Journal of Clinical Virology 122 (2020) 104213

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Prediction of unfavorable outcomes in West Nile virus neuroinvasive infection – Result of a multinational ID-IRI study

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ARTICLE INFO

Keywords:
West Nile virus
WNV
Meningitis
Encephalitis
Neuroinvasive
Death

ABSTRACT

Background: WNV causes 1.4% of all central nervous system infections and is the most common cause of epidemic neuro-invasive disease in humans.
Objectives: Our main objective was to investigate retrospectively West Nile virus neuroinvasive disease (WNND) cases hospitalized during 2010–2017 and identified factors that can influence prognosis.
Study design: We documented the demographic, epidemiologic, clinical and laboratory data of WNND and identified factors that can influence prognosis. The data were recruited through Infectious Diseases International Research Initiative (ID-IRI), which serves as a network for clinical researches.
Results: We investigated 165 patients with WNND in 10 countries from three continents. 27 patients died and the mortality rate was 16.4%. In an univariate analysis age, congestive heart failure, neoplasm and ischemic heart disease ($p < 0.001$), neuropsychiatric disorders ($p = 0.011$), chronic hepatitis ($p = 0.024$) and hypertension ($p = 0.043$) were risk factors for death. Fatal evolution was also correlated with ICU addmission, disorientation, speech disorders, change in consciousnes, coma, a low Glasgow coma score, obtundation, confusion

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https://doi.org/10.1016/j.jcv.2019.104213
Received 30 August 2019; Received in revised form 31 October 2019; Accepted 7 November 2019

($p < 0.001$), history of syncope ($p = 0.002$) and history of unconsciousness ($p = 0.037$). In a binomial logistic regresssion analysis only age and coma remained independent prediction factors for death. We created an equation that was calculated according to age, co-morbidities and clinical manifestations that may be used to establish the prognosis of WNND patients.

Conclusions: WNND remain an important factor for morbidity and mortality worldwide, evolution to death or survival with sequelae are not rare. Our study creates an equation that may be used in the future to establish the prognosis of WNND patients.

1. Background

West Nile virus (WNV) is an arbovirus of the genus *Flavivirus*, family *Flaviviridae* that is mostly transmitted through bites of *Culex* spp. mosquitoes. It has a worldwide distribution, originating from Africa where it was first documented and spreding to Middle East in the 1950s (Israel), in Europe in the 60 s (France), with a first major outbreak in Romania in 1996 (352 confirmed cases), and in United States in 1999, New York (59 cases) ([1–3]). Concordant - vectorborne WNV and Toscana virus central nervous system (CNS) infections are even reported in Southeast Europe [4]. Various patterns of virus spreading across Europe compared to United States have been observed. WNV disseminated rapidly in North America, but in Europe it remained only in the South and South Eastern parts.

Basically, 80% of infected people are asymptomatic, 15–20% developing a mild disease (West Nile fever) and less than 1% develops neurological manifestations like encephalitis, meningitis, meningoencephalitis, acute flaccid paralysis, ataxia and extrapyramidal signs, polyradiculitis, seizures, and eye nevritis. WNV causes 1.4% of all central nervous system (CNS) infections [5] and is the most common cause of epidemic neuro-invasive disease in humans [6]. Among patients with neurological manifestations, mortality rates varies (4.8% in 1996 in Romania, around 9% in United States, and more than 15% in Europe and Israel [7].

2. Objectives

In this study, our main objective was to investigate retrospectively West Nile virus neuroinvasive disease (WNND) cases hospitalized during 2010–2017 with a focus on factors that influence the prognosis of WNND because until now only few single factors (age, underlying diseases, coma, CCR5 deficiency or WNV virulent strains) were corellated with poor prognosis of WNV. Our aim was to find correlations between clinical data that influence prognosis of WNND and to produce an equation as a risk score that can be use by clinicians for WNND patients.

3. Study Design

We documented the demographic, epidemiologic, clinical and laboratory data of WNND and identified factors that can influence prognosis. The data were recruited through Infectious Diseases International Research Initiative (ID-IRI), which serves as a network for clinical researches (https://infectdisiri.wordpress.com/).

3.1. Case definitions

All cases compatible with a CNS infection and with confirmed laboratory diagnosis of WNV disease in accordance with the definitions below have been included in this study.

The case definition for **encephalitis**of 2013 Consensus Statement of the International Encephalitis Consortium [8] was used.

Case definition for **meningitis** included clinical evidence such as fever, headache, vomiting, nuchal rigidity or other signs of meningeal irritation and CSF WBC count ≥5/cubic mm [9].

When criteria for both meningitis and encephalitis were met, the cases was classified as **meningo-encephalitis**.

Other clinical forms of CNS like acute flaccid paralysis, myelitis or cerebellar ataxia have also been included in the study.

Glasgow coma scale (GCS) scores at admission with value more than 13 were recorded as mild, 9–12 as moderate, and ≤8 as severe [10].

Laboratory criteria were used from European Commission Decision [11]:

Laboratory test for **case confirmation**

At least one of the following four:

o Isolation of WNV from blood or CSF

o Detection of WNV nucleic acid in blood or CSF

o WNV specific antibody response (IgM) in CSF

- WNV IgM high titer AND detection of WNV IgG, AND confirmation by neutralization

Each center participated in the study used their own kits for diagnosis approved by local health authorities.

3.2. Ethical approval

The study was approved by the ethics committee of the Dr Victor Babes" Clinical Hospital of Infectious and Tropical Diseases from Bucharest, decision number 1320/25 Jan 2018 which was approved retrospective collection of data from patients records with anonymization of personal data.

3.3. Statistical analysis

Data were collected in Office Excel 2010 files and statistical processing was performed in IBM SPSS Statistics v.20. Continuous data were analyzed with F-test (for normally distributed data) and non-parametric approach Mann-Whitney-U test (for non-normally distributed data). Comparison of categorical data was assessed using the Pearson chi-squared and Fisher-exact tests, and non-parametric tests were Kruskal-Wallis and median test. The critical probability level of the statistical tests was $p < 0.05$. Risk ratios (RR) were used to compare incidence rates.

4. Results

4.1. Epidemiological and demographic data

165 patients (64.2% males) with WNND from 10 countries (Albania – 14 cases, Croatia - 3, Hungary - 7, Israel - 20, Italy - 2, Romania - 52, Russia -16, Serbia - 14, Turkey - 16, USA - 21) were included. The median age of the cases was 60 years old (IQR 43.5–72.00).

The peak incidence was in 2012 (32 cases) and in 2017 (30 cases) and the lowest number of cases was recorded in 2013 (5 cases). Most cases were reported between July and November (92.1%), most of the cases being diagnosed between August (64 cases – 38.8%) and September (60 cases – 36.4%) (Fig. 1). The median illnes duration until admission was 4 days, and the median hospitalization duration 14 days.

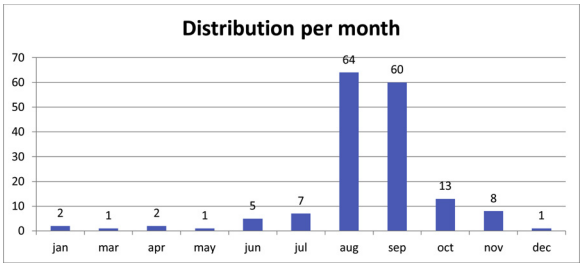

Fig. 1. Month distribution of cases.

4.2. Clinical findings

Of all WNND cases, 64 had encephalitis (38.8%), 60 with meningoencephalitis (36.4%), 34 meningitis (20.6%) and 7 (4.2%) had other types of neurologic manifestations (5 with acute flaccid paralysis, 1 with meningoencephalomyelitis and other with cerebellar ataxia). The most common clinical manifestations were: fever (95.2%), headache (83%), confusion (51.5%), change in consciousness (50.9%), disorientation (48.5%), stiff neck (46.1%), obtundation (43.6%), dizziness (42.4%), nausea and/or vomiting (41.2%), positive Kernig sign (40.6%), positive Brudzinski sign (34.5%), speech disorders (32.7%) and ataxia (24.8%).

In terms of GCS values, 36 patients (21.8%) had severe and 26 had moderate scores (15.8%). Progresion to coma was reported in 45 patients (26.9%) and 22 patients experienced seizures (13.3%). Other registred symptoms were myalgia in 21.8% of patients (36 cases) and rash in 32 cases (19.4%) (Table 1).

At least one underlying chronic medical conditions was reported for 105 patients (63.6%): with the most frequent being hypertension – 65 cases, ischemic hearth disese – 37, diabetes mellitus – 32. (Table 1).

4.3. CSF analysis

Lumbar puncture was performed in 153 patients (92.7%) and the CSF was abnormal with a pleocytosis > 5 cells/mmc in 147 (96%) patients, average of 80 cells/mmc. 85 (55.6%) had more than 20% polymorphonuclear cells, 56 (36.6%) even with values higher than 50%. 112 (73.2%) patients had also an incresed value of proteins in the CSF.

4.4. Virological data

Etiologic diagnosis of WNV infection was based on CSF positive WNV IgM antibodies in 74 patients, CSF positive WNV-RNA detection - 17 cases, blood positive WNV-RNA detection - 11 cases, WNV viral isolation from blood – 10 cases, WNV viral isolation from CSF – 3 cases, high titer of WNV IgM antibodies in blood – 47 cases and in 3 cases we had seroconversion to positive WNV IgM antibodies in the second blood test (2 of these 3 cases had also positive WNV IgG antibodies in the second blood test) (Table 1).

4.5. Cerebral imaging

Computed tomography (CT) scan was performed for 109 patients and magnetic resonance imaging (MRI) for 58 patients, 44 had both CT and MRI performed. Only 15 (13.8%) of CT scans had pathologic aspects and 37 (63.8%) of MRI was abnormal. Most frequently, we observed inflammation (7 cases for CT and 22 cases for MRI) and white matter involvement (5 cases for CT and 12 cases for MRI) (Table 2).

4.6. Treatment

Among 165 patients with WNND, 54 (32.7%) were admitted to the

Table 1
Epidemiological, demographic, clinical and laboratory data.

Variables	WNV N = 165
Age median (IQR)	60(43.5–72.00)
Gender, M/F, (%/%)	106/59(64.2/35.8)
Envinronment, urban/rural, n(%)	92/39 (55.8/23.6) (34 unknown)
Days from onset, median, days (IQR)	4.0(3.0–7.0)
Lenght of stay, median, (IQR)	14.0(9.00–19.00)
ICU admission, yes n(%)	54(32.7)
ICU duration	8.5(3.75–14.0)
Co-morbidities, n(%)	
Hypertension	65(39.4)
Congestive heart failure	12(7.3)
Ischemic heart disease	37(22.4)
Solid organ transplantation	6(3.6)
Cancer	8(4.8)
Diabetes mellitus	32(19.4)
Chronic kidney failure	13(7.9)
Neuropsychiatric disorders	19(11.5)
Sequelae of stroke	6(3.6)
Chronic hepatitis	6(3.6)
Cirrhosis	3(1.8)
Obesity	12(7.3)
Autoimmune disease	1(0.6)
Signs and Simptoms, n(%)	
Disorientation	80(48.5)
Personality changes	22(13.3)
Speech disorders	54(32.7)
Convulsion	20(12.1)
Amnesia	6(3.6)
Hallucination	5(3.0)
Change in consciousness	84(50.9)
History of unconsciousness	12(7.3)
Coma	45(26.9)
Glasgow coma scale score-numeric value median (IQR)	14.0(12.00-15.00)
GCS severe (value 0–8)	36(21.8)
GCS moderate (value 9–12)	26(15.8)
GCS mild (value 13–15)	103(62.4)
Obtundation	72(43.6)
Confusion	85(51.5)
Abulia	25(15.2)
Hemiparesis	21(12.7)
History of syncope	7(4.2)
Ataxia	41(24.8)
Dysmetria	27(16.4)
Dizziness	70(42.4)
Facial and hypoglossal cranial nerve palsy	5(3.0)
Fever	157(95.2)
Headache	137(83.0)
Nausea and vomiting	68(41.2)
Neck stiffness	77(46.1)
Rash	32(19.4)
Myalgia	36(21.8)
Cough	10(6.1)
Kernig sign	67(40.6)
Brudzinski sign	57(34.5)
Laboratory data and other paraclinic investigations	
WNV isolation from blood, n(%)	15(9.1)
WNV isolation from CSF, n(%)	5(3.0)
Detection of WNV nucleic acid from blood (positive), n(%)	13(7.9)
Detection of WNV nucleic acid from CSF (positive), n(%)	17(10.3)
WNV specific antibody response (IgM)-in blood – initial sample, n(%)	147(89.1)
WNV specific antibody response (IgM)-in blood - second sample, n(%)	63(38.2)
WNV specific antibody response (IgM) in CSF, n(%)	74(44.8)
WNV IgG in blood, n(%)	49(29.7)
Leukocyte count, n	9940(7700–12745)
Neutrophile (%)	77(70.75–84.37)

(continued on next page)

Table 1 (*continued*)

Variables	WNV N = 165
Lymphocyte (%)	14.6(9.21–21.00)
Monocyte (%)	6(4.0–9.0)
CRP mg/l	5(1.0–22)
ALT	33.0(24.0–56.0)
Creatinine mg/dl	1.0 (0.9–1.4)
CSF leukocyte count	80(32.5–205.0)
CSF neutrophile (%)	45.5(15.0–70.0)
CSF lympho monocytes (%)	64.5(37.0–92.0)
CSF protein g/l	0.87(0.62–1.14)
CSF glucose g/l	0.61(0.52–0.77)
Glasgow coma score (init)	14(12.0–15.0)
Treatment strategy	
Antiviral treatment (n-acyclovir, %)	94 (57.0)
Antiviral treatment duration (days)	8(3.0–11.0)
Empirical antibiotic treatment, yes(%)	144(87.3)
Duration ab1	7(4.25–10.0)
Corticosteroids, yes n,(%)	119(72.1)
Duration of corticosteroids treatment (days)	7(5.0–10.0)
Corticosteroids dosage (cortisone daily dose)	500(500–750)
Outcome ad follow-up, n(%)	
Survival	138(83.6)
Death	27(16.4)
1 month follow-up	84(50.9)
Alive at 1 month follow-up	109(66.1)
Sequelae at discharge	59(35.8)
Sequelae at 1 month follow-up	32(19.4)

Intensive care unit (ICU) with a median ICU lenght of stay of 8.5 days. Antiviral treatment with Acyclovir was administered in 94 patients with an 8 days median duration. Even if WNND is a viral disease 145 patients received at least one antibiotic (most frequent cephalosporins – 127, vancomycin – 44, penicillines - 31, carbapenems – 20) with a 7 days median duration. Corticosteroids were used for 119 patients (72.1%), with a median dose of 500 mg cortisone daily for a 7 days median duration.

4.7. Outcome

Overall, 27 patients died and the mortality rate was 16.4%. Information at one month follow-up was available for 84 patients with one new death. Of all survivors at discharge 59 (35.8%) had sequelae and at one month follow-up remain only 32 patients with sequelae (19.4%). Weakness at discharge was the most common sequelae in 24 patients, persistents headache was reported in 10 cases, different types of paresis in 8 cases and confusion or disorientation in 5 cases.

In order to establish a corelation between patient's characteristics and evolution to death, we compared the 27 deceased patients with the survivors and defined in an univariate analysis the risk factors for a fatal evolution: age and several comorbidities: congestive heart failure, ischemic heart disease, neoplasm ($p < 0.001$), hypertension ($p = 0.043$) neuropsychiatric disorders ($p = 0.011$), chronic hepatitis ($p = 0.024$). A series of clinical manifestations were associated with a severe evolution: ICU addmission, disorientation, speech disorders, change in consciousnes, coma, a low Glasgow coma score, obtundation, confusion ($p < 0.001$), history of syncope ($p = 0.002$) and history of unconsciousness ($p = 0.037$). On the other hand the presence of rash was correlated with good evolution ($p = 0.027$) (Table 2). At a subsequent binomial logistic regresssion analysis only age, with a threshold of 60 years and coma remained independent prediction factors for death. GCS scores were statistically significant only in univariate analysis for severe and moderate values, and correlated with evolution to death. But, when we compared the categories only comparison between severe and mild or moderate and mild values were significant. The relative risk for death is 2.4 for those aged > 60 years (Pearson test -$\chi 2$ $p = 0.019$), 3.5 at > 65 years and 4.8 at 75 years.

4.8. Risk score

A risk score was calculated according to age, co-morbidities (weighted co-morbidities) and clinical manifestations (also as weighted manifestation). We established weighted index of comorbidities and manifestation related to p-value (probabilities) with significance defined by $p < 0.05$) and RR (risk ratio) values, RR values where noted according to scale: 1 (1.2–1.4), 2 (1.5–2.4) and 3 (2.5–3.5) [12]. From our database we had 3 co-morbidities with $p < 0.05$, the RR was 3 for congestive heart failure and ischemic heart disease and 1 for for hypertension. Out of 6 clinical manifestations with $p < 0.05$ the calculated RR: was 1 for disorentation and confusion, 2 for speech disorders, change in consciousness and obtundation and 3 for coma.

After a binomial regression logistic analysis with the dependent variable survival status (death versus survivors), the following equation was obtained: Log (odss) = (0.041*Age) + (2.479 * total manifestations value > 10) + (1.146 * comorbidities total value > 2) - 4.524. The equation showed a good prediction value (AUROC = 81%,

Table 2
Analysis by evolution (death vs. survivor) - statistically significant data.

Variables (120)	Death at discharge n = 27	Survival at discharge n = 138	p
Age median (IQR)	75(52–80)	59(42.75–69.25)	0.003
ICU admission	19(70.4)	37(25.4)	< 0.001
Co-morbidities, n(%)			
Hypertension	15(57.7)	50(36.5)	0.043
Congestive heart failure	7(26.9)	5(3.7)	< 0.001
Ischemic heart disease	15(55.6)	22(16.3)	< 0.001
Cancer	5(18.5)	3(2.2)	< 0.001
Neuropsychiatric disorders	7(25.9)	12(8.8)	0.011
Chronic hepatitis	3(11.1)	3(2.2)	0.024
Signs and Simptoms n, (%)			
Disorientation	21(91.3)	59(48.8)	< 0.001
Speech disorders	17(77.3)	37(30.6)	< 0.001
Change in consciousness	24(96.0)	60(43.8)	< 0.001
History of unconsciousness	4(22.2)	8(7.0)	0.037
Coma	20(74.1)	23(16.7)	< 0.001
Glasgow coma scale score-numeric value, median (IQR)	9.08(7.58–10.57)	13.49(13.05-13.93)	< 0.001
GCS severe (0-8)*	13(48.1)	23(16.7)	< 0.001*** 0.903**
GCS moderate (9-12)**	9(33.3)	17(12.3)	< 0.001*** 0.903*
GCS mild (13-15)***	5(18.5)	98(71.0)	< 0.001* < 0.001**
Obtundation	20(95.2)	52(44.4)	< 0.001
Confusion	21(95.5)	64(53.3)	< 0.001
History of syncope	4(17.4)	3(2.5)	0.002
Rash	1(4.2)	31(24.2)	0.027
Laboratory data and other investigations Average, CI90%			
Creatinine	1.67(1.03-2.31)	1.22(1.07–1.37)	0.041
CSF neutrophils (%)	60.26(47.99–72.54)	40.70(35.36–46.04)	0.004
Cranial CT scan (admission)	Normal aspect 14(70%) Inflammation 4(20%)	Normal aspect- 80(89.9%) Inflammation 3(3.4%)	0.041
Cranial MRI (admission)	Normal aspect -0(0.0%) Inflammation 8 (100%)	Normal aspect 21(42%) Inflammation 14(28.0%)	0.004

$p < 0.001$). As such we propose the use of this equation in further analysis to determine the risk for severe evolution for a person admitted with WNND.

5. Discussion

This study reports predictive factors of unfavorable outcomes in WNV neuroinvasive infection derived from a multinational retrospective research involving patients with CNS disease. 165 patients were enrolled in the study covering geographically Middle East (Israel), Europe (8 countries) and United States, and to our knowledge is the first study including patients from three continents. Annual number of cases showed two peak periods 2010–2012 (80 cases) and 2016–2017 (57 cases) that can be correlated with climate and with changes in circulating strains of WNV (only Israel and USA remain with initial lineage I and only genotype changes, in the other countries new lineage II strains have been introduced after 2007 in addition to lineage I).

Different patterns of epidemiological spread of WNV have been observed in these 10 countries. In Israel, WNV have been circulated since 1950, but the first large-scale human outbreak occurred in 2000 with more than 400 cases and 40 deaths [13]. After 1999 New York outbreak, WNV has rapidly spread in all country with 24657 cases of WNND in 1999–2018 period with 2199 deaths (9%) [14]. In both countries the same lineage I involved in human cases, the strain from New York outbreak was closely related to a lineage I strain from Israel 1998 [15]. After introduction, a new genotype (WN02) was responsible for increasing efficiency and rapidity of viral transmission in North America mosquitos in USA [13,16]. In Romania, after 1996 lineage I outbreak, only sporadic cases have been reported until 2010 when a new small outbreak occurred, with change of the lineage to Volgograd 2007 Russia lineage II strain and later Nea Santa 2010 Greece lineage II strain [17]. The same replacement from lineage I to lineage II WNV or co-circulation of both lineages have been observed in the other countries [18–27].

Currently, patients with WNND have limited treatment options and the main option remains supportive care. In the treatment of our patients 87.8% received at least one antibiotic and 57% acyclovir, both without effect on WNV infection. This frequent using of antibiotics could explain by the severity of the disease (75.2% had encephalitic involvement), 32.7% admitted in ICU or by the presence of polymorphonuclear cells in more than 20% of the cells in the CSF (45.5%), which makes the differential diagnosis with bacterial infections more difficult at the onset. A rapid laboratory diagnosis of WNV may decrease the period for using antibiotics or acyclovir, which was 7 and 8 days respectively in our study.

In our study, WNND affects especially older persons and males. The association between age and WNND has been well described in literature, but the reason of higher incidence in males are unknown, maybe correlated with underlying diseases that are more frequent in men and also linked with WNND [28].

The outcome of WNND is often characterized by neurologic and cognitive sequelae and long-term excess all-cause mortality. In this study, 27 patients died with 16.4% mortality rate at discharge. This mortality rate is comparable to literature, but inside the study we observed differences between sites with high mortality in Turkey (37%), Romania (25%) and USA (19%) or sites with low Israel (10%), Russia (6.25%), Serbia (7.1%) or zero mortality rates (Albania, Croatia, Hungary and Italy). These may be linked to differences in hospital admission according to hospital level and with the fact that in some countries only more severe cases may have been tested for WNV. On the other hand, it is a low number of cases from Italy, Croatia and Hungary. For Romania, Turkey and USA all hospitals are tertiary centers with intensive care units where the most difficult cases in the region are admitted. All these differences in mortality rates are not important in our analysis because all cases respect the same case definition and we can make a significant statistical analysis for WNND, but there are not representative for countries, where rates can be lower or higher than in our study.

Even if we reported many factors (clinical or co-morbidities) correlated with death in univariate analysis, only age and coma remained independent prediction factors for death at binomial logistic regresssion analysis. This was reported in the literature too [7,29,30]. According to our data, 105 patients had underlying diseases but binominal logistic regression could not estabilish association to outcome. Thus, based on our study data, we calculated a risk factor using age, weighted clinical manifestations and co-morbidities and obtain an equation that may be used in the future to establish the prognosis of WNND patients. Furthermore, 59 of survivors (35.8%) were discharged with sequelae and 32 (19.4%) patients had persisting sequelae at one-month follow-up. Weakness, headache, cognitive disorders and paresis, as the most frequent findings, were consistent with current literature [31–33].

It is well-know that age have been reported as an independent risk factor for WNND and mortality after infection. There are other studies indicating coma or the underlying diseases as prognostic factors of mortality [34–36].

Our study has several limitations; first, this is a retrospective study and is dependent on clinicians who collected data, second the patient group is not homogeneous and there may be differences between hospitals, access to ICU or to brain imaging, and third the high mortality rate in some centers might be related to referral patterns, admission of severe cases and increased physician awareness, and fourth the lineages of WNV may influence the outcomes.

In conclusion, WNND remain an important factor for morbidity and mortality worldwide, evolution to death or survival with sequelae are not rare, and represent a public health priority. Our study creates an equation that may be used in the future to establish the prognosis of WNND patients.

Credit author statement

Corneliu Petru Popescu: conceived the study and coordinated the manuscript, methodology, formal analysis, writing original draft, editing, writing review.

Simin Aysel Florescu: conceptualization, methodology, formal analysis, writing original draft, editing, writing review.

Rodrigo Hasbun, Arjan Harxhi, Razi Evendar, Hasip Kahraman, Ami Neuberger, Mihaela Florentina Zaharia, Selma Tosun, Emanoil Ceausu, Simona Maria Ruta, Gorana Dragovac, Natalia Pshenichnaya, Galina Gopatsa, Olga Shmaylenko, Éva Nagy, Jelena Djekic Malbasa, Mirjana Strba, Nenad Pandak, Husnu Pullukcu, Botond Lakatos, Yasemin Cag, Antonio Cascio, Ilaria Coledan and Serkan Oncu: collected patients' data and critically review of the manuscript.

Hakan Erdem: conceived the study and coordinated the manuscript, methodology, formal analysis, writing original draft, project administration, editing, writing review.

Daniel Codreanu: performed statistical analysis.

All authors critically reviewed the manuscript and provided valuable comments.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

"The research of the Romanian patients was funded through CCCDI-UEFISCDI project PN-III-P1.2-PCCDI-2017-0005/2018, Romania."

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