciones.

6. **Galeradas**. La editorial remitirá al autor responsable de la correspondencia las galeradas del trabajo para su revisión previamente a su publicación. Dicha revisión debe realizarse en cinco días naturales como máximo, puesto que la demora en la devolución de galeradas puede retrasar la publicación del artículo. No se admiten modificaciones en la estructura de los trabajos ya aceptados.

7. **Separatas**. La editorial remitirá a cada uno de los autores que haya facilitado su correo electrónico una copia facsimilar digital en PDF de cada trabajo. El autor puede solicitar, asumiendo su coste económico, la impresión de separatas en el momento de la recepción de las galeradas.

8. **Defensor del Autor**. *Revista de Neurología* dispone de este mecanismo de defensa de los autores en relación con aspectos éticos y del proceso editorial. Las reclamaciones deben dirigirse a: secretaria @viguera.com. Dichas reclamaciones nunca deben remitirse directamente a la persona que en cada momento ocupa esta función, sino a través de la editorial, y deben incluir la demanda de forma razonada en el ámbito de su competencia, así como el número de teléfono, fax o correo electrónico de contacto. El Defensor del Autor en *Revista de Neurología* es un profesional independiente de acreditado prestigio.

Anexo I. Extensión y número de figuras, tablas y referencias bibliográficas máximos, según el tipo de artículo.

	Extensión [a]	Figuras	Tablas	Ref. bibliográficas
Originales	30.000	6	3	50
Revisiones	40.000	6	3	80
Notas clínicas	20.000	3	2	25
Neuroimagen	3.000	2	0	5
Correspondencia	10.000	0	0	15
Cartas al Director	3.000	0	0	5
Crítica de libros	3.000	1 (portada)	0	0

[a] La extensión máxima contempla los espacios en blanco entre caracteres, e incluye bibliografía, resumen, tablas, pies de figuras y anexos.

Anexo II. Ejemplos de referencias bibliográficas:

Artículos: Se relacionarán todos los autores cuando sean seis o menos; si son más de seis, se citan solamente los seis primeros y se añade 'et al' tras una coma.
Más Sesé G, Plaza-Macías I, González-Caballero G, Sola Martínez D, Hernández-Hortelano E, Martín-Bautista D, et al. Análisis de los ingresos evitables en un servicio de neurología. Rev Neurol 2006; 43: 714-8.

Trabajo publicado por un grupo o institución:
Grupo ELEP. Estudio longitudinal de pacientes con enfermedad de Parkinson (ELEP): objetivos y metodología. Rev Neurol 2006; 42: 360-5.

Libro:
Mauri-Llerda JA, Vadillo-Olmo FJ. Crisis y epilepsia en el anciano. Barcelona: Viguera; 2006.

Capítulo de un libro:
Prats JM, Velasco F, García-Nieto ML. Cerebelo y cognición. In Mulas F, ed. Dificultades del aprendizaje. Barcelona: Viguera; 2006. p. 185-93.

Congresos:
Moore WS, Shifrin EG. Intravascular stapler for open or laparoscopic aortic surgery: experimental and early clinical results. In: Vascular Endovascular Issues Techniques Horizons. New York, EE UU, noviembre 2004.

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